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COMPLETE ABSENCE OF LINKAGE DISEQUILIBRIUM BETWEEN ENZYME LOCI IN NATURAL POPULATIONS OF *Drosophila ananassae*

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Linkage disequilibrium has been studied among three linked enzyme loci of second chromosome of *D. ananassae* collected from five natural populations. Each of the three enzyme loci, that is *Acph*1, *Acph* 2 and *Xdh* was represented by two distinct alleles and in three genotypic forms. Thus nine genotypic combinations were recorded for each enzyme pair. The results clearly show absence of non-random occurrence of different genotypic combinations in all the populations studied. The occurrence of all possible genotypic combinations indicates enough frequency of crossing over among these enzyme loci. The absence of linkage disequilibrium in this study thus indicates that selection did not play any role and free recombination among the genes resulted random occurrence of all combinations.

Key words: Allozyme, crossing-over, *D. ananassae*, linkage disequilibrium

INTRODUCTION

A number of animal and plant populations have been analyzed for their genetic characteristics and the results reveal that they are very much polymorphic. When electrophoretic variants of enzymes are taken into consideration, the average proportion of heterozygous loci per individual may be about 20-25% for a majority of plants and animals, vertebrates as well as invertebrates. The amount and pattern of genetic variation in *Drosophila* populations have been of extreme interest to investigators in the area of population and evolutionary genetics, and our knowledge in this area is continuously expanding. Gel electrophoresis of single fly has been carried out in *Drosophila* to study allelic frequency of different enzymes and to observe that to what extent the populations have undergone genetic differentiation (SINGH and RHOMBERG, 1987; MORTON *et al.,* 2004). A large number of allozyme systems have been surveyed in several species of *Drosophila* and it has been concluded that allozyme polymorphism in natural populations of *Drosophila* is maintained by natural selection (AYALA *et al.*, 1972)

The term "linkage disequilibrium" was introduced by LEWONTIN and KOJIMA (1960) to refer to nonrandom association of alleles at two loci. HILL and ROBERTSON

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(1968) and OHTA and KIMURA (1969) demonstrated that linkage disequilibrium may be an indication of genetic drift in populations. LEWONTIN (1974), on the other hand stressed that linkage disequilibrium is theoretically a sensitive indicator of natural selection. A number of workers analyzing allozyme variation in *Drosophila* also attempted to detect linkage disequilibrium between loci, considering that if selection causes genetic polymorphisms to exist, then one might expect linkage disequilibrium for some enzyme loci. The conclusion from all these studies is that virtually no linkage disequilibrium exists among allozyme loci. This would indicate that any epistatic fitness interactions that might exist are not strong enough to overcome the randomizing effect of recombination.

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). Such studies could answer many questions related to enzyme polymorphism, like, are allozyme polymorphisms maintained because the loci coding for the enzymes lie in the region of the chromosome exhibiting inversion polymorphism or do they follow their own dynamics? Japanese populations of *D. melanogaster* were analyzed for enzyme and chromosomal polymorphism by WATANABE and WATANABE (1977) and they reported that significant positive correlation between frequencies of Adh S and *In(2L)B* was caused by linkage. Gametic disequilibrium studies have been done by RODRIGUEZ *et al.* (2001) 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 explained that restriction of recombination in heterokaryotypes seems to be the best explanation for the occurrence of linkage disequilibrium between inversion and loci located inside the rearranged segments. However, epistatic interactions between *Xdh* (outside of the inversion) and loci tightly linked to inversions, is the most likely explanation for the association between *Xdh* and chromosomal inversions.

Drosophila ananassae is a cosmopolitan and domestic species and it belongs to the *ananassae* species complex of the *ananassae* subgroup in the *melanogaster* species group. It occupies a unique status in genus *Drosophila* due to certain peculiarities in its genetic behavior (SINGH, 2000; SINGH, 2010). Chromosomal polymorphism has been extensively studied in natural and laboratory populations of this species. It has also been used in the study of its behaviour especially sexual behavior and pupation height preference (SINGH, 2010). However, *D. ananassae* has not been brought too much in use for studying enzyme polymorphism. Recently, work on the allozyme polymorphism in natural populations of this species has been undertaken to envisage the level of genetic variation among the Indian natural populations (KUMAR and SINGH, 2012a; 2012b; KUMAR and SINGH, 2013a; 2013b; KRISHNAMOORTI and SINGH, 2013; SINGH *et al.* 2013). While working on the allozyme polymorphism in *D ananassae*, we came to know that *Xdh, Acph1* and *Acph2* are the linked enzyme loci and thus could be involved for the study of linkage disequilibrium. The present study aims to detect linkage disequilibrium among three enzyme loci i.e. *Xdh, Acph 1* and *Acph 2,* present on 2L chromosome arm of *Drosophila ananassae* in five natural populations of South India.

MATERIALS AND METHODS

Drosophila ananassae flies were collected from five different places of South India, Washi (WSI), Akola (AKL), Solapur (SLP), all the three from Maharastra state, Bellary (BLY) from Karnataka state and Thrissure (TSR) from Kerala. Naturally impregnated females were

cultured in individual vials to establish isofemale lines. After emergence of progeny, individual flies from isofemale lines were used to analyze allozymes. For this a single fly was homogenized in 50 µl of 20 mM Tris buffer (pH 7.4) and the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes. Supernatant was separated into two aliquots and subjected to 8% native polyacrylamide gel electrophoresis in 25mM Tris and 250 mM Glycine electrode buffer at 200V for 4 hour at 4 °C. In-gel staining for Xanthine dehydrogenase (*Xdh*) and Acid phosphates (*Acph*) enzymes were done according to AYALA *et al.,* (1972).

RESULTS

We are able to record three genotypes for each of the three enzyme loci studied. Since these loci are linked on a single chromosome, intrachromosomal associations among these can be looked into by seeing the association of genotypes related with two enzyme loci. Due to occurrence of three genotypes in all three loci, nine combinations between *Xdh-Acph 1, Xdh-Acph 2* and *Acph1-Acph2* could be ascertained. Under the assumption of random combination of genotypes, their expected numbers have been calculated from the marginal totals of R X C contingency table. The significant deviation from expectation would indicate the non random association between allozyme loci. Observed and expected numbers of different combinations between *Xdh* and *Acph1* loci in five natural populations of *D. ananassae* is shown in Table1. χ^2 analysis shows that there is good agreement between observation and expectation indicating random occurrence of various combinations. Table 2 depicts observed and expected numbers of nine genotypic combinations between *Xdh* and *Acph2* loci. In this case also there is insignificant difference between observed and expected values in all the five populations indicating random association among the different karyotypic combinations. In Table 3, observed and expected number of *Acph*1 and *Acph2* genotypic combinations for these five populations has been shown. Like earlier observation, we were able to record all possible combinations, and there is random occurrence of these combinations in all the five populations.

Table 1. Observed and expected numbers of different combinations between Xdh and Acph1 loci in natural populations of D. ananassae

Populations		Genotype combinations									
	Xdh *Acph 1*	*0.98/0.98 0.96/0.96*	*0.98/0.98 0.96/1.00*	*0.98/0.98 1.00/1.00*	*0.98/1.00 0.96/0.96*	*0.98/1.00 0.96/1.00*	*0.98/1.00 1.00/1.00*	*1.00/1.00 0.96/0.96*	*1.00/1.00 0.96/1.00*	*1.00/1.00 1.00/1.00*	χ^2
WSI	Obs.	2	1	5	0	2	10	1	5	10	6.414*
	Exp.	0.66	1.77	5.55	1	2.66	6.9	1.33	3.55	11.11	
AKL	Obs.	4	3	2	9	1	2	7	2	4	3.363*
	Exp.	5.29	1.58	2.11	7.05	2.11	2.82	7.6	2.29	3.05	
SLP	Obs.	4	3	8	5	2	3	14	5	4	5.968*
	Exp.	7.18	3.12	4.68	4.79	2.08	3.125	11.02	4.79	7.18	
BLY	Obs.	9	1	4	8	4	4	10	5	3	2.597*
	Exp.	7.875	2.91	3.2	9	3.33	3.66	10.125	3.75	4.125	
TSR	Obs.	1	2	8	2	2	8	4	5	16	0.305*
	Exp.	1.6	2.06	7.33	1.75	2.25	8	3.64	4.68	16.66	

df = 4, *Insignificant

Table 2. Observed and expected numbers of different combinations between Xdh and Acph2 loci in natural populations of D. ananassae

Populations		Genotype combinations									
	Xdh	*0.98/0.98*	*0.98/0.98*	*0.98/0.98*	*0.98/1.00*	*0.98/1.00*	*0.98/1.00*	*1.00/1.00*	*1.00/1.00*	*1.00/1.00*	χ^2
	Acph 2	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	
WSI	Obs.	4	2	16	1	1	8	0	2	2	5.537*
	Exp.	3.05	3.05	15.88	1.38	1.38	7.22	0.55	0.55	2.88	
AKL	Obs.	5	2	2	5	2	5	8	3	2	2.38*
	Exp.	4.76	1.8	2.3	6.3	2.47	3.17	6.88	2.67	3.44	
SLP	Obs.	9	3	3	5	0	5	12	4	7	3.822*
	Exp.	8.15	2.18	4.68	5.41	1.45	3.125	12.45	3.35	7.18	
BLY	Obs.	9	1	3	8	7	2	9	3	6	6.541*
	Exp.	7.04	2.97	2.97	9.2	3.89	3.89	9.75	4.15	4.125	
TSR	Obs.	1	1	9	4	1	7	3	3	19	3.325*
	Exp.	1.83	1.14	8.02	2	1.25	8.75	4.16	2.6	18.22	

df = 4, *Insignificant

Table 3. Observed and expected numbers of different combinations between Acph 1 and Acph 2 loci in natural populations of D. ananassae

Populations		Genotype combinations									
	Acph 1	*0.96/0.96*	*0.96/0.96*	*0.96/0.96*	*0.96/1.00*	*0.96/1.00*	*0.96/1.00*	*1.00/1.00*	*1.00/1.00*	*1.00/1.00*	χ^2
	Acph 2	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	*0.96/0.96*	*0.96/1.00*	*1.00/1.00*	
WSI	Obs.	0	0	3	0	2	6	3	3	19	2.701*
	Exp.	0.25	0.41	0.19	0.66	0.71	0.007	0.40	0.063	0.009	
AKL	Obs.	12	4	4	1	1	4	5	2	1	5.621*
	Exp.	10.58	4.11	5.29	2.11	1.23	1.58	4.23	1.64	2.11	
SLP	Obs.	16	2	5	4	3	3	7	2	6	3.203*
	Exp.	12.93	3.35	6.7	5.62	1.45	2.9	8.43	2.18	4.37	
BLY	Obs.	17	5	5	3	3	4	7	2	2	3.61*
	Exp.	15.18	5.62	6.18	5.625	2.08	2.29	6.18	2.29	2.5	
TSR	Obs.	1	1	5	3	2	4	4	2	26	5.003*
	Exp.	1.16	0.72	5.10	1.5	0.93	6.56	5.33	3.33	23.33	

df = 4, *Insignificant

DISCUSSION

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 nil. Indeed recombination is even known to occur within a gene and as suggested by CHARLESWORTH and CHARLESWORTH (1973), there is no correlation between linkage and magnitude of linkage disequilibrium. However, two genes may lie in a low recombination zone of the genome and in such a case linkage disequilibrium may be so absolute that the other possible combinations may have no representation in the population. An extensive work on allozyme-allozyme linkage disequilibrium in natural populations of *D. melanogaster* has been done involving 36 allozyme pairs (LANGELY *et al.,* 1974). Among all these, only three showed significant deviation from expectation and only one of them (*Odh-Ao*) could be established as indeed showing nonrandom association. In another study, LANGLEY *et al.* (1977) found no linkage disequilibrium among even tightly linked loci which are nearly 3cM distant from each other in natural populations of *D. melanogaster*.

When we test association between loci, it is important to realize that random mating does not absolutely restore the population to equilibrium 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. SINGH and SINGH (2010), 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. In the present study, nine genotypic combinations possible between two enzyme loci were found to be distributed randomly in all the three enzyme pairs studied in all the natural populations. *D. ananassae* is a species which exhibits some unique features, one of them being spontaneous male meiotic recombination (SINGH, 2010). Thus, in *D. ananassae* the recombination rate is higher than the other species, where males are not known to have recombination during meiosis. This may be one of the reasons for the absence of linkage disequilibrium in the present study. When these three loci are observed on the polytene chromosome positions it was found that *Xdh* is located on 28A and *Acph* at 37B on 2L arm (STEPHEN *et al.,* 2008; KUMAR and SINGH, 2013b) and there is approximately 11.5 Mb distance between the two. *Acph 1*and *Acph 2* loci are clustered together owing to gene duplication during the course of evolution. The random occurrence of different genotypic combinations in the present case is due to free recombination among the three enzyme loci.

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POTPUNO ODSUSTVO DISEKVILIBRIUMA UKOPČANOSTI IZMEĐU ENZIMATSKIH LOKUSA NA DRUGOM HROMOZOMU *Drosophila ananassae*

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Izvod

Vršena su ispitivanja disekvilibriuma između tri ukopčana enzimatska *D. ananassae* sakupljene iz pet prirodnih populacija. Svaki od tri enzimatska lokusa, *Acph*1, *Acph* 2 and *Xdh* koji su pretstavnici dva različita alela u tri forme genotipa. Utvrđeno je devet kombinacija genotipa za svaki enzimatski par. Rezultati jasno pokazuju odsustvo pravilog događanja različitih kombinacija genotipova u svim ispitivanim populacijama. Pojava svih mogućih kombinacija genotipova ukazuje dovoljnu učestalost krosin – overa između tih enzimatskih lokusa. Odsustvo disekvilibriuma ukopanosti ukazuje da selekcija nije imala ulogu i da je slučajna rekombinacija gena i da su slobodne rekombinacije gena rezultat slučajne pojave svih kombinacija.

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