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Random Genetic Drift Affecting Alcohol Dehydrogenase Polymorphism in Laboratory Populations of *Drosophila ananassae*

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Abstract

A mass culture stock of *Drosophila ananassae* established from Ranchi (Jharkhand) was analyzed for its Adh polymorphism and the stock was observed to be polymorphic at this locus as all the three genotypes (FF, FS and SS) could be recorded. From this stock, five separate lines were established by utilizing five pairs of flies. These five lines were maintained for at least five generations and in each generation twenty pairs were used for starting the next generation. It was observed that *Adh* polymorphism get affected very drastically in all the five lines as compared with the mass culture stock from which they were established. The present study thus clearly indicates the effect of random genetic drift on alcohol dehydrogenase polymorphism in laboratory populations of D. ananassae.

Keywords: Allozyme polymorphism, Alcohol dehydrogenase, genetic drift, *Drosophila ananassae.*

Introduction

Genetic polymorphism is maintained due to higher Darwinian fitness of heterozygotes (Dobzhoansky 1970). The factors which cause changes in the frequencies of alleles and genotypes in a population have been considered as important elemental forces of evolution. Among different elemental forces of evolution, natural selection and genetic drift are important in causing alterations in gene frequencies in populations (Hickey 1979, Hilbish and Koehn 1985). In a given environment, certain alleles or genotypes may be favored due to high adaptive values of their carriers by selection which will lead to gradual enhancement in the frequencies of those alleles in populations. However, in small populations gene frequencies may fluctuate significantly due to random genetic drift. The roles of evolutionary forces like selection and random genetic drift have been demonstrated in several animal species. It is very interesting to understand whether the phenomenon of polymorphism is affected more by random genetic drift or by natural selection (Kimura 1983, Koehn et al 1983).

Allozymes are allelic variants of an enzyme encoded by a particular locus. Numerous studies have been done on allozyme variation and its effect on the mechanism of evolution. It has been observed that allelic frequency changes at a particular allozyme locus in laboratory populations and are often considered as evidence for the occurrence of selection (Berger 1997, Ayala and Anderson 1973, Fontdevila et al 1975). Lewontin and Hubby (1966) undertook the first

extensive analysis of protein polymorphism in natural population of *Drosophila pseudoobscura* and studied 18 gene loci. Allozyme variation is widespread in most organisms including humans in which about 30 percent of all enzyme coding genes are polymorphic (Harris and Hopkinson 1976, Saastamoinen et al 2009, Flippov and Andronova 2011, Korshikov et al 2011).

Alcohol dehydrogenase is one of the most studied enzyme systems among proteins exhibiting allozyme polymorphism (Dickinson and Sullivan 1975). In natural populations of most of the *Drosophila* species, *Adh* gene is polymorphic for two allozymes designated as Slow (S) and Fast (F), on the basis of their electrophoretic mobility. Prakash and Shamina (1994) analyzed ten different geographical populations of *D. ananassae* from India to observe the allelic frequencies of Alcohol dehydrogenase (*Adh*), Octanol dehydrogenase (*Odh*) and Aldehyde oxidase (Ao). They reported genetic divergence pattern at Adh and Odh loci which they believed to be maintained by balancing natural selection varying spatially along the north- south axis at the Indian subcontinent.

Drosophila ananassae belongs to the *melanogaster* species group of the subgenus *Sophophora.* It is a cosmopolitan and domestic species. This species is known to possess many unique genetic properties and it is most prevalent in India. Alcohol dehydrogenase polymorphism has not been studied in the laboratory stocks of this species. In the present study we have analyzed one of the mass culture stocks and five drift lines for Adh polymorphism to study the role of evolutionary forces causing changes in the gene frequency of *Adh.*

Materials and Methods

Ranchi (RN) mass culture stock was established in 2010 from 17 isofemale lines. Polymorphism at Adh locus was tested in this stock and it was found to be polymorphic for all the three genotypes (FF, FS, and SS). From this mass culture stock, five drift lines (A, B, C, D and E) were established and maintained in culture bottles. The mass culture stock and all the five lines were maintained in yeast-agar culture medium in normal laboratory condition (12 hour light-dark cycle at 24 ºC). Drift lines were established by taking five pairs of flies from the mass culture stock and for further generations, only 20 pairs were used. All the five lines were maintained for five generations and then Adh polymorphism was tested. Flies were analyzed from each of the culture bottles for in- gel assay. For this purpose, single fly homogenate was run on 10% native Polyacrylamide gels at100 volts, for 4 hours at 4ºC. Staining procedure of Ayala et al. (1972) was followed, with a little modification in which isopropanol was replaced by 95% ethanol. To know whether the mass culture stock and the drift lines were in Hardy-Weinberg equilibrium for Alcohol dehydrogenase, chi square test was performed.

Results

Table 1 incorporates the observed and expected numbers of three genotypes (FF, FS and SS) and the allelic frequencies of S and F in the Ranchi mass culture stock. Out of 77 individuals analyzed from this stock, the heterozygotes were in maximum number followed by SS homozygotes. The frequency of S and F alleles were found to be 0.57 and 0.43 respectively. Chi-square analysis indicates that this population is not in Hardy-Weinberg equilibrium as there is significant difference between the observed and expected values ($p<0.01$). Table 2 incorporates the observed and expected numbers of genotypes, their allelic frequencies (F and S) and χ^2 values

in 5 different lines maintained in the laboratory for five generations. Populations reared in the bottles serialized as A, D and E were found to be polymorphic since SS and FS genotypes could be recorded in them. However, no individual having genotype FF could be detected in these stocks. Populations raised in bottles C and D became monomorphic at Adh locus since only SS genotypes could be detected. The deviation from Hardy–Weinberg equilibrium was insignificant in all the lines except in line E ($P<0.05$).

Table 1
Genotypic and allelic frequencies of flies analyzed from Ranchi mass culture stock.

Total No. of flies analysed		Genotypes			Allelic Frequencies	
		SS	FS	FF	S	F
77	obs.	19	50	8	0.57	0.43
	exp.	25.2	37.7	14.1		

$\sum\chi^2 = 8.176$, d.f=1, $P<0.01$

Table 2
Genotypic and allelic frequencies of flies analyzed from 5 drift lines established from Ranchi mass culture stock

Lines	Total No. of flies analyzed		Genotypes SS	FS	FF	Allelic Frequency S	F	χ^2	P
A	35	obs.	32	3	0	0.96	0.04	0.09	>0.99
		exp.	32.26	2.68	0.06				
B	21	obs.	21	0	0	1	0	0	>0.99
		exp.	21	_	_				
C	21	obs.	21	0	0	1	0	0	>0.99
		exp.	21	_	_				
D	21	obs.	15	6	0	0.86	0.14	0.60	>0.20
		exp.	15.53	5.06	0.41				
E	21	obs.	7	14	0	0.67	0.33	5.30	<0.05*
		exp.	9.43	9.29	2.28				

* Significant

Discussion

Drosophila ananassae has been well studied for its chromosomal polymorphism but its protein polymorphism has not so substantially been studied (Singh 2000). We have started doing enzyme polymorphism in this species by selecting some of the enzymes which are known to be polymorphic in other species of *Drosophila* (Kumar & Singh 2012). In this study we have tried to see the founder effect on the persistence of enzyme polymorphism in this species. It is well documented that an allele may increase or decrease in its frequency through chance. in

populations, genetic drift favors either the loss or fixation of an allele. The rate at which an allele is lost or becomes fixed is completely dependent on the population size. The impact of random genetic drift is more significant in smaller populations. Random genetic drift may not have any effect in the fitness of a population or it may have a beneficial or detrimental effect on the population. In nature there are several interesting ways by which small population size and genetic drift affects the genetic composition of a population. By using inversions as chromosome markers, the phenomenon of genetic drift has been observed in *D. pseudoobscura* (Dobzhansky and Pavlovsky 1957) and *D. paluistoram* (Powell and Richmond 1974). They observed random changes in the frequencies of chromosome arrangements in the different experimental populations established with small numbers of individuals. Singh (1988) reported evidence for random genetic drift in laboratory populations of *D. ananassae.* He found variations in ST gene arrangement in contrast to its counterpart, AL arrangements in different laboratory lines started with less number of flies.

In this study, the mass culture stock showed polymorphism at Adh locus as all the three genotypes were recorded. The Adh locus in this mass culture stock is not in the Hardy-Weinberg equilibrium as there is significant deviation from expectation. The five founded populations raised from the mass culture stock showed very different genetic composition when analyzed after five generations. The five lines were established by just five founding females and were maintained by transferring only 20 pairs of flies in each generation. Therefore, the changes observed in the frequency of alleles are likely to be caused by random genetic drift. Fixation of the slow (S) allele occurred in bottles B and C in just five generations. In all the five lines, no fast homozygous (FF) individual was observed which indicates the elimination of this genotype. Although, heterozygotes (FS) were scored but their number was found to be less in frequency, indicating that the two alleles are still maintained in the population. It can be stated that the allele which was low in its frequency in the mass culture (i.e. the fast allele) decreased in its frequency in subsequent generations and even got eliminated completely in two of the lines (B and C). In fact the initial frequency of an allele, guides the path of random genetic drift more than it does to the other elemental forces of evolution. This is because, in a small population, where the role of random genetic drift is more pronounced, an allele having a low frequency is more likely to decrease in its frequency in the next generations and vice versa. Thus, in subsequent generations, it is likely to get eliminated and the other one to get fixed in the population. In this study each of these populations is assumed to undergo drift independently of the other populations.

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