INTRODUCTION
CHAPTER I

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(Detection of nature and extent of genetic variation in natural populations of *Drosophila* species is the major thrust of experimental population genetics.) The genetic studies involving electrophoretic phenotypes and morphometrical phenotypic traits in the Indian populations can give information regarding the adaptability of these phenotypic traits. (India is a large subcontinent and covers a vast latitudinal range (8°N to 33°N) and accessible altitudinal range (varying from 10 meter to more than 2500 meter) with significant environmental variability, which provides a better opportunity for such type of studies.)

The gel electrophoresis is a very useful technique to quantify the nature and extent of genetic variability within and between the species populations (Wills, 1981). In this technique, the enzymes/proteins are separated on the basis of their net electric charge and the resulting enzyme banding patterns are genetically interpreted. The allozymes for different enzymatic loci have been used to characterise the genetic structure, nature and extent of genetic variation occurring in natural populations of diverse taxa (Nevo, 1978; Ayala, 1983; Lemeunier *et al.*, 1986). The electrophoretic data have been found useful in analysing genetic differentiation between various populations or related species in relation to their ecological and evolutionary history and to determine the amount of genetic polymorphism between different geographical populations.

The presence of large amount of genetic variability in different taxa raised questions regarding its maintenance in natural populations. Some form of balancing natural selection can maintain the biochemical polymorphism and hence were adaptive in nature as described by selectionists (Ayala, 1983; Spiess, 1989; Chambers, 1988) whereas neutralists argued that genetic variability is adaptively neutral and are maintained by mutational input and random fixation (Nei, 1975; Kimura, 1983). But the evolutionary significance of the genetic diversity in nature remains controversial because none of the theory adequately explains the data. Gel electrophoretic techniques have revolutionised the status of analytical studies in population and evolutionary genetics (Ayala, 1976; Wills, 1981).

The resulting electrophoretic data revealed the nature and extent of genetic variation occurring in natural populations (Nevo, 1978). But only one third of the genetic variation occurring at loci coding for enzymes and soluble proteins can be detected by simple gel electrophoretic technique (Ramshaw *et al.*, 1979; Ayala, 1983). Recently, the introduction of sequential acrylamide gel electrophoresis and heat denaturation technique have enhanced the resolving
power of gel electrophoresis (Trippa et al., 1980). The distribution of genetic variation in subdivided populations is a paramount question in population genetics. Several attempts have been made to describe the total genetic variability into within and between sub-population variation.

One possible procedure to analyse genetic differentiation of natural populations is the use of Wright's F-statistics (Wright, 1965, 1978; Nei, 1977). The degree of genetic differentiation among populations has a certain relationship to gene flow (Wolf and Soltis, 1992). When F-statistics have been calculated for allozyme data of several Drosophila species, it has been shown that $F_{st}$ value fell between 0.007 and 0.067 in most cases (Wright, 1978), indicating little differentiation among local populations. The situation, however, appears to be more complicated in D. melanogaster. Kojima et al. (1970) and Smith et al. (1978) found little differentiation within the species, while O'Brien and McIntyre (1969) found considerably more. The discrepancies among these results has probably resulted from linkage disequilibrium between particular allozyme variants and gene arrangements (Taylor and Powell, 1983). Latitudinal clines for allozyme frequencies were observed in North America (Pipkin et al., 1973; Vigue and Johnson, 1973; Mettler et al., 1977, Voelker et al., 1977) as well as in Europe, tropical Africa, Japan and Australia (Girard et al., 1977; Watanabe and Watanabe, 1977; David, 1982; Singh et al., 1982; Oakeshott et al., 1981, 1983). The latitudinal clines observed in America, Australasia were found to be correlated to climatic variables i.e. Adh (increase of F allele with latitude), $\alpha$-Gpdh (increase of S), G-6-pdh (increase of F) and Est-6 (increase of S) i.e., the variations in climate is responsible for biogeographical clines.

Several climatic parameters such as average annual temperature, minimum monthly temperature, daily thermal amplitude and rainfall, vary in a regular way with altitude. Looking for climatic adaptation in local populations leads, therefore, to a search for regular, clinal, variations according to altitude.

Most of the enzyme loci known to be associated with the metabolism of alcohol (Adh, Odh, $\alpha$-Gpdh and Mdh) are polymorphic in natural populations (Oakeshott et al., 1982, 1983). ADH is a key enzyme in the detoxification of exogenous alcohol (Heinstra, 1993). Consequently, allele frequencies at this locus have been extensively investigated in natural populations living in different habitats. Comparative studies of populations originating from winery (where ethanol is present at higher concentration) and surrounding areas (where ethanol concentrations are lower) have reported higher alcohol tolerance together with higher $Adh^F$ allele frequency in winery populations (Marks et al., 1980; Gibson et al., 1981; Gibson and Wilks, 1988; David et al., 1989). The other alcohol metabolizing enzyme is ODH, whose primary substrates are known to be longer chain alcohols. Although ODH seems to be influenced by environmental ethanol (Pecsenye and Lorincz, 1988; Pecsenye et al., 1995), $\alpha$-GPDH is an important
enzyme in the conversion of ethanol into lipids (Geer et al., 1983). While MDH is indirectly involved in the degradation of ethanol by way of the Kreb's Cycle (Freriksen, 1992).

In *D. melanogaster* more than 90% of the environmental alcohols are metabolized in a pathway initiated by ADH (David et al., 1976; Geer et al., 1985 and Heinstra et al., 1987). *Drosophila* adults are able to use ethanol as a resource and convert it into acetate and its further transformation involves energy production. Ethanol use could, therefore, be the primary target of primary selection, being responsible for the high ADH activity found in *D. melanogaster*. (Many species of the family drosophilidae feed on diverse type of fermenting and rotting fruits, vegetables, plant types such as cacti and even household decaying organic food materials (Carson, 1971; Atkinson and Shorrocks, 1977). The range of primary and secondary alcohols produced in environment depends upon the type of microflora (yeasts and other microbes) involved and the types of organic matter undergoing decomposition (Parsons, 1983). Thus, it can be predicted that the diverse types of drosophilids could reflect interspecific difference in tolerance to different alcoholic resources)

During the last decade the attention of population geneticists has been mainly focussed on the analysis of biochemical polymorphism of living beings (Lewontin, 1974; Ayala, 1975; Powell, 1975). Genetic analysis of morphological traits, although well documented from many selection experiments, has been neglected. This seems to be due to both practical and theoretical difficulties. Such traits are often difficult to evaluate with good accuracy and, they also show continuous variability so that genetic differences are to be described in terms of heritability of the polygenes. Morphological differences are however, the first and the best criteria used by taxonomists for identifying species. It has been argued that morphological evolution and structural gene evolution can proceed at independent rates (King and Wilson, 1975). Quantitative traits of adults are very sensitive to preimaginal conditions such as temperature, food availability or crowding. Wild selected flies are much more variable than the laboratory reared flies, indicating that they are faced with a very variable environment (David, 1979). Two kinds of environmental factors control size during development, larval nutrition and temperature. Among individuals collected at the same time, size differences are mainly due to nutritional effects, although some temperature variation may also occur. Thermal effects, on the other hand are more important when different seasons are compared (Atkinson, 1979). Among natural populations of *Drosophila melanogaster* considerable genetic differentiation in quantitative characters has been demonstrated (Lemeunier et al., 1986; David and Capy, 1988; Singh, 1989). Some morphological and physiological characters exhibit latitudinal clines in this species. One of them is well documented cline for numerous
characters (including wing length) extended from France to tropical Africa (David et al., 1976, 1977).

Phenotypic plasticity has long been recognised and studied in plants. It has been seen that same genotype reacts to different environment in different ways to give rise to different phenotypes (Schmalhausen, 1949). Therefore, any phenotype arising under the influence of any environmental factor found in nature or created artificially by man is necessarily within the range of reaction of the genotype that produces it. In a quantitative genetic approach a significant genotype-environment interaction is generally observed (Via and Lande, 1985). D. melanogaster strains and populations have been known for a long time to be polymorphic, for the occurrence of dark pigmented area on the thorax with a general trident pattern. Morgan and Bridges (1919) already paid some attention to the inheritance of this pigmentation. A trident gene \((tr, 2-55)\) and another gene pentagon were responsible for the same (Plough and Ives, 1934; Lindsley and Grell, 1977). Other genes like, ebony or black (which increase the darkness of the whole body) are also known to enhance the expression of trident patterns. The extension of black pigment on the female abdomen is also a trait which exhibits a broad range of variation in response to gross temperature and for which several genetic effects have been described (Robertson et al., 1977; David et al., 1990) and it has also been demonstrated that variation in body colour in natural populations of Drosophila have some adaptive significance (David et al., 1983, 1985; Capy et al., 1988).

In Drosophila, pupation site preference is also an important aspect of habitat selection and niche partitioning (Grossfield, 1978). The choice of suitable site for pupation by the larvae is considered as an important component of fitness because this behaviour has direct repercussion on viability of pupae. It has been found that considerably additive genetic variation occurs for pupation height in D. melanogaster (Markow, 1979). Significant variability in pupation height trait results from both heritable variation and variation in environmental forces in D. melanogaster (Sokolowski, 1989).

A major problem of community ecology is to understand how species are able to co-exist at the same trophic level. In this respect, a major evolutionary process seems to be a diversification of their ecological niches leading to some kind of specialization (Hutchinson, 1978). Resource partitioning appears to be most general trend explaining species biodiversity, although some other more complex processes are also considered (Rosewell et al., 1990). Reduction of niche breadth is often considered as a consequence of interspecific competition (Tilman, 1982). Evolutionary adaptation to thermal environment can be revealed by the examination of the life history traits of the experimental populations. For example, in Drosophila melanogaster long term (about four years) laboratory culture at low or high temperature resulted in adaptation of development time,
longevity and female fecundity; at each experimental temperature (17°C and 25°C), the superior performance was shown by the lines from the corresponding thermal selection regime (Partridge et al., 1994). Partridge et al. (1994) studied the pre-adult life-history by measuring the viability at two different temperatures and they concluded that pre-adult traits might contribute to differences in pre-adult survival and adult body size. Rapid larval development increases fitness and its advantage seems from a positive effect on larval survival.

*Drosophila* adults are poorly protected against desiccation and water balance is maintained by water ingestion. Variations in desiccation tolerance have been mainly investigated in Australian natural populations (Parsons, 1980; Stanley and Parsons, 1981). Under desiccation conditions only a part of reserves disappears and death presumably occurs due to loss of water. The levels of resistance to desiccation are known to influence the distribution of species in wild (Da Lage et al., 1990).

So *Drosophila* species populations constitute excellent material for the study of population and evolutionary genetics. The Indian subcontinent represents a diverse array of climatically variable habitat and there is no information on the electrophoretic or other studies on the altitudinal populations except a single study on Mexican mountains (Pipkin et al., 1973).

Since natural populations of *Drosophila* occur abundantly throughout the year in most parts of Indian subcontinent they seem to experience altitudinally/latitudinally varying climatic factors (temperature, humidity, rainfall etc.). Therefore, altitudinal as well as latitudinal *Drosophila* populations constitute suitable material for exploring the effect of evolutionary forces on the genetic variability occurring in them. The objective of the proposed studies were to analyse the nature and magnitude of genetic variability in altitudinal and latitudinal populations of *Drosophila* species. These are summed up as under:

1. The species specific isofemale lines of altitudinal populations of *D.melanogaster* from Himachal Pardesh were analysed electrophoretically so as to examine alleles per locus, allele frequency, proportion of polymorphic loci and extent of heterozygosity for some of the gene-enzyme systems. The degree of genetic similarity or differences among altitudinal populations of *D.melanogaster* were examined with the help of Nei's method.

2. The different altitudinal populations of *D.melanogaster* were examined for the occurrence of hidden/cryptic variation at Est-6 locus through post-electrophoretic heat denaturation technique of Trippa et al. (1978).

3. To analyse Adh frequency and extent of alcohol tolerance in altitudinal populations of *D.melanogaster*.

4. To analyse ethanol as well as acetic acid tolerance in latitudinal populations of *D.melanogaster*. 
5. To determine the correlation of $Adh$ allele frequency and ethanol tolerance in *D. melanogaster* with altitude/latitude.

6. To analyse starvation and desiccation tolerance in altitudinal/latitudinal populations of *D. melanogaster*.

7. To analyse the morphological traits (fresh body weight, wing length, thorax length, abdominal bristles, sternopleural bristles and ovariole number) in different altitudinal and latitudinal populations of *D. melanogaster* and latitudinal populations of *D. ananassae* and to correlate such traits with altitude and latitude respectively.

8. To analyse the effect of growth temperature and to estimate heritability for trident pigmentation polymorphism in the altitudinal as well as latitudinal populations of *D. melanogaster*.

9. To determine the patterns of abdominal pigmentation at two growth temperatures (17°C and 25°C) on altitudinal as well as latitudinal populations of *D. melanogaster*.

10. To analyse the effect of biotic factors on the pupation site preference in the latitudinal populations of *D. kikkawai*.

11. To determine the reproductive potential and the life history traits in latitudinal populations of *D. melanogaster*.