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ISBN 978-81-322-2564-5 ISBN 978-81-322-2565-2 (eBook)
DOI 10.1007/978-81-322-2565-2

Library of Congress Control Number: 2015944513

Springer New Delhi Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer (India) Pvt. Ltd. is part of Springer Science+Business Media (www.springer.com)

Dedicated to the fond memory of Papa, Mumy, and Aazi

Preface

Nothing in biology makes sense except in the light of evolution.
Theodosius Dobzhansky (1900–1975) Russian geneticist and evolutionary biologist

As propounded by the legendary Theodosius Dobzhansky and later corroborated by contemporary paleobiologist J. William Schopf, "*Evolution is the* GUT (GRAND UNIFYING THEORY) *of biology. It is the bedrock principle of our scientific understanding of the natural laws that govern life. Furthermore, it is logically necessary for life's survival in a changing world environment. To deny this scientific principle is analogous to believing that the earth is the center of the universe*."

Evolution is truly a "bedrock" of life and living systems, both plants and animals. It makes our understanding of life better and enables us to understand and appreciate the biogenesis leading to the untold diversity of not only the living system but also the geo-climatic environment wrapped around us. This is the beauty of evolution that gives readers the thrill of best-seller equally alike for science and non-science background. Originally this book was written to cater to the needs of science scholars from undergraduates to doctoral candidates or anyone driven with the quest of life and evolution. The present study intends to explore some very basic queries concerning the role of natural selection and genetic drift, two predominant forces of almost opposite nature, in bringing about the changes on Earth and its living and nonliving entities since time immemorial.

An attempt had been made and successfully so to encompass all the relevant topics in population genetics under a single book title. This book has also covered allied disciplines/topics like genetics, evolution, *Drosophila* genetics, population structuring, natural selection, genetic drift, etc., thus providing readers with the comfort of having all the major topics in a single book. Other topics that are given emphasis are as follows: population genetics theory, applications and evolutionary deductions, Nei's gene diversity estimates, F-statistics, gene flow, chromosomal association and disassociation, linkage disequilibrium, etc. The aforementioned topics are explained in light of experimental results, making them easier to understand. This book introduces students to classical population genetics and presents all possible applications of population genetics methods to practical problems, including testing for natural selection, genetic drift, genetic differentiation, population structuring, gene flow, and linkage disequilibrium. *Drosophila* is an important laboratory animal for a variety of research problems, so details of culturing,

maintenance, and the use of *Drosophila ananassae* (genetically unique and most used along with *D. melanogaster*) are also described wherever it is required. A separate chapter dealing with the origin, establishment, and spread of chromosomal aberrations in populations of *Drosophila ananassae* is discussed. This becomes all the more important because similar aberrations in higher animals like humans lead to the death of the fetus in the first trimester, while in *Drosophila*, it aids in adaptations as a response to the environmental milieu. Thus, providing an important "checkpoint" controlling the maintenance and survival of living forms for further research in medical science.

I have tried with the best of my ability and competence to present the work in a holistic and comprehensible manner, but science is a collective activity and readers are welcome with their constructive and creative inputs to make this endeavor a grand reality.

Last but certainly not the least, it is my religious duty to acknowledge the silent, indirect contributions of so many people around me for whom thanking in any form will never be good enough. The current work has secluded me from my family and friends, sometimes at rather critical periods, and it is indeed a special pleasure and privileged opportunity to acknowledge their patience and forbearance particularly my better half, who thankfully did not turn into a "bitter half" because of my overindulgence with the book, and my grown up son, whose incredibly innocent and cute activities I had missed all these times.

Amarkantak, Madhya Pradesh, India Pranveer Singh

Contents

About the Author

Dr. Pranveer Singh was born in Lucknow, Uttar Pradesh, India, on July 11, 1976. Dr. Singh received both his undergraduate degree in Zoology and Botany and postgraduate degree in Zoology (specialization in cytogenetics) from the University of Allahabad, Uttar Pradesh, India, in 1998 and 2000, respectively. Dr. Singh completed his doctoral thesis in evolutionary population genetics from Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India, in 2007.

He pursued his postdoctoral study (2008–2012) as a UGC-DS Kothari Postdoctoral Fellow in Biophysics/Proteomics at the Indian Institute of Science (IISc), Bangalore, Karnataka, India. At IISc, his work involved protein engineering of HIV envelope protein to design an immunogen to be used as a subunit vaccine for HIV-1. This work was funded by the International AIDS Vaccine Initiative (IAVI), USA. Currently, he is working as Assistant Professor in the department of Zoology, Indira Gandhi National Tribal University (IGNTU), Amarkantak, Madhya Pradesh, India. He has already published three books: *Surface Plasmon Resonance*; *Chordates: Comparative Account of Physiology*; and *Chordates: Origin, Affinity and Evolution* with Nova Scientific Publishers, New York, USA. His research interests include biophysics, proteomics, vaccine design, protein folding–unfolding kinetics, epidemiology of diseases, evolutionary genetics, and population genetics.

Dr. Singh is currently a senior member of the Asia-Pacific Chemical, Biological, and Environmental Engineering Society (APCBEES). Dr. Singh is awarded with national-level fellowships for doctoral and postdoctoral studies and has served in various university-level committees.

Population Genetics: An Introduction

1

Abstract

Population genetics deals with the description of genetic variation of population. It provides the experimental and theoretical basis of how that variation changes in time and space. The population genetics investigates both the origin of genetic diversity via agency of mutations and chromosomal variability and the mode of distribution of genetic diversity via selection, drift, and migration. Evolutionary potential of a population is quantitative index of the genetic variation, and studies on population genetics provide fundamental information on the genetic structure of the population of the species concerned. Population variability provides a tool to test the evolutionary variability hypothesis, as analysis from genetic, phenotypic, and physiologic variation throws light on the fundamentals of variation in space and time. Genetic polymorphism is the mechanism that aids in the population adaptability in response to spatial and temporal environmental variation. The phenomenon of polymorphism has been studied extensively by numerous population geneticists due to the fact that the study of genetic polymorphism in populations elucidates the underlying mechanism of interplay of evolutionary forces in maintaining and improving the adaptation of population to their environment.

1.1 History

In 1897 in Tahiti, the French artist Paul Gauguin created an enormous painting with a provocative title "Where do we come from? What are we? Where are we going?" The painting displays a group of Polynesian people, both young and old, reclining, sitting, walking, and few of them seem staring interrogatively at the viewer, posing, as it were those three haunting questions. Gauguin's painting reflects a universal quest for what it means to be human. With the advent of nineteenth century, people began to see this issue in new light, especially with the emergence of evolutionary theories. In his series of magnum opus, "Origin of Species" and "Descent of Man," Darwin had addressed these issues (Edwards 2011). Population genetics is in fact,

P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2_1

the translation of Darwin's principles into precise genetic terms. As such, it deals with the description of genetic variation of population and with the experimental and theoretical determination of how that variation changes in time and space.

1.2 Population Genetics

Population genetics emerged as a branch of genetics in the 1920s and 1930s, with inputs from the likes of Sir Ronald Fisher, Sewall Wright, and JBS Haldane. It was the marriage of convenience between principles of Mendel and Darwin theories that has given birth to what we see today as "population genetics." Hardy–Weinberg principle or binomial square law given independently by G.H. Hardy in Great Britain and by W. Weinberg in Germany in 1908 led to the mathematical foundation of population genetics and of modern evolutionary theory (Singh and Singh 2008). This states that under the conditions of random mating, absence of mutation, selection, and drift, the frequencies of gene and genotypes will remain constant from generation to generation, and this stability of population is called genetic equilibrium or Hardy–Weinberg equilibrium. Evolution occurs when equilibrium is modified. The forces, which modify this equilibrium, are known as elemental forces of evolution (Singh 2013). The chief forces of evolution are mutation, migration, drift, and selection. Evolution is fundamentally the set of variations that can change the genetic composition of a population. Therefore, population genetics is significant in understanding not only the elemental forces of evolution but also their mechanism of action. The population genetics investigates both the origin of genetic diversity via agency of mutations and chromosomal variability and the mode of distribution of genetic diversity via selection, drift, and migration (Singh 1996; Singh and Singh 2008).

Approaches used to investigate phenomena in population genetics, and many other biological disciplines, can be generally separated into three basic types: empirical, experimental, and theoretical. The traditional empirical (or descriptive) approaches in population genetics comprise extensive observation of genetic variation of a particular gene or genes in a population or populations, perhaps over time, and the measurement of related factors such as environmental patterns, which may influence this genetic variation. These data may provide associations between the patterns or levels of genetic variation and other factors, thereby suggesting potential problems for further study. The genetic variants used initially in these empirical investigations included morphological variants, blood group polymorphisms, and chromosomal inversions, and then starting in 1960s, allozyme variation – that is, genetic variation in enzymes and proteins. In recent years, similar empirical examinations have focused on DNA sequence variation, between different species, and between individuals within the same species. In fact, the amount of information in DNA sequences has resulted in a new field called genomics that endeavors to compare and understand the significance of this variation.

Generally, only experimental tests can provide support for hypotheses developed from empirical data about the effect of particular factors on degree and patterns of genetic variation (Pratdesaba and Segarra 2015). However, in recent years, the definition of an experiment in evolutionary genetics becomes broader and includes, for example, comparison of DNA sequences between organisms and genes that have different histories, functions, or other characteristics. Using information obtained either from empirical or experimental studies, one can construct a general theoretical framework to provide conceptual basis for understanding the impact of various factors on the levels and patterns of genetic variation and also to make predictions about future genetic changes and provide past scenarios consistent with present-day genetic variation (Pratdesaba and Segarra 2015). Evolutionary potential of a population is index of the quantitative genetic variation, and studies on population genetics provide fundamental information on the genetic structure of the population of the species concerned. Population variability

provides a tool to test the evolutionary variability hypothesis as analysis from genetic, phenotypic, and physiologic variation throws light on the fundamentals of variation in space and time (Singh 1989).

1.3 Genetic Polymorphism

Genetic polymorphism is the mechanism that aids in the population adaptability in response to environmental variation, spatially and temporally (De Souza et al. 1970). The phenomenon of polymorphism has been studied extensively by numerous population geneticists due to the fact that the study of genetic polymorphism in populations elucidates the underlying mechanism of interplay of evolutionary forces in maintaining and improving the adaptation of population to their environment (Singh and Singh 2008).

A polymorphic population, having many adaptively different genotypes, may be able to utilize many ecological opportunities and to exploit the environment much more successfully than a homogeneous population. A polymorphic population, being genetically very plastic, is also able to respond to temporal changes of the environment than a homogeneous population (Da Cunha 1955). The genetic polymorphism provides natural selection with constellation of genes with favorable epistatic effect on fitness. This helps populations to improvise their adaptability in response to environmental variation in space and time (Singh and Singh 2008). The adaptedness of the populations has been shown to be based mainly on the high adaptive values of heterozygotes than those of homozygotes (Dobzhansky 1951). The capacity of maintaining the degree of heterosis in variable environment varies from individual to individual. It is genotypically conditioned, because heterozygous individuals usually have a better homeostasis than the homozygous ones (Dobzhansky and Wallace 1953). Thus, polymorphism is a mechanism that promotes heterozygosity and is therefore related to the maintenance of homeostasis.

1.4 Chromosomal Inversion Polymorphism

The population genetics of polymorphism due to chromosomal inversions are best studied in *Drosophila*. In evolutionary population genetic studies, chromosomal inversions can be used as a genetic marker, considering them to be the alleles to analyze various population genetic parameters like population subdivision, gene flow, etc. (Powell 1997; Singh and Singh 2010). Chromosomal inversions can also be used for phylogenetic studies via polytene chromosome analysis (Carson 1970; Moltó et al. 1988; Wassermann 1992).

The polymorphism and its role in population adaptability are extensively studied in experimental population biology. Population geneticists from the very beginning have considered natural selection to be the force that actually acts on the genetic variability present in the population. Inversion polymorphism helps in population adaptability via the agency of natural selection, but the actual gene targets on which selection acts are still elusive (Schaeffer et al. 2003). Different types of selection acts in different ways on their gene targets, for instance, adaptive selection acts on its gene target via genetic hitchhiking, directional selection decreases the degree of polymorphism by rapidly fixing new mutations, while balancing selection increases the degree of polymorphism by maintaining the alleles longer under the neutral model (Schaeffer et al. 2003). If genetic variation produces phenotypic differences, then natural selection effects the population adaptation via polymorphism (Ford 1975; Merrel 1981). However, in those cases where genetic variation does not translate into a phenotype (chromosomal or allozyme variations), the role of natural selection and its significance in adaptation remains unknown (Lewontin 1974). To date, the amount and pattern of genetic variations in natural populations have been described for over a thousand species (Lewontin 1974; Powell 1975; Nevo 1984). A major and widely held conclusion that has emerged from these studies is that natural populations possess a substantial amount of genetic variation. The role of

this ubiquitous variation in adaptation and evolution and the mechanism whereby it is maintained have indeed become the source of ongoing controversy in population genetics (Lewontin 1974; Nei 1975; Kimura and Ohta 1974; Kimura 1982; Sisodia and Singh 2005).

1.5 Chromosomal Inversions in *Drosophila*

Natural population of *Drosophila* shows widespread occurrence of chromosomal inversions (Aulard et al. 2004). These inversions were detected indirectly, quite early in history of genetics. Sturtevant (1926) was the first to detect the inversions in *Drosophila melanogaster* via crossover suppression in inversion heterozygotes (Singh 1973). However, the physical reality of chromosome inversions were confirmed with the discovery of giant polytene chromosomes from which one could read the gene order by the morphology of banding patterns (Patau 1935; Painter 1933; Tan 1935; Koller 1936; Zhang et al. 2007). Inversions are inherited intact as single simple Mendelian units. In a population genetic sense, various gene arrangements differing by inverted segments could be considered as alleles at a single locus (Singh and Singh 2010). Flies produced by inversions having different karyotypes cannot be distinguished via external phenotypes. This led many populations geneticist to believe that karyotypes produced by inversions are neutral with respect to adaptation. However, it was later on established that inversion polymorphism in *Drosophila* populations is acted upon by natural selection and therefore an adaptive trait (Singh 2008, 2013). A number of adaptive functions are linked with inversion polymorphism (Day et al. 1983). Inversions can also be used to study geographical clines, temporal cycles, meiotic drive, and natural selection (McAllister 2002; Ananina et al. 2004; Singh and Singh 2008; Klepsatel et al. 2014). These are of interest because of their unique origin and also because of the fact that functional coadaptations are likely to occur within an inversion so that rearrangements might also be involved in an adaptive polymorphism (Dobzhansky 1950; Aulard et al. 2002). The inversion karyotypes may vary in some aspects of fitness such as fecundity, viability, rate of development, fertility, hatchability, and sexual activity (Da Cunha 1960; Banerjee and Singh 1998).

1.6 Chromosomal Polymorphism

In natural populations of Diptera, chromosomal polymorphism is an established fact (Da Cunha 1955, 1960). The availability of polytene chromosomes in the larval salivary gland cells of many Diptera facilitates the study of structural aberrations such as inversions, translocations, deletions, and duplications (Wasserman 1963). Paracentric inversions are the most common chromosomal aberration found in natural populations of Diptera (Da Cunha 1960). Chromosomal polymorphism due to inversions is common in natural populations of *Drosophila* and is an adaptive trait. Chromosomal polymorphism is maintained by selection in variable environment rather than by overdominance (Da Cunha 1960; Dobzhansky 1970; Sperlich and Pfriem 1986; Krimbas and Powell 1992; Iriarte and Hasson 2000; Muntẻ et al. 2005; Kennington et al. 2006; Singh and Singh 2008). Besides, frequency-dependent selection or variable selection in time or space can contribute to the adaptive character of chromosomal polymorphism. Majority of *Drosophila* species analyzed are found to be naturally polymorphic for inversions in single or more chromosome arms (Singh and Singh 2010). This is unusually high for any type of chromosomal variation. Sperlich and Pfriem (1986) have extensively documented the pattern of inversion polymorphism in *Drosophila* (Aulard et al. 2004; Singh and Singh 2008). They listed around 182 species in the subgenera *Drosophila* and *Sophophora* for which at least ten independently derived strains from natural populations have been examined for inversions (excluding Hawaiian *Drosophila*, placed in subgenus *Idiomyia*) (Aulard et al. 2004). Natural populations of nearly 60 % species are polymorphic for inversions (Singh and Singh 2008); subgenus *Sophophora* seems to contain a higher proportion

of polymorphic species than do subgenera *Drosophila* and *Idiomyia*. These inversions have nonrandom pattern of distribution across genus or subgenus (Levitan 1958b). A similar nonrandom pattern is evident with regard to distribution on chromosome arms. All arms of all chromosomes are polymorphic (*D. willistoni*, *D. subobscura*), some have only one arm of one chromosome that is polymorphic (*D. pseudoobscura*, *D. persimilis*), and some species have no inversions in any chromosomes (*D. simulans*). Autosomes and sex chromosomes may be equally or unequally polymorphic. In *D. cardini*, for example, 22 out of the 29 known inversions are in the X chromosome. Several species exhibit the opposite pattern. For example, *D. guaramunu*, *D. mediostriata*, and *D. rubida* have, respectively, a total of 31, 21, and 19 inversions with none in the X-arm (for references, see Powell 1997). However, *D. prosaltans* shows polymorphism due to the presence of paracentric inversions, mostly located in X chromosome (Bicudo 1973; Bicudo et al. 1978).

1.7 Origin of Inversion and Monophyly

The reason behind the origin of inversions in natural populations of *Drosophila* is still obscure (Ladevze et al. 1998). However, there is some evidence suggesting the role of transposable elements. In *D. robusta*, an inversion-inducing mutator stock has been found that is due to unusually frequent movement of transposable elements (Levitan 1992). Lyttle and Haymer (1992) have found a transposable element (*hobo*) near the breakpoints of inversions of *D. melanogaster* endemic to Hawaii. There is presently no direct evidence of involvement of movable elements in the creation of naturally occurring inversions in populations of *Drosophila*; the only direct studies (Wesely and Eanes 1994; Cirera et al. 1995; Cláudia et al. 2005) must be taken as evidence against involvement of mobile elements. Each inversion has a unique origin, having arisen in a single fly, and has since been maintained in the species as a polymorphism or become fixed. The argument was essentially probabilistic (Sturtevant and Dobzhansky 1936). First, the generation of an inversion is a rare event, on the order of 10^{-5} or less. Second, the probability that two inversions will contain exactly the same two breakpoints is also very small, assuming randomness of the breaks along the length of chromosome. Sperlich and Pfriem (1986) calculated this probability to be on the order of 10^{-6}. Third, after being generated, the newly inverted chromosome has a lower possibility of being retained in the population. So, retaining of the identical inversions in the population more than once (polyphyly) requires the concatenation of three rare events, with the overall probability being the product of three small probabilities. In all likelihood, then, inversions are monophyletic. The monophyly hypothesis has been strengthened by molecular studies (Wesely and Eanes 1994).

1.8 Geographical Distribution and Clinal Pattern of Inversions

Inversions display interesting and revealing patterns of geographical distribution. The first species to be studied in this regard remains among the best studied, *D. pseudoobscura*, and its sibling species, *D. persimilis* (Dobzhansky and Epling 1944; Dobzhansky et al. 1966; Powell et al. 1972; Guzman et al. 1975; Arnold 1982). In general, the geographic differentiation corresponds fairly closely to the phylogeny of inversions, i.e., more closely related inversions tend to be geographically clustered. Clines are another feature of these inversions. While initially accepted as evidence that selection operates on these clines, it is now recognized that clines can arise for other reasons, including historical stochastic processes (Endler 1977). These clines track the latitude regardless of hemisphere (Prevosti et al. 1985, 1988). A similar pattern of latitudinal clines in both hemispheres exists for *D. melanogaster* (Lemeunier and Aulard 1992; Colombo 2010; Klepsatel et al. 2014). Levitan (1992) documented north–south and east–west clines in *D. robusta*. Etges (1984) reported altitudinal clines in *D. robusta*. In *D. silvestris*,

geographic differentiation with regard to inversions including altitudinal clines is remarkable (Craddock and Carson 1989). Even populations that have been made extinct by volcanic activity in the past 2100 years and become recolonized have restored the clinal pattern (Carson et al. 1990). However, rare cases of geographic homogeneity are also known in *D. pavani* (Brncic 1973) in Chile. Singh and Das (1990) in their study on *D. melanogaster* have shown that urban populations differ from their rural counterparts with respect to the degree of inversion polymorphism (Das and Singh 1991). Therefore, the geographic pattern of inversion polymorphism depends on the number of different factors (Da Cunha 1955; Brncic 1970).

1.9 Genetic Variability: Classical and Balanced Hypothesis

Genetic variability in populations is maintained by mechanisms which have been defined by Dobzhansky (1955) in two different hypotheses – the classical hypothesis and the balance hypothesis. According to the classical hypothesis, evolutionary changes consist of gradual substitution and eventual fixation of the more favorable alleles and chromosomal structures. According to the balance hypothesis, the adaptive norm is an array of genotypes heterozygous for more or less numerous alleles, gene complexes, and chromosomal structures. Wright and Dobzhansky (1946) have proven that inversion polymorphism in *Drosophila* can be maintained by selective superiority of inversion heterozygotes by conducting experiments with artificially composed populations of *D. pseudoobscura* (Kumar and Gupta 1992). Further investigations in *D. pseudoobscura* not only confirmed the previous results but also showed that frequency equilibria in *D. pseudoobscura* depended on other environmental factors (Dobzhansky and Pavlovsky 1953; Dobzhansky and Spassky 1954; Moos 1955; Levine and Beardmore 1959). Similar frequency equilibria for gene arrangements were also found in experimental populations of other *Drosophila* species (for details see Sperlich and Pfriem 1986; Pegueroles et al. 2013).

1.10 Central versus Marginal Population

Of all the geographic patterns of inversions observed in *Drosophila*, none has raised more interest than the comparison of central populations with those from the margins of species distribution. The general pattern is that, populations sampled from the center of species distribution are high in inversion heterozygosity and as one approaches the margins, it decreases nearly to chromosome monomorphism in many cases. The general assumption is that the center of a species distribution represents the optimal ecology for the species and that approaching the periphery, conditions for the species become progressively less favorable until it is no longer found. Carson (1958a) was among the first to note the distinct decline in inversion heterozygosity near the margins of the distribution of *D. robusta*. Carson introduced what he called the "index of free recombination" or IFR, which is the percentage of euchromatin in an individual that is free to recombine, that is, structurally homozygous. The IFR decreases from 99.7 % in marginal populations to 67 % in geographically central populations (Zivanovic and Mestres 2010). Prakash (1973) studied this same species for allozyme frequencies and found no such pattern for single gene polymorphisms; heterozygosity was as high in marginal populations as in central populations. Similar observations have been reported in the neotropical species *D. willistoni* (Da Cunha and Dobzhansky 1954; Dobzhansky 1957; Da Cunha et al. 1959). In *D. subobscura* also similar results have been found (Krimbas and Loukas 1980; Krimbas 1992). Carson (1958a, b) presented a very different theory emphasizing the role of inversions in reducing the rate of recombination. He reasoned that in central populations, conditions for the species are relatively stable and favorable; therefore, stabilizing selection would act to maintain a modal adaptive phenotype. Inversion heterozygotes represent such stable modal phe-

notypes, which are well buffered from developmental instability and exhibit overall vigor in favorable stable environments ("heteroselection"). The relative lack of recombination in central populations protects these "tried and true" genotypes from disruption. Approaching the margins of distributions, the environment becomes less favorable and predictable, and extreme phenotypes produced by inversion homozygotes may have an advantage. Furthermore, such homozygotes would experience greater recombination of their genomes, increasing the probability of generating phenotypes capable of occupying extreme environments. Carson called this phenomenon "homoselection," whereby chromosome structural homozygosity is actively favored in the margin. Finally, Wallace (1984) postulates the essential difference between central and marginal populations on the basis of presence or absence of intraspecific competition. In the center of species, distribution densities are high and intraspecific competition is strong. This produces density-dependent selection, which favors the maintenance of inversion heterozygosity. At the margins, however, populations are small and density is low, so the advantage of inversion heterozygosity is lost, and chromosome homozygosity is likely to become fixed.

Da Cunha and Dobzhansky (1954) proposed that the levels of inversion polymorphism in populations of *D. willistoni* are directly related to the diversity of the habitat occupied by the populations (Colombo 2008). They devised "habitat diversity index" based on biotic (e.g., number of plant species) and abiotic (e.g., temperature) variation and demonstrate a high positive correlation between inversion heterozygosity and this diversity index. The idea is that the greater environmental diversity a population faces, the more inversions it can maintain due to diversifying selection (*Ludwig effect*) (Colombo 2008).

1.11 Rigid and Flexible Polymorphism

A polymorphic population can react in two different ways to the environmental changes. Dobzhansky (1962) has classified inversion polymorphism into two different kinds – "rigid" inversion polymorphism and "flexible" inversion polymorphism. Seasonal, altitudinal, or long-term changes in environment are regularly accompanied by the changes in the gene pool composition in flexible species, whereas no such relations appear in rigid species (Solé et al. 2002). Seasonal changes in the frequencies of various gene arrangements of *D. pseudoobscura* have been recorded from a number of different localities by Dobzhansky and coworkers (Dobzhansky 1943; Dobzhansky and Ayala 1973; Singh and Singh 1990), whereas no such changes were observed at other localities (Epling et al. 1957). Altitudinal frequency changes were also detected in this species (Dobzhansky et al. 1966; Anderson et al. 1975). *D. pavani*, on the other hand, serves as an extreme example of rigid polymorphism with no geographic, seasonal, and altitudinal variation in the frequency of gene arrangements in local populations (Brncic 1957a; Gosteli 1990). Two other species, *D. mesophragmatica* and *D. orkui*, also seem to possess rigid polymorphism (Brncic 1957b). *D. prosaltans* also serves as an example of rigid polymorphism (Bicudo et al. 1978). Geographic variation in gene arrangements of *D. subobscura* is apparent (Krimbas and Loukas 1980; Prevosti et al. 1985, 1990; Orengo and Prevosti 1996), but cyclic seasonal frequency variation is not (Prevosti 1964) or not strongly expressed (Krimbas and Alevizos 1973). This suggests that *D. subobscura* shows rigid polymorphism. Seasonal frequency changes have been observed in *D. robusta* (Carson 1958a), *D. flavopilosa* (Brncic 1972), *D. willistoni* (Hoenigsberg et al. 1977), *D. nasuta* (Ranganath and Krishnamurthy 1978), *D. rubida* (Mather 1964), *D. funebris* (Dubinin and Tiniakov 1946), and others. Altitudinal variations in inversion polymorphism were recorded for *D. robusta* (Stalker and Carson 1948) and *D. flavopilosa* (Brncic 1972) and *D. nasuta* (Ranganath and Krishnamurthy 1978). Latitudinal clines were observed in Indian populations of *D. melanogaster* (Das and Singh 1991; Singh and Das 1992b). Changes in environment are followed by frequency changes of the various coexisting gene arrangements of a population, depending on

whether the environmental factors are favoring the carriers of certain chromosome structures or not. The flexibility of gene pool will then maximize the population fitness steadily by frequency changes that have been termed by Lerner (1954) as genetic homeostasis. Gene arrangements in homozygous and heterozygous combinations maximize the fitness of the individuals by increasing their buffering ability to the environmental alterations: a kind of rigid polymorphic system should arise which has been termed as developmental homeostasis (Thoday 1953; Lerner 1954) or canalization (Rendel 1967).

1.12 Position Effect and Coadaptation

Why do gene arrangements differing by inversions of chromosomal segments have such strong selective differences? What is the genetic basis for the variation in fitness? Two hypotheses have been advanced: position effect and coadaptation. Sperlich (1966a) has been the main proponent of the view that, at least initially, the only difference between an inverted and non-inverted chromosome is in the position of genes at the breakpoints. If an initial selective advantage is necessary to keep an inversion in the population when it first arises, this could be due to position effect, perhaps one causing heterosis. The alternative is to postulate that when a successful inversion first arose, by chance it captured a favorable set of interacting alleles, which are said to be coadapted (Sperlich had termed this preadaptation hypothesis) (Huynh et al. 2011). The term coadaptation in this context has two aspects. First, the interacting alleles within an inversion are coadapted with one another to form a particularly fit genotype (epistatic fitness effects) (Yadav and Singh 2007). Second, at least initially, the new inversion will be predominantly in the heterozygous state; its nonrecombining block of alleles must be coadapted to those carried on alternative gene arrangements such that heterozygous combinations have high fitness.

1.13 Genetic Coadatation

Genes do not act independently; rather they tend to organize themselves in functional gene complexes or supergenes (Darlington and Mather 1949; Banerjee and Singh 1998), which confer an adaptive advantage to the recipient genotype (Banerjee and Singh 1998). The gene pool, i.e., the collection of all genes in the population, adjusts itself, and this internal adjustment has been called by Dobzhansky (1949) as "genetic coadaptation" (Banerjee and Singh 1998). This adjustment involves the establishment of favorable linkage relations and the selection of genes, which interact to maximize the fitness. Evidence for coadaptation is, therefore, the evidence for interaction (Singh 2008). The chromosomes with different gene arrangements have different complexes of genes (or polygenes) (Singh 2008). These polygenic complexes in the chromosomes found in one geographic population have been mutually adjusted or coadapted through long continued natural selection so that inversion heterozygotes possess higher Darwinian fitness (Singh 2008). But the polygenic complexes in chromosomes with same or different gene arrangements vary from locality to locality (Singh 2008). Since inversion heterozygotes for such foreign gene complexes are not found in nature, there is no coadaptation by natural selection between the polygenic complexes in different localities (Singh 2013). Therefore, heterosis is a consequence of a historical process of adaptation to the varying environmental milieu (Singh 1972, 1991).

The phenomenon of genetic coadaptation associated with inversion polymorphism originally discovered by Dobzhansky in *D. pseudoobscura* has been studied in other species also, viz., *D. willistoni* and *D. paulistorum* (Dobzhansky and Pavlovsky 1958) and *D. pavani* (Brncic 1961a; Singh 1982). In each case, interracial hybridization leads to the breakdown of coadapted polygenic complexes due to crossing-over and a resultant loss of heterosis (Singh 1972). So, it could be said that heterosis associated with inversion polymorphism is due to previous selectional coadaptation. A number of studies have been carried out in various species of *Drosophila*, taking

various components of fitness such as fecundity, viability, longevity, development time, and body size in geographical populations and interpopulation crosses of *D. pseudoobscura*, *D. willistoni*, *D. paulistorum*, and *D. melanogaster* to know the extent of coadaptation in local populations (Stone 1942; Wigan 1944; Vetukhiv 1954, 1957; Wallace 1955; Wallace and Vetukhiv 1955; Singh 1985; Banerjee and Singh 1998). In each case, F_1 heterosis and F_2 breakdown were reported. F_1 heterosis is the result of increased heterozygosity for genes with overdominant effect (Anderson 1968), whereas F_2 breakdown is caused due to disruption of balanced polygenic complexes by recombination (Banerjee and Singh 1998). Genotypes of each local population or race represent an integrated adaptive system, the different parts of which are mutually adjusted or coadapted by natural selection (Vetukhiv 1953). This hypothesis is further supported by the lack of any comparable breakdown in F_2 generation of intralocality hybrids. Similar results were obtained by Brncic (1954) while studying viability under intense competition in *D. pseudoobscura*; Prevosti (1957) in *D. subobscura*; Kitagawa (1967) in *D. pseudoobscura*, taking viability as the component of fitness, and David (1979) in crosses between French and African races of *D. melanogaster* (Robertson 1987). Anderson (1968) tested the hypothesis of genetic coadaptation with respect to body size (wing length) in geographic populations of *D. pseudoobscura* (Banerjee and Singh 1998). He found no strong F_1 heterosis but pronounced F_2 breakdown due to breaking apart of gene complexes by recombination, thus, providing evidence for genetic coadaptation (Anderson 1968).

McFarquhar and Robertson (1963) studied body size, development time, and survival in *D. subobscura* on parental populations and F_1 and F_2 of various crosses involving strains from distant localities (McFarquhar and Robertson 1963). These investigators found F_1's to be always close to midparent value and no evidence of breakdown in F_2 or of increased viability (McFarquhar and Robertson 1963). Thus, there was no evidence for differences in coadaptation in geographic populations of *D. subobscura*. A study by Robertson (1987) involved comparison of phenotypic variation of thorax and wing length of natural and laboratory-bred populations of *D. buzzatii* (Yadav and Singh 2003; Esteban et al. 1995). There was no evidence of heterosis or F_2 breakdown indicating lack of coadaptation in *D. buzzatii* (Yadav and Singh 2003). Thus, the situation apparently conflicts with what has been found in *D. pseudoobscura* and other species (Ayala et al. 1972).

Heterosis associated with chromosome inversions may be simple luxuriance rather than population heterosis (coadaptation) (Singh 2013). This provides evidence against selectional coadaptation hypothesis. These species may be what Mayr (1959) has termed a "wide-open" species showing high incidence of immigration. A population where immigration is coupled with extensive ecological variation shows reduced degree of coadaptation (Singh 1972). It seems that the gene pools of some species are more prone to evolving coadapted gene complexes, showing the evolution of geographic races characterized by its own coadapted gene complexes. So, different species have acquired success by evolving different modes of adjustment within their gene pools (Anderson 1968; Banerjee and Singh 1998). Further, linkage disequilibrium between inversions and also between allozyme loci and inversions has been extensively studied in *Drosophila* to examine the level of coadaptation as it involves interaction of genes at fitness level. Two unlinked inversions of a chromosome may occur together due to epistatic interaction. This mutual adjustment involves the establishment of favorable linkage relations and the selection of genes, which interact to maximize the fitness. Evidence for coadaptation is therefore evidence for interaction (Barker 1979; Gonzalo et al. 1983). The individual mutations that are favorable in some combinations may be unfavorable in others. Thus, selective values can be assigned to genetic system as a whole (Hedrick et al. 1978; Singh 2013).

1.14 Inversion and Selection

The first unambiguous indication that inversions were subject to strong selection came from studies of temporal shifts in inversion frequencies.

Several other studies of seasonal changes in *D. pseudoobscura* inversions have been made (Dobzhansky 1948; Strickberger and Wills 1966; Dobzhansky 1971; Dobzhansky and Ayala 1973; Crumpacker et al 1977). *D. persimilis* (Dobzhansky and Ayala 1973) was also studied for seasonal cycles. Other species in which seasonal changes in inversion frequencies have been reported are *D. melanogaster* (Stalker 1976; Masry 1981; Inoue et al. 1984), *D. robusta* (Carson 1958a; Levitan 1992), *D. subobscura* (Prevosti 1964; Burla and Goetz 1965; Krimbas and Alevizos 1973), *D. flavopilosa* (Brncic 1972), *D. nasuta* (Ranganath and Krishnamurthy 1978), *D. willistoni* (Hoenigsberg et al. 1977), *D. rubida* (Mather 1964), and *D. funebris* (Dubinin and Tiniakov 1946).

1.15 Linkage Disequilibrium

Linkage disequilibrium studies can throw sufficient light on the fundamental problems of population genetics. Linkage disequilibrium occurs due to many factors acting alone or in combination, like epistatic selection, random drift, and gene flow between populations, showing variable frequencies of gene arrangement at more than one locus. Excluding last two factors and taking into account historical and mechanical reasons for association between allele and inversions leaves selection as the only candidate causing linkage disequilibrium. This reason has generated considerable interest in such studies after the pioneering work of Prakash and Lewontin (1968, 1971).

Inversion polymorphism in different species of *Drosophila* provides a good tool for analyzing epistatic interactions. The phenomenon of epistatic interactions between linked inversions are well documented (Levitan 1955, 1958a, b, 1961, 1973; Levitan and Salzano 1959; Brncic 1961b; Mather 1963; Stalker 1964; Prakash 1967; Sperlich and Feuerbach-Mravlag 1974; Singh and Das 1991b, 1992a; Banerjee and Singh 1995, 1996). Levitan (1958b) working on the nonrandom association of linked inversions in *D. robusta* has proposed that nonrandom association (linkage disequilibrium) of linked inversions is attributed to two causative factors acting independently or together, viz., crossover suppression between linked inversions and natural selection discriminating against certain recombinant arrangements. Meiotic drive is also implicated in causing linkage disequilibrium (Dyer et al. 2007). Prakash (1967), working on *D. robusta*, first gave the evidence for interchromosomal interactions. Study by Sperlich and Feuerbach-Mravlag (1974) also supported data on interchromosomal associations and reported random association of various unlinked inversions. Similar study has also been conducted in *D. melanogaster* (Singh and Das 1991a, 1992a) and *D. bipectinata* (Banerjee and Singh 1995, 1996).

1.16 Linkage Disequilibrium and Allozyme Polymorphism

Several studies in *Drosophila* have focused on the allozyme variation with a view to detect linkage disequilibrium between loci as selection affecting these polymorphisms may lead to disequilibrium at least for few loci (Singh and Singh 2008). Prakash and Lewontin (1968) were the first to note association between allozymes and inversions in *D. pseudoobscura*. Inversion–allozyme associations have also been studied in *D. subobscura* (reviewed in Krimbas and Loukas 1980, Krimbas 1992; Mestres et al. 1998). Here, some loci within inversions are in linkage disequilibrium, while other loci in the same inversion are not. *D. melanogaster* too has been studied with respect to linkage disequilibrium between allozyme and inversions (Lemeunier and Aulard 1992). All these studies invariably conclude that no linkage disequilibrium exists among allozyme loci (reviewed in Hedrick et al. 1978; Barker 1979; Rodríguez-Trelles 2003; Singh and Singh 2008). This would indicate that any epistatic fitness interactions that might exist are not strong enough to overcome the randomizing effect of recombination. The exceptions to this generalization are allozyme loci located within or very near the breakpoints of inversions. Suppression of recombination extends beyond

the breakpoints, presumably because of the physical difficulties in pairing due to the contortions associated with the loop formation.

1.17 DNA Polymorphism

Few studies of inversions on the DNA level conducted to date have been highly informative. The detail provided by DNA sequences far exceeds that of allozymes. Aquadro et al. (1991) studied 26 Kb DNA section containing *Amy* locus in *D. pseudoobscura*. Aquadro et al. (1991) used restriction enzymes to detect differentiation among gene arrangements collected at various localities to construct a phylogenetic tree. The significant finding was that the same chromosome arrangements always clustered with another regardless of geographic origin (Wallace et al. 2011). Aguade (1988) made similar studies on *D. melanogaster* and lead to finding that inversion polymorphism predates the allozyme polymorphism. Rozas and Aguade (1990) studied ribosomal protein-coding locus *rp49* in O chromosomes of *D. subobscura*. They detected several shared haplotypes among inversions and thus no support for monophyly.

Carson (1961) hypothesized that a genetically variable gene pool on being subjected to environmental challenges initiates microevolutionary changes. These changes could only be understood by knowing the structural detail and integration of the gene pool before and after the change (Singh and Banerjee 1997). In nature, relatively fluctuating environment exists, but in the laboratory, the environment is quite constant. Thus, with a view to know the response of the gene pool to the environmental change, populations of *Drosophila* often studied after transferring to laboratory environment (Iriarte et al. 1999).

1.18 Inversion Dynamics in Laboratory Populations

Experimental populations in laboratory have several advantages for the empirical population geneticists. One really knows exactly what the population is and can manipulate it more or less at will. L'Heritier and Heritier PL and Teissier (1933)) were the first to develop a population cage for continuous breeding of *Drosophila* populations. Considering the clear evidence for some kind of balancing selection maintaining inversion polymorphism in natural populations, it is not surprising that inversions have served as model systems for studying the nature of selection in experimental laboratory population cages (Soto et al. 2010). Wright and Dobzhansky (1946) for the first time reported the mechanism of inversion selection in laboratory populations of *D. pseudoobscura*. Other species that have been studied in laboratory populations for changes in inversion frequencies are *D. persimilis* (Spiess 1966), *D. robusta* (Carson 1958a, 1961), *D. subobscura* (Krimbas 1992), *D. melanogaster* (Lemeunier and Aulard 1992; Singh and Das 1992a), *D. willistoni* (Dobzhansky and Pavlovsky 1953), *D. paulistorum* (Dobzhansky and Pavlovsky 1953), and *D. tropicalis* (Dobzhansky and Pavlovsky 1955).

Majority of *Drosophila* species show persistence of inversions for many generations under laboratory conditions. Levene and Dobzhansky (1958), working on *D. pseudoobscura*, have shown that inversions persist in laboratory populations for a number of generations due to adaptive superiority of inversion heterozygotes (Singh and Singh 2007). Experiments were performed in different species of *Drosophila*, to find out the genetic mechanisms that maintains the inversion polymorphism under laboratory conditions. Under laboratory environment, inversion frequencies show both decreasing and elimination tendency, e.g., *D. pseudoobscura* (Dobzhansky and Spassky 1962; Watanabe et al. 1970), *D. subobscura* (De Frutos 1978), *D. paulistorum* (Powell and Richmond 1974), and *D. melanogaster* (Inoue 1979; Singh and Das 1992a; Singh and Banerjee 1997; Singh and Singh 2008). Some species showing lower level of variation was also reported in other studies, e.g., *D. persimilis* (Spiess 1950), *D. subobscura* (Sperlich et al. 1976; Krimbas and Loukas 1980), and *D. robusta* (Carson 1961). *D. bipectinata* shows increasing trend in inversion polymorphism when

populations were transferred to laboratory conditions (Singh and Banerjee 1997; Singh and Singh 2008).

1.19 Heterosis (Overdominance) and Frequency-Dependent Selection

What kind of selection maintains the stable equilibria so well documented in these laboratory experiments in *Drosophila* inversions? Two explanations have been offered: heterosis (overdominance) and frequency-dependent selection. Examples in favor of heterosis could be seen in *D. tropicalis* (Dobzhansky and Pavlovsky 1955) and *D. silvestris* (Carson 1987). When initially arising, all new inversions will be found in heterozygous state with other gene arrangements. The adaptive superiority of populations polymorphic for inversions would argue for some kind of general heterotic effect associated with these polymorphisms. In favor of frequency dependence, the strongest argument is the direct evidence for frequency-dependent male-mating success (Ehrman 1967; Spiess 1968) and frequency-dependent viability (Kojima and Tobari 1969; Anderson 1989; Tobari 1993) for different karyotypes. The association of inversions with microhabitat preferences suggests a role for multihabitat selection, which can often be frequency dependent as well as density dependent. Further, selection experiment dynamics are better predicted by frequency-dependent models (Spiess 1957; Pavlovsky and Dobzhansky 1966; Wright 1977; Lewontin et al. 1981).

A classic paper by Levene (1953) has generated considerable interest in the role that heterogeneity of the environment plays in genetic polymorphism (see Hedrick 1990 and references therein for development of theory). Direct laboratory investigation of this issue has taken two forms: monitoring levels of genetic variation in replicate populations held in relatively constant and heterogeneous environments and studies of habitat choice (Nevo 1978). This latter issue, namely, whether different genotypes choose different parts of the niche space, is especially important as it makes the conditions for stable maintenance of a polymorphism much less stringent (Stanton and Thiede 2005). At least, seven experiments manipulating the variation of the environment have been conducted on laboratory populations of *Drosophila*. Of these, positive associations between environmental heterogeneity and genetic heterozygosity have been found (Powell 1971; Mc Donald and Ayala 1974; Powell and Wistrand 1978; Parsons 1980; Hawthorne 1997). However, no correspondence between the level of environmental heterogeneity and genetic variability was noted in other experiments (Minawa and Birely 1978; Oakeshott 1979; Haley and Birley 1983; Yamazaki et al. 1983). What about habitat choice? Given a choice of environments, do different genotypes nonrandomly assort themselves, and if so, is there a positive correlation with their fitness in the chosen environment? De Souza et al. (1970) found that population cages of *D. willistoni* had evolved two types of larvae, those that pupated inside the food cups and those that pupated outside the cups (De Souza et al. 1970; Carracedo 1987). The difference was shown to be genetically determined and positively correlated with survival in the two habitats. Similar observations have been made with natural and laboratory populations of *D. melanogaster* (Sokolowski et al. 1986; Rodriguez et al. 1992; Aulard et al. 2002). Other experiments of similar kind have also shown the same results (Taylor and Condra 1983; Dodd 1984).

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Drosophila ananassae 2

Abstract

Drosophila ananassae is a domestic species with cosmopolitan distribution, found in all six zoogeographic regions. It belongs to the *ananassae* subgroup, which is further divided into *ananassae* complex and *bipectinata* complex. *Drosophila pallidosa* is a sibling species of *Drosophila ananassae* with complete sexual isolation. *Drosophila ananassae* is genetically unique among *Drosophila* species due to certain genetic peculiarities notably spontaneous crossing-over in males, spontaneous bilateral genetic mosaic, segregation distortion (meiotic drive), Y-4 linkage of nucleolus organizer, parthenogenesis, extrachromosomal inheritance, and lack of genetic coadaptation. Natural populations of *Drosophila ananassae* exhibit a large number of inversions. A total of 76 paracentric inversions, 21 pericentric inversions, and 48 translocations are reported so far. Most of these paracentric inversions are transient in nature and have limited distribution, while the three inversions, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, are cosmopolitan in nature and are distributed worldwide. In view of its unique position, several aspects of behavior genetics of *Drosophila ananassae* like phototactic behavior, latitudinal variation in eclosion rhythm in the locomotor activity, oviposition site preference, and pupation site preference, mating propensity, sexual activity, and chromosomal polymorphism were studied. Laboratory populations of *Drosophila ananassae* were also subjected to fluctuating asymmetry (FA) studies to analyze deviation from perfect symmetry of bilaterally symmetrical metrical traits.

2.1 Introduction

Doleschall (1858) first described *D. ananassae* from a small island off the southwestern tip of Ceram in Indonesia known as Ambon (=Ambonia) (Lemeunier et al. 1997). *D. ananassae* is prominently distributed in tropical and subtropical regions of the world. It is one of the eight species which were recorded as occurring in all six zoogeographic regions by Patterson and Stone

P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2_2

(1952). *D. ananassae* is a domestic species, can be easily spotted around places of human dwellings, is a human commensal, and can qualify as a polytypic species (Tobari 1993; Singh and Singh 2010b). Kaneshiro and Wheeler (1970) reported that the ***ananassae* species subgroup** can be divided into ***ananassae* complex (five species)** and the ***bipectinata* complex (six species)** (Singh and Singh 2007a; 2008). Bock and Wheeler (1972) described five species of the *ananassae* complex: *D. pallidosa*, *D. phaeopleura* from Fiji, *D. nesotes* from Palau, *D. atriplex* from Laguna, and *D. varians* from Luzon. Futch (1966) described a light **Samoan fly** ("light form") whose abdominal pigmentation was very yellowish and proposed that it is a second species very closely related to *ananassae*. Bock and Wheeler (1972) classified this light form *ananassae* as new species, ***Drosophila pallidosa*** (Lemeunier et al. 1997). *D. pallidosa* has been found in the same localities as dark form *ananassae*. Reproductive isolation between *D. pallidosa* and *D. ananassae* was reported by Futch (1973). Other studies (Doi et al 2001; Vishalakshi and Singh 2006a) also indicate strong sexual isolation between the sibling species. Isolation between the two species is probably complete in nature. Themitotic chromosome complement of *D. ananassae* is composed of two large pairs, a pair of small V-shaped metacentric autosomes and a pair of medium-sized V-shaped metacentric sex chromosomes in females. One of the two X chromosomes is replaced by J-shaped Y chromosome in males (Kaufmann 1937; Kikkawa 1938; Futch 1966; Hinton and Downs 1975). In the decade leading to World War II, genetic and cytological studies of *D. ananassae* were successfully pursued in Japan and the USA. Kaufmann (1936a, b, 1937) carried out a series of valuable cytological studies on the wild strains of *D. ananassae* collected in Alabama. Kikkawa (1938) summarized that the general structural features of *D. ananassae* are fairly close to *D. melanogaster*. However, there are many slight differences, viz., the eye is comparatively larger, the body color is more dull yellowish, there is no black tip in males, and coastal index of the wing is much smaller than in *D. melanogaster* (Kikkawa 1938). Most prominent among these is the absence of a distinct sex-comb that is present in the males of *D. melanogaster* (Kikkawa 1938). *D. ananassae* is genetically unique among *Drosophila* species due to certain peculiarities. One of the most interesting aspects of the genetics of *D. ananassae* is the existence of spontaneous crossing-over in males (Kikkawa 1937; Moriwaki 1937b, 1940; Kale 1969; Hinton 1970; Moriwaki et al. 1970; Singh and Singh 1987, 1988b; Singh 1996). It is suggested that male crossing-over in *D. ananassae* is due to dominant enhancer (E) located in 3R chromosome and a recessive suppressor (S) mapped on 2L chromosome (Moriwaki 1937a, 1940; Kikkawa 1938; Hinton 1970). Kale (1967) and Matsuda (1986) found that increased temperature and radiation enhanced recombination-frequency in males of *D. ananassae*. The effect of age on male crossing-over in *D. ananassae* has also been studied (Kikkawa 1938; Ray-Chaudhuri and Kale 1965; Matsuda and Tobari 1982). Spontaneous crossing-over in males has meiotic origin (Kale 1969; Hinton 1970; Moriwaki et al. 1970; Moriwaki and Tobari 1973; Singh and Singh 1987). In *D. ananassae*, inversion heterozygosity suppresses crossing-over completely within its limit but enhances the recombination frequency outside the inverted zone (Singh and Singh 1987, 1988a, 1989b; Singh and Mohanty 1990). In *D. ananassae*, chromosome rearrangements, such as pericentric inversions, translocations, transpositions, deficiencies, and extrabands, havdifferentiation of inversion polymorphisme recurrently been found in natural populations, but these types of chromosomal rearrangements are rarely found in other species of *Drosophila*, thus indicating high mutability in *D. ananassae* (Kikkawa 1938; Singh and Banerjee 1997; Singh and Singh 2008). *D. ananassae* genome contains a large number of inversions of which highest are paracentric inversions (76), followed by translocations (48) and pericentric inversions (21) (Kaufmann 1936b; Freire-Maia 1955; Sajjan and Krishnamurthy 1970, 1972; Singh et al. 1971, 1972; Reddy and Krishnamurthy 1972a, b; Hinton and Downs 1975; Singh 1983a, 1996; Hegde and Jayashankar 1992; Tobari 1993). Majority of these paracentric inversions are limited in distribution, but the three inversions, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and

Eta (ET) in 3R, show global distribution pattern (Singh 1970; Singh and Singh 2008). Therefore, Futch (1966) referred these three inversions as cosmopolitan inversions. Different workers have given different nomenclature to these inversions. But for the sake of uniformity, the present work will follow the nomenclature by Ray-Chaudhuri and Jha (1966) as alpha (AL), delta (DE), and eta (ET) (Singh and Singh 2007a; 2008). The presence of pericentric inversions and translocations are among the most unusual features of natural populations of *D. ananassae* (Grieshop and Polak 2012). Among other *Drosophila* species, pericentric inversions have been found only in two species, *D. algonquin* (Miller 1939) and *D. robusta* (Carson and Stalker 1947; Levitan 1951; Carson 1958), and translocations in only three species, *D. prosaltans* (Dobzhansky and Pavan 1943; Cavalcanti 1948; Mather 1962), *D. rubida* (Mather 1962), and *D. pseudoobscura* (Dobzhansky 1970).

While a number of mutator systems are found and studied extensively in *D. ananassae*, the *optic morphology (Om)*, hypermutability system is quite unique among them. Almost all mutants found in early studies of this system show defects in the adult compound eye, and for this reason they were called *Om* mutation (Hinton 1984). The *Om* mutation is especially interesting as nearly all mutations obtained in this system show phenotypes affecting the structure of adult compound eye, thus helping in understanding the mechanisms and specificity of the mutagenic event. Factors that cause these mutations is *tom*, a retrotransposon, which resides in the element itself and is semidominant in nature (expressed poorly in heterozygotes but more extremely in homozygotes); nonlethal, gain of function mutations are found in the whole genome (28 different loci) of *D. ananassae* and are restricted to the eye tissue (Tanda et al. 1993). The mutagenic effect of *tom* could be due to the presence of tissue-specific enhancers within this retrotransposon that affects transcription of nearby genes. Alternatively, *tom* might carry transcription signals of a more general nature that increase transcription from specific enhancers located in the mutant genes.

Other important genetic peculiarities of *D. ananassae* include segregation distortion (meiotic drive), Y-4 linkage of nucleolus organizer, parthenogenesis, extrachromosomal inheritance, and lack of coadaptation (reviewed in Singh 1985a, 1996, 2000).

Singh and Mohanty (1992), while scoring the progeny of a test cross between heterozygous-males and mutant females, identified a spontaneously occurring bilateral genetic mosaic, characterized by three mutant characters (*cu, e, se*) on the left side and all nonmutant (normal) characters on the right side. Spontaneous bilateral genetic mosaic might have occurred through mitotic recombination in heterozygous zygote (Singh 1996). The segregation distortion leads to unequal segregation of two alleles in heterozygotes due to some unusual mechanisms during meiosis (Singh 1996). It is caused by mutant gene (SD factor) and occurs in males only. *D. ananassae* differs from *D. melanogaster* significantly with respect to various aspects of segregation distortion and also the mechanisms causing it (Mukherjee and Das 1971; Singh 1996). Therefore, it leaves a lot to explore regarding high frequency of spontaneous male crossing-over in *D. ananassae* and its relation to segregation distortion (Mukherjee and Das 1971; Singh 1996). Futch (1972) has also reported parthenogenesis light (samoan form, *D. pallidosa*) and dark forms of *D. ananassae*. Carson et al. (1969) have reported a lot of mechanistic similarities between the development of unfertilized eggs in *D. ananassae* and automictic type of parthenogenesis in *D. mercatorum* (Singh 1996). In *Drosophila*, usually X-Y-linked nucleolus organizer is found. In male *D. ananassae*, the nucleolus is associated with the Y chromosome and the pair of chromosome 4 (shortest in size). This cytological observation was supported by the genetic demonstration of Y-4 linkage of bobbed mutation in *D. ananassae.* The Y-4 association of nucleolus organizer indicates a probable translocation of the nucleolus organizer region (NOR) between X and 4 chromosome during speciation of *D. ananassae* (Hinton and Downs 1975; Singh 1996).

2.2 Behavior Genetics of *D. ananassae*

In view of its unique position, several aspects of behavior genetics of *D. ananassae* were studied. The phototactic behavior of *D. ananassae* is controlled by several genes and is affected by additive genetic variation (Markow and Smith 1979). Eclosion rhythm and locomotor activity show latitudinal variation pattern in *D. ananassae* (Joshi 1999; Joshi and Gore 1999). The choice of the oviposition site by the adult female *Drosophila* is an important fitness-related nonsexual trait as the survival and longevity of flies largely decided by oviposition site preference. *D. ananassae* female prefers the margins of the food vials for oviposition and to insert eggs into the medium. Oviposition site preference is largely governed by external factors like temperature, light, and chemicals. Singh and Singh (2003) have reported geographic differentiation pattern among strains of *D. ananassae* regarding oviposition site preference. They found positive response to selection for the choice of oviposition site preference. The preference for pupation site is important in *Drosophila*larval behavior as the site selected by larvae can affect its subsequent survival. Singh and Pandey (1993) have studied the pupation site preference in *D. ananassae* (reviewed in Singh and Singh 2003). Many investigators have shown the correlation between sexual activity and chromosomal polymorphism, for instance Spiess and Langer (1964) in *D. persimilis*, Spiess et al. (1966) in *D. pseudoobscura*, Brncic and Koref-Santibanez (1964) in *D. pavani*, Sperlich (1966) in *D. subobscura*, Prakash (1968) in *D. robusta*, and Singh and Chatterjee (1986, 1988) in *D. ananassae*. Sexual isolation has primary role in causing speciation; therefore, its analysis has the potential to elucidate the genetics behind speciation. Singh and Chatterjee (1985a) studied sexual isolation in natural populations of *D. ananassae* using isofemale lines based on male choice experiments. Majority of the crosses demonstrated that homogamic matings were far more than the heterogamic ones and the deviation from randomness was statistically significant. These findings provided evidence for positive assortative mating within *D. ananassae*. The level of sexual isolation was more prominent in isofemale lines when compared to natural populations and may involve genetic bottlenecks (Singh and Chatterjee 1985b). These findings invariably show the role of genetic divergence in causing the behavioral reproductive isolation in the laboratory strains of *D. ananassae*. Genetic variability was also reported for the quantitative characters, such as sternopleural bristle number in Indian populations of *D. ananassae* (Singh and Mathew 1996), and higher level of fertility was demonstrated for flies with high number of sternopleural bristles (Singh and Mathew 1997).

2.3 Fluctuating Asymmetry (FA) in *D. ananassae*

Fluctuating asymmetry (FA) was examined in the laboratory populations of *D. ananassae* to analyze deviation from perfect symmetry of bilaterally symmetrical metrical traits. Analysis unequivocally confirms the existence of FA under controlled laboratory conditions; its occurrences in sexual and nonsexual traits with males show higher level of FA for sexual traits. Therefore, sexual traits are a good indicator of developmental stress than nonsexual traits (Vishalakshi and Singh 2006b).

2.4 Reproductive Behavior of *D. ananassae*

Mating propensity or success involves the exchange of behavioral, visual, acoustic, tactile, and chemical signals between the partners. Chatterjee and Singh (1986, 1987, 1988) analyzed the mating propensity in mutant and wild-type isofemale strains of *D. ananassae* and demonstrated the genetic basis of the phenomenon. Mating propensity showed positive correlation with fertility in *D. ananassae* (Singh and Chatterjee 1987). Courtship time, duration of copulation, and fertility have been tested in *D. ananassae* and these traits show significant variation among them. The strains with longer copula-

tion duration produced more progeny (reviewed in Singh and Singh 2003). Sisodia and Singh (2004) observed size assortative mating, which provides further evidence for sexual selection in *D. ananassae*. Sisodia and Singh (2002) found that *D. ananassae* females when grown to maturity at 18 °C showed a considerable gain in body weight when compared to females grown at 25 °C. At a given temperature, early productivity was highest when the rearing and test temperature are the same. This shows trade-off between longevity and productivity in *D. ananassae*. Studies have also been done in *D. ananassae* showing the relationship between inversion polymorphism, body size, and life history traits with respect to larval development time, pupal period, etc. The role of polymorphic inversions in maintaining body size by modifying genotype frequency under various selection pressure through bidirectional selection has been demonstrated in *D. ananassae* (Yadav and Singh 2006; Singh and Singh 2008). Correlation between bidirectional selection and thorax length was analyzed for several life history traits and chromosome inversion polymorphisms; the findings have uncovered apparent trade-offs in *D. ananassae* (Yadav and Singh 2007; Singh and Singh 2008).

Som and Singh (2001) reported lack of evidence for rare male mating advantage in wild-type--> strains of *D. ananassae*. However, on analyzing rare male mating advantage involving standard homozygotes (ST/ST) and inversion homozygotes (AL/AL) of *D. ananassae*, the rare male mating advantage goes in favor of inversion homozygotes (AL/AL) and sexual isolation between karyotypically different strains of *D. ananassae* (Singh and Som 2001; Som and Singh 2004).

The results obtained in *D. ananassae* with respect to the phenomenon of genetic coadaptation (Singh 1972, 1974b, 1981, 1985b) conflict with what has been found in other species of *Drosophila*. In *D. ananassae*, the inversion heterozygotes produced by chromosomes derived from distant localities exhibit heterosis (Singh and Singh 2008). Furthermore, interracial hybridization does not lead to the breakdown of heterosis associated with inversions in *D. ananassae*. Based on this, it has been suggested that the evidence for coadaptation in geographic populations of *D. ananassae* is lacking. Heterosis associated with chromosome inversions may be simple luxuriance rather than population heterosis (coadaptation). This provides evidence against selectional coadaptation hypothesis (Singh 2008).

D. ananassae serves as an extreme example of rigid polymorphism with no temporal and seasonal variations in the frequency of gene arrangements. However, Reddy and Krishnamurthy (1974) detected altitudinal changes in inversion frequencies in *D. ananassae*. Thus, the manner in which the genetic system of *D. ananassae* is adjusted to the environment may be different from that of other cosmopolitan species.

2.5 Enzyme Polymorphism in *D. ananassae*

In population genetical studies, enzyme polymorphism is utilized to detect how selection acts on particular loci. This is done to elucidate genetic structure of populations and patterns of geographic differentiation (Singh and Singh 2008). Jha et al. (1978) confined this study to single locus analysis. Another study by Johnson (1971) and Johnson et al. (1969) was confined to seven enzymatic loci, showing little variation in allele frequencies. Findings of amylase electrophoresis in *D. ananassae* (Doane 1969) demonstrated some polymorphism. A significant geographic pattern exists for amylase variants in populations of *D. ananassae* throughout the globe (Da Lage et al. 1989). Enzyme polymorphism has shown that *D. ananassae* populations, irrespective of their global distribution, show moderate degree of genetic variability (Tobari 1993; Singh and Singh 2008). However, chromosomes, which show more variability and differentiation even over short distances, exhibit an entirely different picture regarding geographic differentiation when compared with allozymes, the reason being the relative neutrality of allozymes over chromosome arrangements (Tobari 1993). Detailed studies involving *D. ananassae* and its subgroup have revealed that nearly 30 %

of the loci are polymorphic. Even the most polymorphic (*Estc, Acph, Ca, Pgm*) loci show similar variability pattern in all species (Tobari 1993). In the numerous studies of allozyme variation in *D. ananassae*, investigators have tried to examine linkage disequilibrium between loci because if selection acts on polymorphism, then linkage disequilibrium should occur for some loci. These studies have unequivocally ruled out linkage disequilibrium among allozyme loci (Gillespie and Kojima 1968; Johnson 1971).

Few studies of inversions at the DNA level conducted to date have been highly informative as, the details provided by DNA sequences far exceeds that of allozymes.

2.6 DNA Polymorphism in *D. ananassae*

DNA sequence variation at *fw* gene region between and within four populations of *D. ananassae* has been examined. The results revealed that frequencies of SNPs are homogenized over a large geographic region, but show significant differentiation between north and south regions of the study area (Chen et al. 2000), while at *Om (1D)*, gene region shows "isolation by distance" effect (Chen et al. 2000). In another study (Stephan et al. 1998) DNA sequence variation was estimated at *Om (1D)* and *vermilion (v)*. In each population, levels of nucleotide diversity at *v* are reduced 10–25-fold relative to those at *Om (1D)*. *D. ananassae*, because of its extensive population structure, was also analyzed to determine as to how population substructuring affects molecular genetic variation. However, past molecular analyses are limited to only few loci (Stephan 1989; Stephan and Langley 1989; Stephan and Mitchell 1992; Stephan et al. 1998). Vogl et al. (2003), in the similar kind of study, analyzed nine nuclear DNA fragments in eight populations and found variability in the molecular variation among the studied populations. Das et al. (2004), in another study, found variations in the levels of nucleotide diversity, the number and frequency of haplotypes, and the amount of linkage disequilibrium. In contrast to polymorphism, divergence between *D. ananassae* populations and its sibling species*D. pallidosa* is constant across loci. Schug et al. (2007) analyzed microsatellite repeat length variation among 13 populations of *D. ananassae* and found high degree of heterozygosity and variability in the size of repeat units in the studied populations.

2.7 Chromosomal Polymorphism in *D. ananassae*

Ray-Chaudhuri and Jha (1966, 1967) initiated studies on chromosomal polymorphism in Indian populations of *D. ananassae*. They reported numerous chromosomal aberrations and found geographic differentiation of inversion polymorphism (Singh 1996). This has opened floodgates of studies on inversion polymorphism in Indian populations of *D. ananassae*, and many investigators have reported chromosomal rearrangements like paracentric and pericentric inversions and translocations (Sajjan and Krishnamurthy 1970, 1972; Singh 1970, 1983a, 1996, 2001; Singh et al. 1971, 1972; Reddy and Krishnamurthy 1972a, b; Hegde and Jayashankar 1992). Population genetical studies of inversion polymorphism in Indian natural populations of *D. ananassae* have invariably shown geographic differentiation of inversion polymorphism (reviewed by Singh 1998; Singh and Singh 2010b). Singh (1984a) has reported low level of chromosomal variability in rural populations when compared to urban populations. A study by Singh (1996) has also indicated north–south trends in inversion frequencies. These studies have strongly indicated that Indian natural populations of *D. ananassae* show high degree of genetic divergence at the level of inversion polymorphism and populations from southern regions exhibit more differentiation than populations of northern region (Singh 1996; Singh and Singh 2007a). Pairwise comparisons ruled out any correlation between geographic distance and genetic differentiation though populations separated by smaller geographic distance show high genetic similarity (Singh 1996). Therefore, natural populations of *D. ananassae* show genetic differentiation at the

level of inversion polymorphism. Inversion polymorphism has adaptive importance in a widely distributed domestic species, and populations undergo evolutionary divergence due to their adaptation to varying environmental conditions (reviewed in Singh 1996, 1998; Singh and Singh 2008).

2.8 Population Structuring in *D. ananassae*

Population genetics is chiefly concerned with finding out the origin, population structure, and demographic history of a species. Intra- and interpopulation genetic variation elucidates the pattern of species origin and demography. Natural populations display geographic population substructure, which is the function of genetic differences in populations from different geographic regions (Singh and Singh 2008, 2010b). Natural populations invariably show substructuring as herds, flocks, colonies, etc., due to random mix of favorable areas with the unfavorable ones (Wang and Caballero 1999). Population subdivision leads to genetic differentiation among the subpopulations as they acquire variable allele frequencies that vary among the subpopulations (Wang and Caballero 1999). Population subdivision is centrally important for evolution and affects estimation of all evolutionary parameters from natural and domestic populations (Hartl and Clark 1997; Singh and Singh 2008). In subdivided populations, random genetic drift (causing genetic divergence among subpopulations) acts antagonistically to migration, which holds subpopulations together, and the balance between the two decides the degree of genetic divergence that can occur. As an evolutionary process that brings potentially new alleles into population, migration is qualitatively similar to mutation. The major difference is quantitative, i.e., the rate of migration among subpopulations of a species is higher than the rate of mutation of a gene (Hedrick 2005).

D. ananassae exhibits more population structuring than both *D. melanogaster* and *D. simulans* (Vogl et al. 2003; Das 2005; Singh and Singh 2010b). This species shows high incidence of interpopulation migration (Dobzhansky and Dreyfus 1943). *D. ananassae* is very common and cohabits the human dwellings in tropical and subtropical regions of the world. *D. ananassae* populations are separated by major geographic barriers such as mountains and oceans, but frequent transport by human activity is responsible for inter- and intraspecific genetic exchange. It exists as semi-isolated populations in mainland Southeast Asia and on the islands of the Pacific Ocean around the equator, a suspected place of its origin (Dobzhansky and Dreyfus 1943; Tobari 1993; Das et al. 2004). *D. ananassae* shows extensive population structure in its natural population; thus, it is a good model to study the effect of population subdivision on genetic variation. Past molecular analyses (Stephan 1989; Stephan and Langley 1989; Stephan and Mitchell 1992; Stephan et al. 1998; Das et al. 2004; Schug et al. 2007) showing the effect of population substructuring on genetic variation are limited to few loci and populations. However, Singh and Singh (2010b) have tried to bridge the gap by analyzing 45 populations from across different geo-climatic regions of India, utilizing chromosomal markers to study the population substructuring and gene flow in natural populations of *D. ananassae*.

2.9 Chromosomal Polymorphism in Laboratory Populations of *D. ananassae*

Laboratory populations initiated from the naturally impregnated females show the persistence of inversion polymorphism (Singh 1982a, 1983c, d, 1987; Singh and Singh 2007a, 2008). Populations maintained even for more than 100 generations exhibit the presence of three cosmopolitan inversions, thus showing heterosis or heterotic buffering associated with these three cosmopolitan inversions (Singh and Singh 2007b; Moriwaki et al. 1956; Singh 1982a; Tobari and Moriwaki 1993), although the degree of heterosis shows variation that depends on the allelic contents of the chromosome variants (Singh 1983c; Singh and Singh 2007a, 2008). Singh and Ray-

Chaudhuri (1972) conducted a population cage experiment by using standard (ST) and inverted (AL) gene arrangements of the second chromosome derived from Indian populations (Singh 1996). The results show that the experimental populations remained polymorphic for a number of generations and both the chromosomes were maintained at definite frequencies (Singh 1996). This shows that chromosomal polymorphism is balanced owing to adaptive superiority of inversion heterozygotes (Singh 1996). Studies were also conducted to analyze the persistence of heterosis associated with cosmopolitan inversions in interracial hybridization experiments, involving chromosomally polymorphic and monomorphic strains of *D. ananassae* (Singh 1972, 1974b, 1981, 1985b). Based on these findings it has been suggested that heterosis associated with cosmopolitan inversions in *D. ananassae* appears to be simple luxuriance rather than population heterosis (coadaptation), and thus, luxuriance can function in the adjustment of organisms to their environment (Singh 1985b, 1996; Singh and Singh 2007a, b, 2008).

2.10 Chromosomal Associations in *D. ananassae*

Inversion polymorphism present in species of *Drosophila* provides a good tool to study epistatic interactions. Linkage disequilibrium between linked inversions is reported in *D. ananassae* (Singh1983a, 1984b; Singh and Singh 2010a). Levitan based on his studies in *D. robusta* has proposed that nonrandom association of linked inversions occurs because of suppression of crossing-over between dependent (linked) inversions or natural selection discriminating against certain recombinant arrangements. Dyer et al. (2007) have suggested the role of meiotic drive leading to linkage disequilibrium.

In *D. ananassae*, two inversions, viz., delta (3L) and eta (3R), of the third chromosome are linked. These are cosmopolitan in distribution and persist under laboratory conditions (Singh 1982a, 1983b, c, 1987; Singh and Singh 2010a). A chromosome map (chromosome map of *D. ananassae*) prepared by Ray-Chaudhuri and Jha (1966) shows that delta (DE) inversion spanning from 1A to 8A covers 60 % of the total length of 3L, while eta (ET) inversion (10A–12C) covers 20 % of the total length of 3R. These inversions are separated by distance, which is approximately 25 % of the total length of the third chromosome. These linked inversions (3L–3R) show random association in natural populations (Singh 1974a, 1984b; Singh and Singh 2010a) and nonrandom association in laboratory populations (Singh 1983a, 1984b; Singh and Singh 1988c, 1990, 1991; Yadav and Singh 2007). Interchromosomal associations in *D. robusta* were first reported by Prakash (1967). For *D. ananassae*, there is no evidence for interchromosomal associations for unlinked inversions (2L–3L, 2L–3R) in natural as well as laboratory populations (Singh 1982b, 1983a; Singh and Singh 1989a).

Objectives

The present book intends to explore some very basic queries concerning the role of natural selection and genetic drift on the degree of inversion polymorphism. For doing work of such kind, India with its whole range of diversity in geoclimatic conditions provides a very good platform to conduct such studies. Sampling records of inversion frequencies in Indian natural populations of *D. ananassae* now extend almost to two decades. By combining new data with those from the earlier surveys, a time series was generated that enabled exploration of the evolutionary dynamics of inversion polymorphism. Such long time series are rare but nonetheless crucial for studying the evolutionary dynamics of inversion polymorphism. The present study, being first of its kind due to its enormity on temporal (two decades) and spatial (most regions of India covered) scale, also intends to investigate the patterns of polymorphic system in *D. ananassae* and to see if there is any temporal divergence. Finally, an attempt has been made to present the holistic picture of inversion polymorphism across the country in space and time.

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Geographical Distribution of Inversion Polymorphism

3

Abstract

Population genetics of inversion polymorphism was studied in terms of time and space scale to understand the dynamics of inversion polymorphism employing *Drosophila ananassae* as a model organism. *D. ananassae* harbors three cosmopolitan inversions, namely, alpha (AL), delta (DE), and eta (ET). Singh and Singh conducted a study where 45 natural populations from different geographic localities of the India (spanning the regions from Kashmir to Kanniyakumari and Gujarat to Nagaland) were studied for chromosomal inversions. All the analyzed populations exhibit the three cosmopolitan inversions, albeit in variable frequencies. Correlations among frequencies of the three cosmopolitan inversions and regression analysis of inversion frequencies with latitude, longitude, and altitude were found to be insignificant. This strengthens the theory of rigid polymorphism in *D. ananassae*. Genetic differentiation, both spatial and temporal, was also studied at the level of chromosomal polymorphism. Analysis reveals geographic differentiation but no temporal differences. The results were in conformity with the rigid polymorphic systems of *D. ananassae*, which do not show long-term directional changes as a function of time. Combining the results of the present study with similar studies done earlier, it could be postulated that the three cosmopolitan inversions in *D. ananassae* show nearly similar frequency distribution while, on geographical or spatial level, the pattern of distribution largely remains the same.

3.1 Chromosomal Polymorphism

Population dynamics of chromosomal inversion polymorphism has been studied in some Indian *Drosophila* species (reviewed in Singh and Singh 2008). The most noteworthy is *D. ananassae*, a cosmopolitan and domestic species in which the genetical studies were initiated for the first time in India by Ray-Chaudhuri and his students. In the present study, *D. ananassae* is used as a

P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2_3

model organism. It is a genetically unique species and possesses many unusual genetic features (Singh 1985, 2000; Beaumont and Hoare 2003). Polytene chromosomes occur in certain tissues, such as salivary glands, malpighian tubules, gut epithelium, and fat bodies. Because of their giant size and somatic pairing, they have been found to be useful for the study of various types of structural aberrations such as inversions, translocations, deletions, and duplications.

3.2 *Drosophila* Strains

D. ananassae flies were sampled from 45 different geographical regions of India from Jammu in the north to Kanniyakumari in the south and Dwarka in the west to Dimapur in the east (Table 3.1) (Singh and Singh 2007a, 2010). In close to 2 years of collection of *D. ananassae* flies from different regions of India, it was revealed that the fly's number goes down considerably in northern parts of India during hot and dry summer because of significant seasonal changes in temperature and climatic conditions (Singh and Singh 2007a, 2010; Sisodia and Singh 2010). This minimizes the size of the populations, thus making the collection of flies difficult during this period. However, these flies are available in abundance in southern and northeastern regions of India, due to uniform pattern of temperature and high humidity throughout the year (Sisodia and Singh 2010).

Collections of flies were designed in such a way to incorporate the whole geo-climatic heterogeneity available in India so that the effect of these dynamic factors can be taken into account while studying the dynamics of inversion polymorphism. For example, in provinces with coastline (all South Indian states, Orissa, West Bengal, and Gujarat), sampling was done from both coastal regions as well as the mainland regions. For those states with no coastline, collection was planned from different altitudes (northeast, Uttaranchal and Himachal Pradesh). In Haridwar (Uttaranchal), flies were sampled from the outskirts of the city and also from the city center in Mansa Devi shrine located at the height of 3500 m. In states or provinces like Uttar Pradesh and Madhya Pradesh without any geographical heterogeneity, collection was done from places located far away (about 200 km or more) from each other. In each case, flies were collected from fruit and vegetable markets by "net sweeping" method. Figure 3.1 depicts the geographical details of the whole set of 45 localities (Singh and Singh 2007a).

3.3 Cytological Analyses

To determine frequencies of the three cosmopolitan inversions, females collected from nature were kept singly in food vials and F_1 larvae were squashed by lacto-aceto-orcein method. Quantitative data was obtained by identifying karyotypes of single F_1 larva from each wildfemale. The polytene chromosome map of *D. ananassae* prepared by Ray-Chaudhuri and Jha (1966) was used as a standard reference map to determine the breakpoints of inversion. In addition to the data on these populations, previous data on relevant populations were also utilized to determine temporal changes (Singh 1984a, b, 1989a, 1989b, 1991).

3.4 Geographic Trends in Frequencies of Three Cosmopolitan Inversions and Level of Inversion Heterozygosity

All the natural populations of *D. ananassae* analyzed by Singh and Singh (2007a) exhibited the presence of three cosmopolitan inversions. As given in Table 3.2, alpha (AL) inversion ranges from 48.9 % (Haridwar) to 97.6 % (Shillong); delta, 6.3 % (Lucknow) to 77.7 % (Kanniyakumari); and eta, 3.1 % (Kangra) to 38.3 % (Gangtok) (Singh and Singh 2007a). Chromosomal analysis clearly reveals that inversions are frequent in south and northeastern Indian regions (tropical regions) of study area, whereas populations from the north Indian region of study area show more of standard gene

Table 3.1 Details of collection of *D. ananassae*

Name of the locality	State	Time of collection	Number of females analyzed
Jammu (JU)	Jammu and Kashmir	October 2006	130
Dharamshala (DH)	Himachal Pradesh	October 2006	46
Kangra (KG)	Himachal Pradesh	October 2006	65
Dehradun (DN)	Uttaranchal	October 2005	54
Haridwar (HD)	Uttaranchal	October 2005	45
Mansa Devi (MD)	Uttaranchal	October 2005	30
Gangtok (GT)	Sikkim	June 2006	34
Lucknow (LK)	Uttar Pradesh	August 2005	48
Guwahati (GU)	Assam	June 2006	101
Raidopur (RP)	Uttar Pradesh	September 2005	25
Chowk (CW)	Uttar Pradesh	September 2005	71
Dimapur (DM)	Nagaland	September 2006	211
Shillong (SH)	Meghalaya	June 2006	47
Patna (PN)	Bihar	October 2006	211
Allahabad (AB)	Uttar Pradesh	September 2005	51
Imphal (IM)	Manipur	September 2006	119
Gaya (GY)	Bihar	October 2006	79
Ujjain (UJ)	Madhya Pradesh	November 2005	30
Bhopal (BP)	Madhya Pradesh	November 2005	58
Indore (IN)	Madhya Pradesh	November 2005	101
Jamnagar (JM)	Gujarat	December 2005	52
Howrah (HW)	West Bengal	June 2005	35
Sealdah (SD)	West Bengal	June 2005	11
Kolkata (KL)	West Bengal	June 2005	61
Rajkot (RJ)	Gujarat	December 2005	52
Dwarka (DW)	Gujarat	December 2005	90
Ahmedabad (AD)	Gujarat	December 2005	21
Paradeep (PA)	Orissa	May 2005	33
Bhubaneswar (BN)	Orissa	May 2005	09
Puri (PU)	Orissa	May 2005	16
Shirdi (SI)	Maharashtra	June 2006	103
Nashik (NA)	Maharashtra	June 2006	134
Mumbai (MU)	Maharashtra	January 2006	99
Visakhapatnam (VP)	Andhra Pradesh	June 2005	33
Vijayawada (VD)	Andhra Pradesh	June 2005	26
Panaji (PJ)	Goa	February 2006	33
Madgaon (MA)	Goa	February 2006	78
Gokarna (GK)	Karnataka	February 2006	80
Mangalore (ML)	Karnataka	February 2006	118
Bangalore (BL)	Karnataka	April 2005	36
Yesvantpur (YS)	Karnataka	April 2005	15
Pondicherry (PC)	Tamil Nadu	April 2005	21
Ernakulam (ER)	Kerala	April 2006	58
Thiruvananthapuram (TR)	Kerala	April 2006	54
Kanniyakumari (KR)	Tamil Nadu	April 2006	56

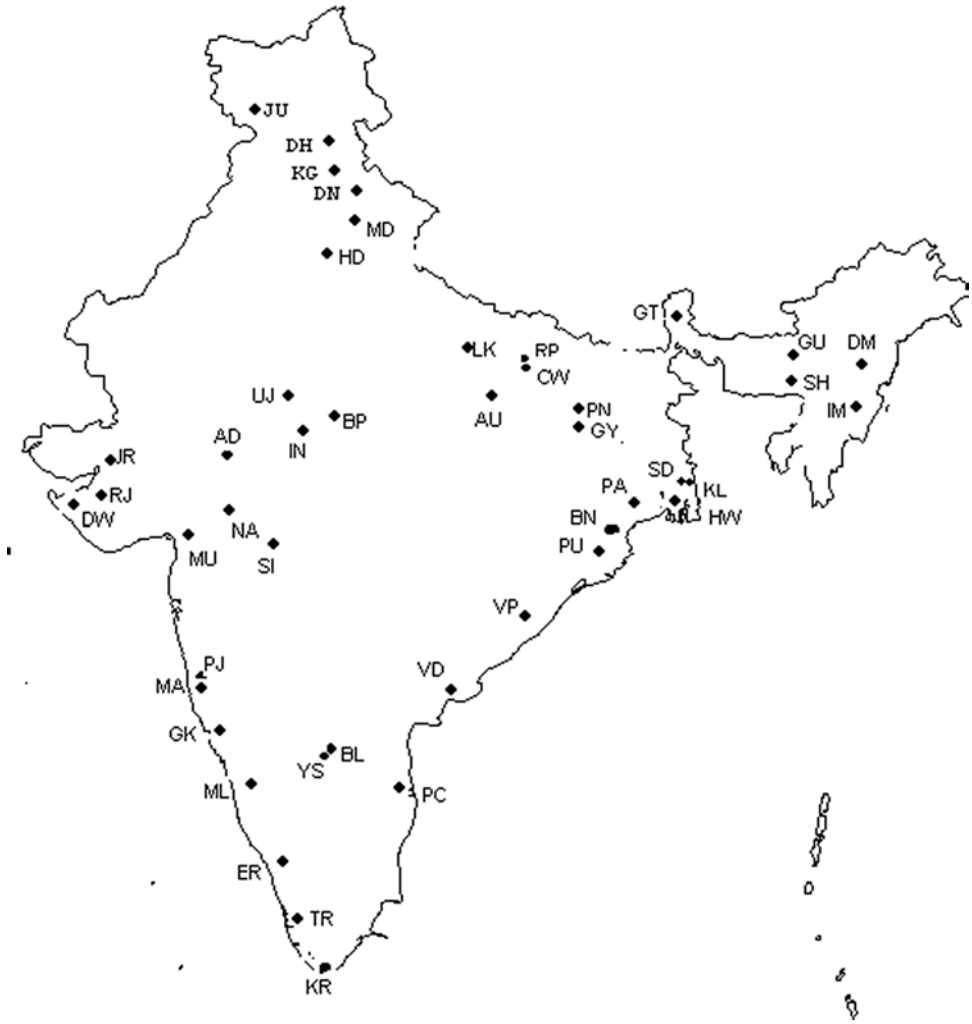

Fig. 3.1 Map of India showing the localities from where *D. ananassae* flies were collected. *JU* Jammu, *DH* Dharamshala, *KG* Kangra, *DN* Dehradun, *HD* Haridwar, *MD* Mansa Devi, *GT* Gangtok, *LK* Lucknow, *GU* Guwahati, *RP* Raidopur, *CW* Chowk, *DM* Dimapur, *SH* Shillong, *PN* Patna, *AB* Allahabad, *IM* Imphal, *GY* Gaya, *UJ* Ujjain, *BP* Bhopal, *IN* Indore, *JR* Jamnagar, *HW* Howrah, *SD* Sealdah, *KL* Kolkata, *RJ* Rajkot, *DW* Dwarka, *AD* Ahmedabad, *PA* Paradeep, *BN* Bhubaneswar, *PU* Puri, *SI* Shirdi, *NA* Nashik, *MU* Mumbai, *VP* Visakhapatnam, *VD* Vijayawada, *PJ* Panaji, *MA* Madgaon, *GK* Gokarna, *ML* Mangalore, *BL* Bangalore, *YS* Yesvantpur, *PC* Pondicherry, *ER* Ernakulam, *TR* Thiruvananthapuram, *KR* Kanniyakumari

arrangements thereby exhibiting a definite north–south pattern in inversion frequencies. Populations from the identical geographical localities (same state or province) reveal an identical pattern in the frequencies of three cosmopolitan inversions. A similar trend is also observed for the level of inversion heterozygosity that shows the range of 0.18 in Sealdah to 1.85 in Pondicherry (Table 3.2) (Singh and Singh 2007a). Such widespread occurrence of three cosmopolitan inversions is a feature of *D. ananassae* populations. Microphotographs of the three cosmopolitan paracentric inversions are depicted in Fig. 3.2a–c. The results of the chromosomal analysis of Indian natural populations of *D. ananassae* clearly reveal significant variability in the frequencies of three cosmopolitan inversions and the degree of inversion heterozygosity (Singh

Table 3.2 Frequencies (in percent) of three inversions, namely, AL (2L), DE (3L), and ET (3R) and level of inversion heterozygosity in Indian natural populations of *D. ananassae*

Populations	Latitude (N°)	Number of chromosomes analyzed	AL	DE	ET	Mean number of heterozygous inversions per individual
JU	34.08	260	61.6	16.2	15.4	0.92
DH	32.22	92	59.8	27.2	4.4	0.95
KG	32.10	130	58.5	39.3	3.1	0.87
DN	30.19	108	63.9	39.9	8.4	0.94
HD	29.98	90	48.9	35.6	6.7	0.84
MD	29.58	60	63.4	38.4	16.7	1.10
GT	27.20	68	95.6	14.8	38.3	0.70
LK	26.50	96	69.8	6.3	20.9	0.72
GU	26.17	202	92.6	11.4	36.2	0.78
RP	26.00	50	60.0	8.0	14.0	0.76
CW	26.00	142	49.3	11.3	16.2	0.88
DM	25.92	422	92.7	20.0	27.3	0.81
SH	25.36	94	97.6	20.8	28.1	0.73
PN	25.35	422	96.5	8.8	22.1	0.57
AB	25.28	102	63.8	18.7	14.8	1.07
IM	24.81	238	84.9	27.4	31.9	0.96
GY	24.75	158	96.3	16.5	23.5	0.74
UJ	23.25	158	68.4	35.0	16.7	0.86
BP	23.16	116	67.3	24.2	5.2	0.75
IN	23.05	202	65.9	38.2	13.4	1.17
JR	22.47	104	89.5	26.0	18.3	0.71
HW	22.45	70	75.8	28.6	5.8	0.77
SD	22.43	22	81.9	27.3	18.2	0.18
KL	22.32	122	84.5	31.2	21.4	0.93
RJ	22.30	104	85.6	24.1	19.3	0.88
DW	22.23	180	92.8	19.5	17.3	0.63
AD	22.03	42	95.3	16.7	16.7	0.47
PA	20.55	66	77.3	28.8	25.8	0.75
BN	20.27	18	88.9	38.9	16.7	0.66
PU	19.50	32	84.4	28.2	28.2	0.56
SI	19.45	206	85.5	18.5	6.8	0.58
NA	19.00	268	82.1	16.8	4.2	0.64
MU	18.96	198	84.9	10.7	20.3	0.65
VP	17.42	66	67.0	25.8	19.7	0.78
VD	16.31	52	67.4	46.2	36.6	0.76
PJ	15.25	66	92.5	45.5	15.2	0.81
MA	15.18	156	87.2	35.9	17.4	0.80
GK	14.48	160	91.3	60.0	17.5	0.82
ML	12.85	236	87.9	8.5	7.3	0.72
BL	12.58	72	68.1	45.9	25.0	1.38
YS	12.58	30	60.0	46.7	13.4	1.46
PC	11.93	42	59.6	50.0	31.0	1.85
ER	10.00	116	80.2	61.3	19.9	0.84
TR	8.53	108	85.2	58.4	14.9	0.90
KR	8.07	112	79.5	77.7	26.8	0.82

Fig. 3.2 (**a–e**) Microphotographs of heterozygous inversions in different chromosomal arms of *D. ananassae*: alpha (AL) in 2L; delta (DE), theta (TH), and iota (IT) in 3L; and eta (ET) in 3R

and Singh 2007a). The three cosmopolitan inversions in *D. ananassae* show a wide distribution pattern, are present in high frequency, and have adaptive significance since the evolutionary history of flies. In the earlier studies of similar kind (reviewed in Singh 1998; Singh and Singh 2008), *D. ananassae* populations were sampled from different geographical regions in India. The results demonstrate significant variation in the frequencies of the three cosmopolitan inversions and the degree of inversion heterozygosity (Singh and Singh 2007b).

Singh and Singh (2007a) analyzed about 45 populations collected from different geographical

regions of India. The analysis revealed the presence of three cosmopolitan inversions in the studied populations. Populations belonging to identical geographical localities exhibit identical patterns of three cosmopolitan inversions and degree of inversion heterozygosity. This could be attributed to similar geo-climatic conditions (Singh and Singh 2007a). It may be postulated that *D. ananassae* populations have acclimated to microhabitat in its current geographic range and suppressors of recombination have evolved to maintain the positive epistatic gene combination within the boundaries of the inversion breakpoints (Schaeffer et al. 2003). There are, however, geographical differences, which could be attributed to the exposure of populations to the differences in habitats or environmental variability since historical past (Singh and Singh 2007a; Singh and Chatterjee 1985).

To determine the pattern of distribution of genetic diversity, populations were grouped by region, and the analysis revealed the existence of genetic variation in the populations coming from different geographical localities than from the populations coming from the similar geographical regions (Singh and Singh 2007a; Singh and Chatterjee 1985).

Among the three cosmopolitan inversions, AL shows maximum frequency of 97.6 % (Shillong) followed by DE, 77.7 % (Kanniyakumari), and ET, 38.3 % (Gangtok), in natural populations of *D. ananassae* (Table 3.2) (Singh and Singh 2007a). This is expected as lengthwise AL is the longest inversion in *D. ananassae* while ET is the shortest. Alpha (AL) inversion being the longest among the three cosmopolitan inversions can capture genes with favorable epistatic effect on fitness, which goes on increasing with the length of the inversion, i.e., the advantage conferred to inversions by selection increases with the distance of recombination between them (Càceres et al. 1999; Schaeffer et al. 2003; Singh and Singh 2007b). Further, AL shows higher frequency in coastal regions and high-altitude regions. In Australian populations of *D. serrata*, a similar pattern of increase was found (Stocker et al. 2004). Various environmental parameters affect the frequencies of inversion. Andjelković et al. (2003) and Etges et al. (2006) focused on the role of temperature in modulating the gene arrangement frequencies. Analysis of time series data for some Palearctic localities points to recent increase in the frequencies of inversions typical of low latitudes (Rodriguez-Trelles and Rodriguez 1998; Solẻ et al. 2002; Balanya et al. 2003; Levitan 2003; Etges et al. 2006; Stamenkovic-Radak et al. 2008) suggesting that contemporary global warming is affecting geographical and temporal patterns of inversion frequencies (Balanya et al. 2003). These studies indicate the indirect role of temperature and implicate the role of a selective agent associated with temperature in modulating the inversion frequencies (Bettencourt et al. 2002; Andjelković et al. 2003). In any case, temperature and humidity are effective microecological agents acting in an indirect manner in affecting the type of vegetation, the composition of existing species, and rates of fermentation and decay of fruits (Inoue et al. 1984).

The degree of inversion heterozygosity shows similar trend among the localities from similar ecogeographical regions (Singh and Singh 2007a). This could be due to similar habitat and geo-climatic conditions, though it does show geographical variations, which could be due to the differences in the habitat conditions (Singh and Singh 2007a). Higher genetic variability in South Indian and northeastern parts of Indian populations could be explained by the "founder principle" originally suggested by Mayr (1942) and widely demonstrated by other studies (Dobzhansky and Pavlovsky 1957; Powell and Richmond 1974; Sperlich et al. 1982; Singh 1987) and "flush and crash" effect by Carson (1970), whereby between the two localities, the one with a uniform distribution of weather and climatic conditions (here, South India and northeastern parts of India) shows higher degree of variability, while for the other locality with extremes of weather and climatic conditions, variability will be low as populations will be exterminated during extreme and hostile weather before it peaks again, thus reducing the variability.

*D. ananassae*populations show geographic differentiation of inversion polymorphism. The three cosmopolitan inversions have become an

indelible part of the genetic composition of the species (for references, see Singh 1985, 1996, 1998, 2001; Yadav and Singh 2006; Singh and Singh 2008). The geographic differentiation pattern of inversion polymorphism must have evolved in response to the ecological heterogeneity prevalent in different geographical regions. The three cosmopolitan inversions in *D. ananassae* show wide distribution pattern, are present in high frequency, and have adaptive significance since the evolutionary history of flies (Singh 1998; Singh and Singh 2008). The genetic differences between these populations may be correlated with the ecological niche variability (Singh 1984a). Frequencies of inversions and the degree of inversion heterozygosity show considerable changes in Indian natural populations of *D. ananassae*. Therefore, Indian natural populations of *D. ananassae* show quantitative variation in the level of chromosomal polymorphism (Singh 1984a; Singh and Banerjee 1997).

Carson (1965) has identified four groups of *Drosophila* species based on the parameters of geographical distribution and geographical characteristics of their chromosomal polymorphisms (Singh 1984a). The first group includes *D. ananassae*, *D. busckii*, *D. hydei*, *D.immigrans*, and *D. melanogaster*. Members of the first group invariably show chromosomal polymorphism but the frequencies of inverted gene arrangements never go beyond 50 %. However, data on inversion frequencies of *D. ananassae* collected from different studies (reviewed by Singh 1984a, 2001) clearly reveal that inverted gene arrangements may go beyond 50 % in natural populations and the degree of chromosomal polymorphism also shows differences in different populations. Therefore, the chromosomal polymorphism patterns shown in *D. ananassae* differs from other cosmopolitan species with which it has been grouped by Carson (1965).

3.5 Statistical Analyses

Simple correlations between frequencies of cosmopolitan inversions and correlation and multiple regression analysis of angularly transformed inversion frequencies with latitude, longitude, and altitude were analyzed (Singh and Singh 2007a).

Correlation of inversion frequencies with latitude, longitude, and altitude was not detected even after multiple regression analysis (Table 3.3). Correlation between frequencies of three cosmopolitan inversions was positively but insignificantly related (Table 3.4) (Singh and Singh 2007a).

Insignificant correlations between inversion frequencies together could be due to the effect of physical length and recombination length on the success of a particular inversion that means the three inversions could not be the same in its distribution and prevalence (Càceres et al. 1999; Soto et al. 2010). Insignificant correlation between frequencies of inversions with latitude, altitude, and longitude even after multiple regression analysis restores the concept of rigid polymorphic systems in *D. ananassae*, whereby genetic constitution is maintained by conferring higher phenotypic plasticity and response to environment is by individual adaptability and not by genetic specialization (Dobzhansky 1962; Brncic and Budnik 1987).

Table 3.3 Pearson correlation coefficients (r) and regression analysis of inversion frequencies with latitude, longitude, and altitude

Simple correlation (r)				Multiple regression (b)			
Inversions	Latitude	Longitude	Altitude	Latitude	Longitude	Altitude	R^2
AL (2L)	−0.237	0.067	−0.296	−0.138	0.378	−0.002	0.097
DE (3L)	−0.004	−0.238	0.031	−1.018	−0.065	0.004	0.323
ET (3R)	−0.039	0.170	−0.144	−0.444	0.687	0.0003	0.441

($p > 0.05$)

Table 3.4 Pearson correlation coefficients (r) between frequencies of different inversions

	AL (2L)	DE (3L)	ET (3R)
AL (2L)	–	0.014	0.367
DE (3L)		–	0.042
ET (3R)			–

($p > 0.05$)

3.6 Success Rate of the Three Cosmopolitan Inversions

Insignificant correlations between inversion frequencies together could be due to the effect of physical length and recombination length on the success of a particular inversion that means the three inversions could not be the same in its distribution and prevalence (Càceres et al. 1999). Insignificant correlation of inversion frequencies with latitude, altitude, and longitude even after multiple regression analysis restores the concept of rigid polymorphic systems in *D. ananassae*, whereby genetic constitution is maintained by conferring higher phenotypic plasticity and response to environment is by individual adaptability and not by genetic specialization (Dobzhansky 1962; Brncic and Budnik 1987; Singh and Singh 2007a).

3.7 Genetic Divergence (Temporal and Spatial) at the Level of Inversion Polymorphism

Genetic distance (D) was estimated to determine the temporal divergence between the same populations that differ by more than two decades (Singh 1984a, b, 1989a, b, 1991; Singh and Singh 2007a).

Genetic identity (I) was estimated among 45 Indian natural populations of *D. ananassae* to determine the genetic differences at the level of inversion polymorphism, using the formula proposed by Nei (1972):

$$I = Ixy / \sqrt{Ix.Iy}$$

where Ix, Iy, and Ixy are the arithmetic means over all loci of Σx_i^2, Σy_i^2, and $\Sigma x_i y_i$ with x_i and y_i being the frequencies of the ith allele in the populations X and Y, respectively (Thorpe 1979; Beaumont and Hoare 2003). Nei (1972) proposed this formula to measure genetic identity with respect to enzyme polymorphism (Das and Singh 1991). Singh and Singh (2007a) applied this to chromosomal polymorphism using chromosome arms and gene arrangements instead of enzyme loci and alleles, respectively. Nei's (1973) gene diversity statistics (H_T, H_S, G_{ST}, and D_M) were also applied to estimate the distribution of genetic diversity within and among populations and the geographic pattern of genetic diversity (Huh et al. 2000). In addition, $2 \times n$ χ^2 differences in karyotypic frequencies among natural populations of *D. ananassae* were also calculated to know population differentiation (Singh and Singh 2007a).

3.8 Genetic Differentiation (Temporal and Spatial) at the Level of Inversion Polymorphism

Temporal divergence between the same populations was analyzed at the interval of more than two decades (Singh 1984a, b, 1989a, b, 1991; Singh and Singh 2007a). This was done by calculating genetic distance (D). Table 3.5 shows the details of collection of the same populations that differ by time of collection along with D values and chi-square values (Singh and Singh 2007a). While comparing a total of twelve of such populations, values of D came closer to zero in each case (0.013–0.051), thus showing no divergence as a function of time (Singh and Singh 2007a). Also, $2 \times n$ χ^2 estimates revealed statistically insignificant differences in karyotypic (2L, 3L, and 3R) frequencies of all the populations analyzed (Singh and Singh 2007a).

3.9 Temporal Divergence and Rigid Polymorphism

Singh and Singh (2007a) conducted one of the longest temporal studies of more than two decades. Temporal divergence in natural populations of

Table 3.5 Values of genetic distance (D) and $2 \times n$ χ^2 analysis between populations of *D. ananassae* analyzed in the present study and the similar populations analyzed earlier

Initial populations	Time of collection	Final populations	Time of collection	Genetic distance (D)	χ^2	df
[a]LK	August 1982	[f]LK	August 2005	0.045	7.710	7
[b]ER	October 1983	ER	April 2006	0.013	9.590	8
TR	October 1983	TR	April 2006	0.030	10.260	8
[c]BN	October 1984	BN	May 2005	0.051	7.930	8
PU	October 1984	PU	May 2005	0.046	10.110	8
MU	March 1985	MU	January 2006	0.023	13.780	8
PJ	March 1985	PJ	February 2006	0.019	10.660	8
KL	October 1985	KL	June 2005	0.020	15.490	8
[d]JU	October 1987	JU	October 2006	0.045	13.710	8
[e]GU	November 1989	GU	June 2006	0.038	13.970	8
SH	November 1989	SH	June 2006	0.030	11.590	7
KR	November 1989	KR	April 2006	0.044	12.990	8

$p > 0.05$
Abbreviations: Refer to localities listed in Table 3.1
[a]Singh (1984a)
[b]Singh (1984c)
[c]Singh (1989a)
[d]Singh (1989b)
[e]Singh (Singh 1991)
[f]Present study

D. ananassae was estimated from D values between the populations analyzed at the interval of more than two decades (Singh 1984a, b, 1989a, b, 1991; Singh and Chatterjee 1988; Singh and Singh 2007a). None of the populations revealed differences as the function of time, i.e., no long-term directional changes (Singh and Singh 2007a, 2008). This strengthens the theory of rigid polymorphism in *D. ananassae,* as rigid polymorphic systems do show variation in terms of time. This could be due to natural selection favoring canalization that represses deviation from phenotype that is optimal in common selecting environment (Eshel and Matessi 1998).

3.10 Spatial (Geographical) Divergence

Singh and Singh (2007a) obtained genetic identity (I) values among 45 natural populations of *D. ananassae* (Table 3.6) (Singh and Singh 2007a). Genetic identity values range from 0.564 (LK vs. GK) to the maximum of 1.000 (DN vs. UJ, MD vs. IN, and UJ vs. IN) (Singh and Singh 2007a). Other lower values are 0.766 (LK vs. KR), 0.767 (YS vs. PN), 0.769 (YS vs. GU), 0.773 (YS vs. GT), 0.776 (ML vs. KR), and 0.777 (KR vs. PN). Nei's gene diversity (see Table 3.7) estimates showed that total gene diversity (H_T) values vary between 0.255 (GY) to 0.506 (JR) with an average of 0.454. Within-population diversity (H_S) value ranges from 0.160 (ML) to 0.461 (PC) with an average of 0.308, while diversity among populations (G_{ST}) ranges from 0.054 (GY) to 0.638 (ML) with an average of 0.333. Distribution of genetic diversity and its geographic pattern was determined by grouping together the populations from a state or province (Table 3.8) (Singh and Singh 2007a). Table 3.8 shows the total diversity (H_T=0.453), within-population diversity (H_S=0.315), and among-population diversity (G_{ST}=0.311), while magnitude of absolute gene differentiation (D_M) came around 0.220. The analysis shows that 31.1 % of genetic differentiation is distributed among populations, which could be attributable to geographic locations of the populations (Petros et al. 2007; Singh and Singh 2007a).

A dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) clustering of genetic identity values is shown in Fig. 3.3. Among various pairwise comparisons, populations from southern region of the study area are genetically more identical among themselves and with the populations from northern region and northeastern region of study area except for PC and BL populations (Singh 1996; Singh and Singh 2007a). Same is true for Orissa and Andhra Pradesh populations, etc. Azamgarh (Uttar Pradesh) populations show lower degree of genetic similarity with Bihar populations but higher with others. Similarly, Madhya Pradesh, Gujarat, and Uttaranchal populations show higher degree of genetic similarity with other pairs. Surprisingly, ML shows very low level of genetic similarity with KR, ER, and TR populations though these regions are not much separated geographically and situated along the same coastline (west coast of India) (Singh and Singh 2007a). Also, ER shows low level of genetic similarity with northeastern and Bihar populations while TR shows higher level of genetic similarity with Bihar and northeastern populations, though both TR and ER lie close to each other and have similar geo-climatic conditions (lying along the western coastline). KR population shows lowermost similarity with PN (Bihar) and northeastern populations, which is quite reasonable. SH population shows low degree of genetic similarity with Himachal Pradesh populations but higher with Jammu populations (Singh and Singh 2007a). Bihar populations exhibit higher genetic similarity with northeastern populations. In totality, population pairs belonging to identical geo-climatic regions (same state or province) and collected in the similar climate reveal higher genetic similarity among each other (Singh and Singh 2007a). Also, there has not been a positive relation between geographic and genetic distances. One important observation that comes out from this study is that seasonality (time of collection) could have a role in influencing the degree of differentiation; for instance, among northeastern populations (GT, SH, GU, IM, DM), the first three populations were collected at the same time while the last two at a different time, as result trends with the first three populations with other pairs are more similar than with the last two populations pairing with others (Singh and Singh 2007a).

The $2 \times n$ χ^2 values were also calculated to obtain the differences in karyotype frequencies (2L, 3L, and 3R) among Indian natural populations of *D. ananassae* (Table 3.9) (Singh and Singh 2007a). Results show that populations from different geographical localities reveal statistically significant differences in karyotypic frequencies while populations from identical geographical localities do not show significant differences in karyotypic frequencies (Singh and Singh 2007a). For instance, LK vs. GK populations show highly significant ($p < 0.001$) differences in karyotypic frequencies, but DN vs. UJ, UJ vs. IN pairs, etc., show insignificant differences ($p > 0.05$). Therefore, from $2 \times n$ χ^2, analysis between populations strengthens the results obtained from genetic identity (I) values (Singh and Singh 2007a).

Results from Nei's G and I estimates clearly reinforces that there is significant genetic divergence at the level of inversion polymorphism in Indian natural populations of *D. ananassae* (Singh and Singh 2007a). Dendrogram based on estimates of genetic identity (I) among 45 natural populations does not reveal any significant pattern except for few cases where populations from different geographical regions show genetic differences, for instance, LK vs. GK 0.564, CW vs. ER 0.567, and CW vs. MA 0.577 population pairs. Here, LK and CW populations are from the plains of Uttar Pradesh while MA, GK, and ER are coastal populations (Singh and Singh 2007a). The maximum genetic identity obtained for KL–SD pair is justifiable as these populations are separated by less than 10 km of geographic distance. For other population pairs, genetic identity or differences do not correlate with geographic distances or the geo-climatic factors, i.e., no correlation between genetic distance and geographic distance is observed (Singh and Singh 2007a). However, African populations of *D. melanogaster* have revealed striking genetic differences over a few kilometers of geographic distance (Aulard et al. 2002). The specific complexes of

Table 3.6 Genetic identity (I) estimated from the gene arrangement frequencies among Indian natural populations

	JU	DH	KG	DN	HD	MD	GT	LK	GU	RP	CW	DM	SH	PN	AB	IM	GY	UJ	BP	IN	JR	HW
JU		1	1	0.9	1	1	0.9	1	0.9	0.8	1	0.9	0.9	0.9	1	1	0.9	1	1	1	1	1
DH			1	1	1	1	0.8	0.9	0.8	1	1	0.9	0.8	0.9	1	0.9	0.9	1	1	1	0.9	1
KG				1	1	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.8	1	0.9	0.8	1	1	1	0.9	1
DN					1	1	0.9	0.9	1	0.9	0.9	1	1	0.9	1	0.9	0.9	1	1	0.9	0.9	0.9
HD						1	0.8	0.9	0.8	1	1	0.9	1	0.8	0.9	0.9	0.9	1	1	1	0.9	1
MD							0.9	0.9	0.9	1	1	0.9	0.9	0.9	1	1	0.9	1	1	1	0.9	1
GT								0.9	1	0.9	0.9	1	1	1	0.9	1	0.9	0.9	0.9	0.9	1	0.9
LK									1	1	1	1	1	1	1	1	0.9	1	1	0.9	0.9	1
GU										0.9	0.9	1	1	1	0.9	1	1	0.9	0.9	0.9	1	0.9
RP											1	0.9	0.9	0.9	1	0.9	0.9	1	1	1	0.9	1
CW												0.9	0.9	0.9	1	0.9	0.9	1	1	1	0.9	0.9
DM													1	1	0.9	1	1	0.9	0.9	0.9	1	0.9
SH														1	0.9	1	1	0.9	0.9	0.9	1	1
PN															0.9	1	1	0.9	0.9	0.9	1	0.9
AB																1	0.9	1	1	1	1	1
IM																	1	1	1	1	1	1
GY																		0.9	0.9	0.9	1	0.9
UJ																			1	1	0.9	1
BP																				1	0.9	1
IN																					0.9	1
JR																						0.9
HW																						
SD																						
KL																						
RJ																						
DW																						
AD																						
PA																						
BN																						
PU																						
SI																						
NA																						
MU																						
VP																						
VP																						
PJ																						
MA																						
GK																						
ML																						
BL																						
YS																						
PC																						
ER																						
TR																						
KR																						

of *D. ananassae*

SD	KL	RJ	DW	AD	PA	BN	PU	SI	NA	MU	VP	VD	PJ	MA	GK	ML	BL	YS	PC	ER	TR	KR
0.9	1	1	0.9	0.9	1	0.9	0.9	1	1	1	1	0.9	0.9	0.9	0.9	1	0.9	0.9	0.9	0.9	1	0.8
0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	0.9	1	0.9	1	0.9	0.9	0.9	0.9	1	0.9	0.9	1	0.8
0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	0.9	1	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	0.9
1	1	0.9	1	0.9	1	1	1	1	0.9	1	1	1	0.9	1	0.9	0.9	1	1	1	1	1	0.9
0.9	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9	1	0.9	1	0.9	0.9	0.9	0.9	0.9	0.9	1	1	0.9	1	0.8
1	1	1	0.9	0.9	1	1	1	0.9	1	0.9	1	1	1	1	0.9	0.9	1	1	1	1	1	0.9
0.9	1	1	1	1	1	0.9	1	0.9	0.9	1	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.9	1	0.8
1	1	1	1	1	1	0.9	1	1	1	1	1	0.9	0.9	0.9	0.6	1	0.9	0.9	0.9	0.9	1	0.8
0.9	1	1	1	1	1	0.9	1	0.9	0.9	1	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.9	1	0.8
1	0.9	1	0.9	0.9	1	0.9	0.9	0.9	0.8	1	1	0.9	0.9	0.9	0.8	1	0.9	0.9	0.9	0.8	1	1
0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	0.9	1	0.9	0.9	0.6	0.8	0.9	0.9	0.9	0.9	0.6	1	0.8
1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	1	0.9	1	0.9	0.8	0.8	0.9	1	0.8
1	1	1	1	1	1	1	1	1	0.9	1	0.9	0.9	1	1	0.9	1	0.9	0.8	0.8	0.9	1	0.8
1	1	0.9	1	1	1	0.9	1	1	1	1	0.9	0.9	0.9	0.9	0.9	1	0.8	0.8	0.9	0.9	1	0.8
0.9	1	1	0.9	0.9	1	0.9	0.9	1	1	1	1	0.9	0.9	0.9	0.9	1	0.9	0.9	0.9	0.9	1	0.8
0.9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	1	0.9	0.9	0.9	1	0.9
1	1	1	1	1	1	1	1	1	1	1	0.9	0.9	1	1	0.9	0.9	0.9	0.8	0.8	0.9	1	0.8
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	0.9
1	1	1	0.9	1	1	1	1	1	1	1	1	0.9	0.9	1	0.9	1	1	1	0.9	0.9	1	0.8
1	1	1	1	0.9	1	1	1	0.9	1	0.9	1	1	1	1	0.9	946	1	1	1	1	1	0.9
0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	1	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1	0.9
1	1	1	1	1	1	1	1	0.7	1	1	1	0.9	1	1	0.9	1	1	1	0.9	0.9	1	0.9
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	1	1	1	0.9	0.9	1	0.8
		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.9	0.9	1	1	0.9
			1	1	1	1	1	1	1	1	1	0.9	1	1	0.9	1	1	0.9	0.9	0.9	1	0.9
				1	1	1	1	1	1	1	1	0.9	1	1	1	1	0.9	0.9	0.9	0.9	1	0.8
					1	0.9	1	1	1	1	1	0.9	1	1	0.9	1	0.9	0.9	0.9	0.9	1	0.8
						1	1	1	1	1	1	1	1	1	0.9	1	1	1	0.9	0.9	1	0.9
							1	0.9	1	1	1	1	1	1	1	1	1	1	0.1	1	1	0.9
								1	1	1	1	1	1	1	0.9	1	1	0.9	0.9	0.9	1	0.9
									1	1	1	0.9	1	1	0.9	1	0.9	0.9	0.8	0.9	1	0.8
										1	1	0.9	1	1	0.9	1	0.9	0.9	0.8	0.9	1	0.8
											0.9	0.9	0.9	1	0.9	1	0.9	0.9	0.9	0.9	1	0.8
												1	1	1	0.9	1	1	1	1	0.9	1	0.9
													0.9	1	0.9	0.9	1	1	1	1	1	0.9
														1	1	0.9	0.9	0.9	0.9	1	1	0.9
															1	1	1	1	0.9	1	1	0.9
																0.9	1	0.9	0.9	1	1	1
																	0.9	0.9	0.8	0.9	1	0.8
																		1	1	1	1	0.9
																			1	0.9	1	0.9
																				0.9	1	0.9
																					1	1
																						1

Table 3.7 Nei's gene diversity statistics and population differentiation parameters across 45 Indian natural populations of *D. ananassae*

Populations	H_T	H_S	G_{ST}
JU	0.431	0.340	0.211
DH	0.427	0.326	0.236
KG	0.446	0.344	0.228
DN	0.446	0.349	0.217
HD	0.400	0.342	0.145
MD	0.468	0.390	0.166
GT	0.490	0.259	0.471
LK	0.415	0.275	0.337
GU	0.487	0.258	0.470
RP	0.388	0.286	0.262
CW	0.365	0.312	0.145
DM	0.490	0.279	0.430
SH	0.488	0.250	0.487
PN	0.490	0.192	0.608
AB	0.415	0.322	0.224
IM	0.484	0.330	0.318
GY	0.255	0.241	0.054
UJ	0.472	0.380	0.194
BP	0.428	0.294	0.313
IN	0.465	0.376	0.191
JR	0.506	0.284	0.438
HW	0.453	0.279	0.384
SD	0.474	0.312	0.341
KL	0.485	0.329	0.321
RJ	0.479	0.300	0.373
DW	0.288	0.236	0.180
AD	0.478	0.202	0.577
PA	0.480	0.362	0.245
N	0.481	0.300	0.376
PU	0.484	0.341	0.295
SI	0.454	0.213	0.530
NA	0.441	0.206	0.532
MU	0.463	0.248	0.464
VP	0.459	0.369	0.196
VD	0.488	0.448	0.081
PJ	0.488	0.288	0.409
MA	0.487	0.311	0.361
GK	0.484	0.301	0.378
ML	0.442	0.160	0.638
BL	0.493	0.431	0.125
YS	0.472	0.343	0.273
PC	0.493	0.461	0.064
ER	0.486	0.356	0.267
TR	0.486	0.316	0.349
KR	0.464	0.343	0.260
Mean	0.454	0.308	0.333

Abbreviations: H_T total diversity, H_S diversity within populations, G_{ST} diversity among populations

ecological factors and genetic structure of local populations could probably cause this (Andjelković et al. 2003). Here, the role of ongoing gene flow and historical associations as cause of genetic similarity between pair of populations is equally important (Futch 1966; Prevosti et al. 1975; Nielsen and Slatkin 2000). The observed genetic similarity may also be due to co-transportation of flies with fruits and vegetables to the regions where they might have started their colony afresh. Since, in each case, flies were collected from fruits and vegetable markets, even the type of fruits and vegetables are the same throughout so similar food niche could have a possible role in translating the genetic similarity between populations situated far away from each other (Singh and Singh 2007a). Although populations may have genetic contact resulting from transportation of flies along with fruits and vegetables and other human goods, genetic divergence in natural populations might have occurred in response to ecological conditions existing in different geographic localities (Singh 1989; Singh and Singh 2008). Thus, the factors leading to adaptation are crucial in defining the distribution of chromosomal inversions in populations of *D. ananassae* (Prevosti et al. 1975; Singh 1986; Singh and Anand 1995). Trade contacts and subsequent transport of *D. ananassae* along with human goods might have led to gene flow between natural populations of *D. ananassae* leading to the homogenization effect and hence the genetic similarity, but this may not be the only and sufficient reason accounting for the observed genetic identity between these populations. This is because there are instances where populations located in a close proximity and having good trade contacts show genetic differences while populations far apart from each other and not having trade contacts show genetic identity. Genetic similarity can be mostly attributed to the identical geo-climatic conditions instead of trade contacts between the regions. This reinforces the role of natural selection leading to genetic similarity or differences (Singh 1986).

Natural populations of *D. ananassae* irrespective of the geographic barriers and distances experience considerable gene flow among its populations (Singh 1986). *D. ananassae*, being a

Table 3.8 Nei's gene diversity statistics and population differentiation parameters when 45 Indian natural populations of *D. ananassae* were grouped by regions

State/province	Number of populations	H_T	H_S	G_{ST}	D_M
Jammu and Kashmir	1	0.431	0.340	0.211	–
Himachal Pradesh	2	0.436	0.335	0.231	0.101
Uttaranchal	3	0.438	0.360	0.178	0.117
Uttar Pradesh	4	0.395	0.298	0.245	0.129
Northeast	5	0.487	0.275	0.435	0.265
Bihar	2	0.372	0.216	0.419	0.312
West Bengal	3	0.470	0.306	0.348	0.246
Madhya Pradesh	3	0.455	0.350	0.230	0.157
Orissa	3	0.481	0.334	0.305	0.220
Andhra Pradesh	2	0.473	0.408	0.137	0.130
Gujarat	4	0.437	0.255	0.416	0.242
Maharashtra	3	0.452	0.222	0.508	0.345
Goa	2	0.487	0.299	0.386	0.376
Karnataka	4	0.472	0.308	0.347	0.218
Kerala	2	0.486	0.336	0.308	0.300
Tamil Nadu	2	0.478	0.402	0.158	0.152
Mean		0.453	0.315	0.311	0.220

Abbreviations: H_T total diversity, H_S diversity within populations, G_{ST} diversity among populations, D_M absolute population differentiation

domestic species, is frequently transported along with the human goods leading to reintroduction of these flies in the same region (Da Cunha 1960; Futch 1966). Therefore, natural populations of *D. ananassae* show high level of genetic divergence irrespective of its high mobility arising out of the co-transportation via human traffic causing gene flow between populations. It could be safe to postulate that genetic divergence obtained in the natural populations of *D. ananassae* is more due to their adaptation to variable environment and natural selection acts to maintain these inversions (Singh 1986).

The holistic picture that comes out after including the present work (Singh and Singh 2007a) with the similar studies done earlier (Singh 1974, 1984a, b, 1989a, b, 1991) establishes substantially that three cosmopolitan inversions show nearly similar distribution patterns in *D. ananassae* populations. The geographic differentiation of inversion polymorphism also shows a similar trend, which could be attributed to the identical geo-climatic conditions, thus providing evidence in favor of natural selection (Singh and Singh 2007a). Also, inversion frequencies do not vary with latitude, longitude, and altitude, the three important signatures of rigid polymorphism. These results demonstrate spatial variation, i.e., differences between habitats sans temporal changes (variation as a function of time) in inversion polymorphism of *D. ananassae*. Spatial and temporal variations in inversion frequencies strengthen the theory that natural selection maintains the inversion polymorphism (Mettler et al. 1977; Stalker 1980; Kennington et al. 2006; Singh and Singh 2007a). A certain degree of this kind of polymorphism with environmental variability corresponds to flexible polymorphism as contrasted to a rigid type of polymorphism (Dobzhansky 1962; Andjelković et al. 2003; Singh and Singh 2007a).

Allopatric populations show genetic differences either due to genetic drift or to founder effect from a long geographical isolation or from natural selection due to environmental variability (Dobzhansky 1970; David 1982; Song et al. 2006). Coming back to the natural selection versus genetic drift debate for their influence on the

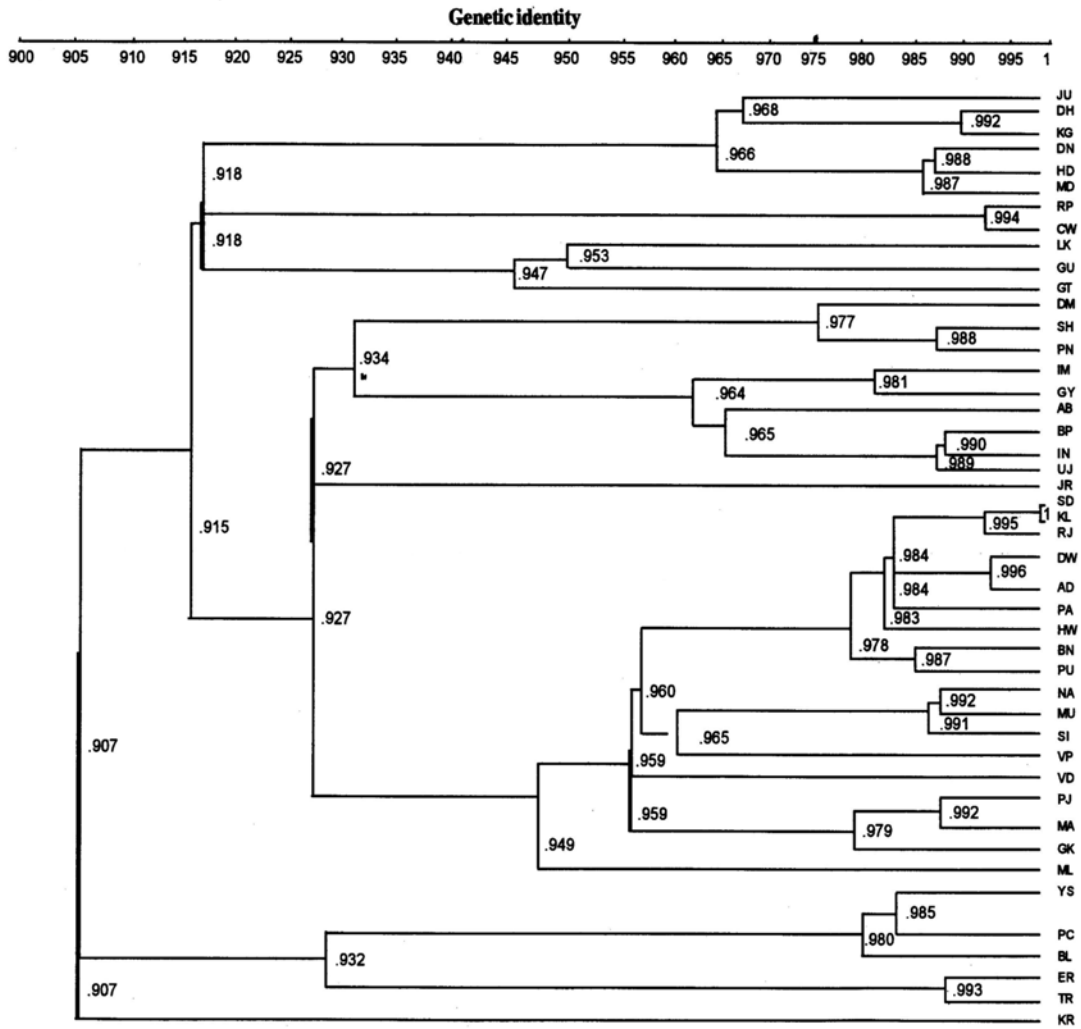

Fig. 3.3 Dendrogram of natural populations of *D. ananassae* based on UPGMA clustering of genetic identity values

degree of inversion polymorphism, it could be said that the size of the observed frequency changes and their regularity make it extremely unlikely that they could be due to random effect, though at the same time absence of clinical variation in the inversion frequencies with respect to latitude, longitude, and altitude also discounts the role of natural selection (Singh and Singh 2007a). So, it could be safe to hold the view that micro-evolutionary processes, being the complicated ones, involve a multitude of factors contributing differentially, spatially, and temporally to the process and that natural selection and genetic drift are not exclusive, but they complement each other in bringing out the evolutionary changes.

3.11 Genetic Differentiation among Natural Populations Using Genetic Distance (D) Approach

Singh and Singh (2007a, 2010) utilized pairwise F_{ST} values and genetic distance (D) methods to reveal the degree of geographic differentiation among Indian natural populations of *D. ananassae* (Singh and Singh 2010). Genetic distance (D) could be defined as the index of genetic differences between populations as the function of gene arrangement frequencies. Many investigators (Powell et al. 1972; Fukatami 1976; Pinsker

Table 3.9 Chi-square values (χ^2) and associated probabilities for different inversion karyotypes among Indian natural populations of *D. ananassae*

Populations	χ^2	Probability	Populations	χ^2	Probability
JU X DH	18.41	<0.02*	JU X SI	42.03	<0.001*
JU X KG	39.85	<0.001*	JU X NA	45.57	<0.001*
JU X DN	30.06	<0.001*	JU X MU	33.75	<0.001*
JU X HD	27.75	<0.001*	JU X VP	13.78	>0.05
JU X MD	25.46	<0.01*	JU X VD	49.33	<0.001*
JU X GT	37.84	<0.001*	JU X PJ	49.63	<0.001*
JU X LK	9.88	>0.05	JU X MA	48.92	<0.001*
JU X GU	72.62	<0.001*	JU X GK	111.56	<0.001*
JU X RP	2.53	>0.05	JU X ML	101.22	<0.001*
JU X CW	7.15	>0.05	JU X BL	34.32	<0.001*
JU X DM	127.20	<0.001*	JU X YS	22.54	<0.01*
JU X SH	43.85	<0.001*	JU X PC	40.11	<0.001*
JU X PN	91.33	<0.001*	JU X ER	79.41	<0.001*
JU X AB	3.64	>0.05	JU X TR	76.70	<0.001*
JU X IM	44.48	<0.001*	JU X KR	120.17	<0.001*
JU X GY	60.69	<0.001*	DH X KG	4.90	>0.05
JU X UJ	19.38	<0.02*	DH X DN	9.89	>0.05
JU X BP	18.12	<0.02*	DH X HD	14.48	>0.05
JU X IN	35.23	<0.001*	DH X MD	15.53	<0.05*
JU X JR	33.35	<0.001*	DH X GT	52.76	<0.001*
JU X HW	15.92	<0.05*	DH X LK	30.93	<0.001*
JU X SD	31.02	<0.001*	DH X GU	84.73	<0.001*
JU X KL	32.18	<0.001*	DH X RP	12.99	>0.05
JU X RJ	25.18	<0.01*	DH X CW	23.34	<0.01*
JU X DW	50.97	<0.001*	DH X DM	94.98	<0.001*
JU X AD	23.54	<0.01*	DH X SH	55.32	<0.001*
JU X PA	29.35	<0.001*	DH X PN	139.53	<0.001*
JU X BN	19.78	<0.02*	DH X AB	14.89	>0.05
JU X PU	25.56	<0.01*	DH X IM	41.51	<0.001*
DH X GY	73.98	<0.001*	KG X GU	118.65	<0.001*
DH X UJ	10.69	>0.05	KG X RP	22.70	<0.01*
DH X BP	6.79	>0.05	KG X CW	40.71	<0.001*
DH X IN	16.04	<0.05*	KG X DM	136.13	<0.001*
DH X JR	33.77	<0.001*	KG X SH	69.18	<0.001*
DH X HW	10.14	>0.05	KG X PN	189.31	<0.001*
DH X SD	22.38	<0.01*	KG X AB	28.08	<0.001*
DH X KL	29.06	<0.001*	KG X IM	60.77	<0.001*
DH X RJ	30.18	<0.001*	KG X GY	96.03	<0.001*
DH X DW	55.46	<0.001*	KG X UJ	12.61	>0.05
DH X AD	29.41	<0.001*	KG X BP	12.36	>0.05
DH X PA	28.10	<0.001*	KG X IN	20.03	<0.02*
DH X BN	10.51	>0.05	KG X JR	42.98	<0.001*
DH X PU	20.57	<0.01*	KG X HW	11.63	>0.05
DH X SI	30.27	<0.001*	KG X SD	22.95	<0.01*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
DH X NA	26.94	<0.001*	KG X KL	38.09	<0.001*
DH X MU	47.48	<0.001*	KG X RJ	42.20	<0.001*
DH X VP	16.78	<0.05*	KG X DW	72.84	<0.001*
DH X VD	32.60	<0.001*	KG X AD	36.95	<0.001*
DH X PJ	33.81	<0.001*	KG X PA	35.69	<0.001*
DH X MA	34.75	<0.001*	KG X BN	10.22	>0.05
DH X GK	67.70	<0.001*	KG X PU	25.21	<0.01*
DH X ML	45.18	<0.001*	KG X SI	46.95	<0.001*
DH X BL	25.21	<0.01*	KG X NA	49.03	<0.001*
DH X YS	8.55	>0.05	KG X MU	75.61	<0.001*
DH X PC	30.17	<0.001*	KG X VP	22.35	<0.01*
DH X ER	41.00	<0.001*	KG X VD	33.23	<0.001*
DH XTR	42.48	<0.001*	KG X PJ	32.89	<0.001*
DH X KR	64.28	<0.001*	KG X MA	39.89	<0.001*
KG X DN	8.45	>0.05	KG X GK	63.08	<0.001*
KG X HD	10.33	>0.05	KG X ML	42.30	<0.001*
KG X MD	19.10	<0.02*	KG X BL	28.37	<0.001*
KG X GT	69.22	<0.001*	KG X YS	10.38	>0.05
KG X LK	48.70	<0.001*	KG X PC	38.66	<0.001*
KG X ER	39.65	<0.001*	DN X PJ	19.21	<0.02*
KG X TR	38.38	<0.001*	DN X MA	22.68	<0.01*
KG X KR	64.08	<0.001*	DN X GK	96.05	<0.001*
DN X HD	10.19	>0.05	DN X ML	28.94	<0.001*
DN X MD	5.20	>0.05	DN X BL	16.45	<0.05*
DN X GT	46.73	<0.001*	DN X YS	11.29	>0.05
DN X LK	31.96	<0.001*	DN X PC	25.63	<0.01*
DN X GU	83.58	<0.001*	DN X ER	20.45	<0.01*
DN X RP	15.69	<0.05*	DN X TR	20.77	<0.01*
DN X CW	31.83	<0.001*	DN X KR	40.59	<0.001*
DN X DM	100.70	<0.001*	HD X MD	13.85	>0.05
DN X SH	47.16	<0.001*	HD X GT	50.29	<0.001*
DN X PN	147.33	<0.001*	HD X LK	35.95	<0.001*
DN X AB	14.66	>0.05	HD X GU	92.49	<0.001*
DN X IM	35.61	<0.001*	HD X RP	17.12	<0.05*
DN X GY	67.89	<0.001*	HD X CW	26.23	<0.001*
DN X UJ	4.82	>0.05	HD X DM	123.29	<0.001*
DN X BP	7.36	>0.05	HD X SH	50.48	<0.001*
DN X IN	4.60	>0.05	HD X PN	160.62	<0.001*
DN X JR	25.40	<0.01*	HD X AB	19.69	<0.02*
DN X HW	8.41	>0.05	HD X IM	56.50	<0.001*
DN X SD	18.36	<0.02*	HD X GY	72.95	<0.001*
DN X KL	21.28	<0.01*	HD X UJ	10.94	>0.05
DN X RJ	23.16	<0.01*	HD X BP	15.86	<0.05*
DN X DW	49.90	<0.001*	HD X IN	20.87	<0.01*
DN X AD	24.35	<0.01*	HD X JR	34.26	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
DN X PA	17.64	<0.05*	HD X HW	10.48	>0.05
DN X BN	10.33	>0.05	HD X SD	20.27	<0.01*
DN X PU	18.19	<0.02*	HD X KL	33.42	<0.001*
DN X SI	32.11	<0.001*	HD X RJ	35.32	<0.001*
DN X NA	37.85	<0.001*	HD X DW	56.91	<0.001*
DN X MU	49.59	<0.001*	HD X AD	28.57	<0.001*
DN X VP	10.09	>0.05	HD X PA	26.53	<0.001*
DN X VD	21.02	<0.01*	HD X BN	12.32	>0.05
HD X PU	21.13	<0.01*	MD X RJ	16.13	<0.05*
HD X SI	49.97	<0.001*	MD X DW	58.58	<0.001*
HD X NA	54.89	<0.001*	MD X AD	19.72	<0.02*
HD X MU	62.41	<0.001*	MD X PA	8.89	>0.05
HD X VP	14.05	>0.05	MD X BN	8.94	>0.05
HD X VD	21.78	<0.01*	MD X PU	12.67	>0.05
HD X PJ	26.58	<0.001*	MD X SI	29.97	<0.001*
HD X MA	36.58	<0.001*	MD X NA	39.60	<0.001*
HD X GK	55.03	<0.001*	MD X MU	32.96	<0.001*
HD X ML	51.94	<0.001*	MD X VP	5.64	>0.05
HD X BL	20.85	<0.01*	MD X VD	13.01	>0.05
HD X YS	14.49	>0.05	MD X PJ	17.37	<0.05*
HD X PC	31.40	<0.001*	MD X MA	18.41	<0.02*
HD X ER	37.52	<0.001*	MD X GK	34.32	<0.001*
HD X TR	34.03	<0.001*	MD X ML	31.50	<0.001*
HD X KR	58.08	<0.001*	MD X BL	10.64	>0.05
MD X GT	32.38	<0.001*	MD X YS	10.96	>0.05
MD X LK	22.38	<0.01*	MD X PC	16.05	<0.05*
MD X GU	54.91	<0.001*	MD X ER	13.98	>0.05
MD X RP	12.71	>0.05	MD X TR	17.26	<0.05*
MD X CW	25.28	<0.01*	MD X KR	27.30	<0.001*
MD X DM	75.22	<0.001*	GT X LK	19.76	<0.02*
MD X SH	35.22	<0.001*	GT X GU	1.48	>0.05
MD X PN	115.24	<0.001*	GT X RP	25.15	<0.01*
MD X AB	10.61	>0.05	GT X CW	46.33	<0.001*
MD X IM	22.49	<0.01*	GT X DM	4.13	>0.05
MD X GY	51.20	<0.001*	GT X SH	2.74	>0.05
MD X UJ	3.90	>0.05	GT X PN	8.46	>0.05
MD X BP	10.74	>0.05	GT X AB	32.59	<0.001*
MD X IN	2.74	>0.05	GT X IM	12.75	>0.05
MD X JR	19.18	<0.02*	GT X GY	5.15	>0.05
MD X HW	11.93	>0.05	GT X UJ	24.42	<0.01*
MD X SD	15.78	<0.05*	GT X BP	42.83	<0.001*
MD X KL	15.35	>0.05	GT X IN	55.71	<0.001*
GT X JR	9.30	>0.05	LK X UJ	17.15	<0.05*
GT X HW	26.81	<0.001*	LK X BP	21.48	<0.01*
GT X SD	17.58	<0.05*	LK X IN	40.62	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
GT X KL	14.94	>0.05	LK X JR	21.24	<0.01*
GT X RJ	12.13	>0.05	LK X HW	21.42	<0.01*
GT X DW	8.49	>0.05	LK X SD	19.95	<0.02*
GT X AD	6.56	>0.05	LK X KL	26.10	<0.001*
GT X PA	14.34	>0.05	LK X RJ	19.15	<0.02*
GT X BN	12.60	>0.05	LK X DW	28.35	<0.001*
GT X PU	8.95	>0.05	LK X AD	13.95	>0.05
GT X SI	32.29	<0.001*	LK X PA	13.07	>0.05
GT X NA	51.41	<0.001*	LK X BN	18.13	<0.05*
GT X MU	10.95	>0.05	LK X PU	14.96	>0.05
GT X VP	19.85	<0.02*	LK X SI	28.62	<0.001*
GT X VD	23.35	<0.01*	LK X NA	37.71	<0.001*
GT X PJ	19.61	<0.02*	LK X MU	9.44	>0.05
GT X MA	18.59	<0.02*	LK X VP	19.85	<0.02*
GT X GK	42.74	<0.001*	LK X VD	29.70	<0.001*
GT X ML	53.95	<0.001*	LK X PJ	37.90	<0.001*
GT X BL	35.83	<0.001*	LK X MA	34.38	<0.001*
GT X YS	38.41	<0.001*	LK X GK	77.18	<0.001*
GT X PC	44.88	<0.001*	LK X ML	74.51	<0.001*
GT X ER	37.64	<0.001*	LK X BL	38.50	<0.001*
GT X TR	39.14	<0.001*	LK X YS	34.41	<0.001*
GT X KR	53.73	<0.001*	LK X PC	46.91	<0.001*
LK X GU	28.71	<0.001*	LK X ER	48.93	<0.001*
LK X RP	14.38	>0.05	LK X TR	55.22	<0.001*
LK X CW	12.01	>0.05	LK X KR	70.28	<0.001*
LK X DM	50.75	<0.001*	GU X RP	37.09	<0.001*
LK X SH	28.11	<0.001*	GU X CW	77.78	<0.001*
LK X PN	58.69	<0.001*	GU X DM	13.31	>0.05
LK X AB	12.70	>0.05	GU X SH	7.58	>0.05
LK X IM	29.92	<0.001*	GU X PN	17.35	<0.05*
LK X GY	34.40	<0.001*	GU X AB	51.35	<0.001*
GU X IM	29.98	<0.001*	RP X AB	6.39	>0.05
GU X GY	10.82	>0.05	RP X IM	28.90	<0.001*
GU X UJ	43.64	<0.001*	RP X GY	40.68	<0.001*
GU X BP	69.52	<0.001*	RP X UJ	9.44	>0.05
GU X IN	101.56	<0.001*	RP X BP	19.13	<0.02*
GU X JR	18.54	<0.02*	RP X IN	22.13	<0.01*
GU X HW	43.27	<0.001*	RP X JR	20.65	<0.01*
GU X SD	31.25	<0.001*	RP X HW	12.79	>0.05
GU X KL	29.55	<0.001*	RP X SD	16.02	<0.05*
GU X RJ	20.80	<0.01*	RP X KL	22.54	<0.01*
GU X DW	18.18	<0.02*	RP X RJ	19.49	<0.02*
GU X AD	8.81	>0.05	RP X DW	30.24	<0.001*
GU X PA	26.30	<0.001*	RP X AD	15.48	>0.05
GU X BN	15.89	<0.05*	RP X PA	11.73	>0.05

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
GU X PU	15.12	>0.05	RP X BN	13.07	>0.05
GU X SI	50.67	<0.001*	RP X PU	12.63	>0.05
GU X NA	75.60	<0.001*	RP X SI	22.05	<0.01*
GU X MU	15.24	>0.05	RP X NA	25.16	<0.01*
GU X VP	36.02	<0.001*	RP X MU	14.60	>0.05
GU X VD	50.33	<0.001*	RP X VP	6.22	>0.05
GU X PJ	38.29	<0.001*	RP X VD	20.97	<0.01*
GU X MA	40.93	<0.001*	RP X PJ	30.41	<0.001*
GU X GK	86.89	<0.001*	RP X MA	27.77	<0.001*
GU X ML	107.23	<0.001*	RP X GK	60.69	<0.001*
GU X BL	63.19	<0.001*	RP X ML	50.69	<0.001*
GU X YS	56.14	<0.001*	RP X BL	26.01	<0.001*
GU X PC	69.84	<0.001*	RP X YS	19.92	<0.02*
GU X ER	77.58	<0.001*	RP X PC	31.66	<0.001*
GU X TR	74.34	<0.001*	RP X ER	34.95	<0.001*
GU X KR	103.59	<0.001*	RP X TR	40.66	<0.001*
RP X CW	2.48	>0.05	RP X KR	53.36	<0.001*
RP X DM	62.17	<0.001*	CW X DM	113.29	<0.001*
RP X SH	32.06	<0.001*	CW X SH	56.01	<0.001*
RP X PN	74.74	<0.001*	CW X PN	137.90	<0.001*
CW X AB	10.64	>0.05	DM X IM	17.19	<0.05*
CW X IM	62.69	<0.001*	DM X GY	7.28	>0.05
CW X GY	73.53	<0.001*	DM X UJ	38.75	<0.001*
CW X UJ	22.68	<0.01*	DM X BP	63.56	<0.001*
CW X BP	23.94	<0.01*	DM X IN	95.37	<0.001*
CW X IN	42.78	<0.001*	DM X JR	12.08	>0.05
CW X JR	45.42	<0.001*	DM X HW	26.90	<0.001*
CW X HW	27.23	<0.001*	DM X SD	30.17	<0.001*
CW X SD	35.31	<0.001*	DM X KL	19.80	<0.02*
CW X KL	45.97	<0.001*	DM X RJ	17.08	<0.05*
CW X RJ	37.91	<0.001*	DM X DW	8.89	>0.05
CW X DW	65.85	<0.001*	DM X AD	17.33	<0.05*
CW X AD	33.35	<0.001*	DM X PA	29.59	<0.001*
CW X PA	31.23	<0.001*	DM X BN	35.54	<0.001*
CW X BN	28.01	<0.001*	DM X PU	16.22	<0.05*
CW X PU	30.59	<0.001*	DM X SI	44.19	<0.001*
CW X SI	56.19	<0.001*	DM X NA	64.28	<0.001*
CW X NA	61.63	<0.001*	DM X MU	15.39	>0.05
CW X MU	43.72	<0.001*	DM X VP	33.13	<0.001*
CW X VP	17.12	<0.05*	DM X VD	61.47	<0.001*
CW X VD	43.10	<0.001*	DM X PJ	40.25	<0.001*
CW X PJ	57.66	<0.001*	DM X MA	30.92	<0.001*
CW X MA	61.45	<0.001*	DM X GK	92.20	<0.001*
CW X GK	113.34	<0.001*	DM X ML	105.02	<0.001*
CW X ML	108.45	<0.001*	DM X BL	49.77	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
CW X BL	41.58	<0.001*	DM X YS	45.38	<0.001*
CW X YS	28.54	<0.001*	DM X PC	64.66	<0.001*
CW X PC	43.33	<0.001*	DM X ER	97.71	<0.001*
CW X ER	72.75	<0.001*	DM X TR	78.65	<0.001*
CW X TR	78.06	<0.001*	DM X KR	149.04	<0.001*
CW X KR	99.90	<0.001*	SH X PN	13.66	>0.05
DM X SH	8.99	>0.05	SH X AB	36.28	<0.001*
DM X PN	26.92	<0.001*	SH X IM	11.79	>0.05
DM X AB	42.40	<0.001*	SH X GY	3.02	>0.05
SH X UJ	26.62	<0.001*	PN X JR	33.61	<0.001*
SH X BP	45.12	<0.001*	PN X HW	70.04	<0.001*
SH X IN	53.85	<0.001*	PN X SD	74.80	<0.001*
SH X JR	8.02	>0.05	PN X KL	61.57	<0.001*
SH X HW	25.51	<0.01*	PN X RJ	42.08	<0.001*
SH X SD	17.73	<0.05*	PN X DW	20.86	<0.01*
SH X KL	13.49	>0.05	PN X AD	19.25	<0.02*
SH X RJ	12.36	>0.05	PN X PA	81.68	<0.001*
SH X DW	5.82	>0.05	PN X BN	51.69	<0.001*
SH X AD	6.70	>0.05	PN X PU	44.75	<0.001*
SH X PA	18.31	<0.05*	PN X SI	60.91	<0.001*
SH X BN	12.71	>0.05	PN X NA	89.67	<0.001*
SH X PU	10.90	>0.05	PN X MU	27.43	<0.001*
SH X SI	31.30	<0.001*	PN X VP	81.65	<0.001*
SH X NA	48.97	<0.001*	PN X VD	126.13	<0.001*
SH X MU	15.81	<0.05*	PN X PJ	67.86	<0.001*
SH X VP	23.44	<0.01*	PN X MA	73.30	<0.001*
SH X VD	26.74	<0.001*	PN X GK	140.27	<0.001*
SH X PJ	15.62	<0.05*	PN X ML	155.59	<0.001*
SH X MA	15.53	<0.05*	PN X BL	127.12	<0.001*
SH X GK	35.61	<0.001*	PN X YS	107.18	<0.001*
SH X ML	46.23	<0.001*	PN X PC	139.75	<0.001*
SH X BL	35.85	<0.001*	PN X ER	150.43	<0.001*
SH X YS	40.69	<0.001*	PN X TR	128.37	<0.001*
SH X PC	48.11	<0.001*	PN X KR	204.39	<0.001*
SH X ER	37.63	<0.001*	AB X IM	26.41	<0.001*
SH X TR	34.62	<0.001*	AB X GY	45.80	<0.001*
SH X KR	56.31	<0.001*	AB X UJ	12.58	>0.05
PN X AB	95.39	<0.001*	AB X BP	12.13	>0.05
PN X IM	66.42	<0.001*	AB X IN	12.27	>0.05
PN X GY	10.19	>0.05	AB X JR	24.50	<0.01*
PN X UJ	93.03	<0.001*	AB X HW	12.28	>0.05
PN X BP	112.58	<0.001*	AB X SD	25.97	<0.01*
PN X IN	175.97	<0.001*	AB X KL	21.53	<0.01*
AB X RJ	17.96	<0.05*	IM X PU	7.71	>0.05
AB X DW	38.17	<0.001*	IM X SI	28.56	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
AB X AD	21.68	<0.01*	IM X NA	45.01	<0.001*
AB X PA	19.63	<0.02*	IM X MU	21.34	<0.01*
AB X BN	17.86	<0.05*	IM X VP	19.14	<0.02*
AB X PU	22.39	<0.01*	IM X VD	34.18	<0.001*
AB X SI	29.70	<0.001*	IM X PJ	12.37	>0.05
AB X NA	29.20	<0.001*	IM X MA	7.41	>0.05
AB X MU	25.75	<0.01*	IM X GK	44.76	<0.001*
AB X VP	10.92	>0.05	IM X ML	47.22	<0.001*
AB X VD	32.40	<0.001*	IM X BL	21.92	<0.01*
AB X PJ	31.72	<0.001*	IM X YS	20.69	<0.01*
AB X MA	30.61	<0.001*	IM X PC	30.05	<0.001*
AB X GK	67.44	<0.001*	IM X ER	38.47	<0.001*
AB X ML	58.45	<0.001*	IM X TR	31.69	<0.001*
AB X BL	21.90	<0.01*	IM X KR	70.67	<0.001*
AB X YS	17.59	<0.05*	GY X UJ	41.24	<0.001*
AB X PC	27.65	<0.001*	GY X BP	59.16	<0.001*
AB X ER	42.16	<0.001*	GY X IN	76.05	<0.001*
AB X TR	42.17	<0.001*	GY X JR	12.16	>0.05
AB X KR	66.38	<0.001*	GY X HW	33.10	<0.001*
IM X GY	21.17	<0.01*	GY X SD	31.21	<0.001*
IM X UJ	15.78	<0.05*	GY X KL	23.26	<0.01*
IM X BP	34.52	<0.001*	GY X RJ	16.27	<0.05*
IM X IN	37.08	<0.001*	GY X DW	6.74	>0.05
IM X JR	4.44	>0.05	GY X AD	8.81	>0.05
IM X HW	16.96	<0.05*	GY X PA	29.43	<0.001*
IM X SD	21.31	<0.01*	GY X BN	21.25	<0.01*
IM X KL	1.23	>0.05	GY X PU	20.64	<0.01*
IM X RJ	3.13	>0.05	GY X SI	36.18	<0.001*
IM X DW	15.06	>0.05	GY X NA	55.17	<0.001*
IM X AD	8.84	>0.05	GY X MU	17.91	<0.05*
IM X PA	12.94	>0.05	GY X VP	35.62	<0.001*
IM X BN	3.88	>0.05	GY X VD	52.55	<0.001*
GY X PJ	25.29	<0.01*	UJ X ER	10.90	>0.05
GY X MA	27.32	<0.001*	UJ X TR	15.30	>0.05
GY X GK	59.11	<0.001*	UJ X KR	25.25	<0.01*
GY X ML	70.55	<0.001*	BP X IN	15.47	>0.05
GY X BL	55.75	<0.001*	BP X JR	22.79	<0.01*
GY X YS	55.58	<0.001*	BP X HW	6.91	>0.05
GY X PC	69.22	<0.001*	BP X SD	17.33	<0.05*
GY X ER	63.83	<0.001*	BP X KL	23.61	<0.01*
GY X TR	53.35	<0.001*	BP X RJ	20.76	<0.01*
GY X KR	93.08	<0.001*	BP X DW	40.13	<0.001*
UJ X BP	7.76	>0.05	BP X AD	18.90	<0.02*
UJ X IN	10.02	>0.05	BP X PA	18.44	<0.02*
UJ X JR	11.10	>0.05	BP X BN	11.22	>0.05

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
UJ X HW	6.69	>0.05	BP X PU	17.36	<0.05*
UJ X SD	9.86	>0.05	BP X SI	15.73	<0.05*
UJ X KL	8.90	>0.05	BP X NA	15.74	<0.05*
UJ X RJ	11.93	>0.05	BP X MU	31.83	<0.001*
UJ X DW	25.96	<0.01*	BP X VP	10.11	>0.05
UJ X AD	13.12	>0.05	BP X VD	30.49	<0.001*
UJ X PA	5.03	>0.05	BP X PJ	26.62	<0.001*
UJ X BN	3.16	>0.05	BP X MA	26.73	<0.001*
UJ X PU	5.09	>0.05	BP X GK	63.36	<0.001*
UJ X SI	20.22	<0.01*	BP X ML	40.54	<0.001*
UJ X NA	31.34	<0.001*	BP X BL	27.66	<0.001*
UJ X MU	23.16	<0.01*	BP X YS	17.89	<0.05*
UJ X VP	2.09	>0.05	BP X PC	40.09	<0.001*
UJ X VD	7.56	>0.05	BP X ER	36.79	<0.001*
UJ X PJ	12.47	>0.05	BP X TR	37.50	<0.001*
UJ X MA	9.77	>0.05	BP X KR	62.62	<0.001*
UJ X GK	27.27	<0.001*	IN X JR	32.38	<0.001*
UJ X ML	21.92	<0.01*	IN X HW	13.95	>0.05
UJ X BL	9.75	>0.05	IN X SD	26.59	<0.001*
UJ X YS	9.61	>0.05	IN X KL	23.93	<0.01*
UJ X PC	18.83	<0.02*	IN X RJ	24.62	<0.01*
IN X DW	62.62	<0.001*	JR X PJ	6.98	>0.05
IN X AD	30.58	<0.001*	JR X MA	3.20	>0.05
IN X PA	23.97	<0.01*	JR X GK	26.97	<0.001*
IN X BN	16.92	<0.05*	JR X ML	24.01	<0.01*
IN X PU	29.33	<0.001*	JR X BL	25.33	<0.01*
IN X SI	48.97	<0.001*	JR X YS	24.84	<0.01*
IN X NA	55.99	<0.001*	JR X PC	37.59	<0.001*
IN X MU	64.17	<0.001*	JR X ER	23.06	<0.01*
IN X VP	18.07	<0.05*	JR X TR	20.60	<0.01*
IN X VD	35.93	<0.001*	JR X KR	44.96	<0.001*
IN X PJ	23.24	<0.01*	HW X SD	14.60	>0.05
IN X MA	29.98	<0.001*	HW X KL	11.32	>0.05
IN X GK	55.70	<0.001*	HW X RJ	13.66	>0.05
IN X ML	46.88	<0.001*	HW X DW	18.25	<0.02*
IN X BL	15.32	>0.05	HW X AD	13.10	>0.05
IN X YS	12.69	>0.05	HW X PA	15.24	>0.05
IN X PC	22.93	<0.01*	HW X BN	6.61	>0.05
IN X ER	32.00	<0.001*	HW X PU	11.38	>0.05
IN X TR	27.13	<0.001*	HW X SI	11.12	>0.05
IN X KR	61.14	<0.001*	HW X NA	9.73	>0.05
JR X HW	11.13	>0.05	HW X MU	22.40	<0.01*
JR X SD	12.29	>0.05	HW X VP	9.44	>0.05
JR X KL	3.53	>0.05	HW X VD	19.68	<0.02*
JR X RJ	3.49	>0.05	HW X PJ	12.88	>0.05

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
JR X DW	3.50	>0.05	HW X MA	10.40	>0.05
JR X AD	3.20	>0.05	HW X GK	30.14	<0.001*
JR X PA	8.08	>0.05	HW X ML	17.27	<0.05*
JR X BN	2.37	>0.05	HW X BL	17.56	<0.05*
JR X PU	4.43	>0.05	HW X YS	14.23	>0.05
JR X SI	11.56	>0.05	HW X PC	30.86	<0.001*
JR X NA	26.10	<0.01*	HW X ER	22.45	<0.01*
JR X MU	10.91	>0.05	HW X TR	18.92	<0.02*
JR X VP	11.26	>0.05	HW X KR	42.93	<0.001*
JR XVD	20.55	<0.01*	SD X KL	16.65	<0.05*
SD X RJ	19.45	<0.02*	KL X GK	26.31	<0.001*
SD X DW	17.04	<0.05*	KL X ML	24.21	<0.01*
SD X AD	8.28	>0.05	KL X BL	14.93	>0.05
SD X PA	6.85	>0.05	KL X YS	15.64	<0.05*
SD X BN	8.67	>0.05	KL X PC	24.52	<0.01*
SD X PU	5.82	>0.05	KL X ER	20.02	<0.02*
SD X SI	20.95	<0.01*	KL X TR	28.39	<0.001*
SD X NA	36.08	<0.001*	KL X KR	41.78	<0.001*
SD X MU	19.18	<0.02*	RJ X DW	10.57	>0.05
SD X VP	8.14	>0.05	RJ X AD	7.67	>0.05
SD X VD	9.92	>0.05	RJ X PA	9.64	>0.05
SD X PJ	16.13	<0.05*	RJ X BN	9.23	>0.05
SD X MA	14.06	>0.05	RJ X PU	9.43	>0.05
SD X GK	24.71	<0.01*	RJ X SI	13.14	>0.05
SD X ML	24.70	<0.01*	RJ X NA	25.96	<0.01*
SD X BL	21.90	<0.02*	RJ X MU	11.01	>0.05
SD X YS	24.25	<0.01*	RJ X VP	11.98	>0.05
SD X PC	32.56	<0.001*	RJ X VD	26.50	<0.001*
SD X ER	16.92	<0.05*	RJ X PJ	11.24	>0.05
SD X TR	20.91	<0.01*	RJ X MA	7.57	>0.05
SD X KR	28.32	<0.001*	RJ X GK	35.99	<0.001*
KL X RJ	3.69	>0.05	RJ X ML	30.91	<0.001*
KL X DW	14.08	>0.05	RJ X BL	22.79	<0.01*
KL X AD	8.87	>0.05	RJ X YS	22.04	<0.01*
KL X PA	9.81	>0.05	RJ X PC	31.07	<0.001*
KL X BN	2.10	>0.05	RJ X ER	25.56	<0.01*
KL X PU	5.62	>0.05	RJ X TR	24.09	<0.01*
KL X SI	20.54	<0.01*	RJ X KR	47.86	<0.001*
KL X NA	34.08	<0.001*	DW X AD	2.93	>0.05
KL X MU	20.71	<0.01*	DW X PA	19.13	<0.01*
KL X VP	12.45	>0.05	DW X BN	6.81	>0.05
KL X VD	20.07	<0.01*	DW X PU	18.00	<0.05*
KL X PJ	7.16	>0.05	DW X SI	18.32	<0.05*
KL X MA	2.51	>0.05	DW X NA	32.98	<0.001*
DW X MU	11.71	>0.05	PA X NA	43.73	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
DW X VP	23.33	<0.01*	PA X MU	14.47	>0.05
DW X VD	39.93	<0.001*	PA X VP	2.76	>0.05
DW X PJ	15.74	<0.05*	PA X VD	8.44	>0.05
DW X MA	14.06	>0.05	PA X PJ	14.65	>0.05
DW X GK	48.33	<0.001*	PA X MA	11.25	>0.05
DW X ML	48.91	<0.001*	PA X GK	32.13	<0.001*
DW X BL	45.14	<0.001*	PA X ML	34.63	<0.001*
DW X YS	42.56	<0.001*	PA X BL	19.58	<0.02*
DW X PC	62.24	<0.001*	PA X YS	22.24	<0.01*
DW X ER	51.24	<0.001*	PA X PC	29.07	<0.001*
DW X TR	41.33	<0.001*	PA X ER	15.27	>0.05
DW X KR	83.30	<0.001*	PA X TR	21.82	<0.01*
AD X PA	7.60	>0.05	PA X KR	29.16	<0.001*
AD X BN	6.86	>0.05	BN X PU	2.10	>0.05
AD X PU	4.92	>0.05	BN X SI	8.39	>0.05
AD X SI	7.56	>0.05	BN X NA	22.60	<0.01*
AD X NA	18.78	<0.02*	BN X MU	11.45	>0.05
AD X MU	4.97	>0.05	BN X VP	4.89	>0.05
AD X VP	10.31	>0.05	BN X VD	4.89	>0.05
AD X VD	17.66	<0.05*	BN X PJ	2.50	>0.05
AD X PJ	10.56	>0.05	BN X MA	1.18	>0.05
AD X MA	8.73	>0.05	BN X GK	5.79	>0.05
AD X GK	28.18	<0.001*	BN X ML	4.37	>0.05
AD X ML	22.94	<0.01*	BN X BL	8.29	>0.05
AD X BL	28.39	<0.001*	BN X YS	8.76	>0.05
AD X YS	28.18	<0.001*	BN X PC	14.85	>0.05
AD X PC	37.67	<0.001*	BN X ER	4.10	>0.05
AD X ER	21.98	<0.01*	BN X TR	5.69	>0.05
AD X TR	22.68	<0.01*	BN X KR	10.77	>0.05
AD X KR	38.41	<0.001*	PU X SI	17.85	<0.05*
PA X BN	4.98	>0.05	PU X NA	35.88	<0.001*
PA X PU	2.42	>0.05	PU X MU	10.84	>0.05
PA X SI	23.90	<0.01*	PU X VP	4.78	>0.05
PU X VD	5.08	>0.05	NA X PC	72.89	<0.001*
PU X PJ	9.87	>0.05	NA X ER	81.92	<0.001*
PU X MA	5.55	>0.05	NA X TR	66.98	<0.001*
PU X GK	19.35	<0.02*	NA X KR	130.19	<0.001*
PU X ML	21.38	<0.01*	MU X VP	16.61	<0.05*
PU X BL	15.25	>0.05	MU X VD	43.25	<0.001*
PU X YS	16.81	<0.05*	MU X PJ	33.18	<0.001*
PU X PC	24.26	<0.01*	MU X MA	28.75	<0.001*
PU X ER	11.47	>0.05	MU X GK	80.59	<0.001*
PU X TR	17.30	<0.05*	MU X ML	78.94	<0.001*
PU X KR	21.81	<0.01*	MU X BL	51.06	<0.001*
SI X NA	5.25	>0.05	MU X YS	40.32	<0.001*

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
SI X MU	18.06	<0.05*	MU X PC	60.30	<0.001*
SI X VP	18.72	<0.02*	MU X ER	61.28	<0.001*
SI X VD	49.69	<0.001*	MU X TR	61.07	<0.001*
SI X PJ	21.94	<0.01*	MU X KR	92.14	<0.001*
SI X MA	21.33	<0.01*	VP X VD	8.64	>0.05
SI X GK	67.69	<0.001*	VP X PJ	16.98	<0.05*
SI X ML	41.63	<0.001*	VP X MA	15.06	>0.05
SI X BL	49.56	<0.001*	VP X GK	39.21	<0.001*
SI X YS	30.18	<0.001*	VP X ML	37.88	<0.001*
SI X PC	61.36	<0.001*	VP X BL	16.05	<0.05*
SI X ER	52.92	<0.001*	VP X YS	16.77	<0.05*
SI XTR	45.27	<0.001*	VP X PC	25.80	<0.01*
SI X KR	90.40	<0.001*	VP X ER	19.28	<0.02*
NA X MU	32.73	<0.001*	VP X TR	24.49	<0.01*
NA X VP	35.49	<0.001*	VP X KR	35.62	<0.001*
NA X VD	80.54	<0.001*	VD X PJ	16.26	<0.05*
NA X PJ	37.83	<0.001*	VD X MA	20.84	<0.01*
NA X MA	37.47	<0.001*	VD X GK	26.55	<0.001*
NA X GK	95.77	<0.001*	VD X ML	44.94	<0.001*
NA X ML	58.86	<0.001*	VD X BL	13.40	>0.05
NA X BL	62.86	<0.001*	VD X YS	18.03	<0.05*
NA X YS	34.27	<0.001*	VD X PC	23.05	<0.01*
VD X ER	13.43	>0.05	BL X PC	5.90	>0.05
VD X TR	20.74	<0.01*	BL X ER	17.32	<0.05*
VD X KR	18.98	<0.02*	BL X TR	21.15	<0.01*
PJ X MA	3.47	>0.05	BL X KR	27.62	<0.001*
PJ X GK	5.08	>0.05	YS X PC	4.40	>0.05
PJ X ML	5.89	>0.05	YS X ER	16.64	<0.05*
PJ X BL	16.68	<0.05*	YS X TR	16.97	<0.05*
PJ X YS	19.61	<0.02*	YS X KR	23.07	<0.01*
PJ X PC	28.47	<0.001*	PC X ER	25.92	<0.01*
PJ X ER	9.65	>0.05	PC X TR	26.95	<0.001*
PJ X TR	4.60	>0.05	PC X KR	30.14	<0.001*
PJ X KR	23.03	<0.01*	ER X TR	4.82	>0.05
MA X GK	18.43	<0.02*	ER X KR	6.59	>0.05
MA X ML	15.15	>0.05	TR X KR	15.62	<0.05*
MA X BL	19.66	<0.02*			
MA X YS	19.73	<0.02*			
MA X PC	33.59	<0.001*			
MA X ER	17.49	<0.05*			
MA X TR	12.83	>0.05			
MA X KR	41.57	<0.001*			
GK X ML	15.07	>0.05			
GK X BL	26.40	<0.001*			
GK X YS	28.41	<0.001*			

(continued)

Table 3.9 (continued)

Populations	χ^2	Probability	Populations	χ^2	Probability
GK X PC	41.08	<0.001*			
GK X ER	10.98	>0.05			
GK X TR	3.56	>0.05			
GK X KR	21.76	<0.01*			
ML X BL	33.85	<0.001*			
ML X YS	22.07	<0.01*			
ML X PC	49.22	<0.001*			
ML X ER	18.32	<0.02*			
ML X TR	8.50	>0.05			
ML X KR	43.61	<0.001*			
BL X YS	5.03	>0.05			

*Significant

and Sperlich 1979; Larruga et al. 1983; Singh 1984a, b, 1986, 1989a, 1991; Singh and Anand 1995) have utilized genetic distance (D) indexes before to determine the genetic differentiation at the level of inversion polymorphism in *Drosophila*. It could be obtained from the genetic identity (I) formula given by Nei (1972), i.e., (D = 1 – I).

Table 3.10 shows the pairwise genetic distance (D) values among Indian natural populations of *D. ananassae*. These values show the range from 0.000 (UJ vs. IN and KL vs. SD) to 0.436 (LK vs. GK). The lowermost D values (close to zero) correspond to geographically closest populations (Singh and Singh 2007a, 2010).

3.12 Laboratory Populations

In a study by Singh and Singh (2007b), 45 laboratory populations of *D. ananassae* were initiated from females collected from different geographical localities in India (Fig. 3.1). Table 3.1 provides the collection details. Laboratory populations were maintained on simple culture medium by transferring nearly 50 flies (equal number of males and females) to fresh food bottles in each generation under normal laboratory conditions. These stocks were maintained for several generations (minimum ten generations). To obtain quantitative data on the frequencies of different gene arrangements, chromosomal analysis of these populations was done by squashing around 100 third-instar larvae taken from each culture bottle following lacto-aceto-orcein method (Singh and Singh 2007b). Employing Nei's (1972) genetic identity (I) formula, genetic divergence at the level of chromosomal polymorphism was analyzed by calculating genetic identity (I) and genetic distance (D) between natural and laboratory populations (Singh 1984b; Singh and Singh 2007b).

Results of chromosomal analysis of laboratory populations of *D. ananassae* show the persistence of all the three cosmopolitan inversions (Singh and Singh 2007b). However, some laboratory populations became monomorphic for standard or inverted gene arrangements. Table 3.1 shows the frequencies (in percent) of different gene arrangements in laboratory populations of *D. ananassae* while the gene arrangement frequencies in corresponding natural populations are given in parentheses (Singh and Singh 2007b). Out of 45 laboratory populations analyzed, 6 populations, namely, DN, GU, PN, DW AD, and BN, became monomorphic in 2L for AL inversion, while 8 populations, namely, LK, GU, RP, UJ, JR, RJ, PU, and MA, became monomorphic in 3L for standard gene arrangement. Nine populations, DN, HD, CW, UJ, IN, PA, NA, MU, and BL, became monomorphic in 3R for standard gene arrangement (Singh and Singh 2007b).

The frequency of AL ranges from 8.0 % (RP) to 100.0 % (DN, GU, PN, DW, AD, and BN)

(Singh and Singh 2007b). Comparison of natural and laboratory populations with respect to alpha (AL) inversion shows that some populations have retained the same inversion frequency of alpha inversion while other populations show decreasing and increasing pattern in the frequencies of alpha inversion. Similar results were obtained for delta (DE) inversion in 3L, which shows the range of 0.0 % (LK, GU, RP, UJ, JR, RJ, PU, and MA) to 91.0 % (KR) (Singh and Singh 2007b). Similarly, ET inversion in 3R shows the range of 0.0 % (DN, HD, CW, UJ, IN, PA, NA, MU, and BL) to 50.5 % (RJ). Of the three cosmopolitan inversions, alpha shows the maximum persistence followed by delta and eta (Singh and Singh 2007b). Level of inversion heterozygosity as the index of mean number of heterozygous inversions per individual ranges from 0.148 in RP to 1.48 in PJ (Table 3.11) (Singh and Singh 2007b). Level of inversion heterozygosity shows both increasing and decreasing trend though difference is not much in most of the populations. Both the frequencies of the three cosmopolitan inversions and the level of inversion heterozygosity show north–south trend (Singh and Singh 2007a, b).

3.13 Genetic Divergence in Laboratory Populations

Singh and Singh (2007b) analyzed the level of genetic divergence, between natural populations and laboratory populations initiated from the same natural populations of *D. ananassae* (Singh 1987). This was to done to examine the behavior of chromosomal inversion polymorphism under constant laboratory environment. Genetic identity (I) and genetic distance(D), which are the indexes of genetic differences, as functions of inversion frequencies, were calculated between the natural and laboratory populations. The genetic identity (I) values were calculated using the formula given by Nei (1972). However, the genetic distance values D were calculated by subtracting genetic identity values (I) from 1 (D = 1 − I). The values of I and D are given in Table 3.12 (Singh and Singh 2007b). Figure 3.4 shows the histogram based on the genetic distances (D) values between natural and laboratory populations. The values of D ranging from 0.006 in (KG) to 0.279 in (BN) clearly reveal the variation in the degree of genetic divergence in *D. ananassae* populations transferred to a laboratory environment. Other populations also show genetic divergence to lower and higher extent during their maintenance in the laboratory environment. Especially, populations coming from similar geographical and climatic conditions and initially showing high level of genetic identity have diverged to different degrees (Singh and Singh 2007b).

The study by Singh and Singh (2007) clearly demonstrates the persistence of inversion polymorphism due to three cosmopolitan inversions when natural populations of *D. ananassae* were transferred and maintained under laboratory environment for minimum ten generations. These results strongly support the idea of heterotic buffering associated with three cosmopolitan inversions in *D. ananassae*, which have also become coextensive with the species distribution (Singh and Singh 2007b). Some of the studied populations, however, became monomorphic for certain arrangements, i.e., show fixation of some inversion. In some populations, a complete elimination of certain gene arrangements was also reported, which could be attributed to its low initial frequency in the sample. Carson (1958a) has postulated that if a particular gene arrangement starts at low initial frequency, it has greater probability of early loss due to the action of random genetic drift provided that population size is limited, which is quite common for laboratory populations (Singh and Singh 2007b). The study by Singh and Singh (2008) supports this assumption, as maximum number of populations became monomorphic for standard (ST) gene arrangement in 3R due to loss of eta chromosome, which usually occurs at low frequency in most of the founding populations. This is also plausible as eta (ET) is the smallest inversion to have lesser tendency of capturing genes with favorable epistatic effect on fitness (Singh and Singh 2007b). On the other hand, alpha (AL) inversion, being the longest in *D. ananassae*, has more possibility

Table 3.10 Pairwise Genetic distance (D) values among Indian populations of *D. ananassae*

	JU	DH	KG	DN	HD	MD	GT	LK	GU	RP	CW	DM	SH	PN	AB	IM	GY	UJ	BP	IN	JR	HW
JU		0	0	0.1	0	0	0.1	0	0.1	0.2	0	0.1	0.1	0.1	0	0	0.1	0	0	0	0	0
DH			0	0	0	0	0.2	0.1	0.2	0	0	0.1	0.2	0.2	0	0.1	0.1	0	0	0	0.1	0
KG				0	0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0	0.1	0.2	0.1	0.1	0.1	0.1	0
DN					0	0	0.1	0.1	0	0.1	0.1	0	0	0.1	0	0.1	0.1	0	0	0.1	0.1	0.1
HD						0	0.2	0.1	0.2	0	0	0.1	0	0.2	0.1	0.1	0.2	0	0	0	0.2	0
MD							0.1	0.1	0.1	0	0	0.1	0.1	0.1	0	0	0.1	0	0	0	0.1	0
GT								0.1	0	0.1	0.1	0	0	0	0.1	0	0.1	0.1	0.1	0.1	0	0.1
LK									0	0	0	0	0	0	0	0	0.1	0	0	0.1	0.1	0
GU										0.1	0.1	0	0	0	0.1	0	0	0.1	0.1	0.1	0	0.1
RP											0	0.1	0.1	0.1	0	0.1	0.1	0	0	0	0.1	0
CW												0.1	0.1	0.1	0	0.1	0.1	0	0	0.1	0.1	0.2
DM													0	0	0.1	0	0	0.1	0.1	0.1	0	0.1
SH														0	0.1	0	0	0.1	0.1	0.1	0	0
PN															0.1	0	0	0.1	0.1	0.1	0	0.1
AB																0	0.1	0	0	0	0	0
IM																	0	0	0	0	0	0
GY																		0.1	0.1	0.1	0	0.1
UJ																			0	0	0.1	0
BP																				0	0.1	0
IN																					0.1	0
JR																						0.1
HW																						
SD																						
KL																						
RJ																						
DW																						
AD																						
PA																						
BN																						
PU																						
SI																						
NA																						
MU																						
VP																						
VP																						
PJ																						
MA																						
GK																						
ML																						
BL																						
YS																						
PC																						
ER																						
TR																						
KR																						

SD	KL	RJ	DW	AD	PA	BN	PU	SI	NA	MU	VP	VD	PJ	MA	GK	ML	BL	YS	PC	ER	TR	KR
0.1	0	0	0.1	0.1	0	0.1	0.1	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0	0.1	0	0.1	0.1	0.1	0.1	0	0.1	0.1	0.1	0.2
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
0	0	0.1	0	0.1	0	0	0	0	0.1	0	0	0	0.1	0	0.1	0.1	0	0	0	0	0.1	0.1
0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0	0.1	0	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0.1	0.1	0.2
0	0	0	0.1	0.1	0	0	0	0.1	0	0.1	0	0	0	0	0.1	0.1	0	0	0	0	0	0.1
0.1	0.1	0	0	0	0	0.1	0	0.1	0.1	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2
0	0	0	0	0	0	0.1	0	0	0	0	0	0.1	0.1	0.1	0.4	0	0.1	0.1	0.1	0.1	0.2	0.2
0.1	0.1	0.5	0	0	0	0.1	0	0.1	0.1	0	0.1	0.1	0.1	0.1	0	0.1	0.1	0.2	0.2	0.1	0.1	0.1
0	0.1	0	0.1	0.1	0	0.1	0.1	0.1	0.2	0	0	0.1	0.1	0.1	0.2	0	0.1	0.1	0.1	0.2	0.2	0
0.1	0.1	0.1	0.1	0.1	0.1	0	0.1	0.1	0	0.1	0	0.1	0.1	0.4	0.2	0.1	0.1	0.1	0.1	0.4	0.2	0.2
0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.1	0	0	0.2	0.2	0	0.1	0.2
0	0	0	0	0	0	0	0	0	0.1	0	0.1	0.1	0	0	0.1	0	0.1	0.2	0.2	0.1	0.1	0.2
0.1	0	0.1	0	0	0	0.1	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0	0.2	0.2	0.1	0.1	0.1	0.2
0.1	0	0	0.1	0.1	0	0.1	0.1	0	0	0	0	0.1	0.1	0.1	0.1	0	0.1	0.1	0	0.1	0.1	0.2
0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.1	0.1	0.1	0	0.1
0	0	0	0	0	0	0	0	0	0	0	0.1	0.1	0	0	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0.3	0	0	0.1
0	0	0	0.1	0	0	0	0	0	0	0	0	0.1	0.1	0	0.1	0	0	0	0.1	0.1	0.1	0.2
0	0	0	0	0.1	0	0	0	0.1	0	0.1	0	0	0	0	0.1	0.1	0	0	0	0	0	0.1
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
0	0	0	0	0	0	0	0	0.3	0	0	0	0.1	0	0	0.1	0	0	0	0.1	0.1	0.1	0.1
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0.1	0.1	0.1	0.2
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1	0	0	0.1
			0	0	0	0	0	0	0	0	0	0.1	0	0	0.1	0	0	0.1	0.1	0.1	0.1	0.1
				0	0	0	0	0	0	0	0	0.1	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0.2
					0	0.1	0	0	0	0	0	0.1	0	0	0.1	0	0.1	0.1	0.1	0.1	0	0.2
						0	0	0	0	0	0	0	0	0	0.1	0	0	0.1	0.1	0.1	0.1	0.1
							0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
								0	0	0	0	0	0	0	0.1	0	0	0.1	0.1	0.1	0.1	0.1
									0	0	0	0.1	0	0	0.1	0	0.1	0.1	0.2	0.1	0.1	0.2
										0	0	0.1	0	0	0.1	0	0.1	0.1	0.2	0.1	0.1	0.2
											0.1	0.1	0.1	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
												0	0	0	0.1	0	0	0	0	0.1	0	0.1
													0	0	0.1	0.1	0	0	0	0	0.1	0.1
														0	0	0.1	0.1	0.1	0.1	0	0.1	0.1
															0	0	0	0	0.1	0	0	0.1
																0.1	0	0.1	0.1	0	0	0
																	0.1	0	0.2	0.1	0.1	0.2
																		0	0	0	0	0.1
																			0	0.1	0.1	0.1
																				0.1	0.1	0.2
																					0	0
																						0

Table 3.11 Frequencies (in percent) of different inversions and mean number heterozygous inversions per individual in different laboratory populations and corresponding natural populations (in parenthesis) of *D. ananassae*

Populations	Total number of chromosomes examined	AL	DE	ET	Mean number of heterozygous inversions per individual
JU	200	47.5 (61.6)	31.0 (16.2)	34.5 (15.4)	1.46 (0.923)
DH	200	60.5 (59.8)	43.5 (27.2)	5.0 (4.4)	1.13 (0.95)
KG	200	53.0 (58.5)	37.5 (39.3)	7.5 (27.3)	1.02 (0.87)
DN	200	100.0 (63.9)	69.0 (39.9)	0.0 (8.4)	0.38 (0.94)
HD	200	58.0 (48.9)	23.5 (35.6)	0.0 (6.7)	0.77 (0.84)
MD	200	59.5 (63.4)	4.0 (38.4)	18.0 (16.7)	0.81 (1.10)
GT	200	86.5 (95.6)	10.0 (14.8)	30.5 (38.3)	0.84 (0.70)
LK	200	34.5 (69.8)	0.0 (6.3)	1.0 (20.9)	0.51 (0.72)
GU	200	100.0 (92.6)	0.0 (11.4)	21.0 (36.2)	0.34 (0.78)
RP	200	8.0 (60.0)	0.0 (8.0)	7.0 (14.0)	0.14 (0.76)
CW	200	28.0 (49.3)	56.0 (11.3)	0.0 (16.2)	0.98 (0.88)
DM	200	81.0 (92.7)	18.5 (20.0)	7.5 (27.3)	0.76 (0.81)
SH	200	80.0 (97.6)	4.0 (20.8)	33.5 (28.1)	0.84 (0.73)
PN	200	100.0 (96.5)	15.5 (8.8)	38.0 (22.1)	0.59 (0.57)
AB	200	63.5 (63.8)	9.0 (18.7)	29.5 (14.8)	0.68 (1.07)
IM	200	91.0 (84.9)	51.0 (27.4)	24.0 (23.6)	1.20 (0.96)
GY	200	89.0 (96.3)	16.5 (16.5)	15.5 (23.5)	0.68 (0.74)
UJ	200	37.0 (68.4)	0.0 (35.0)	0.0 (16.7)	0.50 (0.86)
BP	200	57.5 (67.3)	10.5 (24.2)	0.0 (5.2)	0.70 (0.75)
IN	200	57.5 (67.3)	6.5 (38.2)	0.0 (13.4)	0.68 (1.17)
JR	200	92.5 (89.5)	0.0 (26.0)	4.5 (18.3)	0.18 (0.71)
HW	200	41.5 (75.8)	35.5 (28.6)	24.0 (5.8)	1.24 (0.77)
SD	200	79.5 (81.9)	28.0 (27.3)	7.0 (18.2)	0.89 (0.18)
KL	200	86.5 (84.5)	64.0 (31.2)	17.0 (21.4)	0.91 (0.93)
RJ	200	64.5 (85.6)	0.0 (24.1)	50.5 (19.3)	1.20 (0.88)
DW	200	100.0 (92.8)	54.5 (19.5)	21.5 (17.3)	0.82 (0.63)
AD	200	100.0 (95.3)	8.5 (16.7)	39.5 (16.7)	0.38 (0.47)
PA	200	98.5 (77.3)	19.5 (28.8)	0.0 (25.8)	0.42 (0.75)
BN	200	100.0 (88.9)	0.5 (38.9)	48.5 (16.7)	0.43 (0.66)
PU	200	62.5 (84.4)	0.0 (28.2)	26.5 (28.2)	1.08 (0.56)
SI	200	54.5 (85.5)	32.5 (18.5)	10.0 (11.6)	1.02 (0.58)
NA	200	92.5 (82.1)	30.5 (16.8)	0.0 (4.2)	0.66 (0.64)
MU	200	93.5 (84.9)	6.5 (10.7)	0.0 (20.3)	0.22 (0.65)
VP	200	77.0 (67.0)	8.5 (25.8)	42.0 (19.7)	0.99 (0.78)
VD	200	57.5 (67.4)	25.0 (46.2)	50.0 (36.6)	1.45 (0.76)
PJ	200	72.5 (92.5)	47.0 (45.5)	27.5 (15.2)	1.48 (0.81)
MA	200	91.0 (87.2)	0.0 (35.9)	10.5 (17.4)	1.48 (0.81)
GK	200	99.5 (91.3)	28.5 (60.0)	2.5 (17.5)	0.43 (0.82)
ML	200	60.0 (87.9)	8.5 (8.5)	41.0 (7.3)	1.03 (0.72)
BL	200	35.5 (68.1)	45.0 (45.9)	0.0 (25.0)	0.87 (1.38)
YS	200	76.0 (60.0)	47.0 (46.7)	3.0 (13.4)	1.04 (1.46)
PC	200	41.0 (59.6)	79.5 (50.0)	10.0 (31.0)	1.03 (1.85)
ER	200	75.0 (80.2)	16.5 (61.3)	27.0 (19.9)	1.05 (0.84)
TR	200	69.0 (85.2)	46.0 (58.4)	11.0 (14.9)	1.14 (0.90)
KR	200	55.5 (79.5)	91.0 (77.7)	39.0 (26.8)	1.07 (0.82)

Table 3.12 Genetic identity (I) and genetic distance (D) between natural and laboratory populations of *D. ananassae* based on chromosome arrangement frequencies

Populations	I	D = 1 – I
JU	0.942	0.058
DH	0.972	0.028
KG	0.994	0.006
DN	0.873	0.127
HD	0.983	0.017
MD	0.941	0.059
GT	0.988	0.012
LK	0.914	0.086
GU	0.974	0.026
RP	0.845	0.155
CW	0.867	0.133
DM	0.956	0.044
SH	0.948	0.052
PN	0.985	0.015
AB	0.954	0.046
IM	0.962	0.038
GY	0.984	0.016
UJ	0.879	0.121
BP	0.985	0.015
IN	0.916	0.084
JR	0.946	0.054
HW	0.900	0.100
SD	0.935	0.065
KL	0.952	0.048
RJ	0.872	0.128
DW	0.930	0.070
AD	0.972	0.028
PA	0.908	0.092
BN	0.721	0.279
PU	0.893	0.107
SI	0.930	0.070
NA	0.979	0.021
MU	0.963	0.037
VP	0.953	0.047
VD	0.934	0.066
PJ	0.943	0.057
MA	0.920	0.080
GK	0.802	0.192
ML	0.873	0.127
BL	0.902	0.198
YS	0.929	0.171
PC	0.894	0.106
ER	0.901	0.099
TR	0.967	0.033
KR	0.946	0.054

of capturing genes with favorable epistatic effect on fitness. Therefore, this tendency increases with the size of the inversion because the selective advantage acquired by inversion increases with recombination distance between them (Càceres et al. 1999; Schaeffer et al. 2003; Singh and Singh 2007b). Selective value of an inversion determines its persistence or the elimination from the population (Brncic 1962). However, in small populations, the action of random drift is more predominant leading to the loss or fixation irrespective of selection pressure (Savage 1963). Fixation of these is due to random genetic drift that occurs due to a high initial frequency of these gene arrangements or homozygotes and heterozygotes showing identical selective value (Singh and Das 1992; Singh and Singh 2007b). Carson (1961b) has hypothesized that a particular gene arrangement shows increasing trend in its frequency because of some selective advantage conferred on those individuals carrying it or its linkage with a gene arrangement involved in a major heterotic association. However, there are instances where populations fail to become monomorphic; this could be due to the fact that inversion heterozygotes are adaptively superior to their homozygote counterpart. Also, random variation in gene frequencies follows the Markov rule, where gene frequency of a population is determined by the frequency of that population in the immediately preceding generation and not on the history of the population (Narain 1990; Singh and Singh 2007b).

Comparative analysis of the gene arrangement frequencies of natural and laboratory populations shows considerable variation in some populations. On the other hand, some populations have maintained nearly identical frequencies (Singh and Banerjee 1997; Singh and Singh 2007b). In laboratory populations of *D. bipectinata*, variation reported in the inversion frequencies might have involved reorganization of the genetic system in a uniform laboratory environment (Singh and Banerjee 1997; Singh and Singh 2007b). These results refute the hypothesis of Lewontin (1957) that "polymorphism should be lost in uniform environment" or the "ecological niche hypothesis" of Da Cunha and Dobzhansky (1954)

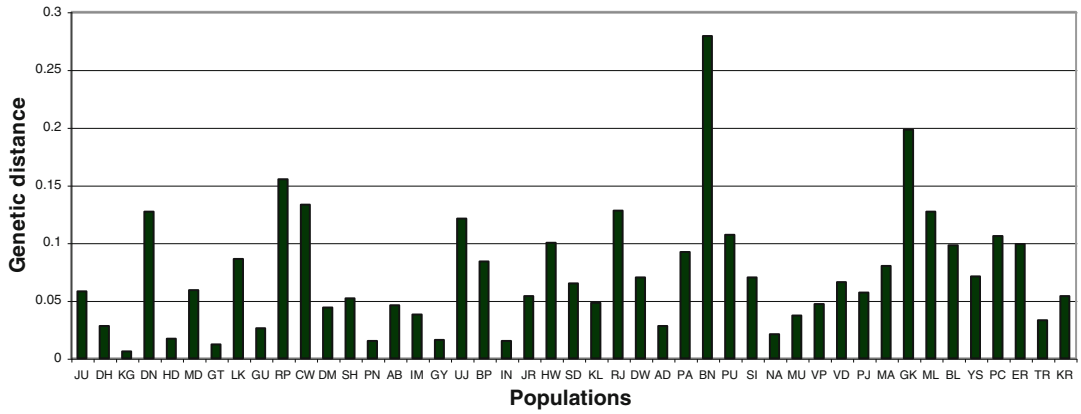

Fig. 3.4 Histogram showing genetic distance between initial (natural) and final (laboratory) populations of *D. ananassae*. *JU* Jammu, *DH* Dharamshala, *KG* Kangra, *DN* Dehradun, *HD* Haridwar, *MD* Mansa Devi, *GT* Gangtok, *LK* Lucknow, *GU* Guwahati, *RP* Raidopur, *CW* Chowk, *DM* Dimapur, *SH* Shillong, *PN* Patna, *AB* Allahabad, *IM* Imphal, *GY* Gaya, *UJ* Ujjain, *BP* Bhopal, *IN* Indore, *JR* Jamnagar, *HW* Howrah, *SD* Sealdah, *KL* Kolkata, *RJ* Rajkot, *DW* Dwarka, *AD* Ahmedabad, *PA* Paradeep, *BN* Bhubaneswar, *PU* Puri, *SI* Shirdi, *NA* Nashik, *MU* Mumbai, *VP* Visakhapatnam, *VD* Vijayawada, *PJ* Panaji, *MA* Madgaon, *GK* Gokarna, *ML* Mangalore, *BL* Bangalore, *YS* Yesvantpur, *PC* Pondicherry, *ER* Ernakulam, *TR* Thiruvananthapuram, *KR* Kanniyakumari

that "the degree of inversion polymorphism is the index of environmental heterogeneity." This, however, strengthens the theory of Carson (1961a) that "genetic polymorphism may be lost in uniform environment only if each heterozygote is especially adapted in nature to some slightly different environmental variable which is not responding in the laboratory conditions." Random associations occurring in founder populations may lead to the conversion of selection process that would cause the founder populations to end up having the gene pool composition different from the founder populations (Sperlich et al. 1982; Singh and Singh 2007b).

Singh and Singh (2008) calculated the genetic identity (I) and genetic distance (D), which are the indexes of genetic differences, as functions of inversion frequencies, between the natural and laboratory populations (Singh 1987). Histogram based on the values of I and D clearly reveals the variation in the degree of genetic divergence in *D. ananassae* populations transferred to laboratory environment. Populations also show genetic divergence to lower and higher extent during their maintenance in the laboratory environment (Singh and Singh 2007b). Especially, populations coming from similar geographical and climatic conditions and initially showing high level of genetic identity have diverged to different degrees. The variation obtained in the degree of genetic divergence in the laboratory populations of *D. ananassae* cannot be simply explained by the process of genetic reconstruction that populations encounter when they were moved from natural to laboratory conditions (Singh and Singh 2007b). This change can be explained by genetic drift. This is reasonable because the populations were reared in culture bottles by transferring about 50 flies in each generation. This small population size when compared to natural populations might have allowed drift to play its role. This is true for small-sized laboratory populations maintained in the culture bottles. Therefore, it is plausible that action of genetic drift in laboratory populations has caused striking differences in the frequencies of different chromosome arrangements of *D. ananassae* (Singh 1987; Singh and Singh 2007b). Singh and Singh (2007b) have observed that populations that had spent more generations under laboratory conditions have diverged more than the populations that have been maintained for lesser number of generations. With increasing time and number of generations a population spent in the laboratory, the chance factor or genetic drift leading to fixation or elimination of a gene arrangement owing

to its high or low initial frequency also increases (Singh and Singh 2007b).

Heterosis (overdominance) frequency-dependent selection maintains the stable equilibrium in laboratory populations of *Drosophila*. The association of inversions with microhabitat preference suggests a role for microhabitat selection, which can often be frequency dependent as well as density dependent. The dependence of equilibrium point on the starting frequency is also indicative of frequency-dependent fitness (Powell 1997). Laboratory experiments were designed to elucidate the role of recombination in providing adaptive flexibility. The results have shown that monomorphic populations for inversions show quicker response to selective pressures than the polymorphic populations. This reinforces the role of recombination in conferring the population the ability to respond to selection (Carson 1958b; Markow 1975; Tabachnick and Powell 1977). Because of the differential action of selection, the affected genotype acquires different adaptive peaks than that occupied by parental populations, thus leading to divergence (Powell 1997).

Establishing laboratory populations invariably leads to the reduction of genetic variation of the population giving rise to founder effects (Pinsker 1981). Since the lines are maintained by transferring lesser number of flies than the total number of flies actually hatched in the bottles, the variation observed in the chromosome arrangement frequencies might be caused by the action of genetic drift (Singh 1988). Sampling errors in small populations may lead to the random fluctuations in the gene frequency across the generation. Sampling errors are more prominent in the small-sized populations, and these have chance to accumulate in the successive generations. Genetic drift is strong enough to diversify genetically similar but isolated populations without the aid of natural selection (Dobzhansky et al. 1976; Singh and Singh 2007b). Flies are usually maintained by transferring around 50 flies from the culture bottles in each generation (Singh 1982). Therefore, it might be possible that, in a polymorphic culture, genetic variants may not be included among the parents of succeeding generation (Singh 1982). This should have caused populations to become monomorphic due to loss of unfixed genetic variants over the period of time but nothing of this sort is observed because of the adaptive superiority of inversion heterozygotes over their homozygote counterparts (Singh 1982).

Among various forces of evolution, natural selection and genetic drift are prominent in causing variations in gene frequencies in populations. Under a given environment condition, selection may favor some alleles or gene combinations for their high adaptive values or discriminates others for their lower adaptive values. The former condition will lead to gradual increase in the frequencies of selected alleles in the populations. However, in small-sized populations, gene frequencies may vary largely due to random genetic drift. The occurrence of selection and drift has been demonstrated in many cases. Thus, it may be postulated that the variation observed in the degree of genetic divergence in populations of *D. ananassae* moved to laboratory conditions is most likely due to genetic drift though inversions in this species are subjected to selection (Singh and Singh 2007b).

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Population Structure and Gene Flow

4

Abstract

Population genetics is chiefly concerned with finding out the origin, population structure, and demographic history of a species. Intra- and interpopulation genetic variation elucidates the pattern of species origin and demography. Natural populations display geographic population substructure, which is the function of genetic differences in populations from different geographic regions. Natural populations invariably show substructuring as herds, flocks, colonies, etc., due to random mix of favorable areas with the unfavorable ones. Population subdivision leads to genetic differentiation among the subpopulations as they acquire variable allele frequencies that vary among the subpopulations. Population subdivision is centrally important for evolution and affects estimation of all evolutionary parameters from natural and domestic populations. In subdivided populations, random genetic drift (cause of genetic divergence among subpopulations) acts antagonistically to migration, which holds a subpopulation together, and the balance between the two decides the degree of genetic divergence that can occur. As an evolutionary process that brings potentially new alleles into a population, migration is qualitatively similar to mutation. The major difference is quantitative, i.e., the rate of migration among subpopulations of a species is higher than the rate of mutation of a gene.

4.1 Population and Gene Dynamics in *Drosophila ananassae*

D. ananassae exhibits more population structuring than both *D. melanogaster* and *D. simulans* (Vogl et al. 2003; Das 2005). This species shows high incidence of interpopulation migration (Dobzhansky and Dreyfus 1943; Singh and Singh 2008). *D. ananassae* is very common and cohabits the human dwellings in tropical and subtropical regions of the world. *D. ananassae* populations are separated by major geographical barriers such as mountains and oceans, but frequent transport by human activity is responsible for inter- and intraspecific genetic exchange. It exists as

P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2_4

semi-isolated populations in mainland Southeast Asia and on the islands of the Pacific Ocean around the equator, a suspected place of its origin (Dobzhansky and Dreyfus 1943; Tobari 1993; Das et al. 2004). *D. ananassae* shows extensive population structure in its natural population; thus, it is a good model to study the effect of population subdivision on genetic variation. Past molecular analyses (Stephan 1989; Stephan and Langely 1989; Stephan and Mitchell 1992; Stephan et al. 1998; Das et al. 2004; Schug et al. 2007) showing the effect of population substructuring on genetic variation are limited to few loci and populations. A study by Singh and Singh (2010) has tried to bridge the gap by analyzing 45 populations from across different geo-climatic regions of India, utilizing chromosomal markers to study the population substructuring and gene flow in natural populations of *D. ananassae*.

4.2 Genetic Variability

Singh and Singh (2010) calculated genetic variability index as mean of observed (H_O) and expected (H_E) heterozygosity and population inbreeding coefficient (F) to deduce the level of inbreeding due to population substructuring.

Estimates of genetic variability are given in Table 4.1 (Singh and Singh 2010). Mean observed heterozygosity (H_O) ranges from 0.15 (AD) to −0.61 (PC); similarly, mean expected heterozygosity (H_E) ranges from 0.15 (ML) to −0.45 (VD). The values of population inbreeding coefficient (F) range from −0.600 (ML) to 0.47 (PU). Other negative values of F are found for other populations: −0.09 (AB), −0.043 (GY), −0.027 (IN), −0.050 (NA), −0.071 (BL), −0.230 (YS), and −0.450 (PC). In all the 45 populations analyzed for F, most of the populations show conformity with the HWE, and in only few cases, deviations from HWE were observed (Singh and Singh 2010).

Values of population inbreeding coefficient range from −0.600 to 0.47. In most of the cases, H_O is almost similar to H_E (indicating populations are in HWE) and, only in few cases, $H_O < H_E$ (indicating inbreeding); in these cases too, H_O is almost similar to H_E and in rest of the populations $H_O > H_E$ (indicating outbreeding) as is the case in most of the natural populations (Singh and Singh 2010). Reduction in heterozygosity due to population substructuring is closely related to a decrease in heterozygosity affected by inbreeding. To understand this, each subpopulation could be treated as sort of "extended family" or populations with related ancestry. Therefore, organisms in the same population will naturally share one or more recent or remote common ancestors, and so, the mating between organisms in the same subpopulation will often be mating between relatives (Hartl and Clark 1997).

4.3 Hierarchical F-Statistics

Wright (1951) defined a set of correlation coefficients, which he called F-statistics (namely, F_{IS}, F_{ST}, and F_{IT}), to partition departures from random mating into components due to nonrandom mating within populations and to population subdivision (Neigel 1997). These were used by Singh and Singh (2010) to analyze the breeding structure of subdivided populations. Pairwise F_{ST} values were also calculated to deduce the level of genetic differentiation among 45 Indian natural populations of *D. ananassae* (Singh and Singh 2010).

Hierarchical F-statistics (namely, F_{IS}, F_{ST}, and F_{IT}) was calculated to know the breeding structure of subdivided populations (Table 4.1) (Singh and Singh 2010). The values of F_{IS} range from −0.53 (ML) to 0.47 (PU). Other negative values are −0.294 (HD), −0.093 (AB), −0.043 (GY), −0.027 (IN), −0.050 (NA), −0.071 (BL), −0.230 (YS), and −0.480 (PC). The values of F_{ST} (see Table 4.1) range from 0.046 (PC) to 0.642 (ML). The values of F_{IT}, which is the most inclusive of all inbreeding coefficients, range from –0.418 (PC) to 0.617 (PN, PU) while other negative values are −0.100 (HD) and −0.040 (YS). In all these cases, values are close to zero or slightly above zero, which indicates populations are mostly outbreeders, which is the case with natural populations (Table 4.1) (Singh and Singh 2010).

Pairwise F_{ST} values (see Table 4.2; Singh and Singh 2010) among populations range from

Table 4.1 Estimates of genetic variability and F-statistics in Indian natural populations of *D. ananassae*

Populations	H_O	H_E	F	F_{IS}	F_{ST}	F_{IT}
JU	0.30	0.33	0.090	0.090	0.214	0.285
DH	0.31	0.32	0.031	0.031	0.200	0.225
KG	0.29	0.34	0.147	0.151	0.250	0.363
DN	0.31	0.35	0.114	0.088	0.227	0.295
HD	0.27	0.34	0.205	–0.294	0.150	–0.100
MD	0.36	0.39	0.076	0.076	0.187	0.250
GT	0.23	0.25	0.080	0.120	0.468	0.531
LK	0.24	0.27	0.111	0.148	0.341	0.439
GU	0.25	0.25	0.000	0.000	0.446	0.468
RP	0.25	0.28	0.107	0.107	0.263	0.342
CW	0.29	0.31	0.064	0.096	0.138	0.222
DM	0.26	0.27	0.037	0.037	0.425	0.446
SH	0.23	0.24	0.041	0.041	0.500	0.520
PN	0.18	0.18	0.000	0.000	0.595	0.617
AB	0.35	0.32	–0.093	–0.093	0.219	0.146
IM	0.31	0.32	0.0310	0.031	0.319	0.340
GY	0.24	0.23	–0.043	–0.043	0.080	0.040
UJ	0.28	0.37	0.243	0.243	0.195	0.391
BP	0.25	0.29	0.137	0.142	0.300	0.400
IN	0.38	0.37	–0.027	–0.027	0.177	0.155
JR	0.23	0.28	0.178	0.148	0.425	0.510
HW	0.25	0.28	0.107	0.074	0.400	0.440
SD	0.18	0.32	0.430	0.410	0.311	0.600
KL	0.30	0.33	0.090	0.062	0.319	0.361
RJ	0.28	0.29	0.034	0.034	0.382	0.404
DW	0.20	0.23	0.130	0.130	0.510	0.570
AD	0.15	0.19	0.210	0.210	0.595	0.680
PA	0.25	0.36	0.305	0.330	0.230	0.480
BN	0.22	0.30	0.260	0.300	0.360	0.553
PU	0.18	0.34	0.470	0.470	0.276	0.617
SI	0.19	0.21	0.095	0.100	0.545	0.590
NA	0.21	0.20	–0.050	–0.050	0.547	0.523
MU	0.21	0.24	0.125	0.125	0.454	0.522
VP	0.26	0.37	0.297	0.305	0.181	0.431
VD	0.25	0.45	0.440	0.450	0.083	0.500
PJ	0.27	0.28	0.035	0.071	0.416	0.458
MA	0.26	0.30	0.133	0.133	0.361	0.446
GK	0.27	0.29	0.068	0.068	0.395	0.437
ML	0.24	0.15	–0.600	–0.533	0.642	0.452
BL	0.45	0.42	–0.071	–0.071	0.106	0.042
YS	0.48	0.39	–0.230	–0.230	0.152	–0.04
PC	0.61	0.42	–0.450	–0.480	0.046	–0.418
ER	0.27	0.35	0.228	0.228	0.225	0.425
TR	0.29	0.31	0.064	0.064	0.340	0.382
KR	0.27	0.33	0.181	0.212	0.266	0.422

Abbreviations: H_O observed average heterozygosity, H_E expected average heterozygosity, F population inbreeding coefficient, F_{IS} inbreeding coefficient due to nonrandom mating, F_{ST} inbreeding coefficient due to population subdivision, F_{IT} inbreeding coefficient due to effect of nonrandom mating with subpopulation and the effect of population subdivision

Table 4.2 Pairwise F_{ST} values calculated among Indian populations of *D. ananassae*

	JU	DH	KG	DN	HD	MD	GT	LK	GU	RP	CW	DM	SH	PN	AB	IM	GY	UJ	BP	IN	JR	HW
JU		0	0.3	0.2	0.2	0.2	0.34	0.24	0.18	0.24	0.2	0.33	0.37	0.42	0.2	0.27	0.16	0.25	0.26	0.2	0.33	0.31
DH			0.2	0.2	0.2	0.2	0.34	0.27	0.34	0.23	0.2	0.32	0.36	0.41	0.2	0.26	0.15	0.2	0.25	0.24	0.32	0.31
KG				0.2	0.2	0.1	0.36	0.29	0.36	0.26	0.2	0.34	0.38	0.43	0.2	0.29	0.19	0.22	0.27	0.21	0.34	0.33
DN					0.2	0.2	0.35	0.28	0.35	0.24	0.2	0.33	0.37	0.42	0.2	0.27	0.17	0.21	0.26	0.2	0.33	0.31
HD						0.2	0.32	0.25	0.32	0.21	0.1	0.3	0.34	0.39	0.2	0.24	0.12	0.17	0.23	0.16	0.3	0.28
MD							0.33	0.26	0.28	0.22	0.2	0.26	0.32	0.39	0.3	0.36	0.15	0.19	0.24	0.18	0.31	0.29
GT								0.41	0.47	0.36	0.3	0.45	0.48	0.53	0.4	0.39	0.33	0.33	0.39	0.33	0.45	0.43
LK									0.41	0.31	0.2	0.39	0.43	0.48	0.3	0.34	0.24	0.26	0.32	0.26	0.39	0.37
GU										0.38	0.3	0.45	0.48	0.53	0.4	0.42	0.33	0.33	0.39	0.32	0.44	0.43
RP												0.3	0.35	0.4	0.2	0.24	0.11	0.17	0.24	0.16	0.3	0.28
CW													0.46	0.51	0.3	0.37	0.31	0.31	0.37	0.3	0.43	0.41
DM														0.55	0.4	0.41	0.36	0.35	0.41	0.34	0.46	0.45
SH															0.4	0.46	0.42	0.4	0.46	0.39	0.51	0.5
PN																0.27	0.17	0.21	0.26	0.2	0.33	0.31
AB																	0.24	0.26	0.31	0.25	0.37	0.36
IM																		0.15	0.22	0.14	0.31	0.29
GY																			0.24	0.19	0.31	0.3
UJ																				0.24	0.37	0.35
BP																					0.3	0.29
IN																						0.41
JR																						
HW																						
SD																						
KL																						
RJ																						
DW																						
AD																						
PA																						
BN																						
PU																						
SI																						
NA																						
MU																						
VP																						
VP																						
PJ																						
MA																						
GK																						
ML																						
BL																						
YS																						
PC																						
ER																						
TR																						
KR																						

SD	KL	RJ	DW	AD	PA	BN	PU	SI	NA	MU	VP	VD	PJ	MA	GK	ML	BL	YS	PC	ER	TR	KR
0.264	0.27	0.3	0.37	0.42	0.22	0.29	0.25	0.38	0.38	0.34	0.2	0.14	0.32	0.29	0.31	0.43	0.16	0.18	0.13	0.2	0.3	0.2
0.258	0.26	0.3	0.37	0.41	0.22	0.29	0.24	0.38	0.38	0.33	0.19	0.14	0.32	0.29	0.31	0.43	0.15	0.17	0.12	0.2	0.3	0.2
0.28	0.29	0.32	0.38	0.43	0.24	0.29	0.26	0.4	0.4	0.35	0.22	0.16	0.34	0.29	0.33	0.44	0.18	0.2	0.15	0.2	0.3	0.3
0.269	0.26	0.31	0.37	0.42	0.23	0.3	0.25	0.39	0.38	0.34	0.2	0.15	0.33	0.3	0.29	0.43	0.16	0.19	0.14	0.2	0.3	0.2
0.235	0.24	0.28	0.34	0.39	0.2	0.26	0.22	0.36	0.35	0.31	0.17	0.11	0.3	0.26	0.26	0.4	0.13	0.15	0.1	0.2	0.3	0.2
0.247	0.25	0.28	0.35	0.39	0.21	0.27	0.23	0.38	0.36	0.29	0.18	0.14	0.31	0.27	0.29	0.4	0.15	0.17	0.12	0.2	0.3	0.2
0.391	0.39	0.43	0.49	0.53	0.35	0.41	0.37	0.49	0.51	0.46	0.33	0.27	0.44	0.41	0.43	0.55	0.29	0.31	0.27	0.4	0.3	0.4
0.325	0.33	0.36	0.45	0.36	0.28	0.33	0.31	0.45	0.33	0.4	0.26	0.2	0.38	0.35	0.37	0.49	0.24	0.24	0.19	0.3	0.3	0.3
0.391	0.39	0.43	0.49	0.53	0.35	0.41	0.37	0.51	0.51	0.46	0.33	0.27	0.44	0.52	0.43	0.55	0.29	0.31	0.27	0.4	0.4	0.3
0.234	0.24	0.27	0.35	0.4	0.19	0.27	0.22	0.36	0.36	0.31	0.16	0.11	0.3	0.27	0.29	0.41	0.12	0.15	0.09	0.2	0.3	0.2
0.269	0.37	0.4	0.47	0.51	0.33	0.39	0.35	0.48	0.48	0.44	0.31	0.25	0.42	0.39	0.41	0.53	0.27	0.29	0.24	0.3	0.4	0.3
0.408	0.41	0.44	0.51	0.55	0.37	0.43	0.39	0.52	0.52	0.48	0.35	0.29	0.46	0.43	0.45	0.57	0.29	0.33	0.29	0.4	0.4	0.4
0.456	0.46	0.49	0.55	0.6	0.41	0.48	0.44	0.57	0.57	0.53	0.4	0.34	0.51	0.48	0.49	0.62	0.35	0.38	0.33	0.4	0.5	0.4
0.267	0.27	0.31	0.38	0.42	0.23	0.3	0.25	0.39	0.39	0.34	0.2	0.15	0.33	0.3	0.31	0.43	0.16	0.18	0.13	0.2	0.3	0.2
0.315	0.32	0.35	0.41	0.46	0.28	0.34	0.3	0.43	0.42	0.38	0.25	0.2	0.37	0.34	0.36	0.47	0.21	0.24	0.19	0.3	0.3	0.3
0.228	0.24	0.28	0.36	0.42	0.18	0.26	0.21	0.38	0.37	0.32	0.14	0.05	0.3	0.26	0.29	0.43	0.11	0.13	0.06	0.2	0.3	0.2
0.252	0.26	0.29	0.35	0.4	0.22	0.28	0.24	0.37	0.36	0.32	0.19	0.14	0.31	0.28	0.3	0.41	0.15	0.17	0.12	0.2	0.3	0.2
0.305	0.31	0.34	0.41	0.46	0.26	0.32	0.29	0.43	0.43	0.38	0.24	0.18	0.36	0.33	0.35	0.48	0.2	0.22	0.17	0.3	0.3	0.3
0.244	0.25	0.28	0.35	0.39	0.21	0.27	0.23	0.36	0.36	0.31	0.18	0.13	0.3	0.27	0.29	0.4	0.14	0.16	0.11	0.2	0.3	0.2
0.369	0.37	0.4	0.47	0.51	0.33	0.39	0.35	0.42	0.48	0.44	0.31	0.25	0.42	0.39	0.41	0.53	0.27	0.29	0.24	0.3	0.4	0.3
0.355	0.36	0.39	0.46	0.5	0.32	0.38	0.23	0.47	0.47	0.43	0.29	0.24	0.41	0.38	0.4	0.52	0.25	0.27	0.23	0.3	0.4	0.3
	0.32	0.35	0.41	0.46	0.27	0.34	0.29	0.43	0.43	0.38	0.25	0.19	0.37	0.34	0.35	0.47	0.21	0.23	0.18	0.3	0.3	0.3
		0.35	0.41	0.46	0.28	0.34	0.3	0.43	0.43	0.38	0.25	0.2	0.37	0.34	0.36	0.47	0.32	0.24	0.19	0.3	0.3	0.3
			0.45	0.49	0.31	0.37	0.33	0.46	0.46	0.41	0.28	0.23	0.4	0.37	0.39	0.45	0.24	0.27	0.22	0.3	0.4	0.3
				0.55	0.37	0.44	0.39	0.52	0.53	0.48	0.35	0.34	0.46	0.44	0.45	0.46	0.31	0.33	0.29	0.4	0.4	0.4
					0.41	0.48	0.44	0.57	0.57	0.42	0.4	0.34	0.51	0.48	0.49	0.62	0.35	0.37	0.33	0.4	0.5	0.4
						0.3	0.26	0.38	0.38	0.34	0.21	0.16	0.33	0.3	0.32	0.43	0.17	0.19	0.14	0.2	0.3	0.3
							0.32	0.45	0.45	0.41	0.27	0.22	0.39	0.36	0.38	0.45	0.23	0.26	0.21	0.3	0.4	0.3
								0.41	0.4	0.36	0.23	0.18	0.35	0.32	0.34	0.45	0.19	0.22	0.17	0.3	0.3	0.3
									0.55	0.5	0.36	0.3	0.48	0.45	0.47	0.59	0.32	0.34	0.3	0.4	0.4	0.3
										0.5	0.36	0.3	0.48	0.45	0.45	0.6	0.31	0.45	0.29	0.4	0.4	0.4
											0.32	0.26	0.43	0.41	0.42	0.55	0.27	0.3	0.25	0.4	0.3	0.4
												0.13	0.3	0.27	0.29	0.41	0.14	0.17	0.11	0.2	0.3	0.2
													0.25	0.22	0.24	0.34	0.11	0.12	0.07	0.2	0.2	0.2
														0.39	0.41	0.52	0.26	0.29	0.24	0.3	0.4	0.3
															0.38	0.49	0.23	0.26	0.21	0.3	0.4	0.3
																0.51	0.25	0.28	0.23	0.3	0.4	0.3
																	0.36	0.39	0.34	0.4	0.5	0.4
																		0.13	0.08	0.2	0.2	0.2
																			0.1	0.2	0.2	0.2
																				0.2	0.2	0.2
																					0.3	0.3
																						0.3

0.054 (GY vs. UJ) to 0.617 (AD vs. ML) showing that Indian populations of *D. ananassae* are heterogenous and show high degree of genetic differentiation (Singh and Singh 2010). Dendrogram based on the genetic identity values also shows the clustering among populations corroborating the findings from F_{ST} values. Based on F_{ST} values, it could be said that northern and southern populations are showing strong genetic differences. This strong genetic differentiation between north and south populations could be further evidenced by strong north–south trend in inversion frequencies (Singh and Singh 2010).

Values of F_{IS} (−0.53 to 0.47) in most of the natural populations are close to zero, indicating random mating in subpopulations (Singh and Singh 2010). Values of F_{IT}, the most inclusive measure of inbreeding (−0.41 to 0.61), are found close to zero in most of the cases. Thus, a set of populations as a whole shows no sign of inbreeding (Singh and Singh 2010). Values of F_{ST} show the range of 0.04–0.64. So, range-wise population subdivision, possibly due to drift, accounts for approximately 4–64 % of the total genetic variation (Singh and Singh 2010). Subpopulation sizes are the chief determinant affecting the values of F_{ST} by influencing the random variation in the allele frequency (Hartl and Clark 1997).

4.4 Gene Flow

Wright's (1951) analysis of nonrandom mating established a quantitative relationship between population subdivision F_{ST} and two other parameters, viz., population size N and rate of migration m (for references, see review by Neigel 1997). A standard approach employing chromosomal markers was utilized to quantify gene flow among subpopulations and estimates of F_{ST} were transformed into estimates of Nm using Wright's island model (Wright 1951; Slatkin 1987). This was an indirect approach as quantification of gene flow was done by the estimation of distribution of chromosomal markers, taking into account population genetic models. Gene flow between populations was therefore an estimation of the number of migrants exchanged between populations per generation at equilibrium (N_em). N_em values were derived utilizing F_{ST} values, following the island model of Wright (1951) with a small level of migration (González-Wangüemert et al. 2004), whereby:

$$\mathbf{N_em = (1 - F_{ST}) / 4F_{ST}}$$

Estimates of gene flow (see Table 4.3; Singh and Singh 2010), using pairwise F_{ST} values, range from 0.155 (PN–ML, AD–ML) to 4.379 (GY–AD). Usually, in other cases, the values of gene flow came around one or above. Other lower values are 0.191 (SH–ML), 0.170 (PN–AD), 0.187 (AD–SI, PN–SI), 0.186 (AD–NA), 0.171 (SI–ML), and 0.163 (NA–ML). Higher values are 2.354 (HD–PC), 2.086 (CW–VD), 2.599 (CW–PC), 3.596 (VD–PJ), 2.996 (BL–PC), and 2.225 (YS–PC) (Singh and Singh 2010).

Population subdivision is central to the mechanism of evolution and influences estimation of different evolutionary parameters from both natural and domestic populations (Forister et al 2004). The relation between population structure and gene flow is central to the number of issues in evolution and ecology, from speciation to local adaptation, to the effect of natural selection on genome and global patterns of diversity (Mayr 1963; Janzen 1967; Ronce and Kirkpatric 2001; Forister et al. 2004).

Due to its extensive population structure, *D. ananassae* is an excellent model to study the effect of population structuring on genetic variation. Earlier studies (Stephan 1989; Stephan and Langely 1989; Stephan and Mitchell 1992; Stephan et al. 1998; Das et al. 2004; Schug et al. 2007; reviewed in Singh and Singh 2008) on the effect of population structuring on genetic variation are essentially molecular studies. The study by Singh and Singh (2010) is unique in utilizing classical genetics approach by using chromosomal markers (inversions), genetic variability parameters (H_O, H_E, F), and F-statistics to infer the population subdivision and gene flow among *D. ananassae* populations from different geoclimatic regions of India.

4.5 Gene Flow and North–South Trends

Pairwise estimates of F_{ST} genetic distance (D) exhibit strong genetic differentiation among natural populations of *D. ananassae*, particularly between populations of north and south regions within the study area, hence showing north–south trends (Singh and Singh 2010). However, Indian natural populations of *D. ananassae* did not show north–south cline pattern with respect to inversion frequencies. *D. melanogaster* though exhibit latitudinal clines in its natural populations (Das and Singh 1991; Singh and Das 1992). These north–south trends (and not the north–south cline) have been reported with respect to the inversion frequencies in natural populations of *D. ananassae.* It may be suggested that persistent north–south differentiation could be due to diversifying selection that prevents homogenization caused by gene flow, as commonly seen among naturally occurring clines (Endler 1977; Arnold 1997). These genetic trends might be the result of a gradual secondary introgression of populations separated in the historical past, equilibrium between dispersal and diversifying selection, or both (Sotka et al. 2004). *D. ananassae* flies have limited dispersal capacity as they cohabit the human dwellings, rich with resources enough to sustain the residents. It may be postulated that on being subjected to forced dispersal, these flies resist to mating thus neutralizing the homogenization due to gene flow on account of mating and maintaining the differentiation. Gene flow between these flies may be limited irrespective of their being broadly sympatric and living in proximal localities thereby leading to sympatric divergence thus providing explanations for the kind of data as has been hypothesized for host races in butterfly genus *Mitoura* (Nice and Shapiro 2001) and *Hesperia comma*, a Holarctic skipper with very high dispersal potential (Forister 2004).

Significantly strong genetic differentiation between north and south parts of the study area indicates limited migration between them and gene flow appears to be severely restricted irrespective of the fact that there is recurrent transportation of these flies along with human goods. It could be postulated that genetic differences observed among *D. ananassae* populations from different geographic localities are chiefly due to geo-climatic heterogeneity, and this difference is canalized via rigid polymorphic system in *D. ananassae*. It is because of this: it resists any change brought through any agency or homogenizing force (Singh and Singh 2010). Also, populations with identical allele frequencies essentially come from similar environmental conditions owing to a similar geographic area, which may give rise to nonrandom distribution of genetic variants (Humeres et al. 1998; Mcrae et al. 2005).

4.6 Gene Flow and Geographic Features

Low pairwise gene flow values as reported by Singh and Singh (2010) (only slightly above the range shown by rat snakes, Lougheed et al. 1999) and conversely high F_{ST} values (Singh and Singh 2010) compared to other studies involving other models (Carmichael et al. 2001; Nice and Shapiro 2001; Schwartz et al. 2002; Bargelloni et al. 2003; Rueness et al. 2003; Mcrae et al. 2005) are surprising because *Drosophila* has very limited dispersal capacity but, since it is co-transported via fruits and vegetables along with human goods, geographic barriers or habitat discontinuity can hardly have any dominating effect on its movement and dispersal (Singh and Singh 2010).

Geographic barriers play a role in causing structuring in populations (Walker 2000; Mcrae et al. 2005), so that where geographic barriers are present, a high degree of population structure is present, but in the case of *Drosophila*, these barriers do not carry much weight as flies are transported along with fruits and vegetables to the different parts of the country and there might be a possibility that few founders have started their colony and, during this population bottleneck, random genetic drift might have led to genetic differentiation. Striking genetic differentiation in

Table 4.3 Estimates of gene flow using pairwise F_{ST} values

	JU	DH	KG	DN	HD	MD	GT	LK	GU	RP	CW	DM	SH	PN	AB	IM	GY	UJ	BP	IN	JR	HW
JU		0.9	0.83	0.83	1.03	1	0.49	0.65	0.49	0.8	1.15	0.52	0.43	0.35	0.91	0.68	1.22	0.75	0.73	1.03	0.52	0.56
DH			0.86	0.92	1.18	0.9	0.48	0.67	0.48	0.84	1.21	0.53	0.44	0.36	0.95	0.7	1.38	1.02	0.75	0.81	0.53	0.57
KG				0.8	0.99	1.5	0.44	0.6	0.44	0.73	1	0.49	0.41	0.33	0.81	0.63	1.08	0.88	0.67	0.92	0.49	0.52
DN					1.07	1	0.46	0.64	0.46	0.8	1.09	0.51	0.43	0.35	0.87	0.66	1.2	0.93	0.71	0.99	0.51	0.55
HD						1.2	0.53	0.97	0.53	0.97	1.49	0.59	0.49	0.39	1.1	0.79	1.78	1.19	0.86	1.27	0.59	0.64
MD							0.52	0.72	0.64	0.89	1.26	0.72	0.53	0.39	0.55	0.45	1.42	1.06	0.8	1.12	0.57	0.61
GT								0.36	0.28	0.44	0.52	0.31	0.31	0.22	0.46	0.39	0.5	0.5	0.39	0.52	0.31	0.33
LK									0.36	0.55	0.77	0.4	0.34	0.27	0.64	0.49	0.78	0.7	0.53	0.73	0.4	0.42
GU										0.91	0.52	0.31	0.27	0.22	0.46	0.3	0.51	0.51	0.39	0.53	0.32	0.33
RP											0.91	0.46	0.39	0.31	0.79	0.6	1.07	0.86	0.64	0.91	0.46	0.49
CW												0.58	0.47	0.38	1.13	0.79	1.94	1.22	0.81	1.31	0.58	0.63
DM													0.29	0.24	0.51	0.42	0.57	0.55	0.43	0.57	0.34	0.36
SH														0.21	0.43	0.36	0.45	0.46	0.36	0.5	0.29	0.3
PN															0.35	0.3	0.35	0.38	0.29	0.39	0.24	0.25
AB																0.67	1.26	0.96	0.72	1.02	0.51	0.55
IM																	0.81	0.72	0.56	0.25	0.42	0.45
GY																		1.37	0.91	1.51	0.57	0.63
UJ																			0.77	1.09	0.55	0.59
BP																				0.81	0.43	0.46
IN																					0.57	0.62
JR																						0.36
HW																						
SD																						
KL																						
RJ																						
DW																						
AD																						
PA																						
BN																						
PU																						
SI																						
NA																						
MU																						
VP																						
VP																						
PJ																						
MA																						
GK																						
ML																						
BL																						
YS																						
PC																						
ER																						
TR																						
KR																						

SD	KL	RJ	DW	AD	PA	BN	PU	SI	NA	MU	VP	VD	PJ	MA	GK	ML	BL	YS	PC	ER	TR	KR
0.7	0.68	0.58	0.43	0.35	0.87	0.61	0.76	0.4	0.41	0.49	1.02	1.49	0.53	0.61	0.55	0.33	1.34	1.1	1.7	0.8	0.64	0.8
0.72	0.7	0.59	0.43	0.36	0.9	0.62	0.79	0.41	0.41	0.5	1.07	1.59	0.54	0.62	0.57	0.34	1.43	1.2	1.8	0.8	0.66	0.8
0.64	0.63	0.54	0.4	0.33	0.79	0.63	0.7	0.4	0.38	0.46	0.91	1.28	0.49	0.63	0.52	0.32	1.18	1	1.4	1.3	0.59	0.7
0.68	0.72	0.56	0.42	0.35	0.84	0.59	0.74	0.4	0.4	0.49	0.98	1.39	0.52	0.59	0.6	0.33	1.27	1.1	1.6	0.8	0.63	0.8
0.81	0.79	0.66	0.48	0.39	1.03	0.7	0.9	0.45	0.46	0.56	1.26	1.96	0.6	0.7	0.71	0.37	1.73	1.4	2.4	1	0.74	0.9
0.76	0.74	0.63	0.47	0.39	0.94	0.67	0.83	0.41	0.45	0.6	1.11	1.6	0.55	0.67	0.61	0.38	1.45	1.2	1.8	0.9	0.7	0.9
0.39	0.39	0.34	0.26	0.22	0.46	0.35	0.42	0.26	0.25	0.29	0.51	0.67	0.32	0.35	0.33	0.2	0.37	0.6	0.7	0.4	0.59	0.4
0.52	0.51	0.44	0.33	0.44	0.63	0.51	0.57	0.31	0.52	0.38	0.72	0.99	0.4	0.46	0.43	0.26	0.8	0.8	1.1	0.6	0.49	0.6
0.39	0.39	0.34	0.26	0.22	0.46	0.35	0.42	0.25	0.25	0.29	0.51	0.67	0.32	0.23	0.33	0.2	0.62	0.6	0.7	0.9	0.38	0.5
0.62	0.6	0.51	0.38	0.31	0.76	0.54	0.68	0.35	0.43	0.89	1.29	0.47	0.54	0.49	0.29	1.17	0.99	1.4	1	0.7	0.57	0.8
0.82	0.79	0.65	0.47	0.38	1.05	0.73	0.91	0.44	0.45	0.55	1.29	2.07	0.59	0.69	0.63	0.36	1.83	1.5	2.6	1	0.74	0.9
0.43	0.42	0.37	0.28	0.24	0.51	0.39	0.46	0.27	0.27	0.32	0.56	0.74	0.34	0.39	0.36	0.22	0.69	0.6	0.8	0.5	0.4	0.5
0.36	0.36	0.32	0.25	0.21	0.43	0.33	0.39	0.23	0.23	0.27	0.47	0.61	0.3	0.33	0.31	0.19	0.63	0.5	0.7	0.4	0.34	0.4
0.3	0.3	0.26	0.2	0.17	0.35	0.27	0.32	0.19	0.19	0.22	0.38	0.49	0.25	0.27	0.26	0.16	0.46	0.4	0.5	0.3	0.28	0.3
0.69	0.67	0.57	0.42	0.35	0.85	0.6	0.75	0.39	0.4	0.48	1	1.46	0.52	0.6	0.55	0.33	1.32	1.1	1.7	0.8	0.63	0.8
0.54	0.53	0.46	0.35	0.3	0.66	0.49	0.59	0.33	0.35	0.4	0.74	1	0.43	0.49	0.45	0.28	0.93	0.8	1.1	0.6	0.51	0.6
0.85	0.81	0.65	0.94	0.35	1.14	0.7	0.95	0.41	0.42	0.54	1.49	4.38	0.58	0.7	0.62	0.33	2.04	1.7	4.1	1	0.75	1
0.74	0.72	0.61	0.46	0.38	0.91	0.65	0.81	0.43	0.44	0.53	1.08	1.56	0.56	0.65	0.59	0.36	1.42	1.2	1.8	0.9	0.68	0.8
0.57	0.56	0.48	0.36	0.29	0.7	0.53	0.62	0.33	0.32	0.41	0.8	1.13	0.44	0.5	0.46	0.28	1.03	0.9	1.2	0.7	0.53	0.6
0.77	0.25	0.64	0.47	0.39	0.96	0.67	0.85	0.45	0.45	0.55	1.15	1.69	0.58	0.67	0.61	0.37	1.52	1.3	1.6	0.9	0.71	0.9
0.43	0.42	0.36	0.28	0.24	0.51	0.39	0.46	0.35	0.27	0.32	0.56	0.74	0.34	0.39	0.36	0.22	0.69	0.6	0.8	0.5	0.4	0.5
0.45	0.45	0.39	0.3	0.25	0.54	0.41	0.85	0.28	0.28	0.34	0.61	0.81	0.36	0.41	0.38	0.23	0.25	0.7	0.8	0.5	0.43	0.5
	0.54	0.47	0.36	0.3	0.67	0.49	0.6	0.34	0.34	0.4	0.76	1.05	0.43	0.49	0.46	0.28	0.96	0.8	1.1	0.6	0.52	0.6
		0.46	0.35	0.3	0.65	0.99	0.59	0.33	0.34	0.4	0.74	1	0.43	0.49	0.45	0.28	0.53	0.8	1.1	0.6	0.51	0.6
			0.31	0.26	0.56	0.47	0.51	0.29	0.29	0.36	0.65	0.83	0.38	0.42	0.39	0.31	0.77	0.7	0.9	0.5	0.49	0.5
				0.2	0.42	0.32	0.39	0.23	0.23	0.27	0.46	0.49	0.29	0.32	0.3	0.29	0.56	0.5	0.6	0.4	0.34	0.4
					0.35	0.27	0.32	0.19	0.19	0.35	0.38	0.49	0.25	0.27	0.26	0.16	0.46	0.4	0.5	0.3	0.28	0.3
						0.59	0.73	0.4	0.4	0.49	0.95	1.34	0.52	0.59	0.59	0.34	1.22	1	1.5	0.8	0.62	0.8
							0.53	0.31	0.31	0.37	0.66	0.88	0.39	0.44	0.41	0.31	0.82	0.7	0.9	0.6	0.46	0.5
								0.37	0.37	0.44	0.84	1.15	0.47	0.53	0.49	0.31	1.06	0.9	1.3	0.7	0.56	0.7
									0.21	0.25	0.44	0.57	0.27	0.31	0.29	0.17	0.54	0.5	0.6	0.4	0.32	0.6
										0.25	0.44	0.53	0.28	0.31	0.29	0.16	0.55	0.3	0.6	0.4	0.32	0.4
											0.54	0.71	0.33	0.37	0.34	0.21	0.66	0.6	0.7	0.5	0.63	0.4
												1.67	0.57	0.66	0.6	0.37	1.51	1.3	1.9	0.9	0.7	0.9
													0.75	0.88	0.8	0.48	1.94	1.9	3.6	1.2	0.94	1.2
														0.39	0.37	0.23	0.7	0.6	0.8	0.5	0.44	0.5
															0.41	0.26	0.82	0.7	0.9	0.6	0.46	0.5
																0.24	0.74	0.6	0.8	0.5	0.43	0.5
																	0.45	0.4	0.5	0.3	0.27	0.3
																		1.7	3	1.1	0.87	1.1
																			2.2	1	0.76	1
																				1.4	1	1.3
																					0.59	0.7
																						0.6

almost all pairwise comparisons (estimates of F_{ST} and genetic distance) among natural populations of *D. ananassae* across the country indicates substantial population structuring irrespective of distance or any other homogenizing factors (Singh and Singh 2010). For *Drosophila* with limited mobility, gene flow may be restricted by distance alone in a population; Wright (1943) called this the "isolation by distance" effect. As shown from D values, populations isolated by larger geographic distance have higher D (genetic distance) values and thus genetically more dissimilar than those situated closer (Singh and Singh 2010). The isolation by distance hypothesis has been tested by Schug et al. (2007) in *D. ananassae* and results of the study conform to the hypothesis.

Populations showing a high degree of structuring indicate that populations in some provinces may not experience frequent gene flow or migration, which leads to populations with allele frequencies strongly divergent to assume functional independence (Moritz 1994; Mcrae et al. 2005). The study by Singh and Singh (2010) (including data from I and D values) shows that some populations may share weaker genetic links with neighboring populations than with more distant populations and vice versa. However, in some cases, genetic similarity between faraway-located populations has been found that could be due to similarity in environmental conditions. *D. ananassae* does not show temporal divergence, i.e., lesser incidence of population intermixing as a function of time via migration or gene flow, leading to genetically nonidentical populations throughout the range, thus keeping *D. ananassae* populations highly substructured and semi-isolated (Singh and Singh 2010).

Gene flow provides the index of comparative strengths of migration and genetic drift. In small-sized populations, genetic drift will have a dominating effect on allele frequencies as compared to other forces. This will strike equilibrium between genetic drift and migration that will be relatively less sensitive to other weaker forces such as selection or mutation (Neigel 1997). Conversely, in large populations, genetic drift is comparatively a weak force; if rate of migration is also small, then equilibria that are determined primarily by genetic drift will be reached gradually, and distributions of chromosomal marker will be more sensitive to selection and mutation (Neigel 1997). However, at low rate of migration, the generation time to reach equilibrium is determined by the effective size of population. In case it approaches the ages of populations, then historical relationships among populations instead of gene flow will determine the distribution of genetic markers (Felsenstein 1982; Neigel 1997).

Similar studies (Vogl et al. 2003; Das et al. 2004; Schug et al. 2007) done earlier at a molecular level in *D. ananassae* have led to an identical conclusion (Singh and Singh 2010). These studies have shown that F_{ST} values of the order of 0.1 (much lower than our F_{ST} estimates) could be applied to Indian populations (Singh and Singh 2010). This difference could be attributed to the markers used. When compared to molecular and allozyme markers, the scenario of geographic differentiation seems to be variable for chromosomes, which are more differentiated even over small distances. This is because molecular and allozyme markers are relatively more "neutral" than chromosomal arrangements (Singh and Singh).

It could be suggested that in a broad spatio-temporal study by Singh and Singh (2010) spanning different geo-climatic localities of the India, the natural population of *D. ananassae* shows a high degree of substructuring as a result of differentiation of their natural populations. In light of restricted gene flow, populations are expected to show genetic divergence due to drift. Lower gene flow in conjugation with high level of genetic differentiation might have occurred in the historical past and is maintained since then. Demographic properties and contemporary and historical events coupled with other factors are relatively more important in shaping the population structuring patterns, genetic differentiation, and gene flow than those caused by characteristics of terrestrial habitat favorable or unfavorable for migration (Singh and Singh 2010).

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5 Chromosomal Associations in Natural and Laboratory Populations of *Drosophila ananassae*

Abstract

Inversion polymorphism in *Drosophila* species provides a good material for analyzing epistatic interactions. Nonrandom associations between linked inversions are already reported in *D. ananassae*. In *D. robusta* nonrandom association (linkage disequilibrium) of linked inversions is attributed to two causative factors acting independently or together, viz., cross-over suppression between linked inversions and natural selection discriminating against certain recombinant arrangements. Meiotic drive is also implicated in causing linkage disequilibrium. In a study by Singh and Singh (Genetika 42:210–222, 2010), natural populations of *D. ananassae* and laboratory stocks initiated from these flies were used to study chromosome inversions. Quantitative data on inversion frequencies were employed to test intra- and interchromosomal associations in *D. ananassae*. In most of the populations (natural and laboratory), no significant departure from random combinations of chromosomes (2L–3L, 2L–3R, 3L–3R) were found, thus conforming random associations, although in some cases deviation from randomness was found to be statistically significant in natural as well as laboratory populations. This could be attributed to the increased number of some combinations, lesser number of others, and complete absence of certain combinations. Tight linkage between linked gene arrangements and genetic drift could have caused this. This corroborates the earlier hypothesis that genetic coadaptation is lacking in *D. ananassae.*

5.1 Chromosomal Associations in *D. ananassae*

Inversion polymorphism present in species of *Drosophila* provides a good tool to study epistatic interactions. Linkage disequilibrium between linked inversions is reported in *D. ananassae* (Singh 1983a, 1984; Singh and Singh 2008, 2010). Levitan (1958b) based on his studies in *D. robusta* has proposed that nonrandom association of linked inversions occurs because of suppression of crossing-over between dependent (linked) inversions or natural selection discriminating against certain recombinant

P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2_5

arrangements. Dyer et al. (2007) have suggested the role of meiotic drive leading to linkage disequilibrium (Singh and Singh 2010).

In *D. ananassae*, two inversions, viz., delta (3L) and eta (3R), of the third chromosome are linked. These are cosmopolitan in distribution and persist under laboratory conditions (Singh 1982a, 1983b, c, 1987; Singh and Singh 2008). A chromosome map (chromosome map of *D. ananassae*) prepared by Ray-Chaudhuri and Jha (1966) shows that delta (DE) inversion spanning from 1A to 8A covers 60 % of the total length of 3L, while eta (ET) inversion (10A–12C) covers 20 % of the total length of 3R (Singh 1986). These inversions are separated by distance, which is approximately 25 % of the total length of the third chromosome (Singh 1983a). These linked inversions (3L–3R) show random association in natural populations (Singh 1974, 1984; Singh and Singh 2010) and nonrandom association in laboratory populations (Singh 1983a, 1984; Singh and Singh 1988, 1990, 1991, 2008). Interchromosomal associations in *D. robusta* were first reported by Prakash (1967). For *D. ananassae*, there is no evidence for interchromosomal associations for unlinked inversions (2L–3L, 2L–3R) in natural as well as laboratory populations (Singh 1982b, 1983a; Singh and Singh 1989, 2008, 2010).

5.2 Methodology

In a study by Singh and Singh (2010), quantitative data on frequencies of various karyotype combinations have been utilized to obtain the absolute number of different intra- and interchromosomal associations in natural populations of *D. ananassae*.

5.3 Intra- and Interchromosomal Associations in Natural Populations

The presence of alpha (AL) in 2L, delta (DE) in 3L, and eta (ET) inversions in 3R gave nine combinations each between 2L–3L, 2L–3R, and 3L–3R karyotypes (Singh and Singh 2010). Assuming the random combination of karyotypes, their expected numbers can be calculated from the marginal totals of R×C contingency table. Statistically significant deviation from expectation would indicate nonrandom association between inversions (Singh and Singh 2010).

In a study by Singh and Singh (2010), 2L and 3L associations were obtained in variable frequency though the deviation from randomness was not significant in the majority of the populations with the exceptions of AD ($p<0.01$), PU ($p<0.05$), VD ($p<0.05$), BL ($p<0.01$), and ER ($p<0.05$) populations, which show significant deviation from expectation (Table 5.1 shows the data of significant deviation from randomness only, Singh and Singh 2010). The reason could be the increased number of some combinations, decreased number of other combinations, and absence of certain combinations as in AD population (Singh and Singh 2010). In the case of 2L–3R karyotype combinations, four natural populations, namely, GU ($p<0.05$), SH ($p<0.001$), GY ($p<0.01$), and BL ($p<0.05$), show significant deviation from expectation (see Table 5.2; Singh and Singh 2010), for the similar reason cited above. In SH and GY natural populations, some combinations were completely absent. In the case of 3L–3R karyotype combinations, only two populations, namely, DH ($p<0.05$) and BL ($p<0.05$), show nonrandom association (Table 5.3; Singh and Singh 2010). DH population shows excess of certain combinations, deficiency of other combinations, and complete absence of certain combinations. BL population analyzed among 45 populations is exceptional in showing significant deviations from randomness for all the three (2L–3L, 2L–3R, and 3L–3R) types of karyotype combinations (Singh and Singh 2010).

The data from the study by Singh and Singh (2010) on intra- and interchromosomal associations in *D. ananassae* provide ample evidence for random association of both linked and unlinked inversions in natural populations of *D. ananassae.* Although some populations show significant deviations from randomness, it cannot be the evidence for chromosomal interactions as some combinations either occurred in very low frequency or were found to be absent (less than 5).

Table 5.1 Observed and expected numbers of different combinations between 2L and 3L karyotypes in Indian natural populations of *D. ananassae*

Populations		Karyotype combinations									
	2L	ST/ST	ST/ST	ST/ST	ST/AL	ST/AL	ST/AL	AL/AL	AL/AL	AL/AL	
	3L	ST/ST	ST/DE	DE/DE	ST/ST	ST/DE	DE/DE	ST/ST	ST/DE	DE/DE	$\chi 2$
AD	Obs.	–	–	–	1	1	2	15	2	0	10.58**
	Exp.				3.04	0.57	0.38	12.95	2.42	1.61	df = 2
PU	Obs.	0	0	1	0	2	1	10	1	1	12.58*
	Exp.	0.62	0.18	0.18	1.80	0.56	0.56	7.50	2.25	2.25	df = 4
VD	Obs.	5	0	1	2	3	0	3	5	7	10.90*
	Exp.	2.30	1.84	1.84	1.92	1.53	1.53	5.76	4.61	4.61	df = 4
BL	Obs.	0	1	3	6	9	0	2	13	2	18.28**
	Exp.	0.88	2.50	0.55	3.33	9.58	2.08	3.77	10.86	2.36	df = 4
ER	Obs.	0	0	3	8	6	3	6	11	21	12.12*
	Exp.	0.72	0.87	1.39	4.10	4.90	7.91	9.10	11.10	17.60	df = 4

*$p < 0.05$; **$p < 0.01$

Table 5.2 Observed and expected numbers of different combinations between 2L and 3R karyotypes in Indian natural populations of *D. ananassae*

Populations		Karyotype combinations									
	2L	ST/ST	ST/ST	ST/ST	ST/AL	ST/AL	ST/AL	AL/AL	AL/AL	AL/AL	
	3R	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	χ 2
GU	Obs.	0	0	1	4	5	4	37	42	8	11.54*
	Exp.	0.40	0.46	0.12	5.27	6.04	1.67	35.31	40.48	11.19	df = 4
SH	Obs.	–	–	–	0	0	2	22	15	2	19.44***
	Exp.				1.07	0.73	0.19	20.92	14.26	3.80	df = 2
GY	Obs.	–	–	–	0	6	0	45	25	3	10.05**
	Exp.				3.41	2.35	0.22	41.58	28.64	2.71	df = 2
BL	Obs.	2	0	2	8	6	1	11	6	0	12.65*
	Exp.	2.33	1.33	0.33	8.75	5.0	1.25	9.91	5.66	1.41	df = 4

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Table 5.3 Observed and expected numbers of different combinations between 3L and 3R karyotypes in Indian natural populations of *D. ananassae*

Populations		Karyotype combinations									
	3L	ST/ST	ST/ST	ST/ST	ST/DE	ST/DE	ST/DE	DE/DE	DE/DE	DE/DE	
	3R	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	χ^2
DH	Obs.	23	2	–	17	0	0	3	0	1	12.40*
	Exp.	23.36	1.08		15.89	0.73	0.36	3.73	0.17	0.11	df = 4
BL	Obs.	4	4	0	14	8	1	3	0	2	10.27*
	Exp.	4.66	2.66	0.66	13.41	7.66	1.91	2.91	1.66	0.41	df = 4

*$p < 0.05$

The variable results in analyzed populations could be attributed to the interplay of different genetic factors operative in different populations of the same species (Singh and Singh 2010).

The total 45 populations analyzed exhibit variable frequency of different interchromosomal associations, but only few populations show significant deviation from randomness (Singh and

Singh 2010). This confirms that natural selection does not favor any of the second and third chromosome karyotype combinations owing to its positive epistatic interaction (Singh 1982b). In the case of intrachromosomal association, only DH ($p<0.05$) and BL ($p<0.05$) populations of the total 45 populations show nonrandom association. This could be due to abundance of certain combinations, deficiency of other combinations, and complete absence of certain combinations as seen in DH population (Singh and Singh 2010).

Mechanical factors owing to twisting of the inversions at the time of synapses may interfere with pairing, therefore reducing the crossing-over (Levitan 1958a). It might be possible that the reduced number of recombinant arrangements in some populations occurs due to suppression of crossing-over between the two inversions (Singh 1983a). In some cases, lack of crossing-over has led to complete association between the two inversions (Singh 1973, 1974). This corroborates the "tight linkage theory" between linked inversions (3L–3R) in *D. ananassae*. Brncic (1961), while working on *D. pavani*, has postulated that abundance of coupling and deficiency of repulsion combinations lead to linkage disequilibrium in natural and laboratory populations (Singh and Singh 2010).

Linkage disequilibrium (nonrandom associations) of unlinked inversions (2L–3L, 2L–3R) of the same chromosome was reported in *D. robusta* (Levitan 1958a, b, 1961, 1973, 1978; Prakash 1967), *D. guaramunu* (Levitan and Salzano 1959), *D. pavani* (Brncic 1961), *D. euronotus* (Stalker 1964), *D. subobscura* (Sperlich and Feuerbach-Mravlag 1974), *D. melanogaster* (Knibb et al. 1981), *D. bipectinata* (Singh and Das 1991b; Banerjee and Singh 1996), and others. These studies reinforce the hypothesis of Levitan (1958b) that the natural selection employing epistatic interaction between linked inversions is the main causative factor for maintaining nonrandom association (linkage disequilibrium) between inversions. Yamazaki et al. (1984) have proposed that the occurrence of nonrandom association (linkage disequilibrium) is common under a biallelic system and it goes on decreasing as the system moves from lesser allele to a polyallelic system. Most of the polymorphic loci have multiple alleles, like chromosomal inversions, which might lead to the nonoccurrence of nonrandom association (linkage disequilibrium) between inversions in natural populations (Singh et al. 1975). Natural selection also plays a role by differentially favoring some combinations over others, hence leading to abundance of selected combinations in a population over the rejected combinations. Tight linkage coupled with genetic drift or epistatic selection or population subdivision could also cause nonrandom association. Kojima and Lewontin (1970) have elaborated the significance of linkage and epistasis in evolutionary processes (Singh 1983a). Different workers have reported linkage disequilibrium between polymorphic inversions and isozyme alleles in some species of *Drosophila* (Mukai et al. 1971; Prakash and Lewontin 1971; Yamaguchi et al. 1980; Singh 1983).

Overall, linkage disequilibrium results from the combine interplay of biological factors like mutation, recombination, history of evolution, and population demography (Mueller 2004; Singh and Singh 2010). Also, population structuring, effective size, mode of selection like selective sweeps, and co-selection of loci are other major players influencing the regional patterns of linkage disequilibrium (nonrandom association) (Singh and Singh 2010).

5.4 Intra- and Interchromosomal Associations in Laboratory Populations

Singh and Singh (2010) also studied the chromosomal interactions in the laboratory populations initiated from the natural populations. Four populations, i.e., HD ($p<0.01$), AB ($p<0.001$), BP ($p<0.05$), and BL ($p<0.01$), out of the total 45 populations analyzed show significant deviation (Table 5.4) from randomness as in the case of (2L–3L) karyotype combinations (Singh and Singh 2010). In these four populations also, the reason was excess, deficiency, and complete absence of certain combinations. For instance, in AB population certain combinations are com-

Table 5.4 Observed and expected numbers of different combinations between 2L and 3L karyotypes in laboratory populations of *D. ananassae*

Populations		Karyotype combinations									
	2L	ST/ST	ST/ST	ST/ST	ST/AL	ST/AL	ST/AL	AL/AL	AL/AL	AL/AL	
	3L	ST/ST	ST/DE	DE/DE	ST/ST	ST/DE	DE/DE	ST/ST	ST/DE	DE/DE	χ^2
HD	Obs.	16	6	1	12	24	2	29	9	1	16.57**
	Exp.	13.11	8.97	0.92	21.66	14.82	1.52	22.23	15.21	1.56	df=4
AB	Obs.	17	14	–	9	2	–	56	2	–	23.79***
	Exp.	25.42	5.58		9.02	1.98		47.56	10.44		df=2
BP	Obs.	12	6	0	45	4	0	23	9	1	9.89*
	Exp.	14.40	3.42	0.18	39.20	9.31	0.49	26.40	6.27	0.33	df=4
BL	Obs.	9	20	16	18	17	4	4	11	1	14.88**
	Exp.	18.45	21.60	9.45	15.99	18.12	8.19	6.56	7.68	3.36	df=4

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 5.5 Observed and expected numbers of different combinations between 2L and 3R karyotypes in laboratory populations of *D. ananassae*

Populations		Karyotype combinations									
	2L	ST/ST	ST/ST	ST/ST	ST/AL	ST/AL	ST/AL	AL/AL	AL/AL	AL/AL	
	3R	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	χ^2
AB	Obs.	25	6	0	6	5	0	20	28	10	20.15***
	Exp.	15.81	12.09	3.10	5.61	4.29	1.10	29.58	22.62	5.80	df=4
JR	Obs.	3	0	–	6	3	–	82	6	–	7.31*
	Exp.	2.73	0.27		8.19	0.81		80.08	7.92		df=2
SI	Obs.	25	1	0	34	5	0	24	8	3	10.98*
	Exp.	21.58	3.64	0.78	32.37	5.46	1.17	29.05	4.90	1.05	df=4
ER	Obs.	0	1	2	21	17	6	34	18	1	18.14**
	Exp.	1.65	1.08	0.27	24.20	15.84	3.96	29.15	19.08	4.77	df=4
KR	Obs.	4	11	4	17	20	14	19	11	0	15.82**
	Exp.	7.60	7.98	3.42	20.04	21.42	9.18	12.0	12.60	5.40	df=4

*$p<0.05$; **$p<0.01$; ***$p<0.001$

pletely absent. In the remaining populations deviation from expectation was not significant. For 2L–3R karyotype combinations (see Table 5.5; Singh and Singh 2010) only five populations, namely, AB ($p<0.001$), JR ($p<0.05$), SI ($p<0.05$), ER ($p<0.01$), and KR ($p<0.01$), of the total 45 populations analyzed show significant deviation from expectation. JR population shows complete absence of certain combinations (Singh and Singh 2010).

Also, for 3L–3R karyotype combinations (see Table 5.6; Singh and Singh 2010) 11 populations out of the 45 analyzed populations show significant deviation from expectations. These populations are JU ($p<0.001$), PN ($p<0.001$), IM ($p<0.001$), HW ($p<0.01$), SD ($p<0.001$), DW ($p<0.001$), VP ($p<0.01$), VD ($p<0.01$), PJ ($p<0.001$), ML ($p<0.05$), and ER ($p<0.001$), the reason again being the excess of some combinations over other combinations (less than 5). For instance, SD population shows complete absence of certain combinations (Singh and Singh 2010).

Most significantly, none of the laboratory populations show the evidence of chromosomal interactions for any of the three karyotype combinations (2L–3L, 2L–3R, and 3L–3R) examined (Singh and Singh 2010). Except BL, which showed deviation from randomness for 2L–3L karyotype combinations in natural as well as laboratory populations, none of the other populations showed significant deviation from randomness in analyzed natural and laboratory

Table 5.6 Observed and expected numbers of different combinations between 3L and 3R karyotypes in laboratory populations of *D. ananassae*

Populations		Karyotype combinations									
	3L	ST/ST	ST/ST	ST/ST	ST/DE	ST/DE	ST/DE	DE/DE	DE/DE	DE/DE	
	3R	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	ST/ST	ST/ET	ET/ET	χ^2
JU	Obs.	17	20	9	15	31	0	8	0	0	25.51***
	Exp.	18.40	23.46	4.14	18.40	23.46	4.14	3.20	4.08	0.72	df=4
PN	Obs.	37	29	10	4	12	1	0	1	6	31.42***
	Exp.	31.16	31.92	12.92	6.97	7.14	2.89	2.87	2.94	1.19	df=4
IM	Obs.	8	9	2	27	33	0	20	0	1	24.81***
	Exp.	10.45	7.98	0.57	33.0	25.20	1.80	11.55	8.82	0.63	df=4
HW	Obs.	26	18	4	13	20	0	17	2	0	13.97**
	Exp.	26.88	19.20	1.92	18.48	13.20	1.32	10.84	7.60	0.76	df=4
SD	Obs.	39	14	–	38	0	–	9	0	–	14.43***
	Exp.	45.58	7.42		32.68	5.32		7.74	1.26		df=2
DW	Obs.	17	0	1	32	23	0	16	4	7	29.12***
	Exp.	11.70	4.86	1.44	35.75	14.85	4.40	17.55	7.29	2.16	df=4
VP	Obs.	36	33	15	0	11	4	0	0	1	14.38**
	Exp.	30.24	36.96	16.80	5.40	6.60	3.0	0.36	0.44	0.20	df=4
VD	Obs.	18	26	8	2	32	12	1	0	1	16.41**
	Exp.	10.92	30.16	10.92	9.66	26.68	9.66	0.42	1.16	0.42	df=4
PJ	Obs.	19	3	1	27	32	1	6	6	5	26.29***
	Exp.	11.96	9.43	1.61	31.20	24.60	4.20	8.84	6.97	1.19	df=4
ML	Obs.	37	33	18	2	9	0	1	0	0	9.75*
	Exp.	35.20	36.96	15.84	4.40	4.62	1.98	0.40	0.42	0.18	df=4
ER	Obs.	51	17	3	3	18	4	1	1	2	35.31***
	Exp.	39.05	25.56	6.39	13.75	9.0	2.25	2.20	1.44	0.36	df=4

*$p<0.05$; **$p<0.01$; ***$p<0.001$

populations (Kumar and Gupta 1991), thus reinforcing and confirming the results from earlier studies that both unlinked and linked inversions show random association in natural as well as laboratory populations of *D. ananassae* (Singh and Singh 2010).

The results from the study by Singh and Singh (2010) show ample evidence that delta and eta, the two linked inversions, in the third chromosome of *D. ananassae* show random association in laboratory populations (Singh 1986). Although some of the populations showed the chi-square values indicating significant deviation from random association, these could not be construed as intrachromosomal interactions, because these results might have arrived due to increased number of some combinations over others (less than 5) (Singh and Singh 2010). Linkage disequilibrium (nonrandom association) of unlinked inversions of the similar chromosome has been reported in *D. robusta* (Levitan 1958a, b, 1961, 1973, 1978; Prakash 1967), *D. guaramunu* (Levitan and Salzano 1959), *D. pavani* (Brncic 1961), *D. euronotus* (Stalker 1964), *D. subobscura* (Sperlich and Feuerbach-Mravlag 1974), *D. melanogaster* (Knibb et al. 1981; Singh and Das 1992), and *D. bipectinata* (Singh and Das 1991b; Banerjee and Singh 1995, 1996). These studies reinforce the hypothesis of Levitan (1958b) that the natural selection employing epistatic interaction between linked inversions is the main causative factor for maintaining nonrandom association (linkage disequilibrium) between inversions. However, in *D. pavani*, selective pressure was implicated for its role in maintaining the nonrandom association of gene sequences.

Studies of intrachromosomal associations by different groups in *D. ananassae* (Singh 1983a,

1984; Singh and Singh 1988; Singh and Mohanty 1990; Singh and Singh 1990, 1991, 2010) have shown that two dependent (linked) inversions (3L–3R) show random association in natural and mass culture laboratory populations but not in laboratory populations initiated from single females (isofemale lines). This implicates the role of random drift in causing nonrandom association in isofemale lines. This is further strengthened by the "tight linkage theory" between the two inversions as confirmed by the analysis of recombination studies done earlier that drift causes the nonrandom association (linkage disequilibrium) (Singh and Singh 1990). The differential existence or selective persistence (having two different inversions in the same homologous chromosomes) of different combinations of linked gene sequences in random equilibrium imparts differential selective and adaptive advantage to the genotypes (Singh and Singh 2010). Natural selection will invariably favor the fittest condition in each region. This is further evidenced by the fact that nonrandom associations are invariably the phenomena of the laboratory populations that have been maintained for a very long time, thus strengthening the selective nature of the phenomenon (Singh and Singh 2010). Further, the culture maintained for longer periods shows higher probability of inversions of the same chromosome to be linked. Even the stocks initiated from the natural population and showing random distribution of inversions show association of inversions of the same chromosome if maintained for longer periods (Singh and Singh 2010). Drift could have possible role owing to tight linkage between inversions, but since stocks are initiated from a large number of flies, the role of drift is very unlikely (Singh 1983a). The reason for linkage disequilibrium varies from one population to another population due to the interplay of variable genetic factors in different populations of a species. The genetic factors might be responsible for the differential pattern of observed chromosomal association or disassociation among the strains (Singh 1974, 1983a).

In addition to epistatic selection, linkage disequilibrium may occur for a variety of other reasons like (i) random drift in a small population (Hill 1976), (ii) migration or intermixing of population with differential allele frequencies (Ohta 1982), and (iii) genetic hitchhiking (Hedrick et al. 1978). These additional factors must be discussed for their role in causing linkage disequilibrium instead of solely attributing epistatic selection to be responsible for the occurrence of linkage disequilibrium (Singh and Singh 2010).

Combining the findings of Singh and Singh (2010) with earlier interchromosomal association studies (Singh 1982b, 1983a; Singh and Singh 1989), it could be suggested that laboratory populations of *D. ananassae* do not show any evidence for interchromosomal interactions. A study by Singh and Singh (2010) objectively discounts the role of natural selection due to positive epistatic interaction selectively favoring some karyotype combinations over others in the case of 2L–3L and 2L–3R karyotype combinations. This finding was corroborated by other groups as well (Sperlich and Feuerbach-Mravlag 1974; Das and Singh 1990; Singh and Das 1991a). Kojima and Tobari (1969) have postulated the role of genetic differences in basic lines, variation in genetic background, and interchromosomal interactions, each acting independently or together causing the variation in the equilibrium frequencies of chromosome arrangements as gene arrangements in one chromosome is largely influenced by the karyotypes in the other chromosome.

In genus *Drosophila*, Prakash (1967) has reported the single instance of interchromosomal interactions in *D. robusta*, where he found significant deviation from random association of independent inversions. He analyzed fitness, variability, and fertility and hypothesized the selective advantage of some combinations by natural selection over others due to association between dependent inversions. Sperlich and Feuerbach-Mravlag (1974) have postulated that selection may favor association between interacting genes and chromosome may be treated as functional and selectional units.

Both natural and laboratory populations show deviation from randomness in certain cases. However, the mechanism varies fundamentally in both cases (Singh and Singh 2010). In natural population, the chi-square test for goodness of fit

between observed and expected numbers was found to be significant in some cases showing interpopulation variability with respect to gene arrangements (Singh and Singh 2010), while in laboratory population, the difference between observed and expected numbers is higher and significant. This could be because of the number of generations populations have been maintained in the laboratory (Levitan et al. 1954).

Based on the findings of a study by Singh and Singh (2010) combined with earlier findings, it could be stated that natural and laboratory populations of *D. ananassae* do not exhibit the evidence for chromosomal interactions, both intra- and interchromosomal types. Most significantly, *D. ananassae* populations show lack of any evidence for genetic coadaptation.

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Chromosomal Aberrations in *Drosophila ananassae*

6

Abstract

In *D. ananassae*, different types of chromosomal rearrangements, like paracentric and pericentric inversions, transpositions, translocations, extra-bands, and deficiencies, have recurrently been found in natural populations, but these chromosome rearrangements are rarely found in other species of *Drosophila*, thus reflecting high mutability in *D. ananassae*. A large number of inversions are the common feature of *D. ananassae* genome. A total of 76 paracentric inversions, 21 pericentric inversions, and 48 translocations so far have been reported in the populations of *D. ananassae*. Paracentric inversions are the predominant feature of *D. ananassae* genome. Majority of these paracentric inversions have limited distribution and are transient in nature except three cosmopolitan inversions, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, which show worldwide distribution. The presence of pericentric inversions and translocations are among the most unusual features of natural populations of *D. ananassae*. A given arrangement may occur in one region but may be absent in other. An explanation to account for such disjunct distribution of gene arrangements may be that all types of arrangements did not occur simultaneously in the past history of the species. The former types, being older, had greater opportunities for migration and thereby at present they have a distribution throughout the world. On the contrary, those rearrangements of relatively recent origin and due to obvious impediments in the means of migration could not migrate from their respective native place to the localities where they have been found wanting.

6.1 Chromosomal Aberrations

Populations of *Drosophila ananassae* show a high level of chromosomal polymorphism. Natural populations of *D. ananassae* show large number of inversions (Singh 1998; Singh and Singh 2008). Most of these are reported from different regions of the world. Majority have limited distribution, while the three inversions, viz., Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, show cosmopolitan distribution (Singh 1998; Singh and Singh 2008).

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DOI 10.1007/978-81-322-2565-2_6

Table 6.1 Pericentric inversions in *D. ananassae*

S. no.	Pericentric inversion	Locality	Reference
1.	In (2LR)a	Brazil	Freire-Maia 1955, 1961
2.	In (2LR)A	Niue	Futch 1966; Seecof 1957; Hinton and Downs 1975
3.	In (2LR)Lo	#	*
4.	In (2LR)	India	Singh et al. 1971
5.	In (2LR)9	India	Reddy and Krishnamurthy 1972b
6.	In (2LR)B	Guam	*
7.	In (2LR)B,Ubx	#	
8.	In (2LR)C	Nouméa	*
9.	In (3LR)a	Brazil	Freire-Maia 1955, 1961
10.	In (3LR)b	Brazil	Freire-Maia 1955, 1961
11.	In (3LR)c	Brazil	Freire-Maia 1955, 1961
12.	In (3LR)D,Bl^2	#	
13.	In (3LR)d	Brazil	Freire-Maia 1955, 1961
14.	In (3LR)E,stw	#	
15.	In (3LR)A	Niue	Seecof 1957; Futch 1966
16.	In (3LR)B	Niue	Seecof 1957; Futch 1966
17.	In (3LR)C	Chiang Mai	*
18.	In (3LR)F	Wau	*
19.	In (3LR)G	Hyderabad	*
20.	In (3LR)H	Coimbatore	*
21.	In (3LR)I	Coimbatore	*

Note: * for references, see, Tobari (1993); # detected from various laboratory stocks; Hinton (unpublished)

Chromosomal polymorphism has been extensively studied in Indian natural populations of *D. ananassae* (reviewed by Singh 1998; Singh and Singh 2008). The studies involving population genetics of chromosomal polymorphism invariably shows geographic differentiation of inversion polymorphism. Present section gives the details of chromosomal aberrations detected from natural and laboratory populations of *D. ananassae*. We have tried to include all detected chromosomal aberrations so far in *D. ananassae* in natural and laboratory populations to give the holistic picture of chromosomal variability as well as unusual mutational property of *D. ananassae* (Singh and Singh 2007a).

Tables 6.1 and 6.2 give the details of pericentric-inversions and translocations detected in *D. ananassae*. The numbers of pericentric inversions and translocations are twenty-one (21) and forty-eight (48), respectively (Singh and Singh 2007a, 2008). The occurrence of pericentric inversions (heterozygotes for pericentric inversions produce unbalanced gametes; their appearance, therefore, is opposed by natural selection) and translocations, extremely rare in other *Drosophila* species, indicates the unusual mutational characteristics of *D. ananassae* (Singh and Singh 2007a, 2008).

The paracentric inversions are depicted in Figs. 6.1, 6.2, 6.3, 6.4, 6.5, and 6.6 via line diagram. We have followed the improved edition of polytene chromosome reference photomap of *D. ananassae* (Moriwaki and Ito 1969; Tobari 1993). As for naming different inversions, to make it uniform, we have numbered them in chronological order as given in Tobari (1993). It is difficult to know the exact number of paracentric inversions, since different investigators have named inversions independently. Also all the new inversions have not been reported or documented in relevant journals. In the present section, despite these limitations we have tried to include all possible inversions, thus taking the total tally to seventy-eight (78) (Singh and Singh 2007a, 2008). The II and III chromosomes carried the

Table 6.2 Translocations in *D. ananassae*

S. no.	Translocation	Locality	Reference
1.	T (XL;2R) A	Niue	Seecof 1957; Futch 1966
2.	T (XR;2R)	India	Sajjan and Krishnmurthy 1970
3.	T (XR;2L) 8	India	Reddy and Krishnamurthy 1972b
4.	T (XL;2L) B	Nauru	*
5.	T (XR;2R)A,M Ubx ca	#	Hinton 1979
6.	T(XL;XR)B,ca Th	#	Hinton 1981
7.	T (1;3)A, Mo	#	
8.	T (Y;2L)A, ca	#	Hinton 1979
9.	T (Y;2L)B, ca	#	Hinton 1979; Hinton and Downs 1975
10.	T (Y;2L)C, ca	#	Hinton 1979, 1980; Hinton and Downs 1975
11.	T (Y;2;3)A,M ca stw	#	Hinton 1980
12.	T (Y;3)A.stw	#	Hinton 1980
13.	T (Y;3R)B	#	Hinton and Downs 1975
14.	T (Y;3L)C,e se;ru	#	
15.	T (Y;3L)pe^{v}	#	*
16.	T (2L;3L)	Brazil	Dobzhansky and Dreyfus 1943
17.	T (2L;3L)66	Honolulu	*
18.	T (2L;3L) 9	India	Reddy and Krishnamurthy 1972a
19.	T (2L;3L)10	India	Sajjan and Krishnamurthy 1972
20.	T (2L;3L)8	India	Reddy and Krishnamurthy 1974
21.	T (2R;3R)	Brazil, India	Freire-Maia 1961; Sajjan and Krishnamurthy 1970
22.	T (2R;3R)AA,Ly	#	
23.	T (2R;3R)A,ca stw	#	Hinton 1979; Hinton and Downs 1975
24.	T (2R;3R)B,M ca stw	#	Hinton 1979; Hinton and Downs 1975
25.	T (2L;3R)C,M ca stw	#	Hinton 1979; Hinton and Downs 1975
26.	T (2R;3R)D,ca stw	#	Hinton 1979; Hinton and Downs 1975
27.	T (2L;3R)E,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
28.	T (2L;3L)F,ca stw	#	Hinton 1979
29.	T (2L;3L)G,ca stw	#	Hinton 1979; Hinton and Downs 1975
30.	T (2L;3R)H,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
31.	T (2R;3R)J, Xa ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
32.	T (2L;3L)K,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
33.	T (2R;3L)L,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
34.	T (2L;3L)M,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975

(continued)

Table 6.2 (continued)

S. no.	Translocation	Locality	Reference
35.	T (2L;3R)N,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
36.	T (2R;3L)O,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
37.	T (2L;3R)P,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
38.	T (2R;3R)Q,ca stw	#	Hinton 1979, 1980; Hinton and Downs 1975
39.	T (2;3)R	#	Hinton 1981
40.	T (2R;3R)S	#	Hinton 1981; Hinton and Downs 1975
41.	T (2L;3R)T	#	Hinton 1981; Hinton and Downs 1975
42.	T (2;3)U,ca cy	#	Hinton 1981; Hinton and Downs 1975
43.	T (2L;3R)V	#	Hinton and Downs 1975
44.	T (2L;3L)W,Cy^{EX}	#	Hinton and Downs 1975
45.	T (2;3)Z,Mot	#	
46.	T (2L;3L)15	India	Singh 1991
47.	T (3L;4)Pm	#	Kikkawa 1938
48.	T (3L;4)	India	Ray-Chaudhuri and Jha 1966

Note: # detected from various laboratory strains; * for references, see Tobari, 1993; Hinton (unpublished)

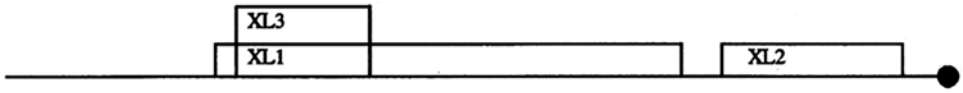
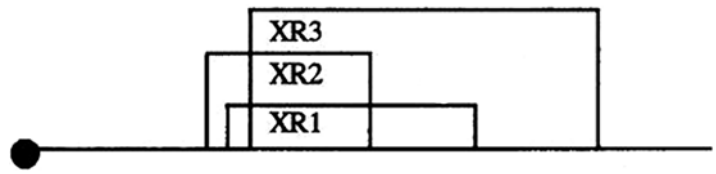


Fig. 6.1 Location of different inversions in XL of *D. ananassae*

Fig. 6.2 Location of different paracentric inversions in XR of *D. ananassae*



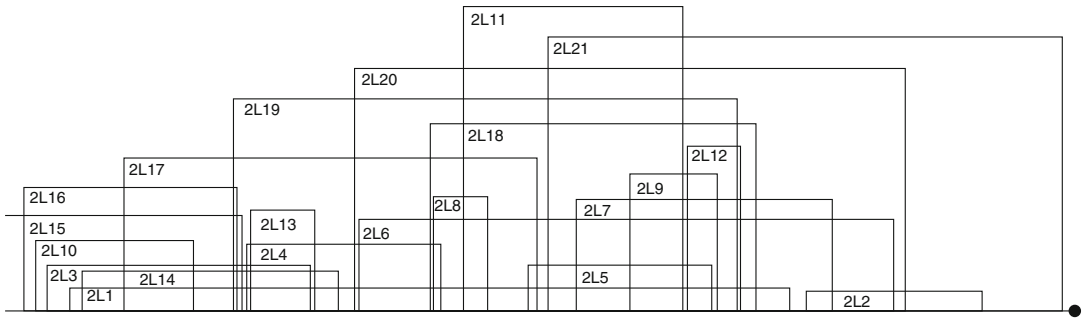


Fig. 6.3 Location of different paracentric inversions in 2L of *D. ananassae*

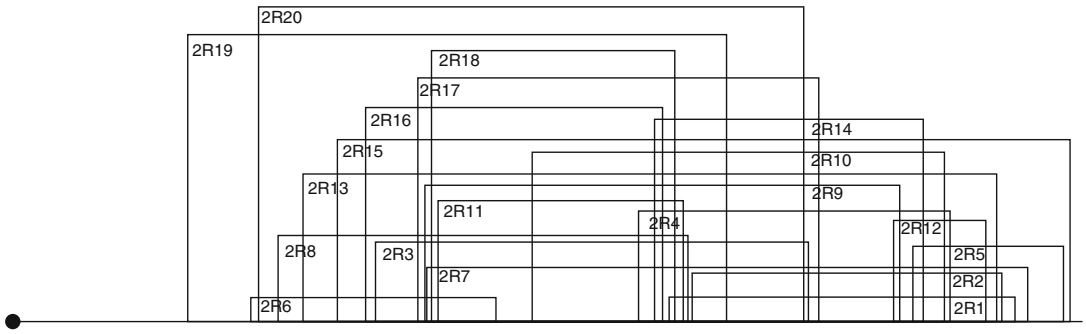


Fig. 6.4 Location of different paracentric inversions in 2R of *D. ananassae*

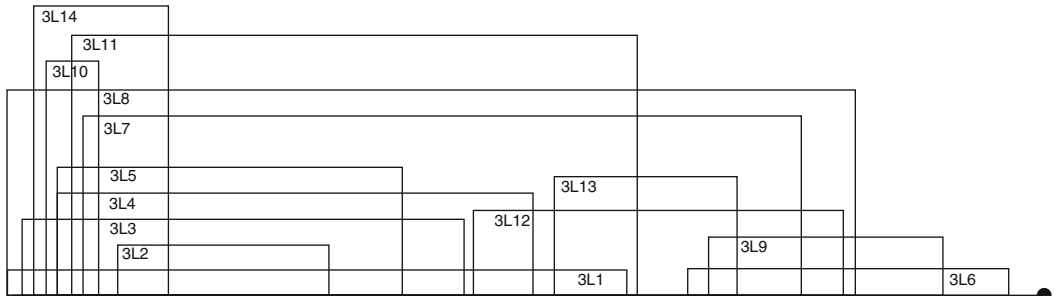


Fig. 6.5 Location of different paracentric inversions in 3L of *D. ananassae*

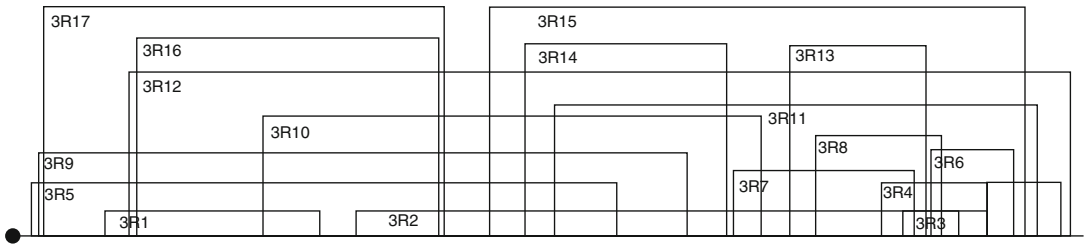


Fig. 6.6 Location of different paracentric inversions in 3R of *D. ananassae*

maximum number of inversions (Singh 1986; Singh and Singh 2007a, 2008). Among these, 2L carried the maximum of twenty-one (21) inversions, followed by 2R (20), 3R (17), 3L (14), and XL and XR, three (3) each. With the exception of few cases, there has been no clustering of breakpoints in any particular region in all the arms. So, it could be said that naturally occurring inversions in *D. ananassae* are randomly distributed in X, second and third chromosomes (Singh 2007a; Singh and Singh 2008).

Populations of *D. ananassae* harbor a large number of paracentric inversions, but only three, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, show cosmopolitan distribution. Majority of these inversions have restricted distribution and have been reported from few individuals. This forms the fundamental feature of the trend of chromosomal polymorphism in *D. ananassae* (Carson 1965; Dasmohpatra et al. 1982; Singh 1988; Sella et al. 2004). A given arrangement may occur in one region but may be

absent in other. An explanation to account for such disjunct distribution of gene arrangements may be that all types of arrangements did not occur simultaneously in the past history of the species. The former types, being older, had greater opportunities for migration and thereby at present they have a distribution throughout the world (Kaufmann 1936a, b; Kikkawa 1938; Dobzhansky and Dreyfus 1943). On the contrary, those rearrangements of relatively recent origin and due to obvious impediments in the means of migration could not migrate from their respective native place to the localities where they have been found wanting (Ray-Chaudhuri and Jha 1967; Charlesworth and Charlesworth 1973).

Disappearance of new sequences suggests that population in question might have developed a kind of resistance to acquire new gene arrangements in its genetic structure, because they could not yield adaptive values or heterotic effects to their carriers. Therefore, natural populations of *D. ananassae* resist any further increase in the amount of chromosomal polymorphism that is not of any adaptive consequence and may well be a genetic load to bear (Singh 1984a, b; Singh and Singh 2008). With the same token, the existing load of chromosome polymorphism due to three cosmopolitan inversions, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET), in 3R in natural populations of *D. ananassae* might be too high to acquire any new gene arrangement as a means of adaptation of population to the extremes of the environmental conditions (White 1958; Illan 1973; Painter 1975).

Freire-Maia's (1961) suggestion that some special mechanism exists in *D. ananassae* to permit the retention of disadvantageous rearrangements in natural populations deserves exploration. Alternatively, the high incidence of such rearrangements may reflect high mutability in this species, a possibility proposed by Kikkawa (1938).

Table 6.3 enumerates the details of geographical distribution of the three cosmopolitan inversions. It is apparent from the table that three cosmopolitan inversions are of frequent occurrence in natural populations and have become cosmopolitan with the species distribution (Singh 1989; Singh and Singh 2007a, 2008).

Dobzhansky and Dreyfus (1943) have reported the probable origin of *D. ananassae* around Eastern and Southeast Asia. It extensively depends on humans for its widespread distribution. *D. ananassae* certainly appears to qualify as a polytypic species (Singh 1984). It shows widespread circum tropical distribution, particularly in the islands of the Pacific Ocean. This geographical isolation has allowed perceptible genetic differences between populations, transforming them into geographic races (Singh 1984; Stephan 1994).

Relatively low number of inversions were observed in dark-form *ananassae*; thus, we suggest that the dark-form *ananassae* was distributed around the Polynesian islands before the cosmopolitan form had a chance to expand its territory throughout the entire tropical and subtropical world. Because no reproductive isolation had developed between the two forms, the cosmopolitan form with its cosmopolitan inversions introgressed into Polynesian populations. Then these cosmopolitan inversions were distributed in many places where the dark-form *ananassae* had been the precedent inhabitants. These widespread cosmopolitan inversions would be maintained in natural populations by the strong superiority of the inversion heterozygotes (Tobari 1993).

6.2 A New Paracentric Inversion in the Left Arm of the Third Chromosome of *Drosophila ananassae*

Singh and Singh (2005a) reported a new paracentric inversion named Theta (TH) in the left arm of the third chromosome (3L). This new inversion was detected in F1 larvae obtained from a cross between wild caught male from Bhubaneswar, Orissa, India, in May 2005 and standard homozygous female from GH-ST stock being maintained in the laboratory (Singh and Singh 2005a, 2007b). According to the chromosome map of polytene chromosomes of *D. ananassae* prepared by Ray-Chaudhuri and Jha (1966), the theta (TH) inversion spans the region from 9A to 10E in the left arm (L) of the third

Table 6.3 Geographical distribution of three cosmopolitaninversions in *D. ananassae*

Area	Subterminal (alpha)	Terminal (delta)	Basal (eta)	Source
Alabama	+	+	+	Kaufmann 1936b
Texas	+	–	+	Shirai and Moriwaki 1952
Hawaii	+	+	+	Shirai and Moriwaki 1952
Majuro resist any further increase in the amount	+	+	+	Seecof 1957
Cuba	+	+	+	Futch 1966
Mexico	+	+	+	Shirai and Moriwaki 1952; Futch 1966
Brazil	+	+	+	Dobzhansky and Dreyfus 1943; Shirai and Moriwaki 1952; Freire-Maia 1955
China	+	+	+	Kikkawa 1938
Formosa (Taiwan)	+	+	+	Kaufmann 1936; Kikkawa 1938
Japan	+	+	+	Kaufmann 1936; Kikkawa 1938
India	+	+	+	Ray-Chaudhuri and Jha 1966; Sajjan and Krishnamurthy 1970; Reddy and Krishnamurthy 1972a, b; Singh 2001
Africa	+	+	+	Shirai and Moriwaki 1952
Micronesia (Caroline Island, Marshal Island, Mariana Island)	+	+	+	Seecof (Stone et al. 1957); Futch 1966
Melanesia (Papua New Guinea, Caledonia Island, Fiji)	+	+	+	Futch 1966
Polynesia (Samoa, Cook Island)	+	+	+	Futch 1966
Mauritius	+	+	+	*
Sri Lanka	+	+	+	*
Myanmar	+	+	+	*
Thailand	+	+	+	*
Malaysia	+	+	+	Singh 1983a, b; *
Borneo	+	+	+	Singh 1983b;*
Philippines	+	+	+	*
Singapore	+	+	+	*

* for references, see Tobari (1993); + indicates presence of inversion; – indicates absence of inversion

(III) chromosome. Figures 6.7 and 6.8 show the theta (TH) inversion in heterozygous condition along with the location of delta (DE) and theta (TH) in 3L and eta (ET) in 3R. Theta (TH) inversion covers almost 24 % region of the left arm of III chromosome (3L), while the delta (DE) inversion covers approximately 60 % of the left arm of III chromosome (3L). The separation between delta (DE) and theta (TH) inversions occupies approximately 6 % of the total length of the left arm of III chromosome 3L (Singh and Singh 2005a, 2007a, 2008).

Fig. 6.7 Microphotograph of a new inversion, Theta (TH) (heterozygous) in 3L of *Drosophila ananassae*

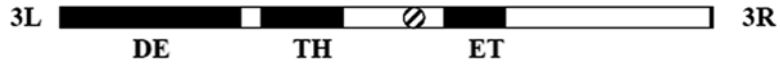


Fig. 6.8 Location of DE, TH (new), and ET inversion in the third chromosome of *D. ananassae*

6.3 A New Inversion in *Drosophila ananassae* Population from Allahabad, Uttar Pradesh

Singh and Singh (2005b) reported a new paracentric inversion named "Iota" (IT) in the left arm of the third chromosome of *D. ananassae. This* was detected from a single F1 larva from a naturally impregnated female collected from fruit and vegetable market in Mumfordganj, Allahabad (Uttar Pradesh), in September 2005. Chromosome map of polytene chromosomes of *D. ananassae* prepared by Ray-Chaudhuri and Jha (1966) was used as a reference map to establish the breakpoints of newfound inversion (Singh and Singh 2005b). This inversion (iota, IT) spans the region from 1C to 4A in 3L and covers approximately 32 % region of 3L, while delta (DE) and theta (TH) inversions occupies approximately 60 % and 24 % region of 3L, respectively (Singh and Singh 2005b). Figure 6.9 shows the microphotograph of the new inversion iota (IT) in heterozygous condition. Figure 6.10 shows the approximate location of iota (IT) inversion in the third chromosome. With the inclusion of new inversion, named iota (IT), the total tally of paracentric inversions in *D. ananassae* goes up to 72 (Singh and Singh 2005b, 2007a, 2008).

6.4 Loss of Paracentric Inversions in Laboratory Stocks of *Drosophila ananassae*

Natural populations of *Drosophilaananassae* show a high level of chromosomal polymorphism. A large number of inversions, particularly paracentric ones, have become integral part of the genome of *D. ananassae* (Singh 1998; Singh and Singh 2007b, 2008). Majority of these are reported from different regions of the world, but most of these have restricted distribution. Only three cosmopolitan inversions, viz., Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, show worldwide cosmopolitan distribution (Singh 1998). Chromosomal polymorphism in natural populations of *D. ananassae* has been extensively studied with respect to population genetics in Indian context (for references see review by Singh 1998; Singh and Singh 2007a). These studies invariably show

Fig. 6.9 Microphotograph of a new inversion, Iota (IT) (heterozygous) in 3L, of *D. ananassae*

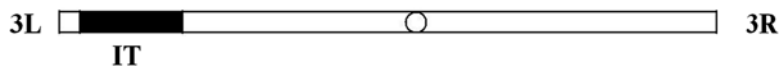


Fig. 6.10 Location of IT inversion (new) in the third chromosome of *D. ananassae*

the pattern of geographic differentiation of inversion polymorphism (Singh and Singh 2007a, b, c).

In the present section, we report about the fate of two new paracentric inversions, namely, theta (TH) and iota (IT) (Singh and Singh 2005a, b), detected from an isofemale line from Bhubaneswar (Orissa) and Allahabad (Uttar Pradesh), respectively. *D. ananassae* flies from these places were collected during May 2005 and September 2005, respectively. In a study, Singh and Singh (2005a, b) maintained these two isofemale lines under laboratory conditions, fed on the simple culture medium. Further, these flies were maintained by transferring 50 flies (equal number of males and females) for several generations. Laboratory stock from Bhubaneswar was analyzed after 18 generations, while laboratory stock from Allahabad was analyzed after 14 generations by squashing more than 50 larvae. In both of the stocks, the two new inversions, namely, theta and iota, were found to be eliminated. Populations of *D. ananassae* carry a large number of paracentric inversions, but only three, namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R, have become coextensive with the species distribution (Sella et al. 2004). Most of these inversions have restricted geographical distribution and were reported from the few individuals only. This forms the characteristic feature of the chromosomal polymorphism pattern in *D. ananassae* (Carson 1965; Singh 1988; Singh and Banerjee 1997; Singh and Singh 2007c).

At its origin, the unique copy of an inversion will be in a single individual in the heterozygous state. In the following generations, if the inversion escapes elimination, it will be found predominantly in the heterozygous state until (and if) it reaches a substantial frequency. Their evolutionary fate, then, depends upon their gene content (Krimbas and Powell 1992; Singh and Singh 2007c; Kirkpatrick 2010).

Usually the evolutionary history of an inversion passes through three critical stages (Nei 1967): (**i**) Origin of inversion as a unique event, this inversion may be lost or survive largely due to random or stochastic events, totally independent of population size (Fisher 1930; Li 1955). (**ii**) After surviving the initial precarious period, it spreads in the population in several copies,

largely due to random or stochastic events or the natural selection, which now completely decides the fate of new inversion. This is particularly true for small populations. (**iii**) A balance is attained for the establishment of stable polymorphism due to the work of selection (Krimbas and Powell 1992; Singh and Singh 2007c).

Two types of model based on computer simulations and analytical procedures exploring the fate of newly arisen inversions can be distinguished, the *additive model* and the *interactive model*. According to the first model (Sturtevant and Mather 1938), there have been several linked genes, each with two alleles, one beneficial and one detrimental. Selection was hypothesized to eliminate unfit phenotypes caused by homozygosity of detrimental alleles in case of dominance or with added selection against heterozygotes in the case of no dominance. The new inversion was introduced in a single copy into a small genetically variable population. Establishment meant either being maintained as stable polymorphism or going to fixation. Inversions with superior allelic content could be established. Gene action (i.e., dominance, absence of dominance, and occasionally overdominance were considered), allelic content of the new inversion, and the initial frequencies of the advantageous alleles in the population were the most important variables in determining the fate of an inversion. Neither overdominance at the genic level nor epistasis was necessary, although they might render easier the establishment of the inversions (Kojima 1967). Thus, the establishment of a new inversion would be a rare event (Singh and Singh 2007c).

The simplest possible *interactive model* consists of two genes with two alleles each (the two-locus system). According to Fisher (1930), in a population where alleles at loci A and B interact such that the combinations AB and ab are selectively advantageous over Ab and aB, any genetic variant reducing recombination between the loci will be favored such that AB/ab heterozygotes would produce fewer unfavorable Ab and aB gametes (Singh and Singh 2007c).

Federer et al. (1967) and Valen et al. (1968) proposed a model in which the expected frequency of generation of an inversion is a linearly decreasing function of its length:

$$h1(v) = 2(c - v)/c2,$$

with a mean c/3 and a variance c2/18 where h1(v) is the frequency of an inversion of length v occurring in a chromosome of total length c.

Van Valen and Levins (1968) also considered a model where the gene content of inversions was crucial in their establishment and that the probability of retention of an inversion is directly related to the number of overdominant loci captured, the expected distribution of lengths favors longer inversions. In *D. ananassae* AL (alpha) inversion is the longest inversion occupying greater part of 2L. By being the longest inversion off the three cosmopolitan inversions (AL, DE, ET), it can probably capture more genes with favorable epistatic effect on fitness. This property increases with the size of inversion. Therefore, it is plausible to state that advantage conferred by selection to the inversion increases with the increase of crossover distance between them (Càceres et al. 1999; Schaeffer et al. 2003; Singh and Singh 2007b, c). Olvera et al (1979) examined the distribution of 34 naturally occurring inversions in a single chromosome (the third) of *D. pseudoobscura* and concluded that the wider the distribution and higher the frequency, the more successful the inversion. Krimbas and Loukas (1980) examined complex inversion combination in *D. subobscura* and concluded that such combinations will protect the chromosomes from recombination as heterozygous inversions are largely crossover suppressors, and this property increases from the single inversion to the complicated inversion combinations. Van Valen (1961) while working on a spontaneously appeared new inversion in a population of *D. pseudoobscura* found both increasing and decreasing trend with latter following the former. He postulated that the exchange of alleles between the new inversion and the already existing gene arrangement causes the shifts in the fitness of a karyotypic. These laboratory-based studies elucidate the fact that an inversion can be retained in a population depending on its ability to combine

with the other chromosomes in the population and its tendency of being heterotic with other gene arrangements (Singh and Singh 2007c).

In the nutshell, it is safe to hypothesize that moderate-sized inversions have better fitness and selective advantage, which could be because of the trade-off between long and short inversions, whereas longer inversions can probably capture more favorable sets of alleles as they cover more of the genome, but at the same time, there might be the risk of losing them (favorable sets of alleles) on account of a double crossover. Small-sized inversions though have a low chance of capturing favorable set of alleles, but once they capture they can retain them more efficiently than do longer inversions (Krimbas and Powell 1992). Gene recombination plays an important role by producing gene combinations that confer rare permanent advantage to the species as happens in the case of *D. ananassae* with three cosmopolitan inversions. Inversion heterozygosity preserves the advantageous gene combinations by suppressing crossing-over, which, if it becomes part of its genetic structure, will be an asset to the career species. Sometimes gene recombination leads to disadvantageous gene combinations as well, which might serve to confer selective advantage in changed environmental condition and help in population adaptation (Singh and Singh 2007c).

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P. Singh, *Evolutionary Population Genetics of Drosophila ananassae*,
DOI 10.1007/978-81-322-2565-2