GENETIC STRUCTURE AND SPECIES DIVERGENCE BETWEEN POPULATIONS OF *Drosophila melanogaster* AND ITS SIBLING SPECIES, *Drosophila simulans*

By

MADHUSUDAN CHOUDHARY, B.Sc. (Hons.),M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree Doctor of Philosophy

GENETIC STRUCTURE AND SPECIES DIVERGENCE IN NATURAL POPULATIONS OF *Drosophila*

DOCTOR OF PHILOSOPHY (1987) McMASTER UNIVERSITY
BIOLOGY

TITLE: Genetic Structure and Species Divergence Between Populations of Drosophila melanogaster and Its Sibling Species, Drosophila simulans

AUTHOR: Madhusudan Choudhary, B.Sc. (Hons), M.Sc. (Patna University)

SUPERVISOR: Professor Rama S. Singh

NUMBER OF PAGES: xv, 214

ABSTRACT

An essential element in the study of evolution is the knowledge about the origin and dynamics of genetic variation within and between populations, and between species. It is for this reason that the experimental population genetic studies always center around the characterization of genetic variation in natural populations. There are two opposing hypotheses about the nature of genetic variation and its role in evolution and speciation. The balanced hypothesis of genetic structure maintains that there is a large amount of genetic variation in natural populations and this variation is maintained by natural selection. The neutral hypothesis agrees with the balance hypothesis with respect to the amount of genetic variation but disagrees with respect to its role in evolution and speciation. The neutral hypothesis assumes that most of the variants are selectively neutral and their fate is governed by balance between neutral mutation and random genetic drift.

The melanogaster subgroup of Drosophila, comprising of eight closely related species, has provided unique materials for studies of evolution. Presently, there is an increasing amount of interest in pursuing molecular evolutionary studies with species of this subgroup. In the past D. melanogaster and its sibling species, D. simulans have been extensively studied for their genetics, cytology, ecology and behaviour. These two sibling species have also been extensively studied for gene-enzyme variation.

However, all previous studies have sampled approximately between 10-30 gene loci and also these studies have emphasized mainly enzymes; very little work has been done with non-enzymatic proteins. The experimental approach taken in this thesis was to score structurally distinguishable gene products by gel electrophoresis within species, and compare the identity of variants at homologous gene loci between species. Over a hundred gene loci representing both enzymes and non-enzymatic proteins were sampled.

The natural populations of Drosophila melanogaster and Drosophila simulans were compared for their geographic structure and genic divergence. A total of 114 gene-protein loci were studied in four mainland (from Europe and Africa) and an island (Seychelle) populations of D. simulans and the results were compared with those obtained on the same set of homologous loci in fifteen worldwide populations of D. melanogaster (Singh and Rhomberg, 1987b). The main results are as follows: (1) D. melanogaster shows a significantly higher proportion of loci polymorphic than D. simulans (52% vs 39%, $p < 0.05$), (2) both species have similar mean heterozygosity and mean number of alleles per locus, (3) the two species share some highly polymorphic loci but they do not share loci that show high geographic differentiation, and (4) D. simulans shows significantly less geographic differentiation than D. melanogaster. The differences in geographic differentiation between the two species are limited to loci located on the X and second chromosomes; loci on the third chromosome show similar level of geographic differentiation in both species. Variation in niche-widths and/or genetic "strategies" of

adaptation appear to be the main causes of differences in the genetic structure of these two species.

The comparison of genetic divergence between species produced the following results: (1) The polymorphic loci between D. melanogaster and D. simulans are significantly correlated, i.e., if a locus is polymorphic in one species, it is likely to be polymorphic in the other species also. (2) The various chromosomes show similar proportion of unique alleles within species but differ between species; D. melanogaster shows more unique alleles than D. simulans. (3) All chromosomes show similar proportions of shared alleles and similar genetic identities between species. (4) The loci that are diverged within species, are not the one that are diverged between species, suggesting no role of population structure to the species divergence. (5) While the present estimate of mean genetic distance, D = 0.179, between D. melanogaster and D. simulans is lower than previously reported values, the proportion of loci showing complete divergence between the two species is higher (10%) than all previously reported values.

These results suggest that possibly many genes are involved in species formation but the question remains whether the generalized enzyme loci sampled in the present as well as in past studies have much relevance to the problem of reproductive isolation and speciation. It is proposed that different kinds of genes or genetic systems may underlie adaptation and speciation, and that genetic and molecular analysis of reproductive characters (e.g. male-female genetalia and reproductive behaviours) would shed more light on the nature of genetic variation for speciation.

ACKNOWLEDGEMENTS

I wish to express my deep gratitude and sincere thanks to my supervisor, Prof. Rama. S. Singh, for his advice and encouragement through the course of this work. Sincere thanks are also due to Profs. R. A. Morton, S. F. H. Threlkeld, T. T. Chen, for many critical and helpful suggestions.

Many thanks must go to my friends, Dr. M. B. Coulthart, Shanta Thomas, Larry Hale, and Bill McMillan, who made my stay in McMaster enjoyable. Without them I would have missed much of scientific discussions. Also, I greatly appreciate the kindness and help of Shanta who taught me a great deal of electrophoretic technique.

Finally, the support and patience of my wife Abha and daughters, Nivedita and Nichiketa were essential to the successful completion of this thesis.

This research work was supported by the Natural Sciences and Engineering Research Council of Canada, through a research grant to Dr. R. S. Singh.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1

INTRODUCTION

One of the unifying concepts of biology is that of the continuity of life through heredity and evolution. Living beings are organized in a hierarchical fashion (molecular, tissue, organism, population, species, community, etc.) and there are complex interactions operating within and between these levels. Furthermore, there is an interaction between organisms and the environment in which they live. These interactions are the basis of organization and evolution of biological systems. Evolution of populations together with origin and evolution of new species comprise the field of population genetics. The characterization of genetic variation within and among populations and the study of natural forces that affect the level and pattern of genetic variation constitute the basic goals of experimental population genetics. In the course of this chapter I will briefly outline the historical developments in evolutionary biology and describe some of the basic paradigms of organic evolution and their formulations in the light of the current state of knowledge. Subsequently the ideas behind the present research will be introduced in the framework of model

themes of evolutionary process and at the end of this chapter the rationale of the present research will be presented.

1.0 Historical Overview:

1.1 Darwin's Theory of Natural Selection: The Conflict Between Mendelians and Naturalists

Charles Darwin's theory of natural selection is central to evolutionary biology. In the "Origin of Species", Darwin (1859) established two things. First, he provided evidence that evolution had in fact occurred (i.e., that all existing forms of life have evolved by a series of changes from a few simple pre-existing forms). Second, he showed that natural selection, acting on randomly occurring slight variations, was the main cause of evolution. Darwin summarized his theory in these words (Darwin, 1859):

> "As many more individuals of each species are born than possibly survive; and as, consequently, there is frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable to itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving and thus be naturally selected. From the strong principle of inheritance, any selected variety will tend to propagate its new and modified form."

Since Darwin proposed his theory of organic evolution by means of natural selection, the whole field

has gone through a series of developments. Additional evidence accumulated after 1859, by Darwin himself in later years and by numerous other naturalists, greatly strengthened the theory of Darwinian selection. These studies have shown that species and varieties in nature possess a large amount of slight variations among individuals. It has also been repeatedly shown that when a character is artificially selected, the population mean can be shifted to a new stable level beyond the original limits of variation. These facts further support the hypothesis that natural selection acts by accumulating slight, successive, and favourable variations. On one hand Darwin's view of gradual and continuous evolution by natural selection was widely supported while on the other it was also severely criticised because there did not exist a consistent theory of heredity to account for the origin of variations on which selection must act. Although Mendel's brilliant work on discrete inheritance was completed about at the time Darwin published his theory, it was unnoticed until rediscovered independently by three scientists, Erich von Tschermark, Carl Correns and Hugo de Vries at the early part of this century.

During 1900-1930 biology was seen with strong conflicts concerning the nature of hereditary variation, the process of evolution and their interrelationship. One

problem concerning the nature of hereditary variations was resolved by the careful research of Nilsson-Ehle, East, Castle, and many others (reviewed by Dunn, 1965) who showed that inheritance can be particulate at the level of the gene but blending or non-blending at the level of the phenotype. However, the role of natural selection in the evolutionary process remained controversial. There were two major groups, Mendelians and naturalists (especially biometricians) opposed to each other. Naturalists believed that evolution proceeded by natural selection acting upon small variations while Mendelians supported the mutation theory (De Vries, 1905) and believed in discontinuous evolution. Genetic discoveries during this period such as the chromosome theory of heredity, polygenic inheritance, pleiotropy and epistatic interactions of genes were interpreted as being antagonistic to Darwinian selection. This conflict was primarily due to opposing views of the two groups, their philosophical attitudes and training rather than alternate interpretations of data. The resolution of this conflict and the synthesis of a coherent theory of evolution which takes into account all pertinent facts of modern biology, has been the work of many biologists during the first fifty years of this century. Leaders among them were R. A. Fisher, J. B. S. Haldane, and Sewall Wright who synthesized the elements of evolutionary

theory. They combined biometric methods, Mendelian inheritance, selection, and systems of mating into quantitative models of the evolutionary process (Pronine, 1977). They showed that continuous, quantitative phenotypic variation could be explained by alternate alleles at many polymorphic loci, and that evolution must depend on changes in the allele frequencies at polymorphic loci in the population. They further argued that evolutionary changes could not be brought by mutation alone, with selection acting only to remove inharmonious gene combinations, but did depend upon the continuous action of natural selection.

1.2 The Modern Synthesis: The Synthesis of Mendelism and Darwinism, and The Emergence of Population Genetics

The work of population genetics prepared the way for "The Modern Synthesis" of evolution biology, developed in the period 1920-1950 by a group of scientists, including Th. Dobzhansky, E. B. Ford, Julian Huxley, E. Mayr, H. J. Muller, B. Rench, G. G. Simpson and G. L. Stebbins. This period is notable for the fusion of Mendelian genetics with mathematical theories of natural selection, systematics and palentology. "The Modern Synthesis", as this phrase was originally termed by Huxley (1942), had the following

characteristics. (i) Gene mutations and their recombinations in sexually breeding population are the ultimate source of genetic variation upon which natural selection acts to cause evolution. (ii) Gradual evolution occurs due to accumulation of small genetic changes in the gene pool, which are constantly under natural selection. (iii) Although migration (gene flow), random genetic drift and deviations from random mating (inbreeding, assortative mating, selfing, etc.) do change the distribution of genotypes in populations they play a minor role in the evolutionary process. (iv) The evolutionary process is not a hierarchical process. The observed macroevolutionary processes, such as speciation, divergence between species and higher taxa can be explained in the same manner as it works within population (Mayr, 1942; Mayr and Provine, 1980).

The Darwinian view is that evolution transforms variation between individuals into variation between populations and species. An essential element in the study of evolution is therefore knowledge about the origin and dynamics of genetic variation within and between populations, and between species. Population genetics sets a much more modest goal (Dobzhansky, 1951, 1970) than general evolutionary theory which Lewontin (1974) described in these words:

> "The subtle changes in cell physiology, developmental processes, behaviour, and morphology that lead to reproductive isolation and ecological differentiation are the observables, but presently the only variables for which we can construct a dynamic theory of evolution are the frequency distribution of genes and genotypes. The sufficient set of state variables for describing an evolutionary process within a population must include some information about the statistical distribution of genotypic frequencies. It is for this reason that the experimental study of population genetics has always begun with and centered around the characterization of genetic variation in populations."

It has long been realized that the evolutionary potential of a population is largely a function of the amount of genetic variation present in a population. Therefore, how much genetic variation exists within populations is of fundamental importance for understanding the evolutionary process.

1.3 Two Models of Population Structure: Classical vs. Balance

In the past many studies addressed the question of the genetic structure of species. Two general models of the genetic structure of populations have been proposed, and are referred to as the "classical" and "balance" hypotheses (Dobzhansky, 1955). The classical model proposed that a typical individual would be homozygous for the wild type allele at most of its gene loci (Muller,

1927, 1950), however, at a very small proportion of its loci the individual would be heterozygous. According to this model, mutant alleles are continuously introduced in the population by mutation, but are generally deleterious to the organism and are therefore gradually removed from the population by natural selection. Occasionally an advantageous mutation arises, conferring higher fitness upon its carrier than the pre-existing wild-type allele. This beneficial allele would gradually increase in frequency as a result of natural selection to become the new wild-type allele, while the former wild-type allele would be eliminated from the population. In contrast to the classical model, the balance model proposes that most individuals are heterozygous for nearly all gene loci. Genic polymorphisms are maintained in population by various forms of balancing selection such as heterosis, frequency dependent selection, etc. (See reviewed by Dobzhansky, 1970; Ford, 1971). Under balancing selection two or more alleles (or chromosomal variants) are conserved in a population at stable equilibrium frequencies. Several forms of balancing selection such as heterosis (Cavallissforza and Bodmer, 1971; Lewontin, 1974), frequency dependent selection (see reviews by Allard and Adams, 1969; Wright, 1969; Kojima, 1971; Ayala and Campbell, 1974), etc., can maintain genetic variation in

natural populations. The balanced model gained support from the experimental observation of large amount of heterozygosity in many populations. A few classical studies, for example the sickle-cell trait, show it is possible for balancing selection to maintain polymorphism. The controversy at present is over what proportion of gene loci are under balancing selection and what proportion are under the purifying selection.

1.4 Balancing Selection vs. Neutrality Hypothesis: An Ongoing Controversy

The early studies of genetic variation in natural populations concentrated on easily detected variation, such as morphological variants (Ford, 1940; Spencer, 1947; for review see Lewontin, 1974), chromosomal inversions Dobzhansky and Spassky, 1953; 1954), or blood groups (Landsteiner and Weiner, 1940). Although important variants as case studies, these did not provide an estimate of the total amount of genetic variation in the genome of the populations studied. This is due to the fact that (i) these variants were not atypical of the majority of loci in the genome, (ii) not every locus produces phenotypic variants, and (iii) most phenotypic traits are polygenic.

Detailed analysis of protein variation did not begin until electrophoresis was introduced to population

genetics in the mid 1960s (Hubby and Lewontin, 1966; Harris, 1966; Markert and Moller, 1959). Since then genetic variation at the protein level has been analysed in natural populations of hundreds of different species (Lewontin, 1974; Powell, 1975; Ayala, 1976; Nevo, 1978, 1984; Selander, 1976; Gottlieb, 1981; Nei and Roychoudhury, 1982). All populations have been shown to be polymorphic for a large number of protein specifying genes. Most biologists now agree that natural populations possess large amounts of genic variation but disagree on the role of this ubiquitous variation in adaptation and evolution. Furthermore, the mechanisms by which genetic variations are maintained have become the source of an ongoing debate among population geneticists (Ayala, 1976; Lewontin, 1974; Nei, 1975; Kimura and Ohta, 1971; Kimura, 1983).

The balance theory has remained a dominant force in evolutionary biology. In this theory, although mutation is regarded as the ultimate source of genetic variation, natural selection plays the creative role in shaping the direction of evolution (Mayr, 1963; Dobzhansky, 1970). Application of molecular and recombinant DNA technologies has produced new kinds of data on genetic variation. The two important points have emerged from molecular biological data: (i) there is a large amount of genetic polymorphism at both the protein and DNA levels in many natural

populations and (ii) there appears to be approximate constancy of the rate of amino acid substitution in each protein (Zuckerkandl and Pauling, 1965; Wilson et. al., 1977). These facts led Kimura (1968) and King and Jukes (1969) to propose the neutral theory. The neutral theory states that random fixation of mutations is the mechanism for maintaining most genetic variation at molecular level (Ohta, 1973; 1974). For these mutant alleles, selection plays a minor role in determining their frequency change. In other words, they are neutral with respect to each other (or functionally equivalent). Nei (1975, 1980) suggested further that mutation is the primary force of evolution even for morphological and physiological characters.

1.5 Divergence Between Species: Theories of Macro-evolution

There are two conceptually contrasting views of species formation which differ in their assumptions about (i) the numbers and kinds of gene mutations that underlie species differentiation, and (ii) the relative role of natural selection and random genetic drift during species formation. Ever since there has been a concept of evolution, there has been the problem how the species arise. Darwin (1859) described this phenomenon in these words:

> "Without variability, nothing can be effected: slight individual differences, however, suffice for the work and are probably the chief or sole means in the production of new species."

In other words, the formation of species is purely quantitative accumulation of genetic change. If one acumulates enough small differences, one would eventually get something that is qualitatively different, an evolutionary novelty. Mayr claims that "all the processes and phenomena of macroevolution and the origin of higher categories can be traced back to intraspecific variation, even though the first steps of such processes are usually very minute". (Mayr, 1942, p. 298).

Mayr in his geographic theory of speciation proposed that speciation starts in a small, peripheral isolated population. Because of inbreeding and random genetic drift, many alleles will lose the advantage of being part of a balanced system and will be selected against. However, such genetically unbalanced populations may be ideally suited to occupy a new niche. The genetic reorganization might be sufficiently rapid which was called by Mayr "genetic revolution" (See Barton and Charlesworth, 1984).

An alternate and opposed view of species formation stated that species appear abruptly and natural selection does not play an important role in guiding the course of

evolution beyond the species level (De Vries, 1905; Goldschmidt, 1940). Recently a modification, "the punctuated equilibrium theory" was proposed (Eldredge and Gould, 1972; Gould, 1977, 1980; Stanley, 1975, 1979; Gould and Eldredge, 1977), having the same basic formulation and features as previously proposed by Goldschmidt. According to this theory evolution is a hierarchical process with complementary, but different, modes of change at its three major levels: variation within populations, speciation, and pattern of macroevolution. They argued that species arise by a process of macromutations rather than substitutions (micromutations) of alleles already present in the population.

2.0 A Study of Closely Related Species: D. melanogaster and D. simulans

2.1 Comparison of Genetic Structure:

A comparison of genetic structure between species can provide information about the relative importance of various evolutionary forces, such as, migration, natural selection and genetic drift, which shape the pattern of genetic variation within species, and about the genetic mechanism or "strategies" of adaptation that are employed to deal with variation in the physical and biotic environments. The causes of gene and protein variation in

natural populations have been analysed in two ways. One approach relies on a detailed biochemical and physiological understanding of genotypic fitness at polymorphic loci (e.g. McDonald, Anderson and Santos, 1980; Hickey, 1977; Eanes, 1984; Richmond et al., 1980; Hilbish and Koehn, 1985; Dykhuizen and Hartl, 1983; Hartl and Dykhuizen, 1981). The second, indirect, approach is based on the correlation between the amount of genetic variation and the life history characteristics of the organism (for a review see Nevo et al., 1984). For a variety of reasons, however, evidence of natural selection based on correlation studies is generally questionable. Many life history parameters affect genetic variation indirectly via their effects on population size. Therefore comparisons of genetic structure are usually done between closely related species (e.g., see Hubby and Throckmorton, 1968; Ayala and Powell, 1972; Lakovaara, Saura and Falt, 1972; Prakash, 1977; Eisses, Van Dijk and Van Delden, 1979; Gonzalez et al., 1982; Ohnishi, Kawanishi and Watanabe, 1983), and if such species are similar in their geographic and climatic distribution, so much the better as this reduces the number of variables to be examined and facilitates the comparison.

The sibling species D. melanogaster and D. simulans are such a species pair. They are the two better known species of the melanogaster subgroup; they are cosmopolitan

(Bock, 1980) and sufficiently versatile to adapt to both temperate and tropical regions (Parsons, 1975; 1983). Their similar geographical distribution increases the probability of similar genetic mechanisms of adaptation. A question of evolutionary significance is whether or not these sibling species have used the same genetic strategies in adapting to their varied environments. This question can be approached by examining patterns of genetic variation over their geographic and climatic distributions.

The above question has been the focus of attention in a number of comparative studies on genetics, morphology, ecology and behaviour of these species (for reviews, see Parsons, 1975; 1983; Parsons and Stanley, 1980). Recent studies have shown that the two species differ in the overall level of genetic variation within populations, and geographic differentiation between populations. For example, there is a striking difference in the degree of chromosomal polymorphism between D. melanogaster and D. simulans. Whereas D. melanogaster contains several chromosomal inversion polymorphisms (Stalker, 1976; Ashburner and Lemeunier, 1976; Mettler, Voelker and Mukai, 1977; Knibb, Oakeshott and Gibson, 1981), D. simulans shows no inversion polymorphism (Ashburner and Lemeunier, 1976). D. simulans has been shown to be substantially less polymorphic than D. melanogaster for allozymes

(O'Brien and MacIntyre, 1969; Berger, 1970; Kojimā, Gillespie and Tobari, 1970; Triantaphyllidis, 1973; Steiner, Sung and Paik, 1976; Triantaphyllidis et al., 1980; 1982; Cabrera et al., 1982; Hyytia et al., 1985; Watada, Tobari and Ohba, 1986; Singh, Choudhary and David, 1987) and mitochondrial DNA (Baba-Aissa and Solignac, 1984; Hale and Singh, 1985). Taken together, these studies show that D. simulans has less genetic variation within populations and less genetic differentiation between populations than D. melanogaster.

One reason for the differences in the genetic structure of these species may simply be that relatively fewer populations and genes have been studied in D. simulans than in D. melanogaster. In fact, as the number of populations and genes studied in D. simulans has increased, the difference in heterozygosity between the two species has decreased. Also, previous studies were limited with respect to the kind of loci sampled (Kojimā, Gillespie, and Tobari, 1970). Several aspects of enzymatic structure and function have been suggested as major determinants of the between-locus pattern of genetic variation (Kojima, 1968; Kojima, Gillespie, and Tobari, 1970; Johnson, 1974; Harris, Hopkinson, and Edwards, 1977; and Koehn and Eanes, 1978). Kojima, Gillespie, and Tobari (1970) found that the enzymes involved in glycolysis were

less variable than others, but in such comparisons, the enzymes from the former group were overrepresented in the sample relative to proportion of all enzymes known in metabolism. Therefore, a diverse sample of loci, not weighted heavily by one or two enzymatic functions, are required to produce meaningful comparison between populations and species. Indeed, in view of the large interlocus variance of heterozygosity observed in most organisms (Fuerst, Chakraborty and Nei, 1977), it is important not only to sample a large number of loci but also preferably the same set of homologous loci should be studied in closely related species.

2.2 Genetic Divergence Between D. Melanogaster and D. Simulans:

In the past, most analyses of genetic differences between species have used morphological characters. More often there appear to be many, but unknown number of gene differences involved in these characters. A second direction has been to examine the genetic basis of male sterility in hybrids between species. By means of marker chromosomes, Dobzhansky (1936, 1951) was able to show that there are at least two genes on each of the large chromosomes influencing testis size in the hybrid males of Drosophila pseudoobscura and Drosophila persimilis, and

that an interaction between the sex chromosome of one species and the autosomes of the other was a predominant effect. Similar finding has recently been reported for the hybrid males from the interspecific crosses between Drosophila simulans and Drosophila mauritiana (Coyne, 1984; Coyne and Kreitman, 1986; Coyne and Charlesworth, 1986). However, these studies have shown that the male sterility in the hybrids is due to many genes, with X-linked loci making the largest contribution to sterility. Also they showed that there was an interaction between X and Y rather than X and autosome as reported earlier (Haldane, 1922; Dobzhansky, 1936).

A new way of looking at the genetic differences between closely related species was begun at the same time the study of protein polymorphism was introduced to analyze genetic variation among populations. In the last two decades, the wide spread application of gel electrophoresis has made it possible to quantify the amount of genetic divergence between closely related species and races (see review Ayala, 1975; Throckmorton, 1977; Avise and Aquadro, 1982; Nei and Roychoudhury, 1982). The first systematic application of gel electrophoresis to the problem of species formation was made by Hubby and Throckmorton (1965). Their study of proteins and enzymes in 9 triads of Drosophila species (two members of each triad being sibling

and the third a non sibling member of the same species group) showed that sibling species differed on the average at about 50% of their loci and the minimum divergence between sibling species was about 16% (Hubby and Throckmorton, 1968). Since then similar studies of genetic divergence between closely related species have been carried out in various species groups of the genus Drosophila (Prakash, 1977; Richmond, 1972; Singh, 1983; Ayala et. al., 1974; Eisses et al., 1979; Gonzalez et al., 1982; Zouros, 1973; Lakovaara, Saura and Falk, 1972). All these studies generally showed very low level of genetic divergence between closely related species. In studies with sibling species there was generally an absence of monomorphic loci which were fixed for alternate alleles (see reviews Lewontin, 1974; Ayala, 1975). The limited extent of genetic differentiation between fully formed species was contrary to the expectation of large genetic differentiation postulated in Mayr's theory of geographic speciation (Mayr, 1954, 1963), which suggested a "genetic revolution" during species formation.

The concept of a genetic revolution originated from the assumption that every gene affects every character because of a pleiotropic effect, that "no gene frequency can be changed, nor any gene added to the gene pool, without an effect on the genotype as a whole, and thus

indirectly on the selective value of other genes" (Mayr, 1963, pp. 269). Although Mayr proposed a genetic revolution during speciation, no quantitative limits can be defined without data for genetic differences between populations at various stages of phenotypic divergence. Since it is almost impossible to determine the proportion of genetic differentiation accompanying species formation, most studies on the genetic basis of species formation have been concerned with enummeration of gene and genotypic frequencies between species that have long been isolated. On the basis of the limited genetic differentiation ($D = 0.230 \pm 0.016$) between various pairs of sub and semi-species in Drosophila willistoni group (Ayala, 1975), it was argued that differentiation during the early stages of speciation must even be smaller than 23%. The general lack of large genetic differentiation among sibling, semi-, and subspecies has provided strong evidence for the hypothesis (Hubby and Throckmorton, 1968) that relatively little genetic differentiation is required in species formation.

It can be argued that previous studies on genetic divergence between species were limited in two respects. First, over 80% of all species examined so far have been sampled for less than 30 gene loci (Nevo, 1984). In view of the fact that the Drosophilia genome has a minimum of about 5000 genes (Judd, Shen and Kaufman, 1972), 30 genes

can hardly be seen as an adequate sample of the total genome. Secondly, many of these studies, especially the later ones, were based on a non-random sample of loci, e.g. loci that were already known to be polymorphic were more likely to be included in the study. Finally, loci which code for enzymatic proteins were over represented in the sample and very few species have been studied for non-enzymatic proteins. It would thus be desirable that a proper analysis of genic differences between closely related species should employ at least 100 genes representing, preferably, a variety of functions.

3.0 Rationale of the Present Research:

The Melanogaster subgroup is comprised of eight closely related species that provide unique materials for evolutionary studies. In the past D. melanogaster and D. simulans have been extensively compared in their morphology, genetics, ecology, and behaviour (Parsons, 1975, 1983). Recently, there has been an increasing interest in pursuing molecular studies with this subgroup of species. Natural populations of D. melanogaster have been examined for nuclear DNA variation (Kreitman, 1983; Aquadro et al., 1986) and mitochondrial DNA restriction fragment length polymorphism (Baba-Aissa and Solignac, 1984; Hale and Singh, 1987). The molecular phylogeny of

these four species based on the DNA sequence comparison of the Alcohol dehydrogenase locus (Bodmer and Ashburner, 1984; Cohn, Thompson and Moore, 1984; Coyne and Kreitman, 1986; Stephan and Nei, 1985) and is consistent with those based on the polytene chromosome banding patterns (Lemeunier and Ashburner, 1976) and electrophoretic protein polymorphisms (Eisses et al., 1979; Gonzalez et al., 1982). This subgroup contains two island endemic species (D. sechellia and D. mauritiana) that may have resulted from founder events. This is why all the four species in the "melanogaster complex" have recently been studied for their reproductive relationships (Coyne, 1983; 1984; 1985; Coyne and Kreitman, 1986; Coyne and Charlesworth, 1986; Lachaise et al., 1986). The present research is concerned with characteristion of genetic variation within and between populations of D. melanogaster and D. simulans. Emphasis is on elucidating the relative importance of the various evolutionary forces that shape the pattern of genetic variation within species and the nature of genetic variation that is the basis of reproductive isolation between species.

The experimental approach taken in this research project was designed to yield relevant data both within-species genic variation and between-species genic divergence. The basic approach was to score structurally

distinguishable protein molecules coded by different alleles of a gene by gel electrophoresis. This technique separates protein variants based on their conformational and charge characteristics. Since one criterion of gel electrophoresis may not detect all the allelic variants segregating at polymorphic loci, and since with exception to few loci, i.e., 6-phosphate gluconate dehydrogenase (Coyne, et. al., 1979); Glucose-6-phosphate dehydrogenase (Eanes, 1983); Xanthine dehydrogenase (Buchanan and Johnson, 1983) and Alcohol dehydrogenase (Kreitman, 1980), very little information exists about the level of hidden variation in this group of species, sequential gel electrophoresis (Coyne, 1977; Singh, Lewontin, and Felton, 1976; Coyne, 1982) was employed to make sure that the total genetic variation was being detected. In the present study a total of 114 protein loci were examined in D. simulans and these results were compared to the studies of D. melanogaster. The details of the data in D. melanogaster have been published (Singh, Hickey, and David, 1982; Singh and Coulthart, 1982; Singh and Rhomberg, 1987a, b).

2

MATERIALS AND METHODS

2.1 Drosophila Stocks

The strains of Drosophila species used in this study are given in Table 2.1. The details about their origin and the sources from which they were obtained are also mentioned.

2.2 Preparation and Maintenance of Drosophila Culture

All the strains were maintained as isofemale lines (i.e. each strain was established from a single, wild caught inseminated female) at 20 ± 1°C, with a diurnal photic cycle of 12 hr. light and 12 hr. dark. Stocks were grown in 25 x 95 mm glass vials on standard cornmeal medium. The receipe of the cornmeal culture medium used is described in Table 2.2. Density was maintained at approximately 20-25 flies per vial. Stocks were subcultured into fresh vials at every 15 days of interval. The old vials were heated for 45 minutes at 65°C, and then washed for reuse.

2.3 Crossing Scheme for the Study of Hidden Genetic Variation

TABLE 2.1

Stocks of *Drosophila* used in this study.

Species/Populations/Lines	Place/Latitude	Time	Source
D. simulans			
France (55 lines)	Porquerolles, France (43°N)	1983	Dr. J.R. David Laboratoire Biologie de, CNRS, France
Tunisia (52 lines)	Nasrallah, Tunisia (35.6°N)	1983	J.R. David
Congo (45 lines)	Brazzaville, Congo (4.3°S)	1983	J.R. David
Cape Town (32 lines)	Cape Town, South Africa (34°S)	1983	J.R. David
Seychelles (26 lines)	Mahe, Seychelles Islands (3.8°S)	1986	J.R. David
D. melanogaster			
France (17 lines)	Villeurbanne, France (46.1°N)	1978	J.R. David
Benin (22 lines)	Benin, West Africa (6.3°N)	1978	J.R. David
Hamilton (28 lines)	Dalewood, Hamilton, Canada (43.3°N)	1977	Dr. R.A Morton Dept. of Biology McMaster Univ. Hamilton, Ont. Canada
Texas (17 lines)	Brownsville, Texas, USA (25.8°N)	1978	Dr. D. Hickey Univ. of Ottawa, Ont. Canada.

TABLE 2.2

Composition of Cornmeal Culture Medium

Yellow Cornmeal	60 g
Dried brewer's yeast	30 g
Agar	10 g
Malt powder	15 g
Sugar	15 g
Corn syrup	10 mL
Water	1 L
Tegosept solution (10 g p-hydroxy benzoic acid, methyl ester + 100 mL 95% ethanol)	24 mL

Keep the mixture boiling and continue stirring for about 5 minutes until the mixture is viscous; cool to 70°C; add Tegosept solution; pour immediately into sterilized culture vials (10 mL/vial) and cap with plugs; store in the cold room until ready to use.

Autosomal loci were studied using inbred or isogenic lines as described by Singh, Lewontin, and Felton (1976). The crossing scheme for studying hidden genetic variation of X-linked loci in D. melanogaster consisted of crossing a single male from each isofemale line to an attached-X female. All male flies resulting from such a cross had the same wild X chromosome and were used for sequential electrophoretic survey.

2.4 Biochemicals and Reagents

The sources of chemicals used in this study are listed in Table 2.3. All reagents used were of reagent grade unless otherwise specified.

2.5 Sample Preparation

2.5.1 Solutions

The extraction buffer used was the same as the electrode buffer (see Section 2.5.3.1) except that when Tris-citrate electrode buffer was used, 0.05 M KPo_4, pH 7.4 was used as extraction buffer. The extraction buffer contained 5% sucrose except for mitochondrial enzymes, where 1% triton-X was also included.

0.05M KPO_4, pH 7.4 extraction buffer

(i)	K_2HPO_4	0.871 g
	Water (glass double distilled)	100 mL

TABLE 2.3

Chemicals Used and their Source

Chemical Name	Abreviation	Source[a]
Acetic Acid (glacial)	--	1
Acetone (50%)	--	
Acetyl thiocholine iodide	--	4
Cis-Aconitic Acid	--	4
Acrylamide (Cyanogum)	--	
Adenosine Diphosphate	ADP	4
Adenosine Triphosphate	ATP	4
Agar	--	
Agarose	--	4
L-Alanine	Ala	4
L-Amino acid oxidase	--	4
Ammonium persulphate	AP	2
L-Arginine	Arg	4
L-Asparatic acid	Asp	4
Boric Acid	--	2
Bromophenol Blue	BPB	4
Citric Acid (monohydrate)	--	2
Cuprous sulphate	$CuSO_4$	2
Coomassie-Blue R	--	4
O-Dianosidine	--	4
2,6-Dichlorophenol-Indophenol	DCIP	4
Diethyl ether	--	1

TABLE 2.3 (Continued)

Chemical Name	Abreviation	Source[a]
DL-AE-3, 4-Dihydroxyphenylalanine	DL-DOPA	4
3,5 Dinitro salicylic acid	--	4
DL-Dithiothreitol	DTT	4
3-[4,5-Dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide	MTT	4
Ethanol	EtoH	
Ethylene diamine tetraacetic acid (Disodium salt)	EDTA	2
Ethylene glycol	--	2
Fast Black K	--	4
Fast Blue BB salt	--	4
Fast Blue RR salt	--	4
Fast Red TR salt	--	4
Ferric Chloride	$FeCl_3$	2
Formaldehyde (37%)	--	3
Formaline (10%)	--	
Fructose 1,6 diphosphate (trisodium salt)	--	4
Fructose 6 phosphate (Barium salt)	--	4
Fumaric acid	--	4
Gluconic acid	--	4
D-Glucose	--	2
Glucose-1-phosphate	--	4

TABLE 2.3 (Continued)

Chemical Name	Abreviation	Source[a]
Glucose-6-phosphate (sodium salt)	--	4
Glucose-6-phosphate dehydrogenase	G-6PD	4
Glutamic acid (sodium salt)	--	4
Glyceraldehyde-3-phosphate dehydrogenase	G-3-PD	4
α-Glycerophosphate (sodium salt)	--	4
Glycine	Gly	4
Hexokinase	HEX	4
Hydrochloric acid	HCl	1
Hydrogen peroxide	H_2O_2	
P-Hydroxybenzoic acid, methyl ester	Tegosept	4
Hypoxanthine	--	4
Isocitrate dehydrogenase	IDH	4
Isocitric acid (trisodium salt)	--	4
Iodine	I_2	2
Isopropanol	--	2
L-Ketoglutaric Acid	--	4
Lactate dehydrogenase	LDH	4
L-leucine-AE naphthyl acid hydrochloride	--	4
Magnesium acetate	$MgCOOCH_3$	2

TABLE 2.3 (Continued)

Chemical Name	Abreviation	Source[a]
Magnesium chloride	$MgCl_2$	2
Malate dehydrogenase	MDH	4
Malic acid (sodium salt)	--	2
Manganese chloride	$MnCl_2$	2
Mannose-6-phosphate	--	4
Methanol	MeOH	
4-Methyl umbelliferyl acetate	--	4
4-Methyl umbelliferyl α-L fucoside	--	4
4-Methyl umbelliferyl AE-galactoside	--	4
4-Methyl umbelliferyl α-D-Glucopyranoside	--	4
4-Methyl umbelliferyl α, D-glucoside	--	4
4-Methyl umbelliferyl AE-D glucoronoside	--	4
4-Methyl umbelliferyl -AE-Mannopyranoside	--	4
Mercaptoacetic Acid	--	
α-Naphthyl Acetate	--	4
AE-Naphthyl Acetate	--	4
α-Naphthyl acid phosphate (sodium salt)	--	4
Nicotinamide adenine dinucleotide	NAD	4

TABLE 2.3 (Continued)

Chemical Name	Abreviation	Source[a]
NAD (reduced form)	NADH	4
Nicotinamide adenine dinucleotide phosphate	NADP	4
NADP (reduced form)	NADPH	4
Octyl alcohol	--	3
Peroxidase		
Phenazine methosulphate	PMS	4
Phosphoenol pyruvate	PEP	4
Phosphoglucose isomerase	PGI	4
6-Phosphogluconate (disodium salt)	--	4
Polyvinyl pyrrolidone	--	4
Potassium chloride	KCl	2
Potassium cyanide	KCN	2
Potassium ferricyanide	--	2
Potassium Iodide	KI	2
Potassium sodium tartarate	--	2
Potassium phosphate (monobasic)	KH_2PO_4	2
Potassim phosphate (dibasic)	K_2HPO_4	2
Pyrazol	--	4
Pyridoxil 5-phosphate	--	4
Pyruvic Acid (sodium salt)	--	4
Pyruvate kinase	--	4

TABLE 2.3 (Continued)

Chemical Name	Abreviation	Source[a]
Silver nitrate	$AgNO_3$	2
Sodium arsanate	--	2
Sodium carbonate (anhydrous)	Na_2CO_3	2
Sodium chloride	NaCl	2
Sodium hydroxide	NaOH	2
Sodium diethyl barbiturate	$NaC_8H_{11}N_2O_3$	3
Sodium nitrate	$NaNO_3$	2
Sorbitol	--	4
Starch	--	2
Succinic Acid	--	4
D-Trehalose (Dihydrate)	--	4
N,N,Nl,Nl-Tetramethyl Ethylenediamine	TEMED	4
Tris (Hydroxymethyl) aminomethane	Tris	4
2,2',5,5'-Tetraphenyl-3, 3'(3,3'-Dimethoxy 4,4'-Biphenylene) Ditetrazolium Chloride	Tetrazolium Blue	4

[a] Sources
1 J.T. Baker Chemical Company, Phillipsburg, New Jersey
2 BDH Chemicals, Toronto, Ontario
3 Fisher Scientific Co., Fairlawn, New Jersey
4 Sigma Chemical Co., St. Louis, Missouri

(ii) KH_2PO_4 0.680 g

Water 100 mL

Make solution (i) and (ii); take the pH of the monobasic solution; then add enough of the dibasic solution until the pH is 7.4.

2.5.2 Procedure

For protein survey, 2-3 day old (since eclosion) flies were collected after anaesthetization with diethyl ether for 2-5 minutes. Sample was prepared by grinding 10 flies in a 400 µL centrifuge tube containing 50 µL of extraction buffer. The crude extract was centrifuged at 16,000 xg for 5 minutes at 4°C (in cold room). The supernatant (10 - 15 µL) was used immediately for electrophoresis. Third instar larvae were used for LAP, APH and Coomassie-stained larval proteins; pupae for EST-10 and GOT; and adults for all other enzymes, Coomassie-stained adult proteins and silver stained proteins.

2.6 One Dimensional Polyacrylamide Gel Electrophoresis

2.6.1 Electrophoretic Apparatus

Allozyme variability was assayed by vertical polyacrylamide slab-gel electrophoresis, using Aardvark gel boxes (Robert and Jones, 1972). The apparatus is 27 cm long X 15 cm wide X 24 cm high, consists of two plexiglass boxes which serve as the anodal and cathodal compartments

and a U-shaped plexiglass spacer. The spacer was placed between the two chambers and the two boxes were clamped together with adjustable, stainless-steel clamps.

2.6.2 Preparation of Gels

All gels for surveying enzymes and coomassie-stained proteins contained 5% commercial grade acrylamide (Cyanogum). For ultrasensitive silver-stained proteins, 7.5% acrylamide (99.9%) was used.

The gel solution (5%) was prepared by dissolving 7.5g polyacrylamide (Cyanogum) thoroughly in 150 mL gel buffer at room temperature. While it was stirring, polymerization was initiated by the addition of 1 mL AP (10%) and 0.3 mL TEMED. After stirring briefly, the solution was immediately poured into the space between gel boxes. Care was taken to get rid of any air bubbles. A pocket former was placed directly into the top of the gel, and was left for 30 minutes to allow polymerization.

2.6.3. Electrode Buffer

0.1 M Tris-Borate EDTA, pH 8.9

Tris	9.825 g
Boric acid	0.765 g
EDTA	0.555 g
Water (distilled)	1 L

Mix them and bring to 1 L with distilled water

0.1 M Tris-Borate, pH 8.9	
Tris	9.825 g
Boric acid	0.765 g
Water (distilled)	1 L

0.02 Tris-Glycine, pH 8.5	
Tris	2 g
Glycine	5 g
Water (distilled)	1 L

0.05 M Tris-Citrate, pH 8.5	
Tris	6.05 g
Citric acid	0.94 g
Water (distilled)	1 L

0.02M Barbitol-acetate, pH 8.6	
Sodium Diethylbarbiturate	4 g
Water (distilled)	1 L

Dissolve sodium diethylbarbiturate in water; pH was adjusted with Acetic acid.

2.6.4 Electrophoretic Procedure

When the gel solution was polymerized, the electrode chambers were filled with electrode buffer to cover the top of the gel. Water channels were connected to the gel boxes which connect a water jacket built into both walls. The gel was cooled for at least half an hour by

running 15% ethylene glycol coolant. Temperature of the circulating coolant was maintained between 3 to 5°C, to remove heat generated during electrophoresis. The pocket former was carefully taken out and 10-15 µL of the samples were layered into the gel pockets with the help of a 100 µL glass syringe.

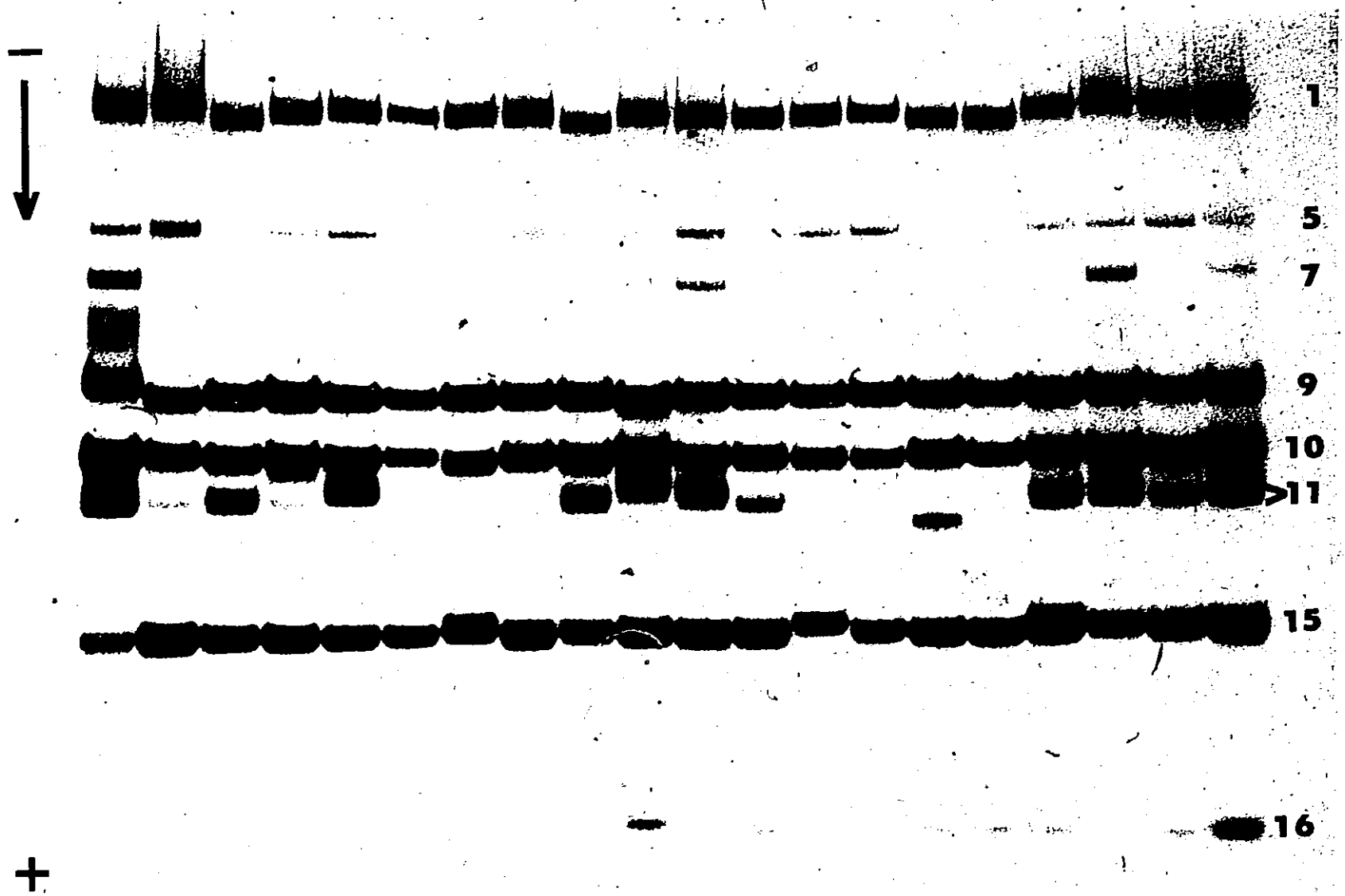
Electrophoresis was carried out at approximately 300-350 V and 50-60 mA. The separation was continued until the tracking dye (Bromopenol blue) had moved out of the gel. Electrophoresis time varied from enzyme to enzyme and were adjusted for optimum separation.

For the silver-stained proteins, electrophoretic separation was carried out following the method described by Orstein (1964) with minor modifications. 7.5% acrylamide gel of 0.75 mm thickness was cast between two glass plates of size 9-1/2" x 7-3/4". The gel was run in the cold room (4°C) for 3 hours at 10 mA.

2.7 Staining for Proteins

The gel was carefully cut out and then washed with distilled tap water in a pyrex glass tray. Except for hydroxyacid dehydrogenase (Cavener, 1980), phenol oxidase (Batterham and McKechnie, 1980), Trehalase and sucrase (Paterson and Hickey, 1982), sorbitol dehydrogenase (Bischoff, 1978), α -Glycerophosphate oxidase (O'Brian and

Figure 2.1 A slab gel, showing mobility variation for larval hemolymph proteins 1, 5, 7, 9, 10, 11, 15 and 16 in 20 isofemale lines of D. melanogaster.


−
+
1
5
7
9
10
11
15
16

MacIntyre, 1972), the staining methods were adapted from Harris and Hopkinson (1976) and Smith (1976). The stained gel was fixed in the fixative which contained Methanol: Acetic acid:Water (5:1:5). Gels were stored for long periods (for more than six months) in "Zip-loc" plastic food storage bags.

2.7.1 Coomassie Blue Staining for Proteins

For protein detection, the gels were placed in 100 mL of a solution of methanol:acetic acid:water (5:1:5) containing 0.01% Coomassie blue R. The gels were agitated by putting tray on the shaker and stained overnight until deeply stained protein bands appeared. Excess stain was then removed and the gel was destained by additional washing in the same solvent, without dye, until the background was clear. The picture of a slab gel, showing mobility variation for larval hemolymph proteins is shown in Figure 2.1.

2.7 Ultrasensitive Silver Straining of Proteins

The proteins were stained following the method developed by Merril, Duncan, and Goldman (1981) with modifications taken from Morrissey (1982). The sequence of steps is given in Table 2.4.

2.8 Sequential Electrophoresis

TABLE 2.4

Silver Staining Steps Employed in This Study

Step	Solution	Time	Comments
1. Fix	Methanol/Acetic Acid/Water (4:1:5)	1/2 an hour to overnight	Do not shake
2. Wash	10% Ethanol	1 hour	Shake
3. Rinse	Water (glass double distilled)		
4. Wash	10% Ethanol	30 minutes	Shake
5. Rinse	Water		
6. Reduce	0.50 mg DTT/100 mL water	30 minutes	Shake; no rinsing; pour DTT
7. Silver binding	0.1% $AgNO_3$	30 minutes	Shake
8. Wash	Water	10 seconds	Rinse rapid
9. Wash	Developer: 3% anhydrous Na_2CO_3+0.5 ml/L	10 seconds	Rinse twice rapidly
	37% formaldehyde		
10. Develop	Developer	10 minutes	Shake (till band appeared)
11. Stop	7.5 mL 2.3% anhydrous Citric acid/150 mL	5 to 30 minutes	Shake

We have examined 84 isofemale lines of D. melanogaster from four geographic locations listed in Table 2.1. A total of 11 allozyme loci were examined. These are Diaphorase-7 (Dia-7), Esterase-6 (Est-6), Est-C, Fumerase (Fum), Hexokinase-3 (Hex-3), Hydroxy acid dehydrogenase (Had), Larval protein-11 (Pt-11), Pt-15, Pt-16, Octanol dehydrogenase (Odh), and 6-phosphogluconate dehydrogenase (6 Pgd). The Est-6, Est-C, and Odh are autosomal and the remaining 8 loci are sex-linked. Electrophoresis was carried out on polyacrylamide slab gels following the standard method, except that two gel concentrations (5% and 8%) and two different pH's (Tris borate EDTA, pH 8.9) and Tris citrate, pH 6.9) were employed. Electrophoresis time was adjusted for optimum separation for each pH and gel concentration. Alleles were designated as described by Singh et al. (1976).

2.9 Criteria for Scoring the Protein Bands on the Gel

A total of 114 gene loci listed in Table 2.5 were studied, of which 79 code for enzymes and the remaining 35 for abundant proteins of unknown functions. Of the latter, 19 were stained by Coomassie Brilliant-Blue, and the others by ultra-sensitive silver stain.

The electrophoretic alleles were designated in the manner described by Singh, Hickey and David (1982). Two

TABLE 2.5

Enzymes, Their International Union of Biochemistry Numbers Genetic Map Position and Electrophoresis Buffers

Enzyme or Proteins	I.U.B. Code	Genetic Map Position[1]	Electro-phoresis Buffer[2]
Chromosome I:			
Fumerase (FUM)	(4.2.1.2)	1-19.9 (Fum)	A
Glucose-6-phosphate dehydrogenase (G-6PD)	(1.1.1.49)	1-63 (Zw)	C
Glutamate pyruvate transaminase (GPT)	(2.6.1.2)	1-42.6 (Gpt)	E
Hexokinase-3 (HEX-3)	(2.7.1.1)	1-29.9 (Hex-A)	A
Hydroxy acid dehydrogenase (HAD)	(1.1.1.45)	1-54.4 (Had)	B
Larval protein-11 (Pt-11)		1-39 (Lsp-1α)	A
6-Phosphogluconate dehydrogenase (6-PGD)	(1.1.1.43)	1-0.9 (Pgd)	A
Chromosome II:			
Alcohol dehydrogenase (ADH)	(1.1.1.1)	2-50.1 (Adh)	A
α-Glycerophosphate dehydrogenase (α-GPD)	(1.1.1.8)	2-20.5 (α-Gpdh)	A
Dipeptidase-3 (Dip-3)	(3.4.11)	2-55.2 (Dip-A)	C
β-Galactosidase (β-GAL)	(3.2.1.23)	2-20+ (β-Gal)	B

Table 2.5 (Cont'd.)

Glyceraldehyde-3-phosphate dehydrogenase (GAPD)	(1.2.1.12)	2-R (Gapdh)	C
Glutamate oxaloacetate transaminase-1 (GOT-1)	(2.6.1.1)	2-75.0 (Got-1)	B
Glutamate oxaloacetate transaminase-2 (GOT-2)	(2.6.1.1)	2-3.0 (Got-2)	B
α-Glycerophosphate oxidase (a-GPO)	(1.1.99.5)	2-75 (α-Gpo)	B
Hexokinase-1 (HEX-1)	(2.7.1.1)	2-73.5 (Hex-C)	A
Larval protein-10 (Pt-10)		2-1.9 (Lsp-1)	A
Malate dehydrogenase-1 (MDH-1)	(1.1.1.37)	2-37.0 (Mdh-1)	A
Phenol ixidase (PHOX)	(1.10.3.1)	2-80.6 (Phox)	A
Phosphoglucose isomerase (PGI)	(5.3.1.9)	2-58.6 (Pgi)	C
3-Phosphoglycerate kinase (PGK)	(2.7.2.3)	2-5.9 (3-Pgk)	A
Succinate dehydrogenase (SDH)	(1.3.99.1)	2-89 (Sdh)	B
Sucrase (SUCR)	(3.2.1.26)	2-L (Sucr)	D
Trehalase (TREH)	(3.2.1.28)	2-R (Treh)	D
Chromosome III:			
Acetylcholinesterase (ACE)	(3.1.1.7)	3-52.2 (Ace)	B
Acid phosphatase (ACPH)	(3.1.3.2)	3-101.3 (Acph-1)	C
Aldehydeoxidase (AO)	(1.2.1.3)	3.56.7 (Aldox)	A

Table 2.5 (Cont'd.)

Aldolase (ALD)	(4.1.2.13)	3-91.5 (Ald)	C
Alkaline phosphatase-larval (APH)	(3.1.3.1)	3-46.3 (Aph) larval	C
Arginine kinase (AK)	(2.7.3.3)	3-L (Ak)	A
Catalase (CAT)	(1.11.1.6)	3-L (Cat)	B
Dipeptidase-1 (DIP-1)	(3.4.11)	3-R (Dip-C)	C
Dipeptidase-2 (DIP-2)	(3.4.11)	3-53.6 (Dip-B)	C
Esterase-C (EST-C)	(3.1.1.1)	3-47.7 (Est-C)	B
Esterase-6 (EST-6)	(3.1.1.1)	3-36.8 (Est-6)	B
α-Fucosidase (α-FUC)	(3.2.1.51)	3-35.5 (α-Fuc)	B
Glucose oxidase (GO)	(1.1.3.4)	3-48.5 (Go)	B
β-Glucoronidase (β-GUS)	(3.2.1.31)	3-R (β-Glu)	B
Isocitrate dehydrogenase-1 (IDH-NADP)	(1.1.1.42)	3-27.1 (Idh-NADP)	C
Isocitrate dehydrogenase-2 (IDH-NAD)	(1.1.1.41)	3-25.4 (Idh-NAD)	C
Leucine aminopeptidase-A (LAP-A)	(3.4.11.1)	3-98.3 (Lap-A)	C
Leucine aminopeptidase-D (LAP-A)	(3.4.11.1)	3-98.3 (Lap-D)	C
Malate dehydrogenase-2 (MDH-2)	(1.1.1.37)	3-62.6 (Mdh-2)	C
Malic enzyme (ME)	(1.1.1.40)	3-51.7 (Men)	A
NAD-Sorbitol dehydrogenase (NAD-SODH)	(1.1.1.14)	3-64.5 (Sodh)	B
Octanol dehydrogenase (ODH)	(1.1.1.73)	3-49.2 (Odh)	A
Phosphoglucomutase (PGM)	(2.7.5.1)	4-43.4 (Pgm)	C

Table 2.5 (Cont'd.)

Larval protein-9 (Pt-9)		3-0.0 (*Lsp-1Y*)	A
Larval protein-15 (Pt-15)		3-37.0 (*Lsp-2*)	A
Tetrazolium oxidase-1 (TO-1)	(1.15.1.1)	3-32 (*To-1*)	A
Xanthine dehydrogenase (XDH)	(1.2.3.2)	3-52.0 (*ry+*)	A
Loci unmapped:			
Aconitase-1 (ACON-1)	(4.2.1.3)	(autosomal)	C
Aconitase-2 (ACON-2)	(4.2.1.3)	(autosomal)	C
β-Acetylglucosaminidase (HEXOS-1)	(3.2.1.30)		A
Adenylate kinase-1 (ADK-1)	(2.7.4.3)	(autosomal)	C
Adenylate kinase-2 (ADK-2)	(2.7.4.3)	(autosomal)	C
Arylsulphatase (ARS)	(3.1.6.1)		D
Carbonic anhydrase-1 (CA-1)	(4.2.1.1)	(autosomal)	B
Carbonic anhydrase-2 (CA-2)	(4.2.1.1)	(autosomal)	B
Carbonic anhydrase-3 (CA-3)	(4.2.1.1)	(sex-linked)	B
Carbonic anhydrase-4 (CA-4)	(4.2.1.1)	(autosomal)	B
Diaphorase-1 (DIA-1)	(1.6.2.2)	(autosomal)	A
Diaphorase-2 (DIA-2)	(1.6.2.2)	(autosomal)	A
Diaphorase-6 (DIA-6)	(1.6.2.2)		A

Table 2.5 (Cont'd.)

Diaphorase-7 (DIA-7)	(1.6.2.2)	(sex-linked)	A
Esterase-9 (EST-9)	(3.1.1.1)		B
Esterase-10 (EST-9)	(3.1.1.1)		A
Glucose dehydrogenase (GDH)	(1.1.1.47)	(autosomal)	B
α-Glucosidase-2 (α-GLU-2)	(3.2.1.20)		B
α-Glucosidase-3 (α-GLU-3)	(3.2.1.20)		B
α-Glucosidase-4 (α-GLU-4)	(3.2.1.20)	(autosomal)	B
Glutamate dehydrogenase (GLUD)	(1.4.1.3)	(autosomal)	C
Hexokinase-2 (HEX-2)	(2.7.1.1)		A
Lactate dehydrogenase (LDH)	(1.1.1.27)		B
Leucine inopeptidase-6 (LAP-6)	(3.4.1.1)		C
Mannose phosphate isomerase (MPI)	(5.3.1.8)	(autosomal)	B
α-Mannosidase-1 (α-MAN-1)	(3.2.1.24)		B
α-Mannosidase-2 (α-MAN-2)	(3.2.1.24)		B
α-Mannosidase-3 (α-MAN-3)	(3.2.1.24)		B
α-Mannosidase-4 (α-MAN-4)	(3.2.1.24)	(autosomal)	B
NADP-Sorbitol dehydrogenase (NADP-SODH)	(1.1.1.14)		B

Table 2.5 (Cont'd.)

Tetrazolium oxidase-2 (TO-2)	(1.15.1.1)	A
Adult Protein-26 (Pt-26)		A
Adult Protein-37 (Pt-37)		A
Other Protein Loci		
Coomassie-Stained Proteins (16 loci)		A
Silver-Stained Proteins (16 loci)		A

[1]The genetic map positions are taken from Treat-Clemons and Doane (1984).

[2]Buffers: A = 0.1M Tris-Borate EDTA, pH 8.9; B = 0.05M Tris-Glycine, pH 8.5; C = 0.1M Tris-Citrate, pH 8.5; D = 0.02M Barbitol-acetic acid, pH 8.6; E = 0.1M Tris-Borate, pH 8.9.

criteria were employed for the interpretation of variation in the gel patterns. First was independent variation in a protein band relative to the other bands in the profile, suggesting independent genetic control of the band. Secondly, the routine use of pooled samples of individuals from isofemale lines frequently permitted us to observe segregating alleles simultaneously in the same extract. Most of the alleles, which were scored, were observed at least once in the form of such internally heterogeneous extracts from heterozygous single female lines. To score allele frequencies, each isofemale line was taken to represent a sample of two genomes, with a segregating line counted as a single heterozygote. In rare cases where isofemale lines were segregating for three alleles, these lines were taken to represent a sample of more than two genomes. Expected heterozygosities were calculated for each locus as $H = 1 - \Sigma pi2$, where pi is the frequency of the i-th allele.

In the comparative analysis, results from previously published data on D. melanogaster (Singh, Hickey and David 1982; Singh and Coulthart 1982; Singh and Rhomberg 1987a, b) were extensively used, which should be consulted for details.

There were two shortcomings in the estimation of genic differentiation between the two species. First, for

most loci the identity of allozyme variants between species was checked under one electrophoretic condition only (usually 5% gel and pH 8.9). The single criterion for comparison was employed mainly because an earlier study of 11 loci in these two species had shown little hidden variation (Choudhary and Singh 1987a). Secondly, to avoid an inadvertent amount of work, the identity of electrophoretic mobility at highly polymorphic (i.e. multi-allelic) loci was checked mostly for the predominant alleles; the less frequent alleles, for the purpose of present comparison, were assumed to be identical between the two species. For these reasons, the present estimate of genetic divergence between D. melanogaster and D. simulans should be treated as a minimum.

3

RESULTS

3.1 A Sequential Electrophoretic Survey of Protein Variation in Drosophila melanogaster

Because of the inordinate amount of work involved, the sequential electrophoretic survey was applied to only 11 loci in four populations - Hamilton, Texas, France, and West Africa. These loci consisted of some monomorphic and some highly polymorphic loci (Table 3.2).

Only one of the 11 allozyme loci studied in D. melanogaster showed an appreciable amount of genetic variation (Table 3.1). The standard condition (5% gel; TBE, pH 8.9) showed six alleles for larval protein-11 (Pt-11) and the most common allele accounted for 76% of the sample. After four different criteria (combination of two gel concentrations and two pH's) had been applied, the original 6 allelic classes increased to 14, but the most frequent allele still accounted for 63% of the frequency. Like Xdh in D.pseudoobscura (Singh, Lewontin and Felton, 1976), the allele frequency distribution at Pt-11 in D. melanogaster was strongly skewed. About 70% of the lines

TABLE 3.1

Number of Lines Showing Alternate Alleles of Larval Protein-11 in Geographic Populations of *Drosophila melanogaster*

Alleles[a]	HAM	TEX	FRA	WAF	Total/mean
0.95/1.00/1.00/1.00		1		1	2
0.95/1.00/0.97/1.00			1		1
0.97/1.00/1.00/1.00	5	1			6
0.97/1.00/1.00/0.99		1			1
0.99/1.00/1.00/1.00	2		1	1	4
1.00/0.97/1.00/1.00				1	1
1.00/1.00/0.97/1.00			1	1	2
1.00/1.00/1.00/0.97	3	2		1	6
1.00/1.00/1.00/1.00	17	11	13	12	53
1.00/1.00/1.00/1.04				1	1
1.00/1.00/1.04/1.00				1	1
1.02/1.00/1.00/1.00			1	2	3
1.02/1.00/1.04/1.00				1	1
1.04/1.00/1.00/1.00	1	1			2
No. of genes examined	28	17	17	22	84
Total alleles	5	6	5	10	14
Unique alleles	--	1	1	4	6
Heterozygosity	0.582	0.554	0.401	0.678	0.554±0.115

[a]Allelic designation is based on relative mobility under four successive electrophoretic conditions.

contained one allele and all other alleles were rare. The same allele predominanted in all four populations studied. The mean heterozygosity increased from 38% under standard conditions to 55% under sequential electrophoresis. The only other locus, besides Pt-11, that showed any hidden variation was Est-6. A total of seven alleles were found and all but two were rare. The remaining nine loci showed no additional variation by the sequential method.

The number of alleles and mean heterozygosity for all loci examined in the present study are summarized in Table 3.2. Also included in Table 3.2 are the results of previous sequential electrophoretic studies on D. melanogaster. Of the 15 loci summarized, only 2 (Xdh and Pt-11) showed an increase in the level of variation and the increases were substantially smaller than those in D. pseudoobscura (Choudhary and Singh, 1987a). Since a relatively low level of hidden variation was detected in D. melanogaster, we interpreted this to mean that genic variation in this pair of species (i.e., D. melanogaster and D. simulans) could be compared by the standard gel electrophoresis without much loss of information.

3.2 Variation in Genetic Structure Between D. melanogaster and D. simulans:

Of the 114 loci studied, 44 were found to be

TABLE 3.2

Number of Alleles (n) and Mean Heterozygosity (H) at Various Allozyme Loci Studied by Sequential Gel Electrophoresis in *D. melanogaster*

Locus[a]	n[b]	H
Xdh	15	0.80
Est-6*	7	0.40
Est-C*	4	0.25
Odh*	2	0.14
Adh	2	0.23
α-Gpd	2	0.30
Fum*	1	0
Had*	2	0.04
Dia-7*	2	0.04
6-Pgd*	3	0.22
G-6pd	2	0.25
Hex-3*	2	0.03
Pt-11*	14	0.55
Pt-15*	6	0.47
Pt-16*	2	0.03
Total/mean		
Sequential method	4.40	0.25
Standard method[c]	2.80	0.21
% increase	57	19

[a]The data for loci marked with superscript asterisks are from the present study. The data for the remaining loci are from the literature. Buchanan and Johnson (1983), Eanes (1983), Kreitman (1980), and Coyne et al. (1979)

[b]The number of alleles shown are only those having a frequency of at least 1%.

[c]Based on the same set of loci as studied by the sequential method.

polymorphic in D. simulans and 60 in D. melanogaster. The frequencies of alternate alleles of 44 polymorphic loci in D. simulans are given in Table A1 (Appendix). Number of alleles, average heterozygosity and fixation index for polymorphic loci in the two species are shown in Table 3.3. The data for D. melanogaster have been analyzed and reported elsewhere (Singh and Rhomberg 1987a, b). Here we analyse the data for D. simulans and compare the results with those of D. melanogaster.

Table 3.4 shows a summary of genic variation in five geographic populations of D. simulans. An average population is polymorphic for about 30% of its loci and an average individual is heterozygous at 9% of its genes. All five populations are highly heterozygous but the Continental tropical population (Congo) shows higher heterozygosity than the temperate populations. However this difference appears to be limited to only the enzyme loci; the abundant proteins have similar heterozygosity in all populations. The difference in heterozygosity arises from the fact that the Congo population contains a number of polymorphic loci (e.g., 6-Pgd, Hex-1, α-Gpd, Odh) that are monomorphic in the remaining populations. The polymorphic loci are about equally heterozygous in all populations. Similar differences in heterozygosity between temperate and tropical populations are reported in D.

Table 3.3

Number of allele (n_a), mean heterozygosity (Hs) and fixation index (F_{st}) for various allozyme loci in Drosophila melanogaster and Drosophila simulans.

Enzymes or Proteins	D. melanogaster			D. simulans		
	n_a[1]	Hs	F_{st}	n_a[1]	Hs	F_{st}
Chromosome - I:						
Carbonic anhydrase-3 (CA-3)	1	0.018	0.043	4	0.467	0.140
Diaphorase-7 (DIA-7)	2	0.048	0.094	1	0	0
Glucose-6-phosphate dehydrogenase (G-6PD)	3	0.254	0.472	1	0	0
Glutamate pyruvate transaminase (GPT)	3	0.345	0.249	4	0.245	0.077
6-Phosphate gluconate dehydrogenase (6-PGD)	2	0.252	0.249	2	0.040	0.070
Larval protein-11 (Pt-11)	5	0.478	0.259	4	0.411	0.190
Chromosome - II:						
Alcohol dehydrogenase (ADH)	2	0.233	0.503	1	0	0
α-Amylase (α-AMY)	–	–	–	2	0.019	0.020
Dipeptidase-3 (DIP-3)	2	0.087	0.064	1	0	0
β-Galactosidase (β-GAL)	1	0	0	3	0.082	0.175
α-Glycerophosphate dehydrogenase (α-GPD)	2	0.306	0.107	2	0.037	0.066
Hexokinase-1 (HEX-1)	3	0.255	0.242	3	0.094	0.108
Malate dehydrogenase-1 (MDH-1)	2	0.027	0.129	1	0	0
Phenol oxidase (PHOX)	2	0.062	0.209	2	0.064	0.023
Larval protein-10 (Pt-10)	5	0.262	0.205	4	0.232	0.032
Succinate dehydrogenase (SDH)	3	0.143	0.243	3	0.324	0.077
Trehalase (TREH)	3	0.402	0.204	4	0.398	0.100

Table 3.3 (continued)

Enzymes or Proteins	D. melanogaster			D. simulans		
	n_a[1]	Hs	F_{st}	n_a[1]	Hs	F_{st}
Chromosome - III:						
Acid phosphatase (ACPH)	2	0.040	0.025	6	0.464	0.186
Aldehyde oxidase (AO)	8	0.416	0.189	4	0.512	0.129
Alkaline phosphatase (APH)	3	0.085	0.056	2	0.074	0.085
Dipeptidase-1 (DIP-1)	1	0	0	4	0.343	0.382
Esterase-C (EST-C)	4	0.238	0.365	5	0.649	0.110
Esterase-6 (EST-6)	3	0.471	0.098	4	0.517	0.071
α-Fucosidase (α-FUC)	3	0.446	0.116	3	0.259	0.455
Glucose oxidase (GO)	4	0.240	0.107	2	0.028	0.039
β-Glucoronidase (β-GUS)	1	0	0	2	0.039	0.084
Isocitrate dehydrogenase-1 (IDH-1)	2	0.025	0.052	4	0.224	0.094
Leucine aminopeptidase-D (LAP-D)	3	0.335	0.213	1	0	0
Leucine aminopeptidase-A (LAP-A)	3	0.424	0.157	1	0	0
Malate dehydrogenase-2 (MDH-2)	2	0.071	0.121	1	0	0
Octanol dehydrogenase (ODH)	2	0.141	0.152	2	0.104	0.257
Phosphoglucomutase (PGM)	3	0.197	0.077	4	0.244	0.058
Larval protein-9 (Pt-9)	3	0.228	0.433	3	0.145	0.031
Larval protein-15 (Pt-15)	3	0.127	0.585	5	0.553	0.045
Sorbitol dehydrogenase-1 (SODH-1)	2	0.070	0.074	2	0.078	0.181
Tetrazolium oxidase-1 (TO-1)	2	0.122	0.115	1	0	0
Xanthine dehydrogenase (XDH)	5	0.521	0.126	4	0.544	0.073

Table 3.3 (continued)

Enzymes or Proteins	D. melanogaster			D. simulans		
	n_a[1]	Hs	F_{st}	n_a[1]	Hs	F_{st}
Autosomes:						
Aconitase-1 (ACON-1)	2	0.385	0.085	1	0	0
Aconitase-2 (ACON-2)	3	0.245	0.088	–	–	–
Adenylate kinase-1 (ADK-1)	1	0.027	0.066	1	0	0
Carbonic anhydrase-1 (CA-1)	3	0.331	0.236	4	0.457	0.186
Carbonic anhydrase-2 (CA-2)	3	0.108	0.095	5	0.522	0.159
Carbonic anhydrase-4 (CA-4)	2	0.055	0.082	3	0.246	0.230
Diaphorase-1 (DIA-1)	3	0.190	0.087	1	0	0
Diaphorase-2 (DIA-2)	4	0.369	0.125	4	0.437	0.069
Glucose dehydrogenase (GDH)	4	0.527	0.203	3	0.164	0.055
α-Glucosidase-4 (α-GLU-4)	3	0.369	0.141	3	0.152	0.034
Glutamate dehydrogenase (GLUD)	4	0.257	0.350	2	0.141	0.346
α-Mannosidase-4 (α-MAN-4)	2	0.077	0.167	1	0	0
Mannose phosphate isomerase (MPI)	2	0.040	0.050	3	0.044	0.875
Unmapped:						
Esterase-9 (EST-9)	4	0.480	0.092	3	0.229	0.104
Esterase-10 (EST-10)	2	0.046	0.043	4	0.250	0.050
α-Glucosidase-3 (α-GLU-3)	1	0	0	2	0.032	0.040
Lactate dehydrogenase (LDH)	1	0	0	4	0.307	0.112
Leucine aminopeptidase-6 (LAP-6)	3	0.307	0.385	1	0	0
Tetrazolium oxidase-2 (TO-2)	1	0.012	0.053	1	0	0
Larval protein-1 (Pt-1)	3	0.377	0.126	4	0.468	0.187
Pt-2	3	0.077	0.068	1	0	0

Table 3.3 (continued)

Enzymes or Proteins	D. melanogaster			D. simulans		
	n_a[1]	Hs	F_{st}	n_a[1]	Hs	F_{st}
Pt-6	2	0.081	0.107	1	0	0
Pt-12	1	0.024	0.028	1	0	0
Pt-13	4	0.126	0.075	1	0	0
Pt-16	1	0	0	2	0.021	0.044
Adult protein-7A (Pt-7A)	2	0.076	0.277	1	0	0
Pt-9A	2	0.070	0.095	2	0.070	0.043
Pt-10B	2	0.053	0.098	-	-	-
Pt-15A	1	0.011	0.065	2	0.040	0.032
Pt-26	2	0.186	0.461	1	0	0
Pt-37	2	0.368	0.242	1	0	0

Monomorphic Loci:
Chromosome I: Fumerase (FUM), Hydroxy acid dehydrogenase (HAD) and Hexokinase-3 (HEX-3). Chromosome II: Glutamate oxaloacetate transaminase-1 (GOT-1), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Phosphoglucose isomerase (PGI), 3-Phosphoglycerate kinase (PGK), α-Glycerophosphate oxidase (α-GPO) and Sucrase (SUCR). Chromosome III: Acetylcholine esterase (ACE), Aldolase (ALD), Arginine kinase-1 (AK-1), AK-2, Catalase (CAT), Dipeptidase-2 (DIP-2), Isocitrate dehydrogenase-2 (IDH-2) and Malic enzyme (ME). Autosomes: Adenylate kinase-2 (ADK-2). Unmapped: Acetyl glucosaminidase (HEXOS-1), Arylsulphatase (ARS), Diaphorase-6 (DIA-6), α-Glucosidase-2 (α-GLU-2), Hexokinase-2 (HEX-2), α-Mannosidase-1 (α-MAN-1), α-MAN-2, α-MAN-3, Sorbitol dehydrogenase-2 (SODH-2), Triosephosphate isomerase (TPI), Larval protein-3 (Pt-3), Pt-4, Pt-5, Pt-7, Pt-8, Adult protein-10A (Pt-10A) and 14 other adult protein loci.

[1] The number of alleles shown are only those having a frequency of at least 1% in the species.

Table 3.4

Summary of genic variation in various geographic populations of *D. simulans*.

Populations and latitude	No. of loci studied[a]	Proportion polymorphic	No. of alleles (Mean ± S.E.)	Heterozygosity (Mean ± S.E.)
France	79	0.304	1.66±1.12	0.105±0.193
43°N	35	0.228	1.51±1.12	0.076±0.178
	114	0.281	1.61±1.12	0.096±0.188
Tunisia	79	0.304	1.70±1.21	0.105±0.191
35.6°N	35	0.171	1.37±0.91	0.060±0.162
	114	0.263	1.60±1.13	0.091±0.183
Congo	79	0.380	1.80±1.20	0.140±0.225
4.2°S	35	0.200	1.34±0.84	0.056±0.172
	114	0.324	1.66±1.12	0.114±0.213
Capetown	79	0.329	1.67±1.07	0.099±0.183
34°S	35	0.200	1.43±0.95	0.053±0.129
	114	0.289	1.60±1.04	0.085±0.169
Seychelle	79	0.329	1.56±0.94	0.110±0.183
3.8°S	35	0.171	1.26±0.66	0.032±0.097
	114	0.281	1.46±0.87	0.086±0.166
Overall Mean:				
Enzymes	79	0.329±0.031	1.68±0.09	0.112±0.016
Abundant proteins	35	0.194±0.024	1.38±0.10	0.055±0.016
Total	114	0.288±0.022	1.59±0.07	0.094±0.012

[a] For each population the three sets of loci are for enzymes, abundant proteins and their total, respectively.

melanogaster (Singh, Hickey and David, 1982), but here the differences arise mostly from the highly polymorphic loci and seem to disappear when the comparison is made with respect to all loci (Singh and Rhomberg, 1987b). The geographically isolated Seychelle population is less heterozygous than Congo although both are tropical in location.

The frequency distribution of polymorphic loci in Table 3.5 shows that in both species polymorphic loci are mostly di - or tri-allelic. Half of the polymorphic loci (48% in D. Simulans and 50% in D. melanogaster) have only two major alleles with a frequency of 10% or more. Only 11 loci (25%) in D. Simulans and 24 loci (40%) in D. melanogaster have three alleles with frequencies more than 20%, and fewer than 5 loci in both species have three or more alleles with individual frequencies of 10% or more. The distribution of polymorphic loci with respect to number of alleles and the frequency of the most common allele is shown in Figure 3.1. Two points emerge from these data. First, in both species the majority of polymorphic loci have only 2-5 alleles and very few loci have more than 5 alleles. Second, a larger proportion of di- and tri-allelic loci in D. simulans show an uneven distribution of allele frequencies, i.e. one allele is common and the others are rare. The multi-allelic loci, on the other

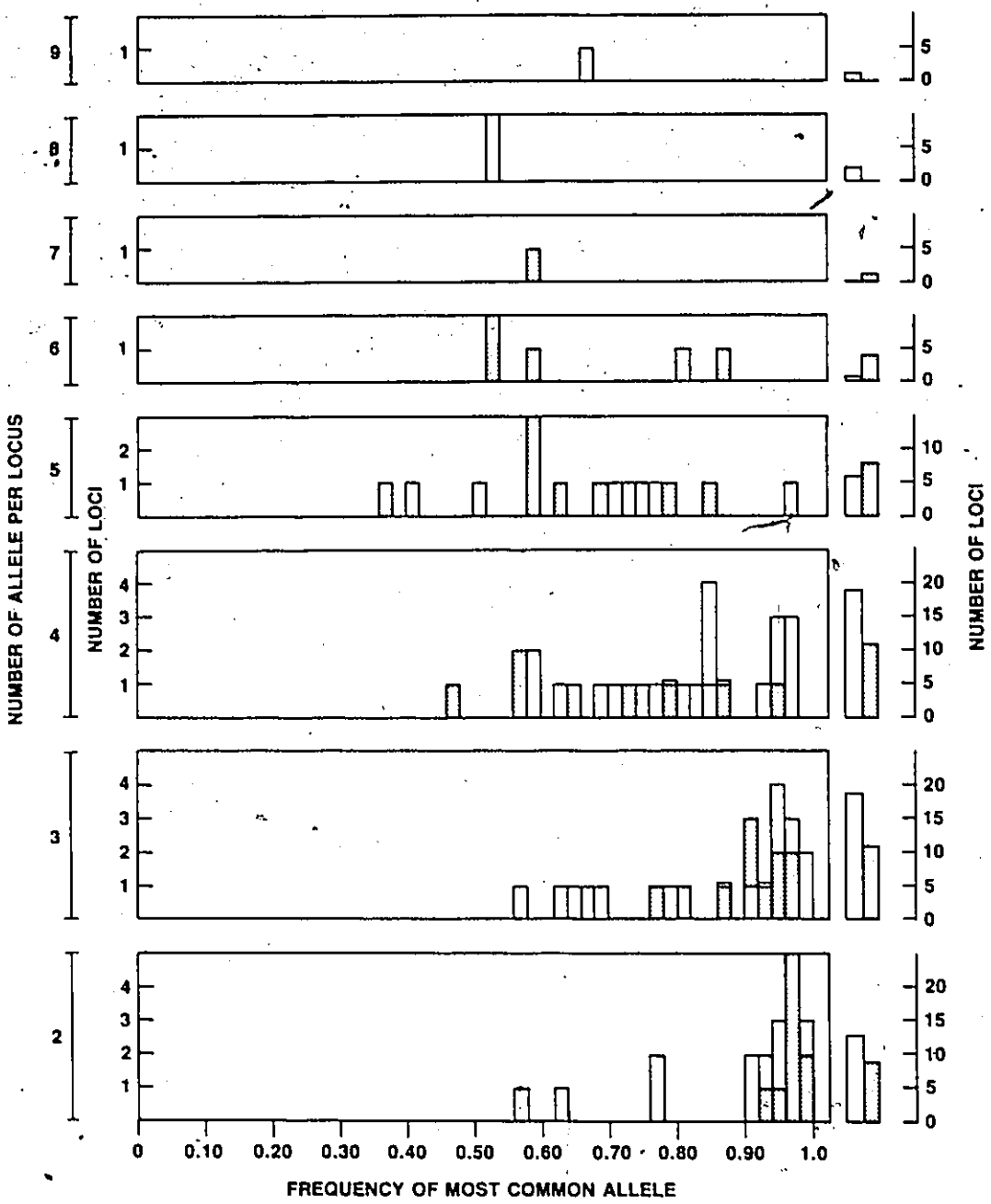
TABLE 3.5

Frequency distribution of polymorphic loci with respect to number of alleles and percentage of total alleles segregating in geographical populations of D. simulans and D. melanogaster. For each statistic the data in the first row are for D. simulans and in the second row for D. melanogaster.

	Number of Alleles segregating per locus								Total	
Criteria	2	3	4	5	6	7	8	9	Loci	Alleles
All alleles included	25.5(9)	25.0(11)	25.0(11)	18.2(8)	9.0(4)	2.3(1)	-	-	44	166
	21.3(13)	31.1(19)	31.(19)	9.8(6)	1.6(1)	-	3.3(2)	1.6(1)	61	220
Alleles with frequency > 0.01	31.8(14)	22.7(10)	36.4(16)	6.8(3)	2.3(1)	-	-	-	44	143
	47.5(29)	34.4(21)	11.5(7)	4.9(3)	-	-	1.6(1)	-	61	172
Two or more major alleles with frequency > 0.10	76.2(16)	19.0(4)	4.8(1)	-	-	-	-	-	21	48
	19.4(6)	45.2(14)	22.6(7)	9.7(3)	-	-	3.2(1)	-	31	62
> 0.20	100.0(11)	-	-	-	-	-	-	-	11	22
	25.0(6)	50.0(12)	16.7(4)	8.3(2)	-	-	-	-	24	48
Three or more major alleles with frequency > 0.10	-	80.0(4)	20.0(1)	-	-	-	-	-	5	16
	-	-	100.0(2)	-	-	-	-	-	2	6
> 0.20	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

Numbers in parentheses are numbers of polymorphic loci.

Figure 3.1 Frequency distribution of polymorphic loci with different numbers of alleles (vertical bars at right-hand side), and the frequency distribution of loci within each of these groups (e.g., those with two alleles, three alleles, etc.) into classes representing the commonness of the most frequent alleles at that locus (box on left-hand side). D. melanogaster (open bars), D. simulans (closed bars). The data on D. melanogaster are from Singh and Rhomberg (1987b).


NUMBER OF ALLELE PER LOCUS
NUMBER OF LOCI
NUMBER OF LOCI
9
8
7
6
5
4
3
2
1
0 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.0
FREQUENCY OF MOST COMMON ALLELE

hand, appear to be more evenly distributed in D. simulans than in D. melanogaster. Thus the lower heterozygosity of the di- and tri-allelic group of loci in D. simulans is offset by the higher heterozygosity of the multi-allelic group of loci. The net result is that D. simulans shows a stronger correlation between number of alleles and heterozygosity than D. melanogaster, although the overall mean heterozygosity is about the same in both species.

3.2.1 Comparison of Genetic Structure Across Homologous Loci:

It is possible that inversions might affect the pattern of genetic variability between the two species. Table 3.6 shows variation statistics for each chromosome separately. As reported earlier for D. melanogaster (Singh and Rhomberg, 1987b) the X chromosome and the autosomes are equally heterozygous within species as well as between species (Wilcoxon rank sum test, $p > 0.05$). However, the mean fixation indices for the X and the second chromosome are significantly higher in D. melanogaster than in D. simulans ($p < 0.05$). The third chromosome loci do not differ in their mean F_{st} but when all autosomal loci are pooled together, the difference between the two species is still significant ($p = 0.02$).

Table 3.6

Proportion of loci polymorphic, average number of alleles, mean heterozygosity and mean fixation index for protein loci located on various chromosomes.

Chromosome	No. of loci Studied (Homologous)	Species	Proportion Polymorphic	No. of Alleles	Heterozygosity	Fixation Index
X	9	D. simulans	0.444	2.111±1.453	0.129±0.193	0.053±0.072
		D. melanogaster	0.667	2.111±1.364	0.155±0.181	0.152±0.165
	Probability[a]			0.893	0.463	0.046
2	16	D. simulans	0.437	1.875±1.147	0.077±0.127	0.036±0.054
		D. melanogaster	0.562	1.937±1.124	0.111±0.136	0.119±0.142
	Probability			0.753	0.110	0.008
3	27	D. simulans	0.555	2.444±1.601	0.174±0.222	0.078±0.118
		D. melanogaster	0.667	2.444±1.577	0.155±0.173	0.113±0.114
	Probability			0.962	0.823	0.231
2+3	56[b]	D. simulans	0.536	2.286±1.436	0.144±0.194	0.083±0.148
		D. melanogaster	0.696	2.339±1.352	0.155±0.164	0.119±0.131
	Probability[a]			0.729	0.553	0.020

[a] Wilcoxon rank sum test

[b] Includes 13 additional autosomal loci that are unmapped.

The comparison of genetic variability of loci associated within or outside of inversions in D. melanogaster with their counterparts in D. simulans (note that D. simulans is not polymorphic for inversions) shows that the difference in the mean F_{st} of loci associated with inversions is on the borderline of statistical significance ($p = 0.05$) and there is no difference in the mean F_{st} of loci that are not associated with inversions (Table 3.7). Similarly, there is no difference in the mean number of alleles or mean heterozygosity of these two groups of loci. This result confirms the earlier conclusion (Singh and Rhomberg, 1987b) that inversions play a minor role in the overall geographic differentiation of associated loci and they do not appear to affect the overall heterozygosity of associated loci.

Figure 3.2 shows the correlation of single locus heterozygosity and interpopulation differentiation between D. melanogaster (France and Benin) and D. simulans (France and Congo). It is interesting to note that while the two species share some highly polymorphic loci ($r^2 = 0.292$, $p < 0.05$), they do not share homologous loci with high genetic differentiation. In fact the loci with high F_{st} in the two species appear to be negatively correlated (Figure 3.2b), although the overall correlation is non-significant ($r^2 = -0.121$, $p > 0.05$). Since homologous loci do not

Table 3.7

Comparison of genic variation for loci located inside and outside inversions in D. melanogaster with homologous loci in D. simulans.

	No. of loci studied	Proportion polymorphic	No. of alleles	Heterozygosity	Fixation Index
			Inside Inversion[a]		
D. melanogaster[b]	12	1.000	2.33±0.49	0.180±0.156	0.182±0.174
D. simulans	12	0.583	2.42±1.50	0.158±0.203	0.068±0.126
	Probability[c]		0.767	0.433	0.050
			Outside Inversion		
D. melanogaster[b]	31	0.484	2.23±1.67	0.123±0.162	0.090±0.121
D. simulans	31	0.484	2.16±1.46	0.130±0.196	0.060±0.090
	Probability[c]		0.730	0.777	0.231

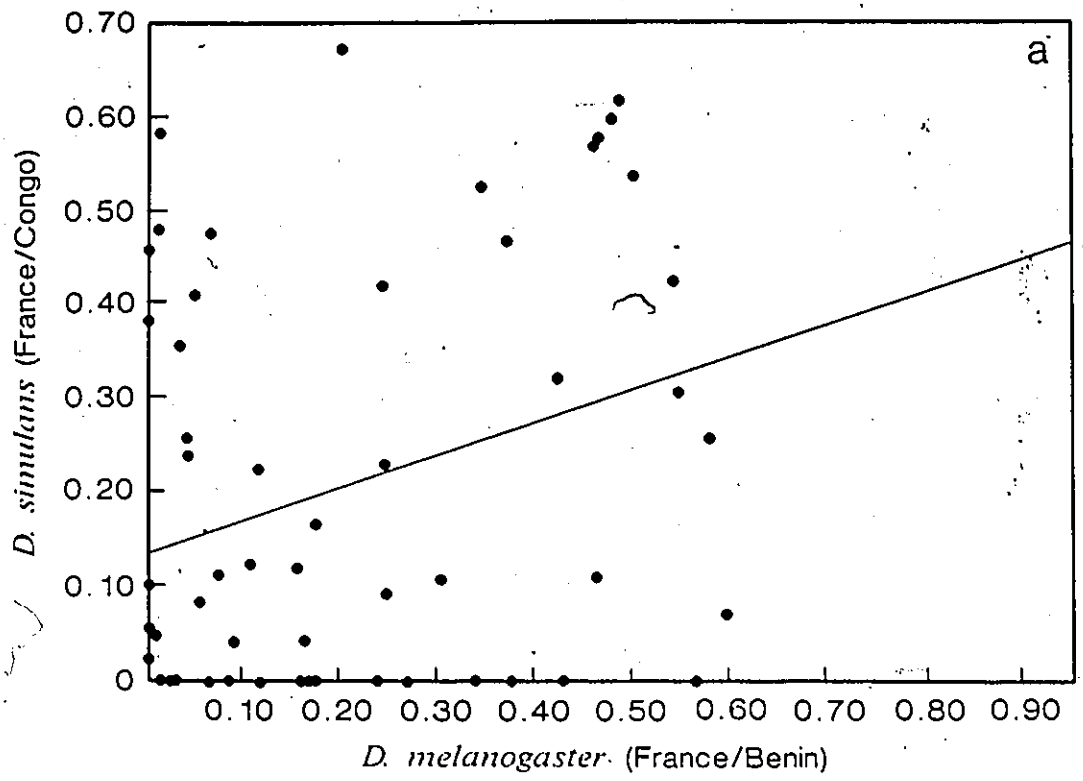
[a] The loci associated with inversions in D. melanogaster are: α-Gpd, Mdh-1, Mdh-2, Adh, α-Amy, Phox, Idh, To-1, α-Fuc, Est-6, Pgm, Sodh-1 and Pt-15 (α-Amy was not included in the analysis). D. simulans has no inversion polymorphism.

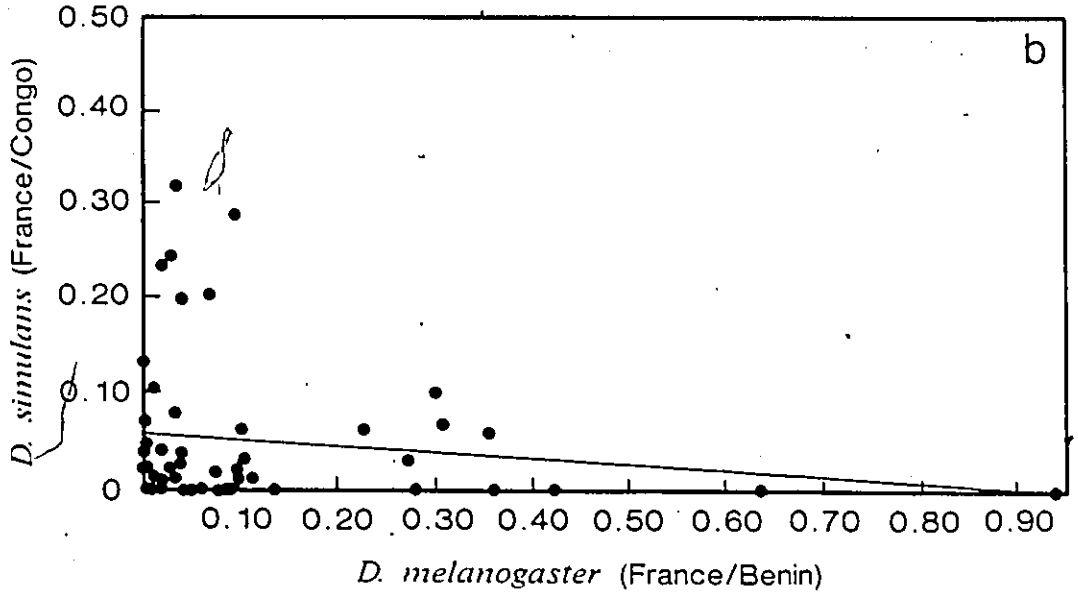
[b] Data of SINGH and RHOMBERG (1987b).

[c] Wilcoxon rank sum test

Figure 3.2a Correlation of single locus heterozygosity between D. melanogaster (France/Benin) and D. simulans (France/Congo): $Y = 0.136 + 0.340X$, Correlation Coefficient (r^2) = 0.292, $p < 0.05$.

Figure 3.2b Correlation for single locus fixation index (F_{st}) between D. melanogaster (France/Benin) and D. simulans (France/Congo): $Y = 0.054 - 0.056X$. Correlation coefficient (r^2) = -0.121, $p > 0.05$.


a
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
D. simulans (France/Congo)
0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90
D. melanogaster (France/Benin)


b
0.50
0.40
0.30
0.20
0.10
0
D. simulans (France/Congo)
0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90
D. melanogaster (France/Benin)

appear to be constrained in the amount or the pattern of variation between species, in the following the comparison is made of the geographic differentiation of all loci across populations between the two species.

3.2.2 Comparison of Genetic Structure Across Populations:

The distribution of average single locus heterozygosity for the 44 polymorphic loci of D. simulans is shown in Figure 3.3. If one takes the 70 additional monomorphic loci into account, the distribution is strongly L shaped. The polymorphic loci are rather widely distributed over the range of heterozygosities observed. However, the high heterozygosity at many loci shown in Figure 3.3 applies to an average population and does not tell us if the total heterozygosity ($H_t = 1 - \Sigma \bar{p}_i^2$) in the species as a whole is greater than the mean heterozygosity per population ($H_s = 1 - \Sigma p_i^2$) shown in Figure 3.3. The species as a whole would have higher total heterozygosity if the geographic populations were differentiated from each other. This is because H_t is based on mean allele frequencies averaged over populations. This procedure increases the evenness of allele frequencies resulting in higher heterozygosity. Figure 3.4 shows the distribution of single locus heterozygosity (H_t) in D. simulans. In contrast to the bimodal distribution of H_t in D.

Figure 3.3 Distribution of single locus heterozygosity in geographic populations of D. simulans.

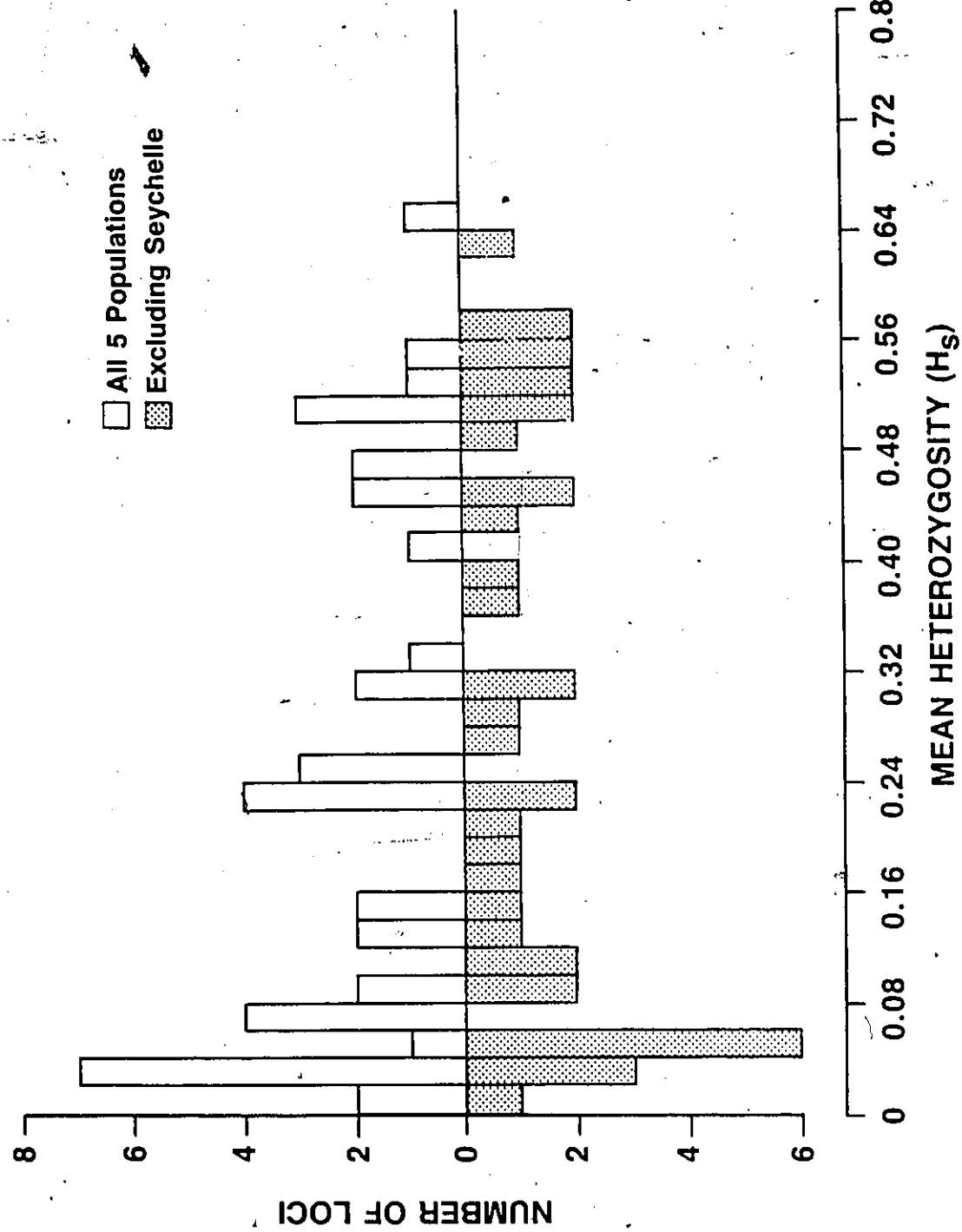
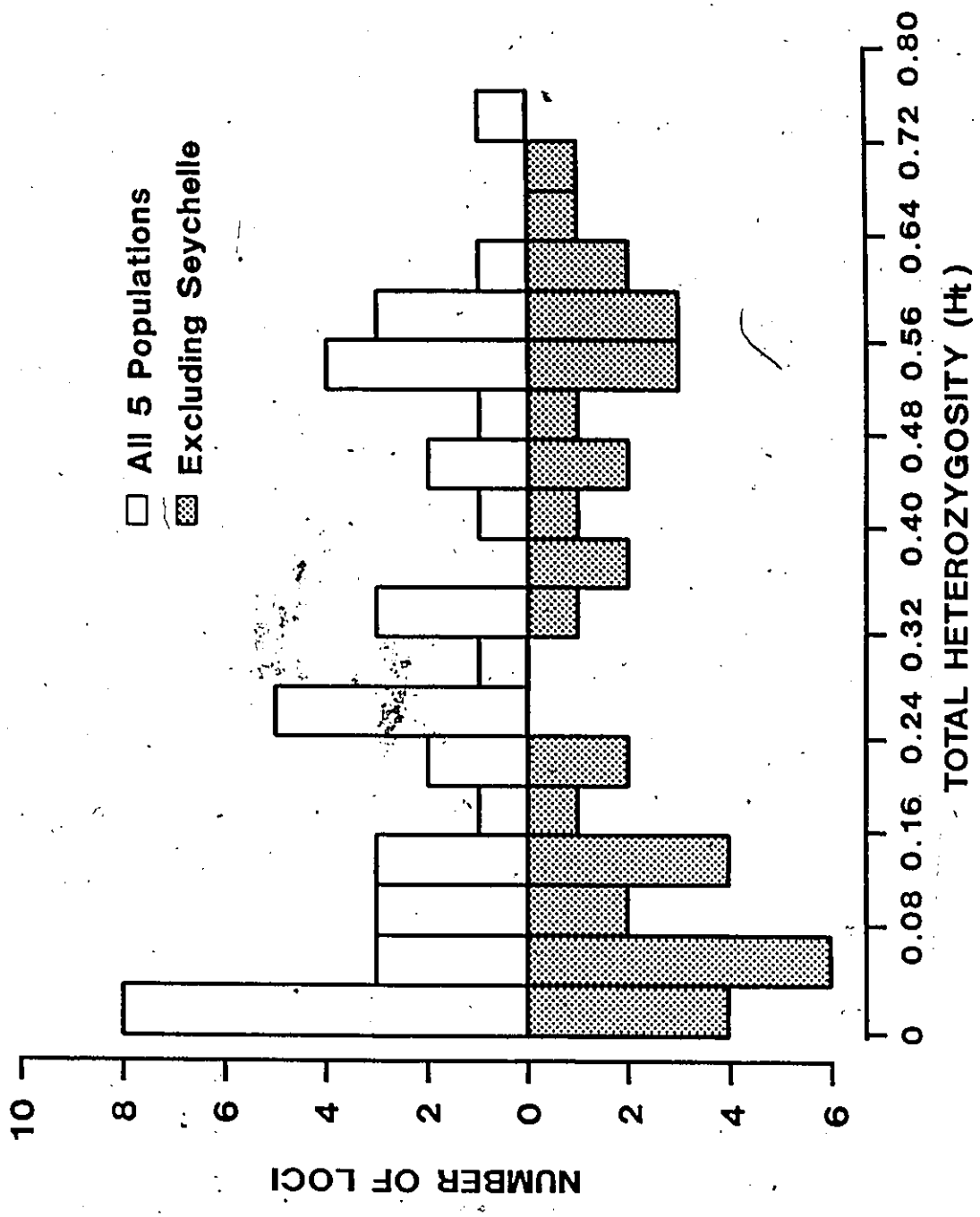

All 5 Populations
Excluding Seychelle
NUMBER OF LOCI
8
6
4
2
0
2
4
6
MEAN HETEROZYGOSITY (H_S)
0
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
0.80

Figure 3.4 Distribution of single locus total heterozygosity (H_t) in D. simulans.


All 5 Populations
Excluding Seychelle
NUMBER OF LOCI
10
8
6
4
2
0
2
4
6
TOTAL HETEROZYGOSITY (Ht)
0
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
0.80

melanogaster (Singh and Rhomberg, 1987b), loci are quite uniformly distributed in D. simulans, reaching up to a maximum of $H_t = 0.76$. The H_s and H_t are related as $F_{st} = (H_t - H_s)/H_t$ where F_{st} is a measure of standardized interpopulation genic differentiation. The distribution of F_{st} for individual polymorphic loci in D. simulans is shown in Figure 3.5 and a diagramatic representation of the algebraic relationship among H_t, H_s and F_{st} is shown in Figure 3.6. The distribution of F_{st} has a prominent mode around 5% and there are only six loci showing $F_{st} > 20\%$ if all populations are considered. These loci are Mpi, α-Fuc, Dip-1, GluD, Odh and Ca-4. A large portion of the interpopulation differentiation is contributed by the Seychelle population as only 2 loci (Odh and α-Fuc) show $F_{st} > 20\%$ if Seychelle is dropped from the computation. In contrast, 21 out of the 60 polymorphic loci of Drosophila melanogaster had $F_{st} > 20\%$ (Singh and Rhomberg, 1987b).

There is no correlation between mean heterozygosity and population differentiation (data not shown). Two-thirds of all polymorphic loci in mainland D. simulans have very little geographic differentiation ($F_{st} < 10\%$), which is not surprising as a large number of these loci (such as α-Gpd, α-Glu-3, 6-Pgd, Phox, α-Amy, Aph, β-Gus, Go) have very little variability ($H_s < 10\%$). A locus must have some variation before it can show geographic differentiation.

Figure 3.5 Distribution of fixation index (F_{st}) at polymorphic loci in geographic populations of D. simulans.

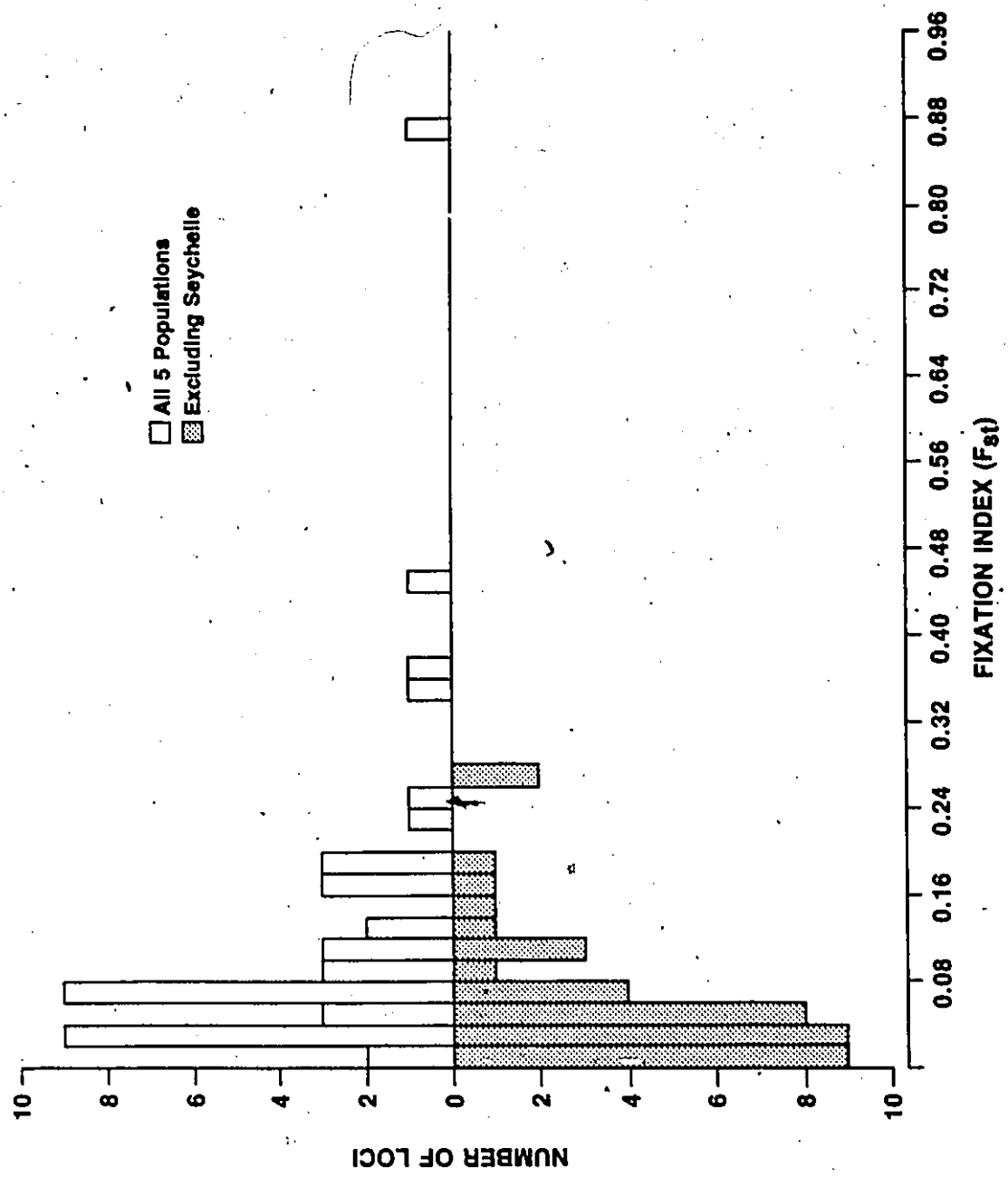
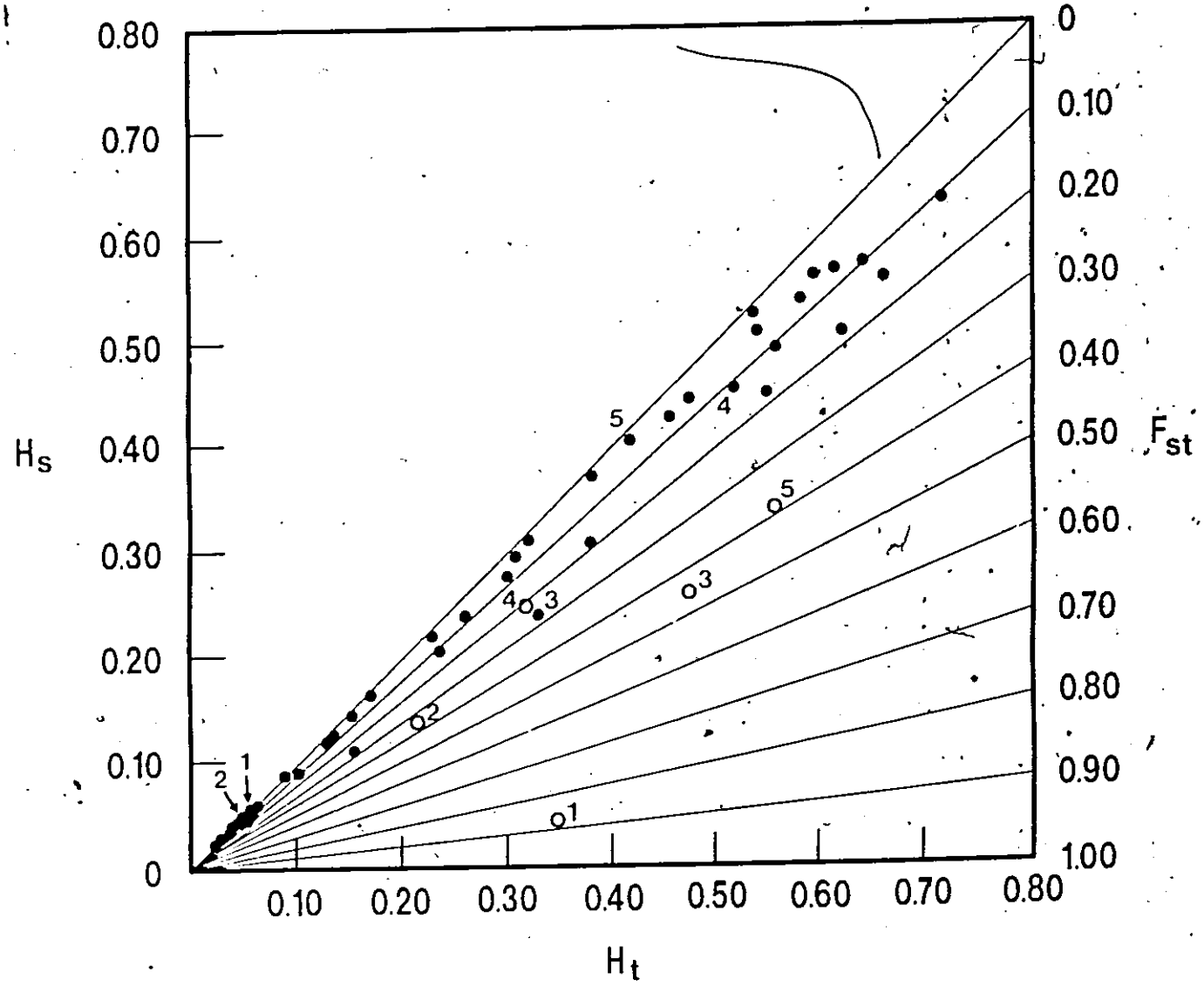

All 5 Populations
Excluding Seychelle
NUMBER OF LOCI
10
8
6
4
2
0
2
4
6
8
10
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
0.80
0.88
0.96
FIXATION INDEX (F_{st})

Figure 3.6 A diagramatic representation of the relationship among H_S, H_t and F_{st} at polymorphic loci in D. simulans. The extent to which points fall below the diagonal line is a measure of interpopulation differentiation. Data are shown for all loci in mainland populations (solid circle) but only for the significantly differentiated loci when Seychelle is included in the analysis (open circle). The loci marked are: 1(Mpi), 2(GluD), 3(α-Fuc), 4(Ca-4) and 5(Dip-1).


0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
H_s
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
H_t
0
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
F_{st}
1
2
3
4
5
o1
o2
o3
o5

Most of these loci (F_{st} < 10%) have one common allele which is the same in all populations (often monomorphic in many populations) and one or two rare alleles. On the other hand, there are several loci (e.g., Ao, Est-6, Xdh, Est-C, Est-10, Pgm, Sdh, Treh) that have high variability and yet show low Fst. Thus, all except two polymorphic loci (Odh and α-Fuc) show F_{st} < 20% and yet they range in heterozygosity from 0 to 64%. Only six loci (Mpi, Odh, GluD, α-Fuc, Ca-4, Dip-1) show significant interpopulation differentiation when Seychelle is included in the analysis (Figure 3.6). It is interesting to note that, like D. melanogaster, the loci showing high F_{st} in D. simulans, when Seychelle is included in the analysis, are mostly di- or tri-allelic and having intermediate levels of heterozygosity. Seychelle is significantly differentiated from the mainland populations at five loci (Mpi, α-Fuc, GluD, Dip-1, Ca-4), and alternately fixed at two loci (Mpi and Dip-1).

Thus, the comparison of genetic structure shows that in both species most of the polymorphic loci have low F_{st} (<20%) and many of them have low genetic variability as well. Both species share a second group of loci (such as, Ao, Est-6 and Xdh) which have high heterozygosity but show little geographic differentiation. A third group of loci show high interpopulation differentiation in both D.

melanogaster and D. simulans, but these loci are not the same in the two species. This group includes about 20 loci in D. melanogaster (such as Adh, G6-pd, Lap-6, GluD, Est-C, Pt-9, Pt-15 and Pt-26) and six loci (Mpi, α-Fuc, Dip-1, GluD, Odh and Ca-4) in D. simulans. Some of the loci that are highly differentiated in D. melanogaster are either monomorphic (such as Adh, G6-pd, Lap-6 and Pt-26) or show little geographic differentiation (Pt-9, Pt-15 and Est-C) in D. simulans.

Table 3.8 shows a overall summary of genic variability in the two species. D. melanogaster has a larger proportion of loci polymorphic than D. simulans ($t = 2.074$, $p < 0.05$). Both species are similar in the mean number of alleles and mean heterozygosity, but they differ in the level of interpopulation differentiation (Wilcoxon rank sum test, $p = 0.001$). D. melanogaster is geographically more differentiated than D. simulans even if we include the highly differentiated Seychelle population in the analysis. However, we must keep in mind that the geographic range of D. simulans sampled in the present study was smaller than that of D. melanogaster.

The general conclusions derived from Table 3.8 and discussed in the previous paragraph can be substantiated by making a more direct comparison between sympatric populations of these two species. Table 3.9 shows a

Table 3.8

Comparison of various genic variation statistics of *D. melanogaster* and *D. simulans*

Statistics	*D. melanogaster*	*D. simulans*[a]	Probability (Wilcoxon rank sum test)
No. of populations	15	5	
No. of loci studied	117	114	
Proportion of loci polymorphic:	0.420±0.070	0.289±0.025 0.288±0.022	
Average number of alleles:	1.48±0.11	1.62±0.03 1.59±0.07	0.626
Heterozygosity	0.102±0.014	0.096±0.012 0.094±0.012	0.149
Fixation index	0.091±0.130	0.025±0.052 0.052±0.113	0.001
Genetic distance[b]	0.031±0.015	0.013±0.006 0.026±0.017	

[a] For *D. simulans*, each statistic shows two values; the first is based on four mainland populations while the second is based on all five populations including Seychelle Island.

[b] The average genetic distance in *D. melanogaster* is based on only 9 populations (see SINGH and RHOMBERG 1987b)

Table 3.9

A summary of allozyme polymorphism in climatically comparable populations of Drosophila melanogaster and Drosophila simulans. Where a statistic has two values, the first is for polymorphic loci and the second for all loci.

Statistics	D. melanogaster		D. simulans	
	France 46.1°N	Benin 6.3°N	France 43°N	Congo 4.2°S
% of loci polymorphic	0.351	0.377	0.281	0.324
Average number of alleles	2.58±0.83 1.53±0.88	2.75±1.26 1.61±1.12	3.19±1.00 1.61±1.12	3.03±1.04 1.66±1.12
Heterozygosity	0.304±0.201 0.107±0.187	0.275±0.215 0.104±0.187	0.342±0.206 0.096±0.188	0.351±0.238 0.114±0.213
Fixation index	0.126±0.179 0.053±0.131		0.075±0.086 0.024±0.060	
Genetic distance	0.050		0.021	
Probability[a]	(D. melanogaster vs. D. simulans): Number of alleles: p = 0.939 Heterozygosity: p = 0.839 Fixation index: p = 0.031			

[a] Wilcoxon rank sum test.

summary of genic variation in the France and Benin populations of D. melanogaster, and France and Congo populations of D. simulans. While both species have similar levels of heterozygosity and number of alleles, D. simulans (France/Congo) shows significantly less geographic differentiation ($p = 0.031$) than D. melanogaster (France/Benin).

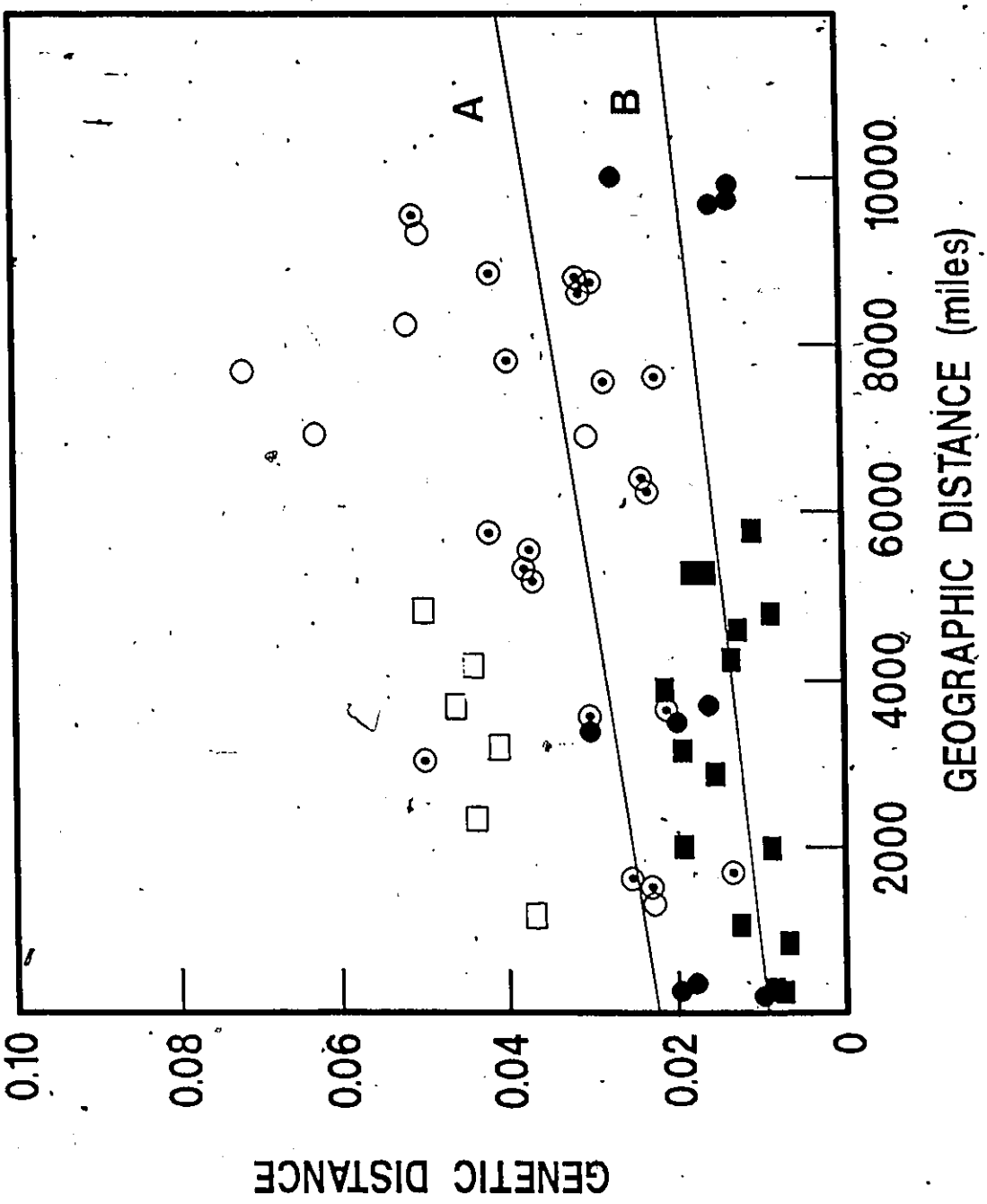
To see if the genetic differentiation between populations is associated with geographic distance, the NEI genetic distance (Nei, 1975) between all pairs of populations (Table 3.10) was plotted against the minimum geographic distance between populations (Figure 3.7). The genetic distances between populations of D. melanogaster are shown for comparison. As would be expected, Seychelle is significantly isolated genetically from the mainland populations of D. simulans. The genetic distances among mainland populations show no correlation with geographic distance, and the mean genetic distance for the mainland D. simulans is significantly lower (t-test; $p < 0.05$) than that for D. melanogaster. D. melanogaster shows only a weak but positive correlation between genetic distance and geographic distance. This is partly due to the fact that all temperate populations, regardless of geographic distance, have similar genetic distances (Singh and Rhomberg, 1987b).

TABLE 3.10

Average genetic identity (above diagonal) and genetic distance (below diagonal) between geographical populations of *D. simulans*

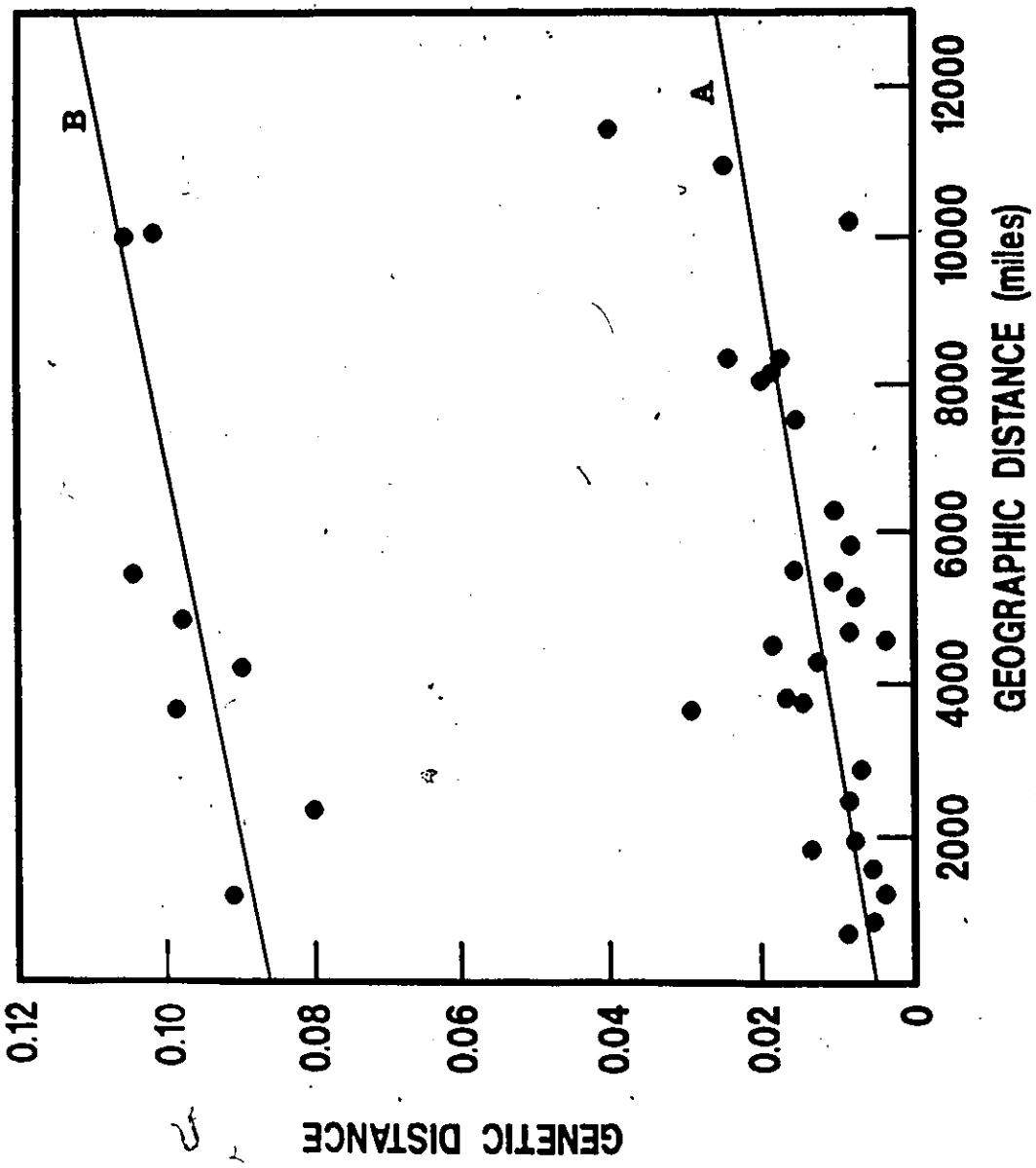
	France	Tunisia	Congo	Capetown	Seychelle
France	--	0.994	0.979	0.990	0.950
Tunisia	0.006	--	0.985	0.992	0.956
Congo	0.021	0.015	--	0.981	0.956
Capetown	0.010	0.008	0.019	--	0.959
Seychelle	0.050	0.044	0.044	0.041	--

Figure 3.7 Correlation between NEI genetic distance and geographic distance in populations of D. melanogaster and D. simulans. D. melanogaster: temperate-temperate (closed circle), tropical-tropical (open circle), and temperate-tropical (circle with dot), regression line A: $Y = 0.022 + 0.002X$, Correlation Coefficient (r^2) = 0.337, $p > 0.05$. Mainland D. simulans (solid square, regression line B): $Y = 0.009 + 0.001X$, Correlation Coefficient (r^2) = 0.351, $p > 0.05$. Seychelle vs. Mainland D. simulans (open square). Data are from Hyytia et al. (1985), Singh and Rhomberg (1987b) and the present study.


A
B
0.10
0.08
0.06
0.04
0.02
0
GENETIC DISTANCE
2000
4000
6000
8000
10000
GEOGRAPHIC DISTANCE (miles)

The populations of D. simulans analyzed in the present study came from a rather narrower geographic range than that of D. melanogaster, and so it is not possible to tell from Figure 3.7 if samples of D. simulans from different continents would increase the genetic distance significantly. To gain an insight into this question, data were gathered from all studies of genic variation on D. simulans in the literature (including the present study) and selected a total of 8 studies that have 8 loci in common. The correlation between genetic distance and geographic distance for these populations is shown in Figure 3.8. Note that here we are not concerned with the absolute amount of genetic distance, since we are looking at a different set of loci, which may have larger or smaller genetic distances than that shown in Figure 3.7. It is the strength of association between genetic distance and geographic distance that we wish to investigate. As noted above, Seychelle again comes out to be significantly isolated from the other populations of D. simulans, and the genetic distances for both groups of populations (i.e., with or without Seychelle) show a weak but positive correlation with the geographic distances ($p < 0.05$). Thus, the data shown in Figure 3.7 and 3.8, together, strengthen the conclusion that on a worldwide scale D. simulans is significantly less differentiated than D.

Figure 3.8 Correlation between genetic distance (based on 8 loci: Acph, Adh, Est-6, α-Gpdh, Mdh, Me, Pgm, and Xdh) and geographic distance in several populations of D. simulans. Mainland populations (regression line A): $Y = 0.004 + 0.001X$, Correlation Coefficient (r^2) = 0.586, $p < 0.05$. Seychelle vs Mainland D. simulans (regression line B): $Y = 0.086 + 0.002X$, Correlation Coefficient (r^2) = 0.739, $p < 0.05$. Data are from Kojima, Gillespie and Tobari (1970); Triantaphyllides (1973); Steiner, Sung and Paik (1976); Triantaphyllides et al. (1980, 1982); Cabrera et al. (1982); Hyytia et al. (1985); Singh, Choudhary and David (1987), and the present study.


B
A
0.12
0.10
0.08
0.06
0.04
0.02
0
GENETIC DISTANCE
2000
4000
6000
8000
10000
12000
GEOGRAPHIC DISTANCE (miles)

melanogaster.

3.3 Genic Divergence Between D.melanogaster and D. simulans:

3.3.1 Polymorphism and Genetic Structure Within Species

Using a 99% criterion of polymorphism (i.e., the most common allele being 99% or less), 58 loci are polymorphic in D. melanogaster, and 44 loci in D. simulans. D. melanogaster shows a significantly higher number of loci polymorphic than D. simulans (t-test, $p < 0.05$). However, if we change the criterion of polymorphism from 99% to 95%, both species show about the same number of loci polymorphic (39 in D. melanogaster and 34 in D. simulans). This shows that the excess of polymorphic loci in D. melanogaster under the 99% criterion is due to many, otherwise monomorphic, loci harboring rare alleles.

Table 3.11 shows data on the joint distribution of polymorphic (or monomorphic) loci in the two species. The polymorphic loci between the species are significantly correlated, i.e., if a locus is polymorphic in one species, it is likely to be polymorphic but not necessarily for the same alleles) in the other species. This result can be explained by giving a historical explanation: the shared polymorphisms (or monomorphisms) predate the speciation event and the two species have not been separated long

TABLE 3.11

Number of loci polymorphic (P) or monomorphic (M) in *D. melanogaster* and *D. simulans* (D.m./D.s) and χ^2 for independence of polymorphism between species.

Criterion for polymorphism*		M/M	M/P	P/M	P/P	χ^2 (Probability)
< 99%	Observed	47	7	21	37	30.29 ($p < 0.005$)
	Expected	33	21	35	22	
< 95%	Observed	62	11	16	23	23.18 ($p < 0.005$)
	Expected	51	22	27	12	

*Frequency of the most common allele.

enough for the initial correlations to disappear (Nei and Li, 1975; Li and Nei, 1977). However, an alternate explanation can be advanced by invoking similar functional constraints (or natural selection) on the monomorphic (or polymorphic) loci between the two species. Functional constraints and the resultant purifying selection can account for homologous loci to remain monomorphic in both species. Similarly, the excess of polymorphic loci in both species may result from accumulation of neutral mutations or from natural selection. On parsimonious ground and without further evidence to support the latter explanation we favour the historical explanation.

3.3.2 Genetic Distance Between Species:

The number of common (i.e., shared between species) and unique (unshared) alleles and genetic identity (Nei 1972) for individual loci between the two species are shown in Table 3.12 and the results are summarized in Table 3.13. The various chromosomes are similar in having proportion of unique alleles within species but differ between species; chromosome X and II, but not III, show more unique alleles in *D. melanogaster* than *D. simulans*. However, due to the small number of loci examined, the differences between species are significant (t-test: $p < 0.05$) only when all autosomes (II + III) or all chromosomes (X + II + III) are

TABLE 3.12

Number of shared (common) and unshared (unique) alleles and genetic identity at allozyme loci in natural populations of D. melanogaster and D. simulans

Enzymes or Proteins	Number of Alleles: Unique to D. melanogaster	Unique to D. simulans	Common	Total	Genetic Identity
Chromosome I (X):					
Carbonic anhydrase-3 (CA-3)	0	2	3	5	0.872
Diaphorase-7 (DIA-7)	3	0	1	4	0.999
Fumerase (FUM)	0	0	1	1	1
Glucose-6-phosphate dehydrogenase (G-6PD)	2	0	1	3	0.872
Glutamate pyruvate transaminase (GPT)	0	1	3	4	0.404
Hexokinase-3 (HEX-3)	0	0	1	1	1
Hydroxyacid dehydrogenase (HAD)	1	1	0	2	0
6-Phosphogluconate dehydrogenase (6 PGD)	0	0	3	3	0.260

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Larval protein-11 (Pt-11)	3	0	5	8	0.977
Chromosome II:					
Alcohol dehydrogenase (ADH)	2	1	0	3	0
α-Amylase (α-AMY)	5	2	0	7	0
Dipeptidase-3 (DIP-3)	2	0	1	3	0.999
β-Galactosidase (β-GAL)	0	2	1	3	0.999
Glutamate oxaloacetate transaminase-1 (GOT-1)	1	1	0	2	0
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0	0	1	1	1
α-Glycerophosphate dehydrogenase (α-GPD)	0	0	2	2	0.968
Hexokinase-1 (HEX-1)	1	0	3	4	0.970
α-Glycerophosphate oxidase (α-GPO)	0	0	1	1	1
Malate dehydrogenase-1 (MDH-1)	1	0	1	2	0.999

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Phenol oxidase (PHOX)	2	0	2	4	0.999
Phosphoglucose isomerase (PGI)	0	0	1	1	1
3-Phosphoglycerate kinase (PGK)	1	1	0	2	0
Larval protein-10 (Pt-10)	0	0	6	6	0.196
Succinate dehydrogenase (SDH)	0	2	3	5	0.984
Sucrase (SUCR)	1	1	0	2	0
Trehalase (TREH)	0	0	4	4	0.616
Chromosome III:					
Acetylcholine esterase (ACE)	0	0	1	1	1
Acid phosphatase (ACPH)	0	4	3	7	0.911
Aldehyde oxidase (AO)	5	0	4	9	0.246
Aldolase (ALD)	0	0	1	1	1
Alkaline phosphatase (APH)	0	0	3	3	0.069

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles				Genetic Identity
	Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	
Arginine kinase-1 (AK-1)	0	0	1	1	1
Catalase (CAT)	0	0	1	1	1
Dipeptidase-1 (DIP-1)	0	3	1	4	0.854
Dipeptidase-2 (DIP-2)	1	1	0	2	0
Esterase-C (EST-C)	0	1	4	5	0.707
Esterase-6 (EST-6)	0	2	4	6	0.859
α-Fucosidase (α-FUC)	1	2	2	5	0.975
Glucose oxidase (GO)	2	0	2	4	0.990
β-Glucoronidase (β-GUS)	0	1	1	2	0.999
Isocitrate dehydrogenase-1 (IDH-1)	1	3	1	5	0.088
Isocitrate dehydrogenase-2 (IDH-2)	0	0	1	1	1
Leucine aminopeptidase-D (LAP-D)	2	0	1	3	0.327

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Leucine aminopeptidase-A (LAP-A)	2	0	1	3	0.917
Malate dehydrogenase-2 (MDH-2)	1	0	1	2	0.999
Malic enzyme (ME)	0	0	1	1	1
Octonal dehydrogenase (ODH)	0	0	2	2	0.999
Phosphoglucomutase (PGM)	1	1	3	5	0.997
Larval protein-9 (Pt-9)	2	1	2	5	0.981
Larval protein-15 (Pt-15)	0	1	4	5	0.952
Sorbitol dehydrogenase-1 (SODH-1)	0	1	3	4	0.999
Tetrazolium oxidase-1 (TO-1)	1	0	1	2	0.997
Xanthine dehydrogenase (XDH)	3	0	5	8	0.948
Autosomes:					
Aconitase-1 (ACON-1)	3	0	1	4	0.921

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Adenylate kinase-1 (ADK-1)	2	0	1	3	0.999
Adenylate kinase-2 (ADK-2)	0	0	1	1	1
Carbonic anhydrase-1 (CA-1)	0	1	3	4	0.926
Carbonic anhydrase-2 (CA-2)	0	3	3	6	0.901
Carbonic anhydrase-4 (CA-4)	2	1	2	5	0.987
Diaphorase-1 (DIA-1)	2	0	1	3	0.995
Diaphorase-2 (DIA-2)	1	1	4	6	0.987
Glucose dehydrogenase (GDH)	2	0	3	5	0.768
α-Glucosidase-4 (α-GLU-4)	2	0	3	5	0.389
Glutamate dehydrogenase (GLU-D)	2	0	3	5	0.992
α-Mannosidase-4 (α-MAN-4)	2	0	1	3	0.046
Mannose phosphate isomerase (MPI)	1	0	3	4	0.034

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Unmapped:					
β-Acetyl glucosaminidase (HEXOS-1)	0	0	1	1	1
Arylsulphatase (ARS)	1	1	0	2	0
Diaphorase-6 (DIA-6)	0	0	1	1	1
Esterase-9 (EST-9)	1	0	4	5	0.759
Esterase-10 (EST-10)	1	1	4	6	0.996
α-Glucosidase-2 (α-GLU-2)	0	0	1	1	1
α -Glucosidase-3 (α-GLU-3)	0	2	1	3	0.999
Hexokinase-2 (HEX-2)	0	0	1	1	1
Lactate dehydrogenase (LDH)	0	3	1	4	0.987
Leucine aminopeptidase-6 (LAP-6)	3	0	1	4	0.898
α -Mannosidase-1 (MAN-1)	0	0	1	1	1

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles				
	Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
α-Mannosidase-2 (MAN-2)	0	0	1	1	1
α-Mannosidase-3 (MAN-3)	0	0	1	1	1
Sorbitol dehydrogenase-2 (SODH-2)	0	0	1	1	1
Tetrazolium oxidase-2 (TO-2)	1	0	1	2	0.999
Larval protein-1 (Pt-1)	0	2	4	6	0.855
Pt-2	3	0	1	4	0.999
Pt-3	0	0	1	1	1
Pt-4	0	0	1	1	1
Pt-5	0	0	1	1	1
Pt-6	1	0	1	2	0.999
Pt-7	0	0	1	1	1
Pt-8	0	0	1	1	1
Pt-12	2	0	1	3	0.999

Table 3.12 (Cont'd.)

Enzymes or Proteins	Number of Alleles: Unique to *D. melanogaster*	Unique to *D. simulans*	Common	Total	Genetic Identity
Pt-13	3	0	1	4	0.999
Pt-16	0	1	1	2	0.999
Adult protein-7A (Pt-7A)	1	0	1	2	0.998
Pt-9A	2	0	2	4	0.999
Pt-10A	0	0	1	1	1
Pt-15A	0	0	2	2	0.999
Pt-26	1	0	1	2	0.962
Pt-37	1	0	1	2	0.816
14 other adult protein loci	0	0	1	1	1

TABLE 3.13

Percent shared (common) and unshared (unique) alleles and mean genetic identity of allozyme loci for various chromosomes between D. melanogaster and D. simulans. Number of alleles are given in the parenthesis.

Chromosome	Number of Loci	% Alleles (number)			Genetic Identity (mean + SE)	Fixation Index	
		Unique to D.m.	Unique to D.s.	Common		D. m.	D. s.
X	9	29.0(9)	12.9(4)	58.1(18)	0.709 ± 0.383	0.152*	0.053*
2	17	29.4(15)	19.6(10)	50.9(26)	0.631 ± 0.467	0.127*	0.035*
3	27	22.7(22)	21.6(21)	55.7(54)	0.774 ± 0.357	0.113	0.084
2 + 3	57**	27.7(56)	18.3(37)	53.9(109)	0.729 ± 0.392	0.121*	0.085*
Total	112	26.9(86)	15.9(51)	57.2(183)	0.832 ± 0.327	0.093*	0.053*

*Wilcoxon rank sum test for species difference: $p < 0.05$

**Includes 13 additional autosomal loci that are unmapped.

considered together. All chromosomes show similar proportion of shared alleles and similar mean genetic identities between the two species. The overall mean genetic identity based on all 112 loci is slightly greater than the values of individual chromosomes due to the fact the former figure includes 47 additional monomorphic loci which are unmapped.

To see whether the unique alleles in each species are common or rare, the frequency distributions of unique as well as total alleles are shown in Figure 3.9. Both species show similar distributions. In both species the majority of the alleles have frequencies less than 0.05 or greater than 0.95. The majority of the unique alleles, on the other hand, have frequencies less than 0.05 in both species. However, the two species differ in absolute number as well as in the proportion of unique alleles which are rare ($\leq$ 0.01).

The distribution of genetic identity for all loci is shown in Figure 3.10. The distribution has two interesting features. First, the majority of loci are huddled at the extremes of the distribution, i.e. they show genetic identity of 0 or 1. Second, eight loci (Adh, α-Amy, Ars, Dip-2, Got-1, Had, Pgk and Sucr) are completely diverged between the two species and all but two of these (i.e., Adh and α-Amy) are <u>alternately</u> fixed between the two

Figure 3.9 Frequency distribution of unique (shaded) and total (open) alleles in D. melanogaster and D. simulans. Inset: frequency distribution of alleles with frequency $\leq$ 5%.

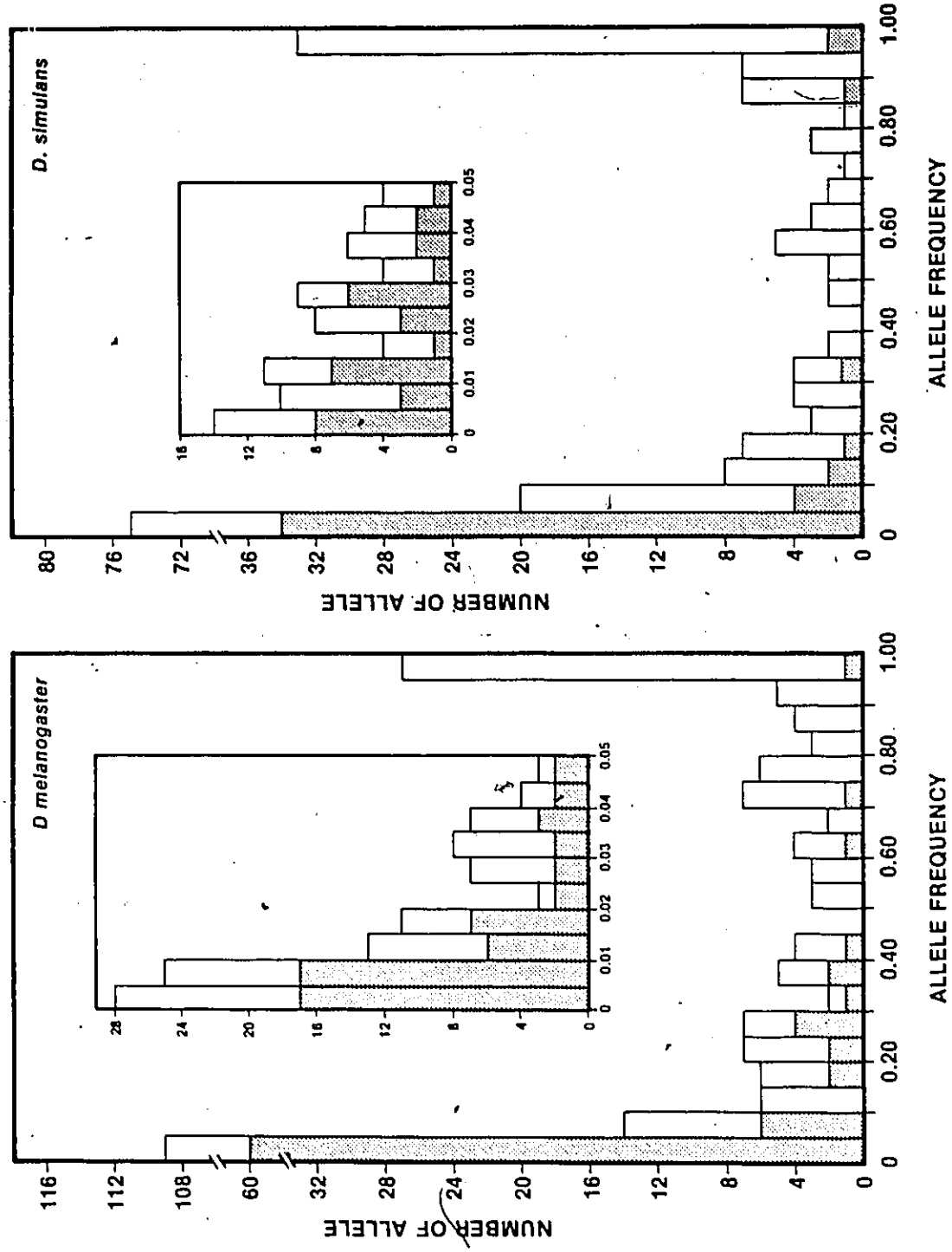
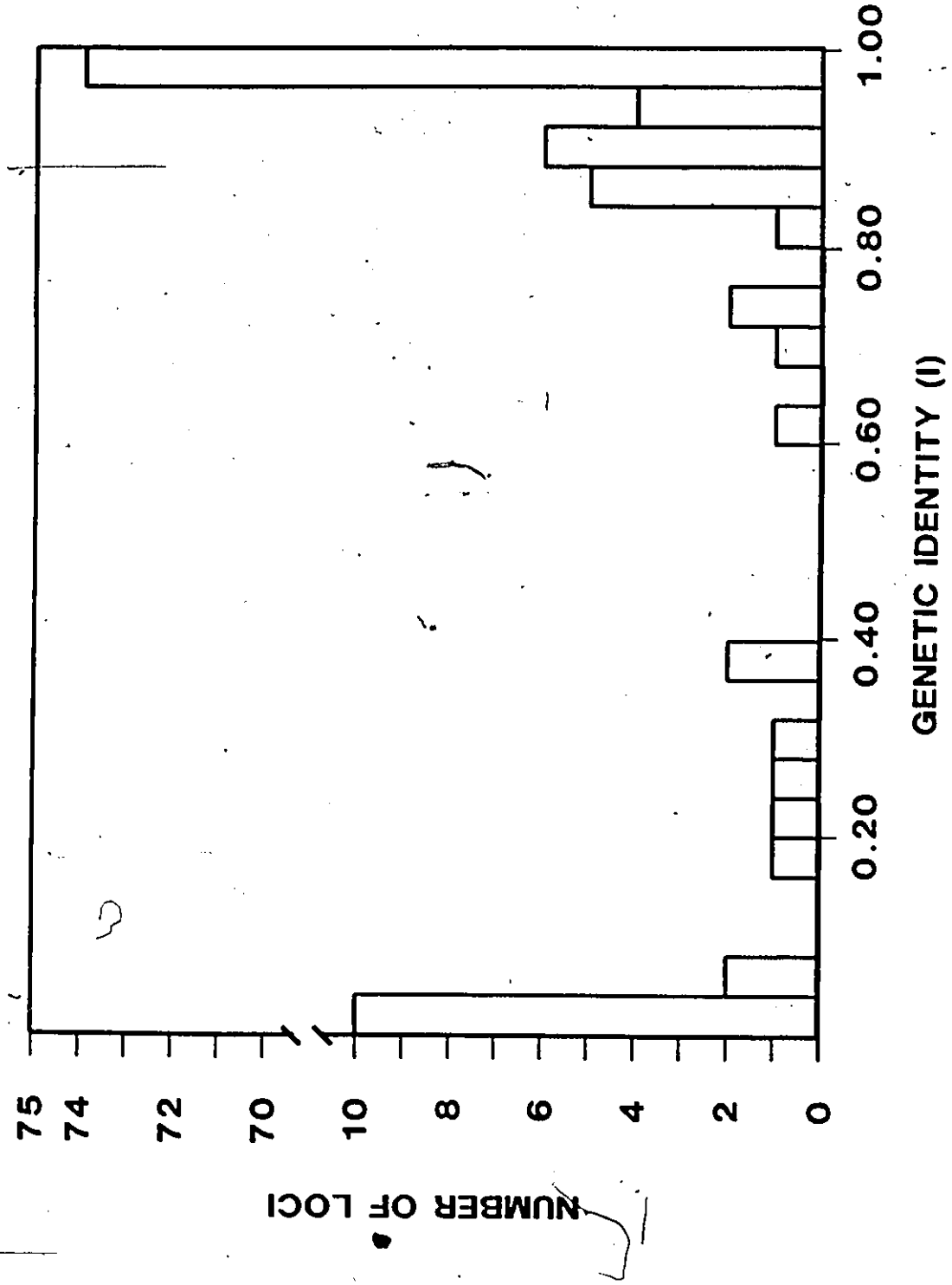

D. simulans
D melanogaster
NUMBER OF ALLELE
ALLELE FREQUENCY

Figure 3.10 Frequency distribution of single locus genetic identity (I) for all loci between D. melanogaster and D. simulans.


NUMBER OF LOCI
75
74
72
70
10
8
6
4
2
0
GENETIC IDENTITY (I)
1.00
0.80
0.60
0.40
0.20

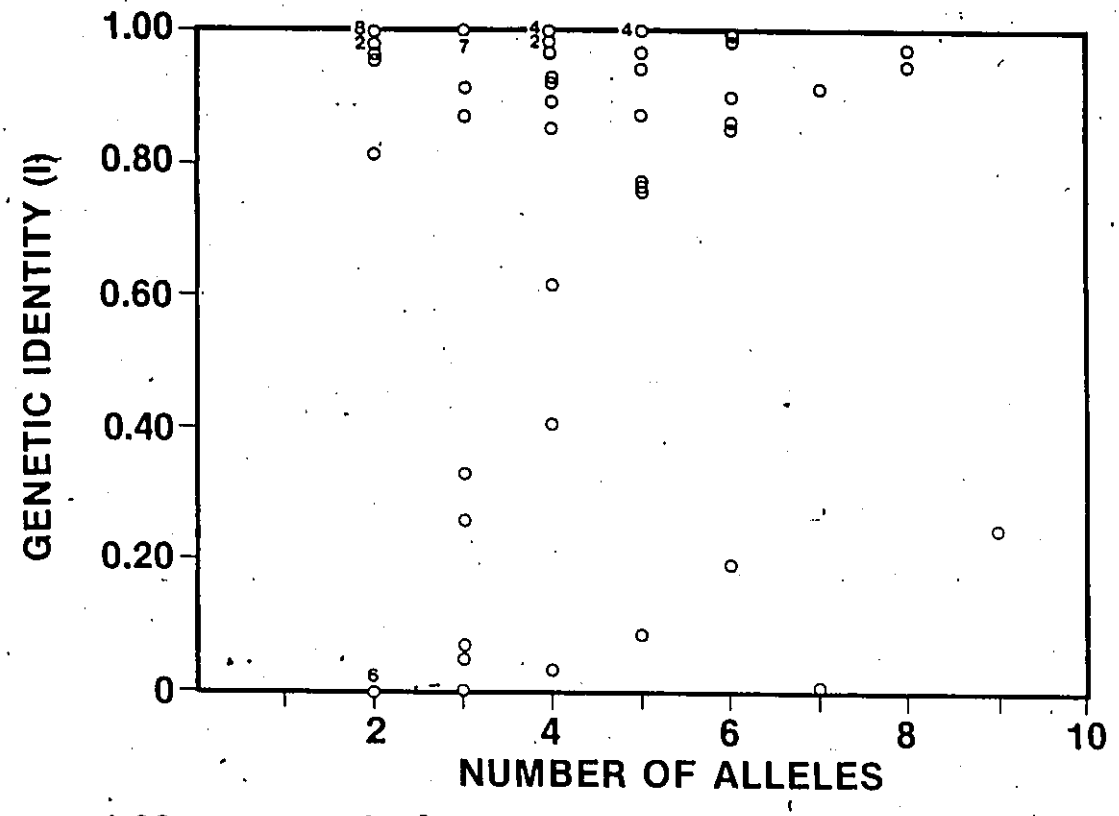
species. The present estimate of genetic distance, D = 0.179, between the two species is lower than the previously reported values (Eisses, Van Dijk, and Van Delden 1979; Gonzalez et al., 1982), but the proportion of loci showing nearly complete divergence between the two species (8%) is higher than all previously reported values.

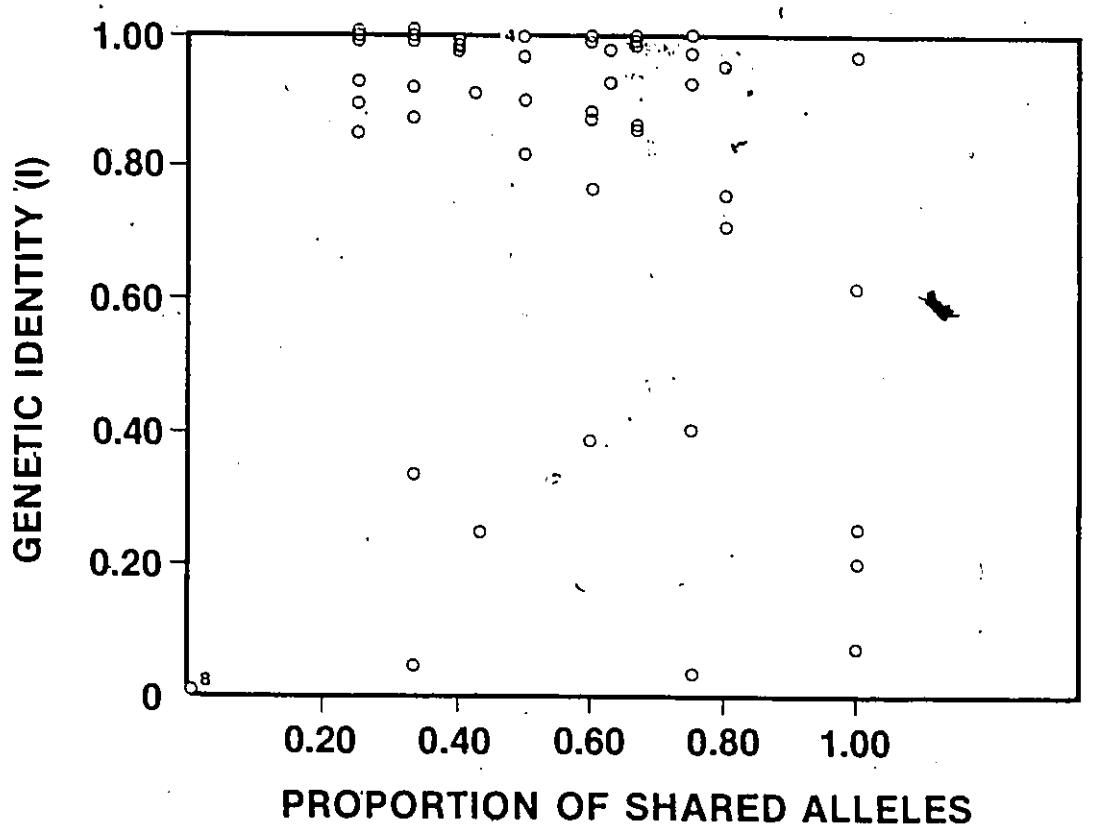
Genetic identity of loci between species can be affected by the number of shared alleles as well as their frequencies. To see whether number of unique alleles or differences in the frequency of shared alleles is the main cause of genic divergence between D. melanogaster and D. simulans, the genetic identity and number of alleles (shared as well as total) for polymorphic loci are plotted in Figure 3.11. The number of alleles (total or shared) seem to have no effect on genetic identity. Polymorphic loci seem to fall into two groups. The loci with high genetic identity are those which share, more or less with similar frequencies, the most common allele and differ only in their less frequent alleles. On the other hand, loci with moderate or low genetic identity exhibit variation in the frequency of the most common allele.

3.3.3 Population Structure and Species Divergence

It was shown that allozyme loci on various chromosomes had dissimilar levels of geographic

Figure 3.11 Correlation for genetic identity (I) and total number of alleles and genetic identity and proportion of shared alleles between D. melanogaster and D. simulans.

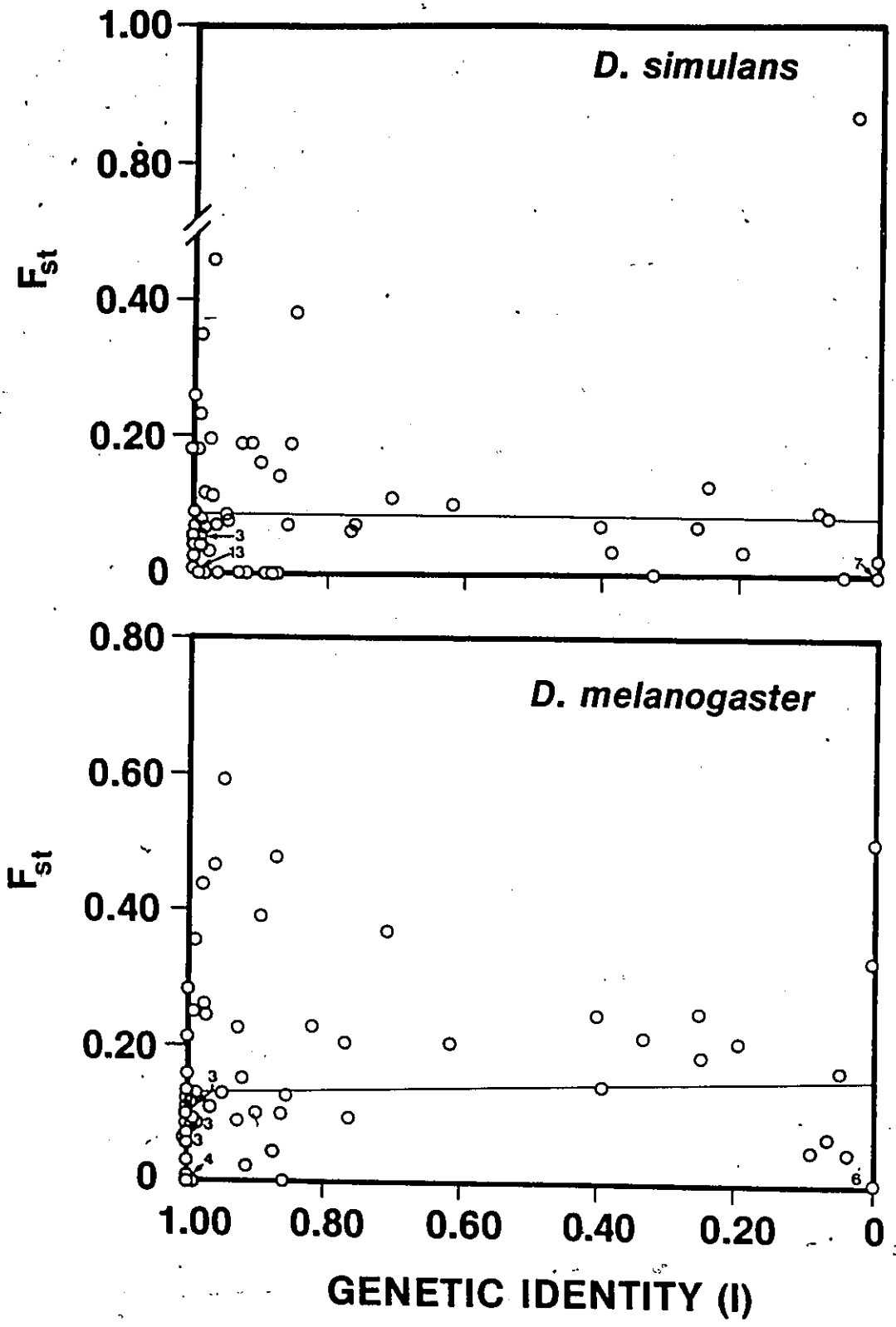

GENETIC IDENTITY (I)
1.00
0.80
0.60
0.40
0.20
0
2
4
6
8
10
NUMBER OF ALLELES


GENETIC IDENTITY (I)
1.00
0.80
0.60
0.40
0.20
0
0.20
0.40
0.60
0.80
1.00
PROPORTION OF SHARED ALLELES

differentiation between D. melanogaster and D. simulans (Table 3.12). The mean Fst for the I and II, but not for the III chromosomes differs significantly between the two species (Wilcoxon rank sum test, $p < 0.05$). The between-species differences in F_{st} remain significant when all loci are considered together. On the other hand, the mean number of alleles and mean heterozygosity for individual chromosomes are similar between the two species. These observations suggest that geographic populations of these two species are probably not experiencing the same evolutionary forces. In any case, differences in genetic structure, regardless of whether they are historical or due to natural selection (see discussion), can be expected to have an important bearing on the level of genic divergence between species.

The fixation indices of polymorphic loci within species and the genetic identities between species are plotted in Figure 3.12. Both species exhibit an identical pattern: polymorphic loci with low genetic divergence between species (or high genetic identity) appear to show high geographic differentiation within species. There are no loci which show both, high geographic differentiation within species and high genetic divergence between species. Of the eight loci that are completely diverged between the two species, two (Adh and α-Amy) are polymorphic in D.

Figure 3.12 Correlation for single locus genetic identity between D. melanogaster and D. simulans and single locus fixation indexes in the two species. For D. melanogaster: $Y = 0.129 + 0.020X$, correlation coefficient (r^2) = 0.057, $p > 0.05$. For D. simulans: $Y = 0.084 - 0.002X$, correlation coefficient (r^2) = 0.006, $p > 0.05$.


D. simulans
1.00
0.80
0.40
0.20
0
F_{st}
D. melanogaster
0.80
0.60
0.40
0.20
0
F_{st}
1.00
0.80
0.60
0.40
0.20
0
GENETIC IDENTITY (I)

melanogaster and only one (α-Amy) in D. simulans.

The patterns of within and between species differentiation shown in Figure 3.13 suggest that the main course of genic divergence between D. melanogaster and D. simulans has been for polymorphic loci to go through a state of monomorphism (alternate fixation) between species probably due to founder effect followed by appearance of new polymorphism in one or both species. Such a pattern of differentiation would strongly suggest that the overall level of genic divergence between D. melanogaster and D. simulans has been influenced less by the evolutionary forces that shape the pattern of variation within species and more by the species history, i.e. the speciation event and the subsequent population sizes. A positive correlation between F_{st} and genetic distance, on the other hand, would have meant that adaptative divergence within species was the cause of genetic divergence between species. The results presented in Figure 3.12 suggest that patterns of population differentiation within species may change rapidly and thus have no long-lasting effect on species divergence. This would appear to be the case in D. melanogaster and D. simulans whose present genetic structure is almost certainly the result of post-speciation and post-colonization geographic divergence.

4

DISCUSSION

4.1 Species Distribution and Their Ecology:

Drosophila melanogaster and Drosophila simulans are two of the eight cosmopolitan species in the genus Drosophila (Bock, 1980). The two species are morphologicaly almost identical except for the posterior process of their male genital arches (Sturtevant, 1920). Although widely spread, both species are restricted in occurrence in most parts of the world to urban or farm environments or other places in which natural habitats have been modified by humans, and therefore they are regarded as "domestic species" (Parsons, 1975, 1983). An exception to their commensal habits with humans is the African continent where the species are widespread in natural habitats, which is consistent with an African origin for the 'melanogaster subgroup' (David and Tsacas, 1981). Although the two species co-occur extensively and show worldwide adaptation to both temperate and tropical conditions (David and Tsacas, 1981; Lemeunier et al., 1985), D. simulans is less successful in the temperate regions (McKenzie 1974; McKenzie and Parsons, 1974a). Neither species is able to

survive over winter and both are reintroduced annually from man made habitats. Their demographic profiles are similar in sympatric but different in allopatric populations, thus apparently showing a parallel response to environmental variations (David et al., 1984). Except for their response to alcohol, both species appear to utilize similar environmental resources (McKenzie and McKechnie, 1979).

In view of these similarities, one would expect to find similar the levels of genetic variation within populations and the geographic differentiation between populations of the two species. While similar latitudinal variations have been reported for a few phenotypic and physiological characters (Tantawy, Mallah and Tewfik, 1964; David and Bocquet, 1975; Parsons, 1980) as well as for allozymes (Anderson and Oakeshott, 1984), previous studies (Hyytia et. al., 1985; Watada, Tobari and Ohba, 1986; Singh, Choudhary and David, 1987) have shown that D. simulans differs from D. melanogaster in having less genic heterozygosity within populations and less geographic differentiation between populations. These results from the present study show that while both species have about the same level of mean heterozygosity and mean number of alleles, D. simulans still shows significantly less geographic differentiation than D. melanogaster. More loci are polymorphic in D. melanogaster than D. simulans (52%

vs. 39%: $t = 1.989$, $p < 0.05$), but the polymorphic loci of former are on the average less heterozygous than those of the latter (Table 3.9). The present results suggest that previous reports of low heterozygosity in D. simulans may have been due to sampling too few loci or populations, although the earlier studies were mostly based on North American populations which are not included in the present study.

The high genetic differentiation between Seychelle and mainland D. simulans requires an explanation. The high heterozygosity and the presence of unique allozyme alleles, together, rule out the possibility that high genetic differentiation of Seychelle is due to a recent founder effect. It has been shown that D. simulans from Madagascar island is also significantly differentiated from the mainland (Hyytia et al., 1985). These results suggest that D. simulans in Seychelle and Madagascar has been isolated from the mainland for a long period of time and may even represent an ancestral race quite different from the one that gave rise to the mainland D. simulans. These speculations are supported by the results from work on mitochondrial DNA (Solignac and Monnerot 1986; Hale and Singh, unpublished). D. simulans from Seychelle has a mitochondrial DNA variant which is quite different from that found in the mainland but very similar to the one

found in Drosophila sechellia and Drosophila mauritiana (Solignac and Monnerot, 1986). The high genetic differentiation between Seychelle and mainland D. simulans appears to be due to their long isolation, and so in the following the comparison of genetic structure between D. simulans and D. melanogaster would be limited to their continental populations.

4.2 Patterns of Gene Flow

The variation in genetic structure between D. melanogaster and D. simulans requires an explanation. The most obvious explanation for differing amount of geographic differentiation between two species is different amount of interpopulation gene flow. All other things being equal, species with less gene flow would show more geographic differentiation. Gene flow should be an important consideraton in the present case as all genetic and phenotypic markers that have been studied, show less geographic differentiation in D. simulans than D. melanogaster. Estimates of Nm based on F_{st} values (F_{st} = 1/4Nm+1: Wright, 1931) show that to explain the differences in the level of geographic differentiation between D. melanogaster and D. simulans would require a 2-4 fold lower gene flow in the former than the latter species. However, the estimates of Nm based on the distribution of rare

alleles (Slatkin, 1985) are every similar in these species, ranging from 1.27 to 3.09 in D. simulans and from 2.74 to 5.83 in D. melanogaster (Singh and Rhomberg, 1987a). Furthermore, D. melanogaster enters human habitat more readily than D. simulans (J. R. David, personal communication) suggesting that the level of gene flow in D. melanogaster should be more, and not less, than in D. simulans. Thus the differences in the level of gene flow in the two species are, in the wrong direction and can not explain the differences of geographic differentiation between the two species. In the following we discuss several hypotheses which individually or in combination can explain the differences of geographic differentiation. A brief description of these hypotheses has been presented elsewhere (Singh, Choudhary and David, 1987); in the following, we consider them in detail.

4.3 Hypotheses of Genetic Structure:

4.3.1 The Mutator-gene Hypothesis:

Dowsett and Young (1982) have recently shown that the genome of D. melanogaster has three times more middle repetitive DNA than that of D. simulans. Most of the repetitive DNA sequences in D. melanogaster are "nomadic", i.e. occupy widely dispersed chromosomal location (Dowsett and Young, 1982) but in D. simulans such sequences are

restricted to single chromosomal locations. A seven fold difference has been observed in the nomadic DNA content between the two species and it is thought that it may contribute to relatively higher mutation rates in D. melanogaster and explain its relatively large amount of genetic variation. In an attempt to explain the lack of inversion polymorphism in D. simulans, Woodruff and Ashburner (1978) have shown that X-ray induced chromosome aberrations and their survival are as frequent in D. simulans as in D. melanogaster. In other words, D. simulans is able to repair X-ray induced breakage and, therefore, does not lack repair enzymes, but it may lack "breakage inducing factors" (Voelker, 1974; Yamaguchi, Cardellino and Mukai, 1976) or mutator loci (Green, 1976) which appear to be common in D. melanogaster. Preponderance of transposable elements in D. melanogaster and their absence or low frequency in D. simulans (Bregliano and Kidwell, 1983; Brookfield, Montgomery and Langley, 1984) would certainly seem to support the view that higher mutation rate in D. melanogaster means greater genic variation. However, while the mutator-gene hypothesis may explain the higher proportion of polymorphic loci in D. melanogaster and lack of inversion polymorphism in D. simulans, it cannot explain why the two species show different degrees of geographic differentiation. Also it

is not obvious how can these genomic mechanisms have an effect on the allozyme variation per se.

4.3.2 The Neutral-mutation Hypothesis:

An expectation to find similar pattern of geographic variation in the two species was based on the implicit assumption that allozyme variation is representative of genes enabling these species to achieve their worldwide distribution and climatic adaptation. But the fact may be otherwise, i.e. allozyme variation may not be representative of the genetic variation which is the basis of similar adaptation in these species. In other words, allozymes may be irrelevant or neutral with respect to climatic adaptation. This hypothesis does not seem tenable as reduction in variability and/or geographic differentiation in D. simulans is also observed for chromosome inversion (Ashburner and Lemeunier, 1976), morphological and quantitative variation (David, Bocquet and De Scheemaeker-Louis, 1977; Hyytia et al., 1985; Watada, Ohba and Tobari, 1986), middle-repetitive DNA variation (Dowsett and Young, 1982), 2DE proteins (Ohnishi et al., 1982) and mitochondrial DNA restriction length polymorphism (Baba-Aissa and Solignac, 1984; Hale and Singh, 1985). Since all genetic elements consistently show, on the average, less geographic differentiation in D.

simulans than D. melanogaster, and since the levels of gene flow appear to be similar in the two species, the neutrality argument, even if accepted for the majority of protein variation within the individual species, cannot adequately explain the varying levels of geographic differentiation between these species.

4.3.3 The Population Bottleneck and Recent Colonization Hypothesis:

Genetic variation in colonizing species appears to be lower than those of comparable non-colonizing species (Parsons, 1983). In the past, a population bottleneck has been invoked to account for low level of variation in a diverse variety of organisms (Haigh and Maynard Smith, 1972; Schwaegerle and Schaal, 1979; Bryant, Van Dijk and Van Delden, 1981; Choudhary and Singh, 1987a). That colonization has reduced the overall amount of genetic variation is evident from the fact that in both species ancestral populations from tropical Africa show more genic diversity and more private alleles (Lemeunier et al., 1985; Singh, Hickey and David, 1982; Hyytia et al., 1985; Singh, Choudhary and David, 1987, and the present study). Theoretical studies show that while loss of alleles largely depends on the bottleneck size, reduction in heterozygosity heavily depends on the rate of population growth after the

bottleneck. If the rate of population growth is very high, a relatively high level of heterozygosity can be maintained even in the face of an extreme reduction in population size (Nei, Maruyama and Chakraborty, 1985; Motro and Thomson 1982; Maruyama and Fuerst, 1985a,b). With two weeks per generation, both D. melanogaster and D. simulans are capable of a rapid population expansion. But neither heterozygosity nor number of alleles per locus give any indication of a recent bottleneck in D. simulans. In this respect, the only relevant difference between the two species is in the proportion of polymorphic loci, which can be satisfactorily explained by assuming higher rate of mutation in D. melanogaster. In fact, if we use a more stringent criterion of polymorphism (i.e. the most common allele being 95% or less), then the proportion of polymorphic loci is about the same in the two species (39 in D. melanogaster and 34 in D. simulans). However, if it could be shown that D. simulans has become cosmopolitan much more recently without entailing a severe bottleneck, then the low geographic differentiation can be explained simply by arguing that D. simulans has had less time to equilibrate with its physical and biotic environment than D. melanogaster.

4.3.4 The Niche-width Hypothesis:

According to this hypothesis the amount of variation in a species is proportional to the niche-width of that species (Van Valen, 1965; Rothstein, 1973). It is argued that a species with a wide spectrum, for example, food resources or environmental ability to adapt to different environmental conditions, is more likely to have more variety of genotypes than one with a narrow spectrum of food resource or environmental adaptation. Thus, arguing for the adaptive significance of chromosomal polymorphisms in Drosophila willistoni, Dobzhansky and his colleagues proposed that populations which exploit a greater variety of ecological niches are more polymorphic than populations restricted to a narrow range of ecological opportunities (Da Cunha and Dobzhansky, 1950; Da Cunha et al., 1959). A laboratory demonstration of association between genetic variation and niche variation was provided by Powell and his colleagues by showing that the mean number of alleles and the mean heterozygosity were higher in populations maintained in heterogeneous environment (variable food types and temperature) than in constant environment (Powell, 1971; McDonald and Ayala, 1974; Powell and Wistrand, 1978). Steiner (1977) has shown an association between the amount of genetic variability and the number of oviposition sites in the Hawaiian Drosophila, and Lacy (1982) has shown an association between

electrophoretic variation and the diversity of host (mushroom) species in the mycophagus Drosophila. Because of its ecological relevance and its ability to maintain genetic variation without genetic load, the niche-width model has a wide appeal among population biologists (e.g. see, Johnson et al., 1969; Hamrick and Allard, 1972; Taylor and Powell, 1977; Cleide, Albuquerque and Napp, 1981) although it is not without criticism (e.g. see Wilson, 1969; Soule and Stewart, 1970; Hallett, 1980, Atkinson and Miller, 1980).

The relationship between genetic variation and niche-width has also been considered in D. melanogaster and D. simulans (Berger, 1971; Johnson and Schaffer, 1973; Minawa and Birley, 1975; Cleide, Albuquerque and Napp, 1981). MacKay, (1981) showed that additive genetic variance of body weight and sternopleural chaeta number (but not of abdominal bristle number) was significantly greater in populations experiencing environmental heterogeneity. Another study showing correlation, although of a tenuous nature, between genetic variation and niche-width is that of Blaylock and Shugart (1972) and Shugart and Blaylock, (1973). Working with inbred strains of D. simulans and D. melanogaster, these investigators showed that the genetic variability induced by low levels of radiation was positively correlated with population density

which they used as a measure of niche-width.

D. melanogaster is reported to be physiologically and behaviourally more flexible than D. simulans (see Parsons, 1975, 1983 for review) but the niche-width variation that appears to be of major significance here is one of a difference in their range of climatic adaptation. Although both species are cosmopolitan and occur together extensively, D. melanogaster is more successful than D. simulans in the temperate regions. Temperature is an important factor for numerical changes in both species, but on a seasonal basis D. melanogaster builds up large populations in the spring or early summer, and D. simulans in the late summer (Parsons, 1975). D. melanogaster can tolerate wider temperature fluctuations than D. simulans (McKenzie, 1974; McKenzie and Parsons, 1974a; Parsons, 1979). Therefore, the difference in the levels of their geographic differentiation could be a result of their varying capacity for macroclimatic adaptation.

4.3.5 The Selection Hypothesis:

In contrast to the niche-width hypothesis, which states that the reduction in the level of geographic differentiation in D. simulans may be due to its comparatively narrow niche, the selection hypothesis states that the low geographic differentiation in D. simulans may

result from a selection 'strategy' favouring broad adaptability of "a single purpose genotype" (cf. Baker, 1965). Under this hypothesis both species are assumed to have similar niches but they differ in their genetic mechanisms of dealing with it. Species with similar niche-width may have qualitatively different genetic strategies (Angus and Schultz, 1979; Jaenike, Parker and Selander, 1980; Lynch, 1983; Parker et al., 1977), i.e. some may respond to the environmental gradient as fine-grained while others react as coarse-grained with resultant selection, respectively, for or against polymorphism independent of niche width. For example, both D. melanogaster and D. simulans may respond to a temperature gradient, however, the former may strongly react to incremental changes and consequently show altered gene frequency but the latter may react less strongly with no significant alteration of gene frequency or may even respond to the same range of temperature as "acceptable" with no consequent polymorphism. The observation of fewer clines in gene frequency in D. simulans than in D. melanogaster provides evidence for this scenario. An environment in which D. melanogaster occurs exclusive of D. simulans is that of alcohol-associated resources (McKenzie, 1974; McKenzie and Parsons, 1974b; McKenzie and McKechnie 1979). On the other hand, in certain places D. simulans has displaced D.

melanogaster for reasons as yet unknown (Hoenigsberg, 1968). However, besides differences in attraction to alcohol and light dependent dispersal (McDonald and Parsons, 1973; Parsons, 1974), little is known about their natural environment, which may not be all that different as the sympatric populations of these species appear to possess similar amount of genetic variation. Therefore, if the selection hypothesis is relevant in this case, the differences in their natural environments must be of spatial nature (e.g. geographic and/or climatic) which would explain why they have similar levels of heterozygosity within populations but different levels of differentiation between populations. An observation in favour of selection hypothesis and against recent bottleneck is that while genes on all the three major chromosomes i.e. X, 2 and 3, are equally heterozygous in both species, only chromosome X and the second chromosomes show significant reduction in geographic differentiation of D. simulans relative to D. melanogaster (Table 3.6).

The above hypotheses are not mutually exclusive but they differ in their role to account for the differences of genetic structure between the two species. In a preliminary report of this study (Singh, Choudhary and David, 1987) we concluded that bottleneck effect and niche-width appear to be the leading hypotheses to account for

the differences in both the amount and the pattern of genetic variation between these species. This conclusion was based on the observation that D. simulans has lower heterozygosity than D. melanogaster. The present results show that the major difference in the genetic variation of these species is not in the heterozygosity or number of alleles per locus but only in the levels of genetic differentiation between populations. These conclusions are supported by the study of 2DE proteins in these species (Coulthart and Singh, 1987a,b). Thus, these results appear to rule out the role of population bottlenecks. If the role of bottleneck can be satisfactorily ruled out, then we must conclude that variation in niche-widths and/or genetic "strategies" of adaptation are the major contributing factors to the varying levels of geographic differentiation in these species. More work is required before we can choose between these two hypotheses.

4.4 Level of Genic Divergence Between D. melanogaster and D. simulans and Its Implications for Genetic Theories of Species Formation

4.4.1 The Genetic Distance and Evolutionary Time:

The genetic distance between D. melanogaster and D. simulans ($D = 0.179$) can be converted to chronological time (t) by using the formula, $t = D/2\alpha$, where α is the rate of

amino acid substitution per year for electrophoretically detectable alleles (Kimura and Ohta, 1971; Nei, 1975). Using $\alpha = 10^{-7}$ (Nei, 1975), the divergence time between D. melanogaster and D. simulans is estimated to be 0.9 Myr, which is lower than the range of values, 2.8 Myr to 13 Myr, previously obtained from a variety of DNA sequence data (Bodmer and Ashburner, 1984; Cohn, Thompson, and Moore, 1984; Easteal and Oakeshott, 1985). The above divergence time based on the DNA data are not corrected for polymorphism within species. Correcting for the effect of polymorphism, Stephens and Nei (1985) calculated the time of divergence between D. melanogaster and D. simulans to be 2.0 - 3.5 Myr, which is still higher than the estimate based on the protein data. The disagreement between the divergence times based on protein vs DNA data may be due to several factors, and as shown below, the divergence time based on protein data may probably be smaller than 0.9 Myr. The mutation rate, α (= $nc\lambda$) depends on three factors, the average number of amino acids per polypeptide (n), proportion of amino acid substitutions that are detected by electrophoresis (c), and the average rate of amino acid substitution per year (λ). All these factors and hence the value of α may vary among loci. For example, the rates of amino acid substitutions between different classes of protein are known to vary thousand fold (McLaughlin and

Dayhoff, 1972). The value of α for intracellular proteins is ten times smaller than that for extracellular proteins (King, 1973). The value of $\alpha = 10^{-7}$ is based on n = 400, $\lambda = 1 \times 10^{-9}$ and c = 0.25 (Nei, 1987). Ramshaw, Coyne and Lewontin (1979) and McLellan (1984), working with allelic variants of proteins of known amino acid sequence, have shown that gel electrophoresis can detect up to 90% of the allelic variants. Thus, if we use a value of c = 0.90, $\alpha = 3.6 \times 10^{-7}$ and the divergence time, t = 0.27 Myr, which is ten times smaller than the mean divergence time based on the DNA sequence data. Non-linearity of D with time would not be a factor in the present case as only a small fraction, if any, of the allelic substitutions between sibling species are likely to be multiple substitutions. The 0.27 Myr estimate of divergence time based on allozyme data may need correcting upward due to the fact that our value of D between *D. melanogaster* and *D. simulans* may be an underestimate, and downward due to the fact these species have gone through bottlenecks that would inflate the estimate of D. Thus, keeping in mind the various factors that can affect D, the estimate of 0.27 Myr divergence time based on allozyme data is significantly smaller than that on the DNA data. These results lead us to conclude that purifying selection has been an important factor in the evolution of proteins.

Nei and Tateno (1975) studied by computer simulation the distribution of single-locus gene identity under the assumption of selective neutrality. Their results show that the distribution of genic identity is inverse L-shaped in the early generations and it becomes U-shaped in the later generations. To see whether the distribution of the genetic identity observed between D. melanogaster and D. simulans (Fig. 3.10) is consistent with the neutral mutation theory, the data was compared with the theoretical distributions obtained by Nei and Tateno (1975). The comparison shows that our result is similar to the expected distribution of genic identity for generation 4N; the observed mean genetic identity between D. melanogaster and D. simulans is 0.832 compared to 0.811 in the simulation study. Of course, the similarity of the observed and theoretical distributions can not be taken as an evidence of selective neutrality because even under natural selection such an agreement is possible except that the attainment of the observed distribution would be delayed (under balancing selection) which would affect the divergence time. Natural selection is not likely to alter the shape of the identity distribution very much. Thus, we cannot test selective neutrality by matching observed and theoretical distributions of genetic identity unless we can obtain independent estimates of effective population size

(or divergence time). Such estimates are presently not available for D. melanogaster and D. simulans.

4.4.2 Genic Divergence and the Geographic Models of Species Formation:

The Neo-Darwinian view of speciation (Charlesworth, Lande, and Slatkin, 1982; Wright, 1980) suggests that reproductive isolation originates by gradual processes when geographic populations are subjected to divergent selective pressures in separate habitats. Such geographic speciation usually results in species that differ in morphology, cytology, and ecology (Mayr, 1942, 1963; Dobzhansky, 1951; Stebbins, 1977). Numerous studies have shown that the levels of genetic divergence among the species of closely related groups generally correlate with their differences in morphology, ecology, and degree of reproductive isolation (e.g., Clausen, 1951; Ayala, 1975; Futuyma and Mayer, 1980; McNeill and Jain, 1983; Warwick and Gottlieb, 1985; Wake and Yanev, 1986). These findings are consistent with the hypothesis that species diverge gradually as they adapt to their geographically isolated habitats.

Species which arise by gradual means are expected to differ at a large number of their gene loci (Mayr, 1954, 1963, 1982). During the last two decades, numerous investigators have used gel electrophoresis to answer the

question: how many gene substitutions are required for a new species to be formed from an ancestral population? Reproductive isolation with little or no changes at allozyme gene loci have been found in birds (Avise and Aquadro, 1982), fish (Avise, Smith, and Ayala, 1975; Kornfield et. al., 1979), Drosophila (Sene and Carson, 1977), and plants (Gottlieb, 1974; Gottlieb and Pilz, 1976; Crowford and Smith, 1983; Snyder and Linton, 1984), indicating that high genetic similarity does not always indicate conspecific status. On the other hand, there are cases where species show extensive amount of allozyme divergence but are not reproductively isolated (Johnson, Stine, and Murrary, 1977; Ochman, 1987). Taken together, these findings suggest that very little genetic differentiation is required in species formation (Hubby and Throckmorton, 1968), although Singh (1983) has argued that in the early studies that the level of genic divergence between species may have been underestimated.

In view of the foregoing what significance can be attached to the 10% of the gene loci that are completely diverged between the two species? Assuming that Drosophila genome has a minimum of 5000 genes (Judd, Shen and Kaufman, 1972), the present results suggest that 500 genes would have been gone through complete substitution between D. melanogaster and D. simulans. Let us further assume that

only a small fraction, say 10%, of the diverged loci are relevant to speciation; this comes to 50 genes. Involvement of 50 genes in the origin and development of reproductive isolation may appear to be a large number, yet it accounts for only 1% of the total genome. So it should not be surprising that in the past in most studies of species divergence, 80% of which have employed less than 30 loci (Singh and Rhomberg, 1987), mostly quantitative and very little qualitative (i.e., alternate fixation) differences have been observed (Hubby and Throckmorton, 1968; Prakash, 1969, 1977; Ayala and Powell, 1972; Ayala, 1975; Avise, 1976; Gonzalez et. al., 1982; Singh, 1983).

There is another factor, geographic differentiation of natural populations, that may affect the level of genic divergence observed between species. Although the potential effects of genetic structure on the speciation ability of a taxon vary with the mode of speciation (Templeton, 1980), population structure is a major determinant in the shifting-balance theory of evolution (Wright, 1931, 1980). Templeton (1980) has argued that if speciation occurs via founder effect or sympatrically, the population structure of the ancestral species may be of great importance. What is not clear, however, is whether geographic structure within species would promote genetic divergence between species. D. melanogaster and D.

simulans differ in their levels of geographic differentiation (Choudhary and Singh, 1987b), but loci with high genetic structure (i.e., high F_{st} value) within each species are not the ones which show high divergence between species (see Fig. 3.12). Thus, at least in this species pair, genetic structure within species appear to have played no role in the level of genetic divergence between species. Such within - vs between -species comparison of genetic divergence are essential before we can choose among the various geographic and genetic models of species formation.

4.4.3 Genetic Models of Species Formation:

The lack of substantial genic divergence between species showing partial and in some cases complete reproductive isolation has led to the view that speciation does not require the large amount of genetic changes envisaged in Mayr's theory of speciation by "genetic revolution" (Mayr, 1954, 1963). The current views appear to be and this is mainly due to the selection-neutrality controversy, that regardless of the level of genetic changes involved the gene-enzyme variation is probably irrelevant to speciation. This view is clearly presented in the Carson's founder-flush theory of speciation (Carson, 1975). Carson (1975) has proposed that a species is

organized into a complex genetic system consisting of two kinds of variability systems, open and closed. The open variability system consists of genetic variation at polymorphic loci, including electrophoretic, polygenic, clinal and subspecific variation which segregate within and between species. The closed variability system, according to Carson, consists of internally balanced blocks of genes locked into strong epistases and strong linkage disequilibrium, barring their segregation within species. Speciation requires changes (disorganization) in the closed variability system by stochastic processes such as a founder effect, a population crash, an interspecific hybridization or sudden release from the constraints of natural selection as in a period of population flush (Carson, 1975). During this phase the closed variability system undergoes genetic recombination over a small number of generations and rapidly produces recombinant genotypes. Soon thereafter, the rebuilding (reorganization) of a new genetic system starts in the isolated populations. The restructuring of the gene pool occurs by natural or sexual selection and requires altered genetic and sexual environments. The altered genetic system would have the potential of forming a novel behavioural syndrome in the population which at some later time might be recognized as a new species (Carson, 1986). Among the Hawaiian

Drosophila for which the founder-flush speciation theory was first invented, there are cases where different species clearly show ethological isolation independent of post mating isolation (Kaneshiro, 1976, 1980; Dodd and Powell, 1985). Other studies also indicate the lability of mate recognition systems in Drosophila when populations experience dramatic genetic changes such as parthenogenesis (Carson, Teramoto, and Templeton, 1977) and interspecific hybridization (Wallace, Timm, and Strambi, 1983).

A similar model, genetic transilience, has been proposed by Templeton (1980, 1981). However, Templeton's model differs from Carson's founder-flush model with respect to the genetic system required for speciation. Templeton's model requires that the ancestral populations be reproductively outcrossed and polymorphic for coadapted gene complexes centered around major loci. The founding event would allow these major genes to be fixed by chance in a founder population. These alterations at a few major loci, would result in a drastic change in fitness due to their altered phenotypic effects. Because of this change in genetic environment (fixation of a few major loci), strong selective forces would come into play shortly after the founding event. However, in order to respond to an altered selection pressure, the founder population uses a large number of genetic variation at numerous modifier

loci. Since in many species including Drosophia founder events are much more common than speciation (Parsons, 1983), the above mechanism is likely only when the founder event is extreme (i.e. a reduction to a very small population size) and is followed by a rapid expansion in population size (Templeton, 1980; Carson and Templeton, 1984). However, biogeographic and ecological conditions favourable to speciation do not seem to be common. Moreover, given appropriate conditions, the chances of a genetic transilience still depend upon several innate traits of the ancestral species. For example, the founders must be drawn from a large panmictic ancestral population, otherwise a founder effect has little genetic impact and speciation is unlikely. Also the founder population must have an open genetic system in order to recognize and respond to the genetic impact. Here Templeton's theory strikingly differs from Carson's theory which assigns no role to open genetic variability system in species formation. Templeton has argued that the simultaneous satisfaction of all these conditions is highly unlikely, and genetic transilience must be regarded as a rare mode of speciation (Templeton, 1986).

While the "founder-flush" and "genetic transilience" theories differ in a fundamental way with respect to the importance of genetic variation within

species to development of reproductive isolation between species, they are similar in invoking roles of coadapted gene complexes consisting of a relatively few major genes. By so doing both theories can account for rapid speciation without much genetic divergence as appear to be the case in the Hawaiian Drosophila (Carson and Templeton, 1984).

4.4.4 Molecular Mechanisms of Speciation:

Recent advances in molecular biology especially the unfolding of the genome (i.e. the study of the structure and organization of the genome), have led to the proposition of a number of molecular mechanisms of speciation (see reviews: Rose and Doolittle, 1983; Krieber and Rose, 1986). These mechanisms can be grouped into three main classes: Genomic Disease Model, Mechanical Incompatibility and Genome Resetting.

In the "Genomic disease" model (Rose and Doolittle, 1983; Ginzburg, Bingham and Yoo, 1984), an isolated population is assumed to lose or fail to acquire mobile genetic elements (transposons) that are present in the rest of the species and the isolated population may lose property in establishing immunity to these elements. Therefore, crosses between individuals from isolated populations could lead to defects in their hybrids, and establish post-zygotic reproductive isolation. This model

is based on the observations of deleterious effects of hybrid dysgenesis in D. melanogaster (Kidwell, Novy and Freeley, 1981; Ginzburg, Bingham and Yoo, 1984). Parallels have been drawn between the P-M hybrid dysgenesis and the D. melanogaster / D. simulans hybrid pathologies (Engels and Preston, 1979; Periquet, 1981) and between the I-R hybrid dysgenesis in D. melanogaster and the hybrid pathologies exhibited by crosses between the sibling species of the D. pseudoobscura group (Kidwell and Novy, 1979).

However, there are striking differences between the dysgenic sterility and the interspecific hybrid sterility. First, dysgenic sterility is normally observed only when flies are grown at high temperatures, while interspecific hybrid sterility occurs at normal rearing temperature. Second, both P-M and I-R dysgenesis in D. melanogaster have more drastic effect in females (Kidwell, Kidwell, and Sved, 1977) while hybrid sterility in Drosophila is much more common in males (Bock, 1984). Furthermore, the parallels break down when other species in the D. melanogaster groups are compared (Coyne, 1985). The genetic divergence leading to developmental anomalies in interspecific hybrids is more advanced in the older species pair, D. melanogaster / D. simulans, than in the younger pair, D. melanogaster / D. mauritiana. Coyne (1986) found no evidence for elevated

rates of male recombination in species hybrids thus negating the role of hybrid dysgenesis like elements in reproductive isolation. In addition, genetic analyses of hybrid male sterility in D. melanogaster (Coyne, 1984, 1985; Coyne and Kreitman, 1986; Coyne and Charlesworth, 1986), D. pseudoobscura (Dobzhansky, 1936, 1951; Orr, 1987), and many other Drosophila (Sturtevant and Novitski, 1941; Naveira and Fontdevila, 1986) and other insects (Curtis, 1982; Grula and Taylor, 1980) has shown that hybrid male sterility is due to many genes, with X-linked loci making the largest contribution to sterility. These results are difficult to explain if mobile genetic elements were the leading cause of reproductive isolation. While still not definitely discarded, the hypothesis that phenomena similar to hybrid dysgenesis cause speciation has little support from recent findings.

The "Mechanical genome incompatibility" model (Burr and Schimke, 1980; Dover, 1982) requires a large amount of interpopulation differences in dispersed repetitive DNA sequences. These differences could result from random amplification of pre-existing sequences initially rare in the parent species, with accidental amplification of different sequences leading to incompatibility between populations. These changes may disrupt chromosomal interaction (i.e., loss or reduction in meiotic recognition

and not the gross chromosomal imbalance) in such a way that hybrids are sterile. There are various molecular processes, such as transposition, unequal crossover and gene conversion, which alter the locations, numbers, and sequences of clustered and dispersed repetitive DNAs (Burr and Schimke, 1980; Murray, Peters and Thompson, 1981). Dover (1982) has integrated them into a single phenomena, called "Molecular drive". The model assumes that rates of repeated-sequence family homogenization within genomes are slower than the rates of randomization between genomes. If this was the case, then new sequence variants may be fixed within large families.

The third model, "Genome resetting", also depends upon non-coding repetitive DNA element but requires at least some of these elements to be functional. They may be regulatory elements whose positions and sequences control the expression of other structural genes in developmental pathways in a complex and coordinated fashion (Britten and Davidson, 1969; Davidson and Britten, 1979; Raff and Kaufman, 1983). The changes in positions and sequences of these regulatory elements could alter early developmental pathways, leading to rapid divergence between populations (Wilson et. al., 1977; Gould, 1977; Hilu, 1983). In this mode of evolution it is obvious that speciation is a macromutational process, regulatory genes play vital role,

and few genes are involved in speciation.

This hypothesis is quite similar to the one proposed by Goldschmidt (1940) in which macromutations were supposed to be the cause of speciation. In present day terminology these macromutations are to be changes in regulatory elements (Wilson, Maxon and Sarich, 1974; Wilson, Carson and White, 1977) and changes in a few regulatory genes account for evolution at and beyond the species level (Gould, 1977). Both chromosomal rearrangements (White, 1973, 1978) and point mutations at critical position in the DNA sequence (Beckwith and Rossow, 1974) have been proposed as mechanisms of altering regulatory genes. Many closely related species differ in their metaphase chromosome in the amount and distribution of heterochromatin (Baimai, Sene, and Pereira, 1983; Ahearn and Baimai, 1987; Gupta and Kumar, 1986). Several studies have shown that rate of change in chromosomal rearrangements is strongly correlated with rate of speciation (White, 1973, 1978; Baker and Bickham, 1986; Thompson and Sits, 1986; Davenport, 1986; Dev and Rai, 1986; Bush, 1981); King, 1981) but this correlation is likely to be due to small population size and hence not a causation as there are many closely related (or sibling) species whose polytene chromosomes are homosequential (Carson and Stalker, 1968; Clayton, Carson, and Sato, 1972;

Chang and Carson, 1985).

4.4.5 Future Prospect: Study of Reproductive Characters

It is very likely that one genetic mechanism may not be sufficient to explain speciation in all diverse groups of organisms, but it is becoming apparent that a fundamental change in our thinking with respect to what characters are relevant to speciation is required. It is essential that we start thinking in terms of separate genetic systems for adaptation and speciation. Irrespective of the controversies of the fewer vs many genes (Hubby and Throckmorton, 1968), structural vs regulatory genes (Wilson, Maxon and Sarich, 1974; King and Wilson, 1975), micro- vs macromutations (Goldschmidt, 1940; Wilson, Carson, and White, 1977; Gould, 1977) and the adaptive vs non-adaptive nature of genetic changes (Mayr, 1963; Muller, 1949), it is important to realize that study of reproductive traits (both reproductive organs and reproductive behaviour) is germane to the question of speciation. There are several old as well as new findings which are the basis of this proposal. First, there is ample evidence to show that animal genitalia diverge much more rapidly than other organs (Eberhard, 1985) and provide critical and in many cases the only, features for species distinction. Second, in most interspecific crosses

involving a closely related species, hybrids are phenotypically normal but have atrophied genitalia and thus are sterile. Third, the male-limited reproductive isolation and frequent hybrid male sterility observed in many old as well as new studies suggest that some sort of epistatic interaction between X and autosomes (Dobzhansky, 1936; Moran, 1979; Naveira and Fontadevila, 1986), between X and Y chromosomes (Coyne, 1984, 1985; Coyne and Kreitman, 1986; Orr, 1987) or between Y and autosomes (Zouros, 1981; Vigneault and Zouros, 1986) is the basis of hybrid male sterility. A direct evidence for X-Y interactions come from molecular data which show that males carrying a large deletion of a specific DNA sequence (2L1) in the long arm of the Y chromosome of D. melanogaster are sterile and exhibit complex molecular phenotype (Hardy et. al., 1984). Studies using D. simulans and D. mauritiana DNA show that the 2L1 sequence is present on the Y chromosome in these species, and that it is transcribed during spermatogenesis (Livak, 1984). Finally, since Y chromosome is haploid, its effective population size is one fourth of that of the autosomes and three fourth of that of the X chromosome. This together with the restricted genetic exchange (Williamson, 1976) and its presence only in males provide Y chromosome with a potential for rapid divergence. Charlesworth, Coyne and Barton, (1987) have shown that the

substitution of selectively favourable mutations often proceeds more rapidly for X and Y linked loci than for the autosomes, provided that mutations are recessive.

The foregoing makes a convincing case for pursuing genetic analyses of the contribution of various chromosomes to reproductive isolation in Drosophila (Cf. Coyne, 1984, 1985) and here I propose a parallel molecular approach to the problem of speciation. While the level of genic divergence observed between D. melanogaster and D. simulans is consistent with the view that many gene loci are involved in species formation, I think that reproductive tract proteins and variation in their expression are more important to speciation than the much widely studied, randomly sampled gene-enzyme loci most of which are involved in intermediary metabolism. This conclusion is based on the results of a systematic comparison of genic divergence in different groups of proteins between D. melanogaster and D. simulans (Table 4.1). The results show that male reproductive tract proteins are more diverged between species than are enzymes (Coulthart and Singh, 1987). In fact a large proportion of the male reproductive tract proteins of the two species shows no homology, i.e., each species has a set of proteins that are absent in the other. The large amount of quantitative difference in the male reproductive tract proteins could be limited to genes

TABLE 4.1

Proportion of loci polymorphic and mean heterozygosity per population within species and proportion of loci showing complete divergence between species of D. melanogaster and D. simulans

Enzymes and Proteins	Species	No. of loci studied	Proportion of loci uniformly monomorphic	Proportion of loci polymorphic (mean ± SE)	Hetero-zygosity (mean±SE)	Proportion of loci diverged	
						Homologous (I = 0)	Non-homologous (showing +/-)
Enzymes (1DE)	D.m.	79[a]	0.418	0.412±0.049	0.127±0.018	0.101	--
	D.s.	79[b]	0.443	0.329±0.031	0.112±0.016		
Abundant soluble proteins (1DE): Larval (hemo-lymph)	D.m.	8[c]	0.375	0.550±0.092	0.179±0.069	0	--
	D.s.	8[b]	0.250	0.650±0.056	0.255±0.081		
Larval (carcass) and adult	D.m.	30[a]	0.667	0.193±0.057	0.037±0.015	0	--
	D.s.	27[b]	0.926	0.059±0.020	0.004±0.003		
Male Reproductive Tract Proteins (2DE)[d]: Common	D.m.	110	0.927	0.041±0.045	0.010±0.008	0.098	0.183
	D.s.	131	0.954	0.038±0.000	0.012±0.005		

TABLE 4.1 (Cont'd.)

Enzymes and Proteins	Species	No. of loci studied	Proportion of loci uniformly monomorphic	Proportion of loci polymorphic (mean ± SE)	Hetero-zygosity (mean±SE)	Proportion of loci diverged	
						Homologous (I = 0)	Non-homologous (showing +/-)
Testis-specific	D.m.	74	0.878	0.101±0.029	0.030±0.007	0.175	0.234
	D.s.	87	0.896	0.092±0.016	0.032±0.010		
Gland-specific	D.m.	26	0.769	0.173±0.082	0.065±0.033	0.261	0.391
	D.s.	25	0.680	0.280±0.000	0.119±0.019		
Testis-elevated	D.m.	34	0.911	0.058±0.042	0.023±0.019	0.035	0.291
	D.s.	28	0.857	0.107±0.051	0.028±0.030		
Total (2DE)	D.m.	244	0.893	0.080±0.044	0.024±0.010	0.132	0.231
	D.s.	271	0.900	0.085±0.000	0.029±0.001		

a - based on 9 populations
b - based on 5 populations
c - based on 15 populations
d - based on 2 populations

which are expressed in a tissue-specific manner, or it could be due to presence of modifiers which control gene expression in a tissue-specific manner. Further research needs to be directed towards (1) characterizing more carefully the testis-specific proteins showing quantitative difference in the sibling species, (2) genetic analysis of interspecific hybrids and backcross progenies to locate and map these genes, (3) genetic analysis of gene expression differences to locate modifiers or regulatory genes, and (4) establishing correlations between specific proteins and the sterility syndrome (e.g., sperm immotility, anomalies in testicular development, etc.). I think that the genetics and molecular studies of reproductive characters are likely to revolutionize studies of speciation in the same manner as the introduction of gel electrophoresis revolutionized population genetics.

5

GENERAL SUMMARY AND CONCLUSIONS

Neo-Darwinism, a synthesis of classical Darwinian adaptationism and Mendelian genetics postulates that randomly generated genetic variation could be amplified and established within populations by forces acting to maintain the adaptively important genetic variation. For half a century the Modern Evolutionary Synthesis has provided elegant set of theories that can satisfactorily answer the biological evolutionary inquiry. From the scheme in Chapter 1 it is evident that descriptions of genetic variation within and between populations are the fundamental observations on which dymamics of evolutionary process depends. Lewontin (1974) described it in these words:

> "The sufficient set of state variables for describing an evolutionary process within a population must include some information about the statistical distribution of genotypic frequencies. It is for this reason that the empirical study of population genetics has always begun with and centered around the characterization of the genetic variation in populations."

Up until the mid 1960's, the analyses of genetic variation within species as well as genetic differences

between species had centered on visible characters (Mayr, 1963; Dobzhansky, 1970; Lewontin, 1974). In addition, inbreeding and selection experiments in laboratory populations were used to study the nature of genetic variation within populations (Lewontin, 1974), and analysis of hybrid sterility in species crosses were used to study the nature of genetic differences between species (Dobzhansky, 1951). Inbreeding and laboratory selection experiments were quite successful in uncovering large amount of genetic variation but the number of genes and alleles remains unknown (Lewontin, 1974). Similarly in the species crosses, usually many but an unknown number of gene differences were found to be involved in the hybrid sterility (Lewontin, 1974; Dobzhansky, 1936; Coyne, 1984) but the actual number of genes and their molecular nature remains unknown.

The problem of measuring genetic variation was resolved as molecular techniques were introduced into population genetics. Since the first application of protein gel electrophoresis to population genetics (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Harris, 1966), a variety of animal and plants have been studied for their genic variation within species (Ayala, 1976, 1982; Lewontin, 1974; Nevo, 1984; Powell, 1975; Nei and Koehn, 1983) and genic divergence between species (Ayala, 1975;

Throckmorton, 1977; Avise and Aquadro, 1982). These studies have shown that natural populations of most organisms possess large amount of genetic variation. The average proportions of polymorphic loci in a population range between 20% - 50% and the average heterozygosity varies between 4% - 8% in vertebraates and between 6% - 15% in invertebrates. Different species show varying degree of geographic structure, which is usually related to the specie's geographic distribution, breeding system and the level of gene flow. Comparisons of closely related species show varying levels of genic divergence depending upon the degree of their genetic relationship. The average genetic identity (I) between closely related taxas are as follows: populations - 0.970 $\pm$ 0.006, subspecies - 0.795 $\pm$ 0.013, semispecies - 0.798 $\pm$ 0.026, sibling species - 0.563 $\pm$ 0.023 (Ayala, 1975). In most studies, sibling and subspecies show a near absence of completely differentiated loci and the species differences occur mostly in terms of differences in allele frequencies (Lewontin, 1974; Ayala, 1975).

The previous studies of gene-enzyme variation within as well as between species have suffered from two main problems. First, over 80% of studies have sampled a small (less than 30 gene loci) and perhaps an unrepresentative set of variable gene loci. Second, the

sample of genes studied have been overrepresented by genes coding for enzymes that are involved in intermediary metabolic functions. The main objective of the present research was to carry out a comparative study of a large number of gene-structure loci, preferably representing a variety of enzymatic functions, in natural populations of D. melanogaster and D. simulans. A total of 114 loci were surveyed in five natural populations of D. simulans. The results were compared with those reported on the same set of homologous loci in fifteen world-wide populations of D. melanogaster (Singh and Rhomberg, 1987a, b).

The experimental approach taken in the present study was designed to yield allele frequency data for various loci within species and the similarity of allele-frequency profiles between species. The basic methodology was to score structurally distinguishable protein molecules, coded by different alleles of a gene, by polyacrylamide gel electrophoresis under non-denaturating conditions. The electrophoretic mobility of a protein is a reliable phenotype that gives discrete, unambiguous difference between genotypes and thus is capable of distinguishing different homozygotes and heterozygotes in a given population sample.

The amount of genic variation within species and the comparison of population structure between species

produced the following results: (1) D. melanogaster shows a significantly higher proportion of loci polymorphic than D. simulans (52% vs. 39%; $p < 0.05$), (2) both species have similar mean heterozygosity and mean number of alleles per locus, (3) the two species share some highly polymorphic loci but they do not share loci that show high geographic differentiation, and (4) D. simulans shows significantly less geographic differentiation than D. melanogaster. The differences in the geographic structure between the two species are limited to loci located on the X and second chromosome only; loci on the third chromosome show similar geographic differentiation in both species.

A number of hypotheses were considered to explain the differences in the proportion of polymorphic loci and geographic structure between the two species. On the 99% criterion of polymorphism (i.e. the most common allele being 99% or less), D. melanogaster shows more loci polymorphic than D. simulans. But this is mostly due to the fact that D. melanogaster harbours low frequency alleles at many loci which are monomorphic in D. simulans. In fact, if we use a more stringent criterion of polymorphism (i.e. the most common allele being 95% or less), the number of polymorphic loci is about the same in the two species (39 in D. melanogaster and 34 in D.

simulans). The presence of more middle repetitive DNA (Dowsett and Young, 1982), transposable elements (Bregliano and Kidwell, 1983; Brookfield, Montgomery and Langley, 1984) and of possibly "breakage inducing factors" (Voelker, 1974) or mutator loci (Green, 1976) in D. melanogaster are suggestive of higher mutation rate in this species and can potentially explain the high proportion of rare alleles and polymorphic loci in this species, but it is not obvious how these genomic mechanisms can have an effect on the allozyme variation per se.

The differences in the level of geographic structure between the two species can be due to gene flow (migration) but this does seem likely as estimates of gene flow (Nm) based on the distribution of rare alleles (Slatkin, 1985) are very similar in these species (Singh and Rhomberg, 1987a). There are several other factors which individually or in combination can explain these results. First, it is possible that the allozymes we have looked are irrelevant or neutral with respect to climatic adaptation. Since all genetic elements (chromosome inversion, morphological and quantitative variation, middle repetitive DNA variation, 2DE proteins, mitochondrial DNA variation) consistently show less geographic differentiation in D. melanogaster than D. simulans, and since the levels of gene flow appear to be similar in the

two species, the neutrality argument cannot adequately explain the varying levels of geographic differentiation between these species. Second, population bottleneck and recent colonization has been invoked to explain differences in the levels of genetic variation within and between populations of various species (Parsons, 1983). If it could be shown that D. simulans has become cosmopolitan much more recently without entailing a severe bottleneck, then its low geographic differentiation can be explained simply by arguing that D. simulans has had less time to equilibrate with its physical and biotic environment than has D. melanogaster. Third, D. melanogaster is reported to be physiologically and behaviourally more flexible than D. simulans (Parsons, 1975, 1983). Although both species are cosmopolitan and co-occur extensively, D. melanogaster is more successful than D. simulans in the temperate regions. Therefore, the difference in the levels of their geographic differentiation could be a result of their varying capacity for macroclimatic adaptation, which would be consistent with the view that the two species have different niche-width. Finally, the low geographic differentiation in D. simulans may result from a selection strategy favouring broad adaptability of a single purpose genotype. In conclusion, variation in nich-width and/or genetic strategies of adaptation appear to be the major

contributing factors to the varying levels of geographic differentiation in these species.

The comparison of allele-frequency profiles at homologous loci between D. melanogaster and D. simulans produced the following results: The polymorphic loci between the two species are significantly correlated, i.e. if a locus is polymorphic in one species, it is likely to be polymorphic in the other species also. The various chromosomes show similar proportion of unique alleles within species but they differ between species; chromosome X and 2, but not 3, show more unique alleles in D. melanogaster than D. simulans. All chromosomes show similar proportion of shared alleles and similar mean genetic identities between species. The majority of loci show genetic identity of 0 or 1. Eight loci (Adh, α -Amy, Ars, Dip-2, Got-1, Had, Pgk, and Sucr) are completely divergd between the two species and all of them except two (Adh and α -Amy) are alternately fixed between them. While the present estimate of mean genetic distance, D = 0.179, between the two species is lower than previously reported values, the proportion of loci showing nearly complete divergence is (10%) higher than all previously reported values. The loci with high genetic identity between species are those that share their most common allele and differ only in the frequency of their less frequent

alleles. On the other hand, the loci showing low genetic identity exhibit variation in the frequency of their most common allele. In both species, most of the allozyme loci that show high genetic identity between species have low geographic differentiation within species; these loci have relatively low variation in both species. However, there are a number of loci which show high geographic differentiation within species inspite of showing high genetic similarity between species. Comparatively there are relatively fewer loci which show the opposite, i.e. low genetic identity between species but low geographic differentiation within species. The observation that loci showing genetic divergence within vs. between species are not the same, leads us to conclude that population structure within species has not played a role in the accumulation of genetic divergence between the present species.

It is argued that in studies of speciation a fundamental change in our thinking with respect to what characters should be studied is required. There are several, old and as well as new findings (Eberhard, 1985; Dobzhansky, 1951; Bock, 1984; Coyne, 1985) which suggest that reproductive characters are the most affected organs in interspecific hybrids and therefore should be the focus of our attention in studies of speciation. Recently,

Coulthart and Singh, (1987a, b) compared different groups of proteins and found male reproductive tract proteins to be more diverged between species than enzymes and other abundant proteins. A molecular approach to the speciation problem is essential and further research in this direction should be directed towards (1) characterizing more carefully testis-specific proteins showing quantitative divergences in the sub- and sibling species, (2) genetic analyses of interspecific hybrids and their back crosses to locate and map these genes, (3) genetic analyses of gene expression differences to locate modifiers or regulatory genes, and (4) establishing correlations between specific proteins and the hybrid pathologies, such as sperm immotility and abnormality in the testicular developments. We think that the level of genic divergence at a randomly chosen sample of genes coding for enzymes, most of which are involved in intermediary metabolism, cannot tell us much about the genetic mechanisms of species formation. Molecular analyses of reproductive characters of potential importance in species hybrids will almost certainly lead to a better understanding of the genetic and molecular basis reproductive isolation in Drosophila.

LITERATURE CITED

Ahearn, J. N. and V. Baimai (1987) Cytogenetic study of three closely related species of Hawaiian Drosophila. Genome 29: 47-57.

Allard, R. W., and J. Adams (1969) The role of intergenotypic interactions in plant breeding. Proc. XII Intern. Congr. Genet. 3: 349-370.

Anderson, P. R. and J. G. OAKESHOTT (1984) Parallel geographical patterns of allozyme variation in two sibling Drosophila species. Nature 308: 729-731.

Angus, R. A. and R. J. Schultz (1979) Clonal diversity of the unisexual fish Poeciliopsis monacha-lucida: a tissue graft analysis. Evolution 33: 27-40.

Aquadro, C. F., S. F. Desse, M. M. Bland, C. H. Langley, and C. C. Laurie-Ahlberg (1986) Molecular population genetics of the alcohol dehydrogenase gene region of Drosophila melanogaster. Genetics 114: 1165-1190.

Ashburner, M. and F. Lemeunier (1976) Relationship within the melanogaster species subgroup of the genus Drosophila (Sophophora). I. Inversion polymorphisms in Drosophila melanogaster and D. simulans. Proc.

R. Soc. London. B. 193: 137-157.

Atkinson, W. D. and J. A. Miller (1980) Lack of habit choice in a natural population of D. subobscura. Heredity 44: 193-200.

Avise, J. C. (1976) Genetic differentiation during speciation. pp. 106-122 in F. J. Ayala (ed.), Molecular Evolution. Sunderland, Massachusetts: Sinauer.

Avise, J. C. and C. F. Aquadro (1982) A comparative summary of genetic distances in the vertebrates: patterns and correlations. Evol. Biol. 15: 151-180.

Avise, J. C., J. J. Smith, and F. J. Ayala (1975) Adaptive differentiation with little genic change between two native California minnows. Evolution 29: 411-426.

Ayala, F. J. (1975) Genetic differentiation during the speciation process. Evol. Biol. 8:1 - 75.

Ayala, F. J. (1976) Molecular Evolution. Sinauer Associates Inc., Sunderland, Mass.

Ayala, F. J. (1982) The genetic structure of species. In: Perspectives on Evolution (ed. by R. Milkman), Sinauer Associates, Inc., Sundarland, Mass.

Ayala, F. J., and C. Campbell (1974) Frequency dependent selection. Ann. Rev. Syst. Ecol. 5: 115-138.

Ayala, F. J., M. L. Tracey, D. Hedgecock and R. C. Richmond

(1974) Genetic differentiation during the speciation process in Drosophila. Evolution 28:576 - 592.

Ayala, F. J. and J. R. Powell (1972) Allozymes as diagnostic characters of sibling species of Drosophila. Proc. Natl. Acad. Sci. 69: 1094-1096.

Baba-Aissa, F., and M. Solignac (1984) La plupart des populations de Drosophila simulans ont probablement pour ancetre une femelle unique dans un passe recent. C. R. Acad. Sci. Paris 299: 289-292.

Baimai, V., F. M. Sene, and M. A. Q. R. Pereira (1983) Heterochromatin and Karyotypic differentiation of some neotropical cactus-breeding species of the Drosophila repleta species group. Genetica 60: 81-92.

Baker, H. G. (1965) Characteristics and modes of origin of weeds. pp. 147-172. In: The Genetics of Colonizing Species, Edited by H. G. Baker and G. L. Stebbins, Academic Press, New York.

Baker, R. J. and J. W. Bickham (1986) Speciation by monobrachial centric fusions. Proc. Natl. Acad. Sci. U.S.A. 83: 8245-8248.

Barton, N. H. and B. Charlesworth (1984) Genetic revolutions, founder effects, and speciation. Ann. Rev. Ecol. Syst. 15:133 - 164.

Batterham, P. and S. W. McKechnie (1980) A phenol oxidase

polymorphism in Drosophila melanogaster. Genetica 54: 121-125.

Beckwith, J. and P. Rossow (1974) Analysis of genetic regulatory mechanisms. Ann. Rev. Genet. 8:1-13.

Berger, E. M. (1970) A comparison of gene-enzyme variation between Drosophila melanogaster and D. simulans. Genetics 66: 677-683.

Berger, E.M. (1971) A temporal survey of allelic variation in natural and laboratory populations of D. melanogaster. Genetics 67: 121-136.

Bischoff, W. L. (1978) Genetic control of soluble NAD-dependent sorbitol dehydrogenase in Drosophila melanogaster. Biochem. Genet. 14: 1019-1038.

Blaylock, B. G. and H. H. Shugart, Jr. (1972) The effect of radiation-induced mutation on the fitness of Drosophila populations. Genetics 72: 469-474.

Bock, I. R. (1980) Current status of the Drosophila melanogaster species-group (Diptera). Syst. Entomol. 5: 341-356.

Bock, I. R. (1984) Interspecific hybridization in the genus Drosophilia. Evol. Biol. 18:41 - 70.

Bodmer, M. and M. Ashburner (1984) Conservation and change in the DNA sequences coding for alcohol dehydrogenase in sibling species of Drosophila. Nature 309:425 - 430.

Bregliano, J. C. and M. Kidwell (1983) Hybrid dysgenesis

determinants. p. 396. In: Mobile Genetic Elements, Edited by J. A. SHAPIRO, Academic Press, New York.

Britten, R. J. and E. H. Davidson (1969) Gene regulation for higher cells: A theory. Science 165: 349-357.

Brookfield, J. F. Y., E. Montgomery and C. H. Langley (1984) Apparent absence of transposable element related to the P elements of D. melanogaster in other species of Drosophila. Nature 310: 330-332.

Burr, H. E. and R. T. Schimke (1980) Intragenomic DNA sequence homologies in the chicken and other members of the class Aves: DNA re-association under reduced stringency conditions. J. Mol. Evol. 15: 291-307.

Bush, G. L. (1981) Stasipatric speciation and rapid evolution in animals. In: Evolution and speciation, essays in honour of M. J. D. White (ed. by W. R. Atchley and D. S. Woodruff), Cambridge University Press, New York.

Bryant, E. H., H. Van Dijk, and W. Van Delden (1981) Genetic variability of the face fly, Musca autumnalis de Geer, in relation to a population bottleneck. Evolution 35: 872-881.

Buchanan, B. A. and D. L. E. Johnson (1983) Hidden electrophoretic variation at the xanthine dehydrogenase locus in a natural population of Drosophila melanogaster. Genetics 104: 301 - 315.

Cabrera, V. M., A. M. Gonzalez, J. M. Larruga, and A. Gullon, (1982) Electrophoretic variability in natural populations of D. melanogaster and D. simulans. Genetica 59: 191-201.

Chang, L. S. and H. L. Carson (1985) Metaphase karyotype identity in four homosequential Drosophila species from Hawaii. Can. J. Genet. Cytol. 27: 308-311.

Carson, H. L. (1975) The genetics of speciation at the diploid level. Am. Natur. 109: 113-139.

Carson, H. L. (1986) Sexual selection and speciation. In: Evolutionary Processes and Theory (ed. by S. Karlin and E. Nevo), Academic Press, Inc.

Carson, H. L. and H. D. Stalker (1968) Polytene chromosome relationships in Hawaiian species of Drosophila. I. The grimshawi subgroup. Univ. of Tex. Publ. 6818: 335-354.

Carson, H. L. and A. R. Templeton (1984) Genetic revolutions in relation to speciation phenomena: The founding of new populations. Ann. Rev. Ecol. Syst. 15:97 - 131.

Carson, H. L., T. Teramoto, and A. R. Templeton (1977) Behavioral differenees among isogenic strains of Drosophila mercatorum. Behav. Genet. 7: 189-197.

Cavalli-Sforza, L. L., and W. F. Bodmer (1971) The genetics of human populations. Freeman, San Francisco.

Cavaner, D. R. (1980) Genetics of male specific glucose oxidase and the identification of other unusual hexose enzymes in Drosophila melanogaster. Biochem. Genet. 18: 929-937.

Charlesworth, B., J. A. Coyne, and N. H. Barton (1987) The relative rates of evolution of sex chromosomes and autosomes. Am. Natur. 130: 113-146.

Charlesworth, B., R. Landé, and M. Slatkin (1982) A New-Darwinian commentary on macroevolution. Evolution, 36: 474-498.

Choudhary, M. and R. S. Singh (1987a) Historical effective size and the level of genetic variation in Drosophila melanogaster and D. pseudoobscura. Biochem. Genet. 25: 41-51.

Choudhary, M. and R. S. Singh (1987b) A comprehensive study of genic variation in natural populations of Drosophila melanogaster III. Variations in genetic structure and their causes between D. melanogaster and D. simulans. Genetics 117 (in press).

Clausen, J. (1951) Stages in the Evolution of Plant Species. Cornell Univ. Press, Ithaca, NY.

Clayton, F. E., H. L. Carson, and J. E. Sato (1972) Polytene chromosome relationships in Hawaiian species of Drosophila. IV. Supplementary data on metaphases and gene sequences. Univ. Tex. Publ. 7213: 163-177.

Cleide, M. R., De Albuquerque and M. Napp, (1981) Genetic variability at the Esterase-6 locus in natural populations of D. simulans in relation to environmental heterogeneity. Genetics 98: 399-407.

Cohn, V. H., M. A. Thompson, and G. P. Moore (1984) Nudeotide sequence comparison of the Adh gene in three drosophilids. J. Mol. Evol. 20: 31-37.

Coulthart, M. B. and R. S. Singh (1987a) Low genic variation in male reproductive tract proteins of D. melanogaster and D. simulans. Mol. Biol. and Evol. (in press).

Coulthart, M.B. and R.S. Singh (1987b) High level of divergence of male reproductive tract proteins between D. melanogaster and its sibling species, D. simulans. Mol. Biol. and Evol. (in press).

Coyne, J. A. (1982) Gel electrophoresis and cryptic protein variation. pp. 1 - 32 in Ratazzi, M. C., J. G. Scandalios and G. S. Whitt (eds.), Isozymes - Current Topics in Biological and Medical Research, Vol. 6. New York: Alan R. Liss.

Coyne, J. A. (1984) Genetic basis of male sterility in hybrids between two closely related species of Drosophila. Proc. Nat. Acad. Sci. USA 81:4444 - 4447.

Coyne, J. A. (1986) Meiotic segregation and male recombination in interspecific hybrids of

Drosophila Genetics 114: 485-494.

Coyne, J. A., W. F. Eanes, J. A. M. Ramshaw, and R. K. Koehn (1979) Electrophoretic heterogeneity of α-glycerophosphate dehydrogenase among many species of Drosophila. Syst. Zool. 28: 164-175.

Coyne, J. A. (1983) Genetic basis of differences in genital morphology among three sibling species of Drosophila. Evolution 37: 1101-1118.

Coyne, J. A. (1985) Genetic studies of three sibling species of Drosophila with relationship to theories of speciation. Genet. Res. Camb. 46: 169-192.

Coyne, J. A. and B. Charlesworth (1986) Location of an X-linked factor causing sterility in male hybrids of Drosophila simulans and D. mauritiana. Heredity 57: 243-273.

Coyne, J. A. and M. Kreitman (1986) Evolutionary genetics of two sibling species Drosophila simulans and D. sechellia. Evolution 40: 673-691.

Crawford, D. J. (1983) Phylogenetic and systematic inferences from electrophoretic studies. In: Isozymes in Plant Genetics and Breeding (ed. by Tanksley, S. D. and T. J. Orton), pp. 257-287, Elsevier, Amsterdam, Neth.

Curtis, C. F. (1982) The mechanism of hybrid male sterility from crosses in the Anopheles gambiae and Glossina morsitans complexes. In: The genetics of insect

disease vectors (ed. by W. M. F. Stein), Stripes, New York.

Da Cunha, A. B., H. Burla, and Th. Dobzhansky (1950) Adaptive chromosomal polymorphism in D. willistoni. Evolution 4: 212-235.

Da Cunha, A. B., Th. Dobzhansky, O. Pavlovsky, and B. Spassky (1959) Genetics of natural populations. XXVIII. Supplementary data on the chromosomal polymorphism in D. willistoni in its relation to the environment. Evolution 13: 389-404.

Darwin, C. R. (1859) On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life (First Edition). Reprinted 1979, New York: Crown.

Davenport, R. (1986) Chromosomal polymorphism and speciation in the genus Eunemobius (Orthopetra: Grylloidea). Genetica 68: 105-107.

David, J. R. and C. Bocquet (1975) Similarities and differences in latitudinal adaptation of two Drosophila sibling species. Nature 257: 588-590.

David, J. R., C. Bocquet and M. De Scheemaeker-Louis (1977) Genetic latitudinal adaptation of D. melanogaster: new discriminative biometrical traits between European and Equitorial Africa populations. Genet. Res. 30: 247-255.

David, J. R. and L. Tsacas (1981) Cosmopolitan,

subcosmopolitan and widespread species: different strategies within the Drosophila family. C. R. Soc. Biogeog. 57: 11-26.

David, J. R., J. Louis, J. A. McKenzie, M. Rocha-Pite, and J. Vouidibio (1984) Comparative demography of the cosmopolitan sibling species, D. melanogaster and D. simulans, under temperate and tropical climate. Annals Soc. Ent. Fr. 20: 135-142.

Davidson, E. H. and R. J. Britten (1979) Regulation of gene expression: possible role of repetitive sequences. Science 204: 1052-1059.

Dev, V. and K. S. Rai (1986) Genetics of speciation in the Aedes scutellaris group (Diptera: Culicidae). V. Chromosomal relationships among five species. Genetica 64: 83-92.

De Vries, H. (1905) Die Mutationstheorie. Lepzie: Veit.

Dobzhansky, Th. (1936) Studies on hybrid sterility. II. Localization of sterility factors in Drosophila pseudoobscura hybrids. Genetics 21: 113-135.

Dobzhansky, Th. (1951) Genetics and the origin of species (3rd edition). Columbia University Press, New York.

Dobzhansky, Th. (1955) A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symp. Quant. Biol. 20:1 - 15.

Dobzhansky, Th. (1970) Genetics of the evolutionary process. Columbia University Press, New York.

Dobzhansky, Th. and B. Spassky (1953) Genetics of natural populations. XXI Concealed variability in two sympatric species of Drosophila. Genetics 38: 471-484.

Dobzhansky, Th. and B. Spassky (1954) Genetics of natural populations. XXII. A comparison of the concealed variability in Drosophila prosaltans with that in other species. Genetics 39: 472-487.

Dodd, D. M. B. and J. R. Powell (1985) Founder-flush speciation: an update of experimental results with Drosophila. Evolution 39: 1388-1392.

Dover, G. (1982) Molecular drive: a cohesive mode of species evolution. Nature 299: 111-117.

Dowsett, A. P. and M. W. Young (1982) Differing levels of dispersed repetitive DNA among closely related species of Drosophila. Proc. Natl. Acad. Sci. USA 79: 4570-4574.

Dunn, L. C. (1965) A short history of genetics. McGraw-Hill, New York.

Dykhuizen, D. E. and D. L. Hartl (1983) Functional effects of PGI allozymes in E. coli. Genetics 105: 1-18.

Eanes, W. F. (1983) Genetic localization and sequential electrophoresis of G-6PD in Drosophila melanogaster. Biochem. Genet. 21: 703-711.

Easteal, S. and J. G. Oakeshott (1985) Estimating divergence times of Drosophila species from DNA sequence comparisons (Letter to the editor). Mol. Biol. and Evol. 2:87 - 91.

Eberhard, W. G. (1985) Sexual selection and animal genitalia. Harvard University Press, Cambridge.

Eisses, K.T., H. Van Dijk, W. Van Delden (1979) Genetic differentiation within the melanogaster species group of the genus Drosophila (Sophophora). Evolution 33: 1063-1068.

Eldredge, N. and S. J. Gould (1972) Punctuated equilibria: an alternative to phyletic gradualism. pp. 82 - 115 in T. J. M. Schopf (ed.), Models in Paleobiology. San Francisco: Freeman, Cooper & Co.

Engels, W. R. and C. R. Preston (1979) Hybrid dysgenesis in D. melanogaster: the biology of female and male sterility. Genetics 92: 161-174.

Ford, E. B. (1940) Polymorphism and taxonomy. In: The new systematics (ed. by J. Huxley), pp. 493-513. Clarendon Press, Oxford.

Ford, H. A. (1971) The degree of mimetic protection gained by new partial mimics. Heredity 27: 227-236.

Futuyma, D. J. and G. C. Mayer (1980) Non-allopatric speciation in animals. Syst. Zool. 29: 254-271.

Fuerst, P. A., R. Chakraborty, and M. Nei (1977)

Statistical studies on protein poly-morphism in natural populations. I. Distribution of single locus heterozygosity. Genetics 86: 455-483.

Gillespie, J. H. and K. I. Kojima (1968) The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two Drosophila ananassae populations. Proc. Nat. Acad. Sci. USA 61: 582 - 585.

Ginzburg, L. R., P. M. Bingham, and S. Yoo (1984) On the theory of speciation induced by transposable elements. Genetics 107: 331-341.

Goldschmidt, R. (1940) The Material Basis of Evolution, Yale University Press, New Haven.

Gonzalez, A. M., V. M. Cabrera, J. M. Larruga and A. Gullon (1982) Genetic distance in the sibling species of Drosophila melanogaster, D. simulans and D. mauritiana. Evolution 36: 517-522.

Gottlieb, L. D. (1974) Genetic confirmation of the origin of Clarkia lingulata. Evolution 28: 244-250.

Gottlieb, L. D. (1981) Electrophoretic evidence and plant populations. Phytochemistry 7: 1-46.

Gottlieb, L. D. and G. Pilz (1976) Genetic similarity between Gaura longiflora and G. demareei. Syst. Bot. 1: 181-187.

Goula, J. W. and O. R. Taylor (1980) Some characteristics of hybrids derived from the sulfur butterflies C.

eurytheme and C. philodice. Evolution 34: 688-695.

Gould, S. J. (1977) Ontogeny and Phylogeny. Harvard University Press, Cambridge, Mass. pp. 501.

Gould, S. J. (1980) Is a new general theory of evolution emerging? Paleobiology 6: 119-130.

Gould, S. J. and N. Eldredge (1977) Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3: 115-151.

Green, M., (1976) Mutable and mutator loci. In: The Genetics and Biology of Drosophila, Edited by M. Ashburner and E. Novitski, Vol. 1(b): 929-944.

Gupta, J. P. and A. Kumar (1986) Characterization and modification of heterochromatin in four species of the immigrans species group of Drosophila. Can. J. Genet. Cytol. 28: 340-347.

Haigh, J.K. and J. Maynard Smith (1972) Population size and protein variation in man. Genet. Res. 19: 73-89.

Haldane, J. B. S. (1922) Sex-ratio and unisexual sterility in hybrid animals. J. Genet. 12: 101-109.

Hale, L. R. and R. S. Singh (1985) Mitochondrial DNA variation in natural populations of D. melanogaster and D. simulans. Genetics 110: s42.

Hallett, J. G. (1980) Niche width and genetic variation in Drosophila reexamined. Am. Natur. 115: 594-595.

Hamrick, J. L. and R. W. Allard (1972) Microgeographic

variation in allozyme frequencies in Avena barbata. Proc. Natl. Acad. Sci. USA 69: 2100-2104.

Hardy, R. W., D. L. Lindsley, K. J. Livak, B. Lewis, A. L. Siversten, G. L. Joslyn, J. Edwards, and S. Bonaccorsi (1984) Cytogenetic analysis of a segment of the Y chromosome of Drosophila melanogaster. Genetics 107: 591-610.

Harris, H. (1966) Enzyme polymorphism in man. Proc. Roy. Soc. London. 164: 298-310.

Harris, H., D. A. Hopkinson, and Y. H. Edwards (1977) Polymorphism and the subunit structure of enzymes - a contribution to the neutralist - selectionist controversy. Proc. Nat. Acad. Sci. USA 74: 698-701.

Harris, H. and D.A. Hopkinson, (1976) Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam.

Hartl, D. L. and D. E. Dykhuizen (1981) Potential for selection among neutral allozymes of 6-phosphogluconate dehydrogenase in E. coli. Proc. Natl. Acad. Sci. USA 78: 6344-6348.

Hickey, D. A. (1977) Selection for amylase alloyzymes in Drosophila melanogaster. Evolution 31: 800-804.

Hilbish, T. J. and R. K. Koehn (1985) The physiological basis of natural selection at the LAP locus. Evolution 39: 1302-1317.

Hilu, K. W. (1983) The role of single-gene mutations in the evolution of flowering plants. Evol. Biol. 16: 97-122.

Hoenigsberg, H. F. (1968) An ecological situation which produced a change in the proportion of D. melanogaster to D. simulans. Am. Natur. 102: 389-390.

Hubby, J. L. and L. H. Throckmorton (1965) Protein differences in Drosophila. I-I. Comparative species genetics and evolutionary problems. Genetics 52: 203-215.

Hubby, J. L. and R. C. Lewontin (1966) A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. Genetics 54: 577 - 594.

Hubby, J. L. and L. H. Throckmorton (1968) Protein differences in Drosophila. IV. A study of sibling species. Am. Natur. 102: 193-205.

Huxley, J. S. (1942) Evolution: The Modern Synthesis London: George Allen and Unwin.

Hyytia, P., P. Capy, J. R. David, and R. S. Singh (1985) Enzyme and quantitative variation in European and African populations of D. simulans. Heredity 54: 209-217.

Jaenike, J., E. D. Parker and R. K. Selander (1980) Clonal

niche structure in the parthenogentic earthworm Octolasion tyrtaeum. Am. Natur. 116: 196-205.

Johnson, F. M., H. E. Schaffer, J. E. Gillaspy, and E. S. Rockwood (1969) Isozyme genotype-environment relationships in natural populations of the harvester ant Pogonomyrmex barbatus from Texas. Biochem. Genet. 3: 429-450.

Johnson, F. M. and H. E. Schaffer (1973) Isozyme variability in species of the genus Drosophila. VII. Genotype-environment relationships in populations of D. melanogaster from the eastern U.S. Biochem. Genet. 10: 149-163.

Johnson, G. B. (1974) Enzyme polymorphism and metabolism. Science 184: 28-37.

Johnson, M. S., O. C. Stine, and J. Murray (1977) Reproductive compatibility despite large-scale genetic divergence in Cepaea nemoralis. Heredity 53: 655-665.

Judd, B. H., M. W. Shen, and T. C. Kaufman (1972) The anatomy of a segment of the X chromosome of Drosophila melanogaster. Genetics 71: 139-156.

Kaneshiro, K. Y. (1976) Ethological isolation and the phylogeny in the planitibia subgroup of Hawaiian Drosophila. Evolution 30: 740-745.

Kaneshiro, K. Y. (1980) Sexual isolation, speciation, and the direction of evolution. Evolution 34: 437-444.

Kidwell, M. G., J. F. Kidwell, and J. A. Sved (1977) Hybrid dysgenesis in Drosophila melanogaster: a syndrome of aberrant traits including mutation, sterility and male recombination. Genetics 86: 813-833.

Kidwell, M. G. and J. B. Novy (1979) Hybrid dysgenesis in D. melanogaster: sterility resulting from gonadal dysgenesis in the P-M system. Genetics 92: 1127-1140.

Kidwell, M. G., J. B. Novy, and S. M. Freeley (1981) Rapid unidirectional change of hybrid dysgenesis potential in Drosophila. J. Heredity 72: 32-38.

Kimura, M. (1968) Evolutionary rate at the molecular level. Nature 217:624 - 626.

Kimura, M. (1983) The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press.

Kimura, M. and T. Ohta (1971) Theoretical Aspects of Population Genetics. Princeton: Princeton University Press.

King, J. L. (1973) The probability of electrophoretic identity of proteins as a function of amino acid divergence. J. Mole. Evol. 2: 317-322.

King, J. L. and T. H. Jukes (1969) Non-Darwinian evolution: Random fixation of selectively neutral mutations. Science 164: 788-798.

King, M. (1981) Chromosomal change and speciation in

lizards. In: Evolution and Speciation (ed. by W. R. Atchley and D. Woodruff). Essays in honour of M. J. D. White. Cambridge Univ. Press, N.Y.

King, M. C. and A. C. Wilson (1975) Evolution at two levels. Molecular similarities and biological differences between humans and chimpanzees. Science 188:107 - 116.

Knibb, W. R., J. G. Oakeshott and J. B. Gibson (1981) Chromosome inversion polymorphisms in D. melanogaster. I. Latitudinal clines and associations between inversions in Australian populations. Genetics 98: 833-847.

Koehn, R. K. and W. F. Eanes (1978) Molecular structure and protein variation within and among populations. Evol. Biol. 11: 39-100.

Kojima, K. (1971) Is there a constant fitness value for a given genotype? No! Evolution 25: 281-285.

Kojima, K. I., J. Gillespie and Y. N. Tobari. (1970) A profile of Drosophila species enzymes assayed by electrophoresis. I. Number of alleles, heterozygosity, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet. 4: 627-637.

Kornfield, I. L., U. Ritte, C. Richler, and J. Wahrman (1979) Biochemical and cytological differentiation among cichlid fishes of the sea of Galilee.

Evolution 33: 1-14.

Kreitman, M. (1980) Assessment of variability within electromorphs of alcohol dehydrogenase in Drosophila melanogaster. Genetics 95: 467-475.

Krieber, M. and M. R. Rose (1986) Molecular aspects of the species barrier. Ann. Rev. Ecol. and Syst. 17: 465-485.

Kreitman, M. (1983) Nucleotide polymorphism at the alcohol dehydrogenase locus of Drosophila melanogaster. Nature 304: 412 - 417.

Lachaise, D., J. R. David, F. Lemeunier, L. Tsacas, and M. Ashburner (1986) The reproductive relationships of Drosophila sechellia with D. mauritiana, D. simulans and D. melanogaster from the afrotropical regions. Evolution 40: 262-271.

Landsteiner, K., and A. S. Weiner (1940) An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc. Soc. Exp. Biol. 43: 223.

Lacy, R. C. (1982) Niche breadth and abundance as determinants of genetic variation in populations of mycophagous Drosophilid flies. Evolution 36: 1265-1275.

Lakovaara, S., A. Saura and C. Falk (1972) Genetic distance and evolutionary relationships in the Drosophila obscura group. Evolution 26: 177-184,

Lemeunier, F. and M. Ashburner (1976) Relationships within

the melanogaster species subgroup of the genus Drosophila (Sophophora). II. Phylogentic relationships between six species based upon polytene chromosome banding sequences. Proc. Roy. Soc. Lond. B 193:275 - 294.

Lemeunier, F., J. R. David, L. Tsacas and M. Ashburner (1985) The Drosophila melanogaster species group. In: The Genetics and Biology of Drosophila, Edited by M. Ashburner H. L. Carson and J.N. Thompson, Jr.), Vol. 3e, Academic Press, New York.

Lewontin, R. C., (1974) The Genetic Basis of Evolutionary Change. New York: Columbia University Press.

Lewontin, R. C. and J. L. Hubby (1966) A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics 54: 595 - 606.

Li, W. and M. Nei (1977) Persistence of common alleles in two related populations or species. Genetics 86: 901-914.

Livak, K. J. (1984) Organization and mapping of a sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. Genetics 107: 611 - 634.

Lynch, M. (1983) Ecological genetics of Daphnia pulex. Evolution 37: 358-374.

MacKay, T. F. C. (1981) Genetic variation in varying environments. Genet. Res. 37: 79-93.

Markert, C. L. and F. Moller (1959) Multiple forms of enzymes: Tissue, ontogenetic and species specific patterns. Proc. Nat. Acad. Sci. USA 45: 753-763.

Maruyama, T. and P. A. Fuerst (1985a) Population bottlenecks and non equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675-689.

Maruyama, T. and P. A. Fuerst (1985b) Population bottlenecks and non equilibrium models in population genetics. III. Genetic homozygosity in populations which experience periodic bottlenecks. Genetics 111: 691-703.

Mayr, E. and W. B. Provine (1980) The Evolutionary Synthesis. Harvard University Press, Cambridge.

Mayr, E. (1942) Systematics and the Origin of Species. Columbia University Press, New York.

Mayr, E. (1954) Change of genetic environment and evolution. pp. 157 - 180 in Huxley, J., A. C. Hardy and E. B. Ford (eds.), Evolution as a Process. London: Allen and Unwin.

Mayr, E. (1963) Animal Species and Evolution. Cambridge, Massachusetts: Harvard University Press.

Mayr, E. (1982a) The Growth of Biological Thought:

Diversity, Evolution, and Inheritance. Cambridge, Massachusetts: Harvard University Press.

Mayr, E. (1982b) Speciation and macroevolution. Evolution 36: 1119 - 1132.

McDonald, J. F., S. M. Anderson, and M. Santos (1980) Biochemical differences between products of the Adh locus in Drosophila. Genetics 95: 1013-1022.

McDonald, J. and P. A. Parsons (1973) Dispersal activities of the sibling species Drosophila melanogaster and D. simulans. Behav. Genet. 3: 293-301.

McDonald, J. F. and F. J. Ayala (1974) Genetic response to environmental hetero-geneity. Nature 250: 572-574.

McKenzie, J. A. (1974) The distribution of vineyard populations of D. melanogaster and D. simulans during vintage and nonvintage periods. Oecologia 15: 1-16.

McKenzie, J. A. and S. W. McKechnie (1979) A comparative study of resource utilization in natural populations of D. melanogaster and D. simulans. Oecologia 40: 299-309.

McKenzie, J.A. and P.A. Parsons, (1974a) Numerical changes and environmental ultilization in natural populations of D. melanogaster and D. simulans. Aust. J. Zool. 22: 175-187.

McKenzie, J. A. and P.A. Parsons (1974b) Microdifferentiation in a natural population of D.

melanogaster to alcohol in the environment. Genetics 77: 385-394.

McLaughlin, P. J. and M. O. Dayhoff (1972) Evolution of species and proteins: a time scale. In: Atlas of protein sequence and structure (ed. by M. O. Dayhoff Vol. 5: 47-66. Natl. Biomed. Res. Found. Washington, D.C.

McLellan, Tracey (1984) Molecular charge and electrophoretic mobility in cetacean myoglobins of known sequence. Biochem. Genet. 22: 181-200.

McNeill, C. I. and S. K. Jain (1983) Genetic differentiation studies and phylogenetic inferences in the plant genus Limnanthes. Theoret. Appl. Genet. 66: 257-269.

Merril, C. R., M. L. Duncan and D. Goldman (1981) A rapid sensitive silver stain for polypeptides in polyacrylamide gels. Anal. Biochem. 110: 201-207.

Mettler, L. E., R. A. Voelker, and T. Mukai (1977) Inverion clines in natural populations of D. melanogaster. Genetics 87: 169-176.

Minawa, A. and A. J. Birley (1975) Genetical and environmental diversity in D. melanogaster. Nature 255: 702-704.

Moran, C. (1979) The structure of the hybrid zone in Caledia captiva. Heredity 42: 13-32.

Morrissey, J. H. (1982) Silver stain for proteins in

polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. 117: 307-310.

Motro, U. and G. Thomson (1982) On heterozygosity and effective size of populations subject to size change. Evolution 36: 1059-1066.

Muller, H. J. (1927) Artificial transmutation of the gene. Science 66: 84-87.

Muller, H. J. (1949) The Darwinian and modern conceptions of natural selection. Proc. Amer. Phil. Soc. 93:459 - 470.

Murray, M. G., D. L. Peters, W. F. Thompson (1981) Ancient repeated sequences in the Pea and Mung Bean genomes and implications for genome evolution. J. Mol. Evol. 17: 31-42.

Naveira, H. and A. Fontdevila (1986) The evolutionary history of Drosophila buzzatti. XII The genetic basis of sterility in hybrids between D. buzzatti and its sibling D. serido from Argentina. Genetics 114: 841-857.

Nei, M. (1975) Molecular population genetics and evolution. North-Holland Publishing Company, Amsterdam, pp. 175-209.

Nei, M. (1980) Stochastic theory of population genetics and evolution (ed. by C. Barigozzi), Springer-Verlag, Berlin, pp. 17-47.

Nei, M. (1987) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei, M. and R. K. Koehn (1983) Evolution of Genes and Proteins pp. 89-114, Sinauer Associates Inc., Sundarland, Mass.

Nei, M. and W. Li (1975) Probability of identical monomorphism in related species. Genet. Res. Camb. 26: 31-43.

Nei, M. and A. K. Roychoudhury (1982) Genetic relationship and evolution of human races. In: Evolutionary Biology edited by M. K. Hecht, W. C. Steere and B. Wallace Vol. 14: 1-59.

Nei, M. and Y. Tateno (1975) Interlocus variation of genetic distance and the neutral mutation theory. Proc. Nat. Acad. Sci., USA, 72(7): 2758-2760.

Nei, M., T. Maruyama and R. Chakraborty, (1975) The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Nevo, E. (1978) Genetic variation in natural populations: patterns and theory. Theoret. Pop. Biol. 13: 121-177.

Nevo, E. (1984) The evolutionary significance of genic diversity: Ecological, demographic and life history correlates. In: Evolutionary Dynamics of Genetic Diversity, edited by G. S. Mani. Lecture notes in Biomathematics 53: 13-213.

O'Brian, S. J. and R. J. MacIntyre (1969) An analysis of gene-enzyme variablity in natural populations of D. melanogaster and D. simulans. Am. Natur. 103: 97-113.

O'Brian, S. J. and R. J. MacIntyre (1972) The α-Glycerophosphate cycle in Drosophila melanogaster. I. Biochemical and developmental aspects. Biochem. Genet. 7:141-161.

Ochman, H., J. S. Jones, and R. K. Selander (1987) Large scale patterns of genetic differentiation at enzyme loci in the land snails Cepaea nemoralis and C. hortensis. Heredity 58: 127-138.

Ohnishi, S., M. Kawanishi and T. K. Watanabe (1983) Biochemical phylogenies of Drosophila protein differences detected by two-dimensional electrophoresis. Genetica 61: 55-63.

Ohnishi, S., A. J. Leigh Brown, R. A. Voelker and C. H. Langley (1982) Estimation of genetic variability in natural populations of D. simulans by two-dimensional and starch gel electrophoresis. Genetics 100: 127-136.

Ohta, T. (1973) Slightly deleterious mutant substitutions in evolution. Nature 246: 96-98.

Ohta, T. (1974) Mutational pressure as the main cause of molecular evolution and polymorphism. Nature 252: 351-354.

Orr, A. H. (1987) Genetics of male and female sterility in hybrids of D. pseudoobscura and D. persimilis. Genetics 116: 555-563.

Orstein, L. (1964) Disc electrophoresis I. Background and theory. Anal. N. Y. Acad. Sci. 121: 321.

Parker, E. D., R. K. Selander, R. O. Hudson and L. J. Lester (1977) Genetic diversity in colonizing parthenogenetic Cockroaches. Evolution 31: 836-842.

Parsons, P. A. (1974) Phototactic responses along a gradient of light intensities for the sibling species D. melanogaster and D. simulans. Behav. Genet. 5: 17-25.

Parsons, P. A., (1975) The comparative evolutionary biology of the sibling species, D. melanogaster and D. simulans. Quar. Rev. Biol. 50: 151-169.

Parsons, P. A. (1979) Resistance of the sibling species D. melanogaster and D. simulans to high temperature in relation to humidity: evolutionary implications. Evolution 33: 131-136.

Parsons, P. A. (1980) Parallel climatic races for tolerances to high temperature desiccation stress in two Drosophila species. J. Biogeog. 7: 97-101.

Parsons, P. A. (1983) The Evolutionary Biology of Colonizing Species. Camb. Univ. Press.

Parsons, P. A. and S. M. Stanley (1980) Spatial ecological studies - domesticated and widespread species In:

Genetics and Biology of Drosophila, Vol. 3a, Edited by M. Ashburner, H. L. Carson and J. N. Thompson, Academic Press, New York, pp. 349-393.

Paterson, R. and D. A. Hickey (1982) A simple method for the localization of Drosophila Trehalase and Sucrase isozymes on polyacrylamide gels. Droso. Inf. Ser. 58: 168-169.

Periquet, G. (1981) Hybrid sterility in Drosophila simulans: Relationship with the hybrid dysgenesis syndrome in D. melanogaster. Heredity 46: 255-261.

Powell, J. R. (1975) Protein variation in natural populations of animals. Evol. Biol. 8: 79 - 119.

Powell, J. R. (1971) Genetic polymorphism in varied environments. Science 174: 1035-1036.

Powell, J. R. and H. Wistrand (1978) The effect of heterogeneous environments and a competitor on genetic variation in Drosophila. Am. Natur. 112: 935-947.

Prakash, S. (1977) Genetic divergence in closely related sibling species Drosophila pseudo- obscura, D. persimilis and D. miranda. Evolution 31: 14-23.

Prakash, S., R. C. Lewontin and J. L. Hubby (1969) A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of D. pseudoobscura. Genetics

61: 841-858.

Provine, W. B. (1971) The Origins of Theoretical Population Genetics. Chicago: University of Chicago Press.

Raff, R. A. and T. C. Kaufman (1983) Embryos, Genes, and Evolution. MacMillan Publishing Co., Inc., New York.

Ramshaw, John A. M., J. A. Coyne, and R. C. Lewontin (1979) The sensitivity of gel electrophoresis as a detector of genetic variation. Genetics 93: 1019-1039.

Richmond, R. C. (1972) Enzyme variability in the Drosophila willistoni group. III. Amounts of variability in the subspecies D. paulistorum. Genetics 71: 87-112.

Richmond, R. C., D. G. Gilbert, K. B. Sheehan, M. H. Gromko, and F. M. Butterworth (1980) Esterase-6 and reproduction in D. melanogaster Science 297: 1483-1485.

Roberts, R. M. and J. S. Jones (1972) Improved apparatus for vertical gel electrophoresis. Analy. Biochemistry 49: 592-597.

Rose, M. R. and W. F. Doolittle (1983) Molecular biological mechanisms of speciation. Science 220: 157-162.

Rothstein, S. I. (1973) The niche-variation model - is it valid? Am. Natur. 107: 598-620.

Schwaegerle, K. E. and B. A. Schaal (1979) Genetic

variability and founder effect in the pitcher plant, Sarracenia purpurea. Evolution 33: 1210-1218.

Selander, R. K. (1976) Genic variation in natural populations. pp. 21 - 45 in F. J. Ayala (ed.), Molecular Evolution. Sunderland, Massachusetts: Sinauer.

Sene, F. M. and H. L. Carson (1977) Genetic variation in Hawaiian Drosophila IV. Allozymic similarity between D. silvestris and D. heteroneura from the island Hawaii. Genetics 86: 187-198.

Shugart, H. H. and B. G. Blaylock (1973) The niche variation hypothesis: an experimental study with Drosophila populations. Am. Natur. 107: 575-579.

Singh, R. S. (1983) Genetic differentiation for allozyme and fitness characters between mainland and Bogota populations of Drosophila pseudoobscura. Can. J. Cytol. Genet. 25: 590-604.

Singh, R. S., M. Choudhary and J. R. David (1987) Contrasting patterns of geographic variation in cosmopolitan sibling species D. melanogaster and D. simulans. Biochem. Genet. 25: 27-40.

Singh, R. S., D. A. Hickey and J. R. David (1982) Genetic differentiation between geographically distant populations of Drosophila melanogaster. Genetics 101: 235-256.

Singh, R. S. and M. B. Coulthart (1982) Genic variation in abundant proteins of Drosophila melanogaster and D. pseudoobscura. Genetics 102: 437-453.

Singh, R. S., R. C. Lewontin, and A. A. Felton (1976) Genetic heterogeneity within electrophoretic 'alleles' of Xanthine dehydrogenase in D. pseudoobscura. Genetics 84: 609-629.

Singh, R. S. and L. R. Rhomberg (1987a) A comprehensive study of genic variation in natural populations of Drosophila melanogaster. I. Estimates of gene flow from rare alleles. Genetics 115: 313-322.

Singh, R. S. and L. R. Rhomberg (1987b) A comprehensive study of genic variation in natural populations of Drosophila melanogaster. II. Estimates of heterozygosity and patterns of geographic differentiation. Genetics (in press).

Slatkin, M. (1985) Rare alleles as indicators of gene flow. Evolution 39: 53-65.

Smith, I. (1976) Chromatographic and electrophoretic techniques. Vol. II: Zone electro-phoresis, Heineman, London.

Snyder, T. P. and M. C. Linton (1984) Population structure in black flies allozymic and morphological estimates for prosimulium mixtum and prosimulium fuscum (Diptera: Simuliidae). Evolution 38: 942-956.

Solignac, M. and M. Monnerot (1986) Race formation, speciation, and introgression within Drosophila simulans, D. mauritiana, and D. sechellia inferred from mitochondrial DNA analysis. Evolution 40: 531-539.

Soule, M. and B. R. Stewart (1970) The niche variation hypothesis: a test and alternatives. Am. Natur. 104: 85-97.

Spencer, W. P. (1947) Mutations in wild populations of Drosophila. Advan. Genet. 1: 359-402.

Stalker, H. D. (1976) Chromosome studies in wild populations of D. melanogaster. Genetics 82: 323-347.

Stanley, S. M. (1975) A theory of evolution above the species level. Proc. Nat. Acad. Sci. USA 72: 646-450.

Stanley, S. M. (1979) Macroevolution: Pattern and Process. W. H. Freeman, San Francisco.

Stebbins, G. L. (1977) Processes of Organic Evolution. Prentice-Hall, Englewood Cliffs, NJ.

Steiner, W. W. M., K. C. Sung and Y. K. Paik (1976) Electrophoretic variability in island populations of D. simulans and D. immigrans. Biochem. Genet. 14: 496-506.

Steiner, W. W. M. (1977) On niche width and genetic variation in Hawaiian Drosophila. Am. Natur. 111:

1037-1045.

Stephens, J. C. and M. Nei (1985) Phylogenetic analysis of polymorphic DNA sequences at the Adh locus in Drosophila melanogaster and its sibling species. J. Mol. Evol. 22: 289-300.

Sturtevant, A. H. (1920) Genetic studies on D. simulans. I. Introduction. Hybrids with D. melanogaster. Genetics 5: 488-500.

Sturtevant, A. H. and E. Novitski (1941) Sterility in crosses of geographical races of Drosophila micromelanica. Proc. Natl. Acad. Sci. USA 21: 392-394.

Tantawy, A. O., G. S. Mallah and H. R. Tewfik (1964) Studies on natural populations of Drosophila. II. Heritability and response to selection for wing length in D. melanogaster and D. simulans at different temperatures. Genetics 49: 938-948.

Taylor, C. E. and J. R. Powell (1977) Microgeograhic differentiation of chromosomal and enzyme polymorphisms in D. persimilis. Genetics 85: 681-695.

Templeton, A. R. (1980) The theory of speciation via the founder principle. Genetics, 94: 1011-1038.

Templeton, A. R. (1981). Mechanisms of speciation - A population genetics approach. Ann. Rev. Ecol. Syst. 12: 23-48.

Templeton, A. (1986) The relation between speciation mechanisms and macroevolutionary patterns. In: Evolutionary processes and Theory (ed. by S. Karlin and E. Nevo), Academic Press, Inc.

Thompson, P. and J. W. Sites (1986) Comparison of population structure in chromosomally polytypic and monotypic species of Sceloporus in relation to chromosomally-mediated speciation. Evolution 40: 303-314.

Throckmorton, L. H. (1977) Drosophila systmeatics and biochemical evolution. Ann. Rev. Ecol. Syst. 8:235 - 254.

Triantaphyllidis, C. D. (1973) Allozyme variation in populations of D. melanogaster and D. simulans from northern Greece. J. Heredity 64: 69-72.

Triantaphyllidis, C. D., J. N. Panourgias, Z. G. Scouras and G. C. Ioannidis (1980) Comparison of gene-enzyme variation between D. melanogaster and D. simulans. Genetica 51: 227-231.

Triantaphyllidis, C. D., Z. G. Scouras, J. N. Panourgias, and G. C. Ioannidis (1982) Allozyme variation in Greek wild populations of D. melanogaster and D. simulans along a North-South gradient. Genetica 58: 129-136.

Van Valen L. (1965) Morphological variation and the width of the ecological niche. Am. Natur. 99: 377-389.

Vigneault, G. and E. Zouros (1986) The genetics of asymmetrical male sterility in Drosophila mojavensis and D. arizonensis hybrids: interactions between the Y-chromosome and autosomes. Evolution 40: 1160 - 1170.

Voelker, R. A. (1974) The genetics and cytology of a mutator factor in D. melanogaster. Mutation Res. 22: 265-276.

Wake, D. B. and K. P. Yanev (1986) Geographic variation in allozymes in a "ring species", The plethodontid salamander Ensatina eschscholtzii of Western North America, Evolution 40: 702-715.

Wallace, B., M. W. Timm, and M. P. P. Strambi (1983) The establishment of novel mate-recognition systems in introgressive hybrid Drosophila populations. Evol. Biol. 16: 467-488.

Warwick, S. I. and L. D. Gottlieb (1985) Genetic divergence and geographic speciation in Layia compositae. Evolution 39: 1236-1241.

Watada, M., Y. N. Tobari and S. Ohba (1986) Genetic differentiation in Japanese populations of D. simulans and D. melanogaster. I. Allozyme polymorphisms. Jpn. J. Genet. 61: 253-269.

Watada, M., S. Ohba and Y. N. Tobari (1986) Genetic differentiation in Japanese populations of D. simulans and D. melanogaster. II. Morphological

variation. Jap. J. Genet. 61: 469-480.

White, M. J. D. (1973) Animal Cytology and Evolution. Cambridge University Press, Cambridge.

White, M. J. D. (1978) Modes of Speciation. W. H. Freeman, San Francisco.

Williamson, J. H. (1976) The genetics of the Y chromosome. In: The Genetics and Biology of Drosophila Vol. 1B (ed. by M. Ashburner and E. Novitski), Academic Press, New York.

Wilson, A. C., G. L. Bush, S. M. Case, and M. C. King (1977) Social structuring of mammalian populations and rate of chromosomal evolution. Proc. Natl. Acad. Sci. USA 72: 5061-5065.

Wilson, A. C., S. S. Carlson, and T. J. White (1977) Biochemical evolution. Ann. Rev. Biochem. 46: 573-639.

Wilson, A. C., L. R. Maxson and V. M. Sarich (1974) Two types of molecular evolution. Evidence from studies of interspecific hybridization. Proc. Nat. Acad. Sci. USA 71:2843 - 2847.

Wilson, M. F. (1969) Avian niche size and morphological variation. Am. Natur. 103: 531-535.

Woodruff, R. C. and M. Ashburner (1978) The frequency of X-ray induced chromosome breakage in the sibling species D. melanogaster and D. simulans. Am. Natur. 12: 456-459.

Wright, S. (1931) Evolution in Mendelian populations. Gentics 16:97 - 159.

Wright, S. (1969) Evolution and genetics of populations. University of Chicago Press, Chicago.

Wright, S. (1980) Character Change, speciation, and the higher taxa. Evolution, 36(3): 427-443.

Yamaguchi, O., R. A. Cardellino and T. Mukai (1976) High rates of spontaneous chromosome aberrations in D. melanogaster. Genetics 83: 409-422.

Zouros, E. (1973) Genic differentiation associated with the early stages of speciation in the Mulleri subgroup of Drosophila. Evolution 27: 601-621.

Zouros, E. (1981) The chromosomal basis of sexual isolation in two sibling species of Drosophila: D. arizonensis and D. mojavensis. Genetics 97: 703-718.

Zuckerkandl, E., and L. Pauling (1965) Evolutionary divergence and convergence in proteins. In: Evolving genes and proteins (ed. by V. Bryson and H. J. Vogel), Academic Press, New York, pp. 97-166.

APPENDIX 1

TABLE A1

Allele frequency, expected heterozygosity (H), and numbers of isofemale lines examined (N) for different enzyme and abundant protein loci in various populations of *Drosophilia simulans*

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E. (for Mainland populations)	SEYCHELLE 3.8°S	MEAN±S.E. (All populations)
ALDEHYDE OXIDASE (Ao)							
1	--	--	0.148	--	0.037	--	0.029
2	0.476	0.692	0.547	0.788	0.626	0.385	0.578
3	0.248	0.058	0.021	0.076	0.101	0.577	0.196
4	0.276	0.250	0.284	0.136	0.236	0.038	0.197
H	0.636	0.455	0.598	0.355	0.511±0.130	0.517	0.512±0.112
ESTERASE-6 (Est-6)							
1	0.009	--	0.212	--	0.055	--	0.044
2	0.333	0.333	0.331	0.323	0.330	0.058	0.276
3	--	--	--	--	-	0.019	0.004
4	0.618	0.528	0.441	0.569	0.539	0.847	0.601
5	0.040	0.139	0.016	0.108	0.076	0.038	0.068
6	--	--	--	--	-	0.038	0.007
H	0.505	0.591	0.651	0.560	0.577±0.061	0.276	0.517±0.144
ESTERASE-9 (Est-9)							
1	--	--	--	--	-	0.075	0.015
2	0.076	0.083	0.350	0.029	0.134	0.113	0.130
3	0.914	0.917	0.650	0.971	0.863	0.812	0.853
4	0.010	--	--	--	0.003	--	0.002
H	0.159	0.152	0.455	0.056	0.205±0.173	0.322	0.229±0.158

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
PHOSPHOGLUCOMUTASE (Pgm)							
1	0.080	0.058	0.009	0.015	0.041	--	0.032
2	0.060	0.175	0.009	0.203	0.112	0.019	0.093
3	0.820	0.767	0.925	0.782	0.823	0.981	0.855
4	0.040	--	0.057	--	0.024	--	0.020
H	0.316	0.378	0.141	0.347	0.295±0.106	0.037	0.244±0.148
ACID PHOSPHATASE (Acph)							
1	0.019	0.063	0.267	--	0.087	--	0.070
2	0.058	--	0.078	--	0.034	--	0.027
3	0.346	0.326	0.611	0.849	0.533	0.846	0.596
4	0.048	0.074	--	0.015	0.034	--	0.027
5	0.500	0.505	0.011	0.136	0.288	0.154	0.261
6	0.029	0.032	0.011	--	0.018	--	0.014
7	--	--	0.022	--	0.006	--	0.005
H	0.623	0.628	0.548	0.260	0.515±0.174	0.260	0.464±0.189
6-PHOSPHOGLUCONATE DEHYDROGENASE (6-Pgd)							
1	--	--	0.073	--	0.018	--	0.015
2	1.000	1.000	0.890	1.000	0.973	1.000	0.978
3	--	--	0.037	--	0.009	--	0.007
H	0	0	0.201	0	0.050±0.100	0	0.040±0.090
HEXOKINASE-1 (Hex-1)							
1	--	--	--	--	--	0.154	0.031
2	1.000	1.000	0.881	1.000	0.970	0.846	0.945
3	--	--	0.119	--	0.030	--	0.024
H	0	0	0.210	0	0.052±0.105	0.260	0.094±0.130

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
α-GLYCEROPHOSPHATE DEHYDROGENASE (α Gpd)							
1	--	--	0.102	--	0.025	--	0.020
2	1.000	1.000	0.898	1.000	0.975	1.00	0.980
H	0	0	0.183	0	0.046±0.091	0	0.037±0.082
OCTANOL DEHYDROGENASE-2 (Odh-2)							
1	--	--	0.340	--	0.085	0.038	0.076
2	1.000	1.000	0.660	1.000	0.915	0.962	0.924
H	0	0	0.449	0	0.112±0.224	0.073	0.104±0.195
β-GALACTOSIDASE (β-Gal)							
1	--	--	--	--	--	0.074	0.015
2	1.00	1.00	1.00	1.00	1.00	0.741	0.948
3	--	--	--	--	-	0.185	0.037
H	0	0	0	0	0	0.411	0.082>0.184
PHENOL OXIDASE (Phox)							
1	0.979	0.911	0.979	1.000	0.967	0.960	0.966
2	0.021	0.089	0.021	--	0.033	0.040	0.034
H	0.041	0.162	0.041	0	0.061±0.070	0.077	0.064±0.061
α-AMYLASE (α-Amy)							
1	1.000	0.979	1.000	0.971	0.988	1.00	0.990
2	--	0.021	--	0.029	0.012	--	0.010
H	0	0.041	0	0.056	0.024±0.029	0	0.019±0.027

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
XANTHINE DEHYDROGENASE (Xdh)							
1 (0.90)	0.164	0.112	0.302	0.135	0.178	--	0.143
2 (0.94)	0.073	0.163	0.205	0.311	0.188	0.100	0.170
3 (1.00)	0.754	0.715	0.388	0.514	0.593	0.620	0.598
4 (1.03)	0.009	0.010	0.105	0.040	0.041	0.240	0.081
5 (1.05)	--	--	--	--	--	0.040	0.008
H	0.399	0.449	0.705	0.619	0.543±0.143	0.546	0.544±0.124
DIPEPTIDASE-1 (Dip-1)							
1 (0.93)	0.019	0.045	0.043	--	0.027	--	0.021
2 (1.00)	0.827	0.795	0.522	0.706	0.712	--	0.570
3 (1.04)	0.019	0.114	0.152	0.059	0.086	--	0.069
4 (1.09)	0.135	0.045	0.283	0.235	0.175	1.00	0.340
H	0.297	0.351	0.622	0.443	0.428±0.142	0	0.343±0.228
MANNOSE 6-PHOSPHATE ISOMERASE (Mpi)							
1 (0.96)	--	--	0.045	--	0.011	1.00	0.209
2 (1.00)	0.981	1.00	0.933	0.971	0.971	--	0.777
3 (1.06)	0.019	--	0.022	0.029	0.018	--	0.014
H	0.037	0	0.127	0.056	0.055±0.053	0	0.044±0.052
β-GLUCORONIDASE (β-Gus)							
1	--	--	--	--	--	0.111	0.022
2	1.00	1.00	1.00	1.00	1.00	0.889	0.978
H	0	0	0	0	0	0.197	0.039>0.088

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
GLUTAMATE DEHYDROGENASE (GluD)							
1 (0.94)	--	0.032	0.011	--	0.011	--	0.009
2 (1.00)	1.00	0.936	0.956	1.00	0.973	0.500	0.878
3 (1.08)	--	0.032	0.033	--	0.016	0.500	0.113
H	0	0.122	0.085	0	0.052±0.062	0.500	0.141±0.207
CARBONIC ANHYDRASE-1 (Ca-1)							
1 (0.75)	--	--	0.152	--	0.038	0.096	0.050
2 (0.88)	0.379	0.416	0.387	0.328	0.378	0.904	0.483
3 (1.00)	0.568	0.577	0.461	0.647	0.563	--	0.450
4 (1.05)	0.053	0.007	--	0.025	0.021	--	0.017
H	0.531	0.494	0.615	0.473	0.528±0.063	0.173	0.457±0.168
CARBONIC ANHYDRASE-2 (Ca-2)							
N (Null)	--	0.086	0.089	0.029	0.051	--	0.041
1 (0.88)	0.019	0.021	--	0.015	0.014	--	0.011
2 (0.94)	0.157	0.355	0.111	0.186	0.202	--	0.162
3 (0.96)	--	--	0.022	--	0.006	--	0.004
4 (0.98)	0.247	0.495	0.778	0.437	0.489	0.750	0.541
5 (1.00)	0.577	0.043	--	0.333	0.238	0.250	0.241
H	0.581	0.619	0.374	0.662	0.559±0.128	0.375	0.522±0.138
CARBONIC ANHYDRASE-3 (Ca-3)							
1 (0.86)	0.043	0.051	0.018	0.152	0.066	0.038	0.060
2 (1.00)	0.904	0.594	0.427	0.627	0.638	0.385	0.588
3 (1.05)	--	0.022	--	--	0.005	--	0.004
4 (1.09)	0.053	0.304	0.527	0.172	0.264	0.577	0.327
5 (1.13)	--	0.029	0.028	0.049	0.027	--	0.021
H	0.178	0.551	0.539	0.552	0.455±0.185	0.517	0.467±0.162

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
CARBONIC ANHYDRASE-4 (Ca-4)							
1 (0.92)	0.043	0.100	0.355	0.020	0.130	--	0.104
2 (0.96)	0.042	0.100	0.248	0,020	0.102	--	0.082
3 (1.00)	0.915	0.800	0.397	0.960	0.768	1.00	0.814
H	0.159	0.340	0.655	0.078	0.308±0.256	0	0.246±0.261
DIAPHORASE-2 (Dia-2)							
1 (0.86)	0.188	0.174	0.467	0.258	0.272	0.039	0.225
2 (0.90)	0.009	0.033	0.011	--	0.013	0.078	0.026
3 (1.00)	0.732	0.793	0.522	0.636	0.671	0.765	0.690
4 (1.08)	0.062	--	--	0.091	0.038	0.118	0.054
5 (1.12)	0.009	--	--	0.015	0.006	--	0.005
H	0.425	0.340	0.509	0.520	0.448±0.084	0.393	0.437±0.077
α-GLUCOSIDASE-3 (α-Glu-3)							
1 (0.95)	--	--	--	0.062	0.015	--	0.012
2 (1.00)	1.00	1.00	0.977	0.938	0.979	1.00	0.983
3 (1.02)	--	--	0.023	--	0.006	--	0.005
H	0	0	0.045	0.116	0.040±0.055	0	0.032±0.051
α-GLUCOSIDASE-4 (α-Glu-4)							
1 (0.95)	0.019	--	0.034	0.062	0.029	0.116	0.046
2 (1.00)	0.944	1.00	0.943	0.844	0.933	0.846	0.916
3 (1.01)	0.037	--	0.023	0.094	0.038	0.038	0.038
H	0.107	0	0.109	0.275	0.123±0.113	0.269	0.152±0.118

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
LACTATE DEHYDROGENASE (Ldh)							
1 (0.98)	--	--	--	--	--	0.308	0.062
2 (1.00)	0.853	0.830	0.645	0.971	0.825	0.692	0.798
3 (1.03)	0.009	0.032	0.108	--	0.037	--	0.030
4 (1.05)	0.138	0.138	0.247	0.029	0.138	--	0.110
H	0.253	0.291	0.511	0.056	0.278±0.186	0.426	0.307±0.175
ESTERASE-10 (Est-10)							
1 (0.97)	0.009	0.021	--	--	0.007	--	0.006
2 (1.00)	0.838	0.833	0.874	0.723	0.817	1.00	0.854
3 (1.02)	0.045	0.031	0.095	0.185	0.089	--	0.071
4 (1.09)	0.054	0.094	--	0.046	0.049	--	0.039
5 (1.19)	0.054	0.021	0.031	0.046	0.038	--	0.030
H	0.290	0.295	0.226	0.439	0.312±0.090	0	0.250±0.160
NADP-SORBITOL DEHYDROGENASE-1 (Sodh-1)							
1	--	--	--	--	--	0.019	0.004
2	--	--	--	--	--	0.019	0.004
3	1.00	1.00	1.00	1.00	1.00	0.750	0.950
4	--	--	--	--	--	0.212	0.042
H	0	0	0	0	0	0.392	0.078>0.175
ALKALINE PHOSPHATE (Aph)							
1 (0.84)	--	--	--	0.031	0.008	--	0.006
2 (0.90)	0.148	--	--	0.031	0.045	--	0.036
3 (1.00)	0.852	1.00	1.00	0.938	0.947	1.00	0.958
H	0.252	0	0	0.118	0.092±0.120	0	0.074±0.112

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
SUCCINIC DEHYDROGENASE (Sdh)							
1 (0.97)	--	--	0.023	--	0.006	--	0.005
2 (1.00)	0.606	0.823	0.739	0.788	0.739	1.00	0.791
3 (1.05)	--	--	0.011	--	0.003	--	0.002
4 (1.10)	0.293	0.104	0.148	0.151	0.174	--	0.139
5 (1.15)	0.101	0.073	0.079	0.061	0.078	--	0.063
H	0.537	0.306	0.425	0.352	0.405±0.101	0	0.324±0.201
TREHALASE (Treh)							
1 (0.94)	0.037	0.104	0.111	0.094	0.086	--	0.069
2 (1.00)	0.741	0.771	0.734	0.844	0.773	0.556	0.729
3 (1.03)	0.222	0.021	0.111	0.031	0.096	--	0.077
4 (1.10)	--	0.104	0.044	0.031	0.045	0.444	0.125
H	0.400	0.383	0.435	0.277	0.374±0.068	0.494	0.398±0.080
GLUCOSE DEHYDROGENASE (Gdh)							
1 (0.92)	0.019	--	--	0.094	0.028	--	0.023
2 (1.00)	0.962	0.935	0.967	0.859	0.931	0.808	0.906
3 (1.20)	0.019	0.065	0.033	0.047	0.041	0.192	0.071
H	0.074	0.121	0.064	0.251	0.127±0.086	0.310	0.164±0.110
α-FUCOSIDASE (α-Fuc)							
1 (0.92)	--	0.034	--	--	0.008	--	0.007
2 (1.00)	1.00	0.852	0.405	0.952	0.802	0.118	0.665
3 (1.20)	--	--	0.143	--	0.036	0.078	0.044
4 (1.48)	--	0.114	0.452	0.048	0.154	0.804	0.284
H	0	0.260	0.611	0.091	0.240±0.269	0.333	0.259±0.237

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
GLUCOSE OXIDASE (Go)							
1	1.00	1.00	1.00	1.00	1.00	0.923	0.985
2	--	--	--	--	--	0.077	0.015
H	0	0	0	0	0	0.142	0.028>0.063
ISOCITRATE DEHYDRO-GENASE-1 (Idh-1)							
1 (0.96)	--	--	--	--	--	0.250	0.050
2 (0.98)	--	--	--	--	--	0.058	0.011
3 (1.00)	0.830	0.963	0.891	0.938	0.905	0.692	0.863
4 (1.07)	0.170	0.037	0.109	0.062	0.095	--	0.076
H	0.282	0.071	0.194	0.116	0.166±0.093	0.455	0.244±0.152
ESTERASE-C (Est-C)							
1	0.179	0.266	--	--	0.111	0.403	0.170
2	0.333	0.276	0.033	0.015	0.164	0.113	0.154
3	0.399	0.330	0.450	0.397	0.394	0.290	0.373
4	0.081	0.110	0.484	0.529	0.301	0.194	0.280
5	0.008	0.018	0.033	0.059	0.030	--	0.023
H	0.691	0.732	0.561	0.559	0.636±0.089	0.703	0.649±0.083
GLUTAMATE PYRUVATE TRANSAMINASE (Gpt)							
1 (0.90)	0.020	--	--	0.063	0.021	--	0.017
2 (1.00)	0.660	0.927	0.922	0.906	0.854	0.846	0.852
3 (1.09)	0.110	0.010	--	--	0.030	0.154	0.055
4 (1.14)	0.210	0.063	0.078	0.031	0.095	--	0.076
H	0.508	0.137	0.144	0.174	0.241±0.179	0.260	0.245±0.155

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
LARVAL PROTEIN-1 (Pt-1)							
1	0.473	0.481	0.578	0.187	0.430	0.037	0.351
2	0.072	--	--	--	0.018	--	0.014
3	0.366	0.404	0.289	0.688	0.437	0.963	0.542
4	0.018	--	--	--	0.004	--	0.004
5	0.071	0.096	0.133	0.125	0.106	--	0.085
6	--	0.019	--	--	0.005	--	0.004
H	0.632	0.596	0.565	0.476	0.567±0.066	0.071	0.468±0.229
LARVAL PROTEIN-9 (Pt-9)							
1	0.073	--	--	0.062	0.034	--	0.027
2	0.891	0.962	0.978	0.844	0.919	0.926	0.920
3	0.036	0.038	0.022	0.094	0.047	0.074	0.053
H	0.199	0.073	0.043	0.275	0.147±0.108	0.137	0.145±0.094
LARVAL PROTEIN-10 (Pt-10)							
1	0.009	--	--	--	0.002	--	0.002
2	0.109	0.087	0.022	0.031	0.062	--	0.050
3	--	0.067	--	0.047	0.028	0.037	0.030
4	0.837	0.788	0.978	0.891	0.874	0.852	0.869
5	0.045	0.058	--	0.031	0.034	0.074	0.042
6	--	--	--	--	--	0.037	0.007
H	0.285	0.364	0.043	0.202	0.22±0.137	0.266	0.232±0.120
LARVAL PROTEIN-11 (Pt-11)							
1	0.018	0.038	0.056	0.063	0.044	--	0.035
2	0.045	--	--	0.063	0.027	--	0.022
3	0.519	0.568	0.322	0.781	0.547	0.962	0.630
4	0.400	0.375	0.622	0.093	0.373	0.038	0.306
5	0.018	0.019	--	--	0.009	--	0.007
H	0.568	0.535	0.506	0.373	0.495±0.085	0.073	0.411±0.203

Appendix 1 (Continued)

LOCUS/ALLELE	FRANCE 43°N	TUNISIA 35.6°N	CONGO 4.2°S	CAPETOWN 34°S	MEAN±S.E.	SEYCHELLE 3.8°S	MEAN±S.E.
LARVAL PROTEIN-15 (Pt-15)							
1	--	--	0.056	0.094	0.037	--	0.030
2	0.218	0.173	0.144	0.062	0.149	0.093	0.138
3	0.528	0.673	0.411	0.750	0.591	0.648	0.602
4	0.218	0.154	0.233	0.094	0.175	0.259	0.192
5	0.036	--	0.156	--	0.048	--	0.038
H	0.625	0.493	0.728	0.416	0.565±0.138	0.504	0.553±0.123
LARVAL PROTEIN-16 (Pt-16)							
1	0.945	1.000	1.000	1.000	0.986	1.00	0.989
2	0.055	--	--	--	0.014	--	0.011
H	0.104	0	0	0	0.026±0.052	0	0.021±0.046
ADULT PROTEIN-9A(A)							
1 (1.00)	0.889	0.979	0.989	0.953	0.953	1.00	0.962
2 (1.05)	0.111	0.021	0.011	0.047	0.047	--	0.038
H	0.197	0.041	0.022	0.089	0.087±0.078	0	0.070±0.078
ADULT PROTEIN-15A(A)							
1 (1.00)	0.972	1.00	0.979	0.984	0.984	0.962	0.979
2 (1.03)	0.028	--	0.021	0.016	0.016	0.038	0.021
H	0.054	0	0.041	0.031	0.031±0.023	0.073	0.040±0.027
NO. OF GENOMES SAMPLED	110	104	90	64		52	

Appendix 1 (Continued)

MONOMORPHIC LOCI: Hexokinase-2,3; Malic enzyme; NAD-Malic dehydrogenase (cytoplasmic), NADP-Malic dehydrogenase (mitochondrial); Alcohol dehydrogenase (ADH); Glucose-6-phosphate dehydrogenase (G-6PD); Leucine aminopeptidase-D; 6 and 7(A); Fumerase, Hydroxyacid dehydrogenase (HAD); Diaphorase-1,6 and 7; a-Mannosidase-1,2,3, and 4; NAD-Sorbitol dehydrogenase (mitochondrial); Adenylate kinase-1 and 2; Tetrazolium oxidase-1 and 2; Dipeptidase-3(A) and 2(B); Phosphoglucose isomerase (PGI); Aldolase; Sucrase; Aconitase; Arylsulphatase; NADP-Isocitrate dehydrogenase (cytoplasmic); Catalase; Phosphoglycero kinase (PGK); Glyceraldehyde phosphate dehydrogenase (GAPDH); β-N Acetylglucosamaminidase (HEXOSE-1); α-Glycerophosphate oxidase (α-GPO); α-Glucosidase-2; Acetylcholine esterase; Triose phosphate isomerase (TPI); Arginine kinase-1 and 2; Glutamate oxaloacetate transaminase-1 (GOT-1); larval protein-2,3,4,5,6,7,8,12,13; 7A(A); 10A(A); and 16 silver stained protein loci.
