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VARIATION IN GENE FREQUENCIES IN RODENT POPULATIONS: THE ROLES OF SELECTIVE AND NONSELECTIVE EVOLUTIONARY FORCES

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VARIATION IN GENE FREQUENCIES IN RODENT POPULATIONS: THE ROLES OF SELECTIVE AND NONSELECTIVE EVOLUTIONARY FORCES

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

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Michael Soulé — 21 June 1981

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ABSTRACT

The study of protein polymorphism in natural populations has stimulated heated controversy over the effects of various evolutionary forces on the observed patterns of genetic variation. One viewpoint is that a majority of the mutations at a locus are selectively equivalent and that variations in gene frequencies in time and space are primarily a result of nonselective evolutionary forces. The opposing view is that most mutations have sufficient effect on individual fitness that variations in gene frequencies are adaptations resulting from the action of natural selection.

I compared gene frequency distributions among various loci to assess the roles of selective and nonselective evolutionary forces in determining patterns of allozyme variation in populations of rodents. I used two versions of the Lewontin-Krakauer test on temporal variation in allozyme frequencies reported for populations of the prairie vole, Microtus ochrogaster. The tests revealed that the changes in gene frequency were homogeneous among loci which suggests that nonselective forces such as genetic drift and migration were the primary cause of gene frequency change within populations.

I also compared the spatial gene frequency distributions reported for 17 species of rodents to assess which evolutionary factors account for the genetic differentiation of populations within each species. Most loci showed similar degrees of differentiation, a pattern expected if nonselective forces operated in population differentiation.

I found a positive relationship between the amount of differentiation of populations and the magnitude of positive association among rare alleles. This result suggests an active role of genetic drift in population differentiation within rodent species.

The analysis of allozyme distributions in populations of rodents indicates that nonselective evolutionary forces play a substantial role in determining patterns of genetic variation. According to Wright's Shifting Balance Theory, the random differentiation of populations may actually accelerate adaptive evolution, which may account for the rapid evolutionary rates found in rodents.

CHAPTER 1

INTRODUCTION

In the first half of this century, evolutionary biology went through two phases of major change. The first stage was the birth of mathematical population genetics, signaled by the virtually concurrent publication of Fisher's (1930) The Genetical Theory of Natural Selection, Wright's (1931) "Evolution in Mendelian Populations," and Haldane's (1932) The Causes of Evolution. Although these men emphasized different aspects of the evolutionary process, they each began with a simplified population of genes and, using mathematics, deduced the effects of various evolutionary forces on the change in gene frequency. The second stage in development entailed the interpretation and synthesis of a wide body of biological knowledge including cytogenetics (e.g. Dobzhansky 1937), systematics (Huxley 1942; Mayr 1942), paleontology (Simpson 1944), and behavior (Wilson 1975), each using the theoretical framework of population genetics.

Despite the synthesis of evolutionary biology, there coexist today many different views of the evolutionary process. There are two primary areas of conceptual disagreement; the relative magnitude of the mechanisms of evolutionary change and the evolutionary unit upon which the process operates.

The Mechanisms of Evolutionary Change

Wright (1948, 1956) classified the processes of evolutionary change which affect gene frequencies in a population into directed forces and random factors (Table 1). The directed forces include mutation, migration, and selection. In principle, one can measure an average change in gene frequency resulting from each force that is greater than zero and which determines both the rate and direction of evolutionary change.

Both mutation and migration represent the introduction of new genetic material either through mistakes in replication of genes or through the incorporation of genes from outside the local population, respectively. These two directional forces can operate in the absence of local genetic variation (i.e., $p = 0$ or $p = 1$).

In contrast, selection operates only in the presence of variation through differential transmission of genes from generation to generation. Thus, selection in the most general sense, "includes all cause of directed change in gene frequency that do not involve mutation or introduction without" (Wright 1956). (This general definition of selection includes non-Mendelian processes like meiotic drive, segregation distortion, and nondisjunction which alter the segregation ratio at gametogenesis.) Random fluctuations in the directed forces of evolution cause random fluctuations in gene frequency that accompany directional change. Theoretically, one could determine the variance in the effect of a directed force. Other sources of random fluctuation in gene frequency have no mean effect on average gene frequencies and include sampling variation due to finite effective population size

Table 1. The mechanisms of evolutionary change. -- The mechanisms of evolutionary change are divided into three groups: directed forces, random factors, and unique events.

Mechanism	Definition	Antithetic Condition
Directed Forces		
Mutation	Change in genetic material	Perfect Replication
Migration	Introduction of genes from without	Closed population
Selection	Differential transmission of genes	Equal transmission probabilities
Random Factors		
Fluctuation in Directed forces	Random variation in migration, mutation and selection	Constant environment
Finite effective population size	Sampling drift	Infinite population size
Chaotic behavior	Pseudo-stochastic process possible with discrete generations	Continuous, overlapping generations
Unique Events		

and choatic dynamics (Table 1). For diploid organisms, the accidents of sampling at reproduction due to finite population size impart a binomial variance in gene frequency that is a function of gene frequency and effective population size (e.g., Crow and Kimura 1970). Choatic dynamics resemble a stochastic process and can result (at least theoretically) from special circumstances if populations have discrete, nonoverlapping generations (May 1974; Asmussen 1979).

The final category of evolutionary change includes unique evolutionary events that are so rare and unpredictable they can be considered unique. These types of events are not recurrent and, consequently, one cannot estimate a mean and variance. Unique events include novel or very rare favorable mutations, hybridizations, swamping by mass immigration, effects of improbable long-distance migration, bottlenecks in numbers, and selective incidents (Wright 1956).

Through the historical development of evolutionary biology, most investigators have emphasized only some of these factors categorized by Wright as playing major roles in the evolutionary process. For instance, de Vries (1906) and later Willis (1940) stressed mutation as the primary force determining both the direction and rate of evolution. Fisher, Haldane and Wright each had different views of the relative magnitude and level of selection (Provine 1977; Wright 1931) Haldane (1932) viewed the organism as a mosiac of unit characters and populations as nearly homoallelic, and he was primarily interested in the rate of fixation of favorable mutations under constant selection. Fisher (1930) investigated weaker selective forces operating

on polygenic variation in large essentially panmictic populations. Wright (1931), however, emphasized a more holistic balance between evolutionary forces operating on groups of genes (i.e., interaction systems) in structured populations.

Fisher, supported by Ford (Fisher and Ford 1947), interpreted Wright's view of the evolutionary process (as have many others) as primarily determined by genetic drift (i.e., random factors) in small populations. However, Wright (1948) responded that his view encompassed the balance among evolutionary forces and that a population subdivided into numerous finite local populations was most conducive to adaptive change. This is due to the fact that "oscillations in the position of the optimum (of a character) in local populations provide an important mechanism by which all gene combinations with approximately the same effect in respect to the character consideration come to be tried out with respect to secondary effects" (Wright 1948, p. 281).

Recently, the dispute over the relative roles of directed and random processes has been revived with the discovery of extensive protein polymorphism in natural populations. Some have attributed this variation to selective mechanisms whereas others have argued that that it is essentially neutral and the results of random processes (Lewontin 1974). In the following sections I review the history of the selection-neutrality controversy and discuss methods of assessing the roles of selective and nonselective processes in determining genetic variation at loci coding for structural proteins.

The Selectionist-neutralist Controversy

The dynamics of the development of a scientific field depend on the interactions between empirical observation and theory (Lakatos 1978). Occasionally, a new empirical technique reveals unanticipated observations and science goes through a period of controversy, construction and testing of competing hypotheses, and eventually synthesis of new concepts. With the application of electrophoretic techniques to the study of genetic variation in natural populations (Harris 1966; Lewontin and Hubby 1966), new data challenged the established view of the nature of genetic variation in natural populations.

Before 1966, in the synthesis of genetic processes with the Darwin-Wallace concept of natural selection, two interpretations of the genetic structure of populations emerged. Geneticists, working mostly in the laboratory with mutations (Muller 1950), found that most mutations were deleterious, reducing the viability and fecundity of individuals. This research led to the belief that individuals in natural populations are homozygous for wild type alleles which confer high fitness. In contrast, Dobzhansky and his collegues found extensive genic and chromosomal polymorphism and heterozygosity in natural populations and concluded that heterozygous individuals had some advantage in natural situations. These two contrasting schools of thought have been called the classical and balance schools, respectively (Dobzhansky 1955). The schools also differed in their views of natural selection. The classical view is that selection is a purifying process, rejecting most variants. The balance school views selection as a process that maintains variation.

The arrival of electrophoretic techniques for directly assessing genetic variation of structural proteins in natural populations appeared to provide the key for resolving the controversy between the classical and balance views. However, this was not the case. The discovery of a large amount of genetic variation within populations, although appearing initially to support the balance hypothesis, actually exacerbated the controversy. The classicists interpreted the new findings as follows: Genetic loci are of two types. One type of locus is homozygous for alleles subject to natural selection and mutations at these loci are generally deleterious. The other type has numerous alleles that are effectively equal in function and essentially selectively neutral. The variation detected by electrophoresis is of the neutral variety. This new interpretation has been labeled the neoclassical or neutralist point of view (Lewontin 1974). The balance hypothesis, boosted by the variation discovered, was now forced to explain more polymorphism than it had predicted. The problem with large amounts of heterozygosity being maintained by some form of balancing selection is the large genetic load incurred by the population; that is the reduction in average fitness of the population of each generation owing to the continued segregation of less fit combinations of alleles (Lewontin 1974).

The Neutral Hypothesis

Since pioneering work of Fisher (1930) and Wright (1931), population geneticists have used stochastic models to deal with random changes in gene frequencies. Within the last decade, there

has been a proliferation of models that consider the stochastic behavior of mutant genes in finite populations, these models have been motivated by the hypothesis of selective neutrality of polymorphic alleles.

One set of observations that has been interpreted as supporting the neutral hypothesis is the amino comparative acid sequences in proteins such as hemoglobin and cytochrome C in a diverse group of species for which amino acid substitutions have been determined. Kimura (1968) and King and Jukes (1969) argued that the uniformity in substitution rates over evolutionary time (estimated from presumed dates of phylogenetic divergence) is most easily explained by the random fixation of mutant alleles. The neutralists contend that selection models cannot account for this evolutionary rate. Their argument is based on the genetic load incurred by the population in the process of allelic substitution; that is, the difference in the population's average fitness and the fitness of an individual which is homozygous for the alleles being fixed (Kimura and Ohta 1971).

The supporters of the balance hypothesis have challenged the assumption that the allelic substitution rate has been uniform. The time periods involved in the calculations are so long (200 million years) that the <u>average</u> substitution rate yields little information about the actual tempo of evolution (Lewontin 1974). Also the genetic load argument that evolution through natural selection is too costly has been countered by the development of models where the load is substantially reduced (Milkman 1967).

The Balance Hypothesis

The competing school has developed a variety of models in which selection maintains the genetic variation have been developed including the overdominance of heterozygote advantage model and models of density and frequency dependence--soft selection models (Nei 1975 reviewed models of balancing selection). In these models either the heterozygous individual has an advantage in fitness over the homozygous individuals or the polymorphisms is "protected" because fixation is not a stable condition. Observations of the role of natural selection in maintaining polymorphisms come from the classical examples of adaptive coloration in Biston (Kettlewell 1956) and Cepea (Cain and Sheppard 1954) and the wellworn example of hemoglobin polymorphism in man (Allison 1955). Rigorous analysis of these cases depends on estimating genotypic fitnesses in different environments (Hendrick, Ginevan, and Ewing 1976). One example of detecting the direct effect of natural selection on a particular polymorphic protein is the work on Drosophila melanogaster and the alcohol dehydrogenase locus (Clark 1975).

The basic problem with estimating selection in natural populations is the relatively small magnitude of realistic selection coefficients. Even the most ardent selectionists expect relative genotypic fitnesses to vary by only a few percent (Lewontin and Krakauer 1973). (This is undoubtedly the reason why our classical examples of natural selection and polymorphism deal with conspicious polymorphisms and clear cut, relatively strong selective pressures such as predation.) Moreover, estimating genotypic fitness for a particular electrophoretic

locus is frought with difficulties as Lewontin (1974, p. 236) stated: "To the present moment no one has succeeded in measuring with any accuracy the net fitnesses of genotypes for any locus in any species in any environment in nature." However, many authors have attempted to estimate components of fitness, such as viability and fecundity, during an organism's life-cycle (Prout 1971; Gaines, Myers, and Krebs 1971; Bundgaard and Christiansen 1972; Clegg and Allard 1973; Allard, Kahler, and Clegg 1977).

Another approach to the selectionist-neutralist controversy has emerged that does not depend on estimating genotypic fitness components. Researchers have started to test each data set against the null hypothesis of neutrality and to use sophisticated statistical techniques to accept or reject this null hypothesis (Ewens 1972; Johnson and Feldman 1973; Lewontin and Krakauer 1973; Yardley, Anderson, and Schaffer 1977; Watterson 1977; Wilson 1980 and others). This approach relies on the theoretical predictions generated by several classes of models and not on estimating genotypic fitnesses.

The most formal models for testing neutrality fall into four categories (Ewens and Feldman 1976; Ewens 1977): classical models, infinite allele models, infinite site models, and charge-state models. The classical models originated with Fisher and Wright, but were made most flexible with Kimura's (1964) diffusion equation treatment. These models include both directed and random forces, and they focus on the equilibrium distribution of allele frequencies. A variation of the model is the infinite-allele model (Kimura and Crow 1964) where mutation leads to a unique allele. This concept of mutation is more

consistent with the modern view revealed in molecular genetic studies. Infinite site models (Kimura 1971) assume a gene consists of an infinite sequence of sites where each is monomorphic or polymorphic and mutations arise at previously monomorphic sites. These models theoretically capture the nature of the gene in that each site represents a particular nucleotide.

The above-listed three models depend on complete identification of all alleles at each locus and also on the assumption of equilibrium of the evolutionary process. The Charge-state models (Ohta and Kimura 1973) were designed specifically with electrophoresis in mind, where the electric charge on a protein is increased or decreased by a discrete unit mutation. These models are most applicable to electrophoretic data as they now exist.

Two general predictions from neutral models concern the theoretical relationship between effective population size, mutation rate, and heterozygosity, and also the relationship between average heterozygosity in a population and the proportion of polymorphic loci (Nei 1975). To test for neutrality using the theoretical predictions of one of the models, test statistics are derived for comparison of theoretical and empirical allele frequency distributions. The theoretical results of the model depend on the assumption of stationary behavior and usually involve unknown parameters like the effective population size and the per generation mutation rate.

Several statistical tests of neutral models use allelic frequency distributions (Ewens 1972; Johnson and Feldman 1973; Yamazaki and Maruyama 1972; Yardley, Anderson, and Schaffer 1977; Watterson 1977;

Wilson 1980; and others). The most thorough analysis of allele frequency data to date is a series of papers by Nei and his
Using infinite allelic models, they examined the relationship between the mean and variance in heterozygosity among different species (Fuerst, Chakraborty, and Nei 1977), the mean and variations of genetic distance in the differentiation of populations (Chakraborty, Fuerst, and Nei 1978) and the distribution of allele frequencies and the number of alleles per locus in many species of vertebrates and invertebrates (Chakraborty, Fuerst, and Nei 1980). The data used for these analyses included most of the electrophoretic data collected for multiple populations from each species. Their results are consistent with the expectations of the neutral hypothesis. However, their tests rely on certain assumptions about the data (e.g., stationarity, complete identification of alleles) which may not be met. Furthermore, these tests usually consist of an over assessment of neutrality, and usually do not indicate which particular locus (or loci) deviates from the neutral predictions.

To avoid the constrictions of the formal tests, other researchers have devised less rigorous tests that do not depend on a specific model. For example, Aspinwall (1974) examined allelic frequencies in populations of salmon that migrate in alternate years. Since the cohorts return to the same river to spawn on alternate years, there is essentially no gene flow between cohorts. Aspinwall argued that since the two cohorts live under similar selective regimes (same river and ocean), the populations represent natural replications and any allelic differences should be attributable to random drift. The

results indicate a significant difference in the allele distribution between cohorts and the author concluded that random genetic drift accounts for this variation.

Penny and Zimmerman (1973) studied allelic frequencies in pocket gophers (Geomys) and found alternate alleles fixed in different populations along a latitudinal gradient. They tested the pattern of allele fixation for departures from randomness by a runs test, and found that the fixation of alleles at five loci was independent of latitude. They concluded that selection did not account for this pattern.

These results illustrate the statistical approach to the selectionist-neutralist controversy that does not rely on estimating genotypic fitnesses. The interest is in detecting the main effect of selection by examining frequencies of alleles at polymorphic loci and comparing the observed distributions to those predicted from a neutral model. A more general statistical test for the selective neutrality of polymorphisms was developed by Lewontin and Krakauer (1973). The test depends on standardized allelic variances and comparison of these variances among loci and does not depend on a specific model of the mutation process. Although the test has been criticized on statistical grounds (Ewens and Feldman 1976), many of the problems may be avoided with cautious application of the test. (This test will be discussed in more detail in Chapters 2 and 3.)

To summarize, the controversy between the selectionists and neutralists schools stimulated a development of theoretical models

and statistical tests for assessing the role of selective and non-selective forces in biochemical evolution. The theory is hopefully leading to an understanding of the interacting processes responsible for the maintenence of genetic variation. But to generalize that most allelic variation is adaptive or nonadaptive is unnecessary and unwarranted.

In the following two chapters, I applied statistical techniques to allozyme data from natural populations of rodents in order to assess the roles of selective and nonselective forces in determining gene frequency distributions in time and space. In Chapter 2, I analyzed temporal variation in gene frequencies in populations of voles. In Chapter 3, I analyzed spatial variation in gene frequencies within 17 species of rodents. In the final chapter, I discuss the effect of population structure on the rate of adaptive change.

CHAPTER 2

GENETIC CHANGES WITHIN POPULATIONS OF VOLES

Microtine rodents have been the workhorse of mammalian ecologists interested in factors controlling population density. The reason microtines have received so much attention is their wide fluctuations in local population densities through time (for a review see Krebs and Myers 1974). These density fluctuations appear to be periodic in nature with crashes in density occurring every 3-4 years.

In conjunction with the fluctuations in density, demographic, and behaviorial characteristics of vole populations vary through time. For instance, Boonstra and Krebs (1979) reviewed the reported 20-30% increase in the mean body weight of individuals in peak populations as compared to those in low-density populations. They suggested the variation to be genetic and attempted to test two competing hypotheses: Selection may favor greater reproductive ability during increases in density (r selection) or selection may favor greater aggressive ability for interference competition (α selection) at high densities. Rose (1979) discussed variation in the levels of wounding (an indication of aggression levels) through time and finds higher levels at periods of low density.

These recent studies were motivated by the Chitty (1960, 1967) hypothesis which grew from earlier empirical studies of microtines. This hypothesis proposed a mechanism of r and K selection to drive

density cycles. The mechanism is a type of soft selection (sensu Wallace 1975) in which the fitness of an individual depends on both the density and relative frequency of different genotypes in the population. An essential component of the hypothesis is an assumed trade-off in fitness between reproductive capability at low density and competitive ability at high densities (Chitty 1971).

To demonstrate the active role of selection predicted from the Chitty hypothesis, researchers in the late 1960s began to monitor allozymic frequencies using the techniques of electrophoresis. The first studies by Tamarin and Krebs (1969), Gaines and Krebs (1971), and Gaines, Myers, and Krebs (1971) considered only two or three electrophoretic loci which were polymorphic. These studies were designed to test the Chitty hypothesis by monitoring the genetic structure of the populations through time.

These initial studies relied on two methods for inferring the role of natural selection in gene frequency change. First, comparisons were made between genotypes in different physiological components of fitness, i.e., viability and fecundity. Second, statistical tests of single locus gene frequency changes were used to examine the relationship between the rate of frequency change and gene frequency. A more recent study involving multiple loci (Gaines, McClenaghan, and Rose 1978) allows a more sophisticated test of selection which depends on interlocus comparisons.

Genotypic Differences in Components of Fitness

The most direct method for demonstrating selection is to measure the net fitnesses of the genotypes for a particular locus and to show their relationship with particular environments. However, in age-structured populations such as those of voles, genotypic fitness is identified with the genotypic intrinsic rate of increase (Charlesworth 1980). Thus, to estimate net genotypic fitness, one must specify age and genotype specific mortality and fecundity schedules. Because of the difficulties in measuring complete life history parameters, researchers resort to estimating a few components believed to be related to fitness.

Gaines et al. (1978) studied four populations of the prairie vole Microtus ochrogaster in Kansas using five polymorphic loci detected by electrophoresis. They monitored survivorship, reproductive condition, and growth rates of individual genotypes over a 3-year period. During this period the vole population went through dramatic density fluctuations.

Two results of this study appeared to support the role of natural selection in the density cycles. First, gene frequencies at all loci varied through the density cycle, and gene freqnencies at certain loci (Tf and Lap) were correlated with the population density. Second, there were significant differences in genotypic survivorship and reproductive activity for Tf and Lap genotypes. My analysis of the data indicates a possible trade-off between survivorship and fecundity for these genotypes which was one of the basic premises of the Chitty hypothesis.

Table 2 gives the genotypic differences in rates of survivorship and reproductive activity in males for the transferrin (Tf) and leucine aminopepdidase (Lap) loci during periods of density increase. Female genotypes for an esterase locus (Est-4) also showed a difference in reproductive activity. A contingency table analysis of numbers of individuals in each category shows the proportions are not independent of genotype (Table 2). I used an adjusted residual analysis (Everitt 1977) for a two-way contingency table to test which individual genotypes deviated from independence (Table 2). The signs in Table 2 indicate significant deviations from independence for particular genotypes. Note that at the Tf locus the EE genotype has a positive deviation in survival and the FF genotype has a positive deviation in reproductive activity. The heterozygotes are depressed in both survivorship and reproductive activity.

The demonstration of significant differences between genotypes in survivorship and reproductive activity suggests that natural selection plays an active role in the density cycle of voles. Moreover, the apparent trade-off between these two components of individual fitness is consistent with the Chitty hypothesis and the mechanism of selective change in density regulation. To examine more closely the role between these genotypic attributes and the observed fluctuations in gene frequency, statistical tests of gene frequency are necessary.

Statistical Tests of Gene Frequency Changes through Time

Do the differences between genotypes in the components of fitness actually result in gene frequency changes through time? Even with

Table 2. Genotypic differences in components of fitness. -- Differences in the rate of survivorship and the proportion of voles in reproductive condition for the Tf, Lap, and Est-4 genotypes found by Gaines et al. (1978) during periods of density increase. Sample sizes are in parenthesis. The signs indicate significant deviations as revealed by the analysis of residuals.

Locus	Genotype	Survivorship Rates 2 weeks	Reproductive Activity
Male			
Tf	EE	0.82 + (728)	0.37 - (169)
	EF	0.72 - (174)	0.58 - (48)
	FF	0.86 (22)	1.00 + (313)
Lap	FF	0.80 (75)	0.55 (29)
	SF	0.88 + (256)	0.26 - (50)
	SS	0.80 - (473)	0.43 (138)
Female			
Est-4	FF		0.44 - (204)
	SF		0.52 (204)
	SS		0.60 + (171)

significant differences in some components of fitness between genotypes, other unmeasured parts of the life cycle may determine actual fitness differences. Also, gene frequency changes due to selection may be overcome by nonselective evolutionary forces such as migration or drift.

To assess the role of selective and nonselective forces in gene frequency change, two general methods can be used in vole studies. First, Tamarin and Krebs (1969) and Kohn and Tamarin (1978) used the regression of change in gene frequency vs. gene frequency as evidence of balancing selection maintaining polymorphism in populations of Microtus. This method was suggested and used by Wright and Dobzhansky (1946) in the analysis of Drosophila chromosome frequencies in the laboratory. Second, one can compare measures of gene frequency change among five loci as a test of selection. In the following sections, I review these two methods and compare their results to the previous interpretations of the role of selection in gene frequency dynamics of voles.

Wright's Regression Method

Wright and Dobshansky (1946) first used the regression of rate of gene frequency change vs. gene frequency as statistical test of selection. Working with labratory populations of Drosophila pseudoobscura, they recorded changes in the frequency of certain chromosomal rearrangements. Dobzhansky designed the experiments to test for the influence of temperature and food as selective agents on the chromosomal frequencies of the populations. These experiments followed the observation that there are seasonal fluctuations in the frequency of different

arrangements in natural populations of D. pseudoobscura on Mt. San Jacinto in California.

Wright analyzed Dobzhansky's laboratory data by considering both the average rate of change per generation and the regression of rate of change on chromosome frequency. A significant average rate of change would indicate directional change due to selection, whereas zero change coupled with a significant regression would indicate a type of balancing selection; e.g., heterozygote superiority (Figure 1). Such statistical inferences are permissible owning to the control of other forces (e.g., migration) made possible in the laboratory populations.

In the following section, I reanalyze the data of Gaines et al. (1978) using Wright's method. Recall that significant genotypic differences in components of fitness were found in the earlier analysis. If the genotypic differences in survivorship and reproductive activity translate into genotypic differences in fitness, then gene frequencies should be varying by natural selection at these particular loci.

Table 3 give gene frequencies for seven sampling periods in four populations of Microtus. The samples are from 14-week intervals which is sufficient time to ensure new individuals in each sample. Fourteen weeks is twice the highest average survivorship of individuals on a grid (Gaines et al. 1978).

For each locus, I calculated the change in gene frequency between each of seven samples. I lumped the data over the four grids which yielded 24 observed changes in gene frequencies for each locus.

Following the method of Wright (Wright and Dobzhansky 1946), I tested for a significant mean change in gene frequency at each locus

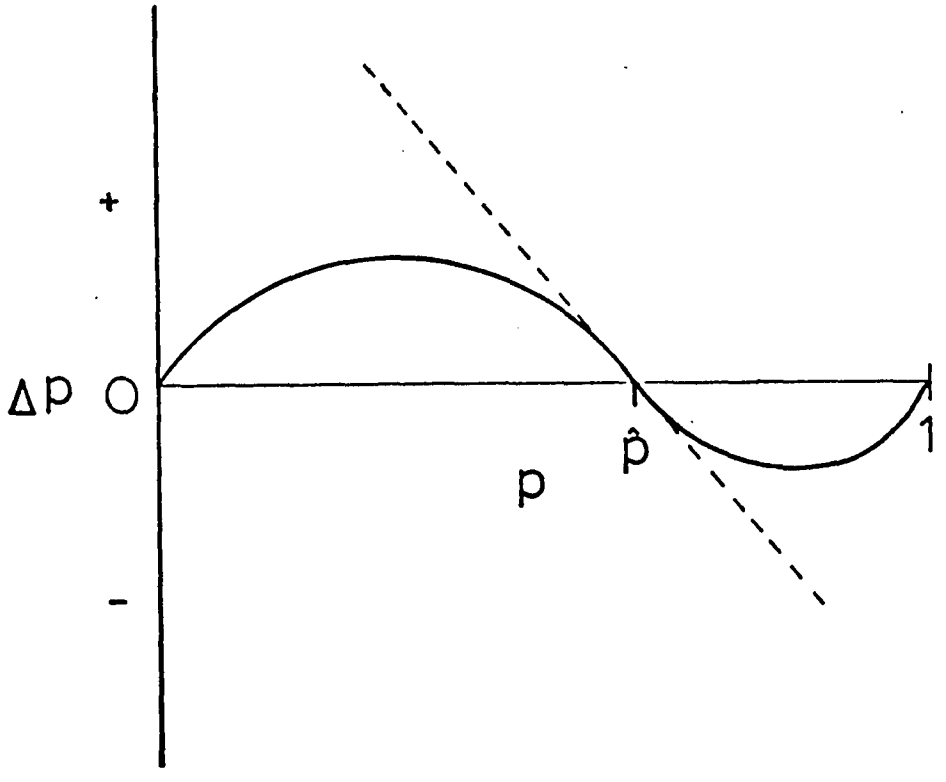

Figure 1. Rate of change in gene frequency vs. gene frequency with heterozygote superiority in fitness. -- The plot of the rate of change in gene frequency, Δp, vs. gene frequency predicted with heterozygote superiority in fitness. The equilibrium gene frequency, $\hat{p}$, represents a stable polymorphism for this locus.

Table 3. Common allele frequencies for voles sampled through time. -- Gene frequencies (p_{ij}) of the common allele at five loci for seven time periods on four grids. Sample sizes (n_{ij}) are in parentheses. Last two columns are the weighted averages with their variances.

Grid and Locus	1971	1972				1973		$\bar{p}_j$	$(s^2_p)_j$
	September	January	April	August	November	March	June		
Grid A									
Tf	0.781 (15)	0.877 (57)	0.839 (62)	0.872 (39)	0.740 (39)	0.725 (60)	0.909 (11)	0.817	0.004
Lap	0.767 (15)	0.737 (57)	0.750 (62)	0.705 (39)	0.700 (25)	0.758 (60)	0.955 (11)	0.747	0.002
Est-1	0.625 (8)	0.727 (44)	0.794 (51)	0.667 (36)	0.600 (25)	0.589 (62)	0.438 (8)	0.669	0.008
Est-4	0.308 (13)	0.500 (54)	0.525 (59)	0.554 (37)	0.577 (26)	0.721 (68)	0.654 (13)	0.574	0.011
6-Pgd	0.938 (8)	0.898 (49)	0.927 (55)	0.917 (36)	0.880 (25)	0.911 (62)	0.938 (8)	0.991	0.000
Grid B									
Tf	1.000 (4)	1.000 (20)	0.946 (37)	0.889 (54)	0.890 (54)	0.848 (23)	0.876 (20)	0.901	0.002
Lap	0.500 (3)	0.700 (20)	0.790 (38)	0.750 (54)	0.732 (41)	0.783 (23)	0.775 (20)	0.751	0.001
Est-1	0.667 (3)	0.850 (20)	0.885 (39)	0.830 (50)	0.805 (41)	0.646 (24)	0.524 (21)	0.716	0.013
Est-4	0.750 (4)	0.850 (20)	0.645 (38)	0.673 (52)	0.663 (40)	0.841 (22)	0.725 (20)	0.780	0.006
6-Pgd	1.000 (3)	0.975 (20)	0.987 (39)	0.927 (55)	0.890 (41)	0.771 (24)	0.786 (21)	0.904	0.006
Grid C									
Tf	0.893 (14)	0.850 (60)	0.818 (33)	0.857 (28)	0.857 (7)	0.900 (10)	0.500 (8)	0.834	0.006
Lap	0.714 (14)	0.842 (60)	0.833 (33)	0.911 (28)	0.857 (7)	0.950 (10)	0.625 (8)	0.838	0.006
Est-1	1.000 (4)	0.804 (60)	0.833 (30)	0.648 (27)	0.667 (6)	0.682 (11)	0.667 (3)	0.765	0.007
Est-4	0.500 (13)	0.492 (59)	0.500 (32)	0.611 (27)	0.667 (6)	0.400 (10)	0.500 (8)	0.516	0.003
6-pgd	0.833 (3)	0.865 (52)	0.924 (33)	0.889 (27)	0.833 (6)	0.889 (9)	0.833 (3)	0.884	0.001
Grid D									
Tf	0.933 (15)	0.935 (23)	0.952 (21)	0.943 (35)	0.813 (8)	0.750 (4)	0.778 (18)	0.903	0.005
Lap	0.833 (15)	0.739 (23)	0.810 (21)	0.871 (35)	0.857 (8)	0.500 (4)	0.639 (18)	0.786	0.009
Est-1	0.813 (8)	0.850 (20)	0.875 (20)	0.778 (36)	0.786 (7)	0.725 (8)	0.444 (18)	0.748	0.026
Est-4	0.600 (15)	0.587 (23)	0.619 (21)	0.643 (35)	0.500 (7)	0.750 (4)	0.694 (18)	0.626	0.003
6-Pgd	0.962 (13)	0.938 (24)	0.925 (20)	0.931 (36)	0.938 (8)	0.875 (8)	0.972 (18)	0.937	0.001

and a significant regression of the rate of change vs. gene frequency. The formulae for these tests are summarized in Table 4. The mean gene frequency change for one locus, $\overline{\Delta p}$ was calculated as an unweighted average of the 24 time periods. Using the estimate of its standard error, I tested a significant mean; i.e., difference from zero using a t test with 22 df. The results are summarized in Table 5. A significant t value would indicate a directional change in gene frequency at a locus through time. However, none of the five loci showed a significant mean change.

Using the regression formulae in Table 4, each locus was tested for a significant relationship between the rate of change and gene frequency in each time interval. The regression lines for all five loci are plotted in Figure 2. All five regression coefficients, $b_{\Delta p.p}$, were negative but only the Est-4 locus was significantly different from zero (Table 5).

The negative relationship found for the Est-4 locus may indicate one of two factors affecting gene frequencies at that locus. First, selection may be operating and maintaining a polymorphism through some mechanism like heterozygote advantage or frequency dependence. A selective mechanism is consistent with the genotypic differences in reproduction rate found for females (see Table 1). The Est-4 locus was the only that showed difference for females.

Second, the negative regression line may indicate immigration or emigration of Est-4 genotypes. This may be a form of selection if the differences in migration affected survivorship and fecundity schedules.

Table 4. Regression formulae for the temporal test of gene frequency

Means

$$\bar{p} = \Sigma p/n, \quad \bar{\Delta p} = \Sigma p/n$$

Sum of Squares

$$SS_p = \Sigma p^2 - \bar{p}\Sigma p$$

$$SS_{\Delta p} = \Sigma \Delta p^2 - \bar{\Delta p}\Sigma \Delta p$$

Sum of Products

$$SP = \Sigma(p \cdot \Delta p) - \frac{(\Sigma p)(\Sigma \Delta p)}{n}$$

Regression Coefficient

$$b_{\Delta p \cdot p} = SP/SS_p$$

Unexplained Mean Square

$$s^2_{\Delta p \cdot p} = \{\Sigma \Delta p - \frac{(SP)^2}{SS_p}\}\frac{1}{(n-2)}$$

Standard Errors

$$s_b = \{\frac{s^2_{\Delta p \cdot p}}{SS_p}\}^{-\frac{1}{2}}$$

$$s_{\bar{\Delta P}} = \{\frac{s^2_{\Delta p \cdot p}}{n}\}^{-\frac{1}{2}}$$

Table 5. Results of the regression analysis for five loci. -- Entries in the table are calculated from the formulae in Table 4. The last two rows are the statistical tests for the regression coefficient and average change in gene frequency, respectively, using a t test. $p < 0.05$

	Locus				
	Tf	Lap	Est-1	Est-4	6 Pgd
$\bar{p}$	0.871	0.619	0.752	0.603	0.909
$\bar{\Delta p}$	-0.018	-0.012	-0.028	0.009	-0.009
$b_{\Delta p \cdot p}$	-0.601	-0.055	-0.385	-0.638	-0.025
SE_b	0.362	0.079	0.350	0.084	0.114
$SE_{\Delta p}$	0.030	0.023	0.372	0.011	0.033
t_b	1.663	0.698	1.099	7.596*	0.216
$t_{\bar{\Delta p}}$	0.589	0.517	0.747	0.849	0.281

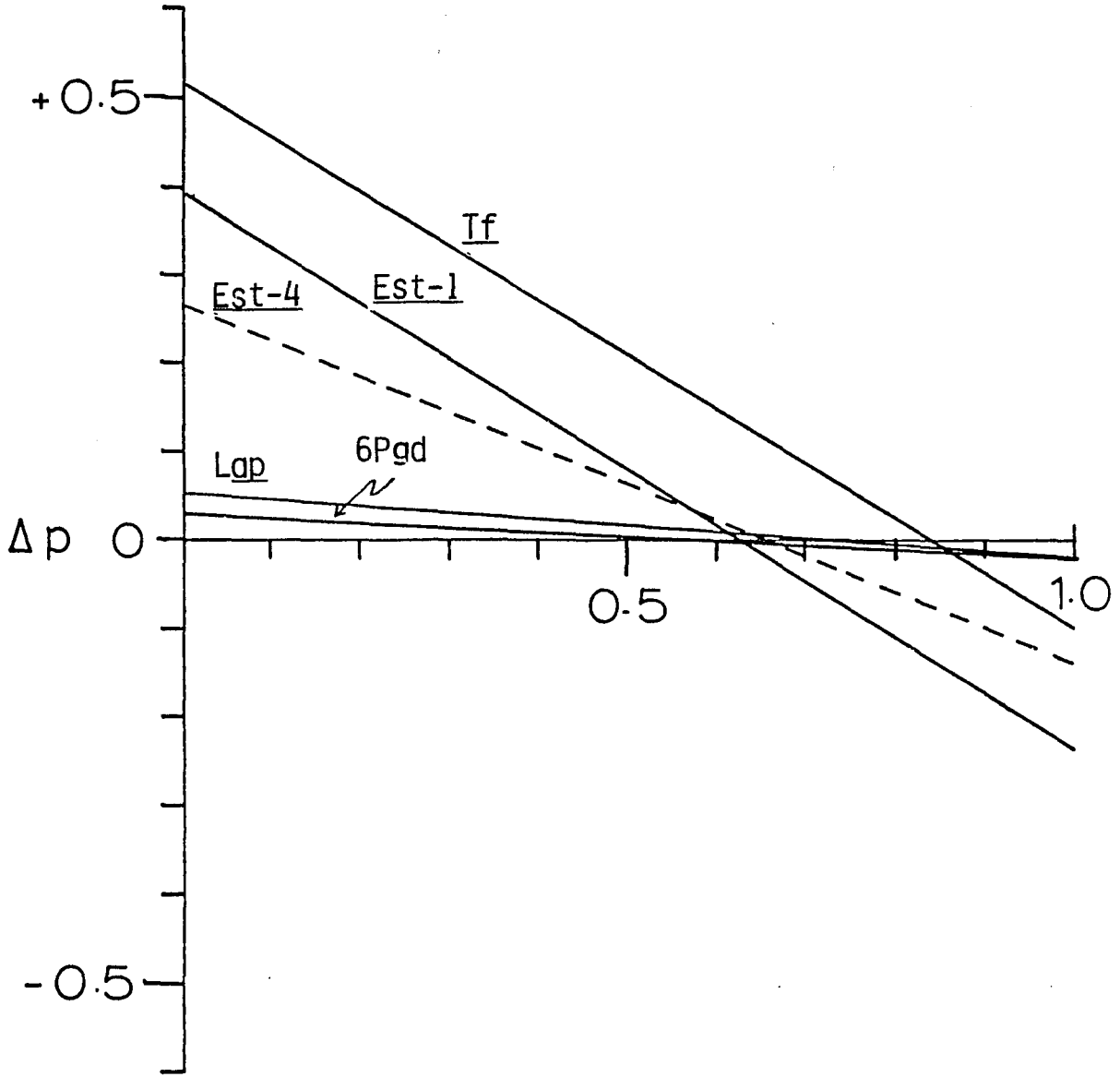

Figure 2. Regression lines for Δp vs. p for five loci. -- For each locus, the rate of change in gene frequency between samples, Δp, was plotted against gene frequency, p, for seven time periods on four grids. The regression lines are plotted in the figure. Only the line for the Est-4 locus was significant (see Table 5).

To summarize the results thus far: Fitness component analysis indicates genotypic differences for males at the Tf and Lap loci and for females at the Est-4 locus. Further analysis of gene frequency changes through time indicate that only the Est-4 locus shows a significant relationship between the rate of gene frequency change and gene frequency. However, the interpretation of this relationship is obscured in a natural population where migration could yield a negative regression.

The gene frequency changes observed at the remaining four loci appear to show no significant directional change in gene frequency and no significant linear relationship to gene frequency. However, if selection coefficients varied through a density cycle, as supposed by the Chitty hypothesis, the regression of Δp vs. p may not be sensitive to this variation.

The Regression of Δp on p as Evidence of Selection

A recent paper by Kohn and Tamarin (1978) and an earlier paper by Tamrin and Krebs (1969) examined the temporal changes in allele frequency between successive samples. When $\Delta p_i (=p_{i+1} - p_i)$, was plotted against p_i, the gene frequency in the ith sample, a significant negative linear regression was obtained. The authors inferred that the predicted gene frequency, $\hat{p}$, when Δp equals zero, estimates an equilibrium gene frequency (Figure 2). Furthermore, they concluded that $\hat{p}$ is a stable, nontrivial equilibrium maintained by some form of balancing selection. A stable, nontrivial equilibrium is a predicted result from the standard deterministic model where selection favors the heterozygote (Li

1955). In this case the relationship between p and Δp near the equilibrium is negative (see Figure 2), such that Δp restores p to the equilibrium point. However, there are other reasons for expecting a negative relationship between Δp and p.

The relationship between Δp and p depends on the underlying distribution of gene frequencies from which one samples. Consider a case where gene frequency is a random variable with uniform probability distribution. All gene frequencies in the interval (0,1) are equiprobable and there is no equilibrium point. If gene frequency in the ith sample, is an independent sample from the uniform distribution, and $\Delta p_i = p_{i+1} - p_i$, then the conditional expectation of Δp_i given p_i is

$$E(\Delta p/p_i) = E(p_{i+1}) - p_i \qquad (2.1)$$

With the uniform distribution over the interval (0,1), $E(p_{i+1}) = 0.5$ so that Δp has a negative regression of p_i with a slope of -1.0 and an intercept of 0.5. Figure 3 shows the results of a simulation where gene frequencies are a random sequence from the interval (0,1). The negative regression coefficient is significantly different from zero and the line explains about 60% of the variance in Δp (n = 100). In the analysis of allele frequencies in natural vole populations, Kohn and Tamarin (1978, Tables 5 and 6), reported that all the regression coefficients were negative and many were significantly different from zero. A comparison of their results and the confidence limits for the regression coefficient generated from random gene frequencies (Figure 1)

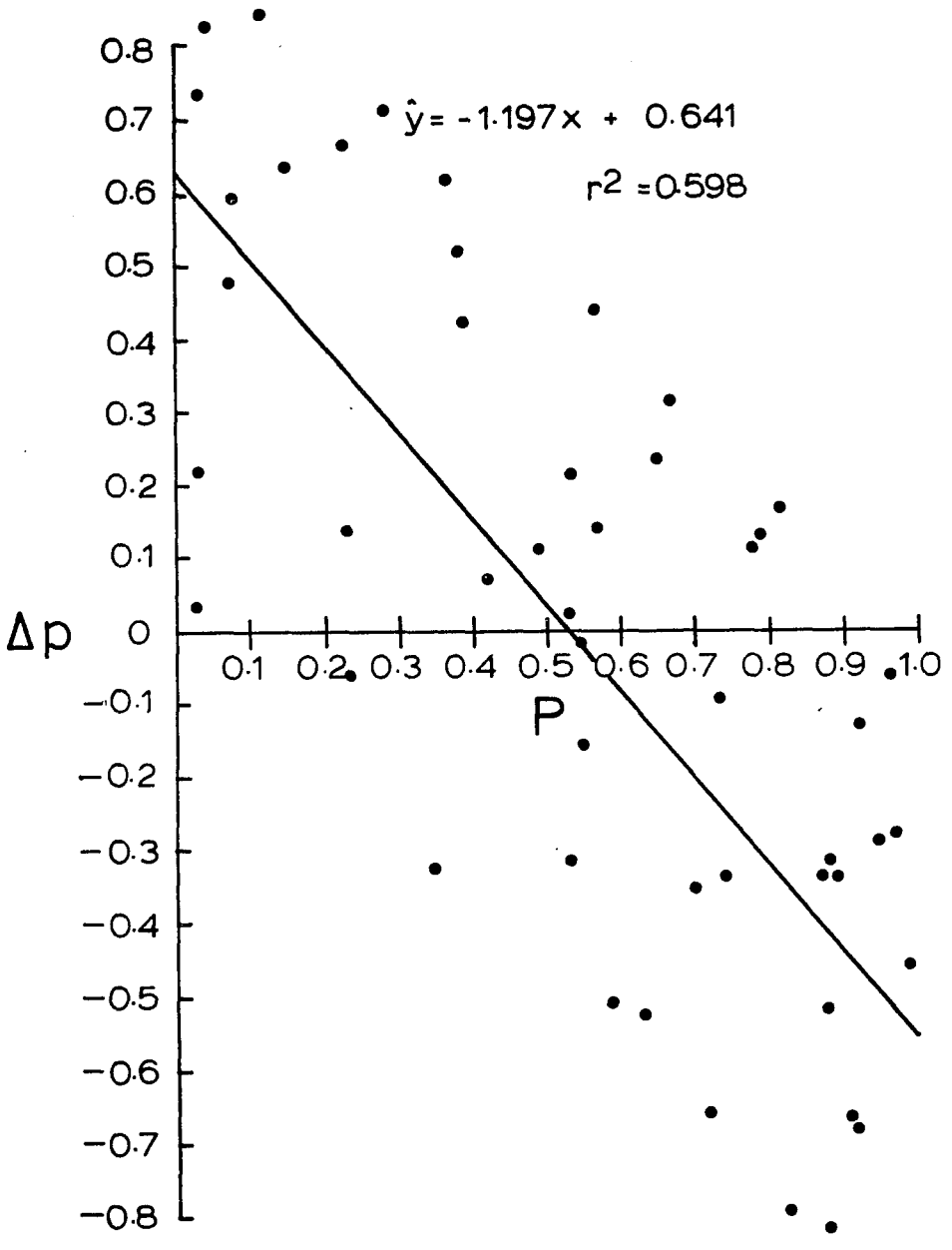

Figure 3. Regression line of Δp vs. p when gene frequencies are sampled from a uniform probability distribution. -- Plot of p and Δp when gene frequencies are sampled form a uniform probability distribution in the interval (0,1). The regression line is based on a sample of 100 realizations (50 are plotted) and has a regression coefficient of -1.197 (0.196) which is significantly different from 0 (F_s = 145.29). The predicted $\hat{p}$ when $\Delta p = 0$ is 0.536 ($\pm$0.026).

indicates about half their results do not differ significantly from the negative slope in Figure 3. The interpretation of a negative slope as evidence of selection maintaining a polymorphism is questionable since a negative slope is expected in the null case where gene frequencies are uniformly distributed.

The uniform distribution of gene frequencies is difficult to relate to the population biology of voles. A more realistic null hypotheses for the relationship between Δp and p can be derived from the relationship between population size and genetic drift. In a randomly mating population of N individuals with frequencies p_i and $(1 - p_i)$ of alleles A_1 and A_2, respectively, in the ith generation, the number of A_1 alleles in the next generation follows a binomial distribution, that is, the expansion of $\{p_i + (1-p_i)\}^{2N}$ (Crow and Kimura, 1970). The expected gene frequency, $E(p_{i+1})$, in the next generation is p_i and, therefore (using equation 1) $E(\Delta p/p_i) = 0$. In this case, where stochastic fluctuations of gene frequency are due only to finite population size, the null hypothesis of zero regression coefficient is appropriate.

It makes more sense, however, to consider genetic drift in conjunction with migration, since drift acting alone eventually leads to a fixation of one of the alleles. In the study of local populations of voles, fixation of an allele has been observed (Gaines et al. 1978), but the polymorphism was restored (presumably by migration) in subsequent generations.

To see if small amounts of migration affect the relationship between p and Δp, I conducted simulations of population undergoing

genetic drift with gene frequencies restored to 0.98 in the subsequent generation after fixation. The model is not meant to portray accurately the interaction between drift and migration (or reversible mutation), but rather the model represents a situation where genetic drift is the predominant cause of gene frequency change but the polymorphism remains. For different population sizes (10, 20, ..., 100) and initial gene frequencies (0.1, 0.2, ..., 0.5) each simulation was run for 51 generations and was replicated 20 times. I expected 1 in 20 replicates to yield a significant regression coefficient (i.e., $p < 0.05$) if there was no relationship between p and Δp. I calculated the regression of Δp_i on p_i ($i = 1, 2 \ldots, 50$) for each replicate and found that most regression coefficients were negative and 357 out of 1,000 simulations were significant (Table 6). The results indicate that for a situation constructed so that fixation is a temporary state and gene frequency change is primarily stochastic (due to genetic drift), the regression coefficient of Δp on p is significantly negative much more than 5% of the time.

Stable polymorphism can result from a variety of evolutionary forces besides balancing selection (e.g, reversible mutation, gene flow). If a population has a constant gene frequency p, and an investigator draws samples of size N, the number of A_1 alleles in the sample follows a binomial distribution. Letting x_i be a random variable representing the gene frequency in the ith sample, the expected difference in gene frequency between successive samples Δx_i ($x_{i+1} - x_i$) conditioned on the gene frequency in the ith sample is

Table 6. Simulations of gene frequency change under genetic drift and migration. -- Entires in the table are the number of significant regression coefficients for each effective population size and initial gene frequency. Each simulation was replicated 20 times for a total of 1,000 simulations.

Effective Population Size	Initial Gene Frequency					
	0.10	0.20	0.30	0.40	0.50	Total
10	10	12	9	13	6	50
20	8	4	4	4	9	29
30	7	7	10	9	10	43
40	8	11	5	9	10	43
50	7	5	8	3	1	24
60	10	7	10	7	7	41
70	10	9	4	8	7	38
80	6	2	7	7	7	29
90	4	3	6	4	5	22
100	7	8	9	7	7	38
						357

$$E(\Delta x/x_i) = \hat{p} - x_i$$

yielding a regression coefficient of -1 and an intercept of $\hat{p}$. Thus, a negative relationship between Δp and p is expected from sampling variation alone when gene frequencies are constant.

One must be cautious when using the negative regression of Δp on p as evidence for natural selection maintaining polymorphisms in a natural population. In the null case where gene frequencies represent individuals sampled from a uniform distribution, a negative regression coefficient is expected and an equilibrium is predicted when there is no equilibrium gene frequency. When sampling without error from a population undergoing genetic drift, a zero regression coefficient is expected, but significant negative regressions are found more often than expected when genetic drift is coupled with other nonselective forces such as migration. When gene frequency is constant over generations, sampling variation by the investigator yields a negative relationship between Δp and p, so that one cannot distinguish between evolutionary forces that maintain constant gene frequency.

Interlocus Comparisons of Gene Frequency Change

Lewontin and Krakauer (1973) developed an original suggestion of Cavalli-Sforza (1966) into a statistical test for selection. They focused on the difference between selective and nonselective forces on gene frequency distributions. They reasoned that if alleles at each polymorphic locus are selectively neutral, then the effects of

nonselective forces, such as genetic drift and migration, should be uniform over loci. However, natural selection, operating through differential fitnesses between genotypes, affects gene frequencies only at specific loci. Therefore, a measure of genetic variation at each polymorphic locus over a number of populations should be similar if only nonselective forces are operating, and dissimilar if selective forces are operating on a subset of loci.

The measure chosen by Lewontin and Krakauer (1973) is a standardized variance of gene frequencies called the effective imbreeding coefficient F_e. They deduced that if alleles at each locus are selectively neutral, the F_e values calculated for each of many loci over an ensemble of populations will be statistically homogeneous whereas, if selection is occurring, there will be statistical heterogeneity in F_e values. The statistical test of standardized variances has been called the Lewontin-Krakauer test (L-K test).

In the following sections I apply two versions of the L-K test to the temporal gene frequency changes found in Microtus ochrogaster (Table 3). The first version is a heterogeneity test over all time periods. In this version, I calculated the effective imbreeding coefficient, F_e, for each locus using the weighted mean and variance of gene frequency over seven subpopulations (time periods). The weighted mean frequency of the common allele at the jth locus, p_j, and the variance, $(s_p^2)_j$ over n subpopulations are calculated as

$$\bar{p}_j = \Sigma \frac{N_{ij}}{N_j} p_j, \quad (s_p^2)_j = \Sigma \frac{N_{ij}}{N_j} (p_{ij} - \bar{p})^2 \qquad (2.3)$$

where N_{ij} is the sample size of the jth allele in the ith subpopulation, p_{ij} is frequency of the jth allele in the ith subpopulation, N_j is total sample size of the jth allele ($N_j = \Sigma_j N_{ij}$) allele is given by

$$F_j = \frac{(s_p^2)_j}{\bar{p}_j(1-\bar{p}_j)} \tag{2.4}$$

This value represents the sample variance standardized by the maxiumum possible variance among subpopulations. (Note that F_e is the same as Wright's (1965) theoretical F_{ST}). Using the F_e calculated for each locus, one can test for heterogeneity among loci using the ratio of the observed variance over all loci to the theoretical variance, s_F^2/σ_F^2. The weighted mean ($\bar{F}_e$) and variance (s_F^2) over m loci are estimated by

$$\bar{F}_e = \Sigma \frac{N_j}{N} F_j, \quad (s_F^2 = \Sigma \frac{N_j}{N} (F_j - \bar{F}_e)^2 \tag{2.5}$$

where N is the total population size. Lewontin and Krakauer (1973) found the relationship between the theoretical variance of F_e and the mean F_e to be

$$\sigma_F^2 = \frac{k\bar{F}_e^2}{(n-1)} \tag{2.6}$$

Through extensive computer simulation, the limiting value of k = 2 was found for various underlying frequency distributions of neutral genes

The variance ratio s_F^2/σ_F^2 is compared with $F(n_{\infty}^{-1})$ distribution following the method of Charkravarti (1977). If the sample variance is significantly greater than the theoretical variance, one can infer heterogeneity in F_e's among loci.

Using the data in Table 3, I calculated effective inbreeding coefficients for each of the five loci using equations 2.3-2.5. Because not every individual vole was sampled in each population, I corrected for sampling error by subtracting $\frac{1}{2N}$ from each F_e value, where N is the average sample size used to calculate allele frequencies (Cavalli-Sforza and Bodmer 1971).

The results of the heterogeneity tests are presented in Table 7. They indicated no heterogeneity among loci on each grid. This suggests no selection was operating on any subset of these loci.

It is possible that selection is acting only during a short time interval, for example at high densities. This might not be detected in heterogeneity tests over all time periods. Lewontin and Krakauer (1973) suggested a second test of gene frequencies using one generation changes to calculate another estimate effective inbreeding f, as follows:

$$F_{ij} = \frac{(\Delta p_{ij})^2}{p_{ij}(1-p_{ij})} \qquad i = 1,2, \ldots, 6 \qquad (2.8)$$

where p_{ij} is the frequency of the jth allele in the ith time period and Δp_{ij} is the change in frequency of the jth allele between the i

Table 7. Effective inbreeding coefficients calculated over all time periods. -- Estimates of the effective inbreeding coefficient F_e corrected for the sampling variance for seven time periods on each grid. Below these is a summary of the heterogeneity test of F_e's. $\bar{F}_e$ is the mean estimate of the effective inbreeding coefficient, s_F^2 is the observed variance, σ_F^2 is the theoretical variance and the variance ratio of the observed to theoretical variance is compared to an F_∞^6

	Grid			
Locus	A	B	C	D
Tf	0.026	0.021	0.044	0.050
Lap	0.010	0.007	0.037	0.050
Est-1	0.034	0.072	0.036	0.104
Est-4	0.042	0.063	0.004	0.005
6-Pgd	0.000	0.063	0.004	0.005
$\bar{F}_e$	0.023	0.038	0.027	0.042
s_F^2	0.0002	0.0006	0.0003	0.0006
σ_F^2	0.0002	0.0005	0.0002	0.0006
s_F^2/σ^2	1.000(ns)	1.200(ns)	1.500(ns)	1.000(ns)

and the i + 1 time periods. I calculated the mean $\bar{f}_i$, for the ith time period from the f_{ij} for each locus in that time period. (See Table 8 for some sample calculations.)

Lewontin and Krakauer (1973) have shown that the cumulative frequency distribution of $(n - 1)\ f_{ij}\sqrt{f_i}$ is distributed as χ^2 with $(n - 1)$ degrees of freedom where n is the number of samples. The theoretical variance of this distribution is $2(n - 1)$, the same as the χ^2 distribution. I tested heterogeneity of f_{ij} values using the ratio of the observed variance of $f_{ij}\sqrt{f_i}$ to its theoretical variance (σ_f^2). I performed 24 heterogeneity tests (6 intervals x 4 grids) of f_{ij} values for each pair of successive time periods on each grid (n = 2). All variances ratios were nonsignificant. (I did not correct for sampling error based on Chakravarti's (1977) criticism of Taylor and Gorman's (1975) analysis. He concluded that correcting for sample size decreases f, whereas the variance of f remains the same. Therefore, the variance ratio is inflated.)

In addition, I pooled the data from all grids and compared the observed distribution of 120 $f_{ij}\sqrt{f}$ values (4 grids x 5 loci x 6 time intervals) to the theoretical χ^2. Intervals were chosen to include 10% of the probability mass and hence have an expected value of 12 (Table 9). The test of goodness of fit between the observed and theoretical distributions showed they were not significantly different $(\chi^2_{(11)} = 8.83,\ 0.50 > p > 0.30)$. This analysis suggests that non-selective forces were the major determinant of temporal variation

Table 8. Sample calculation of the effective inbreeding coefficient between two time periods. -- Frequencies of the common allele at five loci for the first two sampling periods for Grid A. The effective inbreeding coefficient, f, is calculated from the change in allele frequency for each locus and corrected by the mean f among loci.

	Locus				
Time Period	Tf	Lap	Est-1	Est-4	6-Pgd
1. Sept 1971	0.781	0.767	0.625	0.308	0.938
2. Jan 1972	0.877	0.737	0.727	0.500	0.898
$\Delta p = p_2 - p_1$	0.096	-0.030	0.102	0.192	-0.040
$f = \frac{(\Delta p)^2}{p_1(1-p_1)}$	0.054	0.005	0.044	0.173	0.028
$f/\bar{f}$	0.885	0.082	0.721	2.836	0.459

Table 9. The observed and theoretical distribution of the effective inbreeding coefficient. -- A comparison of the observed distribution of $f_{ij}\sqrt{f}$ for all loci on all grids and the theoretical distribution of χ^2 with one degree of freedom. The χ^2 intervals were chosen to include 10% of the probability mass and, hence, have an expected value of 12.

$f_{ij}\sqrt{f}$	Observed	Expected	χ^2
0.000-0.016	14	12	0.333
0.017-0.064	14	12	0.333
0.065-0.148	15	12	0.750
0.149-0.275	15	12	0.750
0.276-0.455	6	12	3.000
0.456-0.708	9	12	0.750
0.709-1.074	13	12	0.083
1.075-1.642	7	12	2.083
1.643-2.706	12	12	0.000
>2.707	15	12	0.750
Total	120	120	8.832

$0.50 > p > 0.30$

Comments on the Lewontin-Krakauer Test

Along with the publication of the two versions of their gene frequency test Lewontin and Krakauer (1973) analyzed data on protein polymorphisms in human populations. They used the first version, initially designed for use with spatial gene frequency distributions and found heterogeneity in F_e values. They concluded that natural selection must have caused the variation in F_e's.

This conclusion generated much controversy and criticism (Nei and Maruyama 1975; Robertson 1975a, 1975b; Ewens and Feldman 1976; Ewens 1977; Nei and Chakravarti 1977; Nei, Chakravarti and Tateno 1977). Most of the criticism focused on the interpretation of heterogeneous F_e values calculated from geographically different populations. The interpretation that natural selection is the only cause of heterogenous F_e values in unwarranted because the historical relationships between the population can inflate the variance in F_e (Robertson 1975a, 1975b).

Although most of the criticisms concern the spatial version of the L-K test, some of the points are applicable to the test when used with temporal data. Ewens and Feldman (1976) discussed the assumptions underlying the calculation of the theoretical variance in F_e. They contend that for two to be an upper limit for k one must have independent samples and that correlations between samples may affect the k value. To correct for this effect, I calculated the correlations in gene frequency between time periods (Table 10) and using Ewens and Feldman's (1976) formula, corrected k for the effect of correlation. The corrected k estimate is calculated as follows:

Table 10. The correlation's ρ_{ij}, in gene frequencies between time periods. -- The average correlations, ρ_{ij}, and the variance in correlations, s_ρ^2, are used in Equation 2.7 to recalculate the k parameter in the theoretical variance of F_e.

Time Period	2	3	4	5	6	7	ρ_i	s_ρ^2
1	.954	.950	.907	.881	.545	.626	.811	.032
2		.972	.961	.837	.429	.533	.783	.057
3			.904	.804	.383	.388	.734	.076
4				.924	.612	.643	.825	.024
5					.851	.756	.842	.003
6						.790	.623	.022

$$\bar{\rho}_i = (n - 1)^{-1} \sum_{i+j} \rho_{ij}$$

$$\bar{\rho} = 2\{(n - 1)n\}^{-1} \sum_{i<j} \sum \rho_{ij}$$

$$s_\rho^2 = \Sigma(\bar{\rho}_i - \bar{\rho})^2$$

$$E(k) \simeq 2 + \frac{2}{(n-1)(1-\rho)} \left| \Sigma\Sigma_{i=j}(\rho_{ij} - \bar{\rho}_i) - \frac{(n-1)(n-2)}{n} \Sigma(\bar{\rho}_i - \bar{\rho})^2 \right| \quad (2.9)$$

where the correlations are from Table 10. The corrected k estimate is 2.067. Thus, the correlations in the data appear to have little effect on the estimate of the theoretical variance of F_e.

A second criticism concerns the shape of the gene frequency distributions. Lewontin and Krakauer (1973) considered binomial, uniform, and U-shaped distributions (beta distributions) in computer simulations to determine the limiting value of the k parameter. Ewens and Feldman (1976) and Ewens (1977) emphasized that gene frequencies must be identical normal distributions for $(n - 1)F_e/\bar{F}_e$ to exactly follow a chi-square distribution. I examined the gene frequencies at each locus for two-week samples and found three of the five loci have approximately unimodal distributions. Two loci (Tf, 6Pgd) have skewed or J-shaped distributions which can result in k values that exceed 2 as found in computer simulations by Nei and his coworkers (Nei and Chakravarti 1977; Nei, Chakravarti, and Tateno 1977). The bias introduced by skewing the gene frequency distribution away from unimodal (approximately normal) distribution, given an inflated estimate of the variance ratio and favors rejection of the neutral hypothesis more often than warranted. In the results, I found homogeneous F_e values and accepted the neutral hypothesis. Thus, it appears that the L-K test is not very sensitive to the distribution assumption.

The only factor that could bias this application of the L-K test toward homogeneity in F_e values and neutrality would be linkage

between the loci studied. Standard breeding techniques conducted by Gaines (pers. comm. 1980) in the laboratory showed no structural linkage among the five loci considered here. However, in the natural situation epistatic interactions among the loci could lead to associations between gametic types (linkage disequilibrium). If this were the case, one would expect deviations from independent assortment in the frequencies of genotypes (Franklin and Lewontin 1970) in the population. Table 11 gives the genotypic frequencies where individual voles are cross-classified for all five loci for the data used in the L-K test. Using a log-linear analysis for multi-way contingency tables (Bishop, Fienberg, and Holland 1975) one can test for independence between the variables (loci). In this case, a mutual independence model adequately fits the data ($n = 985$, $\chi^2 = 205.67$, $df = 232$, $p = 0.893$) which indicates independent assortment among the loci. Therefore, there is no evidence for genetic linkage resulting in the natural population.

Summary and Discussion

The genetic studies of fluctuating vole populations were motivated by the Chitty hypothesis which proposed an active role of natural selection in vole density cycles. The initial studies of Krebs, Gaines, Tamarin and their colleagues revealed genotypic differences in viability and fecundity consistent with this hyposhtesis. Furtheremore, a significant regression of the rate of gene frequency change and gene frequency suggested balancing selection operating to maintain a polymorphism at one locus through the density cycles. However, the

Table 11. Genotypic frequencies of voles for five loci. -- Each individual vole is cross-classified by its genotypes at each of five loci for all time periods on all grids.

Locus		Est-1 Tf	FF			SF			SS		
6Pgd	Est-4	Lap	EE	EF	FF	EE	EF	FF	EE	EF	FF
EE	FF	FF	8	3	0	4	1	1	17	4	1
		SF	17	4	0	13	5	1	52	12	1
		SS	31	11	2	45	16	1	116	48	2
	SF	FF	2	1	0	6	2	0	7	3	1
		SF	7	2	1	18	2	1	27	9	0
		SS	20	7	0	25	13	2	101	16	1
	SS	FF	3	1	0	4	1	1	8	2	0
		SF	5	2	1	5	2	0	21	2	0
		SS	19	3	0	27	6	0	79	21	4
EF	FF	FF	1	0	0	0	1	0	1	0	0
		SF	0	0	0	1	0	1	3	0	0
		SS	2	1	0	2	5	0	16	0	0
	SF	FF	0	0	0	0	0	0	0	0	0
		SF	0	0	0	0	1	0	3	1	0
		SS	0	1	0	2	1	0	8	2	0
	SS	FF	0	0	0	0	0	0	0	1	0
		SF	0	1	0	0	1	0	3	1	0
		SS	0	0	0	3	1	0	8	0	0
FF	FF	FF	0	0	0	0	0	0	0	0	0
		SF	0	0	0	0	0	0	0	0	0
		SS	0	0	0	0	0	0	0	0	0
	SF	FF	0	0	0	0	0	0	0	0	0
		SF	0	0	0	0	0	0	0	0	0
		SS	0	0	0	1	0	0	0	0	0
	SS	FF	0	0	0	0	0	0	0	0	0
		SF	0	0	0	0	0	0	0	0	0
		SS	0	0	0	1	0	0	0	0	0

regression analysis may not indicate selection and moreover, the inter-locus comparisons used in the Lewontin-Krakauer test demonstrated no selective differences between loci in the degree of frequency change.

What factors could account for the observed fluctuations in gene frequency? The simplest hypothesis is that crashes in density lead to bottlenecks in effective population size and result in genetic drift (Nei, Maruyama and Chakraborty 1975). Genetic drift due to bottlenecks would be most effective with intermediate gene frequencies and low effective population size. Drift at low density could override any small selective differences between genotypes.

If drift was the primary cause of gene frequency fluctuations, one would expect a greater gene frequency change at low density compared to high densities. To test this hypothesis, I regressed the absolute value of the change in gene frequency, $|\Delta p|$, against the absolute change in density between time periods for all loci in Table 3. I also used f to estimate the effective population size N_e and regressed $|\Delta p|$ against N_e for 7 time periods. Although both regressions had positive slopes, the regression coefficients were insignificant. This suggests that genetic drift alone may not account for the temporal fluctuations in gene frequency.

In a recent study of voles (Gaines, Vivas, and Baker 1979), fluctuations in the densities were accompanied by changes in the numbers of dispersing individuals. Moreover, the number of dispersers was positively correlated with density. For migration between populations to account for the genetic changes, dispersing individuals must become incorporated into the breeding population, and the resultant

gene flow would have to be of sufficient magnitude to change gene frequencies. To be consistent with the results of the L-K test, the migration and gene flow must be uniform among genotypes. (Differential migration patterns between genotypes would be viewed as a form of selection and should be detected by the L-K test.)

Finally, natural selection may have a different role than proposed by the Chitty hypothesis. Rather than the fitnesses of individual genotypes changing through a density cycle, fitnesses may be constant and demographic changes in the population could alter gene frequencies. Charlesworth and Giesel (1972, p.394) analyzed an age structured population model and concluded that "changes in gene frequency produced by changes in population growth rates may occur even if the environmental agents which effect population size have no specific effects on genotypes (in terms of age-specific mortality and fecundity factors)." Thus fluctuations in age composition and population size could account for genetic changes at polymorphic loci without the effects of variation in selection, genetic drift or migration.

In conclusion, statistical tests of temporal gene frequency changes indicate no difference between the genetic variation at five independent polymorphic loci. This result is most easily explained if the alleles are selectively neutral and nonselective evolutionary forces change gene frequencies. A combination of genetic drift, migration and fluctuating age-structure probability accounts for the stochastic fluctuations in allele frequencies in vole populations.

CHAPTER 3

SPATIAL VARIATION IN GENE FREQUENCIES IN RODENT POPULATIONS

The large amount of polymorphism revealed by electrophoresis has stimulated much controversy concerning the roles of different evolutionary forces in the determining of genetic variation. Two principal views have been propounded; they differ in their emphasis on natural selection. The neutral view proposes that alleles at a locus are selectively equivalent so that nonselective evolutionary factors--such as restricted population size, recurrent mutation, and migration--determine variations in gene frequency in time and space. In contrast, the balance view maintains that alleles differ sufficiently in their effects on individual fitness so that natural selection plays an active role in determining genetic variation. Many evolutionists have an intermediate view, expecting both selective and nonselective factors to influence the distribution of gene frequencies.

However, the problem remains: How does one assess the roles of selective and nonselective factors from the observed patterns of genetic variation? One approach to this problem is to generate predictions from the hypothesis of neutral mutations and to test statistically the predictions against the observed patterns (Fisher and Ford; Ewens 1972; Johnson and Feldman 1973; Yardley, Anderson and Schaffer 1977; Watterson 1977; Wilson 1980; and others). The test is designed

so that if the fit between the expected and observed patterns is good, then the hypothesis of neutrality cannot be rejected. If the fit is poor, then selection may be the reason.

Distribution of Gene Frequency, A Test for Natural Selection

Lewontin and Krakauer (1973) developed a test for the selective neutrality of genetic polymorphisms. This test focuses on the difference between selective and nonselective evolutionary forces on the distribution of gene frequencies. It begins with two assumptions:

1. The variation in gene frequency among populations is expected to be a result of the interaction of selective and nonselective factors. The nonselective factors are characteristic of the breeding structure of the species and include the rates of migration between populations, the effective sizes of populations, and the degree of inbreeding within populations.
2. "While natural selection will operate differently for each locus and each allele, the effect of breeding structure is uniform over all loci and all alleles" (Lewontin and Krakauer 1973, p. 197).

Using these two premises, Lewontin and Krakauer (1973) reason that the steady state variation in gene frequencies from population to population for neutral alleles will reflect only the breeding structure of the species. Thus a statistical measure of the breeding structure should be approximately the same for each neutral locus.

However, if natural selection is operating on a particular locus (or subset of loci), the variation in gene frequency will be

determined by the differences in the coefficients of selection. The selected loci would have gene frequency distributions that differ from the distributions of neutral loci, and this will be reflected in discrepancies between the measures of breeding structure for each locus.

The measure of population structure chosen by Lewontin and Krakauer (1973) is the effective inbreeding coefficient, F_e. For a diallelic locus, F_e is estimated by

$$F_e = \frac{s_p^2}{\bar{p}(1-\bar{p})} \qquad (3.1)$$

where

$\bar{p}$ = the average gene frequency among populations

s_p^2 = the variance in gene frequency among populations

The effective inbreeding coefficient is a variance ratio: The numerator is the sample variance and the denominator is the theoretical maximum variance possible for the average gene frequency. Thus, F_e is a measure of relative differentiation (or degree of fixation) of allele frequencies over an ensemble of population, and varies in magnitude between 0 (all population and the same--no differentiation) and 1 (complete differentiation.

The Lewontin-Krakauer test is the statistical test of the homogeneity of F_e values for many loci. The null hypothesis is that all alleles at all loci are selectively neutral, and that each is equally affected by nonselective forces. Thus neutral alleles the same effects of population structure and the F_e values should be

homogenous among loci. However, natural selection, when operating on a subset of the alleles, will affect the gene frequency distributions at these loci and cause heterogeneity in the F_e values calculated for all loci.

Much controversy and criticism has been generated by the Lewontin-Krakauer test (Nei and Maruyama 1975; Robertson 1975a, 1975b; Ewens and Feldman 1976). The criticisms mainly concern the interpretation of heterogenous F_e values. While some of the criticisms focus on the statistical details of the test (Ewens and Feldman 1976), the primary points are that historical differences between populations (Robertson 1975) or temporary periods of extremely small population size can cause heterogeneity in F_e values for neutral alleles. Thus the rejection of the neutral hypothesis in favor of selection because of heterogenous F_e values may be unwarranted.

Despite the criticisms, the logic behind the Lewontin-Krakauer test appears to be sound and, in fact, other authors have proposed similar types of tests. Nei (1965) suggested homogenous F_{st} values (the theoretical equivalent of F_e) are expected if isolated populations differ solely because of genetic drift. He concluded that a test of the heterogeneity of F_{st} is essentially a test to see if differentiation has occurred at random or not. Nei and Imaizumi (1966) found close agreement among six values of F_{st} calculated for human populations. However, Cavalli-Szorfa (1966) calculated F_{st} values for multiple loci over a wide range of human groups and found considerable variation in the values for different loci. It was this result of

Cavalli-Szorfa (1966) that inspired Lewontin and Krakauer to develop their statistical test.

Because the results of the Lewontin-Krakauer test are difficult to interpret when the gene frequencies are sampled from spatially different populations, I used a different method to compare the gene frequency distributions for many loci. This method avoids the difficulties associated with the Lewontin-Krakauer test although its hypothesis is the same: Gene frequency distributions for neutral alleles should reflect the breeding structure of the species and neutral alleles should show similar degrees of variation among populations. However, whereas the Lewontin-Krakauer test is an overall assessment of neutrality, my method allows each locus to be tested individually against the other loci. In this manner, I can classify loci into two groups; those showing similar patterns of variation among populations and those loci showing much different patterns of variation.

In the following analysis, I tested for significant differences among populations within 17 species of rodents, and compared the degree of differentiation among loci. Those loci that show similar amounts of variation among populations are suspected to be neutral and were used to estimate the breeding structure of the species. Those loci with different patterns of variation are suspected to be affected by natural selection, or reflect some historical event.

In the second part of the analysis I considered the effect of genetic drift on the presence or absence of rare alleles in these populations. If genetic drift is operating, rare alleles will tend to be lost in the populations that subject the alleles to the greatest

drift; e.g., small and/or fluctuating populations. If genetic drift is causing gene frequency fluctuations, then rare alleles (whether they are neutral or not) will tend to be lost at many loci. The simultaneous loss of rare alleles at many loci in certain populations will result in a positive association among rare alleles at different loci; rare alleles should tend to co-occur in the same populations. Furthermore, if the difference among the species is such that certain species are more subject to genetic drift, then these species should show high levels of association among the rare alleles. I tested these predictions by calculating a measure of interdependence among rare alleles and compare this measure to the degree of population structure within each species.

Analysis of Gene Frequencies

Data Base

The data used in the analysis of gene frequencies are from published studies of geographic variation in rodents. In each study, investigators used the method of gel electrophoresis to detect protein polymorphisms at specific genetic loci. I included samples from various localities within the geographic range of the species. I restricted my analysis to studies in which at least three polymorphic loci and three different populations were sampled. I found 19 suitable studies representing 4 families, 6 genera, and 17 species of rodents. In most of these studies, the populations sampled represent different subspecies of geographic races within each species.

The following species of rodents exhibit genetic polymorphisms for structural proteins as detected by the methods of electrophoresis.

Family Heteromyidae

Dipodomys heermanni

Patton, MacArthur, and Yang (1976) studied 17 proteins controlled by 22 loci in six populations of Heerman's Kangaroo rat. Four loci were monomorphic for the same allele. The populations sampled included four- and five-toed populations of D. heermanni. These two forms differ in their karotypes: northern four-toed populations are characterized by a diploid number of 52, and southern five-toed populations are characterized by a 2N of 64. Within each population, genetic variation is low so I combined both forms for the overall assessment of genetic variation within the species. I used eight loci and five population from Patton et al. (1976, Table 1) in the analysis.

Dipodomys merriami

Johnson and Selander (1971) examined the proteins at 17 genetic loci in populations of D. merriami from the edge of its geographic range in southern Arizona and New Mexico. I used the data from eight polymorphic loci sampled in seven populations of D. merriami (Johnson and Selander 1971, Table 3).

Dipodomys ordii

Johnson and Selander (1971) found eight polymorphic loci out of 17 examined in populations of D. ordii. I used the gene frequencies

for the eight polymorphic loci and nine populations in the analysis (from Johnson and Selander 1971, Table 4). Three samples are from Utah and Nevada and six samples are from New Mexico and Texas; these populations are also from the edge of the species geographic range.

Family Cricetidae

Peromyscus boylii.

Avise et al. (1974) studied 21 loci in samples of the brush mouse, P. boylii throughout its range in southwestern USA and Mexico. I used nine polymorphic loci from seven populations in the analysis (Avise et al. 1979, Table 3).

Peromyscus californicus

Smith (1979) analyzed 31 loci in 13 populations of P. californicus. I used 16 polymorphic loci in 13 populations in the analysis (from Smith 1979, Table 1).

Peromyscus floridanus

Smith, Selander, and Johnson (1973) analyzed 41 structural gene loci of the Florida deer mouse, P. floridanus. Of the loci studied, 15 were polymorphic in the 4 populations studied.

Peromyscus maniculatus.

Avise, Smith, and Selander (1979) reported allozymic variation at 22 genetic loci for samples of P. maniculatus from most of its immense geographic range. Fifteen loci were monomorphic (or nearly so).

I used allele frequencies at six polymorphic loci in 17 populations from throughout the range (Avise et al. 1979, Table 3).

Peromyscus melanotis

Avise et al. (1979) reported five polymorphic loci in four populations of P. melanotis sampled from southern Arizona and central Mexico (Avise et al., 1979, Table 2).

Peromyscus pectoralis

Avise et al. (1974) studied 22 electrophoretic loci in three populations of P. pectoralis (Advise et al. 1974, Table 3).

Peromyscus polionotus

Selander et al. (1971) studied 30 populations and detected 17 polymorphic loci in the old-field mouse the southeastern U.S.A. I used 25 populations and 14 loci in my analysis (Selander 1971, Table 5-13).

Peromyscus truei

Advise et al. (1979) reported four polymorphic loci in three populations of P. truei sampled from southern California and central Mexico.

Sigmodon hispidus

Two separate studies of the cotton rat, Sigmodon hispidus, are available. McClenaghan (1977) studied electrophoretic variation in marginal (Kansas) and central (Mexico) populations of S. hispidus. He reported six polymorphic loci in 16 populations sampled. Johnson

et al. (1972) found 12 polymorphic loci in eight populations of S. hispidus sampled throughout its geographic range.

Family Geomyidae

Thomomys bottae

Patton and Yang (1977) studied allozymic variation at 23 loci in 50 localities throughout the southwest U.S.A. and Mexico for the western pocket gopher. Of the 23 loci examined, only two were monomorphic throughout the range of the species. I used the 15 major polymorphic loci and 23 populations for my analysis (Patton and Yang, 1977, Table 1).

Thomomys talpoides

Nevo et al. (1974) studied allozymic variation at 31 electrophoretic loci in 10 populations of the valley pocket gopher. The populations sampled are from southern Rocky Mountains representing the southern part of this species range. I used allele frequencies at 23 polymorphic loci in 10 populations (Nevo et al. 1977, Table 2) in my analysis.

Family Muridae

Rattus fuscipes

Schmitt (1978) studied 16 populations of the Australian bush rat and found 12 polymorphic loci of 16 proteins studied (Schmitt 1978, Table 2). Schmitt was particularly interested in isolated island populations of R. f. greyii. His samples include 10 island

populations, three mainland populations and three samples representing three other subspecies found on the coast of Australia.

Rattus rattus

Patton et al. (1975) studied 11 populations of Rattus rattus which inhabit the Galapagos Islands. They detected seven polymorphic loci (Patton et al..1975, Table 1).

Mus musculus

Selander, Hunt, and Yang (1969) studied 36 proteins and 41 electrophoretic loci in two subspecies of the European house mouse. Twenty of the loci were monomorphic. I used 16 polymorphic loci sampled in 4 regions of Northern Jutland for M. m. musculus (Tables 2-5 of Selander et al. 1969). Selander et al. (1971) also studied many populations of Mus musculus occurring throughout North America. I chose 12 populations sampled throughout the range (Selander et al. 1971, Tables 2 and 3) for five polymorphic loci.

Differences among Populations in Common Allele Frequencies

Before assessing the roles of selection and nonselective processes in variation of gene frequencies at polymorphic loci, I needed to determine if the populations sampled within a species are, in fact, genetically different. Do the populations represent random samples from a large homogeneous population or do the populations represent samples from a heterogenous total population structured into genetically different breeding units?

To test for differences among the populations, I used the common allele at each polymorphic locus (i.e., the allele with the greatest average frequency over populations) in a contingency table analysis. For L loci (one allele each) and N populations, each cell in an L x N contingency table contains the absolute gene frequency, f, which equals the product of the number of genes sampled (twice the number of individuals sampled) times the gene frequency in the sample:

$$f_{ij} = 2 \times n_{ij} \times p_{ij}, \qquad \begin{matrix} i = 1,..,L \\ j = 1,..,N \end{matrix} \tag{3.2}$$

where n_{ij} is the sample size and p_{ij} is the relative gene frequency at the ith locus and the jth population. (Note that this differs from Workman and Niswander's (1960) genic contengency table which deals with one locus at a time.)

To test the null hypothesis that the common allele frequencies are independent of the population sampled, I used the standard χ^2 test of independence for a two-way contingency table (Sokal and Rohlf 1969) where the value

$$\chi^2 = \sum_{ij} \frac{(f_{ij} - e_{ij})^2}{e_{ij}} \tag{3.4}$$

is approximately distributed as a theoretical χ^2 distribution with $(L - 1)(N - 1)$ degrees of freedom. The expected number of genes at the ith locus in the jth population is calculated from the marginal sums of the table as follows:

$$e_{ij} = \frac{(n_{+j})(n_{i+})}{N_{++}} \qquad (3.4)$$

where + stands for summation over the appropriate index, (n_{+j}) is the number of genes sampled at all loci in the population, and (n_{i+}) is the number of genes sampled for the ith locus in all populations. The total number of genes sampled is N_{++} which equals twice the number of individuals sampled, i.e., $N_{++} = 2N$.

I compared the value of χ^2 to the theoretical χ^2 distribution using the 5% probability level as the criterion for statistical significance. A large χ^2 value indicates a significant difference in common allele frequencies among the populations. A small χ^2 value indicates no significant differences among allele frequencies in the populations samples.

Sources of Deviation from Independence

A large χ^2 value, indicating heterogeneity in common allele frequencies, can result for three different reasons (Figure 4). In the first case, a particular population may show significant deviations in allele frequencies at all loci (Figure 4a). In this case, the cells in a particular column of the contingency table contribute large values in the calculation of χ^2 (Equation 3.2).

The second case is the opposite extreme, where the allele frequencies at one locus show deviations in many populations (Figure 4b). In this case, the cells in one row of the table contribute large values to the accumulated χ^2 value.

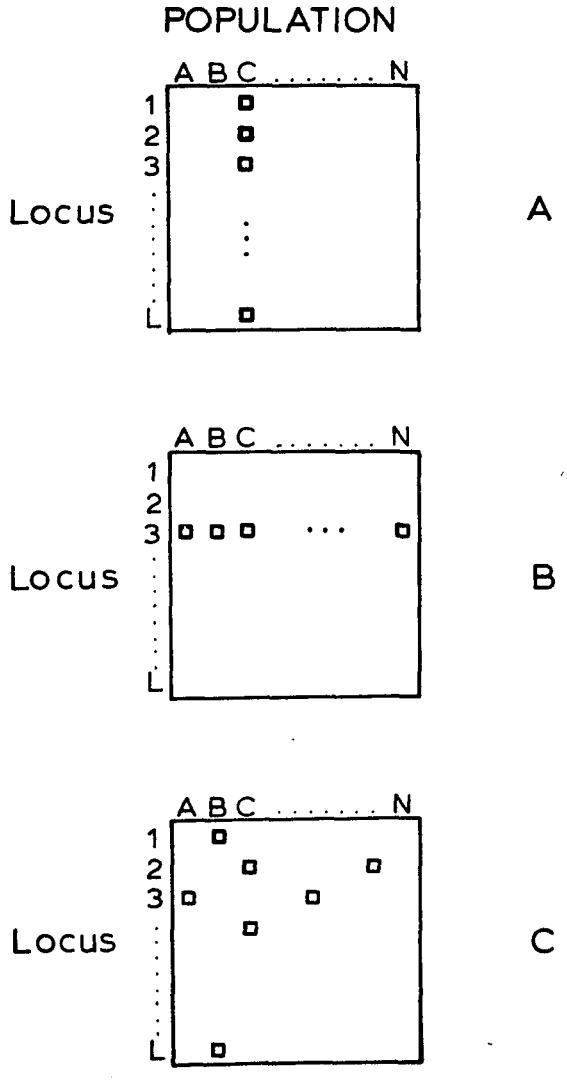

Figure 4. Sources of deviation in the contingency table for common alleles. -- The sources of deviation leading to a large value can result from significant deviations in various cells of the contengency table of common allele frequencies. (A) The boxes designate significant deviations and a large contribution to the overall χ^2 from a particular population. (B) In this example one locus tend to deviate in many populations and contributes significantly to the heterogeneity of the table. (C) In this case different cells in various populations for different loci contribute to the heterogeneity of the table.

The third case is intermediate. The large χ^2 value results from deviations in allele frequencies at different loci and in different populations (Figure 4).

To test which cells show significant deviations from independence and thus contribute large values to the χ^2 value, I used an analysis of residuals for two-way contengency tables. The first step is to calculate the standardized residual (Everitt 1977) for each cell as follows:

$$s_{ij} = \frac{(f_{ij} - e_{ij})}{\sqrt{e_{ij}}} \tag{3.5}$$

(s_{ij} is the square root of the individual terms in (3.2) with the sign intact). An estimate of the variance of s_{ij} is (Haberman 1972):

$$v_{ij} = \frac{(1 - n_{i+})(1 - n_{+j})}{N} \tag{3.6}$$

When the standardized residual is divided by the square root of the variance, as follows:

$$d_{ij} = \frac{s_{ij}}{\sqrt{v_{ij}}} \tag{3.7}$$

then the resulting adjusted residual, d_{ij}, is expected to follow a standard normal distribution with a mean of 0 and a variance of 1. A value of d_{ij} greater than 1.96 or less than -1.96 is in one of the 5% tails of the standard normal distribution and indicates that cell (i,j) contributes significantly to the total χ^2. A significant

d_{ij} means that the observed allele frequency at the ith locus in the jth population deviates significantly from the expected frequency (Equation 3.5) calculated for the null hypothesis (the allele frequency is independent of the population sampled).

Common Alleles: Differences among Loci

The basis of the Lewontin-Krakauer (1973) test of selective-neutrality is the comparison of gene frequency distributions among loci. The steady-state distribution of gene frequencies over populations depends on natural selection and on nonselective aspects of the breeding structure such as effective population size, migration and inbreeding. In the absence of selection, the distribution of gene frequencies over populations will reflect entirely the effect of breeding structure of the species. If the alleles are selectively neutral, then gene frequencies at different loci should show similar degrees of variation from population to population due to the uniform effect of nonselective forces.

To test the null hypothesis that loci show about the same degree of variation over populations, I used a 2 x L contengency table and an adjusted residual analysis. For each locus, I counted the number of populations in each of two categories. The first category is the number of populations with significant deviations in common allele frequencies as measured by the adjusted residual (Equation 3.6). The second category is the number of populations in which the adjusted residual was non-significant (i.e., $-1.96 < d_{ij} < 1.96$)

Another χ^2 test of independence on the 2 x L table tests the null hypothesis that the number of significant deviations is independent of the locus. If the χ^2 value indicates homogeneity over loci, then the loci show similar degrees of variation over populations, a result most easily explained with selectively-neutral alleles. If the χ^2 value indicates heterogeneity among loci in the degree of differentiation, then an adjusted residual analysis may isolate which locus (or subset of loci) shows greater degrees of variation.

Results for Common Alleles

For each species, I first tested for differences among the populations in common allele frequencies. Using the L x N contengency table, I calculated the homogeneity statistic, χ^2, given in (3.2). Of the 19 data sets analyzed, 12 had significantly large χ^2 values (Table 12). A large χ^2 value indicates significant differences in the gene frequencies in different populations within the species.

Because Sigmodon hispidus was the only data set with more than 100 individuals sampled that yielded an insignificant χ^2 value, I expect the remaining species had small χ^2 because of the small sample sizes.

If the genetic differences between the populations are primarily a result of nonselective factors, then all loci should show similar amounts of variation among populations. To measure the variation at a locus I used the analysis of residuals to isolate the populations in which the common allele frequency significantly deviated. I then counted the number of significant deviations for each locus.

Table 12. Heterogeneity tests of gene frequencies. -- The χ^2 values for the test of heterogeneity of common allele frequencies for 17 species of rodents. N is the number of populations sampled, L is the number of loci, and I is the number of individuals sampled. Twelve species had large χ^2 values indicating significant heterogeneity in common allele frequencies among populations. *p < 0.05

Family Species	N	L	I	χ^2
Heteromyidae				
Dipodomys heermanni	5	8	72	165.7*
Dipodomys merriami	7	9	243	42.9
Dipodomys ordii	9	9	392	127.6*
Cricetidae				
Peromyscus boylii	7	9	212	116.3*
Peromyscus californicus	13	16	230	464.3*
Peromyscus floridanus	4	15	71	18.7
Peromyscus maniculatus	18	6	694	501.9*
Peromyscus melanotis	4	5	20	5.4
Peromyscus pectoralis	3	5	35	10.5
Peromyscus polionotus	25	14	666	884.2*
Peromyscus truei	3	4	64	10.9
Sigmodon hispidus(A)	16	6	647	84.8
Sigmodon hispidus(B)	8	12	220	176.1*
Geomyidae				
Thomomys bottae	23	15	654	3,061.9*
Thomomys talpoides	10	23	276	1,004.3*
Muridae				
Rattus fuscipes (islands)	16	12	531	1,743.9*
Rattus rattus (islands)	11	7	137	274.1*
Mus musculus(A) (Europe)	4	14	69	48.7
Mus musculus(B) (NA)	12	5	1,326	366.8*

In the absence of selection, each locus should deviate in about the same number of populations, in response to the similar nonselective factors associated with the breeding structure of the species. I tested the null hypothesis that the number of significant deviations was independent of the locus using a 2 x L contingency table. Of the 12 species tested, 11 species had at least one locus that deviated in more populations than expected. However, most of the loci within each species showed the same degree of differentiation. In five species, at least one locus deviated in fewer populations than expected, indicating the common allele frequencies at these loci are more uniformly distributed in populations than they are at the other loci.

With the analysis of residuals, the loci can be classified into three groups (Table 13): (1) loci which show the same degree of variation among populations (i.e., $-1.96 < d_{lj} < 1.96$); (2) loci which show greater variation among populations (i.e., $1.96 < d_{lj}$); and (3) loci which show less variation among populations (i.e., $d_{lj} < -1.96$). The results for 136 loci (12 species) indicates that 76% of the loci fall into the first group. Because the Group 1 loci show similar degrees of differentiation (i.e., differ significantly in the same number of population), I propose that the alleles at these loci are selectively neutral, and that nonselective evolutionary factors are determining the allele frequency distributions.

In the following section, I use the Group 1 loci to obtain a measure of the genetic structure of each species. Because I suspect that the Group 1 loci are selectively neutral, the measure of population structure should reflect only the role of nonselective

Table 13. The number of loci in each group. -- Entries are the number of loci in each group for 12 species of rodents. See text for group definitions.

Species	Group 1	Group 2	Group 3
Dipodomys heermanni	7	0	1
Dipodomys ordii	8	1	0
Peromyscus boylii	8	1	0
Peromyscus californicus	13	3	0
Peromyscus maniculatus	4	1	1
Peromysecus polionotus	11	2	1
Sigmodon hispidus(B)	6	0	0
Thomomys bottae	8	3	4
Thomomys talpoides	19	4	0
Rattus fuscipes	4	4	4
Rattus rattus	6	1	0
Mus musclus(B)	5	0	0

forces in the differentiation of populations within a species. I will use the measure of population structure in subsequent analysis to compare species and test the hypothesis that a greater degree of differentiation will result in greater positive association among rare alleles.

Before turning to the analysis of rare alleles, I will present a method for measuring the population structure for a species subdivided into numerous populations. This method, developed by Nei (1975), is an alternative to Wright's F statistics (e.g., F_{st}) that allows for the consideration of many alleles at each locus.

Measures of Population Structure

Nei (1975) developed a method for analyzing gene frequencies for loci with many alleles. Nei defined the total genetic diversity, or heterozygosity in an ensemble of populations as:

$$H_t = 1 - \sum_i \bar{p}_i^2 \qquad (3.2)$$

where $\bar{p}_i$ is the average frequency of the ith allele among populations. The term genetic diversity refers to the heterozygosity expected under the Hardy-Weinberg equilibrium regardless of the actual genotypic frequencies in the populations.

The total genetic diversity, H_t can be partitioned into a <u>within</u> population component of genetic diversity, H_s, and a <u>between</u> population component D_{st}. That is

$$H_t = H_s + D_{st} \qquad (3.9)$$

where

$$H_s = 1 - \sum_i \left(\frac{1}{N} \sum_j p_{ij}^2\right) \tag{3.10}$$

$$D_{st} = \left[\sum_{ij} \left(\sum_k \frac{1}{2}(p_{ik} - p_{jk})^2\right)\right]\frac{1}{N^2} \tag{3.11}$$

If the total population is homogeneous and there are no differences among the populations in allele frequencies, then $D_{st} = 0$ and the total genetic diversity is equal to average genetic diversity within populations (i.e., $H_t = H_s$).

Nei (1975) suggests two measures of the degree of differentiation among populations. The first, the average amount of genetic diversity among populations is calculated as

$$\overline{D}_m = \frac{N(D_{st})}{(N - 1)} \tag{3.12}$$

For many loci (both polymorphic and monomorphic loci), $\overline{D}_m$ is an estimate of the minimum number of codon differences between the populations (Nei 1975). $\overline{D}_m$ is a measure of the absolute degree of differentiation among the populations.

A second measure, the relative magnitude of differentiation is calculated as

$$G_{st} = \frac{D_{st}}{H_t} \tag{3.13}$$

G_{st} equals 0 when there is no differentiation among populations (i.e., $D_{st} = 0$) and equals 1 when there is complete differentiation among the populations. Complete differentiation means that the genetic diversity within each population is zero (i.e., $H_s = 0$) and alternate alleles are fixed in different populations.

Results of Genetic Diversity Analysis

To quantify the magnitude of differentiation for each species, I calculated Nei's genetic diversity measures for each locus in Group 1. For the calculations in equations (3.7-3.12), I used the frequencies of all alleles (not just the common one) detected at each locus.

To illustrate how the total genetic diversity is partitioned into within and between population components, I plotted the total genetic diversity, H_t, against the average gene diversity, H_s, within each population for 17 species (Figure 5). The line indicates values of $H_t = H_s$ when $D_{st} = 0$. No points can fall below this line because D_{st} is always greater than or equal to zero. For a particular species (see Peromyscus maniculatus at the right-hand side of Figure 5), the total genetic diversity, H_t, has two components: the vertical distance between the H_s axis and the line is the within population component of genetic diversity (H_s), and the vertical distance above the line is the between population component of genetic diversity D_{st}. The genetic diversity results using all alleles at each Group 1 locus, agree with the results of the heterogeneity tests of common allele frequencies. In the previous analysis of common alleles,

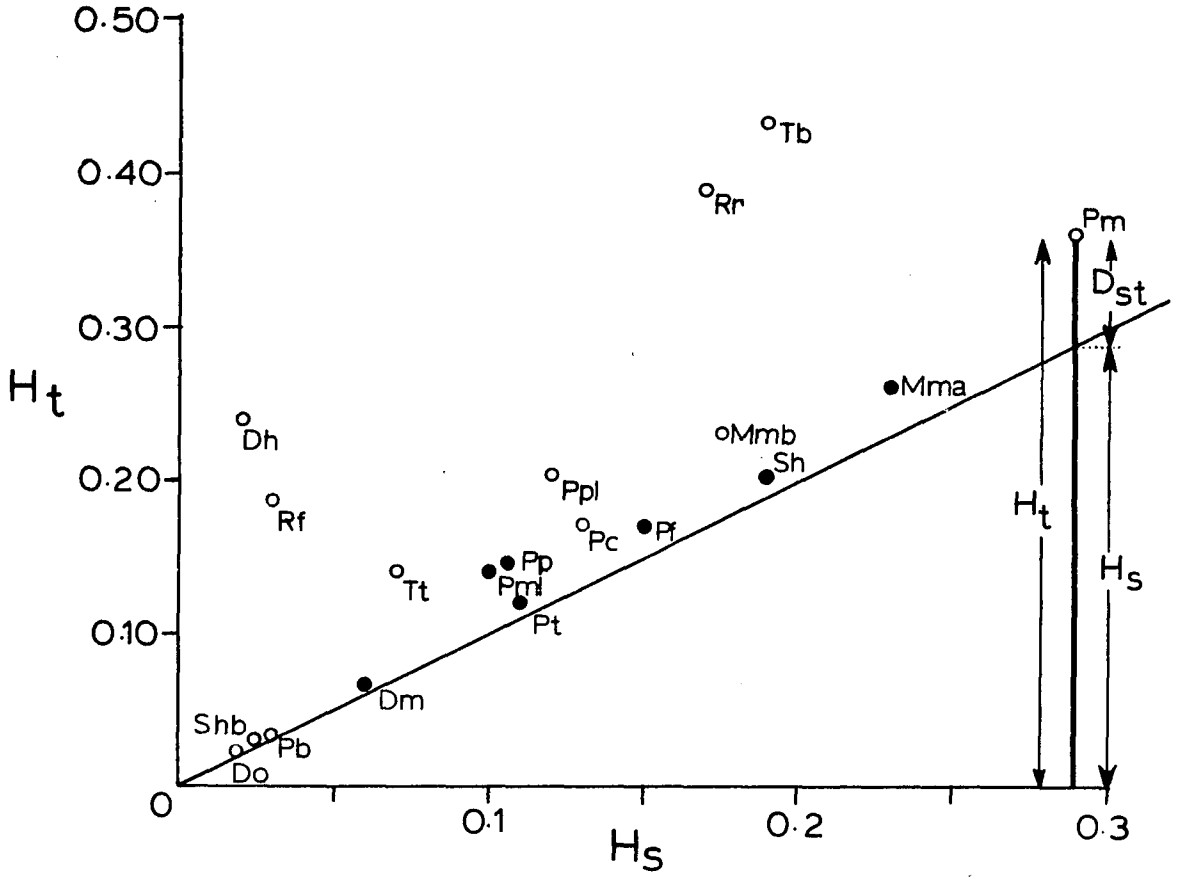

Figure 5. Genetic diversity values for 17 species of rodents. -- The total genetic diversity, H_t, is partitioned into a within population component, H_s, and a between population component, D_{st}. The magnitude of H_s for a given species (see Pm on the right hand side of the figure) is represented by the vertical distance to the line, where $H_s = H_t$, and the magnitude of D_{st} is the distance above the line. The species are as follows: Dh = Dipodomys heermanni, Dm = D. merriami, Do = D. ordii, Pb = Peromyscus boylii Pc = P. californicus, Pf = P. floridanus, Pm = P. maniculatus, Pml = P. melanotis, Ppc = P. pectoralis, Pp = P. polionotus, Pt = P. truei, Sha = Sigmodon hispidus (A), Shb = S. hispidus (B), Tb = Thomomys bottae, Tt = T. talpoides, Rf = Rattus fuscipes, Rr = Rattus rattus, Mma = Mus musculus (A), Mmb = M. musculus (B).

12 species showed significant differences in allele frequencies among populations. These 12 species are designated with open circles in Figure 6. Most of the species that showed significant heterogeneity of common allele frequencies, have large values of D_{st}, the between population component of genetic diversity. Two species, however, that showed significant differences among populations in common allele frequencies, have relatively small D_{st} values. These species, D. ordii and P. boylii, fall on the bottom left-hand part of Figure 6 and have relatively small components of genetic diversity. The remaining species showed homogeneity in common allele frequencies and have small between population components of genetic diversity.

Using only the 12 species that have heterogeneous common allele frequencies, I calculated Nei's two measures of population structure. Table 14 presents the absolute measure of population differentiation, $\overline{D}_m$, and the relative measure of population differentiation, G_{st} for each species. The relationship between the two measures is evident with an examination of Figure 5 and Table 14. For instance, Rattus fuscipes (Rf) and Rattus rattus (Rr) both have relatively large components of between population genetic diversity (Figure 5). When the D_{st} values are corrected for the number of populations sampled (Equation 3.12) the resulting $\overline{D}_m$ value for R. rattus is greater than the $\overline{D}_m$ value for R. fuscipes (Table 14). However, the relative degree of differentiation, as measured by G_{st}, is much greater for R. fuscipes than for R. rattus (Table 14). This result reflects the fact the R. fuscipes has a much smaller within population component of genetic diversity.

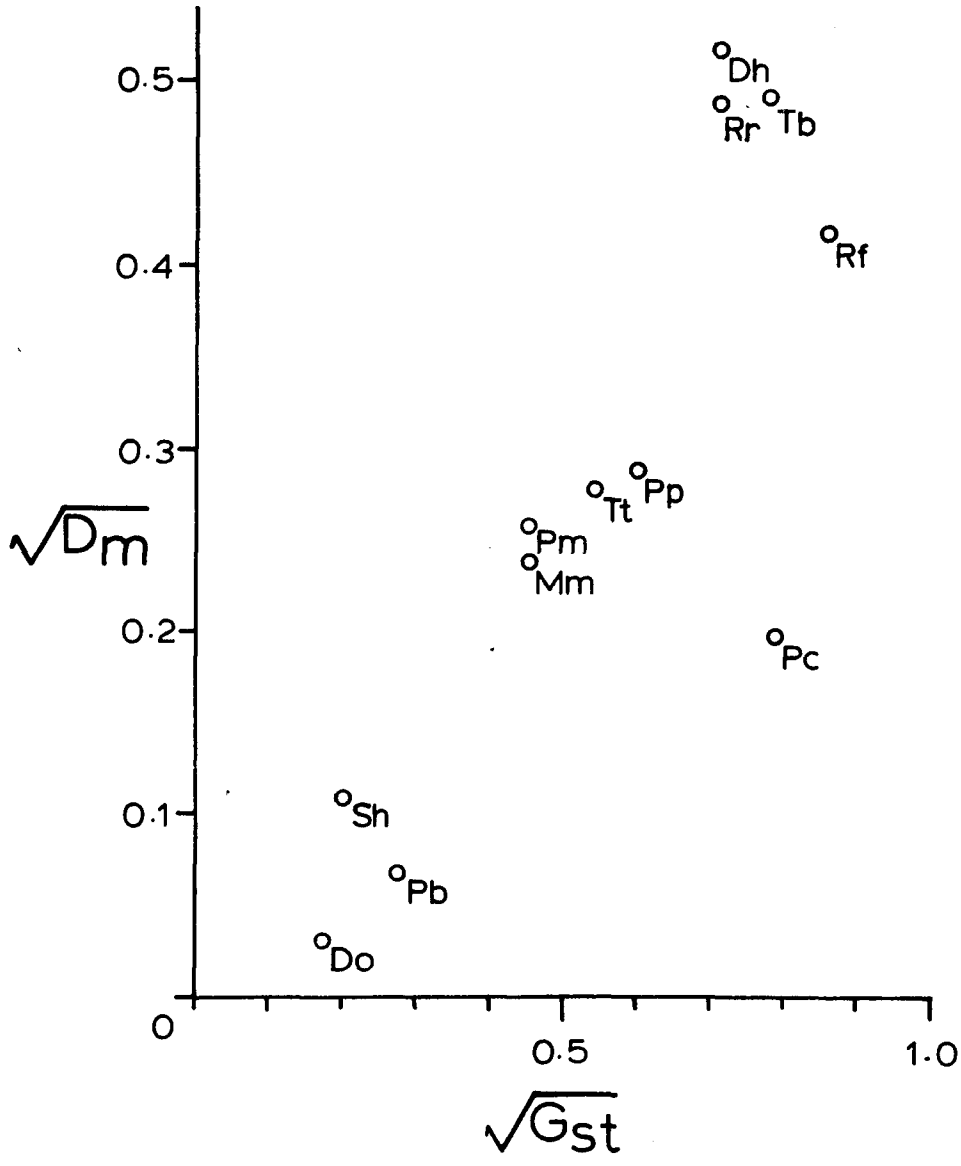

Figure 6. Two measures of population differentiation for 12 rodent species. -- Each point plotted in the figure represents one of the rodent species that showed significant heterogeneity in common alleles frequencies. The location of the points is determined by the values of G_{st} and $\overline{D}_m$ for each species. I used square roots to reduce the variance in each measure.

Table 14. Measures of population differentiation for 12 rodent species. -- The values of G_{st}, the relative amount of population differentiation, and D_m, the absolute degree of differentiation for 12 species of rodents. The measures are calculated using only Group 1 loci for each species.

Species	Number of Populations	Number of Loci	G_{st}	$\overline{D}_m$
Dipodomys heermanni	5	7	0.4987	0.2713
Dipodomys ordii	9	8	0.0319	0.0010
Peromyscus boylii	7	8	0.076	0.0049
Peromyscus californicus	13	13	0.1634	0.0420
Peromyscus maniculatus	18	4	0.2067	0.0689
Peromyscus polionotus	25	11	0.3579	0.0835
Sigmodon hispidus B	8	11	0.0423	0.0018
Thomomys bottae	23	8	0.5870	0.2475
Thomomys talpoides	10	19	0.3013	0.0772
Rattus fuscipes	16	4	0.7254	0.1767
Rattus rattus	11	6	0.4967	0.2425
Mus musculus B	12	5	0.1962	0.0583

Because both $\overline{D}_m$ and G_{st} depend on the genetic diversity between populations they are not independent. Figure 6 shows the relationship between the two measures of population differentiation for 12 species. Species in the upper right-hand corner of the figure have the greatest degree of differentiation among populations. The species exhibiting the greatest amount of differentiation probably have relatively little migration between populations. These species include isolated island populations of R. fuscipes and R. rattus, chromosomal races of D. heermanni, and populations of fossorial pocket gophers, Thomomys bottae.

Analysis of Rare Alleles

The results so far indicate that 12 of 17 species examined showed significant heterogeneity in gene frequencies. A comparison among loci within each species reveals that most loci (about 76% of all loci in 12 species) have similar amounts of variation among populations. The similarity among loci in the degree of differentiation is an expected result if the alleles are selectively neutral and subject to similar nonselective aspects of the breeding of the species. Using only those loci that measure the breeding structure (Group 1 loci), I measured the differentiation of the population using Nei's genetic diversity indices. The results indicate that there are differences among the mammal species in the measures of population structure, and that most differentiated species include those with island populations and different chromosomal races in the samples.

In the subsequent analysis, I will analyze the presence and absence of rare alleles in different populations. If the populations differentiated because of nonselective processes such as genetic drift, rare alleles at all loci could be lost in those populations most subject to drift, for instance in a population which suffered a sudden reduction in its numbers. If this is the case, the presence of rare alleles should tend to be interdependent among loci and positively associated within populations.

A second prediction concerns the differences in the breeding structures of various species. If the breeding structure of a species facilitates the random differentiation of populations, then rare alleles should tend to be positively associated. Thus, breeding structures that promote random differentiation of populations will also promote the positive association of rare alleles. If this is the case, species with large between population components of genetic diversity should also exhibit strong positive associations among rare alleles. I will test these predictions using an overall measure of association developed by Pielou (1972) for presence/absence data. I will compare this measure of association with the measures of genetic diversity calculated for Group I loci among species of small mammals.

Interdependence among Rare Alleles

To measure the interdependence among rare alleles, I constructed a presence/absence matrix for N populations and k alleles including rare alleles from all loci. The matrix is filled with 0's and 1's, where 0 in cell (i,j) represents absence (i.e., $p_{ij} = 0$) of the jth

allele in the ith population, and a 1 denotes the presence (i.e., $p_{ij} > 0$) of the ith allele in the jth population.

For a matrix of this type, Pielou (1972) derived a measure of overall association that depends only on the interdependence among the alleles (in Pielou's case, they were species). The measure of interdependence, D, is calculated from the difference between the observed variance in the number of alleles per population and the theoretical variance expected if the alleles were independently distributed among populations. If D is positive, the observed variance is greater than the theoretical variance and the alleles are positively associated in populations. If D is negative, the observed variance is less than the theoretical variance and the alleles are negatively associated in populations.

Table 15 illustrates a presence/absence matrix for rare alleles. The number of alleles occurring in the ith population is a_i and the number of populations in which the jth allele occurs is n_j.

$$A = \sum_{i}^{n} a_i = \sum_{j}^{k} n_j \qquad (3.14)$$

If the alleles are independently distributed among the populations then the variance in the number of alleles per population can be shown to be

$$\mathrm{Var}(a) = \sum_{i} p_j(1 - p_j) = k(\bar{p}(1 - \bar{p}) - \mathrm{Var}(p)) \qquad (3.15)$$

Table 15. The presence/absence matrix for rare alleles. -- For each population and each rare allele, each entry in the matrix designates the presence (1) or absence (0) of the allele in the population. The row total are the number of alleles in each population and the column totals are the number of populations occupied by an allele. (A) represents the total number of allele occupancies observed. Adapted from Pielou (1972).

	Allele Number				
Population	1	2	3	k	
1	1	0	1	1	a_1
2	0	0	0	0	a_2
3	1	1	1	1	
N	0	1	0	1	a_n
	n_1	n_2	n_3	n_k	A

where $p_j = \frac{n_j}{N}$, the proportion of populations occupied by the jth allele.

Var(a) is the expected variance in the number of alleles per population (row totals in Table 15) which can be calculated from the distribution of occurrences per allele (column totals in Table 15) assuming the alleles are independently distributed.

The mean proportion of populations in which rare alleles occur is given by

$$\bar{p} = \frac{1}{k}\Sigma\frac{n_i}{N} = \frac{A}{kN} \tag{3.16}$$

and has a variance given by

$$\text{Var}(p) = \frac{1}{k}\sum_j(\frac{n_i}{N})^2 - (\frac{A}{kN})^2 = \frac{1}{k^2N^2}\{k\Sigma n_j^2 - A^2\} \tag{3.18}$$

where A is the total number of occurrences (i.e., $A = \Sigma n_j$). Using Equations (3.16) and (3.17), Equation (3.15) can be rewritten in terms of the number of occurrences per allele as follows (pielou 1972):

$$\text{Var}(a) = \frac{A}{N} - \frac{1}{N^2}\sum_j n_j^2 \tag{3.18}$$

The difference between the theoretical variance in the number of alleles per population and the observed variance, s_a^2, is used in the calculation of the interdependence statistic, D, as follows (Pielou 1972):

$$D = \frac{1}{2}\{s_a^2 - \text{Var}(a)\} \tag{3.19}$$

where

$$s_a^2 = \frac{1}{N} \Sigma a_i^2 - \frac{A^2}{N^2}. \qquad (3.20)$$

D values greater than zero indicate overall positive association among alleles, whereas values less than zero indicate negative associations among alleles.

Results for Rare Alleles

I calculated the interdependence statistic D for rare alleles in 12 species of rodents. The results are presented in Table 16. Nine of the 12 species have D values greater than zero which reflects positive associations among the rare alleles at all loci within the species. The average $\overline{D}$ over all species is 1.675 with a standard error of 0.052. These results suggest that rare alleles tend to occur together or be absent together in the same population.

Does this result indicate that nonselective factors are the major influence determining the genetic structure of the populations? The positive association among rare alleles is expected if certain populations have characteristics that promote genetic drift. For instance, populations that go through bottlenecks in density (i.e., the population size is suddenly reduced) suffer a reduced number of alleles per locus and also a reduced heterozygosity (Nei et al. 1975). Those populations that are more likely to undergo reductions in density (e.g., ecologically marginal populations) will tend simultaneously to lose alleles at many loci. The loss of alleles in certain populations means that rare alleles will co-occur in other populations

Table 16. Measures of association for rare alleles in 12 species. -- For each species of rodent that showed significant heterogeneity in common allele frequencies among populations, I calculated D, the association among rare alleles. Positive values indicate a positive association among rare alleles in populations. Negative values indicate a negative association among rare alleles. The average and standard error of D are given below.

Species	D value
Dipodomys heermanni	3.440
Dipodomys ordii	-0.086
Peromyscus boylii	-0.204
Peromyscus californicus	6.189
Peromyscus maniculatus	0.596
Peromyscus polionotus	4.160
Sigmodon hispidus (B)	1.328
Thomomys bottae	1.540
Thomomys talpoides	0.879
Rattus fuscipes	0.785
Rattus rattus	-0.025
Mus musculus (B)	1.500
$\overline{D}$	1.675
SE(D)	0.052

that are less likely to go through conditions promoting genetic drift, such as bottlenecks in density.

The tendency toward co-occurrence of rare alleles in the same populations is indicated by the positive average interdependence value D. However, the sampling distribution of D is not derivable (even in principle) without an underlying hypothesis intended to describe the inter-relations among alleles (Pielou 1972). This means that I cannot test if individual D values represent significant interdependence among the alleles. But the D values can be used in nonparameteric analysis to test the following hypothesis. If the positive association among rare alleles is a result of genetic drift, then those species with breeding structures that promote random differentiation of populations should have greater positive associations among rare alleles.

To test this hypothesis, I compared the measure of association among rare alleles to the measures of population structure calculated with genetic diversity components for Group I loci. The association among rare alleles, D, does not appear to be correlated with the absolute amounts of genetic diversity among populations as measured by $\overline{D}_m$ (Figure 7). Recall the $\overline{D}_m$ measures the absolute degree of differentiation among populations and is independent of the within population component of diversity.

The association among rare alleles does appear to be positively related to the relative degree of differentation among populations as measured by G_{st} (Figure 8). Those species that have relative large G_{st} values also tend to have a high positive association among rare alleles.

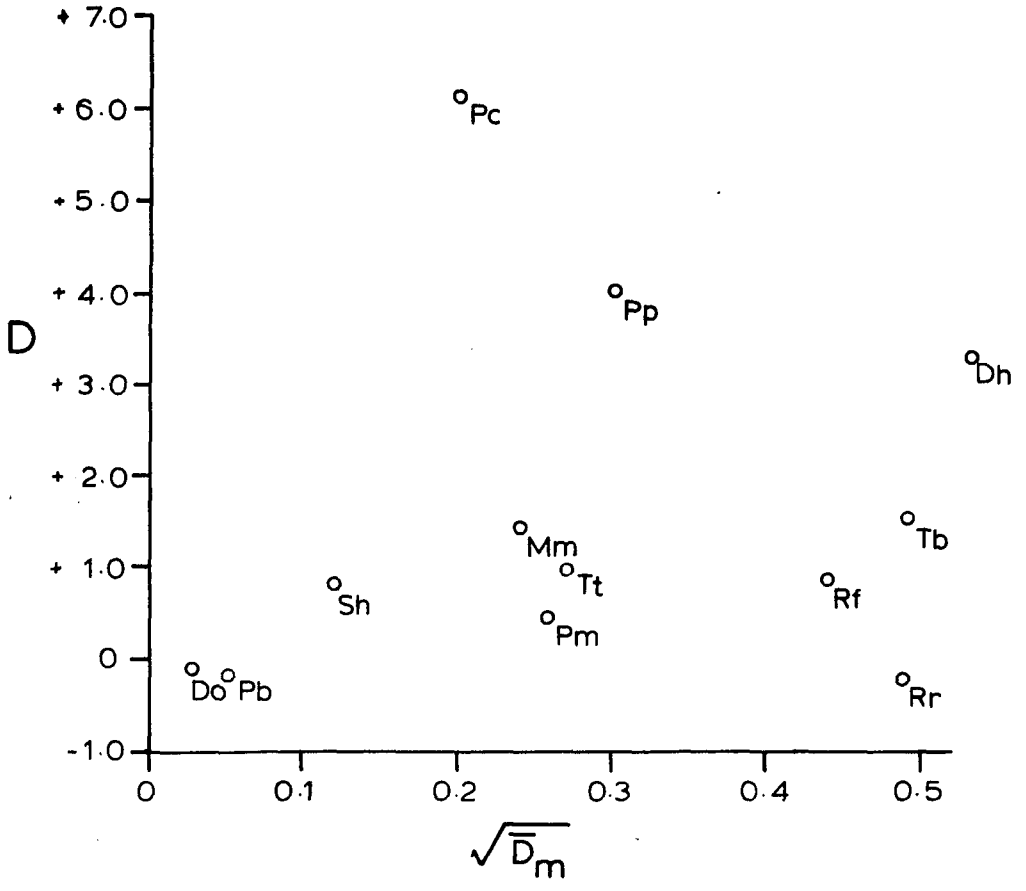

Figure 7. Association of rare alleles vs. the absolute degree of population differentiation for 12 rodent species. -- The overall measure of association, D, among rare allele plotted against the absolute degree of differentiation, D_m, of populations for 12 species of rodents.

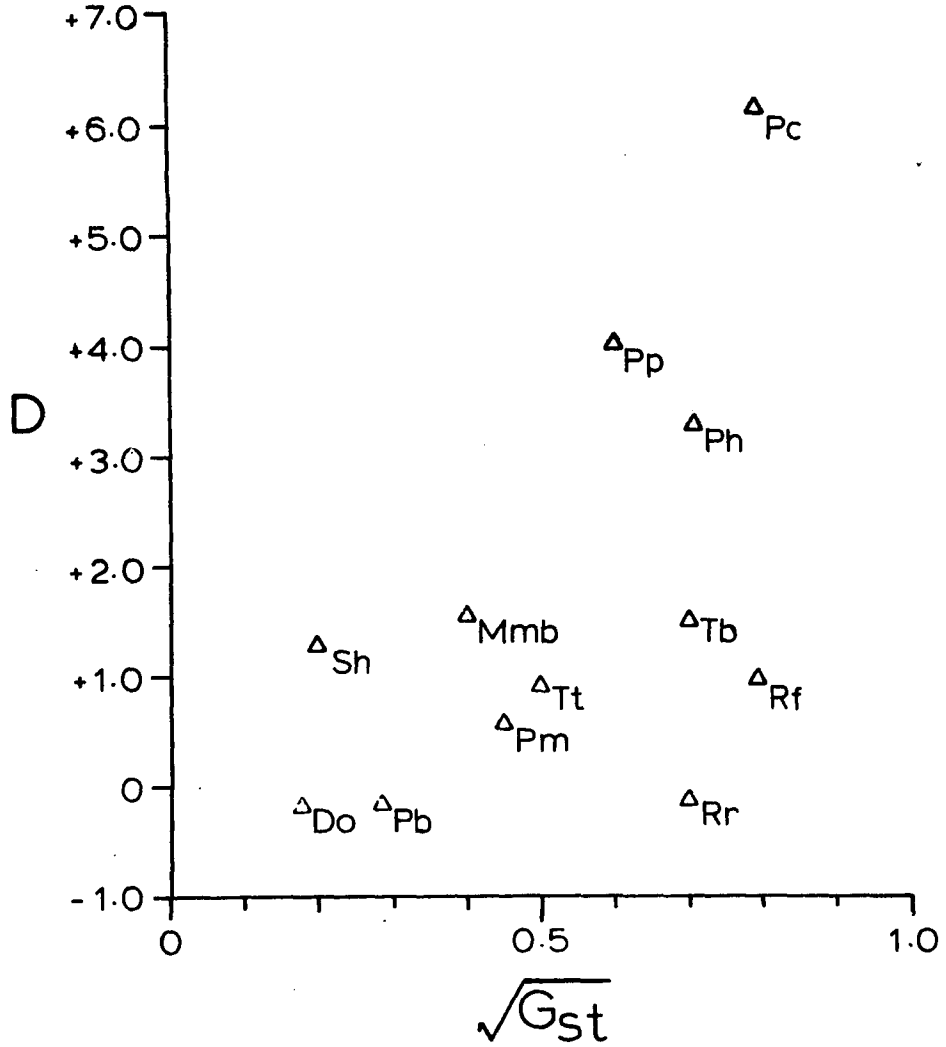

Figure 8. Association of rare alleles vs. the relative degree of population differentiation for 12 rodent species. -- The overall measure of association, D, vs. the relative degree of differentiation of populations, G_{st}, for 12 species of rodents. A nonparametric statistical test reveals a positive relationship between the magnitude of G_{st} and the magnitude of D for pairs of species within a genus.

To test statistically for a relationship between population differentiation and association among rare alleles, I compared values for pairs of species within each genus using a nonparametric sign test (Siegel 1956). The results indicate no significant relationship between D and $\overline{D}_m$. However, for the relative amount of population differentiation, G_{st}, there is a significant trend toward greater positive association among rare alleles (for pairs of species within each genus) with greater degrees of population structure. This suggests that aspects of the breeding structure that differ between species within a genus, aspects which affect genetic divergence of populations, also lead to greater positive association among rare alleles at different loci. This result is consistent with the hypothesis that nonselective forces such as genetic drift are the primary cause of population differentiation within a species.

Summary of Results

I examined polymorphic loci in 17 species of small mammals and within each compared common allele frequencies over numerous populations. Twelve species showed significant population differentiation. A comparison of common allele frequencies at each locus indicated most loci showed a similar amount of variation among populations within a species. Using this similar loci, I calculated measures of the between population component of genetic diversity. I found that the relative amount of genetic diversity is positively correlated with the degree of positive association among rare alleles for pairs of congeneric species. These results suggest that

nonselective evolutionary processes may play an active role in the genetic differentation of rodent populations.

Discussion

Comparisons of gene frequency distributions for many loci have been the basis for inferring the role of selection in biochemical polymorphisms (Selander and Johnson 1973; Lewontin and Krakauer 1973; Chakraborty et al. 1978, 1980). Lewontin and Krakauer (1973) developed a test of selective neutrality for spatial distributions of gene frequencies. The hypothesis tested is that neutral alleles at various loci will be uniformly affected by the breeding structure of the species and should have homogeneous measures of differentiation among populations.

To avoid some of the problems of the Lewontin-Krakauer test for spatial gene frequency data, I devised a method that allows for each locus to be tested against all other loci. With this method, I divided the loci into three groups. Group 1 loci exhibit similar patterns of genetic variation among populations in the frequency of their common alleles. Most loci fell into this group. I used these loci to measure the population structure of the species because they showed similar patterns of differentiation.

Although I focused on Group 1 loci, loci in Groups 2 and 3 reveal some interesting patterns. Group 2 loci exhibited greater degrees of variation among populations than Group 1 loci. Group 3 loci showed less variation than most loci in the common alleles frequencies among the populations. Loci in these two groups included

about 24% of all loci studied in 12 species of rodents. Because I used the 5% tails of the standard normal distribution to define the group classes, I expect 10% of all loci to fall in Groups 2 and 3 with sampling variation alone (i.e., with no real differences among the loci). This means that at least 14% of the loci studied show patterns of genetic variation that differ conspicuously from the patterns exhibited by the majority of loci examined. Natural selection may be operating at these particular loci and accounting for the difference in their gene frequencies distributions.

One example suggests the role of natural selection in determining gene frequency distributions is the north-south clines in gene frequency reported by Smith (1979) for Peromyscus californicus (see Smith's Figure 2). Smith found four loci that showed an abrupt shift in gene frequency in a narrow contact zone between two geographic races. Three of these loci were classified as Group 2 loci by the analysis of residuals. Since these loci differ from the majority of loci in the degree of differentiation and also exhibit similar dramatic changes in gene frequency in a contact zone between geographic races, it appears likely that natural selection is affecting (either directly or indirectly) the gene frequency distributions at these particular loci. If the alleles at Group 2 and Group 3 loci are subject to the action of natural selection, or are reflecting some historical incident, such as an extreme bottleneck in numbers or the splitting of populations, it may be revealing to perform the Lewontin-Krakauer test without these loci. In this case using only

Group I loci, Lewontin and Krakauer's test should yield a result consistent with neutrality.

Lewontin and Krakauer's test compares the observed variance in the effective inbreeding coefficient, F_e, to the expected theoretical variance. To calculate the theoretical variance Var (F_e), Lewontin and Krakauer (1973) used the following formula:

$$Var(F_e) = \frac{k(E^2(Fe))}{(N-1)} \qquad (3.21)$$

where $E^2(F_e)$ = the squared F_e over loci

N = number of populations considered

k = a parameter to be determined

For a variety of gene frequency distributions Lewontin and Krakauer found a limiting value of k = 2 using extensive computer simulations. The use of k = 2 in the calculation of the theoretical variance of F_e was suggested as the appropriate k value for a test of selective-neutrality. However, other investigators criticized this conclusion and found k values larger than 2 for neutral alleles in cases of extremely small effective population sizes (Nei et al. 1977) and also in cases when the populations are historically related in certain ways (Robertson 1975b).

To examine the magnitude of the k parameter in the Lewontin-Krakauer test, I calculated k for Group I loci (i.e., those loci with similar degrees of variation). I expected k to be approximately 2 if the conditions for the Lewontin-Krakauer tests are met.

I calculated the k parameter from the sample mean and variance of F_e over Group I loci by rearranging Equation 3.21) as follows:

$$k = \frac{(N - 1)s_F^2}{(\bar{F}_e)^2} \qquad (3.22)$$

The results are presented in Table 17. The k values range from 0.346 for the T. bottae to 3667 for S. hispidus. Most of the k values are greater than 20. These results indicate that using a value of k = 2 will greatly underestimate the theoretical variance in F_e.

The source of the greater variance may be the historical relationships among populations. Lewontin and Krakauer assume in the test that a large population simultaneously splits into isolated populations that undergo genetic divergence (Figure 9a). Robertson (1975b) points out that if the populations diverge at different times (Figure 9b), the variance of F_e values will be inflated. It appears likely that small mammal species follow Robertson's scheme and go through a series of branchings of populations. This type of hierarchical subdivision could result from populations separating and rejoining in historical times (e.g., T. bottae, Patton and Yang 1977 and P. californicus, Smith 1979) or from species colonizing new localities (e.g., S. hispidus, McClenaghan 1977).

Table 17. Estimates of Lewontin and Krakauer's k parameter. -- For each species, the estimate of the effective inbreeding coefficient, F_e, its variance, s^2_F and the k parameter is given. Lewontin and Krakauer's k parameter is calculated from equation 3.22 and has an expected value of 2 for neutral alleles under certain conditions (see text).

Species	N	L	$\overline{F}_e$	s^2_F	k
Dipodomys heermanni	5	7	0.502	0.485	0.609
Dipodomys ordii	9	8	0.029	0.016	2.097
Peromyscus boylii	7	8	0.070	0.066	6.211
Peromyscus californicus	13	13	0.153	0.090	4.153
Peromyscus maniculatus	18	4	0.174	0.132	1.725
Peromyscus polionotes	25	12	0.374	0.276	46.423
Sigmodon hispidus	8	12	0.037	0.030	3,677.847
Thomomys bottae	23	8	0.563	0.125	0.346
Thomomys talpoides	10	19	0.308	0.281	15.106
Rattus fuscipes	16	4	0.757	0.350	0.642
Rattus rattus	11	6	0.380	0.156	0.839
Mus musculus B	12	5	0.131	0.033	117.212

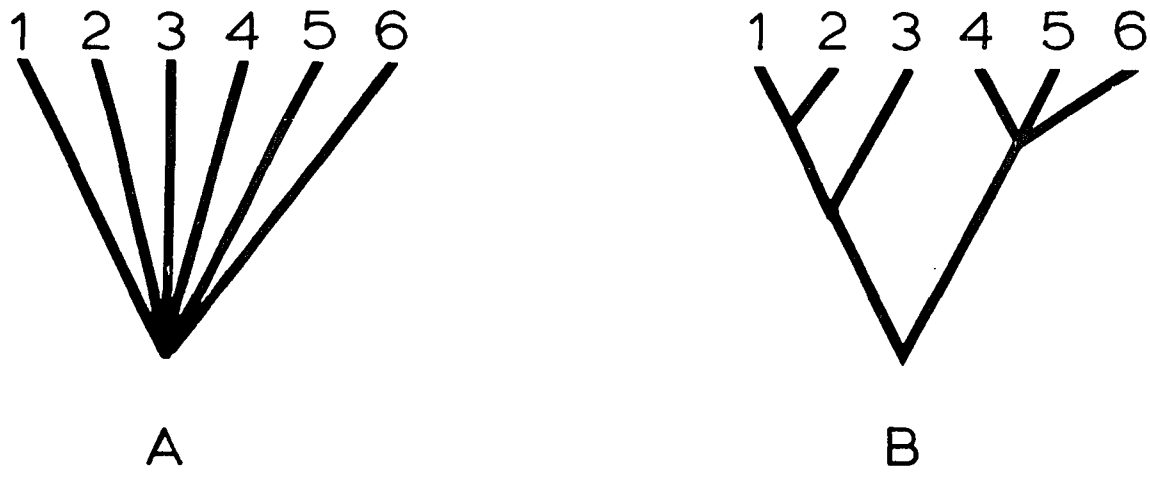

Figure 9. Two hypothetical schemes for the relationship of populations within a species. -- (A) The simultaneous splitting of populations assumed for the Lewontin-Krakauer test. (B) A hierarchical subdivided set of populations that can inflate the variance in the effective inbreeding coefficient (adapted from Robertson 1975).

Associations among Rare Alleles

I argued in the previous sections that rare alleles would be lost in populations during periods of extreme genetic drift (e.g., bottlenecks in density). Two results for rare alleles support this claim. First, rare alleles were found to be positively associated as measured by the D statistic of interdependence. This positive association would result if rare alleles were absent together in the same populations and present together in others. Second, the degree of positive association is positively correlated with the relative magnitude of population differentiation as measured by G_{st} for pairs of species within genus. Thus the more structured species exhibited greater co-occurrence of rare alleles within populations.

The positive association among rare alleles represent the loss of genetic variability in certain populations. Soulé (1973) discussed the loss of genetic variability in marginal populations. He defines a marginal population in terms of population dynamics: "a marginal population is one characterized by relatively great fluctuations in numbers and a relatively high probability of extinction" (Soulé 1973). This ecological definition of a marginal population contrasts with the geographic definition of a peripheral populations. Ecologically marginal populations need not be geographically peripheral and likewise peripheral populations are not always ecologically marginal.

The loss of genetic variability in marginal populations of some mammals has been reported in Peromyscus polionotus (Selander

et al. 1971) and in Dipodomys merriami (Johnson and Selander 1971). I used both these data sets in my analysis. P. polionotus showed a high degree of positive association among rare alleles (Figure 3.5). However, I found no statistical difference among common alleles for D. merriami and therefore did not include it in the analysis of rare alleles.

Soulé (1973) listed several factors which may be responsible for the reduced genetic (and morphological) variability observed in marginal population. The factors include nonselective processes like inbreeding, reduced gene flow, and small effective population size. The other factors invoke selection. The nichewidth-variation hypothesis predicts a correlation between ecological amplitude and genetic variation. It predicts less genetic variability because a narrower range of resources and habitats is typically available in marginal environments (Soulé 1973). The second hypothes is that directional selection is operating in marginal populations in contrast to the stabilizing selection found in central, optimal environments. This directional selection will tend to reduce rather than maintain genetic variation in marginal populations.

Although these hypotheses make the same predictions about genetic variation in marginal populations, my results support the role of nonselective factors. The analysis of common alleles indicates most loci have the same degree of differentiation among populations, an unexpected result if natural selection were operating on a subset of the loci. The analysis of genetic diversity using only similar loci revealed the greatest differentiation in isolated populations.

Finally, the degree of positive association among rare alleles is correlated with the relative degree of population differentiation among species. Since the measure of differentiation included only Group 1 alleles (suspected to be neutral), it reflects the nonselective aspects of the breeding structure of the species. The nonselective factors, determined in part by the breeding structure of the species, appear to play a substantial role in the determining patterns of genetic variation in rodents.

CHAPTER 4

CONCLUSIONS

The study of protein polymorphism in natural populations has stimulated much controversy over the effects of different evolutionary mechanisms on the observed patterns of genetic variation (e.g., Lewontin 1974, Ayala 1976). One viewpoint is that a majority of the mutations of genes are selectively equivalent (Kimura 1968, Nei 1975), and that the observed variation in gene frequencies at polymorphic loci are primarily a result of nonselective evolutionary forces. The opposing view is that mutations have sufficient effects on individual fitness that variations in gene frequencies result from natural selection and represent adaptations at the biochemical level.

During the last decade the applications of electrophoretic techniques to rodent populations have revealed variation in allozyme frequencies in both time and space within a species. Temporal variations in allozyme frequencies have been reported for several vole species in the genus Microtus (Tamarin and Krebs 1969; Gaines and Krebs 1971; Kohn and Tamarin 1978; Gaines et al. 1978). Spatial variation in allozyme frequencies among populations of a species has been reported for numerous mammal species including pocket gophers (Nevo et al. 1974; Patton and Yang 1977), kangaroo rats (Johnson and Selander 1971), deermice (Avise et al. 1974; Selander et al. 1971), cotton rats (Johnson et al. 1972; McClenaghan 1977), mole

rats (Nevo and Shaw 1972), and bats (Greenbaum and Baker 1976). In light of the selective-neutrality controversy, investigators have tried to assess the roles of selective and nonselective evolutionary forces in determining the observed patterns of genetic variation.

In the preceeding two chapters, I compared gene frequency distributions among various loci to assess the roles selective and nonselective forces in patterns of allozyme variation. The rationale behind the comparison is that in the absence of selective differences among alleles, alleles at all loci will reflect the breeding structure of the species (i.e., local effective population sizes, migration rates, and inbreeding). These selectively neutral alleles should have similar patterns of variation in time and space. In contrast, natural selection operates on alleles differently, therefore selected alleles will show much different patterns of variation in time and space, due to both the breeding structure of the species and the variation in the coefficients of selection.

In Chapter 2, I used two versions of the Lewontin-Krakauer test to assess the roles of selective and nonselective forces in temporal gene frequency changes observed in Microtus populations. Gaines et al. (1978) reported fluctuations in allele frequencies at five polymorphic loci in local populations of Microtus ochrogaster. They found significant differences in genotypic survivorship and fecundity measures for three loci, and hypothesized an active role of natural selection in gene frequency change. I tested this hypothesis by comparing gene frequency distributions among loci using estimates of the effective inbreeding coefficient. I found

homogeneity among loci in both comparisons of inbreeding coefficients calculated both ways: over all time periods and between time periods. The conclusion from the homogeneity tests among loci was that non-selective forces like genetic drift and migration were the primary causes of gene frequency change within populations.

In Chapter 3, I compared the spatial gene frequency distributions among populations within 17 species of rodents to see what evolutionary factors account for the genetic differentiation of populations. The hypothesis was that selectively neutral alleles should exhibit similar patterns of spatial variation because non-selective forces will act uniformly over loci with neutral alleles. The first step was a comparison of common allele frequencies among populations in which a test of independence indicated significant genetic differences among populations for 12 of the 17 species. A comparison of the degree of differentiation among loci revealed that for most loci (about 76%) the common alleles showed similar degrees of variation among populations.

The second step in the analysis focused on rare alleles. I tested the hypothesis that populations undergoing substantial amounts of genetic drift (e.g., those populations susceptible to fluctuations in density) will lose rare alleles at many loci. This loss of rare alleles in some populations will result in a positive association of rare alleles in other populations. I found a positive association among rare alleles for nine species of rodents. Because rare alleles tend to co-occur in the same populations, it is likely that genetic drift has played a substantial role in their genetic differentiation.

I also compared the measure of association among rare alleles with the degree of genetic subdivision of the species. The hypothesis that nonselective factors promoting population differentiation within a species should also promote the co-occurrence of rare alleles was supported by the results. I found a positive relationship between population differentiation within a species and the magnitude of the positive association among rare alleles.

The results for both the temporal and spatial analysis of allozymic variation in rodent populations suggests an active role of nonselective evolutionary forces. In the following section, I discuss why rodent populations may be so susceptible to genetic drift.

Factors Promoting Random Genetic Drift

Wright (1948) defined random genetic drift as the fluctuation in gene frequency caused by those evolutionary mechanisms that have no directed effect on gene frequency (i.e., factors that do not cause a mean change in gene frequency). Theoretically the major mechanisms causing genetic drift are (1) sampling variation at gametogenesis due to finite population size, (2) variation in the rate of mutation, (3) variation in the amount of migration, and (4) fluctuations in the magnitudes of genotypic selective values. These random processes cause no mean change in gene frequency but they do cause gene frequency to vary from generation to generation.

Another theoretically possible source of random fluctuation in gene frequency not discussed by Wright concerns age-structured populations. Charlesworth and Giesel (1972) and Charlesworth (1980)

emphasized that in population divided into different age classes, genetic equilibrium is generally possible only if the populations is at constant size or growing at a constant rate, and has a stable age structure. In populations subject to fluctuation in growth rate and age structure, gene frequencies may vary randomly even at loci undergoing selection, Thus, observed changes in gene frequency in age-structured population "may be due to a purely mechanical shift in genotypic fitness as a result of changing demography, and not necessarily caused by changes in the selection regime at the level of age-specific survival probabilities and fecundities" (Charlesworth 1980, p. 145.)

The demographic characteristics of rodents may actually promote genetic drift in various ways. First, populations of rodents vary in density within and between seasons (Table 18). These fluctuations in density may cause random changes in gene frequencies as hypothesized by Charlesworth because of demographic instability of the populations. Furthermore, the effective size of a population that fluctuates in density is calculated as a harmonic mean, that is the average effective population size is weighted towards the smaller values (Crow and Kimura 1970). The effect of the harmonic mean is that the actual average effective population size may be much smaller than the average census size. When the effective population size is small, there is greater variation in gene frequency from one generation to the next due to the sampling effects at reproduction.

Density variation can also affect the variation in the rates of migration and the magnitude of selection. Dispersal rates have been shown to vary with density in a variety of small mammal species

Table 18. Density variation for four groups of rodents. The mean and range in the numbers of individuals per hectare within or between seasons for four groups of rodents. The values are only rough approximations because they represent averages for various species that have been studied throughout the world. From French et al. 1975.

Group	Density	
	Seasonal	Interseasonal
Heteromyidae	8.6 (1-30)	8.5 (0-31)
Cricetinae	15.4 (0-40)	10.6 (0-50)
Fossorial[1]	31.0 (5-82)	23.9 (2-84)
Muridae	117.7 (0-623)	15.7 (2-50)

[1]Geomyidae and other fossorial rodents

(see Gaines and McClenaghan 1980 for a review). This fluctuation in the amount of dispersal may result in variation in the amount of gene flow among populations and thus cause random fluctuations in gene frequency.

Finally, the magnitude of the selection coefficients may vary with density (e.g. Charles worth 1980). If the selection coefficients vary with density, random density variation may result in random variation of the genotypic selection coefficients and produce a change in gene frequency similar to that caused by sampling drift. For example, Wright (1948) found that for a particular model a standard deviation of 0.05 in selective value was roughly equivalent to sampling error in a population of 1,000 individuals.

Thus, density variation experienced by most rodent populations may promote random genetic drift of gene frequencies. Density variation may result in small effective populations sizes, unstable age structure, and random fluctuations in the rate of migration and the direction and severity of selection. In the following section, I will discuss how the action of genetic drift in a subdivided population may actually be conducive to adaptive change.

Rate of Adaptive Evolution

Wright (1931, 1949, 1970, 1977) advocated an evolutionary process that emphasized the role of population structure in the rate of adaptive evolution. Wright (1977) constructed his "Shifting Balance Process" on three basic premises. Two premises concern the phenotypic effects of different alleles. First, the quantitative

variability of a phenotpic character is determined by an extensive number of polymorphic genetic loci. The alleles at the loci differ only slightly in their selective value. Second, there are pleiotropic effects of most allele differences; there are manifold phenotypic effects of a particular mutant allele. The third premise concerns the relationship between the organism and the environment as measured by the ultimate quantitative character, fitness. Wright pictured the combinations of different genes as a multidimensional field of genotypic fitnesses with multiple adaptive peaks and valleys.

The evolutionary problems in Wright's view (as well as many others e.g., Simpson 1944, Rosenzweig 1978) is how a species could move one adaptive peak to another higher peak. In a homogeneous population the rate of adaptive evolution is limited by the additive genetic variance in fitness. When the additive variance is exhausted, the rate of evolution depends solely on the rate of favorable mutations. Furthermore, mass selection can strand a population on an adaptive peak, because there is no mechanism to move across valleys in the adaptive topography against the action of natural selection (but see Rosenzweig 1978).

However, in a heterogeneous population i.e., a species subdivided into numerous, partially isolated, local populations, there is a continually shifting differentiation of the populations by non-selective forces like genetic drift and local selective pressures. The differentiation of populations by whatever reason leads to a store of genetic variability that is greater than in a homogeneous population of the same size. Moreover a local population has

access to the variability through immigration from surrounding populations.

The random differentiation of populations allows for a "trial and error" process in which different combinations of genes arise. If a local population arrives upon a particularly favorable genetic combination (i.e., interaction system) under the control of a higher adaptive peak, this population will produce a surplus of individuals that will disperse, and systematically shift equilibrium gene frequencies toward a higher adaptive peak. Wright called this process of differential migration from local populations "intergroup selection" (Wright 1977).

The rate of adaptive evolution i.e., the time required to move to successively higher adaptive peaks is acclerated in a subdivided species because immigration, rather than mutation, is the source of new genetic combinations, and the immigration is most likely orders of magnitude greater than the rate of mutation.

The shifting balance process is a third alternative view of evolution to the neutral and balance views (Wright 1978). It depends, like the neutral view, on there being an enormous number of nearly neutral polymorphic loci, and emphasizes an active role of genetic drift in gene frequency change. It differs from the neutral view in that genetic drift operates primarily in local populations which evolve somewhat independently. However, genetic drift is not the primary mechanism for fixation or near fixation of an allele at a single locus in the species as a whole. Drift simply allows for the

random combination of alleles at many loci so that a favorable interaction system may emerge in a single locality.

Fixation or near-fixation under the shifting balance process is due to selection, as hypothesized in the balance view. However, individual selection operates primarily in the local population to establish favorable genetic combinations (Wright 1978). These then spread through the species via intergroup selection, i.e., the asymmetrical diffusion of genes.

The rodent species I analyzed in the previous chapters conform to Wright's Shifting Balance view of evolution. Analysis of structural proteins revealed convincing evidence that rodent species are structured into numerous local populations that differentiated at the biochemical level primarily through nonselective evolutionary mechanisms like genetic drift.

Does this population structure of rodents accelerate the rate of formation of favorable genetic combinations? There is little evidence available to answer this question, but karyotypic patterns are very suggestive. Wilson et al. (1975) found an extraordinarily high rate of chromosomal evolution for placental mammals compared to other vertebrate lineages. Rodents, in particular, had the highest rate of karyotypic rearrangement (Wilson et al. 1975, Table 1). This rate of chromosomal rearrangement may reflect the high rate of formation of new genetic combinations in structured rodent populations due to the Shifting Balance process.

However, the rate of karyotypic evolution also reflects the rate of speciation and not simply the formation of genetic combinations

within a species. Interestingly enough, structured populations may be the least amenable to speciation via the founder effect (Templeton 1980).

In conclusion, my analysis of allozyme data for numerous rodent species revealed an active role of nonselective evolutionary mechanisms in the variation of gene frequencies in time and space. The results suggest that many mutations for structural proteins may differ only slightly (if at all) in their selective value. However, the population structure of rodents may actually be conducive to adaptive evolution, with the rate of formation of favorable genetic combinations accelerated by the Shifting Balance process.

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