A GENETICAL STUDY OF ISOLATED POPULATIONS OF THE AUSTRALIAN BUSH-RAT, *RATTUS FUSCIPES*

by

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APPENDIX 1 NOTES ON THE ECOLOGY AND REPRODUCTION OF *RATTUS FUSCIPES GREYII*

APPENDIX 2 PUBLISHED PAPERS

1. Genetic evidence for the existence of two separate populations of *Rattus fuscipes greyii* on Pearson Island, South Australia.

2. Genetic variation in isolated populations of the Australian bush-rat, *Rattus fuscipes*.

3. Mitochondrial iso-citrate dehydrogenase variation in the Australian bush-rat, *Rattus fuscipes greyii*.

4. An electrophoretic investigation of the binding of $3\text{-}^{14}C$ coumarin to rat serum proteins.

ABBREVIATIONS

BIBLIOGRAPHY

SUMMARY

A study has been made of electrophoretically detectable protein variation in fourteen isolated populations of the Australian bush-rat, *Rattus fuscipes greyii*. A small number of individuals from each of the other three *R. fuscipes* subspecies were examined. Family data from about 50 laboratory matings suggest that the thirteen proteins examined represent the products of genes at sixteen different loci.

The *R. f. greyii* populations inhabit several small off-shore islands (less than 300 ha), a large island (about 400,000 ha), and three separate mainland areas (each about 400,000 ha). Seven small-island populations are monomorphic for all loci and two other small-island populations are each polymorphic at one locus only. The mean heterozygosity for the small-island populations is 0.007. These populations are genetically less variable than most mammalian populations. The three mainland and the large-island populations are polymorphic at two to four genetic loci and the mean heterozygosity in these populations is 0.042.

The *R. f. greyii* populations studied are reproductively isolated from one another and the time of isolation can be estimated at between 5,000 and 14,000 years. It is presumed that prior to isolation these populations shared a common gene pool. The variation at several genetic loci throws light on the composition of this ancestral gene pool. Some genes, found only in one population, are apparently recent mutants, while others are more widespread, indicating that they were probably present in the ancestral population. For some loci it seems likely that clines in gene frequency were present in the ancestral population. These inferences are supported by evidence from protein variation in the three other *R. fuscipes* subspecies. It is suggested that in the past, *R. f. greyii* has been a link between the eastern and western Australian subspecies of *R. fuscipes*.

The gene frequency data are summarised by a modification of the method of principal component analysis for contingency tables and by other genetic distance measures. The genetic distances between *R. f. greyii* populations are on average, greater than the genetic distances reported hitherto between other mammalian populations belonging to the same subspecies and the genetic distances between *R. fuscipes* subspecies are greater than the distances between other mammalian subspecies. The genetic dissimilarity between *R. f. greyii* populations appears to have evolved extremely rapidly, by the fixation of different genes present in the ancestral population and by the incorporation of new mutants since isolation.

There is evidence that both natural selection and random genetic drift have played important roles in determining the gene frequencies in the *R. fuscipes* populations.

A study of metrical variation of eighteen skull and body characters in *R. f. greyii* populations revealed significant heterogeneity between populations for all characters. For most characters there is no significant difference between small island and large populations. The variance in each character is less in small populations than in large populations, but this difference in most cases is not statistically significant. The relationships between the populations as described by multivariate analyses of the metric data is similar to that described by the protein variation.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and that to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made.

L. H. Schmitt

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CHAPTER 1

INTRODUCTION

Investigations into genetic variation within and between natural populations are usually concerned with determining: (i) the amount, extent and nature of the variation, (ii) the forces governing these characteristics, and (iii) the evolutionary significance of the variation. Until about ten years ago, most of the genetic variation detected involved differences in blood group, karyotype or morphology. With the introduction of the technique of gel electrophoresis and the adaptation of histochemical procedures, the study of genetic variation in natural populations has been revolutionised. The technique enables the identification of genetically determined variation in the primary structure of proteins, and given certain assumptions, it allows an estimate of the total amount of genetic variation at all structural gene loci in natural populations. Furthermore, since the variation is in proteins it is possible to look for *in vitro* differences between the products of allelic genes, which might reflect *in vivo* differences of selective importance.

However, with some notable exceptions (e.g. the human sickle-cell haemoglobin polymorphism) it has remained difficult to determine either the forces maintaining genetic variation in any particular case or its evolutionary significance. The difficulty is in many cases due to inadequate knowledge of ecological and demographic statistics of the populations concerned. For example, in comparing the observed heterozygosity with that predicted by random genetic drift it is necessary to have estimates of the effective population size and the mutation rate. Furthermore, to understand the evolution of genetic differences between populations it is necessary to measure not only population size and mutation rates, but also the time of isolation and

migration rates.

Many of these problems can be overcome or avoided, at least to some extent, by careful selection of the populations to be studied. In this respect, populations inhabiting small islands may have some useful characteristics including, for example, their reproductive isolation from one another, small population size, and a restricted well-defined habitat. In addition, the period of isolation can sometimes be estimated and small islands often occur in clusters, providing natural replicates of evolutionary "experiments". These features may make the study of small island populations less complex and more informative than those of larger and less well-defined mainland populations, and often valuable comparisons can be made between island and mainland populations.

This thesis describes an investigation into genetic variation in a series of isolated populations of an Australian mammal, the southern bush-rat (*Rattus fuscipes*). This species was chosen because: i) it occurs as a series of island and mainland populations with features similar to those listed above, ii) it is easy to collect samples of reasonable size, and iii) animals breed and are easy to maintain in the laboratory.

There are four recognised subspecies of the southern bush-rat, and each inhabits a separate coastal region of Australia (see Fig. 3.2). This study is primarily concerned with the South Australian subspecies, *R. fuscipes greyii*, which inhabits three geographically isolated areas on the mainland and at least thirteen off-shore islands. The three mainland populations and one island population occupy areas of similar size, about 400,000 ha. The other populations investigated inhabit islands which range in area from about 50 to 300 ha. This contrast in the extent of the areas occupied by different populations is probably accompanied by a corresponding difference in the numbers of individuals in the populations, although no studies of population size have been made.

During the last ice-age in the late Pleistocene, all of the islands in question were part of the mainland. As the ice retreated, there was an eustatic rise in the sea level and the islands were formed. The time of isolation of each population can be estimated from data on the mean sea level during the last 20,000 years and the present topography of the sea bed.

It is postulated that the *R. f. greyii* island populations are relics from the time when the islands were connected to the mainland rather than being the result of subsequent colonisation over-water. *R. f. greyii* was probably distributed across most of central southern Australia during the last ice-age, when at times the climate was wetter than at present, and it is reasonable to assume that suitable habitat for the species was more widespread. The existence of populations of *R. f. greyii* on Dog Island and Goat Island, with no contemporary population on the adjacent mainland, also suggests a previously wider distribution. While it is recognised that this distribution may not have been continuous it is expedient to consider that the founders of the present day populations came from an "ancestral population". The present day discontinuity in the distribution of *R. f. greyii* on the mainland presumably has resulted from recent climatic changes (see, for example, Twidale, 1969) and human habitation which led to the destruction of suitable bushland habitat. Early European settlers brought competitors and predators of *R. f. greyii* with them, and consequently *R. f. greyii* has been forced to live in the more densely vegetated habitats which afford the best protection.

It is unlikely that there has been much, if any, migration between these island populations, since their isolation. Small mammals, except those commensal with Man, are poor dispersers over water barriers. It seems likely then that a widespread ancestral population of *R. f. greyii* was fragmented by environmental changes into a series of populations, some

occupying small areas and others more widespread, and between which no recent migration has occurred.

This study is concerned with determining: (i) the amounts of genetic variation in island and mainland populations, (ii) the patterns of geographic variation in gene frequencies and the genetic distance between populations, and (iii) the evolution of these differences, including the importance of natural selection and random genetic drift in determining (i) and (ii). Four widespread and ten small island populations of *R. f. greyii* have been studied, along with some individuals from the other three subspecies (*R. f. fuscipes*, *R. f. assimilis* and *R. f. coracius*). Starch gel electrophoresis and histochemical staining were used to investigate genetic variation of thirteen proteins which are probably controlled by sixteen different genetic loci. In addition a small study was made of metrical variation in skull and body characters.

CHAPTER 2

GENETIC VARIATION IN NATURAL POPULATIONS

The following discussion is primarily concerned with genetic variation determining electrophoretically distinct proteins.

2.1 GEL ELECTROPHORESIS AND THE FREQUENCY OF POLYMORPHISMS

The first extensive surveys of electrophoretically detectable variation were in Man (Harris, 1966) and *Drosophila pseudoobscura* (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966) and revealed an unexpectedly large number of protein variants. In Man, three out of ten proteins were found to be polymorphic and seven out of eighteen were polymorphic in *Drosophila pseudoobscura*. Since these first reports, data on protein variation in many species have been collected and have generally supported the initial findings in terms of the proportion of polymorphic proteins (e.g. Harris and Hopkinson, 1972; Selander and Johnson, 1973; Lewontin, 1974; Powell, 1975). If, for convenience, a polymorphic locus is defined "as one at which the most commonly occurring allele in the particular population has a frequency which is less than 0.99" (Harris, 1975), then on average, populations seem to be polymorphic for about 30% of their loci. The amount of genetic variation in a population can also be measured by the average proportion of heterozygous loci *per* individual. Unlike the proportion of polymorphic loci, this measure does not require the setting of arbitrary limits for its definition and is less dependent on the sample size than is a measure of the proportion of polymorphic loci. The average heterozygosity *per* locus *per* individual in most natural populations which have been studied is about 10%.

2.1.1 Limitations of the technique

Genetic variation detected by electrophoresis most probably underestimates the total variation in genes coding for proteins. Because of the redundancy of the genetic code, allelic differences in the DNA may not be manifested in the encoded proteins. Furthermore, proteins which differ by one amino-acid substitution will, in general, have distinct electrophoretic mobilities only if the two amino-acids concerned differ in their net electrostatic charge.

From a consideration of the genetic code and the frequency with which the different amino-acids are found in proteins, it has been calculated that gel electrophoresis is capable of distinguishing about 30% of all proteins differing by one amino-acid substitution (Lewontin, 1974; King and Wilson, 1975). However, Markert (1968) suggested that substitutions involving amino-acids of different net charge may not be as well tolerated as those involving amino-acids with the same charge, because the charge properties of proteins may be important for their localisation within the cell. The observed frequency of substitutions involving amino-acids of different electrostatic charge appears to be less than that expected on the basis of random nucleotide substitutions. For example, in an analysis of amino-acid sequences of primate haemoglobins, Boyer *et al.* (1972) found that only about 15% of all variants were the result of a substitution of one amino-acid for a second which differed in net charge, whereas 30% are expected to be of this type.

The existence of more protein variation than that detected by electrophoresis is indicated by such amino-acid sequence studies and also by *in vitro* heat denaturation studies. Studies of the latter type have found that enzymes identical with respect to electrophoretic mobility, differ in their resistance to heat denaturation (Bernstein *et al.*, 1973). Some of these differences in heat stability have been shown to be under

the control of allelic genes (Thorig *et al.*, 1975; Singh *et al.*, 1974). Recently, it has been shown that by using multiple electrophoretic conditions (two different pH's and two gel concentrations) much more variation could be detected than when one condition was used (Coyne, 1976; Singh *et al.*, 1976).

Another important limitation of the electrophoretic method, in estimating the overall level of genetic variation, is that it is not known how typical of the genome (in the amount of variation) are the structural genes detected by electrophoresis. Until methods are developed which can detect many more genes or gene products which are of a different nature to those detected by electrophoresis (e.g. regulatory genes), this limitation will remain.

2.1.2 Levels of variability in different taxa

While the data collected so far have generally supported the initial findings of a large number of protein polymorphisms in natural populations, some substantial differences in the amount of variation have been found between species. The diversity in mean heterozygosity between species is illustrated in Fig. 2.1. Among animal species, invertebrates have about three times the average heterozygosity of vertebrates (15% and 5% respectively - Selander and Kaufman, 1975; Powell, 1975). However, there is also considerable variation among species within these broad groupings. For example, vertebrate subspecies have heterozygosities ranging from 0% to 18% (Powell, 1975). This is illustrated in Fig. 2.2, which also displays the range of heterozygosities found within mammalian subspecies. These histograms should be treated with some caution, since some species are represented by many subspecies, while others are represented by only one population, and of course most species are not represented at all. It should also be noted that the standard errors associated with these estimates are unknown and therefore strict

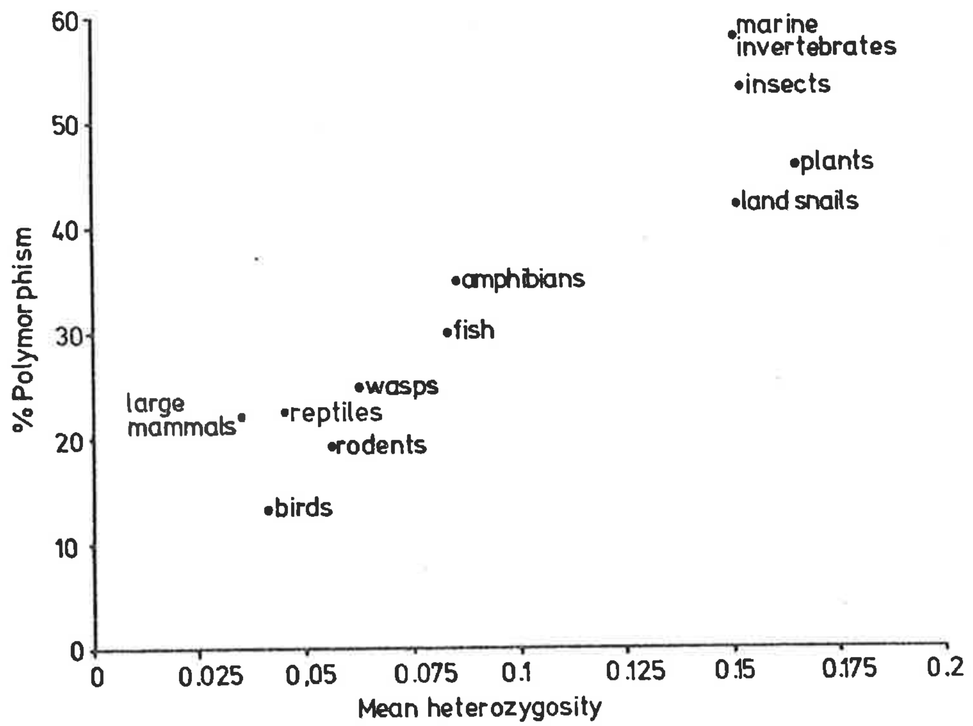
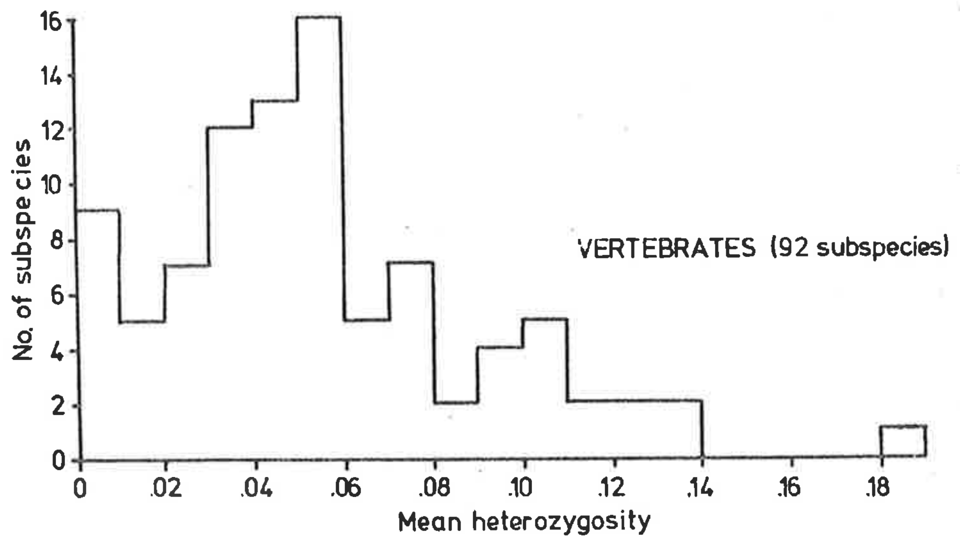


Figure 2.1 Mean heterozygosity and percent polymorphism in various groups of animals and plants (from Selander, 1976).



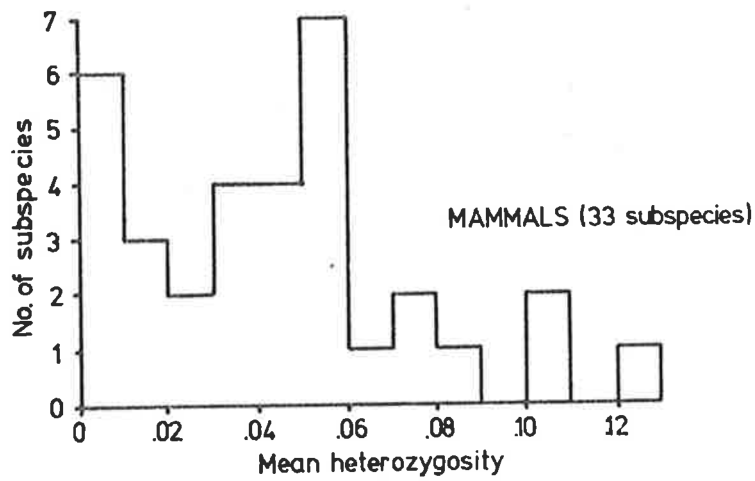


Figure 2.2 Frequency distribution of mean heterozygosity in subspecies of vertebrates (top) and mammals (bottom). Data from Powell (1975).

statistical comparisons are not possible.

The apparent difference in the level of heterozygosity between vertebrates and invertebrates cannot be explained in terms of differences in population size or dispersal abilities, since there are no consistent differences in these qualities between the groups (Selander and Kaufman, 1975). There are theories which could account for the difference (e.g. Levins, 1968; Gillespie, 1974; Gillespie and Langley, 1974; Powell, 1975), these theories being based on a causal relationship between genetic and environmental variability. However, the evidence supporting these theories is tenuous (see Section 2.3.5).

2.1.3 Levels of variability in different proteins

Surveys of enzyme variation in natural populations have indicated that in general, genes coding for enzymes involved in energy production (glucose-metabolising or Group I enzymes) are less often polymorphic than those coding for other enzymes (nonspecific or Group II enzymes) (Gillespie and Kojima, 1968; Kojima *et al.*, 1970; Cohen *et al.*, 1973). These results must be treated with some caution since it is difficult to determine to which group many enzymes belong and the Group II data are based on only a few enzymes. Genes coding for non-enzymatic proteins detectable by electrophoresis (Group III), appear to be less often polymorphic than Group II enzymes (Selander, 1976), although Selander and Johnson (1973) found no differences between Group I, Group II and Group III proteins, in their level of variability.

In seeking an explanation for the apparent difference in the level of variability of Group I and Group II enzymes, Gillespie and Langley (1974) redefined Group I enzymes as those characterised by a single physiological substrate which is usually generated and utilised intracellularly, while Group II enzymes were defined as those with multiple substrates. While these new definitions result in a few enzymes being

reclassified from Group II to Group I, the difference in variability between the two groups is still evident (Gillespie and Langley, 1974; Zouros, 1975). Gillespie and Langley (1974) showed theoretically, that "polymorphisms will be more likely to occur in more variable environments" and suggested that the Group II enzymes experience greater environmental diversity because of their substrate variability, than do Group I enzymes. Hence Group II enzymes have a higher level of polymorphisms than Group I enzymes.

It has been suggested that rate limiting enzymes in metabolic pathways should be more often polymorphic than non-regulatory enzymes (G.B. Johnson, 1971, 1974). However, both the theoretical grounds on which this suggestion has been made and the agreement of the data with the expectation have been criticised (Ayala and Powell, 1972; Selander, 1976).

2.2 GEOGRAPHIC VARIATION IN GENE FREQUENCIES

Investigations into protein variability in very large widespread populations, or groups of populations between which there is some migration, usually reveal two common patterns of variation. Some proteins show no variation within or between populations and all individuals, with rare exceptions, have the same phenotype. The other pattern of variation involves proteins which are polymorphic and the genes controlling this variation have similar frequencies in all populations. Protein variation in *Drosophila willistoni* and Man provide good illustrations of this latter type of variation (see Ayala, 1972 and Mourant *et al.*, 1976 respectively, for reviews).

Apart from these two more common situations, genetic variation takes other forms. Koehn and Rasmussen (1967) showed that the *esterase-1* gene frequencies in *Catostomus clarkii* are linearly correlated with latitude. One allele, $Es\text{-}1^{a}$, shows a steady increase in frequency from 0.18 to 1.00 over a distance of about 525 miles. Examples of such clinal variation

in gene frequencies have been found for other proteins in other species (e.g. Merritt, 1972; Pipkin *et al.*, 1973; Boyer, 1974; Christiansen and Frydenberg, 1974; Guttman, 1975; Johnson, 1976).

Occasionally a gene will occur in an appreciable frequency in one part of a population, but will be absent or rare in the rest of the population. The human haemoglobin C variant in Africa is an example of this (Lehmann and Huntsman, 1974).

There are some examples where the frequencies of two or more genes fluctuate considerably in the range of a species (e.g. Dessauer and Nevo, 1969; F.M. Johnson, 1971; Selander *et al.*, 1971). However, cases of geographic variation in gene frequencies are usually associated with geographic isolation of populations.

Other examples of variation in gene frequencies include cyclical changes associated with changes in population size (Tamarin and Krebs, 1969; Berry and Murphy, 1970; Gaines and Krebs, 1971) and age dependent gene frequencies (Fujino and Kang, 1968; Tinkle and Selander, 1973; Berry and Peters, 1975).

2.3 FORCES RESPONSIBLE FOR GENETIC POLYMORPHISMS

Theoretically, there are several mechanisms by which a genetic polymorphism can be maintained. Some of these mechanisms, together with examples will be presented. However, it should be made clear, that in practice it is usually extremely difficult to determine what mechanism is maintaining a particular polymorphism, if indeed it is being "maintained" by deterministic forces.

2.3.1 Selective replacement of a gene

If, in a population, a rare gene becomes selectively advantageous, then this gene will tend to increase in frequency and replace its allele. During the process of replacement a polymorphism will exist. Pigment

variation within several species of British moths provided good examples of this type of polymorphism (for a review see Kettlewell, 1973). Such polymorphisms are said to be transient, since gene frequencies continually change and ultimately the polymorphisms are lost. It has been suggested by Ford (1975) that these polymorphisms only represent a small proportion of all polymorphisms observed.

2.3.2 Genetic drift

If, in a finite population, a new mutant gene is selectively equivalent to the common allele in the population, then the mutant's frequency may increase due to genetic drift and a polymorphism may result. Alleles can be considered to be selectively equivalent if the difference between their selective values is of a lower order of magnitude than the mutation rates or the reciprocal of the effective population size (Crow and Kimura, 1970). The small range of selective values over which alleles can be considered to be selectively equivalent led Fisher (1930) to propose that such a situation is uncommon because environmental fluctuations would only allow it to occur for short periods. The importance of this mechanism in "maintaining" naturally occurring polymorphisms is controversial and is discussed further in Section 2.3.6.

Polymorphisms where the gene frequencies are determined by genetic drift are also said to be transient because the gene frequencies will change continually and ultimately one of the genes will be lost.

2.3.3 Heterozygote advantage

Fisher (1930) developed the concept of differential selective forces maintaining genetic polymorphisms by the superiority in fitness of the heterozygote over the corresponding homozygotes. This mechanism, more than any other, has been invoked to explain how the genetic polymorphisms observed in natural populations are maintained. Allison (1955, 1964)

showed that the human sickle-cell polymorphism in East Africa is maintained because homozygotes are either highly susceptible to malarial infection or severely anaemic, while heterozygotes do not suffer (at least to the same extent) from either of these disadvantages. There are very few other polymorphisms, where the selective forces have been so thoroughly elucidated.

If heterozygote advantage acts through the differential survival of individuals with particular genotypes, then there will be in adults an excess of heterozygotes over the proportion expected by the Hardy-Weinberg principle. However, unless the selection difference is large, an extremely large sample is needed to reveal a significant difference. Also, most populations of small mammals will include individuals of very different ages which are indistinguishable, and this will add to the difficulties of detecting any heterozygote excess associated with increased age.

This latter disadvantage can be overcome in parthenogenetic species, since a heterozygote excess will be amplified through repeated generations of mitotic reproduction. A study of *Daphnia magna* (Hebert *et al.*, 1972; Hebert, 1974a, 1974b, 1974c) a parthenogenetic species, revealed several examples of heterozygote excess. Furthermore, as expected, this excess increased as populations went through a series of asexual generations following sexual reproduction.

Fincham (1972) proposed two ways in which heterozygosity may confer a selective advantage at the molecular level. Either the quantity of enzyme in heterozygotes may be closer to the optimum than in homozygotes, or heterozygotes may have an enzyme which is qualitatively distinct and which is selectively advantageous. This latter mechanism could work in at least two ways. The heterozygotes may have a "hybrid enzyme" which has biochemical properties outside the range of the enzymes produced by the homozygotes. There are many examples of heterozygotes with hybrid

enzymes and in several of these cases it has been shown that *in vitro*, the hybrid enzymes have properties outside the range of the enzymes produced by homozygotes (e.g. Vigue and Johnson, 1973; Berger, 1974; Singh *et al.*, 1974). Alternatively, a number of distinctive gene products may be found in heterozygotes, which confer greater biochemical diversity to cope with environmental variation.

Zouros (1976) showed that monomeric proteins are more often polymorphic in natural populations than polymeric proteins. He interpreted this to mean that hybrid proteins in heterozygotes were, *per se*, not responsible for much of the variation in natural populations. However, it does not appear to be necessary for proteins to be polymeric in order that they can exhibit distinctive properties in heterozygotes. Heterozygotes for monomeric proteins have been shown to have proteins with properties outside the range of either homozygote (e.g. Koehn, 1969; Frelinger, 1972).

2.3.4 Frequency dependent selection

A balanced polymorphism may be maintained because the fitness of genotypes is a function of their frequencies. This mechanism does not rely upon the selective superiority of heterozygotes and hence overcomes the problem of molecular overdominance. There is evidence that the esterase-6 polymorphism in *Drosophila melanogaster* is maintained by frequency dependent selection (Kojima and Yarbrough, 1967; Huang *et al.*, 1971). Negative assortative mating with respect to colour morphs in *Panaxia dominula* (Sheppard and Cook, 1962) and with respect to karyotypes in *Drosophila pseudoobscura* (Spiess, 1968) are also examples of frequency dependent selection.

2.3.5 Heterogeneous environments

Spatially or temporally varying environments can theoretically, maintain genetic polymorphisms. The environmental variation may be regular (cyclical) or irregular.

Levene (1953) showed that it is possible to maintain a genetic polymorphism if one homozygote is favoured in one niche and the other homozygote is favoured in another. Two well known examples of spatial environmental heterogeneity maintaining polymorphisms are shell colour and banding pattern in *Cepaea nemoralis* and industrial melanism in Lepidoptera (see Clarke, 1968 and Kettlewell, 1973 respectively, for reviews).

If selection favours different homozygotes in different generations (because of environmental fluctuations), then a stable polymorphism may result (Haldane and Jayaker, 1963).

The concept of heterogeneous environments (in space and time) maintaining genetic polymorphisms has been developed further by Levins and MacArthur (1966), Levins (1968), Prout (1968), Gillespie and Langley (1974) and others. If some polymorphisms are maintained as a result of heterogeneous environments, then environmental heterogeneity and genetic heterozygosity will be correlated. Observational and experimental evidence on this point is equivocal. Powell (1971) found that average heterozygosity in cage populations of *Drosophila willistoni*, maintained under variable conditions, was greater than that in populations held in relatively constant environments. However, King (1972) suggested that it may be chromosomal inversion polymorphisms which were the target of selection, not single genetic loci. McDonald and Ayala (1974) made a similar finding to Powell, but in *Drosophila pseudoobscura* which does not have such extensive chromosomal inversions.

Nevo and Shaw (1972) and Nevo *et al.* (1974) examined genetic heterogeneity in natural populations thought to experience unusually

low levels of environmental variability and found genetic heterozygosity to be unusually low. Levinton (1973) found a positive correlation between the effective number of alleles at each of two loci and environmental variability for six marine molluscs. Dessauer *et al.* (1975) found high genetic variability in *Bufo viridis*, a species thought to experience an ecologically variable environment. However, studies of deep-sea invertebrate species, thought to live in extremely constant environments, reveal that such species have levels of genetic heterozygosity similar to those in related terrestrial species (Gooch and Schopf, 1972; Ayala *et al.*, 1975). Somero and Soule (1974) found no association between the level of heterozygosity and the range in temperature experienced by several species of teleost fishes.

One of the major difficulties in studying the relationship between genetic and environmental heterogeneity, is that it is very difficult to quantify environmental heterogeneity, and to identify the specific environmental parameters which may be affecting the organism most strongly.

2.3.6 Natural selection *versus* the neutral mutation-random drift theory

There is considerable controversy over the relative proportion of polymorphisms in natural populations maintained by random drift of selectively neutral alleles (see Section 2.3.2) and the proportion maintained by natural selection (see Sections 2.3.1 and 2.3.3 to 2.3.5). It has been proposed that most allelic genes, detected by electrophoresis, in natural populations are selectively equivalent ("neutral alleles") and therefore the frequencies of these genes are largely determined by stochastic processes (e.g. Kimura and Ohta, 1971a, 1974). According to this neutral mutation-random drift theory (the "neutral theory"), selection acts mainly in a negative fashion, removing deleterious mutants

from populations and only occasionally maintaining genetic polymorphisms.

The arguments presented in support of the neutral theory are mainly concerned with the amount of genetic variation in natural populations (Kimura, 1968; Kimura and Ohta, 1971b), the rate and nature of amino-acid substitutions (Kimura, 1969; King and Jukes, 1969; Dickerson, 1971; Kimura and Ohta, 1973), and the observed patterns of variation between populations (Kimura and Maruyama, 1971; Maruyama and Kimura, 1974).

These arguments have been challenged by many authors (e.g. Sved *et al.*, 1967; King, 1967; Richmond, 1970; Clarke, 1970; Bulmer, 1971; Stebbins and Lewontin, 1972) and the details of these discussions will not be presented here. These authors have refuted the arguments presented as support for the neutral theory and have illustrated specific examples of the action of natural selection and the functional non-equivalence of gene products.

The fact that much of the population data can be accounted for by either stochastic or deterministic theories, is largely due to the absence of any reliable estimates of effective population sizes and mutation and migration rates. For example, the finding of populations with similar frequencies for given genes is often taken as evidence for similar selective pressures, while the observation of different frequencies in different populations is attributed to the action of differential selection in different environments. The neutral mutation-random drift theory interprets these observations as being indicative of the presence and absence respectively, of enough migration to maintain similar gene frequencies in all populations.

2.4 GENETIC VARIATION IN ISLAND POPULATIONS

2.4.1 The level of heterozygosity

Darwin (1859), commenting on the diversity of plant varieties, noted that "wide-ranging, much diffused, and common species vary most". More

recently the implications of this statement have been thoroughly examined and it has become apparent that population size, migration and environmental variation are three important characteristics of populations, which may influence their level of genetic variation. Because populations are finite in size, genetic drift will promote a decrease in genetic heterozygosity (Wright, 1931), and populations with small numbers of individuals are expected to have less genetic variation than those with larger numbers. Furthermore, migration allows populations to exchange genes, and isolation will mean that any genes lost due to genetic drift, cannot be replaced (except by mutation). Some polymorphisms may be maintained because of differential selection in alternate niches or through adaptation to a heterogeneous environment (see Section 2.3.5). Populations occupying small areas may experience a more uniform environment than populations occupying large areas and consequently there may be fewer polymorphisms in the former than in the latter type of populations.

Since Darwin's comment, other studies have found that population size is in fact correlated with the degree of morphological variation (e.g. Fisher and Ford, 1928; Fisher, 1937) and with chromosomal variability (Dobzhansky *et al.*, 1950; da Cunha *et al.*, 1953). The technique of gel electrophoresis provides the opportunity to investigate possible associations of population size, migration and the extent of the area occupied with the level of genetic heterozygosity. Island and mainland populations often differ with respect to the foregoing characteristics and therefore may be suitable for testing for these associations.

A comparison of genetic heterozygosity in species which have both island and mainland populations, and for which relevant data are available, is shown in Table 2.1. For some species there appears to be little difference in the amount of heterozygosity in the two types of populations. Four species (*Philaenus spumarius*, *Bufo viridis*, *Lacerta sicula* and

Table 2.1 Mean heterozygosity *per* locus *per* individual in mainland and island populations.

Species	Mean heterozygosity		Reference
	Mainland	Island	
Philaenus spumarius	0.15	0.08	Saura *et al.*, 1973
Drosophila equinoxialis	0.22	0.17	Ayala *et al.*, 1974a
Drosophila nebulosa	0.20	0.17	Ayala *et al.*, 1974b; Ayala & Tracey, 1974
Drosophila tropicalis	0.14	0.17	Ayala *et al.*, 1974b; Ayala & Tracey, 1974
Drosophila willistoni	0.18	0.16	Ayala *et al.*, 1971
Bufo viridis	0.13	0.03	Dessauer *et al.*, 1975
Uta stansburiana	0.05	0.05	McKinney *et al.*, 1972; Soule & Yang, 1973
Anolis carolinensis	0.05	0.06	Webster *et al.*, 1972
Lacerta sicula	0.09	0.04	Gorman *et al.*, 1975
Peromyscus polionotus	0.06	0.05	Selander *et al.*, 1971
Peromyscus eremicus	0.04	0.01	Avise *et al.*, 1974
Peromyscus leucopus	0.08	0.07	Browne, 1977

Peromyscus eremicus) have island populations which are markedly less variable than their mainland counterparts. The studies summarised in Table 2.1 vary widely with respect to species, population size, ecological context, and the number and type of loci examined, so it is not possible to subject the data to any rigorous statistical analysis.

While island populations are typified by small population size, reproductive isolation and homogeneous environment, these will not be characteristics of all island populations. Nor will all mainland populations be widespread with large population size. For some ecological characteristics, mainland and island populations may be quite similar. For example, Ayala *et al.* (1971) point out that population numbers of *Drosophila willistoni* on small West Indian islands are "probably in the millions". Hence these populations will not be influenced greatly by genetic drift.

The data in Table 2.1 concern species with indigenous island populations. There are several island populations of *Mus musculus*, which were established within the last 200 years. The amount of genetic variability in all these island populations is similar to that in mainland populations (Berry and Murphy, 1970; Wheeler and Selander, 1972; Berry and Peters, 1975). These findings possibly reflect the fact that *Mus musculus* individuals are good colonizers (commensal with Man) and that island populations may have been founded by enough immigrants to prevent any significant founder effect. Indeed, there is evidence that several subspecies have contributed founders to the Hawaiian Islands' populations (Wheeler and Selander, 1972). However, Berry and Peters (1976) note that the Isle of May population of *Mus musculus* (colonization date unknown) is monomorphic at all 17 protein loci examined.

Some populations which do not inhabit islands do, however, exhibit characteristics of island populations, such as small population size, isolation or small habitat area. Studies of such populations are

instructive, since they can give some indication of the importance of these characteristics with respect to their effect on the level of genetic variation. For example, the northern elephant seal (*Mirounga angustirostris*) was commercially exploited until about 1880, when the species was probably represented by less than 100 individuals. The species now numbers more than 30,000. Bonnell and Selander (1974) found no genetic variation at 24 loci in five samples totalling 159 individuals. There is no reason to believe the level of genetic heterozygosity in the species, prior to exploitation, was unusually low, but it is plausible to propose that the present low level resulted from the loss of genes during the bottleneck.

The Bogota (Columbia) population of *Drosophila pseudoobscura* is thought to have arisen by colonization as late as 1960 (Prakash, 1972), and is only about one half as heterozygous as North American populations (Prakash *et al.*, 1969).

Reduced levels of heterozygosity in cave populations of the fish, *Astyanax mexicanus*, when compared with surface populations have been attributed to the small population size of the cave populations (estimated at 200-500), although the effect of selection in a constant environment may be a contributing factor (Avise and Selander, 1972).

2.4.2 Other aspects of island populations

Studies of electrophoretically detectable variation in island populations can be classified into one of three types: i) the sampling of one or two island populations as part of an overall study of genetic variation in a species, ii) intensive investigations of genetic variation in a particular island population, and iii) studies of genetic variation within and between a series of island populations. All three types of studies have made important contributions in the fields of population and evolutionary genetics. Studies of the first type are primarily

concerned with measuring and comparing the levels of genetic variation in island populations with those in mainland populations. The results of many of these studies are summarised in Table 2.1. Examples of each of the latter two types of studies will be discussed to illustrate their value.

Berry and co-workers (Berry and Murphy, 1970; Berry and Jakobson, 1974, 1975; Berry and Peters, 1976) have studied the ecological genetics of *Mus musculus* on the Welsh island of Skokholm. The population was found to be polymorphic at six protein loci, and for three of these, temporal changes in genotype or gene frequencies were observed, which were interpreted as indicating the action of natural selection. For two loci, *Hbb* and *Es-2*, annual cyclical variation in genotype frequencies were observed. These took the form of an excess of heterozygotes over the Hardy-Weinberg equilibrium expectations during one season and a return to expectations six months later. The change in genotype frequencies were associated with periodic fluctuations in breeding activity, survival and environmental stress. At the *Dip-1* locus, directional selection against the $Dip\text{-}1^{b}$ gene resulted in a decrease in its frequency from 92% to 66% in two years, but subsequently it returned to a frequency of 98%. Cyclical variations in the frequency of skeletal variants were also observed.

In terms of gene frequencies, the Skokholm sample differs from samples from two mainland populations nearby more than the mainland samples differ from each other. Despite these differences in gene frequencies, the Skokholm population possesses 14 of the 15 genes commonly found at the loci examined in mainland populations. These observations can be attributed to one or more of founder effect, genetic drift or selection. If the founder effect resulted in the initial colonizers lacking genes common on the mainland, then it must be postulated that selection has favoured the rapid incorporation of mutants.

F.M. Johnson (1971) analysed genetic variation at seven loci in 17 Pacific island populations of *Drosophila ananassae*. These islands can be classified into nine different groups, reflecting geographic proximity. For each of three loci, a low level of genetic heterozygosity was observed and the same gene was the most common one found in all populations. For the other four loci, the frequency of heterozygotes was higher. Islands within a group had similar gene frequencies, but sometimes there were considerable differences between island groups. Johnson argued that this reflected adaptations to different environments.

Genetic variation in Adriatic island populations of the lizards *Lacerta melisellensis* and *L. sicula* was studied by Gorman *et al.* (1975). All but one of the islands studied were probably part of the Yugoslav mainland during the last glacial period and were formed by the eustatic rise in sea level accompanying deglaciation (beginning about 18,000 years BP). Thus the *Lacerta* populations are relic, rather than colonized populations. Mainland *L. sicula* populations were more heterozygous than island populations (0.09 *vs* 0.04) and *L. melisellensis* populations on large islands (53-279 sq km) were more heterozygous than those on small islands (<6 sq km). An association between island area and mean heterozygosity has been noted in populations of a lizard (Soule and Yang, 1973) and an insect (Saura *et al.*, 1973). This observation in itself is in agreement with either or both selection and genetic drift being responsible for reduced heterozygosity.

Two *L. melisellensis* island populations which were intermediate in total area and had intermediate heterozygosity values, were estimated to have population sizes around 10^4 to 10^5 and the authors suggest that such populations have been too large to be subject to genetic drift. According to Wright (1938), the important parameter in determining the effectiveness of genetic drift is the harmonic mean of the population size over previous generations. This may be considerably less than

the estimated population size at any one time, especially if bottlenecks have occurred previously.

The geographic pattern of variation in *Lacerta* species is similar to that in many mainland species, in that for each locus, one gene predominates in almost all populations. Gorman *et al.* used the distribution of gene frequencies and the test of Lewontin and Krakauer (1973) to determine if natural selection has played an important role in determining gene frequencies in the island populations of *Lacerta melisellensis*. They concluded that natural selection acted on genes at many of the loci studied. However, the sensitivity of the test has been severely criticised (e.g. Nei and Maruyama, 1975; Robertson, 1975; Ewens and Feldman, 1976).

Other studies of electrophoretic variation in island populations have been used to clarify evolutionary affinities (e.g. Avise *et al.*, 1974), to determine colonization sequences (Yang *et al.*, 1974; Gorman and Kim, 1976), to determine the importance of island area and other correlates of environmental complexity on heterozygosity (Soule and Yang, 1973), and to measure the concordance between electrophoretic, metric and skeletal variation (Patton *et al.*, 1975).

CHAPTER 3

THE BIOLOGY OF *RATTUS FUSCIPES*

Rattus fuscipes is a native Australian rodent belonging to the family Muridae. Morphological measurements and features vary among populations. On average, adults have a head and body length of about 15 cm, a slightly shorter tail and weigh between 80 and 130 g (Fig. 3.1). Pelage colour also varies both ventrally (from light grey to buffy) and dorsally (from grey to brown). Soft fur and long guard hairs give animals a fluffy appearance. Females have one or two pairs of pectoral mammae and three inguinal pairs (Taylor and Horner, 1973b).

R. fuscipes is one of five recognised species of *Rattus*, native to Australia. This species and all but one of the others, are believed to have evolved their specific status in Australia (Taylor and Horner, 1973a). From evidence on the divergence in morphological characters between species of *Rattus*, Simpson (1961) suggested that the ancestral *Rattus* arrived in Australia, via New Guinea, no later than the early Pleistocene (about 1-2 million years BP). This estimate of the time of arrival remains unsubstantiated, mainly because of the lack of any informative fossil evidence. The *Rattus* fossils that have been found are of relatively recent origin and can be placed in existing species (Watts, 1974).

R. fuscipes is believed to have diverged from the other Australian *Rattus*, soon after their introduction to Australia (Taylor and Horner, 1973a), but again the lack of any fossil evidence prevents a clear assessment of the evolutionary relationships. A karyotypic study of Australian *Rattus*, by Baverstock *et al.* (1977) also fails to clarify this point.

Figure 3.1 Photograph of a southern bush-rat, *Rattus fuscipes greyii* (about $\frac{3}{4}$ actual size).

There are four recognised subspecies of *R. fuscipes*, each inhabiting separate coastal and sub-coastal regions of Australia (Fig. 3.2). Until recently it was considered that *R. f. fuscipes*, *R. f. greyii* and *R. f. assimilis* were separate species and *R. f. coracius* a subspecies of "*R. assimilis*". However, Horner and Taylor (1965) found that animals from the different "species" interbred in captivity and produced fertile offspring. The similar morphology, behaviour, ecology and reproductive biology of the different forms (Horner and Taylor, 1965) also suggests conspecificity. Furthermore, Baverstock *et al.* (1977) have found that all *R. fuscipes* subspecies have the same chromosome number, 2n=48 (XX♀, XY♂) and have two Robertsonian fusions not found in any other *Rattus* species. These observations are evidence that the *R. fuscipes* subspecies are a group distinct from the other Australian *Rattus* species.

Taylor and Horner (1973a) have proposed that the present coastal distribution of *R. fuscipes* resulted from the introduction of an ancestral stock into north-eastern Australia, followed by a southerly colonisation down the eastern seaboard (following suitable habitats). Then during favourable climatic conditions (Jennings, 1971) it is proposed that a westerly colonisation occurred across a coastal corridor south of the Nullarbor Plain to south-western Australia. Fragmentation of a distribution along a southern coastal corridor has been proposed to explain other discontinuities in the distribution of Australian flora and fauna (e.g. Parsons, 1969). It is also possible that the south-western subspecies, *R. f. fuscipes*, resulted from a migration from the north-west, with the south-eastern populations as the end-point of a west to east colonisation. Jennings (1971) considers that as far as the south-western subspecies is concerned, a southerly migration down the west coast would have been climatically less likely than a westerly migration along the south coast.

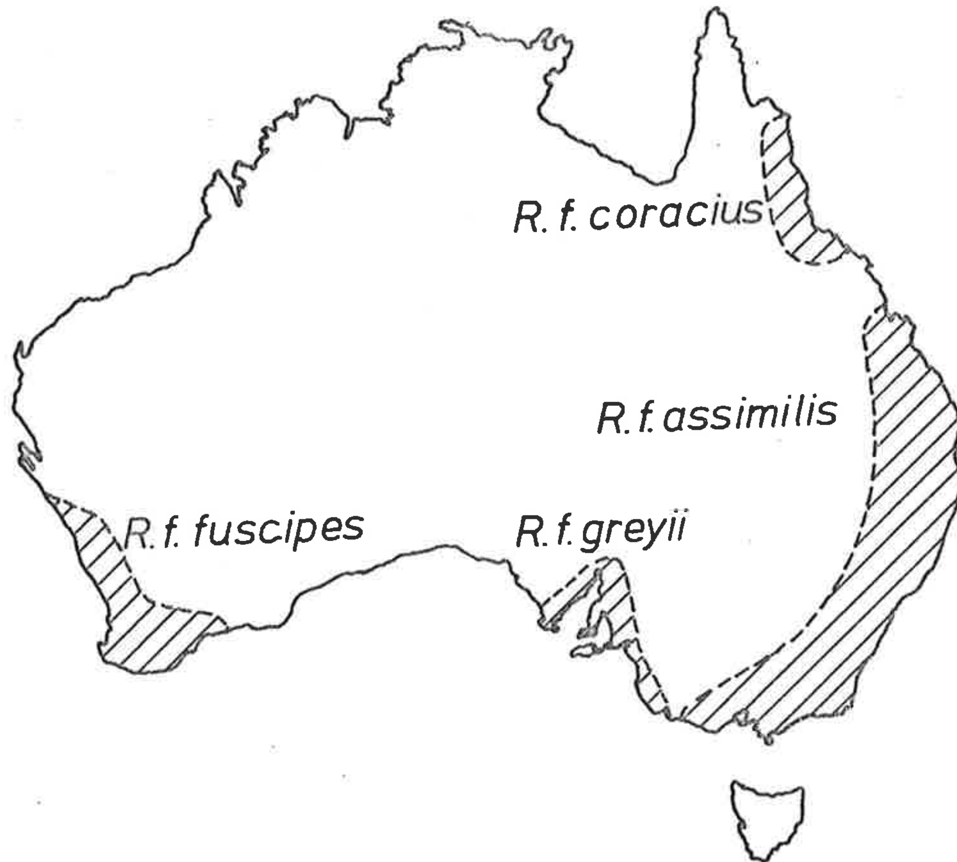


Figure 3.2 Simplified distributional map of the four *Rattus fuscipes* subspecies (from Taylor and Horner, 1973a). For a more detailed map of the distribution of *R. f. greyii* see Fig. 4.1.

R. fuscipes inhabits the sclerophyll and rain forests and brushlands associated with Australian coastal regions. Present day discontinuities in the distribution of *R. fuscipes* are generally associated with interruptions in suitable habitat. The absence of *R. fuscipes* in Tasmania is anomalous, since, when Tasmania was last connected to mainland Australia by a land bridge approximately 12,500 years ago (Jennings, 1971), the species was undoubtedly present in south-eastern Australia (Taylor and Horner, 1973a).

Each of the four subspecies have island forms which are probably relic populations, having been isolated from the mainland when the sea level rose during the last 20,000 years (Godwin *et al.*, 1958; Fairbridge, 1960, 1961; Thom and Chappell, 1975). These island populations often have quite different habitats from those of mainland populations.

There are only a few studies on the ecology of *R. fuscipes*, most being concerned with *R. f. assimilis* (Warneke, 1971; Hobbs, 1971; Wood, 1971; Leonard, 1973; Robinson, 1976) and only one on *R. f. greyii* (Wheeler, 1970). Animals belonging to any of these subspecies are nocturnal, unobtrusive and live in shallow burrows. Population densities of animals are low on the mainland. Taylor and Horner (1973b) reported capture rates (number of animals caught *per* trap set *per* night) of 2.4% (*R. f. fuscipes*), 2.8% (*R. f. greyii*), 4.8% (*R. f. assimilis*) and 1.2% (*R. f. coracius*). Warneke (1971) reported a capture rate of 2.3% in a mainland population of *R. f. assimilis* and estimated there were 4.8 rats *per* acre. Studies of island populations indicate they have much higher population densities of animals. Hobbs (1971) estimated there were 30 specimens of *R. f. assimilis per* acre on Greater Glennie Island; Robinson (1976) found that the Glennie Island population contained a much higher density of individuals than a nearby mainland population. Kirsch (cited in Taylor and Horner, 1973b) obtained a 17% capture rate on East Wallabi Island (*R. f. fuscipes*).

Population numbers generally increase in late spring and summer and decline during winter (Wheeler, 1970; Warneke, 1971; Wood, 1971; Robinson, 1976). These fluctuations in population size appear to be associated with a breeding season in spring and early summer and lower survival rates in winter. While there is a definite reproductive peak in spring and summer, Taylor (1961) and Wood (1971) found pregnant or lactating females (*R. f. assimilis*) at most times of the year. A change of diet, from seeds and insects in spring and summer to fungi and leaves in winter, may account for the poor survival during the latter period (Wheeler, 1970; Robinson, 1976).

Both males and females are territorial and most individuals have a range no more than a few hundred feet in diameter (Taylor, 1961; Wheeler, 1970; Warneke, 1971; Wood, 1971; Robinson, 1976). Robinson (1976) found that the home ranges of Glennie Island rats overlap to a considerably greater extent than mainland rats. Members of mainland populations exhibit significantly more agonistic behaviour than island rats. Just prior to the breeding season the male territories break down and the males disperse (Robinson, 1976).

Only rarely do animals survive to reproduce in two consecutive breeding seasons (Taylor, 1961; Wood, 1971). Warneke (1971) observed females which had up to four litters. Some females born early in a breeding season may reproduce later in that season.

Under laboratory conditions, male *R. f. assimilis* reach sexual maturity at about 11 weeks of age and females at 7 to 9 weeks (Taylor and Horner, 1971; Warneke, 1971), but in field populations, individuals probably take at least a month longer before they are able to breed (Taylor, 1971). Breed (1976) reported that the first oestrus in laboratory bred females (*R. f. coracius*) is at about 14 weeks. The gestation period is 23 days for *R. f. greyii* and about the same for *R. f. fuscipes* and *R. f. assimilis* (Taylor and Horner, 1972) Horner

and Taylor (1965) observed one case of delayed implantation in *R. f. greyii*, resulting in a gestation period of at least 33 days. Litter size usually varies between 3 and 6 (Taylor and Horner, 1972, 1973b).

During the course of this study some miscellaneous observations were made on the ecology and reproductive biology of *Rattus fuscipes greyii*. These observations are described in the first appendix.

Other than the chromosomal studies of Kennedy (1969) and Baverstock (1977), no genetic studies of *R. fuscipes* have been reported.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHODS

4.1 COLLECTING LOCALITIES AND TECHNIQUE

The sampling localities are given in Table 4.1 and Figure 4.1. Animals were captured using "Sherman" traps, baited with a mixture containing rolled oats, honey and peanut paste. In general, four or five lines, each of 25 to 50 traps placed about five metres apart, were set during the day and checked early the next morning for captures. When trapping on the small islands, the trap lines were positioned in such a way that animals would be collected from all the different habitats occupied by the population (e.g. beach, cliff, plateau). The Norton Summit sample was taken from an area of about 10 hectares, although the total amount of suitable habitat contiguous with this area was much greater. The samples from the South-East, Eyre Peninsula and Kangaroo Island consisted of two to four sub-samples collected within 40 kilometres of one another.

4.2 MAINTENANCE OF LABORATORY COLONY

Most of the animals collected were transported alive to Adelaide but some (most *R. f. assimilis* and 11 *R. f. greyii* from Eyre Peninsula) were bled and released in the field. Like-sexed pairs were housed in the laboratory in plastic cages (about 40 cm x 30 cm x 15 cm) with wire-mesh tops. The floors of these cages were covered with saw-dust and shredded paper was provided for nesting. "Mouse-cubes" (containing a mixture of protein, vitamins and minerals), a bird-seed mixture (canary seed, white millet, Japanese millet and pannicum seed in equal parts) and water were provided *ad lib*.

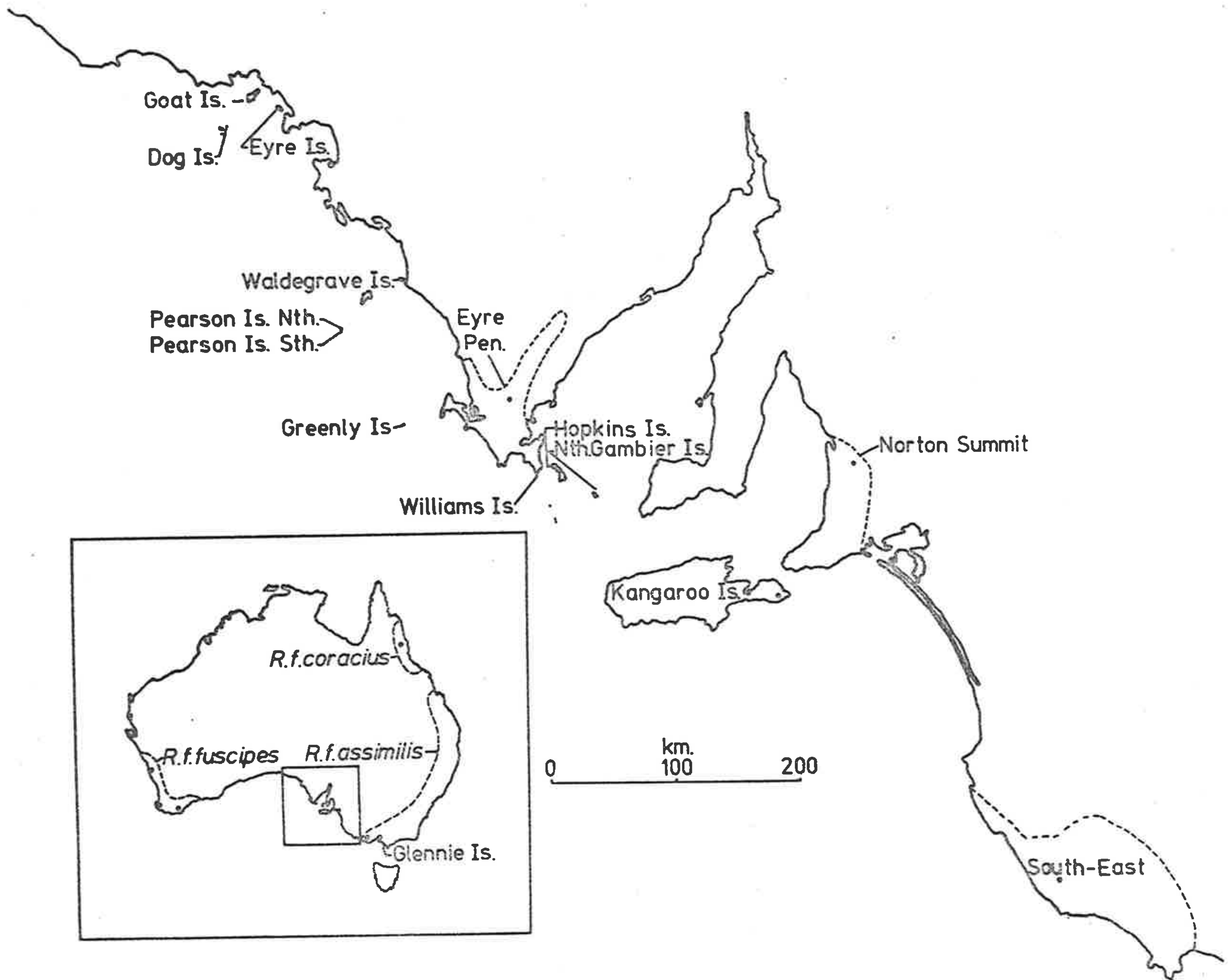
Table 4.1 Sampling information for *R. fuscipes*. The area given is the approximate area occupied by the entire population. No area estimate is given for mainland populations (see Fig. 4.1).

Subspecies	Population	Area (ha)	Approximate latitude and longitude	Sample size	Collecting dates
R. f. fuscipes	Jurien	-	30°08'S; 115°08'E	2	March-April, 1975
"	Augusta	-	34°04'S; 115°02'E	3	" " "
"	Waychinicup Valley	-	34°50'S; 118°15'E	1	" " "
R. f. greyii	Dog I.	60	32°29'S; 133°22'E	29	June, 1975
"	Goat I.	300	32°18'S; 133°28'E	39	" "
"	Eyre I.	1100	32°20'S; 133°50'E	2	March, 1976
"	Pearson I. North	190	33°57'S; 134°18'E	50	February, 1974
"	Pearson I. South	55	33°57'S; 134°18'E	24	" "
"	Greenly I.	200	34°39'S; 134°49'E	50	March, 1974
"	Waldegrave I.	220	33°36'S; 134°49'E	50	February, 1975
"	Williams I.	140	35°01'S; 135°59'E	50	April, 1975
"	Hopkins I.	160	34°57'S; 136°04'E	31	June, 1974
"	North Gambier I.	65	35°09'S; 136°28'E	50	April, 1975
"	Eyre Peninsula	-	34°30'S; 135°50'E	31	August, November, 1973
"	Kangaroo I.	∿400000	35°52'S; 138°00'E	49	December 1973-January 1974
"	Norton Summit	-	34°53'S; 138°35'E	79	August, October, 1973; June, 1975; June, 1976
"	South-East	-	37°30'S; 140°30'E	42	December 1974-January 1975
R. f. assimilis	Glennie I.	70	39°06'S; 146°15'E	10	1973
"	Otway Ranges	-	38°47'S; 143°32'E	9	September, 1973
"	Sherbrooke Forest	-	37°53'S; 145°22'E	19	" "
R. f. coracius	Cairns	-	17°15'S; 145°56'E	3[1]	November, 1974
"	Atherton	-	17°15'S; 145°37'E	3[2]	" "
"	unknown	-	- -	1	not known

[1] One of the three individuals was the offspring of a female, pregnant when caught at the given locality.

[2] One of the three individuals was the offspring of a female caught at the given locality and a *R. f. coracius* male of unknown origin.

Figure 4.1 Distribution and sample locations of *Rattus fuscipes greyii*. Broken lines indicate the approximate extent of the mainland populations. Sample locations in the large populations are indicated by dots. The inset shows the approximate distribution of the other *R. fuscipes* subspecies and their sampling localities.


Goat Is.
Dog Is.
Eyre Is.
Waldegrave Is.
Pearson Is. Nth.
Pearson Is. Sth.
Eyre
Pen.
Greenly Is
Hopkins Is.
Nth.Gambier Is.
Williams Is.
Norton Summit
Kangaroo Is.
South-East
km.
0
100
200
R.f.coracius
R.f.fuscipes
R.f.assimilis
Glennie Is.

For mating, a male and female were placed in a cage for 21 days (the gestation period is approximately 23 days; Taylor and Horner, 1972), after which the male was removed and the female was kept in isolation for a further 21 days. This cycle was repeated (up to a maximum of four times) until the female produced a litter. It was important to remove the male before the birth of a litter, otherwise the parents killed any newborn animals. Offspring were weaned at about 30 days after birth and kept in like-sexed pairs. Sometimes, if adult females were thought to be pregnant when captured, they were caged individually for the first three weeks after capture.

4.3 TISSUE EXTRACTION PROCEDURES

Most specimens were killed within two months of capture, by placing them in a jar containing ether. While the animals were still alive, but unconscious, about two to three ml of blood were taken by cardiac puncture with a 23 gauge needle and syringe. Fifty units of heparin *per* ml of blood were used as an anticoagulant. If a blood sample was required from an animal which was not to be killed, the animal was lightly anaesthetised and up to one ml of blood taken from the suborbital sinus, using a Pasteur pipette.

Whole blood was centrifuged at 2500*g* for 10 minutes in a bench centrifuge and the plasma removed and stored at $-30^{o}C$. The packed red cells were washed two or three times in an excess of physiological saline (0.87 g sodium chloride in 100 ml distilled water), each time centrifuging at 2500*g* for 4 minutes to remove the saline. The packed cells were then lysed by adding an equal volume of distilled water and one-fifth volume of toluene and shaking the mixture vigorously for about one minute. The solution was then centrifuged at 40,000*g* for 20 minutes (at $4^{o}C$) and the supernatant was stored at $-30^{o}C$. The liver, heart and kidneys were excised, the excess fat trimmed off the kidneys and each tissue

homogenised in 2 ml water *per* g tissue using an "Ultra-Turrax" blender (Janke and Kunkel, West Germany). The homogenate was centrifuged at 40,000*g* for 20 minutes (at 4°C) and the supernatant stored at -30°C. All tubes containing blood or tissue were kept in ice, where practicable, during these procedures. The carcasses were stored at -5°C.

4.4 ELECTROPHORETIC PROCEDURES

Electrophoresis using "Electrostarch" (Electrostarch Co., U.S.A.) was carried out in gel beds 30 cm x 15.5 cm x 6 mm. Hot starch was poured into the trays and plastic or stainless steel "slot-formers" were inserted into the liquid starch. These provided wells, where the samples to be electrophoresed were added. Gels were poured and allowed to set overnight before electrophoresis. Except for the electrophoresis of haemoglobin, all gels were run vertically. The gels were cooled by circulating water through a brass jacket attached to the base of each gel bed. A constant electric current, about 5-10 mA below that at which the gel became warm to touch, was applied, although for the first ten minutes of electrophoresis a current 10 to 20 mA below the normal running current was applied. The voltage slowly increased as electrophoresis continued. On completion, piano wire was used to cut the gel into two pieces. Each piece was placed, cut surface up, in a tray and freshly made staining solution added. For the staining of enzymes, the tray and solution were put on a shaker in a dark 37°C constant temperature room. When amido black was used as a general protein stain (for haemoglobin and albumin), staining was done at room temperature in normal light.

The electrophoresis buffers were as follows:-

Buffer I   O. Smithies (cited in Huehns and Shooter, 1965) pH 8.6

A stock solution consisting of -

109 g tris[1]
5.84 g disodium EDTA[2]
30.9 g boric acid

was made up to one litre with distilled water. The gel buffer was a one in twenty dilution of this stock and the electrolyte buffer a one in seven dilution.

Buffer II   Gahne (1966) pH 8.5

Two stock solutions were required. The "electrolyte" stock consisted of -

2.518 g lithium hydroxide
14.15 g boric acid

made up to one litre with water, and the "gel" stock was

9.567 g tris
1.471 g citric acid

made up to one litre with water. The gel buffer was a mixture of one volume of "electrolyte" stock and 5.4 volumes of "gel" stock. The undiluted "electrolyte" stock was used as the electrolyte buffer.

Buffer III   Shaw and Prasad (1970) pH 8.0

The electrolyte buffer was -

83.2 g tris
33.0 g citric acid

made up to one litre with water and the gel buffer was a one in thirty dilution of this.

---

[1] tris(hydroxymethyl)aminomethane

[2] ethylenediamine tetraacetic acid

Buffer IV   Poulik (1957)

The electrolyte buffer consisted of -

18.55 g boric acid
60 ml 1N sodium hydroxide

per litre of water (pH 8.2). The gel buffer was

9.21 g tris
1.05 g citric acid

made up to one litre with water (pH 8.65).

Buffer V   Shaw and Prasad (1970) pH 7.0

The electrolyte buffer consisted of -

16.35 g tris
9.04 g citric acid

made up to one litre with water. The gel buffer was a one in fifteen dilution of the electrolyte buffer.

The electrophoretic conditions and staining mixture for each protein are given below. The tris-HCl/$MgCl_2$ (pH 8.0) buffer contained

12.1 g tris
57.5 ml 1N hydrochloric acid
1.0 g magnesium chloride $6 \cdot H_2O$

made up to one litre with water. Except where noted, stains were modified from Brewer (1970), Shaw and Prasad (1970) and Selander *et al.* (1971). The quantity of staining mixture cited is about that used to stain one slice of gel with 16 to 24 samples.

4.4.1 Acid phosphatase (ACP) (EC No. 3.1.3.2)

tissue	red blood cell haemolysates
buffer system	I
starch concentration	13.0%
initial current	60 mA
final current	80 mA
initial voltage	9 V/cm
final voltage	23 V/cm
time	3 h
stain:-	10 ml 0.05M citrate buffer pH 6.0 1 mg 4-methyl umbelliferyl dihydrogen phosphate (Swallow *et al.*, 1973)

After about 30 minutes, the gel was observed under ultra-violet light and acid phosphatase activity appeared as fluorescent bands on a non-fluorescent background.

4.4.2 Albumin (ALB)

tissue	plasma (dilute $\frac{1}{14}$ with H_2O)
buffer system	II
starch concentration	12.0%
initial current	65 mA
final current	75 mA
initial voltage	7 V/cm
final voltage	13 V/cm
time	$3\frac{3}{4}$ h

stain:- 3.7 g amido black 10B
250 ml H_2O
250 ml methanol
50 ml glacial acetic acid

A few millilitres of the staining solution were applied for two minutes, then the gel was washed for 24 hours in several changes of a mixture of water, methanol and glacial acetic acid in the ratio 5:5:1 respectively.

4.4.3 Esterase (ES) (EC No. 3.1.1.1)

The electrophoretic conditions were the same as for albumin. The stain consisted of -

10 ml acetate buffer pH 5.4
1 mg 4-methyl umbelliferyl acetate
(Hopkinson *et al.*, 1973)

The acetate buffer contained

6.786 g glacial acetic acid
4 g sodium hydroxide

per litre of water. After staining for five to fifteen minutes, esterase activity was seen as fluorescent bands when the gel was viewed under ultra-violet light.

4.4.4 Glucose-6-phosphate dehydrogenase (G6PD) (EC No. 1.1.1.49)

tissue	kidney
buffer system	IV
starch concentration	12.0%
initial current	60 mA
final current	50 mA
initial voltage	7 V/cm
final voltage	19 V/cm
time	3 h

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
20 mg disodium glucose-6-phosphate
5 mg NADP
5 mg MTT
1 mg PMS

As the electrolyte buffer front passed through, the gel behind the front heated if the current was greater than 50 mA. A current of 60 mA was applied until the buffer front reached the sample wells, when it was reduced to 50 mA.

4.4.5 Glutamate oxaloacetate transaminase (GOT) (EC No. 2.6.1.1)

tissue	heart
buffer system	III
starch concentration	12.0%
initial current	90 mA
final current	105 mA
initial voltage	11 V/cm
final voltage	14 V/cm
time	4 h

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
2 mg pyridoxal-5'-phosphate
150 mg α-aspartic acid
75 mg α-ketoglutaric acid
100 mg fast blue BB salt

4.4.6 Haemoglobin (HB)

tissue	red blood cell haemolysates (dilute $\frac{1}{16}$ with H_2O)
buffer system	I
starch concentration	11.0%
initial current	60 mA
final current	80 mA
initial voltage	10 V/cm
final voltage	21 V/cm
time	3½ h

stain:- same as for albumin (amido black)

The packed red cells were haemolysed in an equal volume of water and this haemolysate was diluted one in sixteen in water, just prior to electrophoresis. Hence the total dilution from packed red blood cells was $\frac{1}{32}$. The stain was applied for about 40 seconds and then the gel was destained for 24 hours as for albumin.

4.4.7 Isocitrate dehydrogenase (ICD) (EC No. 1.1.1.42)

tissue	heart or kidney
buffer system	V
starch concentration	12.5%
initial current	60 mA
final current	90 mA
initial voltage	9 V/cm
final voltage	17 V/cm
time	3 h

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
10 mg disodium isocitrate
5 mg NADP
5 mg MTT
1 mg PMS

4.4.8 Lactate dehydrogenase (LDH) (EC No. 1.1.1.27)

tissue	heart or kidney
buffer system	I
starch concentration	13.0%
initial current	60 mA
final current	90 mA
initial voltage	8 V/cm
final voltage	23 V/cm
time	2 h

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
0.25 ml sodium lactate (70% solution)
5 mg NAD
4 mg MTT
1 mg PMS

4.4.9 Malate dehydrogenase (MDH) (EC No. 1.1.1.37)

The electrophoretic conditions were the same as for isocitrate dehydrogenase.

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
100 mg sodium carbonate monohydrate
100 mg malic acid
5 mg NAD
5 mg MTT
1 mg PMS

4.4.10 Malic enzyme (ME) (EC No. 1.1.1.40)

The electrophoretic conditions were the same as for isocitrate dehydrogenase and malate dehydrogenase. The stain was the same as for malate dehydrogenase except that NADP was substituted for NAD. Sometimes both malate dehydrogenase and malic enzyme was stained on the same gel by pooling the staining mixtures.

4.4.11 Phosphoglucomutase (PGM) (EC No. 2.7.5.1)

The electrophoretic conditions were the same as for glutamate oxaloacetate transaminase.

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
15 mg disodium glucose-1-phosphate
6 U glucose-6-phosphate dehydrogenase
3 mg NADP
3 mg MTT
1 mg PMS

The cofactor glucose-1,6-diphosphate, which was required for phosphoglucomutase activity, was present as a contaminant in the disodium glucose-1-phosphate.

4.4.12 6-Phosphogluconate dehydrogenase (6PGD) (EC No. 1.1.1.44)

Electrophoresis was carried out in the same manner as for glucose-6-phosphate dehydrogenase.

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
5 mg disodium 6-phosphogluconate
5 mg NADP
5 mg MTT
1 mg PMS

4.4.13 Superoxide dismutase (SOD) (EC No. 1.15.1.1)

This enzyme was detected in either the lactate dehydrogenase or the glucose-6-phosphate dehydrogenase electrophoretic systems.

stain:- 50 ml tris-HCl/$MgCl_2$ buffer
4 mg MTT
1 mg PMS

The gel was incubated at 37°C in the light and the enzyme appeared as white bands on a blue background.

4.5 SUBCELLULAR LOCALISATION

To determine the subcellular locality of some isozymes, a modification of the techniques of Henderson (1965) and Hogeboom (1955) was used. Tissues were gently homogenised in a buffer (10 ml *per* gm tissue) containing

85.58	g	sucrose	(0.25M)
3.48	g	K_2HPO_4	(0.02M)
0.7445	g	Na_2 EDTA	(2 mM)

made up to one litre with water and brought to pH 7.2 with 1N hydrochloric acid. The homogenate was centrifuged three times at 600*g* for 30 minutes ($4^{\circ}C$), discarding the pellet each time. The supernatant was then centrifuged at 7,500*g* for ten minutes. This supernatant is referred to as the cytoplasmic, soluble or supernatant fraction. It was centrifuged at 40,000*g* for 30 minutes and the supernatant stored at $-30^{\circ}C$ ready for electrophoresis. The pellet from the 7,500*g* spin was washed three times by gently resuspending in an excess of the sucrose buffer, centrifuging at 7,500*g* and discarding the supernatant. After the final spin, the pellet was resuspended in the sucrose buffer (1 ml *per* g original tissue) and the solution was sonicated or frozen and thawed three times. This extract is referred to as the mitochondrial fraction.

4.6 NOMENCLATURE

A symbol has been assigned to each protein studied, based on the recommendations of Giblett *et al.* (1976) and written in non-italicised upper case letters (e.g. ACP for acid phosphatase). The locus coding for the protein is given the same symbol except that it is italicised (e.g. *ACP*). Where there are two or more forms of the protein or two or more coding loci, subscripts are added to distinguish these forms. For example, GOT_M and GOT_S are the two loci coding for the mitochondrial and supernatant forms of glutamate oxaloacetate transaminase. Allelic genes are distinguished by adding superscripts to the gene symbol

(e.g. GOT_M^1 and GOT_M^2). Phenotypic designations consist of the locus symbol (not italicised) followed by the appropriate allele symbol(s). For example, a heterozygote for two codominant genes GOT_M^1 and GOT_M^2 would phenotypically be GOT_M 2-1.

Where electrophoresis reveals more than one form of an enzyme, these forms are referred to as "isozymes" (Markert and Moller, 1959). In the particular case where multiple electrophoretic forms of an enzyme are the result of allelic variation at a genetic locus, they are referred to as "allozymes" (Prakash *et al.*, 1969).

CHAPTER 5

RESULTS

5.1 PHENOTYPES, FAMILY DATA AND GEOGRAPHIC VARIATION FOR EACH PROTEIN

5.1.1 Acid phosphatase

Acid phosphatase activity always appeared as a single band after electrophoresis and the mobility of this band was the same for all individuals examined. For the purposes of the multivariate analyses, it is assumed that all populations are monomorphic for the same gene coding for the primary amino-acid sequence of acid phosphatase.

5.1.2 Albumin

Figure 5.1 shows the three albumin phenotypes which appeared on gels stained with amido black. While each individual was classified into one of three phenotypes, there were slight mobility differences between individuals with the same phenotype. These differences were not investigated and it is not known if they have a genetic basis.

It is proposed that the albumin phenotypes are controlled by two autosomal codominant allelic genes, ALB^F and ALB^S, with the three phenotypes ALB F, ALB F-S and ALB S corresponding to the genotypes ALB^F/ALB^F ALB^F/ALB^S and ALB^S/ALB^S respectively. Breeding data from 44 families are consistent with this proposed mode of inheritance (Table 5.1). The simple mode of inheritance and the two banded phenotype characteristic of heterozygotes are similar to albumin variation in other mammals such as cattle (Ashton, 1964) and Man (Adams, 1966; Weitkamp *et al.*, 1967). No satellite bands were observed with either fresh or stored samples (cf. Gahne, 1966; Selander *et al.*, 1971).

Estimated frequencies of albumin genes in populations of *R. fuscipes* are shown in Table 5.2. No single population was found to be polymorphic

Figure 5.1 Photograph of albumin variation in *Rattus fuscipes*.

Phenotypes from left to right are: ALB S, ALB S, ALB F-S, ALB F, ALB F.

origin —

Table 5.1 Family data on the inheritance of albumin phenotypes. Incomplete family data have been included when the unrecorded parent came from a population known to be monomorphic for a particular albumin phenotype. An asterisk is placed next to the presumed phenotypes of the unrecorded parents.

Parental phenotypes ♀		♂	Number of matings	Offspring phenotypes ALB F	ALB F-S	ALB S	χ^2_1
ALB F	x	ALB F	3	31	-	-	
ALB F	x	ALB F*	2	3	-	-	
ALB F*	x	ALB F*	2	5	-	-	
TOTAL			7	39	-	-	
ALB F	x	ALB F-S	1	1	4	-	
ALB F-S	x	ALB F	1	1	-	-	
TOTAL			2	2	4	-	
ALB F	x	ALB S	3	-	21	-	
ALB S	x	ALB F	5	-	26	-	
ALB S*	x	ALB F	2	-	10	-	
TOTAL			10	-	57	-	
ALB F-S	x	ALB S*	1	-	1	1	
ALB S	x	ALB F-S	3	-	5	5	
TOTAL			4	-	6	6	0.00
ALB F-S	x	ALB F-S	4	8	16	11	0.77
ALB S	x	ALB S	6	-	-	28	
ALB S	x	ALB S*	15	-	-	61	
TOTAL			21	-	-	89	

Table 5.2 Albumin phenotype numbers and gene frequencies in *R. fuscipes* populations.[1]

Population	Phenotype numbers			Gene frequencies	
	ALB F	ALB F-S	ALB S	ALB^F	ALB^S
R. f. fuscipes	6	–	–	1.00	–
Dog I.	–	–	26	–	1.00
Goat I.	39	–	–	1.00	–
Eyre I.	1	–	–	1.00	–
Pearson I. North	50	–	–	1.00	–
Pearson I. South	24	–	–	1.00	–
Greenly I.	48	–	–	1.00	–
Waldegrave I.	–	–	47	–	1.00
Williams I.	–	–	50	–	1.00
Hopkins I.	–	–	29	–	1.00
North Gambier I.	–	–	49	–	1.00
Eyre Peninsula	–	–	19	–	1.00
Kangaroo I.	–	–	48	–	1.00
Norton Summit	–	–	37	–	1.00
South-East	–	–	41	–	1.00
R. f. assimilis	–	–	4	–	1.00
R. f. coracius	–	–	7	–	1.00

[1] Unless noted otherwise, the samples of each subspecies other than *R. f. greyii*, have been pooled and each subspecies treated as a single population.

for albumin, despite the widespread occurrence of the two genes in *R. f. greyii* populations. For albumin and other proteins showing variation between populations, a test of the homogeneity of gene frequencies was made using the contingency table of gene counts. In general, the *R. f. fuscipes, R. f. assimilis, R. f. coracius* and Eyre Island samples had to be omitted because cells for these populations had expectations less than five. The thirteen *R. f. greyii* populations are heterogeneous for albumin gene counts (χ^2_{12} = 1014, P<<0.001) and all pairs of populations with different genes present have significantly different gene frequencies.

5.1.3 Esterase

All *R. f. greyii* and *R. f. assimilis* individuals sampled had a single fluorescent band of esterase activity, termed the "presence" phenotype (ES 1). All *R. f. fuscipes* and most *R. f. coracius* individuals had no detectable plasma esterase (fluorescent). These individuals are said to exhibit the "null" phenotype (ES 0). One specimen of *R. f. coracius*, for which the place of capture and sex were unrecorded, showed the presence phenotype. This individual was only recently typed and was not available for the multivariate analyses (described later). As in the case of albumin, there were slight mobility differences in the presence band. However, these were ignored and the variation considered was limited to that of the presence or absence of a single band.

There are no breeding data on esterase variation because all laboratory matings involved only *R. f. greyii* individuals. However, in other cases of esterase variation of the presence/absence type (e.g. *Mus musculus*, Petras and Biddle, 1967; *Rattus norvegicus*, Womack, 1972), the phenotypes are controlled by two autosomal allelic genes (ES^1 and ES^0), with the presence phenotype dominant to null. Assuming this mode of inheritance, it is possible to estimate the upper

limits of the frequency of the null gene, if present in *R. f. greyii* populations. For example, the upper bound of the 95% confidence limit of the absence gene frequency is 0.24 in the Williams Island population, from which 50 individuals were typed for esterase, and 0.38 in the Eyre Peninsula population from which 19 individuals were scored.

5.1.4 Glutamate oxaloacetate transaminase

Two regions of GOT activity were seen on gels, one migrating towards the anode (GOT_S), the other to the cathode (GOT_M) (Fig. 5.2). Attempts to determine the subcellular localities of the two isozymes in heart and kidney cells were only partially successful. The supernatant cell fraction contained the GOT_S isozyme, but no GOT activity was recovered from the mitochondrial fraction, probably because of the small quantity of tissue used and the loss of material in the preparative steps. Mitochondrial extracts from liver tissue contained predominantly GOT_M, but some GOT_S activity was present. Boyd (1961) also found traces of GOT_S in mitochondrial extracts from rat liver. The subcellular locality of the GOT isozymes is similar to that found in *Mus musculus* (De Lorenzo and Ruddle, 1970) and *Rattus* (Boyd, 1961). As well as the differences in subcellular locality and electrophoretic mobility, the two GOT isozymes have been shown to differ in biochemical characteristics such as substrate affinities and optimum pH's (Boyd, 1961). These enzymes correspond to separate genetic loci (Chen and Giblett, 1971; Davidson *et al.*, 1970; De Lorenzo and Ruddle, 1970), which are not linked to one another in *Mus musculus* (Chapman and Ruddle, 1972).

5.1.4.1 Supernatant glutamate oxaloacetate transaminase

Four GOT_S phenotypes were observed (Fig. 5.2), three (GOT_S A, GOT_S B and GOT_S C) consisting of a single major band of activity and a fourth (GOT_S A-B) with three main bands. If gels were stained, not for

Figure 5.2 Photographs of glutamate oxaloacetate transaminase variation in *Rattus fuscipes*.

Top: GOT_S phenotypes from left to right are: GOT_S A-B, GOT_S A, GOT_S B, GOT_S A-B, GOT_S A, GOT_S A, GOT_S A. The first five samples are GOT_M 2 and the last two are GOT_M 2-1.

Bottom: GOT_S phenotypes from left to right are: GOT_S A-B, GOT_S A-B, GOT_S A-B, GOT_S A-B, GOT_S A, GOT_S C.

+
origin

+
origin

just the usual one hour but for about two hours (to develop the weaker staining GOT_M isozyme), minor satellite bands appeared, anodal to the main bands. Minor sub-bands have been observed previously in *Mus musculus* by De Lorenzo and Ruddle (1970) who suggested that they probably represent alternate molecular forms of GOT_S.

Breeding data on the inheritance of the GOT_S A, GOT_S A-B and GOT_S B phenotypes are presented in Table 5.3. There is a significant excess of GOT_S A offspring from GOT_S A x GOT_S A-B matings (0.05>P>0.025). Apart from this, the data are in agreement with the hypothesis that the phenotypes are determined by two autosomal codominant alleles. These have been designated GOT_S^A and GOT_S^B with the phenotypes GOT_S A, GOT_S A-B and GOT_S B corresponding to the genotypes GOT_S^A/GOT_S^A, GOT_S^A/GOT_S^B and GOT_S^B/GOT_S^B respectively. The phenotype of putative heterozygotes consist of both parental bands of activity as well as a more intensely staining intermediate "hybrid" band which suggests that the active enzyme is a dimer. Supernatant GOT variation in Man (Chen and Giblett, 1971) and *Peromyscus polionotus* (Selander *et al.*, 1971) is also simply inherited with a three banded heterozygote pattern. No data on the inheritance of the GOT_S C phenotype are available. This phenotype was only found in *R. f. assimilis*, but it is presumed that it is controlled by a third codominant allele, GOT_S^C.

Table 5.4 shows the estimated GOT_S gene frequencies in *R. fuscipes* populations. Most populations are monomorphic for the GOT_S^A gene. The GOT_S^B gene was only found in the Pearson Islands' populations. Pearson Island North is monomorphic for the GOT_S^B gene, while Pearson Island South is polymorphic, with two alleles present, GOT_S^A and GOT_S^B. The genotype frequencies in this latter population agree with those expected on the Hardy-Weinberg principle (χ_1^2 = 0.012). The two Pearson Islands have significantly different GOT_S gene frequencies and each is significantly different from all other *R. f. greyii* populations.

Table 5.3 Family data on the inheritance of GOT_S phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀	x	♂	Number of matings	Offspring phenotypes GOT_S A	GOT_S A-B	GOT_S B	χ^2
GOT_S A	x	GOT_S A	14	69	-	-	
GOT_S A	x	GOT_S A*	18	66	-	-	
TOTAL			32	135	-	-	
GOT_S A	x	GOT_S A-B	6	14	9	-	
GOT_S A*	x	GOT_S A-B	1	3	-	-	
GOT_S A-B	x	GOT_S A	1	4	1	-	
TOTAL			8	21	10	-	3.90(1 d.f.)
GOT_S A-B	x	GOT_S A-B	2	1	11	4	3.38(2 d.f.)
GOT_S A	x	GOT_S B	3	-	25	-	
GOT_S A*	x	GOT_S B	2	-	5	-	
GOT_S B	x	GOT_S A	1	-	9	-	
TOTAL			6	-	39	-	
GOT_S B	x	GOT_S A-B	1	-	7	10	0.53(1 d.f.)

Table 5.4 Supernatant glutamate oxaloacetate transaminase phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers				Gene frequencies		
	GOT_S A	GOT_S A-B	GOT_S B	GOT_S C	GOT_S^A	GOT_S^B	GOT_S^C
R. f. fuscipes	6	–	–	–	1.00	–	–
Dog I.	26	–	–	–	1.00	–	–
Goat I.	39	–	–	–	1.00	–	–
Eyre I.	1	–	–	–	1.00	–	–
Pearson I. North	–	–	50	–	–	1.00	–
Pearson I. South	10	10	4	–	0.63	0.38	–
Greenly I.	48	–	–	–	1.00	–	–
Waldegrave I.	47	–	–	–	1.00	–	–
Williams I.	50	–	–	–	1.00	–	–
Hopkins I.	30	–	–	–	1.00	–	–
North Gambier I.	49	–	–	–	1.00	–	–
Eyre Peninsula	19	–	–	–	1.00	–	–
Kangaroo I.	48	–	–	–	1.00	–	–
Norton Summit	42	–	–	–	1.00	–	–
South-East	41	–	–	–	1.00	–	–
R. f. assimilis							
(a) mainland	–	–	–	2	–	–	1.00
(b) Glennie I.	3	–	–	–	1.00	–	–
R. f. coracius	7	–	–	–	1.00	–	–

The two mainland *R. f. assimilis* individuals examined were homozygous for the GOT_S^C gene and were the only animals in which this gene was found. The GOT_S^C gene is probably a common gene in *R. f. assimilis* since the two individuals typed were collected about 200 km apart and their respective populations are probably geographically isolated. However, the three specimens of *R. f. assimilis* from Glennie Island were GOT_S^A/GOT_S^A homozygotes, indicating that the GOT_S^C gene is not universal in *R. f. assimilis*.

5.1.4.2 Mitochondrial glutamate oxaloacetate transaminase

Almost all individuals scored had the GOT_M 2 phenotype, shown in Fig. 5.2. The exceptions were two *R. f. coracius* specimens which were GOT_M 2-1. The GOT_M 2 phenotype has one main band and the GOT_M 2-1 phenotype has three main bands, one corresponding to the GOT_M 2 band, a second more cathodal band of similar staining intensity, and an intermediate darker staining band between these two. Although the GOT_M isozymes stained much less intensely than the GOT_S region, the former also had satellite bands migrating anodal to the main bands.

It is proposed that the variation is controlled by two autosomal codominant allelic genes, GOT_M^1 and GOT_M^2, the GOT_M 2 phenotype being the result of homozygosity for the GOT_M^2 gene and heterozygous GOT_M^1/GOT_M^2 individuals being phenotypically GOT_M 2-1. GOT_M^1/GOT_M^1 homozygotes would presumably have only one main band, corresponding to the most cathodal band of the GOT_M 2-1 phenotype. There are no family data to support the proposed mode of inheritance, but the phenotypes and postulated inheritance are similar to GOT_M variation in Man (Davidson *et al.*, 1970) and *Mus musculus* (De Lorenzo and Ruddle, 1970). The *R. fuscipes* data also support the evidence that the supernatant and mitochondrial GOT isozymes are coded by genes at separate loci.

The observation of two heterozygotes at the GOT_M locus in seven *R. f. coracius* specimens (one of these two was the specimen of unknown locality) suggests that this polymorphism may be widespread in that subspecies. Johnson and Selander (1971) found that GOT_M is less variable than GOT_S in several North American rodent species they studied.

5.1.5 Glucose-6-phosphate dehydrogenase

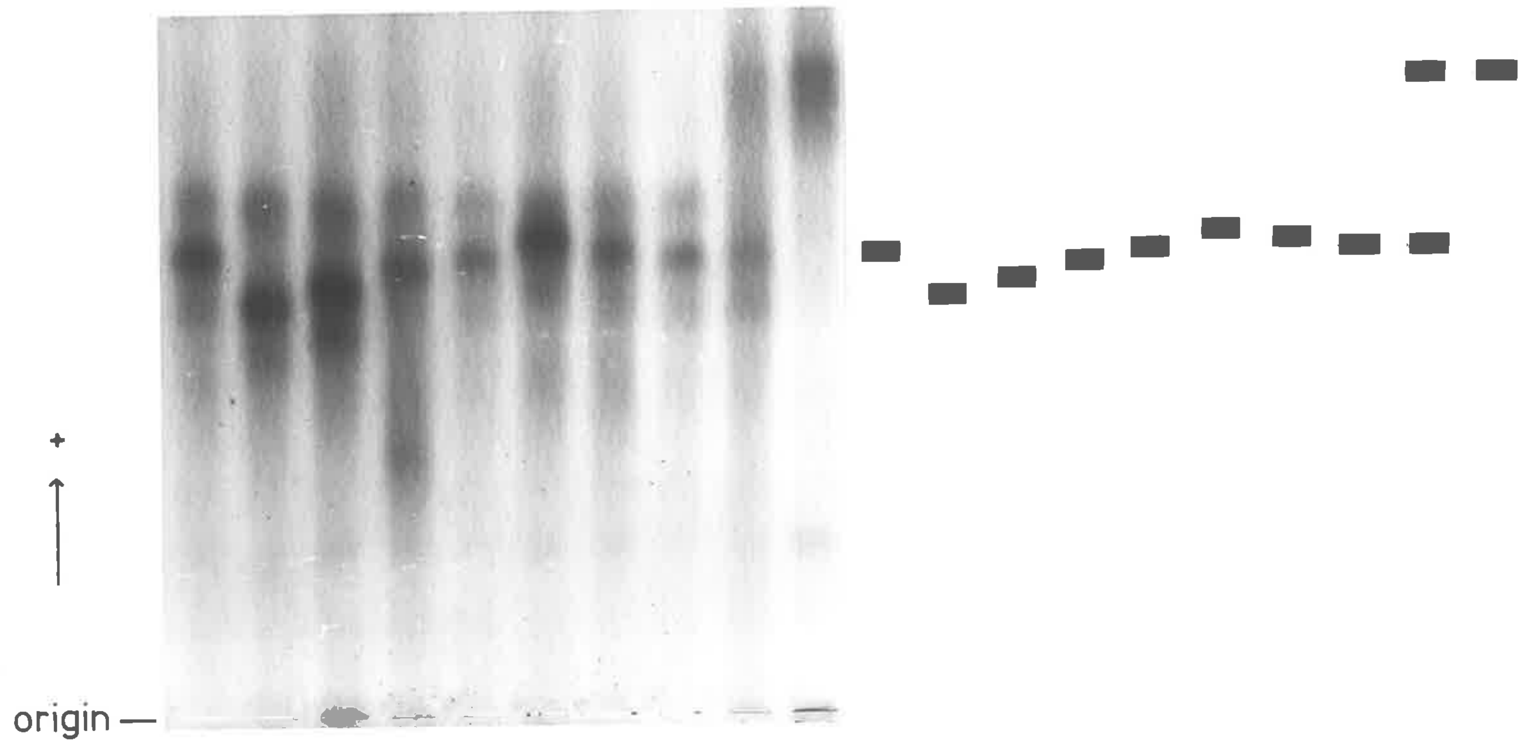
No variation was found in the electrophoretic mobility of G6PD. All individuals scored had a single band of G6PD activity. For the multivariate analyses it was presumed that all females were homozygous and males hemizygous for the same gene (presumably sex-linked) coding for G6PD.

5.1.6 Haemoglobin

Electrophoresis of *R. fuscipes* haemoglobin revealed considerable variation, particularly between populations. Ten phenotypes were found in natural populations and another eight in laboratory bred animals. Eight of the naturally occurring phenotypes are shown in Fig. 5.3. Each of the eighteen phenotypes consisted of one or two main bands and are shown in diagrammatic form in Fig. 5.4. Fresh samples had a weak subsidiary band, but on electrophoresis of samples stored for more than about two months, the main haemoglobin bands became weak and diffuse and bands both anodal and cathodal to the main bands appeared. HB A and HB D were difficult to distinguish, although they could be reliably scored if fresh samples were used and the two types run side by side. Only one HB A-D individual was observed and this phenotype appeared as a band which was virtually indistinguishable from HB A or HB D. The individual concerned was classified as HB A-D because of its parents' phenotypes (see Table 5.5) and HB A-D cannot be regarded as a distinctive phenotype. HB D was not recognised as a unique phenotype until after

Figure 5.3 Photograph and diagram of haemoglobin variation in natural populations of *Rattus fuscipes*.

Phenotypes from left to right are: HB A, HB P, HB K, HB Q, HB A, HB S, HB D, HB A, HB A-B, HB B.


+
origin

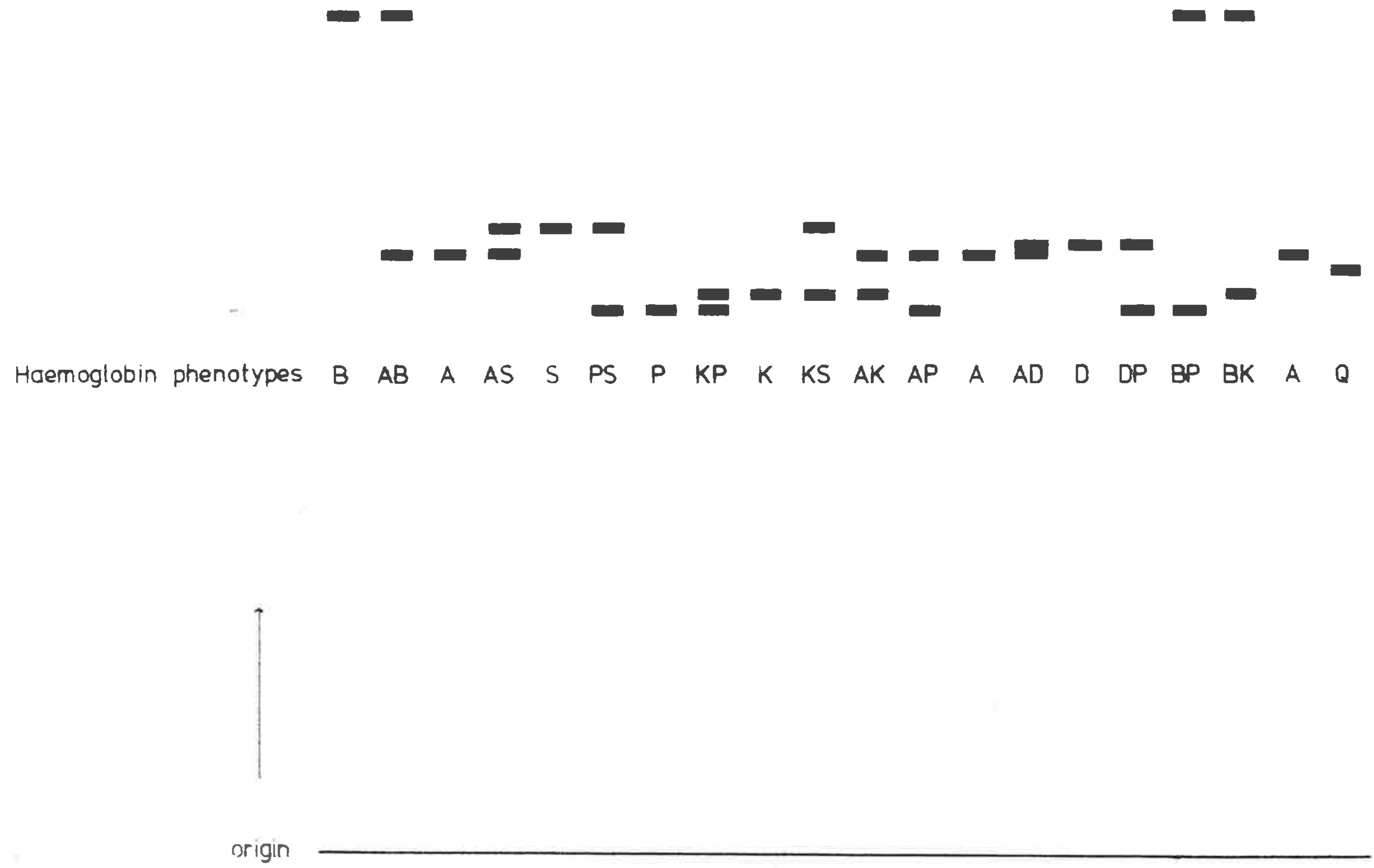


Figure 5.4 Diagram of the eighteen haemoglobin phenotypes found in *Rattus fuscipes*. Minor bands have been omitted for clarity.

most of the population and family data had been collected and it is possible that some individuals scored as HB A were in fact HB A-D or HB D. This problem will be discussed further, when the population data are presented.

Data on the inheritance of the haemoglobin phenotypes are presented in Table 5.5. Although information on only a small proportion of all possible matings has been collected, the data agree with the hypothesis that the variation is controlled by six autosomal codominant allelic genes. This does not include the HB Q phenotype for which no family data are available.

Two problems hinder determination of the mode of inheritance of the haemoglobin variation. First, it is difficult to determine the number of loci coding for haemoglobin polypeptides. In adult mammals, the haemoglobin molecule consists of two different polypeptides, α and β, which are coded by genes at separate genetic loci. In some species, genes at more than one locus code for either the α or β polypeptides (e.g. Man, Hollan *et al.*, 1972; echidna, Thompson *et al.*, 1973; sheep, Boyer *et al.*, 1967). The weak satellite band found in fresh samples may indicate that this situation exists in *R. fuscipes*. Alternatively, the satellite band may merely be another non-genetically determined molecular form of the main haemoglobin band. The phenotypic patterns and family data can not easily be accounted for by a multiplicity of α or β loci.

Second, if only one locus codes for each of the α and β polypeptides, then it is necessary to determine whether the observed variation is due to multiple allelism at one or both of these loci. Individuals heterozygous at just one of these loci should have a two-banded phenotype, while double heterozygotes should have four bands. This expectation is a result of the electrophoretic method and the rapid dissociation-association equilibrium between the haemoglobin tetramer and $\alpha\beta$ dimers

Table 5.5 Family data on the inheritance of haemoglobin phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀		Parental phenotypes ♂	Number of matings	Number and phenotypes of offspring	χ^2
HB A	x	HB A	2	6 HB A	
HB A	x	HB A*	19	41 HB A	
HB A	x	HB K	3	16 HB A-K	
HB A*	x	HB P	3	5 HB A-P	
HB P	x	HB A	3	24 HB A-P	
HB A	x	HB A-K	1	1 HB A + 4 HB A-K	
HB A	x	HB A-P	2	6 HB A + 3 HB A-P	
HB A-K	x	HB D*	1	1 HB A-D[1]	
HB P-S	x	HB D*	1	2 HB D-P	
HB K	x	HB P	1	10 HB K-P	
HB A-B	x	HB K	2	8 HB A-K + 3 HB B-K	2.27(1 d.f.)
HB P	x	HB P	2	28 HB P	
HB P	x	HB P*	2	3 HB P	
HB P	x	HB A-B	1	3 HB A-P	
HB A-B	x	HB P	1	2 HB A-P + 3 HB B-P	
HB S	x	HB S*	4	9 HB S	
HB S	x	HB A-K	1	3 HB A-S + 1 HB K-S	
HB S	x	HB A-P	1	1 HB P-S	
HB S*	x	HB A-P	1	1 HB A-S + 2 HB P-S	
HB A-B	x	HB A-B	1	1 HB A + 4 HB A-B + 1 HB B	
HB A-K	x	HB A-K	2	5 HB A + 4 HB A-K + 1 HB K	
HB A-K	x	HB A-P	2	2 HB A + 2 HB A-P	
HB A-P	x	HB A-P	3	5 HB A + 16 HB A-P + 4 HB P	2.04(2 d.f.)
HB K-P	x	HB K-P	1	3 HB K + 7 HB K-P + 5 HB P	

[1] See text for a discussion of this phenotype.

(Benesch *et al.*, 1966). While no individual appeared to have more than two bands, the resolution was insufficient to eliminate the possibility that some individuals actually had four main bands. Haemoglobin phenotypes HB A-D and HB K-P were particularly difficult to resolve. To compare populations it will be presumed that the variation is under the control of seven allelic genes, HB^{A}, HB^{B}, HB^{D}, HB^{K}, HB^{P}, HB^{Q} and HB^{S}. Unfortunately it was not possible to substantiate this mode of inheritance by using different methods (e.g. "finger-printing") to detect the phenotypes.

Haemoglobin variation found in natural populations is detailed in Table 5.6. Three populations, *R. f. fuscipes*, *R. f. assimilis* and Norton Summit, are polymorphic for haemoglobin. The HB A phenotype is the most widespread geographically, being found in nine populations (and is probably also present in *R. f. assimilis*). Four genes, HB^{B} (Norton Summit), HB^{D} (Dog Island), HB^{K} (Kangaroo Island) and HB^{Q} (*R. f. coracius*) are each found in only one population. The genotype frequencies in the Norton Summit population are in agreement with the Hardy-Weinberg expectations ($\chi_1^2 = 0.587$). The HB^{A} and HB^{S} genes appear to be fairly widespread, being found in the three most southern sub-species. Because the haemoglobin phenotypes were difficult to score if the samples were stored for more than about two months (and especially if the mobility differences were small), it was not possible to check old samples against HB D, which was found after most of the family and population data had been collected. However, one or two individuals from each population of type HB A or their offspring were checked against HB D and found to be HB A. Nevertheless it is possible that the HB^{D} gene is present in samples of populations scored as type HB A.

Table 5.6 Haemoglobin phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers										Gene frequencies						
	HB A	HB A-B	HB B	HB D	HB K	HB P	HB A-P	HB Q	HB S	HB A-S	HB^A	HB^B	HB^D	HB^K	HB^P	HB^Q	HB^S
R. f. fuscipes	1	-	-	-	-	-	2	-	2	1	0.42	-	-	-	0.17	-	0.42
Dog I.	-	-	-	26	-	-	-	-	-	-	-	-	1.00	-	-	-	-
Goat I.	-	-	-	-	-	39	-	-	-	-	-	-	-	-	1.00	-	-
Eyre I.	1	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Pearson I. North	-	-	-	-	-	50	-	-	-	-	-	-	-	-	1.00	-	-
Pearson I. South	-	-	-	-	-	24	-	-	-	-	-	-	-	-	1.00	-	-
Greenly I.	48	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Waldegrave I.	47	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Williams I.	50	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Hopkins I.	29	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
North Gambier I.	49	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Eyre Peninsula	30	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
Kangaroo I.	-	-	-	-	48	-	-	-	-	-	-	-	-	1.00	-	-	-
Norton Summit	19	41	10	-	-	-	-	-	-	-	0.56	0.44	-	-	-	-	-
South-East	-	-	-	-	-	-	-	-	41	-	-	-	-	-	-	-	1.00
R. f. assimilis	-	-	-	-	-	-	-	-	11	*	*	-	-	-	-	-	1.00*
R. f. coracius	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	1.00	-

* A *R. f. assimilis* female which was not typed for haemoglobin produced a litter which included some HB A-S individuals.

5.1.7 Isocitrate dehydrogenase

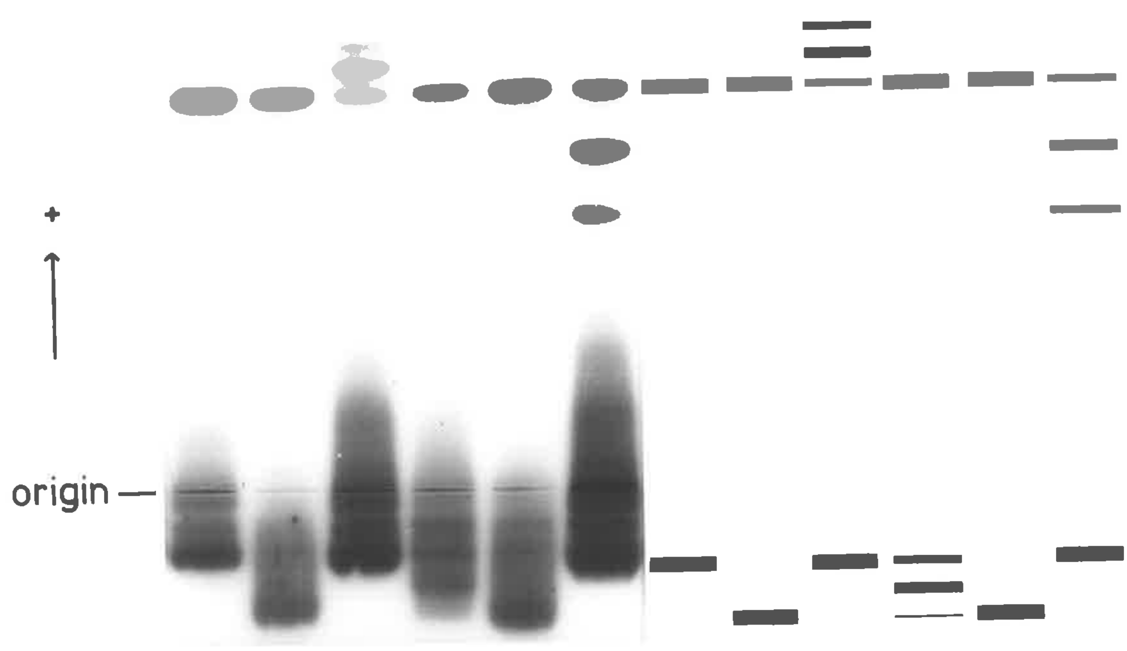
Electrophoresis of all tissue extracts revealed two distinct ICD regions, one migrating anodally, the other cathodally (Fig. 5.5). No NAD-dependent ICD activity was recovered from the tissues examined. On fractionation of liver cells, the anodal isozyme was found to be predominant in the supernatant fraction, but no ICD activity was recovered from the mitochondrial fraction. The reason for the absence of ICD activity in the mitochondrial fraction is not known, but it is possibly due to a low concentration of ICD in liver mitochondria (Bell and Baron, 1968). It will be presumed, by analogy with other mammals such as Man (van Heyningen *et al.*, 1973), *Mus musculus* (Henderson, 1965) and *Rattus norvegicus* (Bell and Baron, 1968) that the anodal isozyme is the supernatant form and the cathodal isozyme the mitochondrial form. It should be noted that these isozymes are not located exclusively in the cytoplasm or mitochondria, but the designated localities reflect the site in the cell where they are predominantly found (Henderson, 1968). The two ICD isozymes, which are coded by non-allelic autosomal genes (Henderson, 1965; Chen *et al.*, 1972), have been shown to differ in characteristics such as their optimum pH and kinetic properties (Plaut, 1963).

5.1.7.1 Supernatant isocitrate dehydrogenase

Three distinct ICD_S phenotypes were observed, ICD_S 1, which has a single band of activity and ICD_S 2-1 and ICD_S 3-1 which have three bands (Fig. 5.5).

Apart from specimens of *R. f. coracius*, all individuals examined were phenotypically ICD_S 1. One specimen of *R. f. coracius* was ICD_S 2-1, two were ICD_S 3-1, and the other four ICD_S 1. From the electrophoretic patterns it is presumed that the variation is controlled by three autosomal codominant alleles, ICD_S^1, ICD_S^2 and ICD_S^3, the three genotypes

Figure 5.5 Photograph and diagram of isocitrate dehydrogenase variation in *Rattus fuscipes*. ICD_S phenotypes from left to right are: ICD_S 1, ICD_S 1, ICD_S 3-1, ICD_S 1, ICD_S 1, ICD_S 2-1. ICD_M phenotypes from left to right are: ICD_M 2, ICD_M 1, ICD_M 2, ICD_M 2-1, ICD_M 1, ICD_M 2.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with their respective phenotypes in parenthesis being ICD_S^1/ICD_S^1 (ICD_S 1), ICD_S^1/ICD_S^2 (ICD_S 2-1) and ICD_S^1/ICD_S^3 (ICD_S 3-1). There are no breeding data to support this mode of inheritance, although about fifty matings of the type ICD_S 1 x ICD_S 1 gave all ICD_S 1 offspring. The three-banded phenotype of postulated heterozygotes is the same as supernatant ICD variation in Man (Chen *et al.*, 1972), *Mus musculus* (Henderson, 1965) and *Peromyscus maniculatus* (Selander *et al.*, 1971) and indicates the active enzyme is a dimer.

5.1.7.2 Mitochondrial isocitrate dehydrogenase

The ICD_M phenotypes are shown in Fig. 5.5. Three phenotypes were observed, ICD_M 1 and ICD_M 2 each with one main band, and ICD_M 2-1 with three bands, two corresponding to the bands of the other two phenotypes and a third band of intermediate mobility. One or two minor bands appeared between the least cathodal main band and the origin. Fresh tissue extracts could not be scored reliably because an unidentified protein migrated with about the same mobility as the ICD_M 2 band. This enzyme appeared even if the substrate (disodium isocitrate) was omitted from the staining mixture and is presumed not to be an ICD. If samples were frozen and thawed two or three times, the activity of this enzyme was negligible and the ICD_M phenotypes could then be easily seen and reliably scored.

Only a small amount of family data on the inheritance of the ICD_M phenotypes is available (Table 5.7). It is proposed that the variation is under the control of two autosomal codominant alleles, which have been designated ICD_M^1 and ICD_M^2. The three phenotypes, ICD_M 1, ICD_M 2-1 and ICD_M 2 are presumed to correspond to the genotypes ICD_M^1/ICD_M^1, ICD_M^1/ICD_M^2 and ICD_M^2/ICD_M^2 respectively. The three banded phenotype of heterozygotes is similar to the supernatant ICD heterozygotes and indicates the active enzyme is a dimer. The homodimer product of the ICD_M^1 gene (ICD_M 1) has

Table 5.7 Family data on the inheritance of ICD_M phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀		♂	Number of matings	Offspring phenotypes		
				ICD_M 1	ICD_M 2-1	ICD_M 2
ICD_M 2-1	x	unknown[1]	1	1	3	1
ICD_M 2-1	x	ICD_M 2-1	1	–	–	2
ICD_M 2	x	ICD_M 2	25	–	–	157
ICD_M 2	x	ICD_M 2*	3	–	–	5
ICD_M 2*	x	ICD_M 2	2	–	–	5

[1] The female was collected from the Eyre Peninsula population and was pregnant when caught. The male is presumed to be heterozygous.

considerably less ICD_M activity than the ICD_M^2 gene product. Consequently, in heterozygous individuals, the ratio of the activity of the three bands is not 1:2:1. Instead, the most cathodal band is barely visible, but it is obviously present because of the "streaky" background staining (see Fig. 5.5).

The ICD_M gene frequencies in the sampled populations are given in Table 5.8. Most populations are monomorphic for the ICD_M^2 gene, but four *R. f. greyii* populations are polymorphic with both the ICD_M^1 and ICD_M^2 genes present. Three of these populations are widely distributed while the other, Waldegrave Island, occupies about 220 ha. This latter population has a relatively large number of heterozygotes. To test for agreement with the genotypic expectations based on the Hardy-Weinberg principle, Fisher's exact test (Fisher, 1973) was used, yielding a probability of 0.045. In the samples from the other three polymorphic populations, no ICD_M 1 individuals and only a few ICD_M 2-1 individuals were found. The fixation index within each of these samples (F_{IS}) was calculated and tested for agreement with zero (Kirby, 1975). Each gave a small negative value for F_{IS}, with an overall mean of -0.08 ($0.05<P<0.1$), indicating a non-significant excess of heterozygotes.

Excluding Waldegrave Island and Kangaroo Island, all populations are homogeneous with respect to ICD_M gene frequencies when compared pairwise. Waldegrave Island has a significantly higher ICD_M^1 gene frequency than all other populations. The ICD_M gene frequencies on Kangaroo Island are significantly different from those in all other populations except Eyre Peninsula and Norton Summit.

5.1.8 Lactate dehydrogenase

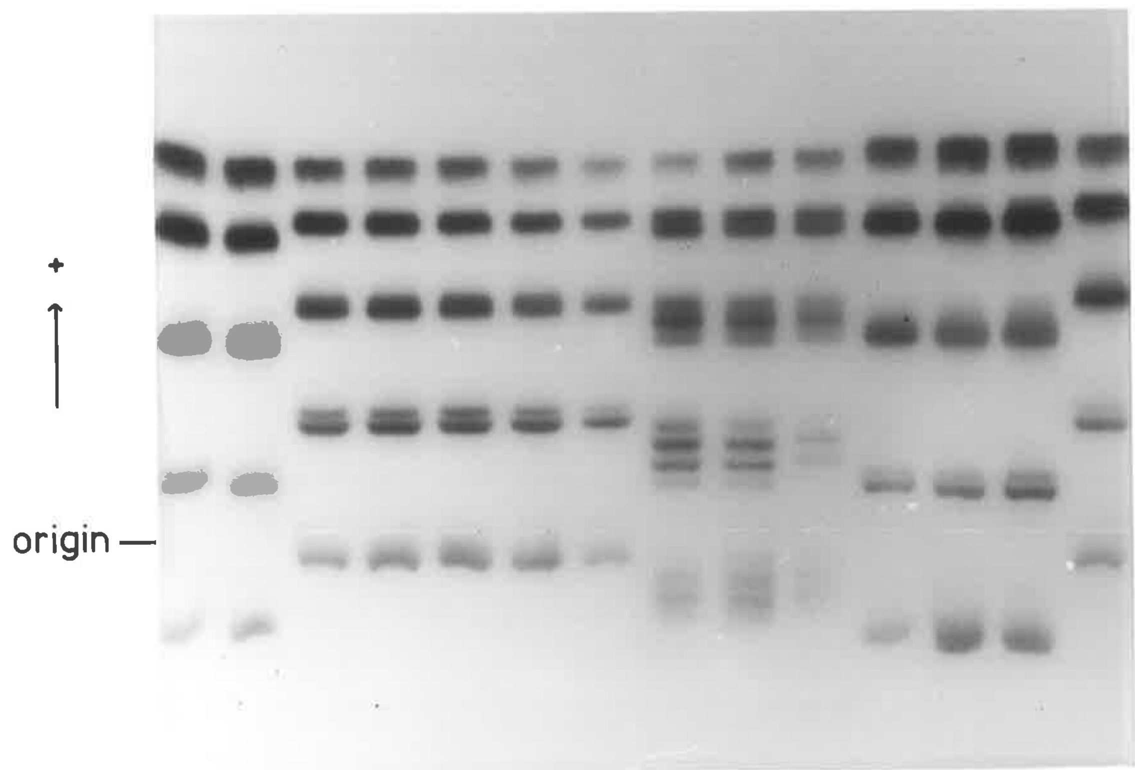
Electrophoresis of kidney extracts yielded electrophoretic patterns shown in Figs. 5.6 and 5.7. Mammalian LDH is a tetrameric enzyme consisting of two different subunits, A and B, which combine to produce

Table 5.8 Mitochondrial isocitrate dehydrogenase phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers			Gene frequencies	
	ICD_M 1	ICD_M 2-1	ICD_M 2	ICD_M^1	ICD_M^2
R. f. fuscipes	-	-	6	-	1.00
Dog I.	-	-	26	-	1.00
Goat I.	-	-	39	-	1.00
Eyre I.	-	-	1	-	1.00
Pearson I. North	-	-	24	-	1.00
Pearson I. South	-	-	50	-	1.00
Greenly I.	-	-	48	-	1.00
Waldegrave I.	3	28	16	0.36	0.64
Williams I.	-	-	49	-	1.00
Hopkins I.	-	-	30	-	1.00
North Gambier I.	-	-	49	-	1.00
Eyre Peninsula	-	2	17	0.05	0.95
Kangaroo I.	-	11	37	0.11	0.89
Norton Summit	-	3	39	0.04	0.96
South-East	-	-	41	-	1.00
R. f. assimilis	-	-	4	-	1.00
R. f. coracius	-	-	7	-	1.00

Figure 5.6 Photograph of lactate dehydrogenase variation in *Rattus fuscipes*. The LDH_B phenotypes are all LDH_B 1. The LDH_A phenotypes from left to right are: LDH_A 1, LDH_A 1, LDH_A 2, LDH_A 2, LDH_A 2, LDH_A 2, LDH_A 2, LDH_A 2-1, LDH_A 2-1, LDH_A 2-1, LDH_A 1, LDH_A 1, LDH_A 1, LDH_A 2.

Figure 5.7 Photograph of lactate dehydrogenase variation in *Rattus fuscipes*. The LDH_B phenotypes are all LDH_B 1 except the third and seventh samples from the left, which are LDH_B 2-1. The LDH_A phenotypes from left to right are: LDH_A 2, LDH_A 3-2, LDH_A 2, LDH_A 1, LDH_A 2-1, LDH_A 2, LDH_A 2, LDH_A 2, LDH_A 3-2, LDH_A 2, LDH_A 2-1, LDH_A 1.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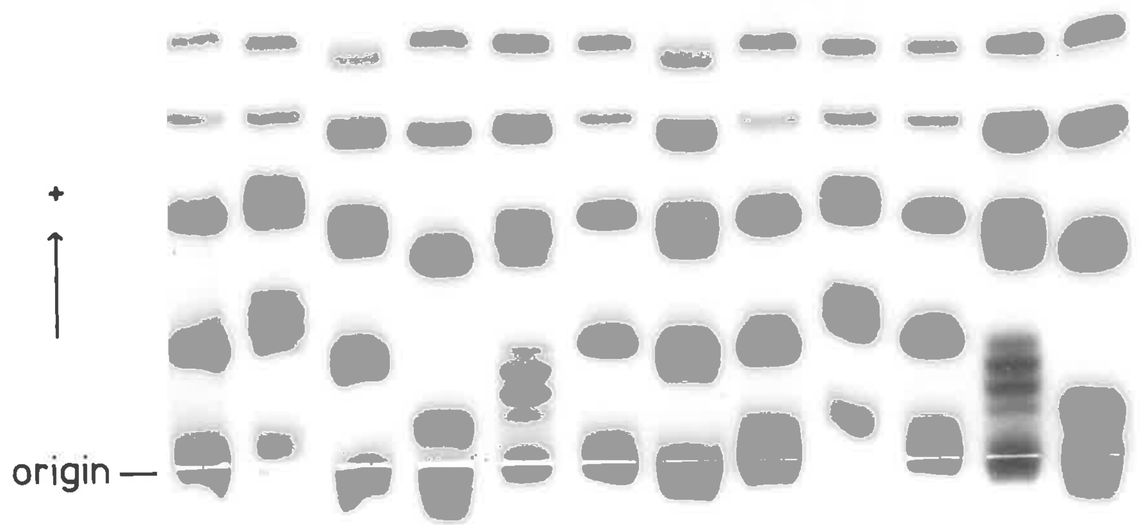
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five electrophoretically distinct forms (Markert, 1968). The five isozymes will be referred to as LDH 1 (B_4), LDH 2 (AB_3), LDH 3 (A_2B_2), LDH 4 (A_3B) and LDH 5 (A_4) with decreasing anodal mobility. Variation in the B subunit will result in changes to LDH 1, LDH 2, LDH 3 and LDH 4 but not LDH 5 and similarly, variation in the A subunit will be detected in all forms except LDH 1.

In mammalian species, the two subunits are coded by non-allelic autosomal genes (e.g. *Peromyscus maniculatus*, Shaw and Barto, 1963; Man, Das *et al.*, 1970). The gene coding the B subunit (the LDH 1 homotetramer) will be designated LDH_B and that coding the A subunit (LDH 5 homotetramer) will be referred to as LDH_A. These two genes are not linked in Man (Boone and Ruddle, 1969). Genetic variation in a regulatory gene controlling the amount of LDH 4 isozyme in *Mus musculus* has been reported (Shows and Ruddle, 1968a).

During the first two years of this study, the five LDH isozymes appeared after electrophoresis as shown in Fig. 5.6, with patterns like those found in most other mammalian species. However, since that time, the LDH 5 isozyme has appeared as a diffuse, more anodal band in all samples and the marked variation previously detected in this isozyme was virtually indistinguishable (Fig. 5.7). No change in the four other isozymes was detected. It is not known what caused this change in LDH 5, but it is presumably a reflection of the differential physical and chemical properties of the LDH isozymes (see Wilkinson, 1970 for a review) and subtle changes in the storage or electrophoretic conditions. Fortunately the A subunit variation is also reflected in the mobility of three other LDH isozymes and it was possible to continue scoring all the LDH phenotypes.

Satellite LDH bands were observed, especially for the LDH 2, LDH 3 and LDH 4 isozymes. These may possibly be due to the presence or absence of bound coenzymes or they may be conformational rearrangements (Wilkinson,

1970; Markert, 1968) such as have been observed in other mammals (e.g. *Peromyscus maniculatus*, Shaw and Barto, 1963).

5.1.8.1 Lactate dehydrogenase: B subunit

Variation in the B subunit is shown in Fig. 5.7. Individuals possessing two different B subunits (*LDH_B* heterozygotes) will have five LDH 1 bands, four LDH 2 bands, three LDH 3 bands, two LDH 4 bands and one LDH 5 band. Two phenotypes were observed, the most common involving a single LDH 1 band (LDH_B 1). The other phenotype (LDH_B 2-1) was a result of two different B subunits. The LDH 3 and LDH 4 isozymes clearly consisted of three and two bands respectively, but the mobility differences were not great enough to distinguish the four and five bands expected of the LDH 2 and LDH 1 isozymes. It is proposed that the variation is under the control of two autosomal codominant genes LDH_B^1 and LDH_B^2 such that the phenotypes LDH_B 1 and LDH_B 2-1 correspond to the genotypes LDH_B^1/LDH_B^1 and LDH_B^1/LDH_B^2 respectively. Except for two *R. f. fuscipes* individuals which were LDH_B 2-1, all animals examined were LDH_B 1. No breeding data are available for *R. f. fuscipes* to substantiate the postulated mode of inheritance. The two LDH_B 2-1 individuals were collected from points over 200 km apart, which suggests that this polymorphism may be widespread in *R. f. fuscipes*.

5.1.8.2 Lactate dehydrogenase: A subunit

Four phenotypes, reflecting variation in the A subunit were detected (Figs. 5.6 and 5.7). Three of these phenotypes, LDH_A 1, LDH_A 2-1 and LDH_A 2 show the classic banding patterns of LDH variation controlled by two codominant allelic genes. The family data presented in Table 5.9 support this mode of inheritance and the genotypes corresponding to the three phenotypes are LDH_A^1/LDH_A^1, LDH_A^1/LDH_A^2 and LDH_A^2/LDH_A^2. A fourth phenotype, LDH_A 3-2 is presumed to be due to a third codominant

Table 5.9 Family data on the inheritance of LDH_A phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀		♂	Number of matings	Offspring phenotypes LDH_A 1	LDH_A 2-1	LDH_A 2	χ^2_1
LDH_A 1	x	LDH_A 1	12	45	-	-	
LDH_A 1	x	LDH_A 1*	5	25	-	-	
TOTAL			17	70	-	-	
LDH_A 1	x	LDH_A 2-1	2	3	2	-	
LDH_A 1*	x	LDH_A 2-1	1	1	2	-	
LDH_A 2-1	x	LDH_A 1	1	3	5	-	
TOTAL			4	7	9	-	0.250
LDH_A 1	x	LDH_A 2	6	-	27	-	
LDH_A 1*	x	LDH_A 2	2	-	5	-	
LDH_A 2	x	LDH_A 1	2	-	11	-	
TOTAL			10	-	43	-	
LDH_A 2-1	x	LDH_A 2-1	1	1	8	4	
LDH_A 2-1	x	LDH_A 2	2	-	15	8	
LDH_A 2-1	x	LDH_A 2*	2	-	2	1	
LDH_A 2	x	LDH_A 2-1	1	-	2	1	
TOTAL			5	-	19	10	2.793
LDH_A 2	x	LDH_A 2	8	-	-	56	
LDH_A 2	x	LDH_A 2*	2	-	-	3	
TOTAL			10	-	-	59	

allele, LDH_A^3, heterozygous with LDH_A^2. No breeding data are available for the LDH_A^3 gene. The LDH_A 3-2 phenotype was unusual in that the LDH 2 isozyme did not have two bands, as expected. Instead, LDH 2 consisted of one main band and a very weak second band which did not appear to migrate at the rate expected of the second band. This is presumably a satellite band. It is not known why the second LDH 2 band was not detected in the LDH_A 3-2 phenotype.

The frequencies of the LDH_A genes in *R. fuscipes* populations are shown in Table 5.10. The LDH_A^3 gene was found in only one individual, from the *R. f. fuscipes* subspecies. Apart from the *R. f. fuscipes* population, Norton Summit is the only population polymorphic at the LDH_A locus. In this population the genotypic frequencies are in agreement with those expected by the Hardy-Weinberg principle ($\chi_1^2 = 0.039$). All other populations are monomorphic for either LDH_A^1 or LDH_A^2. There is significant heterogeneity in LDH_A gene frequencies between *R. f. greyii* populations ($\chi_{12}^2 = 968.27$, $P<<0.001$). Norton Summit is significantly different, with respect to LDH_A gene frequencies, from all other populations and each population with LDH_A^1 has a significantly different LDH_A gene frequency from those with LDH_A^2.

5.1.9 Malate dehydrogenase

Two regions of MDH activity were observed on gels, one migrating towards the anode, the other to the cathode. Fractionation of liver cells revealed the presence of both isozymes in the supernatant fraction, although the cathodal isozyme was barely detectable. Mitochondria contained only the cathodal isozyme. The localisation of the isozymes is similar to that found in pig heart by Thorne *et al.* (1963). The supernatant form (anodal) could not be resolved into sharp bands and so no phenotypic variation was recorded. The mitochondrial form (MDH_M) could be resolved into five bands, the most cathodal being the most

Table 5.10 Lactate dehydrogenase (A subunit) phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers				Gene frequencies		
	LDH_A 1	LDH_A 2-1	LDH_A 2	LDH_A 3-2	LDH_A^1	LDH_A^2	LDH_A^3
R. f. fuscipes	-	-	5	1	-	0.92	0.08
Dog I.	-	-	36	-	-	1.00	-
Goat I.	-	-	39	-	-	1.00	-
Eyre I.	1	-	-	-	1.00	-	-
Pearson I. North	-	-	50	-	-	1.00	-
Pearson I. South	-	-	24	-	-	1.00	-
Greenly I.	48	-	-	-	1.00	-	-
Waldegrave I.	47	-	-	-	1.00	-	-
Williams I.	50	-	-	-	1.00	-	-
Hopkins I.	30	-	-	-	1.00	-	-
North Gambier I.	49	-	-	-	1.00	-	-
Eyre Peninsula	30	-	-	-	1.00	-	-
Kangaroo I.	48	-	-	-	1.00	-	-
Norton Summit	16	28	11	-	0.55	0.45	-
South-East	41	-	-	-	1.00	-	-
R. f. assimilis	39	-	-	-	1.00	-	-
R. f. coracius	7	-	-	-	1.00	-	-

intense and a slow moving anodal band being the weakest. This banding pattern is similar to that found by Thorne *et al.* (1963) and Kitto *et al.* (1966a,b) in *Sus scrofa*, *Gallus gallus* and *Neothunnus macropterus*.

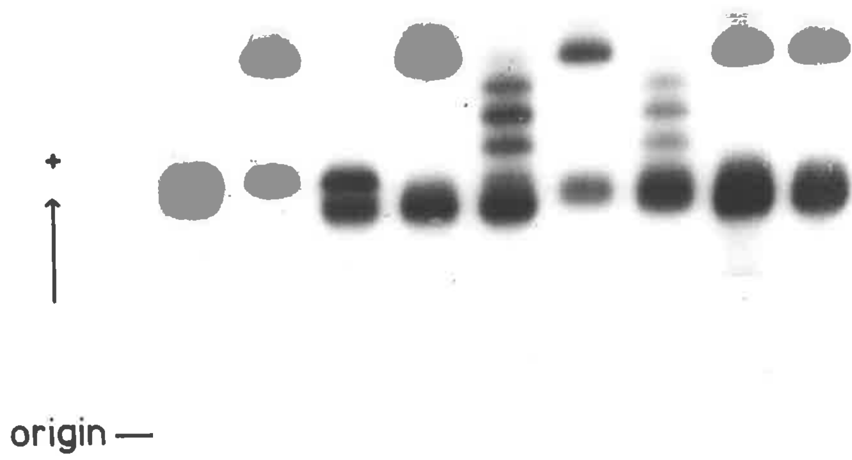
All individuals were recorded as having the same MDH_M phenotype. Animals from the Pearson Islands had an MDH_M which appeared to migrate at a slightly different rate but this difference was extremely difficult to detect and hybrids between the two forms were phenotypically indistinguishable from either of their parents. Consequently for the purposes of this thesis, this difference has been disregarded.

5.1.10 Malic enzyme

The results of electrophoresis of *R. fuscipes* heart tissue followed by staining for ME activity are shown in Fig. 5.8. The weak enzyme activity seen at the anodal end of the figure is ICD_S. Two overlapping regions of ME activity were seen on gels and within both regions there was variation between individuals. Three phenotypes of the more anodal isozyme (ME_S) were observed, two consisting of a single band (ME_S 1 and ME_S 2) and a third consisting of five bands (ME_S 2-1). The electrophoretic mobility of the other ME (ME_M) was about the same or slower than the band of ME_S 2 individuals. ME_M generally did not resolve into sharp bands and was often low in activity. Most of the ME_M activity was lost after about a month of storage or if samples were frozen and thawed two or three times. The appearance of ME_M in Fig. 5.8 is much darker and sharper than that generally observed. Family segregation data and cellular localisation data confirm that the two isozymes are different.

The supernatant fraction of liver cells contained only the anodal (ME_S) isozyme. Mitochondrial extracts had no ME activity. However, it is presumed by analogy with other mammalian species that ME_M is a mitochondrially bound enzyme. Its absence in *R. fuscipes* mitochondrial extracts may be partly due to its poor stability. Two ME's have been

Figure 5.8 Photograph of malic enzyme variation in *Rattus fuscipes*. The ME_S phenotypes from left to right are: ME_S 2, ME_S 1, ME_S 2, ME_S 1, ME_S 2-1, ME_S 1, ME_S 2-1, ME_S 1, ME_S 1. The two most extreme bands of the ME_S 2-1 phenotype are very weak.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found in *Mus musculus* (Henderson, 1968; Shows *et al.*, 1970), Man (Cohen and Omenn, 1972b) and the monkey *Macaca nemestrina* (Cohen and Omenn, 1972a). In *Mus musculus* the mitochondrial form is the more anodal, but in Man and *Macaca nemestrina* the supernatant form migrates more rapidly to the anode than the mitochondrial enzyme. The difference in the electrophoretic mobilities of the two ME's is not as marked as with the two forms of GOT, ICD or MDH in *R. fuscipes*.

Most individuals had ME_M phenotypes like the third, fourth and fifth samples in Fig. 5.8, while Hopkins Island animals (sample two in Fig. 5.8) had an ME_M band which migrated at about the same rate as the band of ME_S 2 individuals. Hybrids with parents of different ME_M phenotypes had bands of intermediate mobility (samples six and seven in Fig. 5.8). However, because of the difficulties mentioned previously, the ME_M phenotype of many individuals could not be reliably scored and this system was not used in any further analyses.

Variation in supernatant ME has previously been reported in other mammals (e.g. *Mus musculus*, Shows and Ruddle, 1968b; *Macaca nemestrina*, Cohen and Omenn, 1972a). Family data presented in Table 5.11 suggest that the variation in *R. fuscipes* ME_S is controlled by two autosomal codominant alleles, which have been designated ME_S^1 and ME_S^2. The three phenotypes, ME_S 1, ME_S 2-1 and ME_S 2 are presumed to correspond with the genotypes ME_S^1/ME_S^1, ME_S^1/ME_S^2 and ME_S^2/ME_S^2 respectively. The electrophoretic pattern of variation in *Mus musculus* (Shows and Ruddle, 1968b) and Man (Povey *et al.*, 1975) is similar to the pattern found in *R. fuscipes* and suggests a tetrameric structure for the active enzyme.

The ME_S gene frequencies in *R. fuscipes* populations are shown in Table 5.12. The four geographically extensive populations of *R. f. greyii* are all polymorphic for ME_S. In three of these populations, Eyre Peninsula, Kangaroo Island and Norton Summit, the genotypic numbers are in agreement with the expectations based on the Hardy-Weinberg

Table 5.11 Family data on the inheritance of ME_S phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀	♂	Number of matings	Offspring phenotypes ME_S 1	ME_S 2-1	ME_S 2	χ^2
ME_S 1 x	ME_S 1	4	21	-	-	
ME_S 1 x	ME_S 2-1	5	5	9	-	
ME_S 2-1 x	ME_S 1	2	9	3	-	
TOTAL		7	14	12	-	0.154(1 d.f.)
ME_S 1 x	ME_S 2	2	-	17	-	
ME_S 1* x	ME_S 2	2	-	5	-	
ME_S 2 x	ME_S 1	4	-	24	-	
TOTAL		8	-	46	-	
ME_S 2-1 x	ME_S 2-1	4	8	22	8	0.947(1 d.f.)
ME_S 2-1 x	ME_S 2	1	-	2	1	
ME_S 2-1 x	ME_S 2*	1	-	-	1	
ME_S 2 x	ME_S 2-1	2	-	2	2	
TOTAL		4	-	4	4	
ME_S 2 x	ME_S 2	2	-	-	32	
ME_S 2 x	ME_S 2*	3	-	-	5	
TOTAL		5	-	-	37	

Table 5.12 Supernatant malic enzyme phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers			Gene frequencies	
	ME_S 1	ME_S 2-1	ME_S 2	ME_S^1	ME_S^2
R. f. fuscipes	–	–	6	–	1.00
Dog I.	–	–	26	–	1.00
Goat I.	–	–	39	–	1.00
Eyre I.	–	–	1	–	1.00
Pearson I. North	–	–	50	–	1.00
Pearson I. South	–	–	24	–	1.00
Greenly I.	48	–	–	1.00	–
Waldegrave I.	–	–	47	–	1.00
Williams I.	–	–	50	–	1.00
Hopkins I.	30	–	–	1.00	–
North Gambier I.	49	–	–	1.00	–
Eyre Peninsula	15	4	–	0.89	0.11
Kangaroo I.	43	5	–	0.95	0.05
Norton Summit	–	15	26	0.18	0.82
South-East	8	10	23	0.32	0.68
R. f. assimilis	5	–	–	1.00	–
R. f. coracius	7	–	–	1.00	–

principle (χ_1^2 = 0.497, 1.169 and 0.830 respectively), but there is a significant deficiency of heterozygotes in the South-East population (χ_1^2 = 5.94, 0.025>P>0.01). This deficiency is probably not due to the pooling of samples from different areas in the South-East population, since most of the sample (32 individuals out of 42) was collected from within an area of only a few hectares with no obvious physical barriers to animal movements. This area was a plantation of *Pinus radiata* and the deficiency of heterozygous individuals from this area was also significant (χ_1^2 = 5.56, 0.025>P>0.01). Apart from these four polymorphic populations, all other populations of *R. fuscipes* were monomorphic for either the ME_S^1 or ME_S^2 gene. The results of the pairwise tests for homogeneity of ME_S gene frequencies are shown in Table 5.13. Populations on the left-hand side of the table are listed in order of increasing frequency of the ME_S^1 gene.

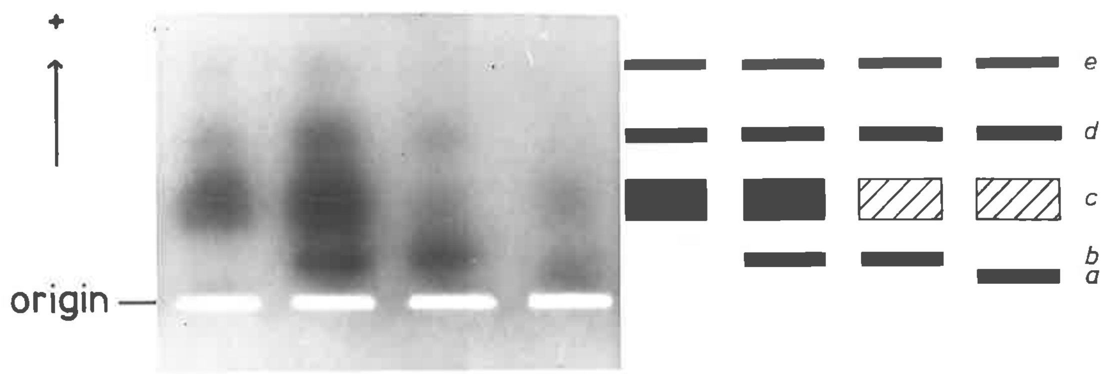
5.1.11 Phosphoglucomutase

The four PGM electrophoretic phenotypes of *R. fuscipes* are shown in Fig. 5.9. These patterns are explicable if it is presumed that genes at two loci code for distinct PGM's, as is found in Man (Spencer *et al.*, 1964; Hopkinson and Harris, 1966). (In Man there is a third, more anodal PGM system and a similar system was seen in *R. fuscipes*. However, it only appeared very faintly on gels.) It is proposed that one PGM system (PGM_2), which is invariant in *R. fuscipes*, results in three bands of activity (*c*, *d*, *e*; Fig. 5.9). A second PGM system (PGM_1) involves the less anodal bands *a* and *b*, as well as *c*. In Fig. 5.9, the third and fourth samples from the left only stain faintly and do not show the more anodal bands of the PGM_2 system. Soon after the staining mixture was added, band *c* appeared as two distinct bands (for PGM_1 1 and PGM_1 2-1 individuals) but these bands quickly merged to appear as a single large band. Thus it is proposed that for PGM_1, individuals have either the

Table 5.13 Results of pair-wise comparisons of populations for homogeneity of ME_S gene frequencies. An '*' indicates a significant ($P<0.05$) difference in gene frequency while 'NS' is a non-significant difference. The samples from *R. f. fuscipes*, *R. f. assimilis*, *R. f. coracius* and Eyre Island have been omitted because of the small number of animals recorded.

	Dog I.	Goat I.	Pearson I. North	Pearson I. South	Waldegrave I.	Williams I.	Norton Summit	South-East	Eyre Peninsula	Kangaroo I.	Greenly I.	Hopkins I.
Dog I.												
Goat I.	NS											
Pearson I. North	NS	NS										
Pearson I. South	NS	NS	NS									
Waldegrave I.	NS	NS	NS	NS								
Williams I.	NS	NS	NS	NS	NS							
Norton Summit	*	*	*	*	*	*						
South-East	*	*	*	*	*	*	NS					
Eyre Peninsula	*	*	*	*	*	*	*	*				
Kangaroo I.	*	*	*	*	*	*	*	*	NS			
Greenly I.	*	*	*	*	*	*	*	*	*	NS		
Hopkins I.	*	*	*	*	*	*	*	*	*	NS	NS	
North Gambier I.	*	*	*	*	*	*	*	*	*	NS	NS	NS

Figure 5.9 Photograph and diagram of phosphoglucomutase variation in *Rattus fuscipes*. The PGM_1 phenotypes from left to right are: PGM_1 1, PGM_1 2-1, PGM_1 2, PGM_1 3.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c band (PGM_1 1) or bands *b* and *c* (PGM_1 2-1 and PGM_1 2). It was easy to distinguish two phenotypes both with the *b* and *c* bands, one with the *c* band slightly stronger than the *b* band (PGM_1 2-1) and the other where the *b* band was much stronger than the *c* band (PGM_1 2). The PGM_1 3 phenotype has the *a* and *c* bands with the former stronger than the latter. The variation is similar to that found in Man (Spencer *et al.*, 1964), *Peromyscus polionotus* (Selander *et al.*, 1971) and *Mus musculus* (Selander *et al.*, 1969).

It is proposed that the PGM_1 phenotypes are under the control of three autosomal codominant allelic genes, PGM_1^1, PGM_1^2 and PGM_1^3. The phenotypes, with their corresponding genotypes in parenthesis, are PGM_1 1 (PGM_1^1/PGM_1^1), PGM_1 2-1 (PGM_1^1/PGM_1^2), PGM_1 2 (PGM_1^2/PGM_1^2) and PGM_1 3 (PGM_1^3/PGM_1^3). Family data supporting the mode of inheritance proposed for the first three phenotypes are shown in Table 5.14. There are no family data involving the PGM_1 3 phenotype, of which only one specimen was found.

Population data presented in Table 5.15 show the geographic distribution of the PGM_1 phenotypes. Kangaroo Island was the only population found to be polymorphic and the PGM_1 genotypic frequencies in that population are in agreement with the expectations based on the Hardy-Weinberg principle ($\chi_1^2 = 0.041$). The one individual sampled from Eyre Island had the PGM_1 3 phenotype and is the only record of this phenotype. All other individuals scored were PGM_1^1/PGM_1^1 homozygotes. The PGM_1 gene frequencies on Kangaroo Island are significantly different from all other *R. f. greyii* populations except Eyre Peninsula. Eyre Island is significantly different from all other populations (using the exact method for 2 x 2 contingency tables).

The PGM_2 system was not scored as an invariant system because many of the samples were not scored until they had been stored for a considerable time, and therefore it was difficult to type reliably for PGM_2.

Table 5.14 Family data on the inheritance of PGM_1 phenotypes. Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀	Parental phenotypes ♂	Number of matings	Offspring phenotypes PGM_1 1	Offspring phenotypes PGM_1 2-1	Offspring phenotypes PGM_1 2	X_1^2
PGM_1 1 x	PGM_1 1	21	135	-	-	
PGM_1 1* x	PGM_1 1	3	8	-	-	
PGM_1 1 x	PGM_1 1*	15	51			
TOTAL		39	194	-	-	
PGM_1 1 x	PGM_1 2-1	4	10	16	-	
PGM_1 2-1 x	PGM_1 1	2	1	3	-	
TOTAL		6	11	19	-	2.133
PGM_1 2-1 x	unknown[1]	1	1	3	1	

[1] This male was an offspring of a PGM_1 1 x PGM_1 2-1 mating and is presumably PGM_1 2-1.

Table 5.15 Phosphoglucomutase-1 phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers				Gene frequencies		
	PGM_1 1	PGM_1 2-1	PGM_1 2	PGM_1 3	PGM_1^1	PGM_1^2	PGM_1^3
R. f. fuscipes	6	-	-	-	1.00	-	-
Dog I.	26	-	-	-	1.00	-	-
Goat I.	39	-	-	-	1.00	-	-
Eyre I.	-	-	-	1	-	-	1.00
Pearson I. North	50	-	-	-	1.00	-	-
Pearson I. South	24	-	-	-	1.00	-	-
Greenly I.	48	-	-	-	1.00	-	-
Waldegrave I.	47	-	-	-	1.00	-	-
Williams I.	49	-	-	-	1.00	-	-
Hopkins I.	30	-	-	-	1.00	-	-
North Gambier I.	49	-	-	-	1.00	-	-
Eyre Peninsula	15	-	-	-	1.00	-	-
Kangaroo I.	37	9	1	-	0.88	0.12	-
Norton Summit	29	-	-	-	1.00	-	-
South-East	41	-	-	-	1.00	-	-
R. f. assimilis	4	-	-	-	1.00	-	-
R. f. coracius	7	-	-	-	1.00	-	-

5.1.12 6-Phosphogluconate dehydrogenase

Four 6PGD phenotypes were detected in natural populations of *R. fuscipes* (Fig. 5.10). Three phenotypes had single bands of activity (6PGD 1, 6PGD 2 and 6PGD 3) and a fourth had three bands (6PGD 2-1). Similar 6PGD variation has been detected in *Felis catus* (Thuline *et al.*, 1967), the marsupial mouse, *Sminthopsis crassicaudata* (Cooper and Hope, 1971) and *Rattus norvegicus* (Carter and Parr, 1969).

It is proposed that the 6PGD variation in *R. fuscipes* is under the control of three autosomal codominant allelic genes, $6PGD^1$, $6PGD^2$ and $6PGD^3$. Family data on the inheritance of the 6PGD 1, 6PGD 2-1 and 6PGD 2 phenotypes support the proposed mode of inheritance (Table 5.16). Heterozygotes have three bands of 6PGD activity indicating that the active enzyme is a dimer. In general, the band of 6PGD 2 individuals was weaker than the band of 6PGD 1 individuals. Also, the most anodal band of heterozygotes was stronger than the least anodal band. This difference is not obvious in Fig. 5.10, since the 6PGD 1 sample represented there was older than the other samples and had lost much of its activity.

Population data are presented in Table 5.17. The South-East population is polymorphic for 6PGD, with both the $6PGD^1$ and $6PGD^2$ alleles present. The genotypic frequencies in this population are in agreement with the expectations based on the Hardy-Weinberg principle (χ^2_1 = 0.038). The 6PGD 3 phenotype was found in one *R. f. coracius* individual (locality unrecorded); all other *R. f. coracius* sampled were $6PGD^2/6PGD^2$ homozygotes. Most populations are monomorphic for the 6PGD 1 phenotype. The *6PGD* gene frequencies in the South-East population are significantly different from the frequencies in the other *R. f. greyii* populations.

Figure 5.10 Photograph of 6-phosphogluconate dehydrogenase variation in *Rattus fuscipes*. Phenotypes from left to right are: 6PGD 2, 6PGD 3, 6PGD 2-1, 6PGD 2, 6PGD 2-1, 6PGD 1.

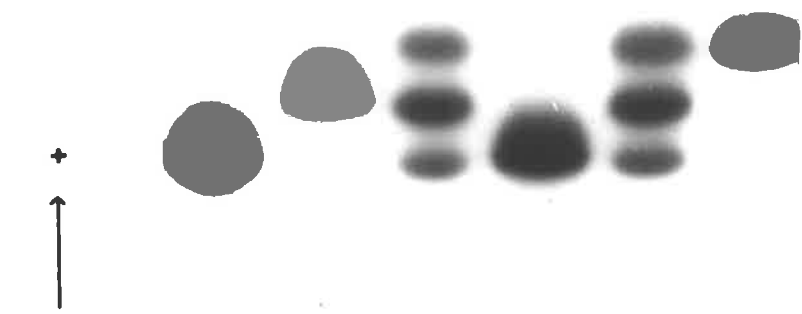

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Table 5.16 Family data on the inheritance of 6PGD phenotypes.

Presumed phenotypes are marked with an asterisk (see Table 5.1).

Parental phenotypes ♀		♂	Number of matings	Offspring phenotypes 6PGD 1	6PGD 2-1	6PGD 2
6PGD 1	x	6PGD 1	25	160	-	-
6PGD 1*	x	6PGD 1	1	2	-	-
6PGD 1	x	6PGD 1*	15	57	-	-
TOTAL			41	219	-	-
6PGD 2-1	x	6PGD 1	1	1	3	-
6PGD 2-1	x	6PGD 1*	1	1	1	-
TOTAL			2	2	4	-
6PGD 2	x	6PGD 1	1	-	1	-
6PGD 2*[1]	x	6PGD 1	1	-	3	-
TOTAL			2	-	4	-
6PGD 2	x	unknown[2]	2	-	-	5
6PGD 2	x	unknown[2]	1	-	1	2
6PGD 2	x	unknown[2]	1	-	1	-

[1] The mother and two siblings of this individual were 6PGD 2; the father was from the South-East population (see Table 5.15).

[2] These males were all from the South-East population (see Table 5.15).

Table 5.17 6-Phosphogluconate dehydrogenase phenotype numbers and gene frequencies in *R. fuscipes* populations.

Population	Phenotype numbers				Gene frequencies		
	6PGD 1	6PGD 2-1	6PGD 2	6PGD 3	$6PGD^1$	$6PGD^2$	$6PGD^3$
R. f. fuscipes	6	-	-	-	1.00	-	-
Dog I.	26	-	-	-	1.00	-	-
Goat I.	39	-	-	-	1.00	-	-
Eyre I.	1	-	-	-	1.00	-	-
Pearson I. North	50	-	-	-	1.00	-	-
Pearson I. South	24	-	-	-	1.00	-	-
Greenly I.	47	-	-	-	1.00	-	-
Waldegrave I.	47	-	-	-	1.00	-	-
Williams I.	50	-	-	-	1.00	-	-
Hopkins I.	30	-	-	-	1.00	-	-
North Gambier I.	49	-	-	-	1.00	-	-
Eyre Peninsula	18	-	-	-	1.00	-	-
Kangaroo I.	48	-	-	-	1.00	-	-
Norton Summit	40	-	-	-	1.00	-	-
South-East	1	6	34	-	0.10	0.90	-
R. f. assimilis	-	-	5	-	-	1.00	-
R. f. coracius	-	-	6	1	-	0.86	0.14*

* see text for a discussion

5.1.13 Superoxide dismutase

Two regions of SOD activity were observed on gels, one migrating to the anode and one to the cathode. The resolution of the cathodal isozyme was poor and no phenotypes were recorded. The anodal isozyme (SOD_1) appeared as a single invariant band of activity. For the purpose of the multivariate analyses it will be considered that this invariance reflects the presence of only one gene, SOD_1^1 coding for the primary amino-acid sequence of SOD_1.

5.2 SUMMARY OF GEOGRAPHIC VARIATION

A summary of the gene frequencies for all loci and all populations is given in Table 5.18. The geographic distribution of the electrophoretic variation can be summarised as follows:

Four enzymes, ACP, G6PD, MDH_M and SOD_1 show no variation within or between populations.

Of the remaining twelve proteins, four, ES, GOT_M, ICD_S and LDH_B, do not vary within *R. f. greyii*, the only extensively studied subspecies, although they show some variation within either *R. f. fuscipes* or *R. f. coracius*. There is very little information on the extent of the variation in subspecies other than *R. f. greyii*, although there is some evidence that the LDH_B polymorphism is geographically widespread in *R. f. fuscipes*.

Eight other proteins show variation within or between *R. f. greyii* populations. With three of these proteins, PGM_1, GOT_S and 6PGD, most populations are monomorphic for the same phenotype but one or two populations contain another phenotype. Most populations are monomorphic for the PGM_1^1 allele, but the Kangaroo Island population has the PGM_1^2 allele as well, while a third allele, PGM_1^3 is probably fixed in the Eyre Island population (Fig. 5.11a). Apart from the Pearson Islands, all *R. f. greyii* populations are monomorphic for the GOT_S^A gene. The

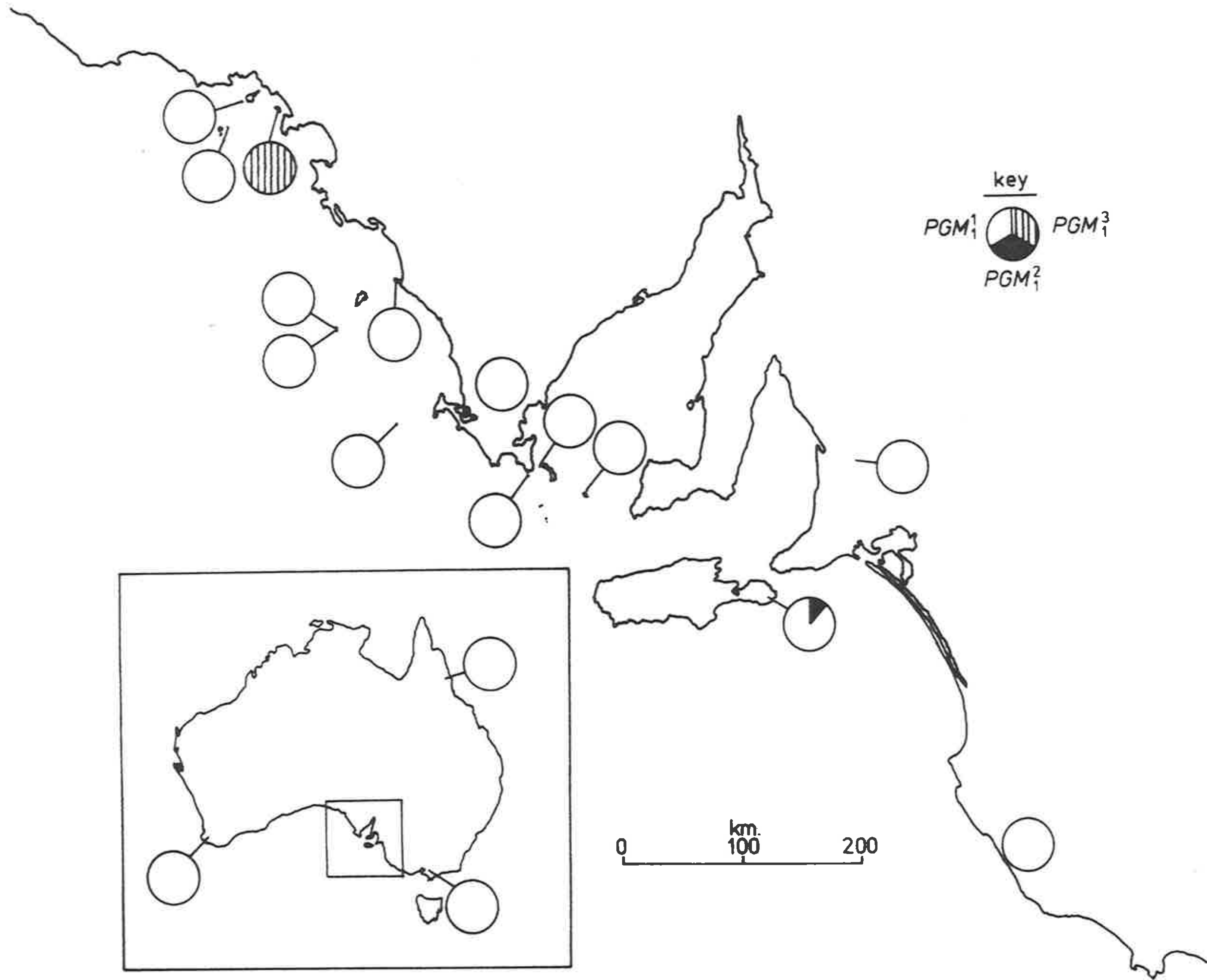
Table 5.18 Approximate sample sizes and gene frequencies in populations of *R. fuscipes*. A dash indicates the gene was not detected in that population.

Locus	Population / Allele	RFF	DI	GOI	EI	PIN	PIS	GRI	WGI	WMI	HI	NGI	EP	KI	NS	SE	RFA	RFC
	Sample size	6	27	39	1	50	24	48	47	50	30	49	19	48	42	41	5	7
ACP	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ALB	F	1.00	-	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-
	S	-	1.00	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ES	1	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.14
	0	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.86
GOT_S	A	1.00	1.00	1.00	1.00	-	0.62	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.60†	1.00
	B	-	-	-	-	1.00	0.38	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40†	-
GOT_M	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.86
G6PD	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HB	A	0.42	-	-	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00	-	0.50	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	-	-
	D	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	K	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-
	P	0.17	-	1.00	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-
	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
	S	0.42	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00*	-
ICD_S	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.79
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14
ICD_M	1	-	-	-	-	-	-	-	0.36	-	-	-	0.05	0.11	0.04	-	-	-
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00	1.00	0.95	0.89	0.96	1.00	1.00	1.00
LDH_B	1	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDH_A	1	-	-	-	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.61	1.00	1.00	1.00
	2	0.92	1.00	1.00	-	1.00	1.00	-	-	-	-	-	-	-	0.39	-	-	-
	3	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDH_M	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ME_S	1	-	-	-	-	-	-	1.00	-	-	1.00	1.00	0.89	0.95	0.18	0.32	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	-	-	0.11	0.05	0.82	0.68	-	-
PGM_1	1	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00	1.00	1.00
	2	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-
	3	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
6PGD	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.10	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.90	1.00	0.86
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.14
SOD_1	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

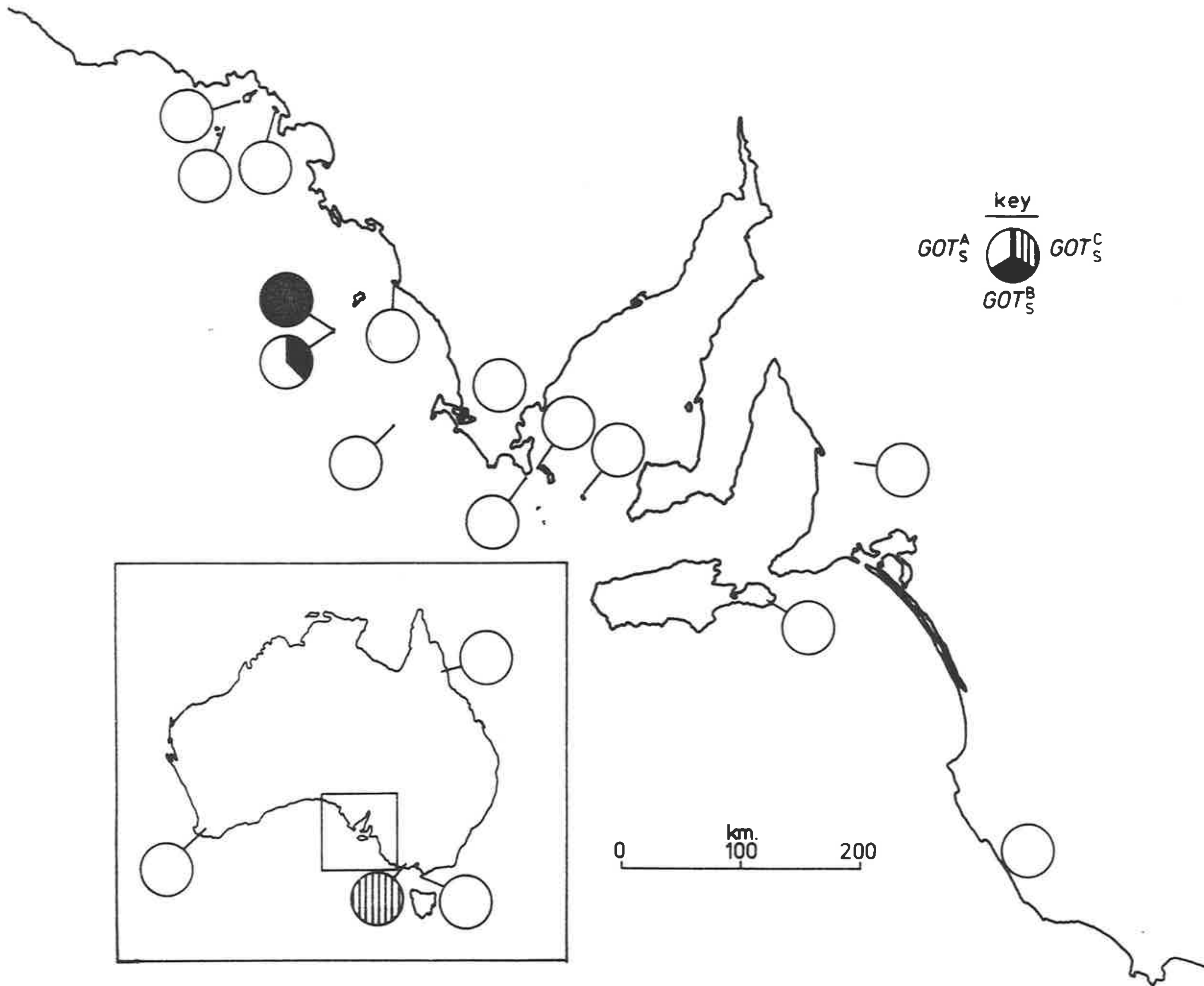
† The three individuals from Glennie Island were homozygous for GOT_S^A. The two mainland animals were homozygous for GOT_S^C.

* Offspring of a *R. f. assimilis* female, with an unknown haemoglobin phenotype, showed segregation for HB^A and HB^S.

Figure 5.11 Pie diagrams showing gene frequencies in samples from different populations of *Rattus fuscipes*. (a) PGM_1, (b) GOT_S, (c) *6PGD*, (d) ICD_M, (e) ME_S, (f) LDH_A, (g) *ALB*, (h) *HB*.



5.11 a



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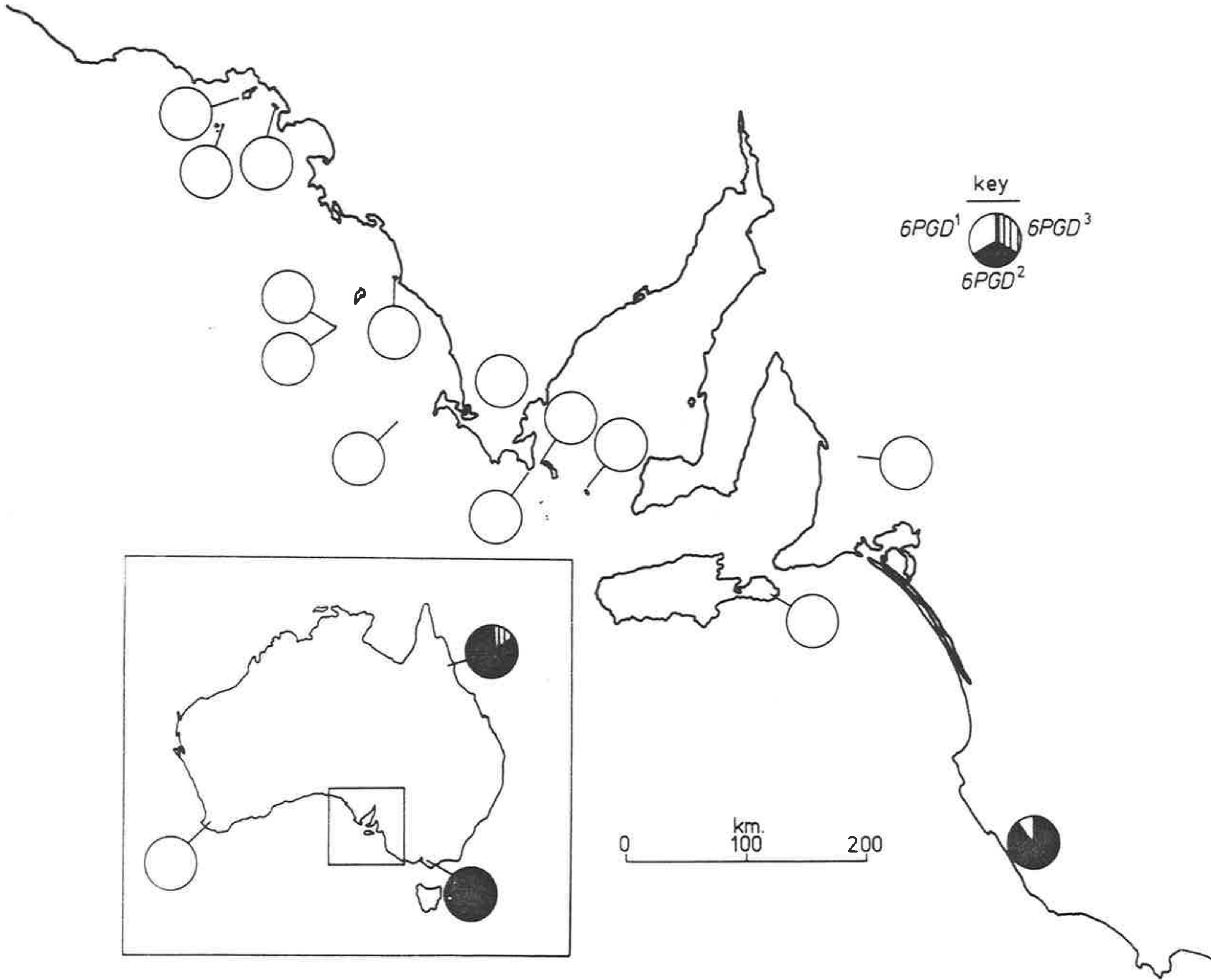

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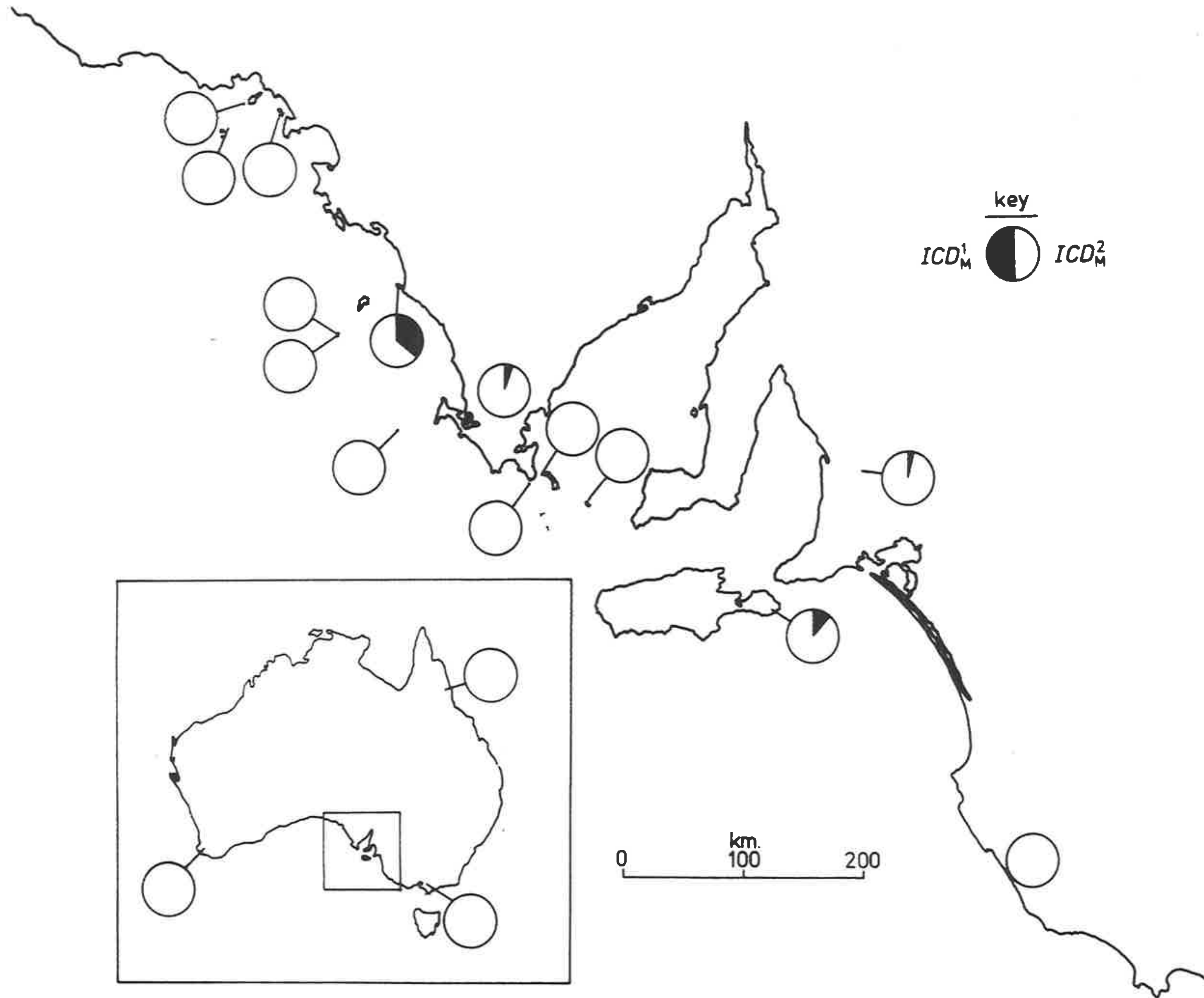

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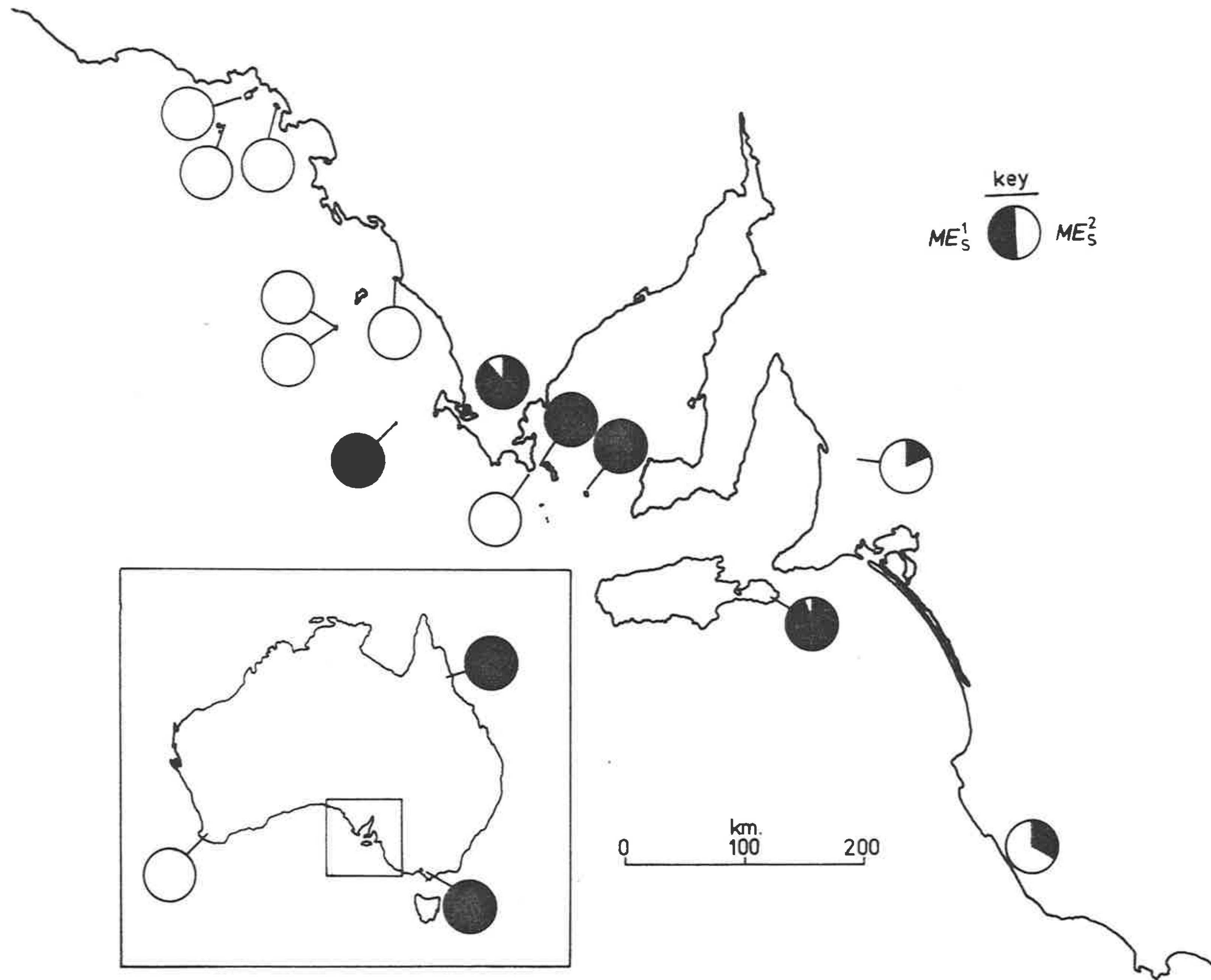

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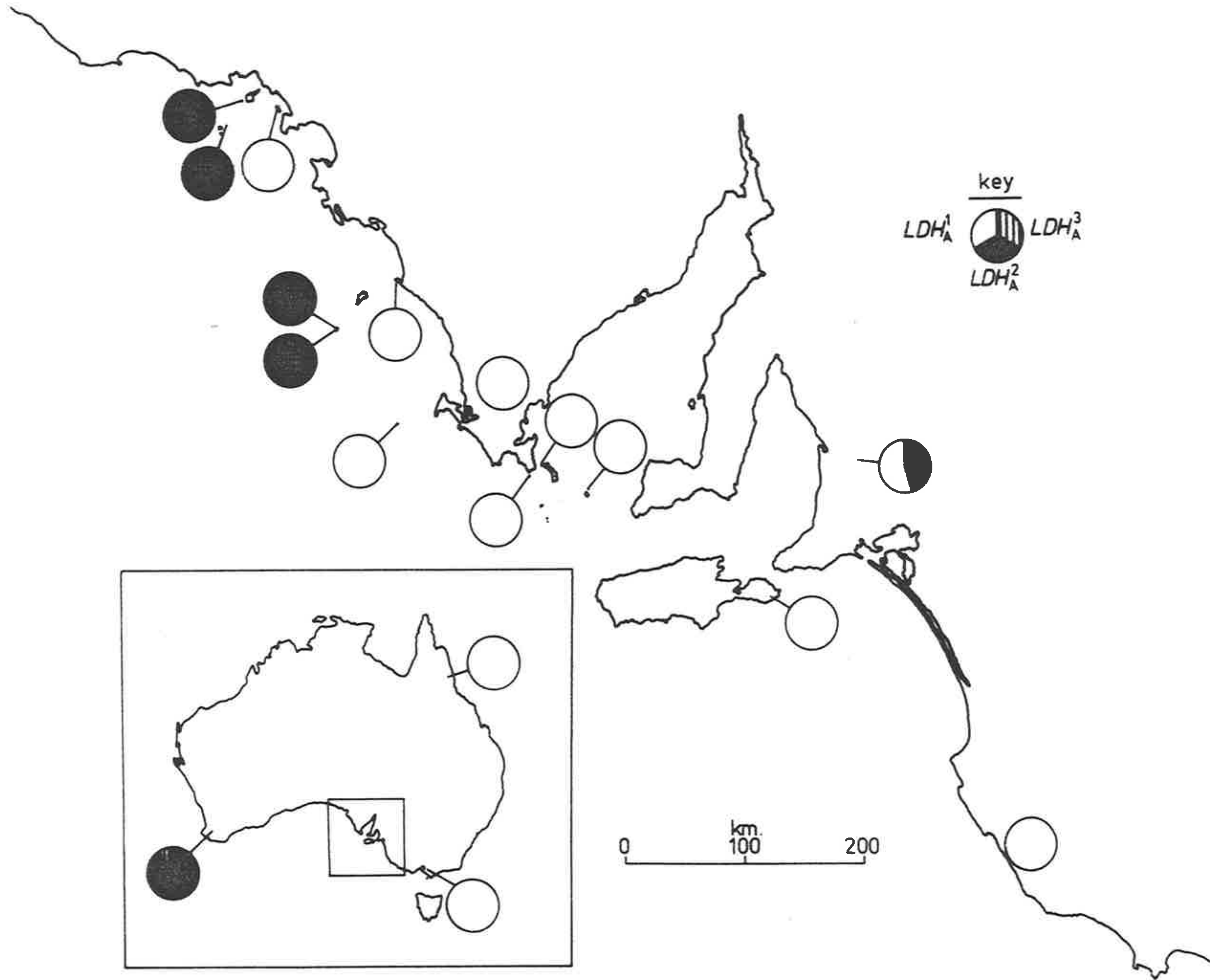
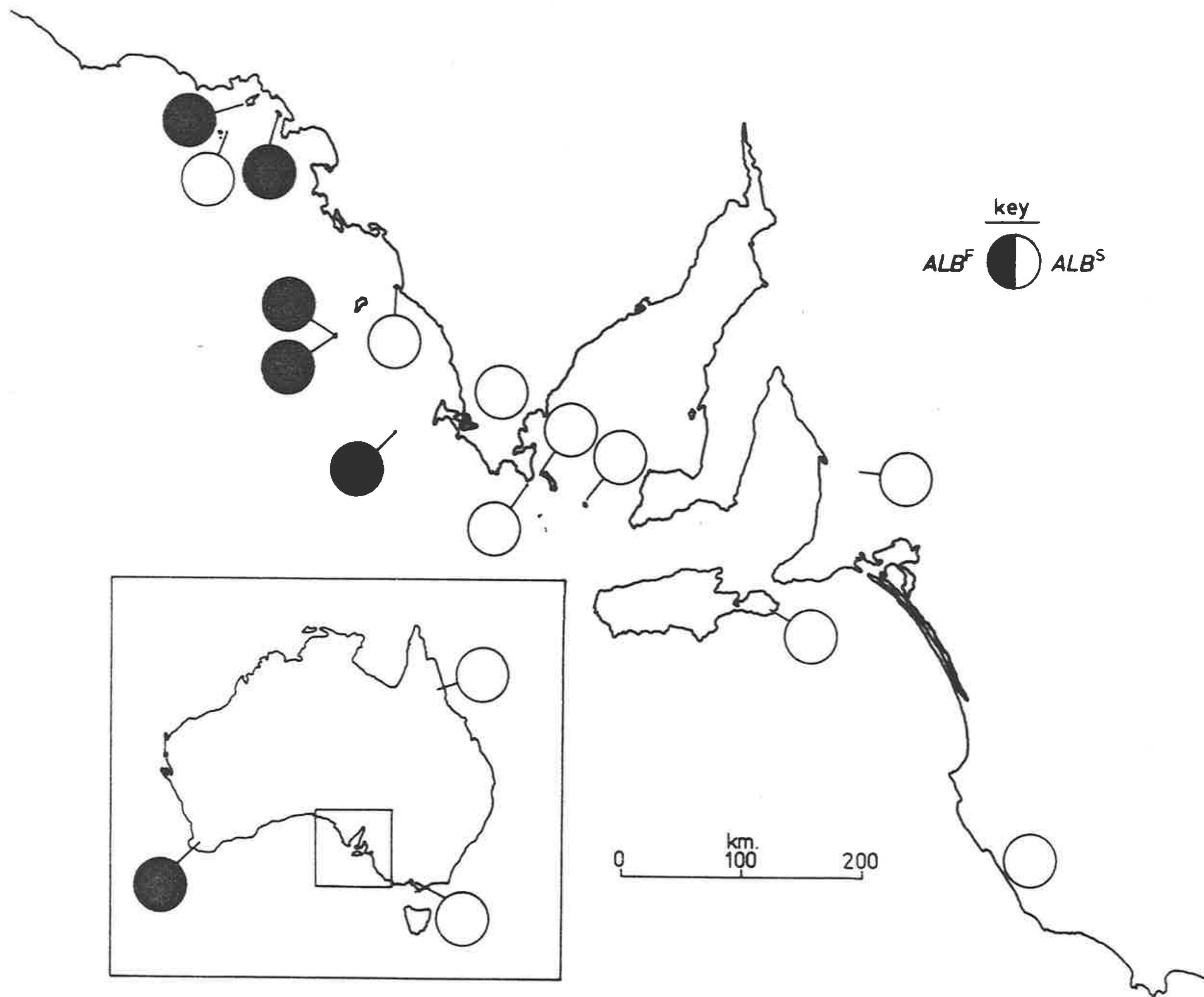
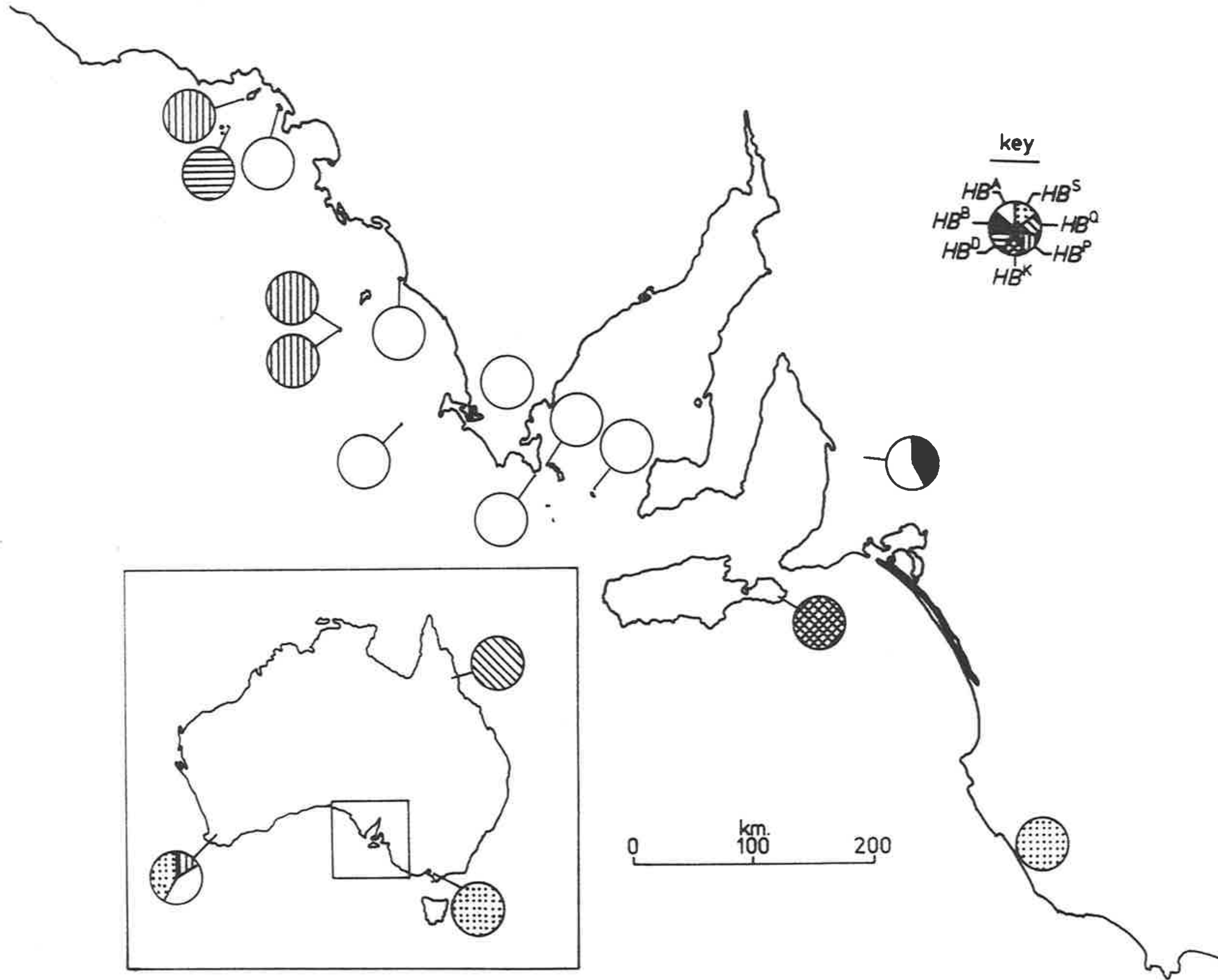


5.11f



5.11g



5.11h

GOT_S^B gene is found only in the two Pearson Island populations (Fig. 5.11b). A third gene, GOT_S^C is present in mainland *R. f. assimilis* samples. All individuals from *R. f. greyii* populations are $6PGD^1/6PGD^1$ homozygotes except some from the South-East where another allele, $6PGD^2$ is also present (Fig. 5.11c). The $6PGD^2$ gene is also found in the two eastern subspecies, together with a third gene, $6PGD^3$ in *R. f. coracius*.

Five proteins, ICD_M, ME_S, ALB, LDH_A and HB show extensive variation in *R. f. greyii*. The mitochondrial form of ICD is polymorphic in three of the four widespread populations as well as on Waldegrave Island (Fig. 5.11d). The ICD_M^1 gene is less frequent than its allele, ICD_M^2, in these polymorphic populations and ICD_M^2 is fixed in all other populations. The ME_S enzyme is polymorphic in all widespread *R. f. greyii* populations whilst all small island populations are fixed for one of two alleles, as are the samples from the other subspecies (Fig. 5.11e). At the LDH_A locus, each population, except Norton Summit and *R. f. fuscipes* which are polymorphic, is fixed for one of two alleles (Fig. 5.11f). ALB is similar in that all populations are monomorphic for one or other of two types (Fig. 5.11g). Four of the seven HB "alleles", HB^B, HB^D, HB^K and HB^Q are each restricted to one population, while HB^A, HB^P and HB^S are each found in several populations (Fig. 5.11h).

A perusal of 6PGD, ME_S, LDH_A and ALB variation in *R. f. greyii* (Figs. 5.11c, 5.11e, 5.11f and 5.11g) show that generally, the most common genes in the north-western populations are also the most common genes in *R. f. fuscipes* and the most common genes in south-eastern populations are the common genes in *R. f. assimilis* and *R. f. coracius*.

5.3 ESTIMATES OF VARIATION WITHIN POPULATIONS

Table 5.19 shows the number of polymorphic loci and mean heterozygosities in each population. It should be stressed that the estimates for the Eyre Island, *R. f. fuscipes*, *R. f. assimilis* and *R. f. coracius* populations are each based on a maximum of seven specimens (with exceptions for some loci for *R. f. assimilis*) and these variability estimates must be treated with particular caution.

Each of the large *R. f. greyii* populations (Eyre Peninsula, Kangaroo Island, Norton Summit and South-East) are polymorphic at two to four loci with mean heterozygosity values in the range between 0.020 to 0.104 (mean 0.045). Two small island populations, Pearson Island South and Waldegrave Island, have one polymorphic locus, while within in all other small islands no variation was detected. The average mean heterozygosity within all small island populations of *R. f. greyii* (except Eyre Island) is 0.007. Excluding the four populations with small sample sizes, the following table illustrates the greater proportion of polymorphic loci in widespread populations compared with populations occupying small islands:

		Number of proteins	
		polymorphic	monomorphic
Population area	large	11	53
	small	2	142

An interesting feature of the average heterozygosity estimates is that in the two small island populations which have polymorphic proteins (Pearson Island South and Waldegrave Island), the proportion of heterozygotes for the polymorphic loci is generally high (0.417 and 0.596 respectively) compared with the large populations (see the right-hand column of Table 5.19). As a result of this, these two small populations

Table 5.19 Estimates of genetic variation in *R. fuscipes*, based on 16 proteins. The standard deviations of mean heterozygosity were calculated according to the method of Nei and Roychoudhury (1974).

Population	Approximate sample size	Polymorphic proteins		Average heterozygosity	
		Number	Proportion	Per locus ± S.D.	Per polymorphic locus
R. f. fuscipes	6	3	0.19	0.063 ± 0.037	0.333
Dog I.	27	0	-	-	-
Goat I.	39	0	-	-	-
Eyre I.	1	0	-	-	-
Pearson I. North	50	0	-	-	-
Pearson I. South	24	1	0.06	0.026 ± 0.026	0.417
Greenly I.	48	0	-	-	-
Waldegrave I.	47	1	0.06	0.037 ± 0.037	0.596
Williams I.	50	0	-	-	-
Hopkins I.	30	0	-	-	-
North Gambier I.	49	0	-	-	-
Eyre Peninsula	19	2	0.13	0.020 ± 0.014	0.158
Kangaroo I.	48	3	0.19	0.033 ± 0.019	0.175
Norton Summit	42	4	0.25	0.104 ± 0.055	0.415
South-East	41	2	0.13	0.024 ± 0.017	0.195
R. f. assimilis	5	2*	0.13	-	-
R. f. coracius	7	4	0.25	0.031 ± 0.023	0.250†

* One "polymorphism" was a difference between mainland and Glennie Island individuals (GOT_S) and the other was HB (see Section 5.1.6).

† Based on the two polymorphisms (GOT_M and ICD_S) where heterozygotes were observed. Two other polymorphisms were differences between the individual of unknown locality and the other six specimens (*6PGD* and *ES*).

have similar heterozygosities, averaged over all loci, to the large populations.

5.4 INTRAPOPULATION ASSOCIATIONS

Where two or more polymorphisms occurred within an *R. f. greyii* population, a test was made for random association between the phenotypes in all pairwise combinations of polymorphisms within that population. Initially each comparison took the form of a 3 x 3 contingency table, but because many cells had expected numbers less than five, these tables were condensed to 3 x 2 or 2 x 2 tables. If, after reduction to a 2 x 2 table, any cells had expected values less than five, Fisher's exact method was used to determine the probability. Otherwise a χ^2 value with Yates' correction was calculated. One pairwise comparison was made for each of the Eyre Peninsula and South-East populations, three comparisons for the Kangaroo Island population and six for the Norton Summit population. No associations significant at the 5% level were detected.

For each polymorphism in a *R. f. greyii* population, a test was made for random association between phenotypes and sex. In most comparisons it was necessary to reduce the 3 x 2 contingency table to a 2 x 2 table. Of the thirteen tests carried out, the ME_S polymorphism on Kangaroo Island was the only one to show a significant non-random association of sex and phenotype. The data are given below:

		ME_S Phenotype	
		ME_S 1	ME_S 2-1
Sex	female	20	5
	male	23	0

The exact probability is 0.031. In view of the number of tests carried out (thirteen), it seems that little weight can be given to this association. More data are required to confirm or disprove the significance of the association.

5.5 MULTIVARIATE ANALYSES OF ISOZYMIC DATA

5.5.1 Genetic distance

One way of comparing populations, based on all the isozymic information available, is to calculate a "genetic distance". Many methods have been advanced for estimating the genetic distance between two populations. Several of these methods have been used for estimating the genetic distances between *R. fuscipes* populations.

In the following section, m will denote the number of alleles at a locus and p_{i1} and p_{i2} the frequencies of the ith allele in populations 1 and 2 respectively.

a) Rogers (1972) proposed a genetic distance

$$D = \sqrt{\tfrac{1}{2}\sum_{i=1}^{m}(p_{i1} - p_{i2})^2} \qquad \ldots (1)$$

where information from several loci, both polymorphic and monomorphic, are combined by calculating an average value of D.

b) Latter (1972, 1973) has suggested two measures of genetic distance,

$$\gamma = (H_B - H)/(1 - H) \qquad \ldots (2)$$

and

$$\phi^* = 1 - \frac{H}{H_B} \qquad \ldots (3)$$

where $$H = 1 - \tfrac{1}{2}\sum_{i=1}^{m}(p_{i1}^2 + p_{i2}^2)$$

and $$H_B = 1 - \sum_{i=1}^{m} p_{i1}\, p_{i2}$$

Information from both polymorphic and monomorphic loci are used to calculate γ, but only polymorphic loci are used to estimate ϕ^*. Latter has indicated that several loci may be combined by using mean values of H and H_B in equations (2) and (3), but Kirby (1974 and pers. commun.) has suggested taking average values of γ and ϕ^*. The second method has the added advantage of allowing an estimate of the standard error to be made.

c) Nei (1972) has defined a genetic distance between two populations as

$$D = -\log_e \frac{j_{12}}{\sqrt{j_1\, j_2}} \qquad \ldots (4)$$

where $j_{12} = \sum_{i=1}^{m} p_{i1}\, p_{i2}$, $j_1 = \sum_{i=1}^{m} p_{i1}^2$ and $j_2 = \sum_{i=1}^{m} p_{i2}^2$.

Information from several loci (polymorphic and monomorphic) can be combined by using mean values of j_{12}, j_1 and j_2. Nei and Roychoudhury (1974) have derived the formulae for estimating the variance of D.

d) Cavalli-Sforza (1969) has defined a genetic distance

$$f_\theta = 4\sum\left(1 - \sum_{i=1}^{m}\sqrt{p_{i1}\, p_{i2}}\right)\Big/\sum(A - 1) \qquad \ldots (5)$$

where the two unmarked summations are taken over polymorphic loci.

e) Balakrishnan and Sanghvi (1968) have proposed the following measure of genetic distance

$$G^2 = \sum_{i=1}^{m} \frac{(p_{i1} - p_{i2})^2}{\bar{p}_i} \qquad \ldots (6)$$

where $\bar{p}_i = \frac{1}{2}(p_{i1} + p_{i2})$ and a mean G^2 for polymorphic loci may be calculated.

f) Jacquard (1974) has defined a genetic distance χ^2, defined in the same way as G^2 except that $\bar{p}_i$ is the weighted (according to sample size) mean frequency of the ith allele over all populations sampled. This measure is considered here because by including monomorphic loci (where $\chi^2 = 0$) and dividing the sum of χ^2 for all loci, by the number of loci and then taking the square root, a value is obtained which is the same as that found in the correspondence analysis to be described in Section 5.5.2.

Genetic distances were computed between all *R. fuscipes* populations, excluding those involving *R. f. assimilis* and Eyre Island. (Note also, that for all multivariate analyses, the *R. f. coracius* specimen of unknown locality was not included. Consequently the *R. f. coracius* gene frequencies used were not exactly the same as shown in Table 5.18.) The γ and ϕ^* distances of Latter were each determined by two methods: (i) using mean values of H and H_B $(\bar{H}, \bar{H}_B)$, and (ii) taking the mean value of γ and ϕ^* for each locus $(\overline{\text{Loci}})$. Table 5.20 shows the correlation coefficients between the various genetic distance measures. These correlations are based on 15 populations giving 105 estimates of distance. All correlations are highly significant and, except those involving ϕ^* (using mean H and H_B) or χ^2, all are 0.99 or 1.00. It is interesting to note that the two methods of estimating γ give almost identical results, while the two estimates of ϕ^* are quite different.

Table 5.20 Correlations of various estimates of genetic distance based on 105 pair-wise comparisons.

	ROGERS D	LATTER γ $(\bar{H},\bar{H}_B)$	LATTER γ $(\overline{Loci})$	LATTER ϕ^* $(\bar{H},\bar{H}_B)$	LATTER ϕ^* $(\overline{Loci})$	NEI D	CAVALLI-SFORZA f_θ	BALAKRISHNAN & SANGHVI G^2
LATTER γ $(\bar{H},\bar{H}_B)$	0.99							
LATTER γ $(\overline{Loci})$	1.00	1.00						
LATTER ϕ^* $(\bar{H},\bar{H}_B)$	0.55	0.60	0.59					
LATTER ϕ^* $(\overline{Loci})$	1.00	0.99	1.00	0.56				
NEI D	0.99	1.00	0.99	0.57	0.99			
CAVALLI-SFORZA f_θ	0.99	1.00	0.99	0.59	0.99	0.99		
BALAKRISHNAN & SANGHVI G^2	1.00	1.00	1.00	0.58	1.00	0.99	1.00	
JACQUARD χ^2	0.70	0.67	0.67	0.34	0.69	0.67	0.69	0.69

It was decided to use only Nei's measure of genetic distance in further analyses in this thesis, because a standard error of the distance could be computed and the distance is highly correlated with most other measures. Furthermore, distances between populations of other species have been calculated and published using Nei's measure (Nei, 1975) and it has been suggested that this measure of distance is closely related to the number of gene substitutions involved (Nei, 1972).

The distance matrix between *R. fuscipes* populations, based on Nei's distance measure is shown in Table 5.21. The standard errors are high, generally being about half of the estimate, although for small distances the standard error is about the same as the distance. It can be seen that the genetic distances between subspecies are generally greater than those between different *R. f. greyii* populations. A comparison of the genetic distances between the populations studied here and the distances of comparable taxonomic groups of mammals was made using data from Nei (1975) (Table 5.22). It shows that the range of genetic distances between races (defined here as populations belonging to the same subspecies) and between subspecies of *R. fuscipes* are, in general, greater than the range of distances between races and subspecies of other mammals.

A dendrogram was produced from the Nei genetic distances, by the unweighted pair-group method of cluster analysis using arithmetic averages (UPGMA, Sneath and Sokal, 1973) (Fig. 5.12). Two distinct population groups are evident, one consisting of the Pearson Islands, Goat Island, Dog Island and *R. f. fuscipes* and the other group containing all other populations. The *R. f. fuscipes* population clusters with an *R. f. greyii* group at a distance of about 0.14, but *R. f. coracius* clusters with another *R. f. greyii* group at a greater distance, about 0.24. However, *R. f. fuscipes* and *R. f. coracius* cluster with *R. f. greyii* groups before the clustering of all the populations making up the latter subspecies.

Table 5.21 Genetic distances with standard errors, between populations of *R. fuscipes*, calculated according to Nei (1972).

	RFC	SE	NS	KI	EP	NGI	HI	WMI	WGI	GRI	PIS	PIN	GOI	DI
SE	.18±.11													
NS	.27±.14	.12±.08												
KI	.22±.13	.16±.10	.11±.07											
EP	.22±.13	.15±.09	.06±.04	.07±.07										
NGI	.21±.13	.16±.10	.07±.05	.07±.07	.00±.00									
HI	.21±.13	.16±.10	.07±.05	.07±.07	.00±.00	.00±.00								
WMI	.29±.15	.13±.09	.03±.02	.13±.09	.05±.05	.06±.07	.06±.07							
WGI	.31±.15	.14±.09	.04±.02	.14±.10	.06±.05	.07±.07	.07±.07	.01±.01						
GRI	.29±.15	.23±.12	.14±.09	.14±.10	.07±.07	.06±.07	.06±.07	.13±.10	.15±.10					
PIS	.51±.21	.30±.15	.16±.10	.30±.15	.29±.15	.30±.15	.30±.15	.22±.13	.24±.13	.22±.13				
PIN	.59±.23	.38±.17	.23±.12	.38±.17	.36±.17	.37±.17	.37±.17	.29±.15	.30±.15	.29±.15	.02±.03			
GOI	.48±.20	.29±.14	.15±.09	.29±.15	.27±.14	.29±.15	.29±.15	.21±.12	.22±.13	.21±.12	.01±.01	.06±.07		
DI	.38±.18	.21±.12	.08±.06	.21±.12	.19±.12	.21±.12	.21±.12	.13±.10	.15±.10	.29±.15	.15±.10	.21±.12	.13±.10	
RFF	.37±.17	.32±.15	.19±.11	.36±.17	.30±.15	.32±.15	.32±.15	.23±.13	.25±.13	.23±.13	.12±.08	.18±.11	.11±.08	.19±.12

Table 5.22 Genetic distances between populations within the *R. fuscipes* group, and between other populations of similar taxonomic rank (from Nei, 1975).

Species	Genetic distance between races	Genetic distance between subspecies
Man	0.011 - 0.019	-
Mus musculus	0.010 - 0.024	0.194
Kangaroo rats	0.000 - 0.058	-
Pocket gophers	-	0.004 - 0.262
Gophers	-	0.009 - 0.054
R. f. greyii	0.00 - 0.38	-
R. fuscipes	-	0.11 - 0.59

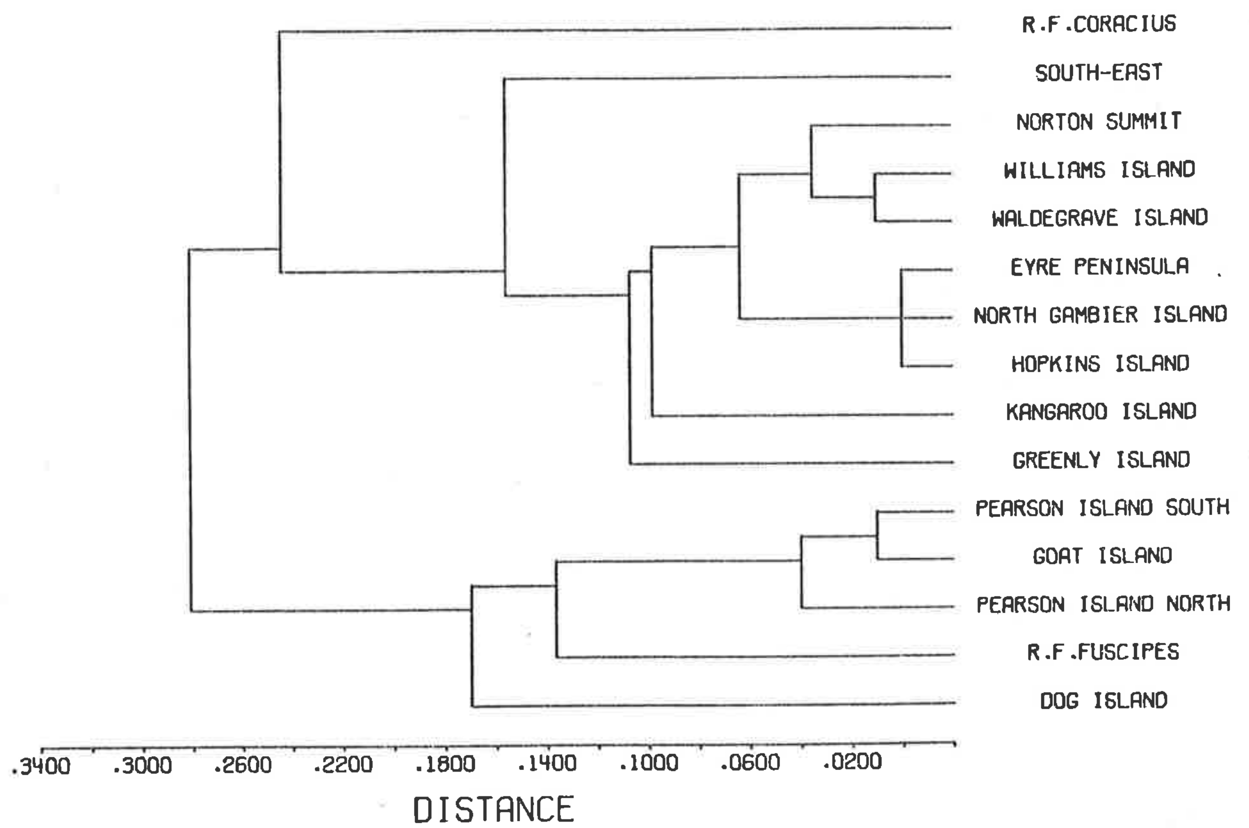


Figure 5.12 Cluster analysis of *Rattus fuscipes* populations. The dendrogram is derived from the genetic distance between populations, measured according to Nei (1972).

Of the *R. f. greyii* populations, Eyre Peninsula, North Gambier Island and Hopkins Island cluster at 0.00, while South-East and Dog Island do not appear to be closely associated with any *R. f. greyii* populations.

5.5.2 Correspondence analysis

Correspondence analysis ("analyse factorielle de correspondances"; Benzecri, 1970) is a modification of principal components analysis, applied to contingency tables. The data are represented in the form of a large contingency table, the populations corresponding to the rows and gene numbers to columns. The value of each cell in the table is then transformed by multiplying it by the square root of the product of its row and column totals. A principal component analysis on the transformed data leads to the arrangement of the populations (and genes) in a multidimensional space. I am most grateful to Dr. R.J. White (University of Southampton) who wrote a computer program and performed the correspondence analysis on the *R. fuscipes* data.

Table 5.23 shows the distance between the populations in the multi-dimensional space. This will be referred to as the CA distance or Jacquard's genetic distance, since it is the same as the genetic distance of Jacquard (1974) as described in the previous section. As shown in Table 5.20, Jacquard's genetic distance is not as highly correlated with the other distance measures as the others are amongst themselves. One way of illustrating this difference is to compare the dendrogram produced from a cluster analysis on the CA distances (Fig. 5.13) with the dendrogram from Nei's genetic distance (Fig. 5.12). The most striking difference is the clustering of all *R. f. greyii* populations without the other subspecies when Jacquard's distance measure is used, whereas two distinct groups form, one containing *R. f. fuscipes* and the other *R. f. coracius*, when Nei's measure is used.

Table 5.23 Genetic distances between populations of *R. fuscipes*, calculated by the correspondence analysis. This is the same as the genetic distance measure of Jacquard (1974), described in Section 5.5.1.

	RFC	SE	NS	KI	EP	NGI	HI	WMI	WGI	GRI	PIS	PIN	GOI	DI
SE	3.22													
NS	3.32	1.39												
KI	3.33	1.51	1.19											
EP	3.23	1.28	0.80	0.94										
NGI	3.23	1.29	0.83	0.95	0.08									
HI	3.23	1.29	0.83	0.95	0.08	0.00								
WMI	3.27	1.25	0.72	1.06	0.46	0.51	0.51							
WGI	3.30	1.32	0.82	1.09	0.58	0.66	0.66	0.42						
GRI	3.28	1.39	0.99	1.09	0.54	0.53	0.53	0.74	0.85					
PIS	3.40	1.55	1.12	1.40	1.13	1.15	1.15	1.04	1.12	1.02				
PIN	3.47	1.71	1.33	1.58	1.35	1.37	1.37	1.27	1.34	1.26	0.49			
GOI	3.38	1.52	1.08	1.37	1.09	1.12	1.12	0.99	1.08	0.98	0.30	0.79		
DI	3.49	1.74	1.37	1.61	1.39	1.40	1.40	1.31	1.37	1.50	1.40	1.58	1.36	
RFF	3.14	2.38	2.25	2.41	2.25	2.26	2.26	2.20	2.24	2.20	2.13	2.26	2.11	2.42

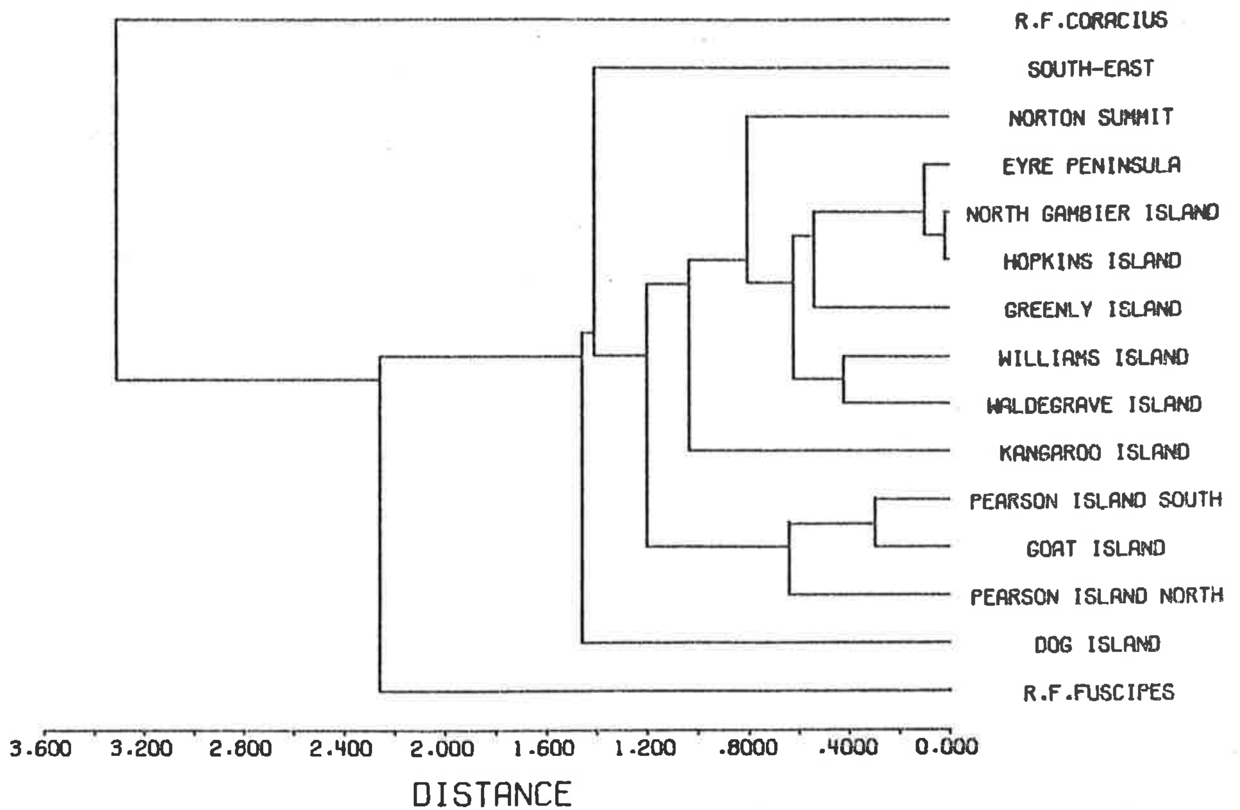


Figure 5.13 Cluster analysis of *Rattus fuscipes* populations. The dendrogram is derived from the distances between populations in the multidimensional space defined by all the principal axes of the correspondence analysis.

The correspondence analysis allows the populations to be plotted on a scatter diagram defined by the principal axes. Fig. 5.14 shows the populations plotted on the first and second principal axes. The plane defined by these two axes displays 48% of the variation in gene frequencies between populations. The first axis results in a largely indiscriminant spreading of the populations, while axis two clearly separates *R. f. fuscipes* and *R. f. coracius* from the other populations, as does the dendrogram (Fig. 5.13). In an attempt to clarify the relationship between the *R. f. greyii* populations, the analysis was repeated without the *R. f. fuscipes* and *R. f. coracius* populations. The plane defined by axes one and two for such an analysis is shown in Fig. 5.15, and the plane defined by axes one and three in Fig. 5.16. The first three axes contain 61% of the total variation in gene frequencies between *R. f. greyii* populations. It is clear that axes two and three separate the South-East and Dog Island populations respectively, the two populations revealed by the dendrogram (Fig. 5.13) as being the most divergent *R. f. greyii* populations. The dendrogram and scatter diagram also reveal three clusters, one consisting of Eyre Peninsula, Hopkins Island and North Gambier Island, a second of Waldegrave and Williams Island, and a third loose cluster of the Pearson Islands and Goat Island. The dendrogram derived from Nei's genetic distances (Fig. 5.12) shows similar clusters.

5.5.3 Genetic distance, geographic distance and the time since isolation

There is some indication from the distribution of individual genes and the multivariate analyses, that there is a relationship between geographic and genetic distance. Four regression analyses were performed to investigate whether there is a relationship between genetic and

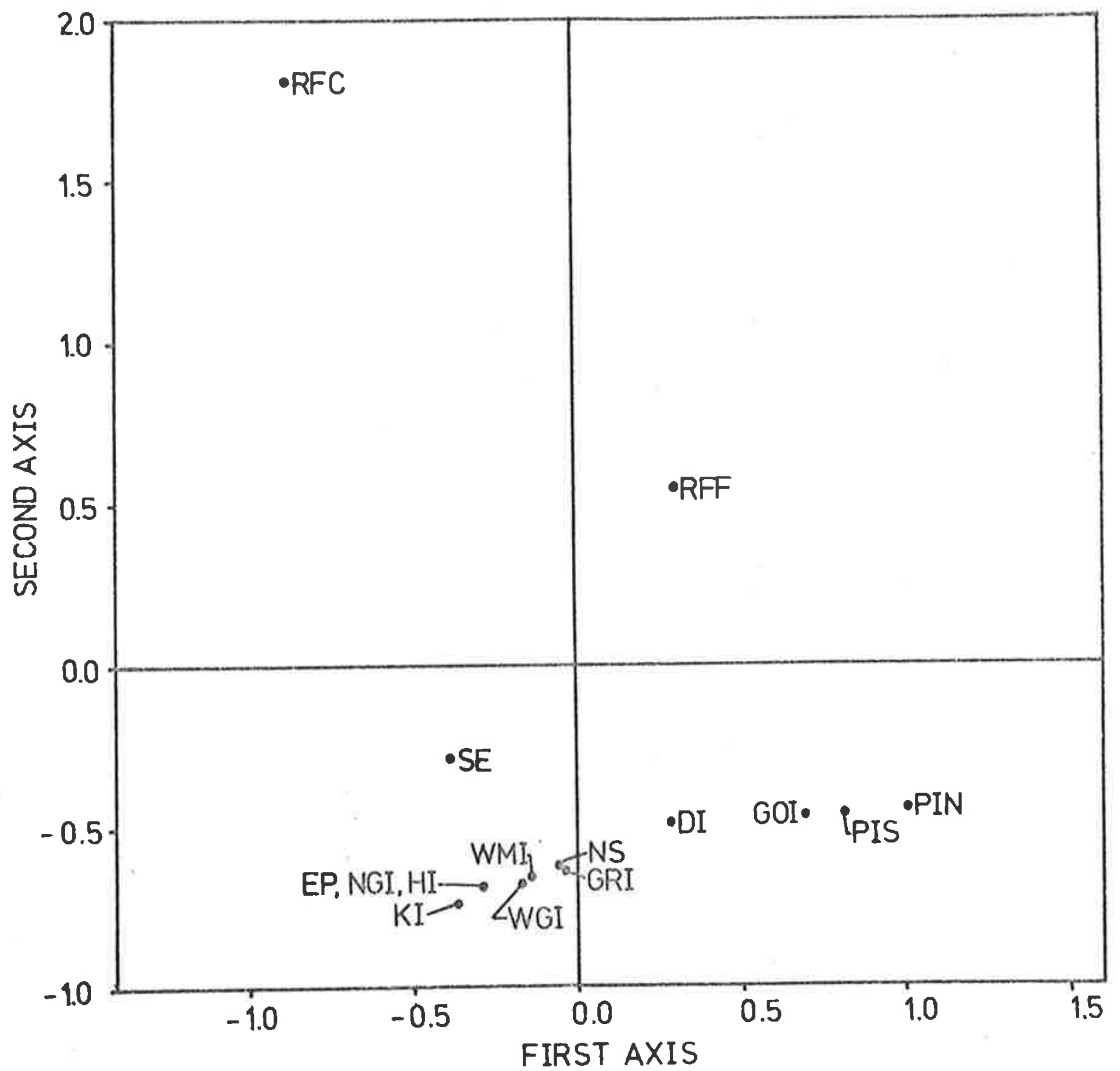


Figure 5.14 *Rattus fuscipes* populations plotted on the first and second principal axes of a correspondence analysis.

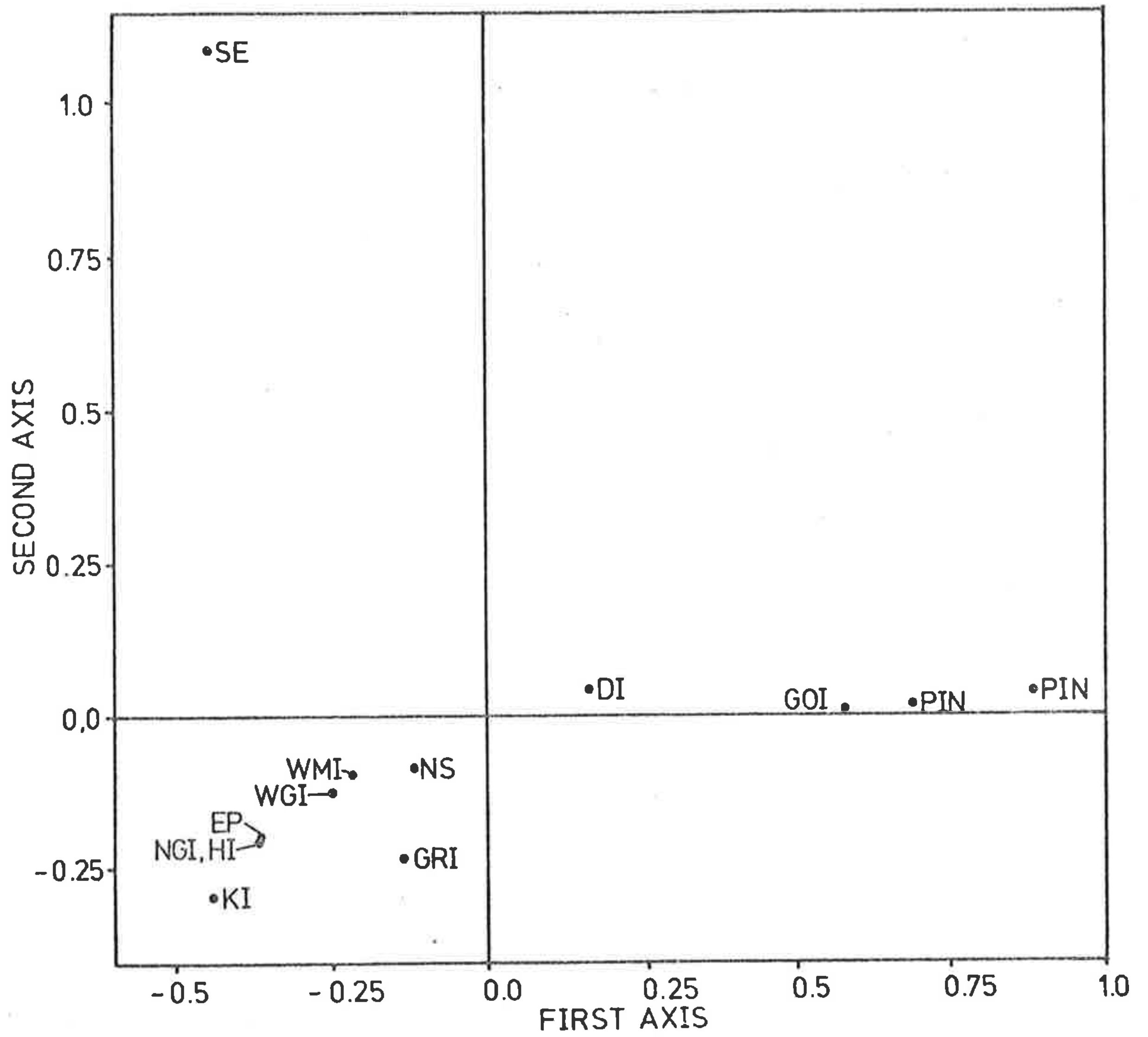


Figure 5.15 *Rattus fuscipes greyii* populations plotted on the first and second principal axes of a correspondence analysis.

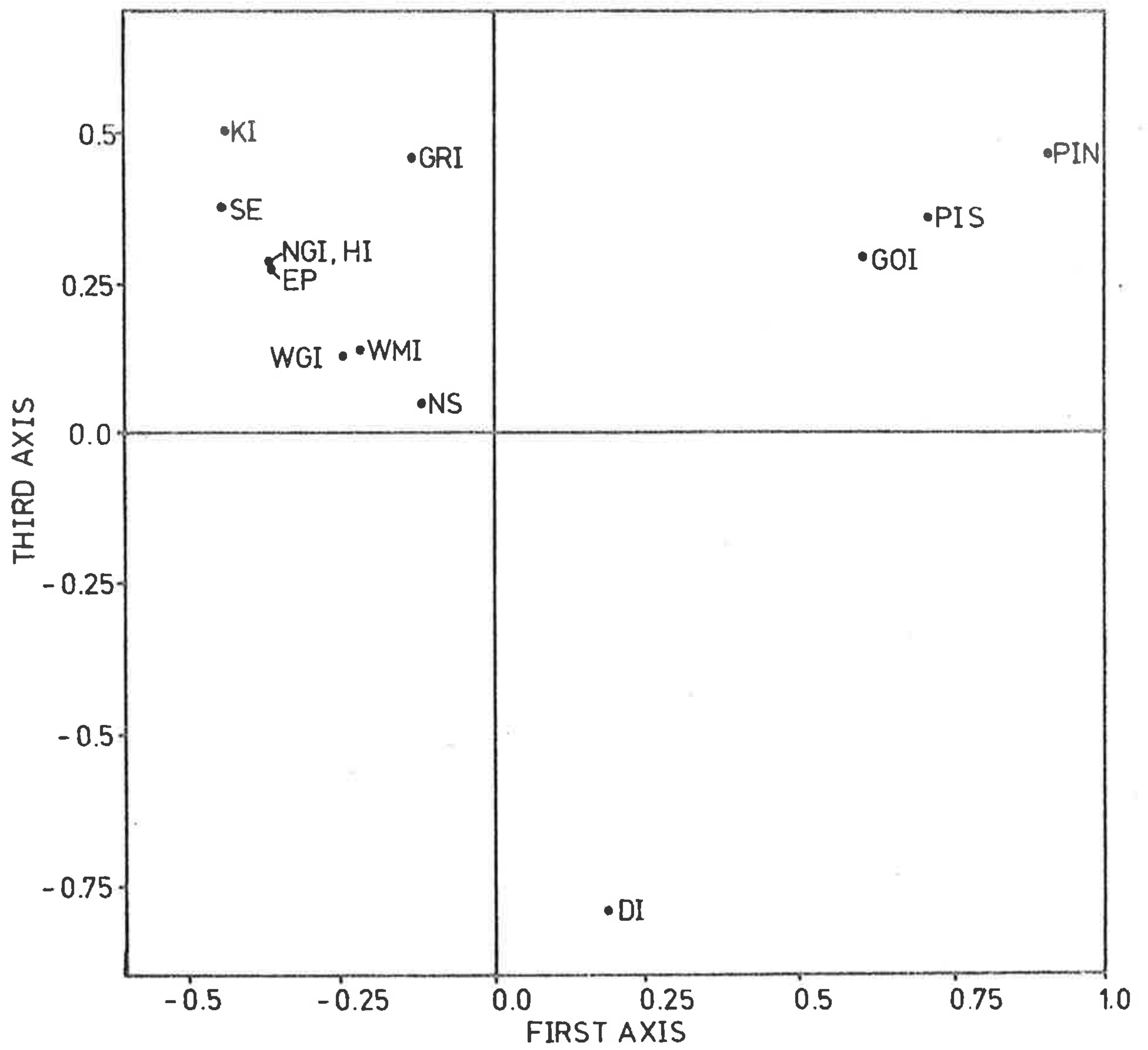


Figure 5.16 *Rattus fuscipes greyii* populations plotted on the first and third principal axes of a correspondence analysis.

geographic distance. Two sets of data were used, one containing all *R. fuscipes* populations except *R. f. assimilis* and Eyre Island and the other with *R. f. greyii* populations only. For each set of data a separate regression of Jacquard's and Nei's genetic distances on geographic distance was computed. The geographic distance between *R. f. greyii* populations was taken as the distance in a straight line between them, for the large populations the centre of the collecting area serving as the position for these populations. The two Pearson Islands were given a geographic separation of 0 km. The distances between *R. f. fuscipes* and *R. f. greyii* populations were determined by adding the least distance between the most eastern *R. f. fuscipes* collection point and Dog Island to the distance between the *R. f. greyii* population and Dog Island. A similar method was used for *R. f. coracius* except that the South-East was used as the base population and the distance from *R. f. coracius* to the South-East was taken along the mid-line of the current distribution of *R. f. assimilis* (see Fig. 3.2).

The regression coefficients of genetic distance on geographic distance (in units of 10,000 km) and the tests of significance are shown below:-

Genetic distance measure	Populations in analysis	b	SE_b	Significance of b
Nei	all	0.503	0.080	$P<0.001$
Nei	*R. f. greyii*	1.850	0.568	$0.001<P<0.01$
Jacquard	all	6.588	0.260	$P<0.001$
Jacquard	*R. f. greyii*	12.152	1.800	$P<0.001$

It is clear that there is a highly significant regression of genetic distance on geographic distance for both Nei's and Jacquard's genetic distance measures. For both measures the regression coefficient is

greater when only the *R. f. greyii* populations are considered. A comparison of the four regressions is best illustrated in Figs. 5.17 to 5.20, in which the estimates of genetic distance are plotted against geographic distance.

During the last ice age, all the islands with *R. f. greyii* populations were part of the mainland. As the ice retreated, there was a eustatic rise in sea level and the islands were formed. It is possible to estimate the time of isolation from data on the mean sea level during the last 20,000 years (Godwin *et al.*, 1958; Fairbridge, 1960, 1961; Thom and Chappell, 1975) and the present topography of the sea bed. Table 5.24 shows these estimated times. Using this information and assuming that the mainland *R. f. greyii* populations have been isolated for 5,000 years (presumably because of a climatic change - see Chapter 1) and the two Pearson Islands for 100 years, it is possible to determine the regression of genetic distance on time since isolation for the *R. f. greyii* populations. The results are set out below (time in units of 1,000 years):

Genetic distance measure	b	SE_b	Significance of b
Nei	0.01136	0.00387	$0.001<P<0.01$
Jacquard	0.01476	0.01523	$0.2<P<0.4$

There is a highly significant regression of Nei's genetic distance on time since isolation, but the regression is not significant when Jacquard's measure is used.

Finally, multiple regressions were performed using both geographic distance and time since isolation as independent variables. In different regression analyses, Nei's and Jacquard's measures were used as dependent

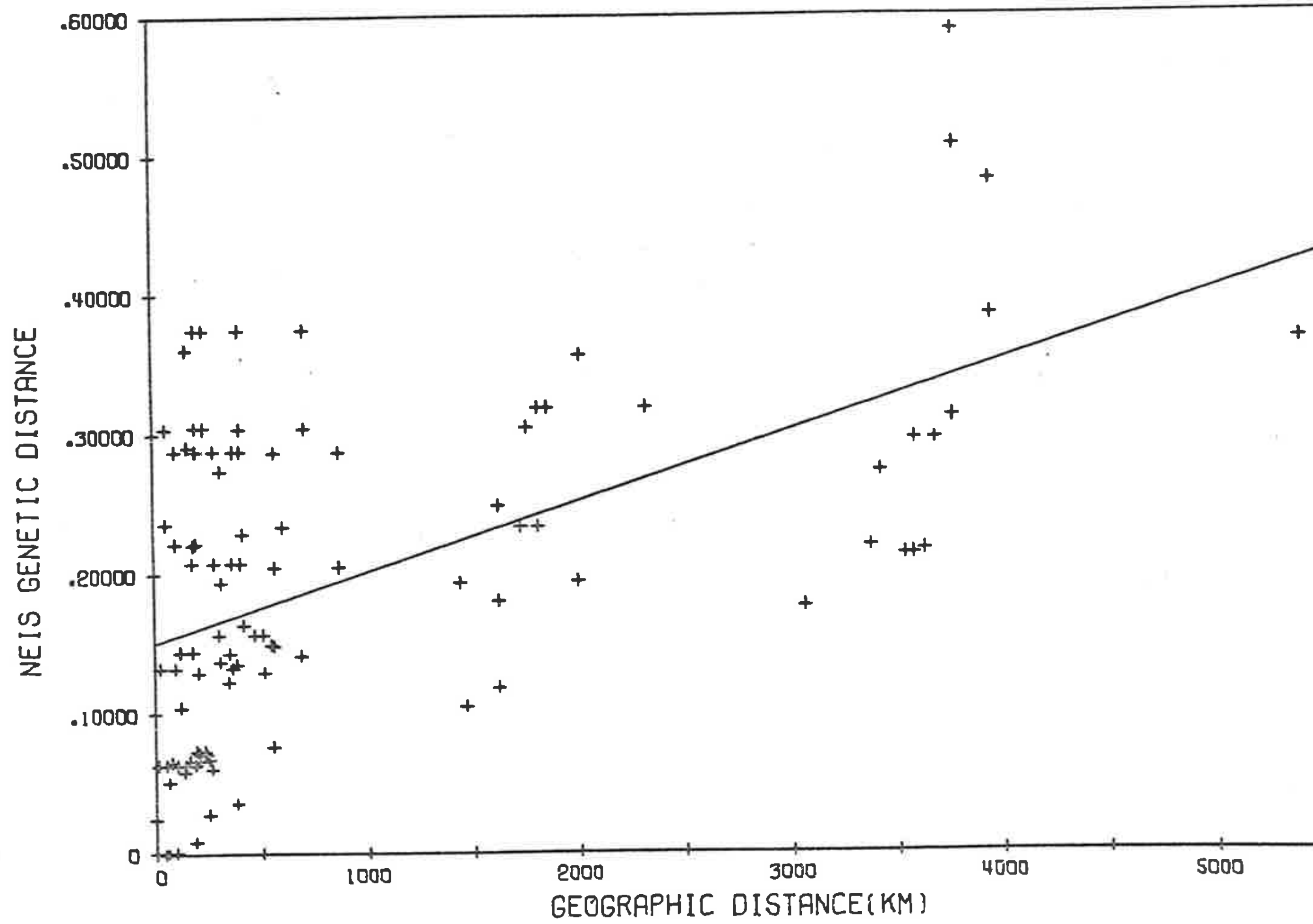


Figure 5.17 Genetic distance, calculated according to Nei (1972), plotted against geographic distance, for all *R. fuscipes* populations sampled. The regression line is included.

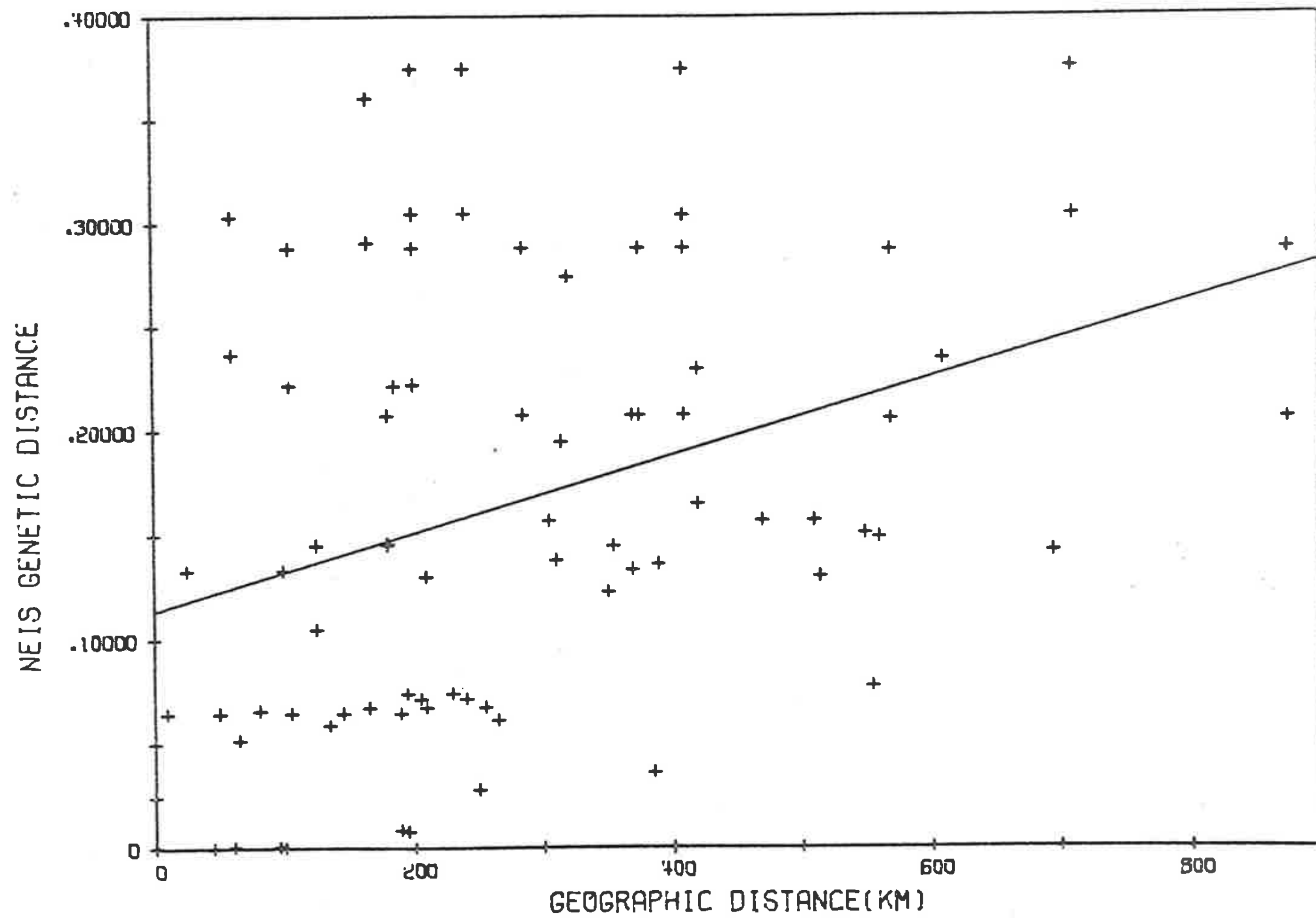


Figure 5.18 Genetic distance, calculated according to Nei (1972), plotted against geographic distance, for the *R. f. greyii* populations sampled. The regression line is included.

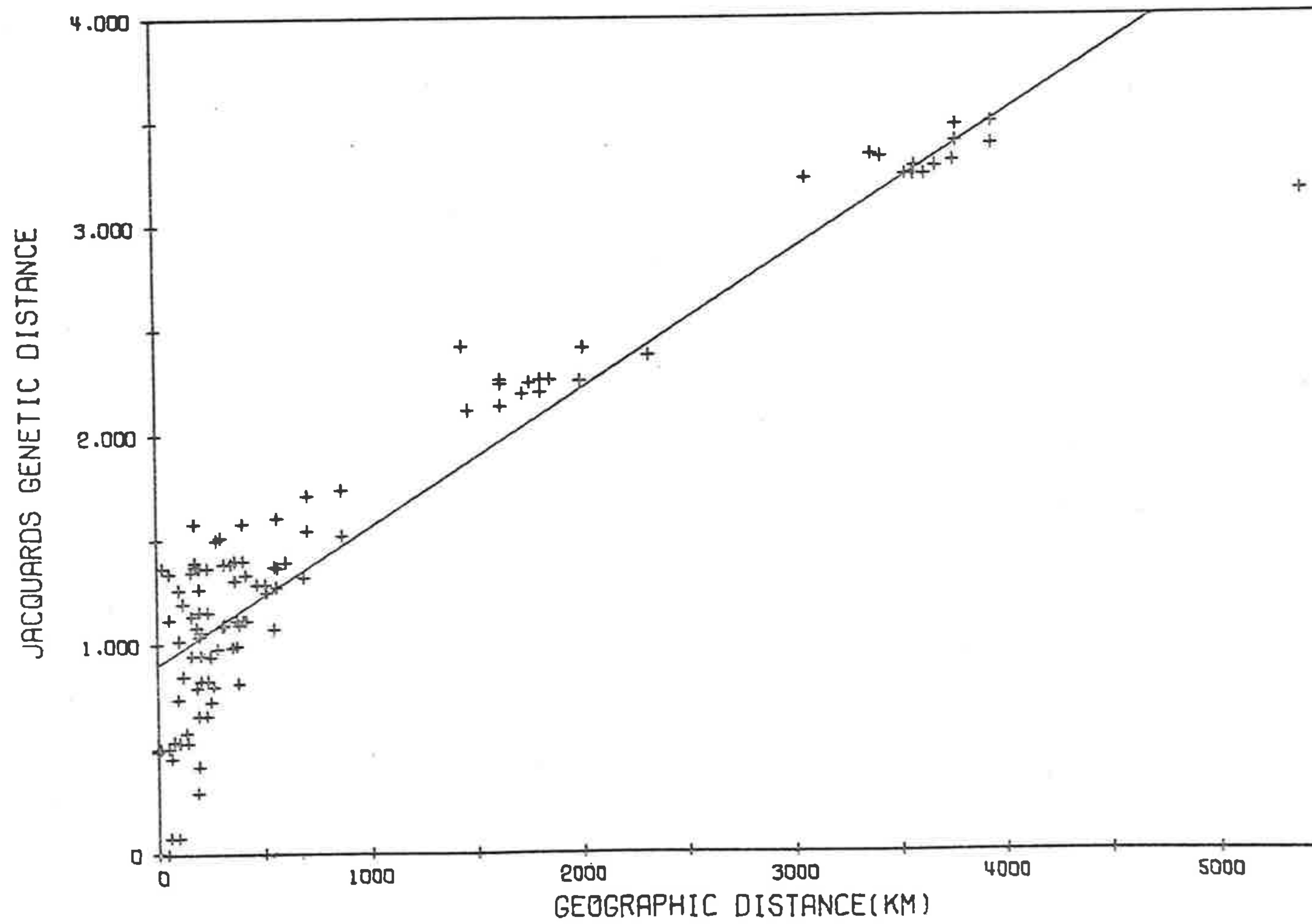


Figure 5.19 Genetic distance, calculated from the correspondence analysis, plotted against geographic distance, for all *R. fuscipes* populations sampled. The regression line is included.

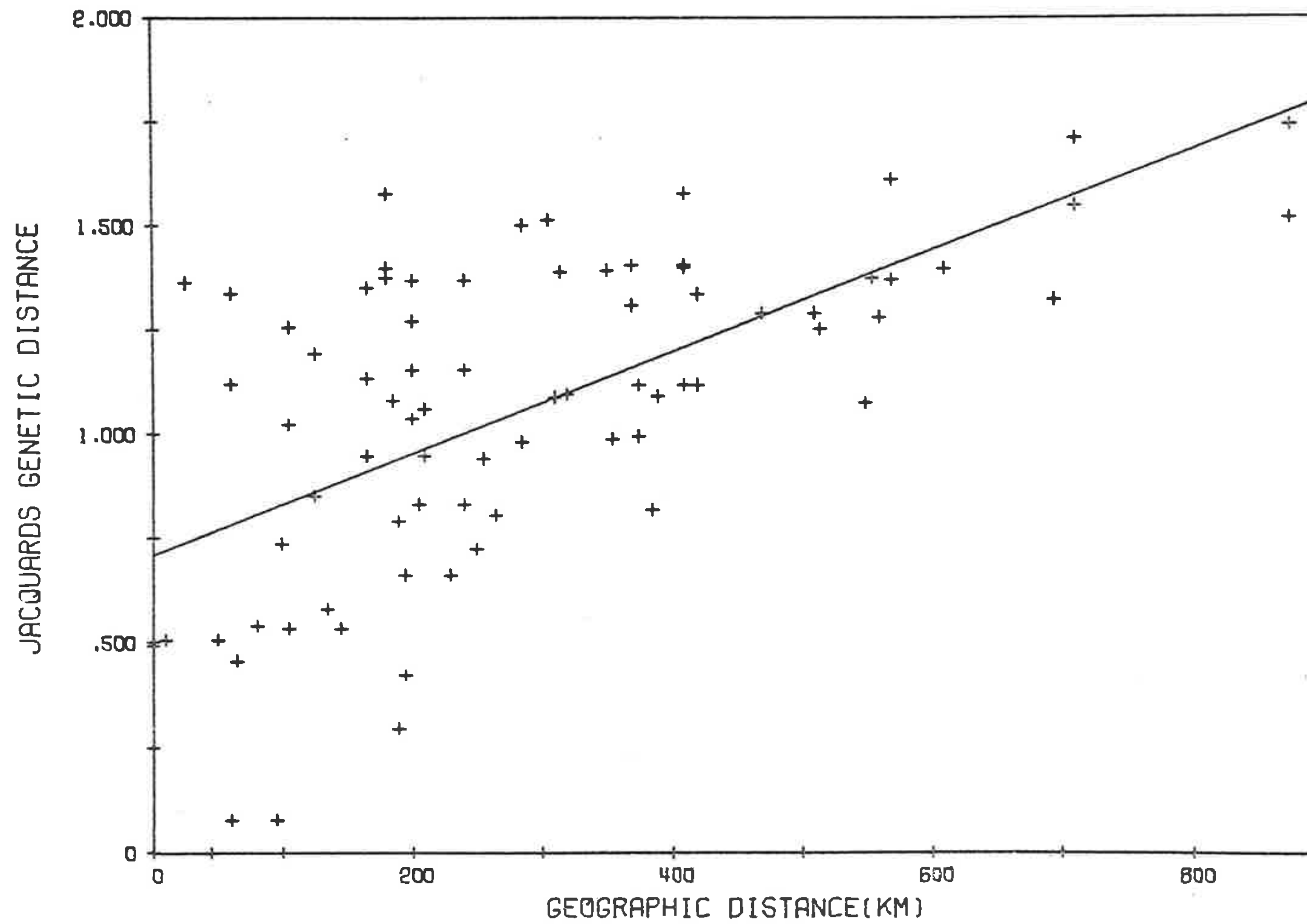


Figure 5.20 Genetic distance, calculated from the correspondence analysis, plotted against geographic distance, for the *R. f. greyii* populations sampled. The regression line is included.

Table 5.24 Estimated time of isolation of various islands from mainland. The depths shown are the changes in sea level (relative to the present level) that would result in a land bridge joining the various islands to the mainland. Data from navigational charts (B.A. 1061; B.A. 3359; B.A. 1762; Aus. 134) and Royal Australian Navy survey sheets (V4/40; V4/41; V4/66; V5/204; V5/205; V5/206) were used.

Populations	Depth (metres)	Years (B.P.)
Eyre Peninsula - Dog Island	37	11,500
Eyre Peninsula - Goat Island	6	6,500
Eyre Peninsula - Eyre Island	≃ 1-3	<6,000[1]
Eyre Peninsula - Pearson Islands	75	14,000
Eyre Peninsula - Greenly Island	75	14,000
Eyre Peninsula - Waldegrave Island	6	6,500
Eyre Peninsula - Williams Island	50	12,000
Eyre Peninsula - Hopkins Island	22	9,500
Eyre Peninsula - North Gambier Island	50	12,000
Norton Summit - Kangaroo Island	31	10,000

[1] Eyre Island is separated from the mainland by a sea channel only a few hundred metres wide. Minor fluctuations in sea level thought to have occurred during the past 6,000 years and changes in the conformation of the channel have probably resulted in Eyre Island being connected by a land bridge to the mainland during that time.

variables. Both regression analyses revealed geographic distance and time since isolation as highly significant predictors of genetic distance. The results are set out below:

Genetic distance measure	Dependent variable	b	Significance of b
Nei	geographic	2.195	P<0.001
	time	0.013844	P<0.001
Jacquard	geographic	12.884	P<0.001
	time	0.029287	0.001<P<0.005

CHAPTER 6

DISCUSSION

6.1 SOLUBLE GLUTAMATE OXALOACETATE TRANSAMINASE IN THE PEARSON ISLANDS

Fig. 6.1 is a map of the Pearson Islands, showing the areas where animals were collected. The two islands are separated by a narrow, shallow sea channel (Fig. 6.2). The *R. f. greyii* populations on the two islands differ markedly in GOT_S gene frequencies as shown by the gene counts tabulated below:

	GOT_S^A	GOT_S^B
Pearson Island South	30	14
Pearson Island North	0	98

A test for homogeneity in the contingency table above gives a $\chi_1^2 = 80.67$ ($P \ll 0.001$). If the GOT_S^A gene is present in the Pearson Island North population, then its frequency is less than 3% (with 95% confidence).

Both Pearson Islands appear to provide very similar habitats for *R. f. greyii*, and therefore it seems unlikely that the marked difference in gene frequencies is maintained by selection. The observed absence of the GOT_S^A gene in the Pearson Island North sample suggests that the movement of animals between the two islands is severely restricted.

There has been a similar restriction in the movement of the Pearson Island wallaby (*Petrogale* sp.), the only other mammalian species inhabiting the islands. Until 1960 the Pearson Island wallaby was only found on Pearson Island North. No evidence could be found to suggest that before 1960, this species inhabited Pearson Island South, although a suitable habitat was available there. In 1960 six wallabies were accidently released on Pearson Island South and the species is now

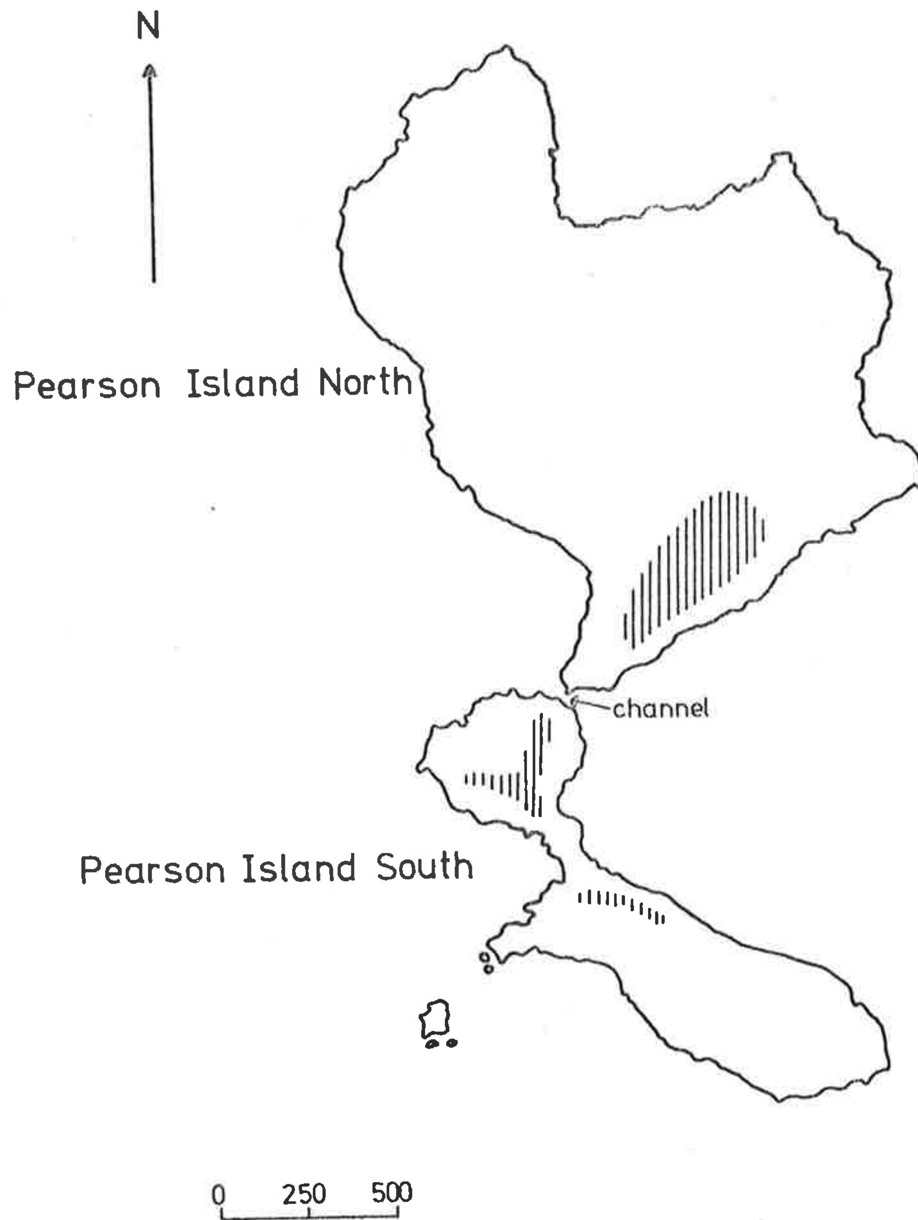


Figure 6.1 Map of the Pearson Islands. Areas where animals were captured are indicated by shading.

Figure 6.2 Aerial photograph of the channel separating Pearson Island North and Pearson Island South. The arrow marks the area which is sometimes dry at spring low tides and provides a land-bridge between the two islands. Scale: 50 mm ≃ 70 m.

abundant there (Thomas and Delroy, 1971).

It appears unlikely that the rats and wallabies are physically incapable of crossing the channel between the two islands. When the sea is calm and the tide is low, it is easy for a man to wade or step from rock to rock between the two sections. Indeed, at very low spring tides, the two islands are sometimes joined by a dry sand bar (J. Forrest, pers. comm.). However, it is possible that the occasional joining of the two islands by a land-bridge is a recent phenomenon. There is some evidence to suggest that since the isolation of the Pearson Islands from the mainland (about 14,000 years BP), the mean sea level on two or more occasions has been about six metres above its present level (Twidale, 1971 and pers. comm.). During these times of high mean sea level, the channel would have been a much more formidable barrier to migration than it is at present.

Thomas and Delroy (1971) suggested that the wallaby did not cross the channel because it found the sea water distasteful. Another possibility is that because the sea is often very rough, selection may favour animals predisposed to keeping away from the shoreline. Such a behavioural trait would limit any migration between the two islands.

The absence of the GOT_S^B gene in animals from all other *R. fuscipes* populations, suggests that either this gene has arisen by mutation in the Pearson Islands populations since their isolation from other populations or else the GOT_S^B gene was not widespread beforehand. If the GOT_S^B gene was widespread in the ancestral population, then it would be expected to occur in some other present-day populations. The GOT_S polymorphism on Pearson Island South may have been present on both Pearson Islands before the channel was formed, in which case it must be presumed that the GOT_S^A gene was lost from Pearson Island North, probably due to genetic drift.

6.2 GEOGRAPHIC VARIATION IN MITOCHONDRIAL ISOCITRATE DEHYDROGENASE

Three of the four large *R. f. greyii* populations as well as the Waldegrave Island population are polymorphic for ICD_M (Table 5.8, Fig. 5.5). ICD_M is unusual in that it is only very rarely found to be polymorphic in mammalian populations. Selander and Johnson (1973) found that the mean heterozygosity per individual at the ICD_M locus of 19 vertebrate species is 0.0031. When only those species polymorphic for ICD_M are considered the mean heterozygosity is 0.0140. For all glucose metabolising enzymes, the estimates are 0.0491 and 0.1043 respectively. Thus ICD_M is not only polymorphic in fewer populations than most other glucose metabolising enzymes surveyed, but where it is polymorphic, the mean heterozygosity is low. However, these differences have not been shown to be statistically significant.

It has been suggested by van Heyningen *et al.* (1973) that there may be a strong conservation of the charge properties of mitochondrially bound enzymes compared with other enzymes, possibly because the charge of mitochondrial enzymes may be important for their localisation in mitochondria. There are two other enzymes, glutamate oxaloacetate transaminase and malate dehydrogenase which have mitochondrial and soluble forms, and for which there are extensive data on their variability in vertebrate populations. The mitochondrial form of GOT has a similar level of variability to ICD_M. While MDH_M has not as low variability levels as the other two mitochondrial enzymes, it is less than average (Selander and Johnson, 1973).

It is difficult to determine if selective forces are maintaining the ICD_M polymorphisms observed. There appears to be a *prima facie* case for the ICD_M polymorphism on Waldegrave Island being maintained by heterozygote advantage at the *ICD_M* locus or a closely linked locus in linkage disequilibrium with it. This is based on the observation of a significant excess of heterozygotes (Section 5.1.7.2). If

heterozygote advantage acts through differential mortality between zygote formation and adulthood, and if equilibrium has been achieved in this population, then it is possible to estimate the relative fitnesses of the three genotypes. These estimates are derived from the ratios of the observed numbers of the different phenotypes to the numbers expected by the Hardy-Weinberg principle. For the ICD_M polymorphism, the ICD_M 1 phenotype has a fitness of 0.38 and the ICD_M 2 phenotype a fitness of 0.64 relative to the heterozygote.

The difference in activity of the allozymes determined by the two ICD_M genes (see Section 5.1.7.2) may reflect *in vivo* differences which are selectively important.

The present widespread geographic distribution of ICD_M genes suggests that these genes were present in the postulated ancestral population. The fact that the Waldegrave Island population has remained polymorphic for ICD_M while other small island populations have become monomorphic, also suggests that selection may have played some part in the maintenance of the polymorphism on Waldegrave Island.

6.3 GENETIC VARIABILITY WITHIN POPULATIONS

On average, mammalian populations are polymorphic at about one-third of their electrophoretically detectable loci and an individual is heterozygous at about 5% of its loci (Harris and Hopkinson, 1972; Selander and Johnson, 1973; Lewontin, 1974; Powell, 1975). The populations of *R. f. greyii* studied here, with the exception of Norton Summit, have much lower values, for the proportion of polymorphic loci and for mean heterozygosity, than the average mammalian population (Table 5.19). The results also indicate that widespread populations of *R. f. greyii* are more variable than those on small offshore islands.

It may be argued that the low levels of variability in most *R. f. greyii* populations are related to the choice of proteins examined.

Kojima *et al.* (1970) and others have demonstrated differences between proteins in the frequency with which they exist in the polymorphic state. It is possible that the low genetic variability found in *R. f. greyii* populations is a result of choosing many proteins, which, in mammals, are generally invariant. In fact, proteins were chosen on the criterion that suitable conditions for satisfactory electrophoretic resolution could be found. This criterion, which is the usual criterion for such studies, seems unlikely to be related to the degree of protein variability.

As a result of the widespread occurrence of two ICD_M genes in the contemporary populations, it was inferred in the previous section that the ancestral *R. f. greyii* population was probably polymorphic for ICD_M. If there was not a widespread ICD_M polymorphism in the ancestral population, then it is difficult to explain the current ICD_M gene distributions (given the absence of migration between populations). On the same grounds, it can be argued that the ancestral population was polymorphic for four other proteins (ALB, HB, LDH_A and ME_S) - this is discussed further in Section 6.4. In the case of haemoglobin, three genes were probably widespread (HB^A, HB^P and HB^S), while three others (HB^B, HB^D and HB^K) were either restricted in distribution or arose by mutation after the isolation of their particular populations. Thus, it can be argued that the ancestral population was polymorphic for at least five of the proteins examined and hence had a level of genetic variability similar to that found generally in present-day mammalian populations. The gene distributions also suggest that the paucity of genetic variation in the small island populations is due to the loss of polymorphisms subsequent to isolation. The large populations have on the whole, retained a greater proportion of polymorphisms than the small island populations.

While some studies show lower levels of genetic variability in island populations compared to mainland populations (e.g. Saura *et al.*, 1973; Soule and Yang, 1973; Avise *et al.*, 1974; Gorman *et al.*, 1975), others show no significant differences (e.g. Berry and Murphy, 1970; Ayala *et al.*, 1971; Berry and Peters, 1975). Reduced genetic variation has also been found in populations which resemble island populations, with respect to low effective population size, a recent bottleneck in numbers, or small habitat area (e.g. Avise and Selander, 1972; Bonnell and Selander, 1974).

The paucity of genetic variation in the small *R. f. greyii* populations can be attributed to one or more of three causes. First, genetic drift may have a more marked effect in the island populations than in the large populations because of the smaller number of animals in the former populations. This suggestion can be tested, as a rough estimate can be made of the population sizes of the small islands and mainland populations. Evidence presented in Chapter 3 suggests that the density of individuals on Glennie Island is about 75 animals *per* ha (30 *per* acre), while on the mainland, the corresponding figure is about 10 *per* ha (4 *per* acre). These relative densities of the two population types are about the same as those indicated by the trap rates (number of individuals caught *per* trap *per* night) found in this and other studies (50% on islands *versus* 5% on the mainland). The mainland populations of *R. f. greyii* each occupy about 400,000 ha while most islands are less than 300 ha, which gives total numbers of animals as 4×10^6 and 2.25×10^4 respectively. Even if only one-tenth of the area of mainland populations is actually inhabited, their population sizes are at least an order of magnitude larger than those of island populations.

Using the estimated population size on small islands, it is possible to compare the observed heterozygosity with that expected, on the assumption that the population is in equilibrium (the loss of genes due

to drift equals the gain from mutation) and selection is ignored. The expected mean heterozygosity H is related to the population size N and mutation rate μ by

$$H = \frac{4N\mu}{4N\mu + 1} \quad \ldots (1)$$

(Kimura and Crow, 1964). For island populations, using $N = 2 \times 10^4$ and $\mu = 1 \times 10^{-6}$, the mean heterozygosity is expected to be about 0.07. If μ is set lower, say to 1×10^{-7}, the mean heterozygosity at equilibrium is about 0.01, which is closer to the observed value (0.007). Since the effective population size has, if anything, probably been over-estimated, it is possible that genetic drift can account for the low levels of heterozygosity in the small island populations (especially if μ is about 1×10^{-7} or less).

However, it is possible that equilibrium between mutation and drift has not been reached. Assuming that at the time of isolation (say, on average, 10,000 years BP) each population was in equilibrium with a mean heterozygosity of 0.05, the mean heterozygosity now at time t is related to the initial heterozygosity by

$$H_t = H_o\left(1 - \frac{1}{2N}\right)^t \quad \ldots (2)$$

(Crow and Kimura, 1970). Since *R. fuscipes* has a generation time of about one year (see Chapter 3), the predicted heterozygosity today (using $H_o = 0.05$, $t = 10{,}000$, $N = 20{,}000$) is 0.04. It appears as if genetic drift would have little effect unless the effective population size has been considerably less than 20,000. An effective population size of about 2,500 would reduce heterozygosity from a value of 0.05 to 0.007 in 10,000 generations.

When the population size varies between generations, the effective size is the harmonic mean over the generations (Wright, 1938). This introduces a second possible cause of lowered heterozygosity on the small islands, namely intermittent drift. A catastrophic event such as fire or famine, is likely to have a more devastating effect on population size on the small islands than it is on the mainland. Such catastrophies could affect the whole or large part of an island population, reducing the population to only a small proportion of its original size. Similar events would occur in the large populations but would only affect a relatively small area. Loss of genetic variability in the affected part of a large population could be replenished by migration from adjacent areas. However, any genetic loss on the small islands could not be replenished by migration and the whole population would pass through a bottleneck. The harmonic mean of population size is especially affected by small values and hence the mean for small populations may be considerably less than 20,000. This could greatly reduce the expected mean heterozygosity from the value predicted by equations (1) and (2) above and could account for the low heterozygosity observed in the small *R. f. greyii* populations.

In summary, it is certainly possible to explain the low levels of heterozygosity on the small islands, on the basis of genetic drift, if equilibrium is assumed. Even if equilibrium is not assumed the results could be explained by genetic drift, providing the effective population size has been, on average, about 2,500. This population size may be a reasonable estimate, given intermittent catastrophic events.

A third force which can influence the level of genetic variation in a population is environmental variation. Several authors (e.g. Levene, 1967; Levins, 1968; Gillespie and Langley, 1974) have suggested that genetic polymorphisms should be more frequent in more variable environments than in less variable environments (see Section 2.3.5).

From several considerations (e.g. number of species sharing the habitat, temperature fluctuations, habitat diversity) it might be thought that individuals in island populations experience less varied environments than those in mainland populations. Accordingly, it is possible that small island populations have less genetic heterozygosity than widespread mainland populations because the former experience a less varied environment than the latter.

There is the possibility that the loci studied here do not give a good indication of the level of genetic variation in *R. f. greyii* populations, because the loci are only a small sample of the total genome. However, there is no reason to believe there has not been a proportionate decrease in variability within the whole genome. It appears then, that in some environments it is possible for populations to exist with considerably less genetic variation than that usually found in most mammalian populations. Since genetic variability is essential for evolution, including adaptation to a changing environment, then the small island populations of *R. f. greyii* do not appear to be as well equipped for evolutionary change as mainland populations. This does not imply that these populations are not well adapted to their current environment.

It is possible that the small island populations do have similar levels of genetic heterozygosity to mainland populations, but on the islands the heterozygosity is not as evenly distributed within the genome. That is, the proportion of polymorphic loci may be much less than normal, but the average heterozygosity for polymorphic loci may be high. Two polymorphisms were detected in small island populations of *R. f. greyii*. The observed heterozygosity at the GOT_S locus in the Pearson Island South population was 0.417, and at the ICD_M locus in the Waldegrave Island population it was 0.596. These observations might reflect the fact that in small island populations, polymorphisms with

one gene at a low frequency are not expected to be maintained as long as polymorphisms with genes approximately equal in frequency. This is because the time to fixation depends upon the initial gene frequencies (Kimura and Ohta, 1969) and for the former type of polymorphism, heterozygote advantage may actually accelerate the fixation of the most frequent gene (Robertson, 1962).

6.4 GEOGRAPHIC VARIATION IN GENE FREQUENCIES

Among the eight proteins polymorphic in *R. f. greyii* (Fig. 5.11), four broad patterns of geographic variation can be discerned. These patterns can be interpreted as follows:

(i) For the PGM_1 locus, it is unlikely that the PGM_1^2 and PGM_1^3 alleles were even moderately widespread before the isolation of Kangaroo Island and Eyre Island from the other populations. If either gene was widespread it might be expected to be found in some present-day populations nearby. By a similar argument, it is likely that the GOT_S^B gene, although present in two populations, arose by mutation after the isolation of the Pearson Islands (see Section 6.1).

An examination of the distribution of *6PGD* genes in the *R. f. greyii* populations only, suggests that the $6PGD^2$ gene arose by mutation, since the isolation of the South-East population, or at least was not widespread in the ancestral population. However, all specimens of *R. f. assimilis* and most *R. f. coracius* are $6PGD^2/6PGD^2$ homozygotes, which raises the possibility that the $6PGD^2$ gene is present in the South-East population of *R. f. greyii* as a result of introgression of that population with *R. f. assimilis*. Alternatively, the presence of $6PGD^2$ in the South-East population may be a relic from a time when *R. f. greyii* and *R. f. assimilis* were sympatric and were indistinguishable at the level of subspecies. It is interesting to note that the GOT_S^C gene, present in mainland

R. f. assimilis, was not detected in the South-East population. Either this gene, unlike *6PGD*^{2}, did not survive in the South-East population or was not present in the region at a time when the two populations were sympatric.

(ii) Because both ICD_M alleles are found in several populations there is a good case for arguing that the two alleles were present in the ancestral *R. f. greyii* population. This polymorphism is discussed in more detail in Section 6.2.

(iii) The distribution of variation at the ME_S, LDH_A and *ALB* loci suggests that all the alleles found at these loci in *R. f. greyii* were present before fragmentation of the ancestral population.

The north-western populations are monomorphic for the ME_S^2 gene while most of the islands towards the south-east are monomorphic for ME_S^1. These observations indicate that there may have been a cline in ME_S gene frequencies in the ancestral population, the ME_S^2 gene being the most frequent gene in the north-west, with ME_S^1 increasing in frequency to the south-east end of the distribution. The fact that all *R. f. fuscipes* individuals are homozygous ME_S^2/ME_S^2, while *R. f. assimilis* and *R. f. coracius* individuals are homozygous ME_S^1/ME_S^1, gives added support to this suggestion.

Similarly, the contemporary distribution of genes at the *ALB* and LDH_A loci suggests that there may have been clines with respect to these genes in the ancestral population. In both cases, the genes most frequent towards the south-eastern end of the *R. f. greyii* distribution are the only genes at these loci found in *R. f. assimilis* and *R. f. coracius*, while the genes most common towards the north-western end of *R. f. greyii* are also present in *R. f. fuscipes*.

(iv) The pattern of haemoglobin variation also throws some light on the possible composition of the ancestral population. Three "alleles", HB^{B}, HB^{D} and HB^{K} are each found separately in only one population and may be considered to be recent mutants. HB^{A}, HB^{P} and HB^{S} have a more widespread distribution, the first two being found in more than one population of *R. f. greyii* and all three are present in at least one other subspecies. HB^{S}, like $6PGD^{2}$, appears from a consideration of *R. f. greyii* populations only, to be a recent mutant.

From these patterns of gene frequency, it seems likely that the ancestral population was polymorphic for genes at at least five loci (*ALB*, *HB*, ICD_{M}, LDH_{A} and ME_{S}) out of a total of 16 loci examined and therefore had a level of genetic variability similar to that in most modern mammalian populations.

6.5 MULTIVARIATE ANALYSES OF GENETIC DATA

The general relationships between populations as seen in the genetic distance matrices, dendrograms and correspondence analysis scatter-diagrams (Tables 5.21 and 5.23, and Figs. 5.12 to 5.16) are in agreement with the locus by locus trends just discussed. These methods of analysis reveal two main groups within *R. f. greyii*, one consisting of Goat Island, Pearson Island South and Pearson Island North and a second consisting of all other populations except Dog Island and South-East. The latter two populations, occupying marginal geographic positions, are apparently not closely associated with either of the two main groups. While Dog Island is geographically close to Goat Island and Eyre Island, it has been isolated from them for about 11,500 years, whereas Goat and Eyre Islands have had more recent connections (up until about 6,500 years BP) with Eyre Peninsula. The distinctiveness of the South-East and Dog Island populations is clearly demonstrated in the correspondence analysis scatter-diagrams which separate these two

populations from other *R. f. greyii* populations on the second and third axes respectively (when *R. f. greyii* populations only are analysed).

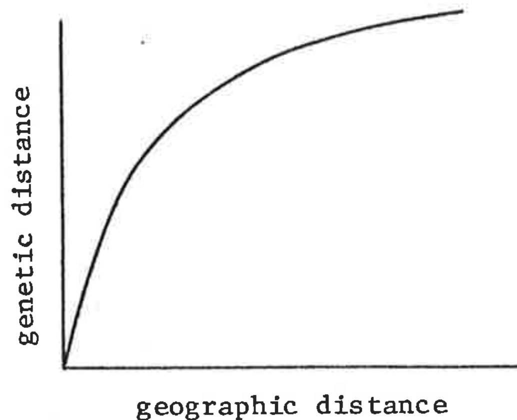
If, for the present, selective forces are ignored, it would be expected that the genetic distance between any two populations will be related to the time of their isolation from one another and the geographic distance between them. For example, it is expected that Waldegrave Island will show a greater affinity to the Eyre Peninsula population than to the Pearson Islands, despite being about equidistant from both. This is because Waldegrave Island has been isolated from Eyre Peninsula for about 6,500 years, but from the Pearson Islands for 14,000 years. Similarly, North Gambier Island has been isolated from Dog Island and Eyre Peninsula for about the same time, but has more genetic similarity to the latter population presumably because of geographic proximity.

An exception to this predicted relationship between genetic distance, geographic distance and the time of separation is Goat Island, which would be expected to show more similarity to Eyre Peninsula than to the Pearson Islands. This is because Goat Island's most recent connection has been with Eyre Peninsula. Goat Island's greater similarity to the Pearson Islands than to Eyre Peninsula may be related to the clines in gene frequency, postulated to have existed in the ancestral population. It is possible that genes with a high frequency in the north-west were fixed on Goat Island and the Pearson Islands, but not in the Eyre Peninsula population, resulting in the first two populations having more genetic similarity to one another than either has to the latter population.

Superimposed upon the effects of geographic distance and time of isolation on genetic similarity, is natural selection, which may either enhance these effects or work to counteract them. For example, natural selection may favour similar gene frequencies in isolated populations

and hence lessen the effects of both geographic separation and time of isolation.

Regression analyses revealed significant relationships between genetic distance and both geographic distance and time since isolation. From theoretical considerations, the rate of increase of genetic distance with respect to geographic distance is expected to be higher for smaller distances than it is for larger distances (Kimura and Weiss, 1964). This is illustrated, in an approximate fashion, below:



The data on *R. fuscipes* generally agree with this prediction, as can be seen in Figs. 5.17 to 5.20. The regression of genetic distance on geographic distance gives a higher regression coefficient (b) when *R. f. greyii* populations only are considered (i.e. short distances), than when *R. f. fuscipes* and *R. f. coracius* are included (i.e. long distances). This difference is statistically significant for both the Nei and Jacquard measures of genetic distance. It is interesting to note that Kirby (1974), analysing data on *Mus musculus* populations, found no clear relationship between genetic distance and geographic distance until the geographic distance was about 120 km or more.

It is not known why the regression of genetic distance on time since separation accounts for a significant proportion of the variation between populations for the Nei genetic distance but not for the Jacquard genetic distance. However, Nei's measure, D, is expected to be linearly related to time since separation, t, by the formula

$$D \simeq 2\alpha t \quad \ldots (3)$$

where α is the rate of gene substitution *per* locus *per* year (Nei, 1972; 1975). Nei (1975) has suggested that a reasonable estimate of α is 1×10^{-7}. Using this value and the mean value of D between *R. f. greyii* populations of 0.17 in equation (3) gives an average time of separation of 850,000 years. From Table 5.24, it would appear that all the South Australian islands have been isolated from the mainland within the last 14,000 years and the separation of the mainland populations probably occurred within the same period (see Chapter 1). There is clearly a large discrepancy between the date of separation of the populations as indicated by the biogeographic information and the prediction by equation (3).

Two factors may be contributing to this discrepancy. First, either equation (3) or the value of α chosen are incorrect. These seem unlikely explanations since the formula has been used to estimate the time of divergence between other populations and these times agree with evidence from other sources (e.g. Nevo *et al.*, 1974; Nei, 1975). However, estimating α is extremely difficult and by using a value of $\alpha = 7 \times 10^{-6}$ an average time of divergence in agreement with the biogeographic data is obtained. The second contributing factor is that the *R. f. greyii* populations may have differentiated very rapidly and have reached a level of differentiation comparable to other mammalian populations separated for much longer periods. Many of the differences between *R. f. greyii* populations have resulted from the fixation of alternate genes which are presumed to have been present in the ancestral population. Such divergence (due to one or both of genetic drift or directional selection) can occur rapidly, especially in small isolated populations.

Recently, Sarich (1977) has used what he calls an "electrophoretic clock" based on the Nei genetic distance metric to estimate the time of

divergence of populations. This electrophoretic clock was calibrated by two steps. First, it was found that 100 albumin immunological distance units (AID's), a measure of dissimilarity in albumin antigenicity, correspond to a time of separation of about 60 million years (e.g. Sarich and Wilson, 1967; Wallace *et al.*, 1971; Maxson *et al.*, 1975). Then Sarich demonstrated a high correlation (r = 0.82) between AID and D, the distance measure of Nei, one AID on the average being the equivalent of 35D. Hence one unit of D corresponds to about 20 million years of separation. The situation is slightly more complex than this, because Sarich showed the calibration depends upon the relative numbers of plasma and "intracellular" proteins used to estimate D. Taking this into account, a genetic distance of 0.17 represents, using Sarich's clock, a divergence time of about 1.5 million years. Clearly this is about two orders of magnitude too large, in the case of the *R. f. greyii* populations.

Apart from the fact that the *R. f. greyii* populations may have diverged rapidly it is possible that Sarich's clock has been calibrated incorrectly. The first step in the calibration involved species which had been separated for long periods of time, corresponding to AID's of about 100 units. However, the relationship between AID and D was only shown at distances up to about 50 AID units. It is possible that this correlation does not hold, or at least the relationship is not linear when extended to about 100 AID. For example, distantly related species may have many albumin amino-acid differences and a new substitution may have relatively little influence on their AID. Recently separated species or populations may have very similar albumin amino-acid sequences and one amino-acid substitution may result in a greater change in AID than the same substitution between the distantly related species. Furthermore, in distantly related populations, there is a greater chance that a substitution in one population is at the site of a previous

substitution and no change in AID will occur. Thus the "setting" of the clock with species separated for long periods may be inappropriate for recently separated populations.

Despite the possible error in the calibration of Sarich's clock, it would appear that the *R. f. greyii* populations have become different rather rapidly. This is probably due to the fact that the divergence has been by the fixation of genes present in the ancestral population as well as the accumulation of new mutants after bifurcations. Clearly, the former mechanism of differentiation can be much more rapid than the latter mechanism.

6.6 GENETIC DISTANCE, TAXONOMIC RANK, AND THE RELATIONSHIP BETWEEN SUBSPECIES

Genetic distances (using the Nei metric) between populations of *R. fuscipes* are, in general, greater than the distances between other populations of the same taxonomic rank reported by other workers (see Table 5.22). It should be emphasised that these genetic distance estimates for *R. fuscipes* are based on about sixteen genetic loci and have standard errors of the order of one half or more of the actual estimate. Two extensive comparative studies of genetic and taxonomic distance (Ayala, 1975; Nei, 1975) have shown that the genetic distances are similar (for the same taxonomic rank) for mammals, insects, fish and reptiles. For mammalian species, races are usually no more than about 0.05 units distant. (While the term "races" has no taxonomic meaning, it is used here to indicate different populations belonging to the same subspecies. These are often referred to as "local populations".) Populations belonging to separate subspecies are generally found to have genetic distances of about 0.1 to 0.15 units, with a maximum around 0.25 units.

One of the main factors contributing to the larger than normal estimates of genetic distance between *R. fuscipes* populations is the low level of variability within small populations. It is clear that there are forces (genetic drift and selection) which can reduce individual variation within populations. Such a reduction in intrapopulation variation (especially when it is due to drift) tends to increase the variation between populations.

There are other examples of low intrapopulation variation and high interpopulation variation. A pocket gopher, *Geomys tropicalis*, which is confined to a much smaller area (and presumably has a lower population size) than three other *Geomys* species examined, has a lower mean heterozygosity and less chromosomal variation than the other species (Selander *et al.*, 1974). *G. tropicalis* is also less similar to the other three species than they are to each other. The least heterozygous populations of *Peromyscus polionotus* are also the most divergent from other populations (Selander *et al.*, 1971; Selander and Jonnson, 1973). Avise and Selander (1972) found that cave populations of *Astyanax mexicanus* have lower levels of variability than surface populations and the genetic distances between cave populations are greater than distances between surface populations.

As a corollary, it is expected that those forces maintaining intrapopulation variation (mutation, selection, migration) will tend to decrease variation between populations. Thus the highly variable *Drosophila* "local populations" are genetically very similar (Ayala, 1975).

It may be appropriate to consider the various *R. f. greyii* populations as each having distinct subspecific status, because they are allopatric populations of the same species. Even if this is taxonomically correct, the distances between these "subspecies" would, on the whole, be higher than the average for many mammals, although less than the distances between species.

Speciation is thought to occur most commonly as the result of geographic isolation followed by the accumulation of genetic differences which ultimately lead to reproductive isolation (Mayr, 1963; Dobzhansky, 1970). *R. fuscipes* populations are geographically isolated and in most cases they have been shown to be genetically different. The genetic distances between *R. f. greyii* races are about the same as the genetic distances between mammalian subspecies, while *R. fuscipes* subspecies are about the same genetic distance apart as many mammalian species. However, there appears to be no significant barrier to reproduction between individuals from the various populations (as measured by reproduction in the laboratory). Since it is unlikely that different populations of *R. fuscipes* will come into contact in the near future, it is not very useful to speculate on the degree of reproductive differentiation reached. What can be said is that the populations have the potential (both geographically and genetically) to diverge into separate species.

The genetic distances which involve *R. f. fuscipes* or *R. f. coracius* must be considered with a good deal of caution, each being based on the genes of only six individuals. The dendrograms derived from Jacquard's genetic distance measure and Nei's measure differ in their placement of *R. f. fuscipes* and *R. f. coracius* with respect to *R. f. greyii*. From a consideration of the current taxonomy of the species, the dendrogram based on Jacquard's distance measure gives a more satisfactory arrangement than that based on Nei's measure, since the former places *R. f. greyii* populations in one group before any clustering with the other subspecies. However, the dendrogram based on Nei's measure emphasises the close relationship between *R. f. fuscipes* and the north-western populations of *R. f. greyii*, and between *R. f. coracius* and the south-eastern *R. f. greyii* populations. These relationships imply that *R. f. greyii* has, in the past, been a link between the other subspecies and may have

been part of a large, roughly linear population, stretching from south-western Australia along the southern and eastern coasts to north-eastern Australia. Whether this population was continuous at any one time is problematical. The evidence presented here is in favour of *R. fuscipes* colonising Australia along one route, rather than spreading out from a central point. That is, the ancestors of the species either migrated down the east coast and across the south of the continent to western Australia or the colonisation was in the opposite direction (see Chapter 3). It is not possible, with these data, to distinguish between these alternatives. If *R. f. fuscipes*, *R. f. greyii* and *R. f. assimilis* are the result of independent colonisations from an ancestral stock, then it is most likely that the patterns in gene frequencies (at the *6PGD*, ME_S, LDH_A, *ALB*, and *HB* loci) in *R. f. greyii* are the result of introgression with the other subspecies.

6.7 EVOLUTIONARY FORCES

Studies of genetic variation in natural populations are always faced with the problem of determining the nature and relative roles of selective and non-selective forces in maintaining the variation. There are very few examples in which the forces have been precisely determined and measured. In order to enhance the possibility of distinguishing the forces, populations with special features are studied. This study was undertaken in the hope that the unusual distributional characteristics of *R. f. greyii* might enable data relevant to this problem to be obtained.

While it is difficult to assess the roles of selection and drift in determining the genetic structure of *R. f. greyii* populations, it seems likely that drift has been a major influence affecting the gene frequencies on the small islands. For example, it seems most unlikely that selection is responsible for the existence of different allelic genes on islands only a short distance apart and apparently similar in habitat

(e.g. GOT_S genes on the Pearson Islands and ME_S genes on Hopkins and Williams Islands).

Perhaps the strongest evidence in favour of genetic drift being the prime factor in determining the genetic differences between the *R. f. greyii* populations is the general agreement between the theoretical expectations based on a model of neutral genes and genetic drift, and the observations. When genes are selectively neutral, large populations will have more variation than small populations and this is what is observed. Furthermore, when there is no migration between populations, the neutral theory predicts that the probability of fixation of genes depends upon the gene frequencies. Hence two allelic genes, both initially common in several populations which are then subjected to genetic drift, will result in the fixation of one or other gene in each population. Again, this is what appears to have happened in the *R. f. greyii* populations. Indeed, there has been no other study reported where, over such a large number of populations, such low intrapopulation variation and large interpopulation variation has been observed. The correlation between genetic distance and geographic distance is also in agreement with most genes being selectively "neutral".

However, as pointed out in Section 2.3.6, variation in gene frequencies can also be accounted for by selection being the main force determining the gene frequencies. The observations on *R. fuscipes*, suggested in the preceding paragraph as in agreement with genetic drift and mutation determining gene frequencies, are also compatible with selection being the main force. If this is the case, two points can be made. First, selection may well have acted to decrease the genetic variation in island populations compared with mainland populations. Environmental diversity is likely to be greater on the mainland than on an island because islands will experience a moderate marine climate

expected to influence the whole island, and furthermore, environmental diversity is probably proportional to area. Hence it is expected that selective processes will maintain more polymorphisms in mainland populations than in island populations. Second, different allelic genes may well have been favoured in different islands, presuming that there have been differences in environment between islands. Such differences may be difficult to detect because the author found most islands to be superficially very similar. In addition, the environmental differences which may have led to the fixation of different alleles in different populations, may not be present now.

There is some circumstantial evidence that selection has been important. The ICD_M polymorphism on Waldegrave Island has already been discussed in this regard. Selection may have played an important role in producing and maintaining the suggested clines in gene frequency in the ancestral population.

Wills and Nichols (1971; 1972) found heterosis at the *Odh* locus in *Drosophila pseudoobscura* which was conditional upon a sufficiently homozygous genome. As a result of their study they concluded that as inbreeding increases, the loci that remain polymorphic should play a progressively more important role in determining an organism's fitness. Alternatively, only those polymorphisms which are already maintained by marked heterozygote advantage will tend to remain polymorphic with continued inbreeding. In either case, it can be argued that in the abnormally homozygous island populations of *R. f. greyii*, selection may be important for the maintenance of the polymorphisms that are now present in those populations.

Lewontin and Krakauer (1973) proposed a test for the action of selection or drift in maintaining polymorphisms, based on the geographic distribution of genes. The test is for the homogeneity between loci, of the estimated effective inbreeding coefficient, $F = s_p^2/\bar{p}(1-\bar{p})$,

where s_p^2 = between population variance for one of two alleles and $\bar{p}$ = the mean frequency of the allele over all populations. This test was performed using 13 *R. f. greyii* populations (i.e. all those sampled except Eyre Island) and the eight polymorphic loci. The results are shown in Table 6.1 and indicate that the effective inbreeding coefficients are homogeneous. Since the haemoglobin variation may not be due to allelic variation at a single locus, the test was repeated without the haemoglobin variation and a similar result was obtained. The results, if interpreted the way Lewontin and Krakauer suggest, imply that the variation is either due to selection or drift, but not both. However, the sensitivity of the test has been questioned (e.g. Nei and Maruyama, 1975; Robertson, 1975; Nicholas and Robertson, 1976; Ewens and Feldman, 1976). Two points arise from the test on the variability in the effective inbreeding coefficients for the *R. f. greyii* populations. First, most values of F are very high (about 1), indicating considerable heterogeneity in gene frequencies between populations. Second, the values for ICD_M and PGM_1 are much lower than those for the other polymorphisms. These two polymorphisms have maintained similar gene frequencies in all populations despite the absence of migration. Given the insensitivity of the Lewontin and Krakauer test, the small values of F may in fact be indicative of the action of natural selection.

In summary, there is evidence that both natural selection and random genetic drift have played important roles in determining the gene frequencies in the *R. fuscipes* populations.

6.8 FUTURE STUDIES

To help clarify the questions raised by the work described in this thesis several other aspects of the *R. fuscipes* populations need to be investigated. First, more ecological information on the populations must be obtained. For example, accurate estimates of population sizes,

Table 6.1 Effective inbreeding coefficients in *R. f. greyii* populations and the Lewontin and Krakauer test.

Locus	Allele	$\hat{F}$
ALB	ALB^F	1.08
GOT_S	GOT_S^A	0.88
HB	HB^A	1.00
	HB^B	0.43
	HB^D	1.08
	HB^K	1.08
	HB^P	1.08
	HB^S	1.08
ICD_M	ICD_M^1	0.25
LDH_A	LDH_A^1	0.99
ME_S	ME_S^1	0.91
6PGD	$6PGD^1$	0.97
PGM_1	PGM_1^1	0.12

$$\bar{F} = 0.84$$

$$s_F^2 = 0.12 \text{ (observed variance)}$$

$$\sigma_F^2 = 0.12 \text{ (expected variance)}$$

$$\frac{s_F^2}{\sigma_F^2} = 0.99 \qquad \chi_{12}^2 = 11.8 \quad (P \simeq 0.5)$$

especially if monitored over an extended period may give a much better indication of whether drift due to small population size has been important. Simultaneous collection of demographic and genetic information may give some indication of the selective differences between phenotypes in terms of survival, reproduction etc.

Second, investigations into the physiological aspects of the protein variants (such as K_m's, optimum pH's, substrate affinities) may give some clue as to a basis for differential selection of variants. An investigation of this sort may be particularly useful in the case of the ICD_M and HB variants. There is already evidence that selection maintains the ICD_M polymorphism. There is ample evidence, from a wide variety of species such as Man (Allison, 1955, 1964), house-mouse (Berry and Murphy, 1970) and sheep (Pant and Pandey, 1975), that selection acts differentially on some haemoglobin phenotypes. In an attempt to determine a physiological basis for possible differential selection of haemoglobin types, a preliminary investigation into the oxygen affinities of the various haemoglobins has been initiated by Dr. M. Coates (Department of Zoology, University of Adelaide) and the author. Individuals with phenotypes HB A, HB B and HB P had haemoglobins with similar oxygen affinities ($P_{50} \simeq 15$ mm Hg at pH 6.7), while the haemoglobins of HB K and HB S individuals had considerably less affinity for oxygen ($P_{50} \simeq$ 22 mm Hg at pH 6.7). One HB P-S individual was similar to the first group with respect to haemoglobin oxygen affinity, while a HB A-K individual was similar to the second group.

It would be useful to have more information on the habitat of populations. In particular, a comprehensive survey of climatic conditions and flora would be helpful from two points of view. It may give some indication of the relative environmental diversity and help determine whether or not large mainland populations do have more diverse environments than small island populations. Also, it would be interesting

to screen for associations between gene frequencies and environmental variables. This may give a clue to the proteins and their properties that should be investigated.

The tiger snake, *Notechis scutatis*, is the only animal, apart from *R. f. greyii*, which is found on many off-shore islands of southern Australia. It is found on some islands inhabited by *R. f. greyii* as well as some others. An electrophoretic study of *Notechis* would provide an excellent opportunity to compare genetic variation in populations living in similar conditions and having been isolated for similar periods to the *R. f. greyii* populations. *Notechis* is also found on the mainland of Australia and a comparison of large mainland and small island populations would be possible. If intermittent drift is responsible for the low level of genetic variation in *R. f. greyii* island populations, then it is likely that *Notechis* would have experienced bottlenecks in population size and therefore would have been subjected to genetic drift.

Finally, the loci studied here are only a small selection of the total genome. It is important that more electrophoretically detectable genes are examined to see if the trends observed are representative. If island populations do have a low proportion of polymorphic loci, but these loci exhibit high levels of heterozygosity, then this should be confirmed when more loci are examined. In addition to expanding the electrophoretic information, it would be valuable to investigate forms of genetic variation that are undetected by electrophoresis. Drift and other stochastic processes act equally over the entire genome, whereas selection may act on specific genes or groups of genes. In the following chapter a preliminary investigation into variation in body and skull measurements is described.

CHAPTER 7

METRIC VARIATION IN *RATTUS FUSCIPES GREYII*

7.1 INTRODUCTION

In this chapter, metric variation in *R. f. greyii* populations is discussed and the geographic patterns with respect to this variation are compared with those reported for the electrophoretic variation in the previous chapters.

While there have been many published accounts of isozymic or metric studies, there have been very few which make a direct comparison of isozymic and metric variability in the same populations. Three studies which do make this comparison will be discussed, because they most resemble the present study, in that they investigate variation of island populations.

Soule (1971) studied isozymic variation (based on about 20 electrophoretic loci) and metric variation (based on eight morphological characters) in the lizard *Uta stansburiana* on islands in the Gulf of California. Two methods were used to estimate the level of variability from the metric data. All eight characters were used to determine a single measure of generalised variance, while for each of five of the characters, the coefficient of variation was calculated and the overall mean of these coefficients then used. The generalised variance was positively correlated with the area of islands, the largest islands being about as variable as mainland populations. The mean coefficient of variation was found to be significantly correlated (by rank-order correlation) with heterozygosity, estimated from the isozymic data. No attempt was made to compare the geographic patterns in metric and isozymic variation.

Soule *et al.* (1973) reported an association between the percent heterozygosity (based on about 21 loci) and the coefficient of variation for one morphological metric character in species of *Anolis* lizards in the West Indies.

Patton *et al.* (1975) investigated variation at 37 electrophoretic loci, 11 cranial and 4 external metric characters, and 5 presence-absence cranial, "epigenetic" characters among *Rattus rattus* populations on seven islands in the Galapagos Archipelago. Significant positive correlations between island area and both mean heterozygosity and the mean coefficient of variation were reported. For each measure of variation an estimate of the distance between pairs of populations was made and a dendrogram was produced by a cluster analysis of the distance matrix. This resulted in a remarkable similarity in the clustering of populations when either the metric, epigenetic or isozymic data were used.

In summary then, the studies of Soule (1971), Soule *et al.* (1973) and Patton *et al.* (1975) suggest that isozymic, metric and epigenetic data all reveal similar geographic patterns of variation and similar estimates of the comparative levels of variation within populations.

7.2 MATERIALS AND EXPERIMENTAL METHODS

Only samples from *R. f. greyii* populations (excluding Eyre Island) were used for this study (nine individuals from Pearson Island South and ten from each of the other populations). These animals were chosen from those used for the electrophoretic study, so as to ensure that the time between capture and killing was as short as possible, and only animals which appeared to be sexually mature were included. Where possible, each population was represented by equal numbers of males and females. Unequal sex ratios were present in the samples from Eyre Peninsula (7♀♀, 3♂♂), Pearson Island South (4♀♀, 5♂♂) and Pearson Island North (3♀♀, 7♂♂). The frozen bodies were thawed and four external

characters measured. Skulls were cleaned by soaking in water for a few days and removing any remaining flesh with forceps.

Fourteen skull characters were chosen from a sample of 48. These 48 characters were measured on a sample (10 individuals) of skulls housed in the South Australian Museum. Some characters were eliminated because they were difficult to measure and repeated measurements gave inconsistent results. From the remainder, the eleven least correlated characters as well as three characters measuring the overall height, width and length of skull and four external characters, were chosen for inclusion in the main study. These characters, except height of skull and the external characters, are shown in Fig. 7.1. All measurements were made with dial calipers, accurate to the nearest 0.1 mm.

1. Greatest length of skull. From the anteriormost point of nasals to the posteriormost point of occipital.
2. Orbit length (left). From anteriormost point to posteriormost point.
3. Interparietal width. As measured from its points of junction with parietals and occipital.
4. Interparietal length. From point of juncture with sagittal suture of parietals to most posterior point near the midline.
5. Interorbital width. Least distance between orbits, across the frontals.
6. Premaxillae width. Greatest distance across posteriormost tongues.
7. Braincase width. Distance across skull, along a line passing through the posteriormost points of the orbits.
8. Foramen magnum depth. Maximum vertical distance across.
9. Foramen magnum width. Maximum horizontal distance across.

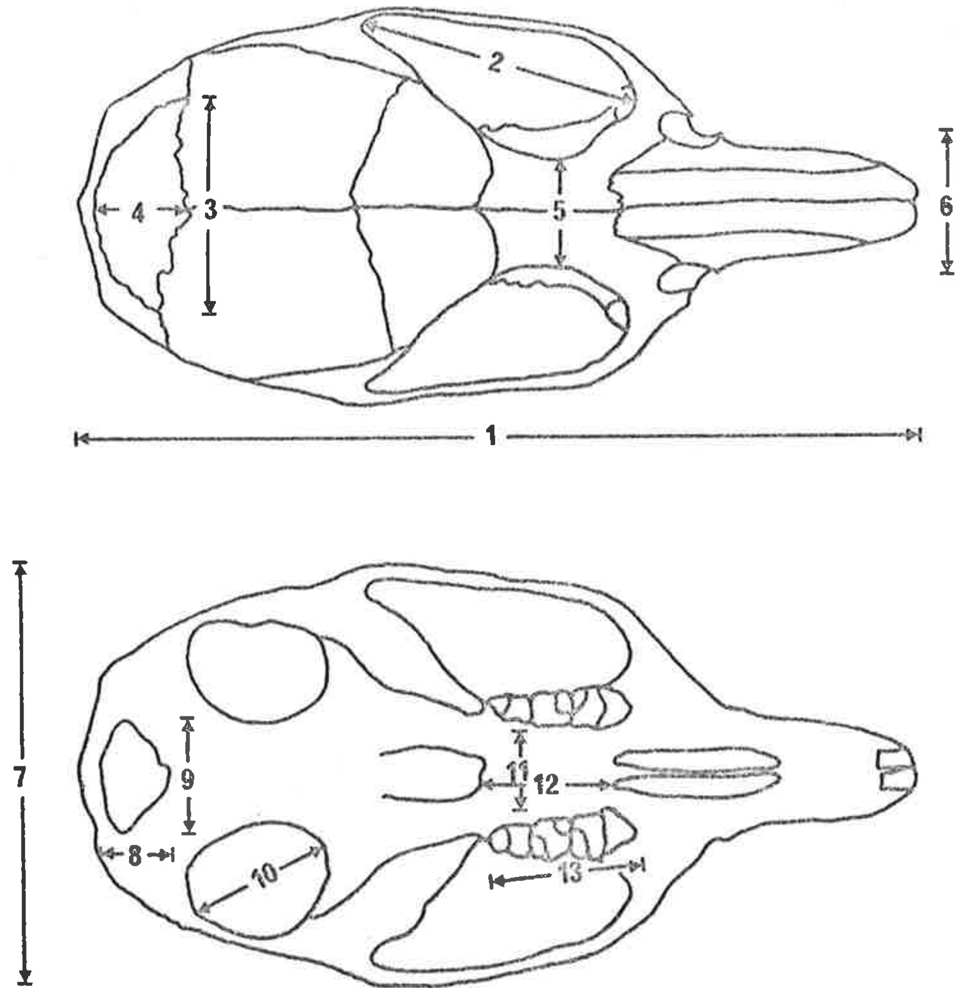


Figure 7.1 Diagrams of dorsal (top) and ventral (bottom) aspects of the skull of a *Rattus fuscipes greyii*. The numbers refer to the characters measured. For a full description see the text.

10. Bulla length. From anteriormost point to posteriormost point.
11. Inside M^{1-1} width. Least width between crowns of first upper molars.
12. Length of palatal bridge. From the anteriormost point of interpterygoid fossa to the most posterior point of the anterior palative foramen.
13. M^{1-3} length (left). Distance along molar row.
14. Height of skull. Measured by resting the skull, ventral surface down, on a glass slide and taking a vertical measurement of the distance between the bottom of the slide and the uppermost point of the skull (and subtracting the thickness of the slide).
15. Head and body length.
16. Tail length.
17. Hind foot length.
18. Ear length.

Each individual was classified into one of seven categories, reflecting the amount of molar tooth-wear. The categories were based, with minor adaptations, on those of Lidicker (1966) for *Mus musculus*.

Class 1. Virtually no tooth wear on posterior cusps of M^2 and M^3. Lobes of second cusp of M^2 not joined.

Class 2. Lobes of second cusp of M^2 joined. Lobes of M^1 anterior cusp not joined.

Class 3. Lobes of anterior cusp of M^1 touching, but lakes not joined.

Class 4. The lobes of the anterior cusp of M^1 joined. Lakes becoming more developed. M^1 consists of three distinct lakes.

Class 5. Lakes well developed. Anterior cusps of M^2 joined.

Class 6. M^2 cusps may be joined and those of M^1 just joining.

Class 7. All cusps of M^1 joined to form one large lake. Ridges between M^1 cusps absent or greatly worn.

Warneke (1971) devised a set of eight tooth-wear categories in *R. f. assimilis*. Unfortunately the author was not aware of Warneke's classifications until after the work on *R. f. greyii* had been completed. However, the categories of Warneke and those above are similar.

7.3 STATISTICAL METHODS

For all characters, the mean scores for males were greater than those for females. An analysis of variance showed that these differences were, in most cases, statistically significant and were present whether or not the differences between populations were taken into account by using a two-way analysis of variance (Table 7.1). These differences between the sexes, and the fact that the numbers of males and females differed between samples, necessitated an adjustment of an individual's score for each character according to the individual's sex. The adjustments were determined from the multiple regression of a pseudo-variate for sex and 12 pseudo-variates for populations, on each character (Table 7.1).

It was also found that individuals with greater tooth-wear had higher scores for most characters than those with less wear (Table 7.1) This suggests that, for most characters, older animals (since Warneke (1971) showed tooth-wear and age to be positively correlated) have greater scores and therefore each individual's score was adjusted according to its tooth-wear category (age). This adjustment was made in a similar fashion to that for sex, except that six pseudo-variates for the seven age categories were required.

A canonical discriminant analysis (CDA) was used to distinguish the populations (see Rao, 1952). The distance between populations, in the multi-dimensional space defined by the canonical variates (CDA distance), was used to perform a cluster analysis by the UPGMA method (Sneath and Sokal, 1973).

Table 7.1 Average deviation of sex and tooth-wear categories from the grand mean, calculated after removing population differences. These deviations were subtracted from each individual's score to remove differences due to sex and age. The significance of the observed differences are given. All measurements are in mm.

		VARIABLE																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Average deviation of sex from grand mean	♀	-0.54	-0.19	-0.08	-0.06	-0.07	-0.12	-0.18	-0.02	-0.03	-0.07	-0.06	-0.08	-0.03	-0.11	-2.86	-1.34	-0.49	-0.36
	♂	+0.54	+0.19	+0.07	+0.06	+0.07	+0.11	+0.19	+0.02	+0.03	+0.07	+0.06	+0.08	+0.03	+0.11	+2.82	+1.44	+0.49	+0.35
Significance		*	*	NS	*	*	*	*	*	*	*	*	*	NS	*	*	*	*	*
Average deviation of tooth-wear categories from grand mean	1	-2.59	-0.95	-0.19	-0.08	-0.22	-0.63	-1.13	+0.22	-0.07	-0.31	-0.41	-0.51	-0.20	-0.47	-17.01	-0.05	-0.11	-0.67
	2	-1.24	-0.43	-0.05	-0.07	-0.07	-0.26	-0.46	+0.03	-0.06	-0.20	-0.20	-0.14	-0.18	-0.23	-5.64	-5.64	-0.36	-0.61
	3	-0.08	-0.06	+0.08	+0.01	-0.04	+0.06	-0.14	-0.07	+0.03	+0.03	-0.07	-0.04	-0.07	-0.06	-1.79	+0.38	-0.14	-0.11
	4	+1.03	+0.37	-0.01	+0.05	+0.09	+0.26	+0.38	-0.03	+0.05	+0.15	+0.17	+0.17	+0.12	+0.19	+4.56	+4.53	+0.33	+0.38
	5	+1.46	+0.52	+0.08	+0.12	+0.08	+0.52	+0.61	-0.03	+0.03	+0.14	+0.29	+0.17	+0.32	+0.19	+7.38	+8.06	+0.23	+0.90
	6	+1.57	+0.56	+0.03	-0.04	+0.11	+0.54	+0.68	-0.01	0.00	+0.23	+0.24	+0.25	+0.29	+0.45	+16.00	-0.03	+0.12	+0.71
	7	+2.06	+0.77	+0.12	+0.25	+0.09	-	+0.58	+0.04	+0.04	+0.13	+0.19	+0.33	+0.24	+0.48	+7.38	+4.01	+1.17	+1.45
Significance		*	*	NS	NS	*	*	*	*	NS	*	*	*	*	*	*	*	NS	*

* P < 0.05

NS Not significant

7.4 RESULTS

The within-populations correlation matrix for the eighteen characters is shown in Table 7.2 and the population means and within-groups standard deviations are given in Table 7.3. These statistics were computed from the measurements adjusted for sex and tooth-wear. A univariate analysis of variance for each character revealed highly significant (all $P<<0.001$) heterogeneity between populations, when either the raw or adjusted data were used.

A nested analysis of variance was used to test for differences in each character, between populations occupying large areas and those confined to small areas. Populations were nested into one of two types (large *versus* small). For fifteen characters, there was no significant difference between the population types. Individuals from populations occupying large areas have significantly greater interparietal width (character 3, $0.05>P>0.01$), bulla length (character 10, $0.05>P>0.01$) and length of molar row (character 13, $0.01>P>0.001$) than those for small populations.

The variability in large and small populations was compared using the standard deviation of each character in each population. Since the standard deviation may be dependent upon its mean, and there is significant heterogeneity between means, a correction is necessary to allow for this variation. Accordingly, for each character an analysis of covariance was carried out with the standard deviation as the dependent variable and the mean as the independent variable, each population being classified as large or small (in area). The two population types are significantly different for only one character, foramen magnum width (character 9, $0.05>P>0.01$), large populations having greater variability than small populations. However, for all but two characters (numbers 2 and 18), the average adjusted standard deviation (standard deviations adjusted to the same mean by using the

Table 7.2 Within-groups correlation matrix for eighteen metric characters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	0.81	1.00																
3	0.18	0.11	1.00															
4	0.30	0.22	0.29	1.00														
5	0.42	0.41	0.23	0.29	1.00													
6	0.54	0.47	0.22	0.19	0.12	1.00												
7	0.64	0.63	0.14	0.23	0.46	0.48	1.00											
8	0.03	-0.04	0.17	0.05	0.10	0.05	0.18	1.00										
9	0.44	0.43	0.11	0.11	0.31	0.17	0.46	0.35	1.00									
10	0.59	0.53	-0.03	0.02	0.19	0.35	0.41	0.11	0.28	1.00								
11	0.49	0.36	0.08	0.20	0.38	0.28	0.38	0.04	0.29	0.32	1.00							
12	0.42	0.39	0.05	0.12	0.26	0.19	0.53	0.07	0.21	0.30	0.17	1.00						
13	0.32	0.31	0.05	0.22	0.26	0.15	0.26	0.14	0.19	0.29	0.28	0.26	1.00					
14	0.61	0.54	0.15	0.28	0.44	0.33	0.47	0.03	0.28	0.39	0.40	0.24	0.27	1.00				
15	0.49	0.47	0.21	0.12	0.26	0.25	0.31	-0.07	0.25	0.28	0.27	0.16	0.08	0.37	1.00			
16	0.50	0.41	0.01	-0.05	0.16	0.32	0.42	0.05	0.28	0.37	0.21	0.19	0.04	0.25	0.22	1.00		
17	0.58	0.33	0.14	0.19	0.32	0.25	0.32	0.12	0.28	0.41	0.48	0.24	0.17	0.41	0.34	0.31	1.00	
18	0.41	0.43	-0.11	0.08	0.28	0.26	0.18	0.00	0.20	0.31	0.42	0.06	0.12	0.29	0.31	0.22	0.20	1.00

Table 7.3 Means of eighteen characters in thirteen populations of *R. f. greyii*, based on data adjusted for sex and tooth-wear. All measurements are in mm. The within-groups standard deviations are also shown.

POPULATION	VARIABLE																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
South-East	36.13	12.86	9.79	4.95	5.26	6.14	18.31	5.01	6.03	6.22	3.64	5.86	6.65	13.46	132.83	128.05	29.06	21.29
Norton Summit	34.49	12.56	9.87	4.87	4.80	6.08	17.20	5.19	6.27	6.17	3.47	5.68	6.38	12.76	133.41	119.19	28.07	19.77
Kangaroo I.	36.13	12.83	9.70	4.98	5.36	6.26	18.03	5.28	6.33	6.40	3.90	5.75	6.64	13.25	138.40	129.17	29.12	21.60
Eyre Peninsula	36.40	13.37	10.08	5.24	5.41	6.19	18.20	5.28	6.36	6.41	3.77	5.66	6.37	13.39	146.49	131.97	28.61	22.21
North Gambier I.	34.28	12.81	8.86	4.49	5.15	5.62	17.03	4.71	5.94	5.78	3.62	5.14	6.01	12.73	133.02	123.67	26.91	20.77
Hopkins I.	35.07	12.50	9.09	4.71	5.17	5.81	17.42	4.72	5.97	5.98	3.42	5.33	6.15	13.10	124.96	123.64	27.62	20.51
Williams I.	36.43	13.22	8.70	4.38	5.36	6.57	17.85	5.38	6.30	6.09	3.64	5.32	6.08	13.79	143.12	137.42	30.08	22.50
Waldegrave I.	36.19	12.97	8.91	4.95	5.33	6.38	18.07	5.11	6.08	6.16	3.79	5.44	6.35	13.54	143.17	130.86	29.97	19.98
Greenly I.	37.33	13.35	8.89	4.86	5.38	6.13	18.65	5.25	6.23	6.22	3.94	6.47	6.32	13.37	146.46	138.24	31.46	21.17
Pearson I. South	34.71	12.36	8.12	4.59	5.38	6.11	17.34	5.24	6.16	6.19	3.40	5.14	5.58	13.06	131.17	111.80	27.22	18.92
Pearson I. North	34.67	12.33	7.56	4.34	5.19	6.06	16.98	5.42	6.10	6.29	3.60	4.99	5.54	12.92	122.65	106.99	27.78	19.20
Goat I.	35.66	12.98	9.42	5.45	5.47	5.84	17.91	5.02	6.13	5.97	3.64	5.38	5.72	13.64	136.84	121.93	27.88	20.98
Dog I.	33.87	12.17	10.06	5.82	5.21	5.90	16.53	5.30	6.52	5.66	3.33	4.78	5.79	12.81	127.51	117.84	26.14	19.50
Within-groups standard deviation	0.83	0.31	0.43	0.29	0.12	0.17	0.38	0.14	0.13	0.14	0.14	0.22	0.20	0.27	8.23	5.10	0.83	0.72

regression equation from the covariance analyses) of the large populations is greater than that for the small populations. This analysis was extended to a multiple analysis of covariance by adding, where appropriate, the means of four other characters as independent variables (characters 1, 7, 14 and 15). These characters were added because they reflect the overall size of the skull and body and it was hoped that the effect of size differences could be removed from the variation of the standard deviation. This analysis revealed that the two population types are significantly different (0.05>P>0.01) for one character, ear length, and it is interesting to note that this is one of two characters where small populations have a greater adjusted standard deviation than the large populations.

The results of a canonical discriminant analysis of the adjusted data are shown in Table 7.4 and Figs. 7.2 and 7.3. Very similar results were obtained when the unadjusted data were used, the only obvious difference being the positioning of Hopkins Island and North Gambier Island in Fig. 7.2. When the unadjusted data were used the position of these two islands on the plane defined by the first two discriminant axes was intermediate between Kangaroo Island and Williams Island. The plane defined by the first two discriminant axes contains 57% of the total variation between populations. The first axis clearly divides the Pearson Islands from the other eleven populations and axis two separates Dog Island from the rest. When axes one and two are considered together, several other populations become differentiated.

The dendrogram produced from a cluster analysis of the distances between populations in the multidimensional space is shown in Fig. 7.3. One cluster contains the four large populations and Hopkins Island. The Pearson Islands and Dog Island are quite distinct from other populations - as they are in the scatter diagram of axes one and two.

Table 7.4 Distances between populations of *R. f. greyii*, in the multidimensional space calculated by the canonical discriminant analysis of the metric data.

	SE	NS	KI	EP	NGI	HI	WMI	WGI	GRI	PIS	PIN	GOI
NS	7.02											
KI	5.14	6.16										
EP	5.85	6.57	3.94									
NGI	7.65	8.49	8.08	7.31								
HI	4.71	7.76	6.80	6.94	5.31							
WMI	9.23	10.32	8.48	8.50	9.60	9.28						
WGI	7.08	8.41	6.27	7.19	7.49	7.12	6.32					
GRI	7.47	8.96	7.33	7.85	8.56	8.80	8.48	6.58				
PIS	12.30	11.98	10.47	10.88	11.15	10.27	10.40	8.60	12.05			
PIN	13.78	12.78	11.74	12.32	12.14	12.00	11.47	9.90	12.82	4.21		
GOI	7.93	9.62	8.08	6.49	6.48	6.10	9.09	7.32	8.85	8.65	10.38	
DI	11.84	9.55	9.84	9.18	10.14	9.84	11.25	10.46	12.99	10.25	12.09	8.42

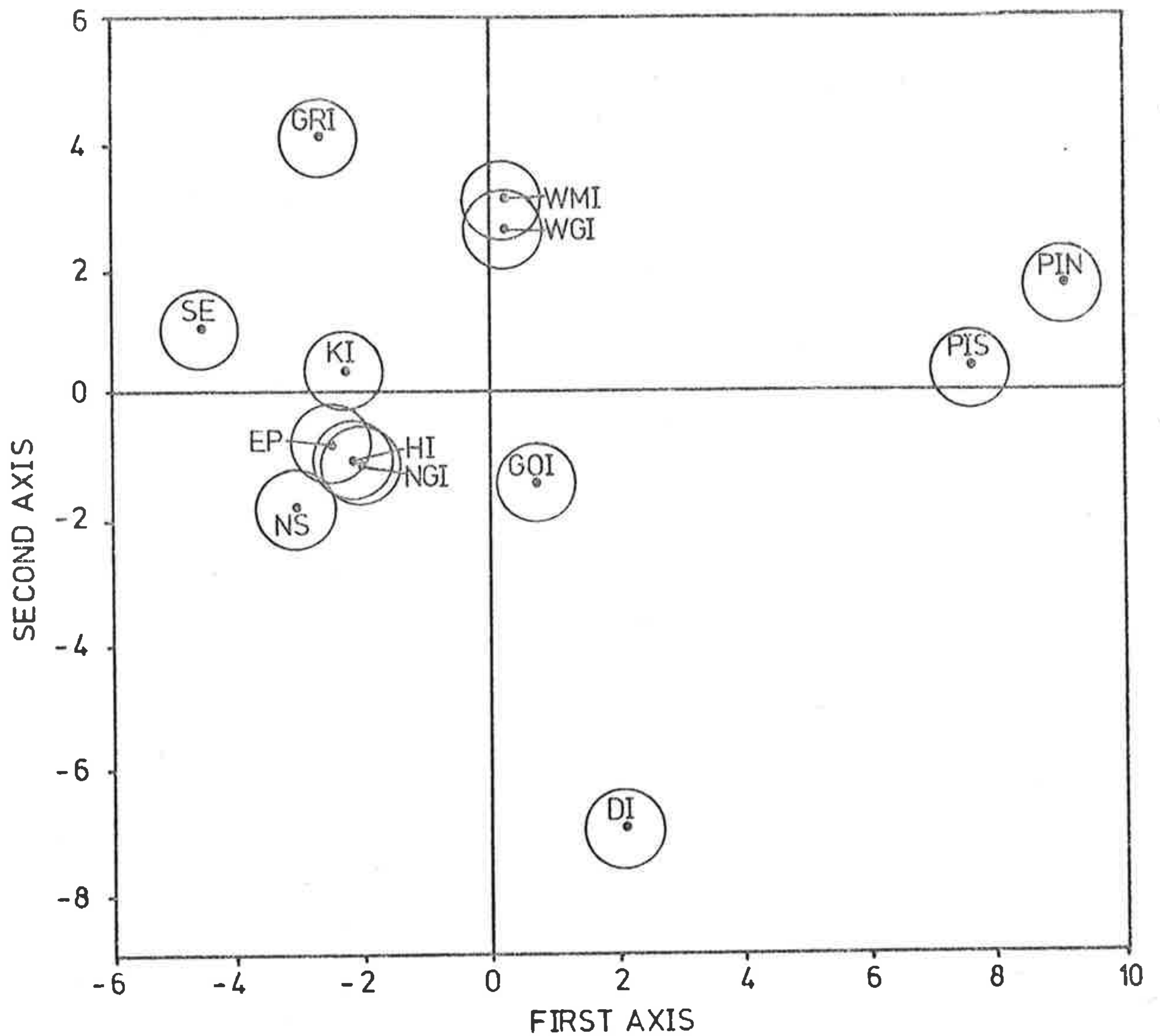


Figure 7.2 *Rattus fuscipes greyii* populations plotted on the first and second discriminant axes of a canonical discriminant analysis. The circles are drawn to mark the 95% confidence limits of the mean of each population.

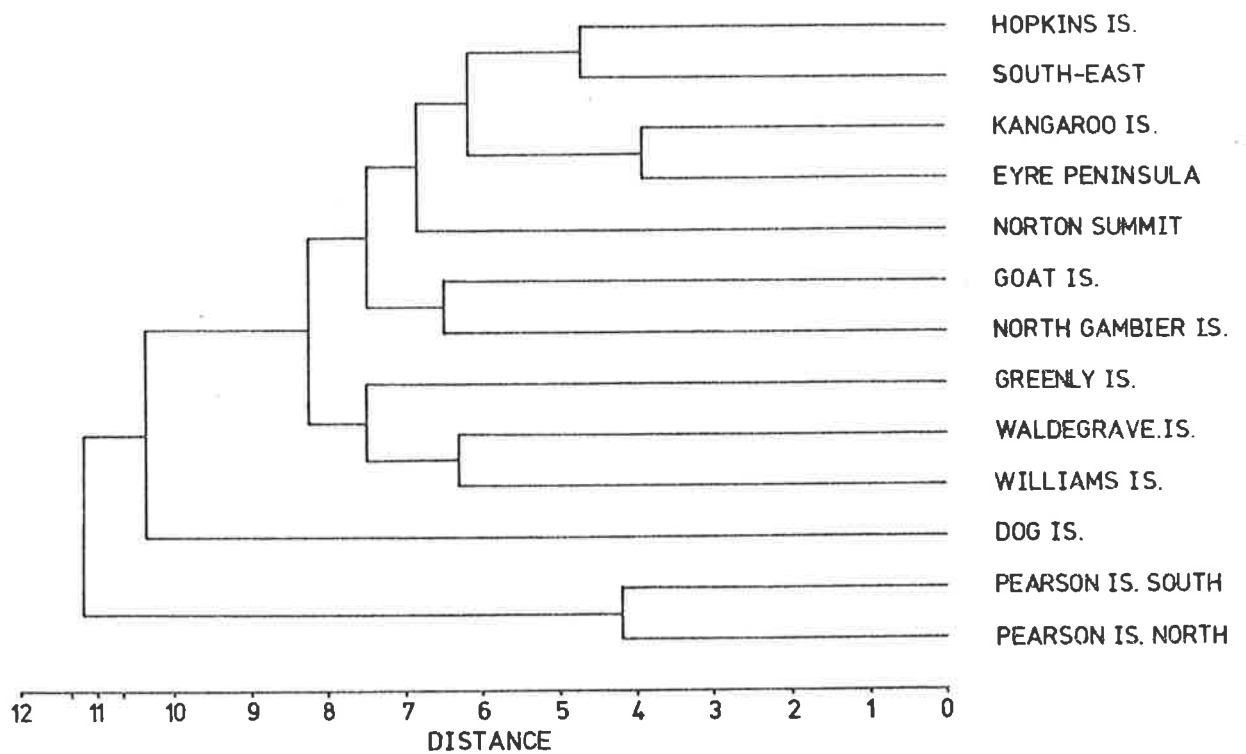


Figure 7.3 Cluster analysis of *Rattus fuscipes greyii* populations. The dendrogram is derived from the distances between populations in the multidimensional space defined by all the principal axes of the canonical discriminant analysis.

Figs. 7.2 and 7.3 should be compared with Figs. 5.12 to 5.16, which are the results of multivariate analyses of the electrophoretic data.

7.5 DISCUSSION

There is considerable metrical variation between populations of *R. f. greyii*. However, the presence of two types of populations in the sample, one occupying small islands and the other relatively large areas, does not, for most characters, significantly contribute to this inter-population variation. This contrasts with some other comparative studies of island and mainland populations of small mammals, which have found that island animals have greater body size than their mainland counterparts (e.g. Delany and Healy, 1967; Berry, 1964). The causes of the discrepancy between these studies are not known.

An important aspect of this work has been the attempt to estimate the amount of genetic variation in the various populations. The isozymic data on *R. f. greyii* show that on the whole, populations occupying large areas have more individual variation than those confined to small areas. For most metric characters, large populations have greater standard deviations than small populations, but the differences are not statistically significant. Darwin (1859) commented that "wide-ranging, much diffused and common species vary most". Fisher and Ford (1926, 1928) showed that "abundant" species of Lepidoptera had more wing colour variability than "common or rare" species. Fisher (1937) studied the size of eggs in birds and found that species classified as "more abundant" had more variability in egg size than those classified as "less abundant". It would appear from this study of *R. f. greyii*, and the work of Soule (1971) and Patton *et al.* (1975), that wide-ranging populations of a species tend to be more variable than populations occupying relatively small areas. Because population size

may be correlated with area occupied, it is difficult, at least in the case of *R. f. greyii*, to distinguish between the contributions to variability, of area and population size.

This study also permits a comparison of the relationships between populations as described by the isozymic and metric characters. The analysis of the isozymic variation by an adaptation of Benzecri's (1970) method for the analysis of contingency tables (correspondence analysis, CA, presented in Chapter 5) reveals a broadly similar pattern of geographic variation to that indicated by the analysis of metric data. Analyses of both sets of data lead to the two Pearson Islands being distinguished from the other populations on the first discriminant axis. Dog Island is displaced from the other populations on the second CDA axis and on the third CA axis. The CA analysis clearly distinguishes the South-East population from the rest (axis two), but the distinctiveness of this population is not as marked by the metric data. Goat Island is positioned differently by the two sets of data. The isozymic data place Goat Island near the Pearson Islands, but the metric data place it nearer the other populations. The scatter diagrams of the first two axes of the CDA and CA display 57% and 54% respectively, of the total interpopulation variation. The dendrograms (Figs. 5.13 and 7.3) illustrate a similar relationship between the populations as their respective scatter diagrams.

That the metric and isozymic data give fairly similar results, in describing the relationships between populations, is further illustrated by a plot of the distance between pairs of populations as measured by the metric data (CDA distance) *versus* the isozymic distance as measured by the correspondence analysis (CA distance) (Fig. 7.4). With the thirteen *R. f. greyii* populations, there are 78 pair-wise comparisons. The correlation coefficient (r) between the two measures of distance is 0.45 ($P<0.001$). The CDA distance is also significantly correlated with

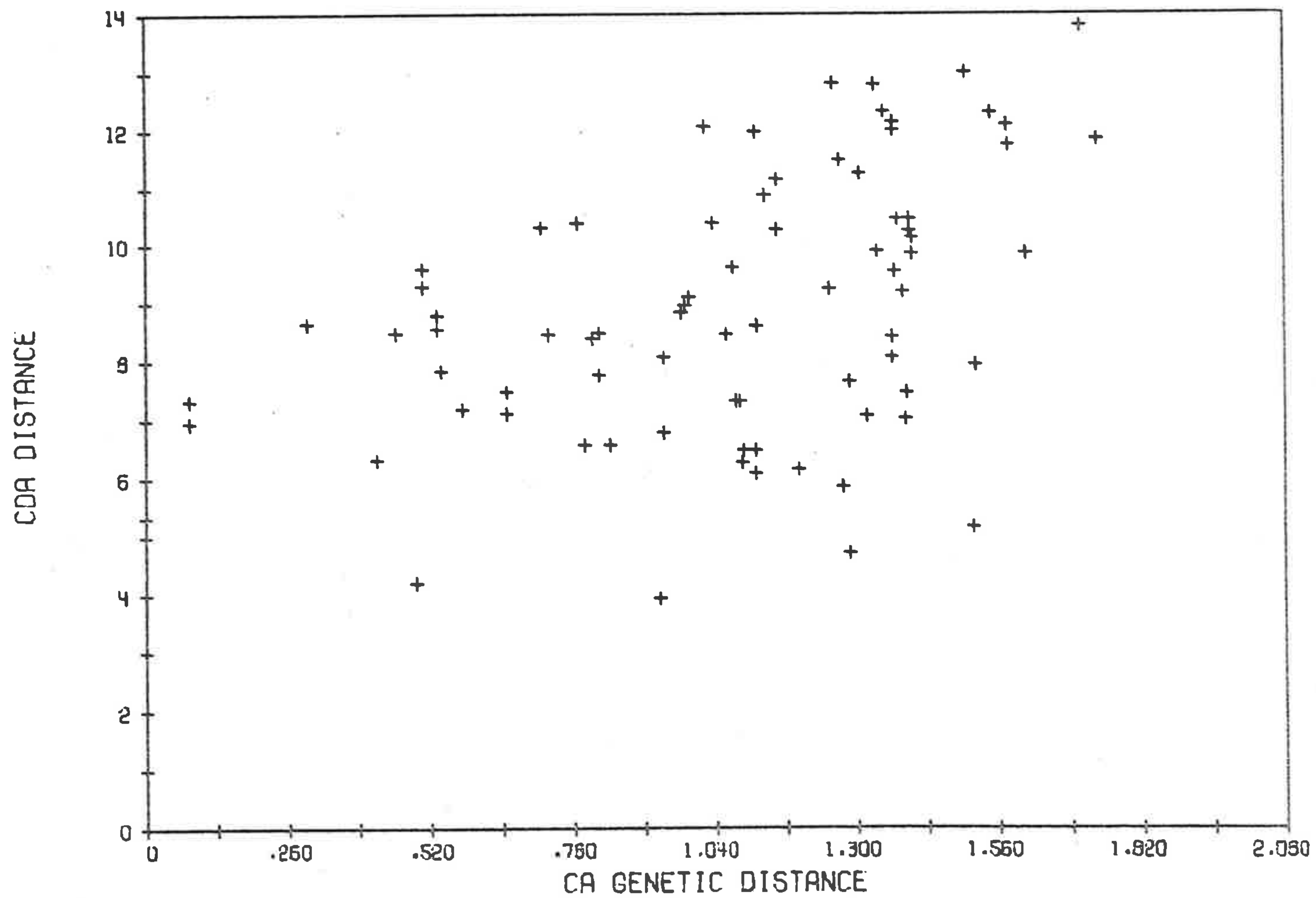


Figure 7.4 Plot of CDA (metric) distance *versus* CA (isozymic) distance for *Rattus fuscipes greyii* populations.

the isozymic distance measured by Nei's method ($r = 0.56$, $P<0.001$).

Unlike the distance measured with the isozymic data, CDA distance does not regress significantly on geographic distance ($b = 14.32$, $0.2<P<0.3$). However, the regression of CDA distance on time of separation gives a statistically significant regression coefficient ($b = 0.46$, $P<0.001$) (Fig. 7.5). The genetic distance from the CA analysis (Jacquard's measure) did not regress significantly on time of separation, but Nei's genetic distance did (see Section 5.5.3). It should also be pointed out that geographic distance and time of separation are not significantly correlated ($r = -0.16$, $0.1<P<0.2$).

It seems difficult to explain these observations on the basis of genetic drift being the major determinant of the gene frequencies for both isozymic and metric characters. If drift was primarily responsible then it could reasonably be expected that the distances between populations, as measured by the two sets of data, should give similar results when regressed onto geographic distance and time of separation. The fact that they don't provides further evidence that natural selection has also been important in determining gene frequencies.

It is difficult to assess the relative merits of the metric and isozymic data in reflecting the true genetical relationships of the populations. The CA is based on variation at eight genetic loci. Populations are distinguished according to differences in gene frequency and there is weighting in favour of genes unique to one population. For example, the distinctiveness of the South-East population is based almost entirely on its monomorphism for a unique haemoglobin gene and the presence of a unique gene coding for 6-phosphogluconate dehydrogenase. On the other hand, the metric variation is presumably controlled by many genes, generally each with small effect, as well as environmental variation.

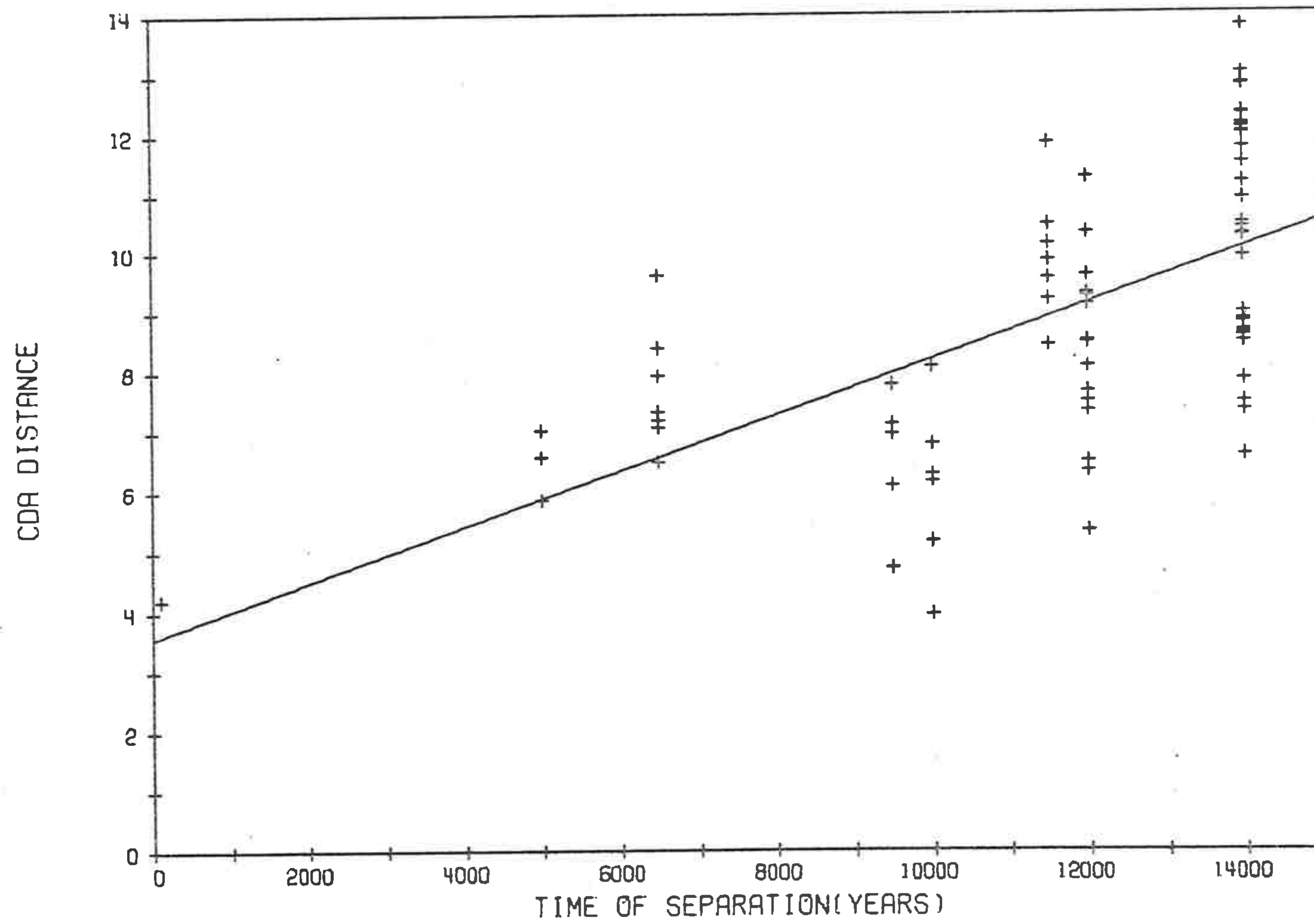


Figure 7.5 Plot of CDA (metric) distance against time of separation for *Rattus fuscipes greyii* populations. The regression line is included.

Despite the differences in the control of the two types of characters, both reveal a similar relationship between the populations. This similarity may be due to one or more of three causes. First, the metric and isozymic characters may be under the control of genes at the same loci. The isozymic variation is presumed to be based on genes at eight loci and it seems unlikely that these are the genes primarily responsible for controlling the metric variation. Furthermore, the regression analyses outlined above do not support this contention. Second, it is possible that some of the genes determining the isozymic and metric characters have been held together in coadapted blocks by linkage disequilibrium. A third explanation is that the divergence of populations with respect to their isozymic and metric characteristics is a function of the time since their isolation from one another. Hence, recently separated populations may have similar isozymic and metric characters because of a recent common ancestry.

In summary, this and other studies have found reasonable concordance between isozymic and metric data in estimating the amount of variation. The two sets of data also describe similar geographic patterns of variation, and this may be due to some of the genetic components for the two types of characters being held together in coadapted blocks or to population divergence being a function of time of separation.

APPENDIX 1

NOTES ON THE ECOLOGY AND REPRODUCTION OF *RATTUS FUSCIPES GREYII*

While trapping and maintaining *R. f. greyii* for this study, some information on the ecology and reproduction of this subspecies was obtained.

Table A1.1 shows the capture rates for the small off-shore islands. No capture rates are available for Hopkins Island, Kangaroo Island or the three mainland populations. The capture rates for small islands, presented in Table A1.1, are considerably higher than those obtained for widespread populations by other investigators (see Chapter 3). In the present study, no records were kept of capture rates from the widespread populations, but the rates were markedly less than those obtained in the small island populations. This difference may not reflect a proportional difference in the population densities of the two types of population. Animals from the two types of populations differ in their behaviour (Robinson, 1976) and this may influence the probability of an animal entering a trap.

Table A1.2 shows the date of birth of litters born to females which were pregnant when captured. These data are not a random sample of breeding activity in the field, being biased according to the time of trapping. Hence, the data do not refute the evidence that a peak in breeding activity occurs during the summer months (e.g. Warneke, 1971; Robinson, 1976). Table A1.2 merely confirms that the observation by Taylor (1961) and Wood (1971) that breeding can occur at all times of the year in *R. f. assimilis*, also applies to *R. f. greyii*.

Two observations on the laboratory colony of *R. f. greyii* deserve mention. First, whilst wild caught animals and their laboratory-born offspring bred readily in the conditions provided, very few matings

Table A1.1 Capture rates (average number of individuals caught *per* trap set *per* night) for eight small island populations of *R. f. greyii*. A pooled estimate is given for the Pearson Islands.

Population	Capture rate
Dog Island	0.73
Goat Island	0.62
Pearson Islands	0.34
Greenly Island	0.22
Waldegrave Island	0.68
Williams Island	0.82
North Gambier Island	0.52

Table A1.2 Date of birth of litters born to females inseminated in the wild.

Population	Date of birth of litter	Number of litters
Eyre Peninsula	13. 8.73	1
"	17.11.73	2
"	24.11.73	1
"	3.12.73	2
"	10.12.73	3
South-East	6. 1.75	2
"	15. 1.75	1
"	17. 1.75	1
"	20. 1.75	1
Waldegrave Island	26. 2.75	1
Goat Island	20. 6.75	1
"	23. 6.75	1
Eyre Island	12. 3.76	1

involving second generation laboratory-bred animals produced litters, and the few successful matings generally included one wild-caught or first generation parent. Taylor (1961) reported a similar loss of reproductive success in two laboratory colonies of *R. f. assimilis* kept in California. Considering only the 74 litters where at least one individual survived until weaning age (4 weeks), the mean litter size at the time of weaning was 4.08 ± 1.51 (SE), with a maximum litter size of eight.

Second, the sex ratio observed in laboratory-reared animals surviving to weaning age showed some interesting variation. From 54 matings (74 litters) a total of 170 females and 132 males reached weaning age, representing a significant excess of females (χ^2_1 = 4.78, $P<0.05$). Table A1.3 shows a breakdown of the data according to the origin of the parents and whether the female parent was inseminated in the wild or in the laboratory. One of the matings of type 2 involved two Norton Summit individuals, while the other two each involved a parent from Pearson Island South and a parent from Pearson Island North. Table A1.3 shows that the excess of daughters was largely confined to litters from females inseminated in the field (type 1) and from the three matings of type 2. Furthermore, matings of types 1 and 2 produced greater average litter sizes than those involving laboratory-bred individuals or parents from different populations (types 3 and 4). One factor possibly contributing to the aberrant sex ratio is that more females than males are conceived, but there is a preferential loss between conception and weaning, of females, in matings of types 3 and 4. Alternatively, females and males are conceived with equal frequency, but female survival is greater in matings of types 1 and 2 than the other two types. It is not known what mechanisms may operate to produce differential conception or survival with respect to sex.

Table A1.3 Numbers of males and females and average litter size at weaning age, in litters of *R. f. greyii* reared in the laboratory.

Mating type	Number of matings	Number of litters	Average litter size	Number of Females	Number of Males	χ^2_1
1. Field inseminated females	17	17	4.41	49	26	7.05**
2. Wild caught parents from same population; laboratory conception	3	6	5.83	26	9	8.26**
3. Wild caught parents from different populations	13	20	3.60	37	35	0.06 ns
4. At least one laboratory bred parent	21	31	3.87	58	62	0.13 ns
Total	54	74	4.08	170	132	4.78*

* $P < 0.05$

** $P < 0.01$

ns not significant

APPENDIX 2

PUBLISHED PAPERS

1. Genetic evidence for the existence of two separate populations of *Rattus fuscipes greyii* on Pearson Island, South Australia. *Transactions of the Royal Society of South Australia* 99: 35-38 (1975).

2. Genetic variation in isolated populations of the Australian bush-rat, *Rattus fuscipes*. *Evolution*: in press.

3. Mitochondrial iso-citrate dehydrogenase variation in the Australian bush-rat, *Rattus fuscipes greyii*. *Animal Blood Groups and Biochemical Genetics*: in press.

4. An electrophoretic investigation of the binding of $3\text{-}^{14}C$ coumarin to rat serum proteins. (with N.B. Piller) *Experientia*: in press.

Schmitt, L. H. (1975). Genetic evidence for the existence of two separate populations of *Rattus fuscipes greyII* on Pearson Island. *Transactions of the Royal Society of South Australia, 99*, 35-38.

NOTE:

This publication is included in the print copy
of the thesis held in the University of Adelaide Library.

Evolution: in press.

GENETIC VARIATION IN ISOLATED POPULATIONS OF THE AUSTRALIAN BUSH-RAT, *RATTUS FUSCIPES*.

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Department of Genetics,
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Running heading: Genetic variation in *Rattus fuscipes*.

Populations inhabiting small islands have some characteristics which make them particularly useful for population genetics studies. In general, the features associated with such populations are: (i) their reproductive isolation, (ii) the small number of individuals and (iii) a well defined and reduced habitat area. These features may make the study of small island populations less complex than those of mainland populations and often a valuable comparison can be made between island and mainland populations.

The study described here is an attempt to genetically characterise a series of isolated populations of the Australian bush-rat, *Rattus fuscipes*. There are four recognised sub-species of the southern bush-rat, each inhabiting a separate coastal region of Australia (Taylor and Horner, 1973) (Fig. 1). This study is primarily concerned with the South Australian sub-species *R. fuscipes greyii*, which inhabits three geographically isolated areas on the mainland and thirteen off-shore islands. The three mainland populations and that on Kangaroo Island occupy areas of similar size, about 400,000 ha, and will be referred to as "large" populations. The other populations inhabit islands which range in area from about 50 to 300 ha and will be referred to as "small" populations. This contrast in the extent of the areas occupied by the two types of populations is probably accompanied by a corresponding difference in the numbers of individuals in the populations, although no studies of population size have been made.

During the last ice-age, all of the islands in question were part of the mainland. As the ice retreated, there was a eustatic rise in the sea level and the islands were formed. It is possible to estimate the time of isolation from data on the mean sea level during the last 20,000 years (e.g. Fairbridge, 1960; Thom and Chappell, 1975) and the present topography of the sea bed. Table 1 shows these estimated times. It is

INSERT TABLE 1

TABLE 1. *Estimated time of isolation of various islands from mainland. The depths shown are the changes in sea level (relative to the present level) that would result in a land bridge joining the various islands to the mainland. Data from navigational charts (B.A.1061; B.A. 3359; B.A. 1762; Aus. 134) and Royal Australian Navy survey sheets (V4/40; V4/41; V4/66; V5/204; V5/205; V5/206) were used.*

Populations	Depth (metres)	Years (B.P.)
Eyre Peninsula - Dog Island	37	11,500
Eyre Peninsula - Goat Island	6	6,500
Eyre Peninsula - Pearson Islands	75	14,000
Eyre Peninsula - Greenly Island	75	14,000
Eyre Peninsula - Waldegrave Island	6	6,500
Eyre Peninsula - Williams Island	50	12,000
Eyre Peninsula - Hopkins Island	22	9,500
Eyre Peninsula - North Gambier Island	50	12,000
Norton Summit - Kangaroo Island	31	10,000

probable that *R. f. greyii* was distributed across most of central southern Australia during the last ice-age, when at times the climate was wetter than at present, and it is reasonable to assume that suitable habitat for the species was much more widespread. The existence of populations of *R. f. greyii* on Dog Island and Goat Island, with no contemporary population on the adjacent mainland, also suggests a previously wider distribution. While it is recognised that this distribution may not have been continuous, the founders of the present day populations will be considered, in this paper, to have come from an "ancestral population". The present day discontinuity in the distribution of *R. f. greyii* on the mainland presumably has resulted from recent climatic changes (e.g. Twidale, 1969) and human habitation which led to the destruction of suitable bushland habitat. Early European settlers certainly brought predators and competitors of *R. f. greyii* with them.

It is unlikely that there has been much, if any, migration between these island populations, since their isolation. Small mammals, except those commensal with man, are poor over-water dispersers. It seems likely then that a widespread ancestral population of *R. f. greyii* was fragmented by environmental changes into a series of large and small populations, between which no recent migration has occurred.

This study is concerned with determining: (i) the relative amounts of genetic variation in insular and mainland populations, (ii) the patterns of geographic variation and the genetic similarity of populations and (iii) the importance of selection and drift on (i) and (ii). Four large and nine small populations of *R. f. greyii* have been studied, along with some individuals from three other sub-species (*R. f. fuscipes, R. f. assimilis* and *R. f. coracius*). Starch gel electrophoresis was used to investigate genetic variation of thirteen proteins presumed to be controlled by sixteen different genetic loci.

MATERIALS AND EXPERIMENTAL METHODS

Specimens of *R. f. greyii* were collected from the following islands (see Fig. 1 for geographic position and Table 2 for sample size): Dog Is. (DI), Goat Is. (GOI), Pearson Is. North (PIN), Pearson Is. South (PIS) Greenly Is. (GRI), Waldegrave Is. (WGI), Williams Is. (WMI), Hopkins Is. (HI), North Gambier Is. (NGI) and Kangaroo Is. (KI) and from the three mainland populations, Eyre Peninsula (EP), Norton Summit (NS) and South-East (SE). In addition, six *R. f. fuscipes* (RFF), five *R. f. assimilis* (RFA) and six *R. f. coracius* (RFC) were obtained. Except for three *R.f. assimilis* from Glennie Island, all the specimens of these sub-species were collected from mainland populations (Fig. 1).

Tissues were homogenised in 1 ml distilled water per gm tissue and centrifuged at 40,000 g for 20 min. The supernatant was stored at -25°C. Vertical starch gel electrophoresis was carried out using water-cooled gel beds. The proteins scored were as follows: supernatant malic enzyme (ME-1), mitochondrial malate dehydrogenase (MDH-2), cytoplasmic isocitrate dehydrogenase (IDH-1), mitochondrial isocitrate dehydrogenase (IDH-2), phosphoglucomutase (PGM), cytoplasmic glutamate oxaloacetate transaminase (GOT-1), mitochondrial glutamate oxaloacetate transaminase (GOT-2), glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), superoxide dismutase (SOD-1), haemoglobin (HB), red cell acid phosphatase (ACP), lactate dehydrogenase 'B' (LDH-1), lactate dehydrogenase 'A' (LDH-2), esterase (ES), and albumin (ALB). The electrophoresis buffers and tissues used for the various proteins were as follows: ME-1, MDH-2, IDH-1 and IDH-2 (heart or kidney), tris-citrate pH 7.0 (Shaw and Prasad, 1970); PGM, GOT-1 and GOT-2 (heart), tris-citrate pH 8.0 (Selander *et al.* 1971); G-6-PD, 6-PGD and SOD-1 (kidney), discontinuous tris-citrate pH 8.7, borate pH 8.2 (Selander *et al.*, 1971); HB and ACP (red cell haemolysates) and LDH-1 and LDH-2 (heart or kidney), tris-borate-EDTA pH 8.6 (O. Smithies,

cited by Huehns and Shooter, 1965); ES and ALB (plasma), discontinuous tris-citrate pH 8.5, lithium-borate pH 8.5 (Gahne, 1966). Staining solutions were essentially the same as those used by Selander *et al.* (1971), except that ES was detected by the fluorescent method of Hopkinson *et al.* (1973) using 4-methyl-umbelliferyl acetate, and ACP using 4-methyl-umbelliferyl di-hydrogen phosphate (Swallow *et al.*, 1973).

RESULTS

The data on gene frequencies and sample sizes for each population are presented in Table 2. Data on the inheritance of all of the protein variants found in *R. f. greyii* (i.e. on all protein variants mentioned except $Idh\text{-}1^{2}$, $Idh\text{-}1^{3}$, Es^{null}, $Got\text{-}1^{c}$, $Got\text{-}2^{1}$, $Ldh\text{-}1^{2}$ and $Ldh\text{-}2^{3}$) have been obtained from forty-five matings. All breeding data are in agreement with the hypothesis that, for each protein, the variation is controlled by alleles at an autosomal locus. The electrophoretic patterns for each protein are the same as those most commonly found in other mammalian species. Individuals heterozygous at the *Alb* and *Pgm* loci have two bands, *Got*, *Idh* and *6-Pgd* heterozygotes have three bands, and *Me* heterozygotes have five bands. Individuals heterozygous at one *Ldh* locus have the characteristic fifteen bands. Esterase variation involves the presence or absence of a single band. The haemoglobin variation is complex and work is in progress to elucidate its genetic basis. All presumed homozygotes have one main band and a minor, more anodal band. All presumed heterozygotes appeared to have only two main bands, but in some cases the resolution was not good enough to eliminate the possibility of four bands being present. For simplicity in the statistical analysis, the haemoglobin variants have been treated as being determined by alleles at a single autosomal locus, although it is quite possible that more than one locus is involved. No variants were detected for the enzymes ACP, G-6-PD, MDH-2 and SOD-1.

Variability estimates

Each of the large *R. f. greyii* populations, Eyre Peninsula, Norton Summit, South-East and Kangaroo Island, are polymorphic at two to four loci, with mean heterozygosity values in the range 0.02 to 0.10 (mean 0.042) (Table 2). No variation was detected within seven of the nine small island

INSERT TABLE 2

populations. Pearson Island South and Waldegrave Island each have one polymorphic locus and mean heterozygosities of 0.03 and 0.04 respectively. The mean heterozygosity for all small populations is 0.007. The small sample sizes of *R. f. fuscipes*, *R. f. assimilis* and *R. f. coracius* do not allow reasonable estimates of variability to be made for them.

Geographic variation in R. f. greyii

The polymorphic loci have been grouped according to similarities in their geographic pattern of variation.

1. For two loci, *Pgm* and *6-Pgd*, all populations but one (Kangaroo Island and South-East respectively) are monomorphic for the same allele. The *Got-1* locus has a similar pattern, with all populations except the Pearson Islands being fixed for the $Got\text{-}1^a$ allele. The allele $Got\text{-}1^b$ is only found in the Pearson Island populations (Schmitt, 1975).

2. The mitochondrial form of IDH, is variable in three of the four large populations (Fig. 2), with the $Idh\text{-}2^1$ allele being less frequent than $Idh\text{-}2^2$ in all populations. On Waldegrave Island, the $Idh\text{-}2^1$ allele has a frequency of 0.36. All other small populations are fixed for the $Idh\text{-}2^2$ allele.

INSERT FIGURES 1 and 2

3. The *Me-1* locus is variable in all large populations (Fig. 2). In each population the same two alleles, $Me\text{-}1^1$ and $Me\text{-}1^2$, are present. All small islands are fixed for either the $Me\text{-}1^1$ or $Me\text{-}1^2$ allele. Similarly, at the *Ldh-2* and *Alb* loci, each small population is fixed for one of two alleles (Fig. 2). No population is polymorphic at the *Alb* locus, while only Norton Summit is polymorphic at the *Ldh-2* locus.

4. Six haemoglobin "alleles" are found in *R. f. greyii* (Fig. 2). Hb^a is the most widespread. Only one population, Norton Summit, is polymorphic

TABLE 2. *Sample size, gene frequencies, proportion of polymorphic loci and mean heterozygosity in populations of R. fuscipes. A dash indicates the gene was not detected.*

Locus	Allele	RFF	BI	GOI	PIN	PIS	GRI	WGI	WMI	HI	NGI	EP	KI	NS	SE	RFA	RFC
	Sample size	6	27	39	50	24	48	47	50	30	49	19	48	42	41	5	6
Alb	*f*	1.00	-	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-
	s	-	1.00	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Es	*p*	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-
	null	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
Got-1	*a*	1.00	1.00	1.00	-	0.62	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.60†	1.00
	b	-	-	-	1.00	0.38	-	-	-	-	-	-	-	-	-	-	-
	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.40	-
Got-2	*1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.92
Hb	*a*	0.42	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	-	0.50	-	-	-
	b	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	-	-
	d	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	k	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-
	p	0.17	-	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-
	q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
	s	0.42	-	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00*	-
Idh-1	*1*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08
Idh-2	*1*	-	-	-	-	-	-	0.36	-	-	-	0.05	0.11	0.04	-	-	-
	2	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00	1.00	0.95	0.89	0.96	1.00	1.00	1.00
Ldh-1	*1*	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ldh-2	*1*	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.61	1.00	1.00	1.00
	2	0.92	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	0.39	-	-	-
	3	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Me-1	*1*	-	-	-	-	-	1.00	-	-	1.00	1.00	0.89	0.95	0.18	0.32	1.00	1.00
	2	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	-	-	0.11	0.05	0.82	0.68	-	-
6-Pgd	*1*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.10	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	0.90	1.00	1.00
Pgm	*1*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00	1.00	1.00
	2	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-	-	-
Proportion polymorphic loci			0.00	0.00	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.13	0.19	0.25	0.13		
Mean heterozygosity			0.00	0.00	0.00	0.03	0.00	0.04	0.00	0.00	0.00	0.02	0.03	0.10	0.02		

No variants were detected for the enzymes ACP, G-6-PD, MDH-2 and SOD-1.

† The three individuals from Glennie Island were homozygous for *Got-1*^{a}. The mainland animals were homozygous for *Got-1*^{c}.

* Offspring of a *R. f. assimilis* female, with an unknown HB type, showed segregation for *Hb*^{a} and

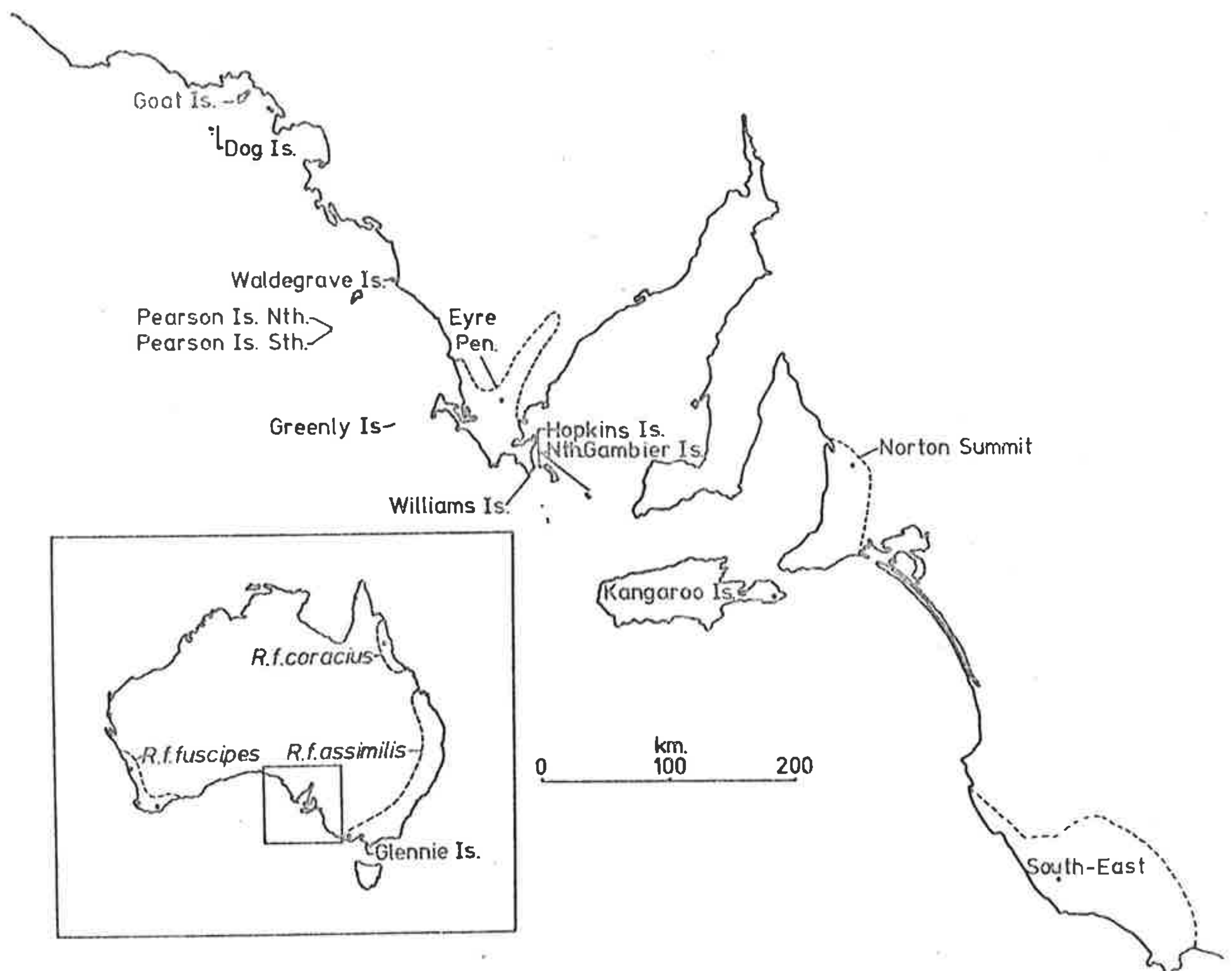


Figure 1 Distribution and sample locations of *R. f. greyii*. Broken lines indicate the extent of the mainland populations. Sample locations in the large populations are indicated by dots. The inset shows the approximate distribution of the other *R. fuscipes* subspecies and their sampling localities.

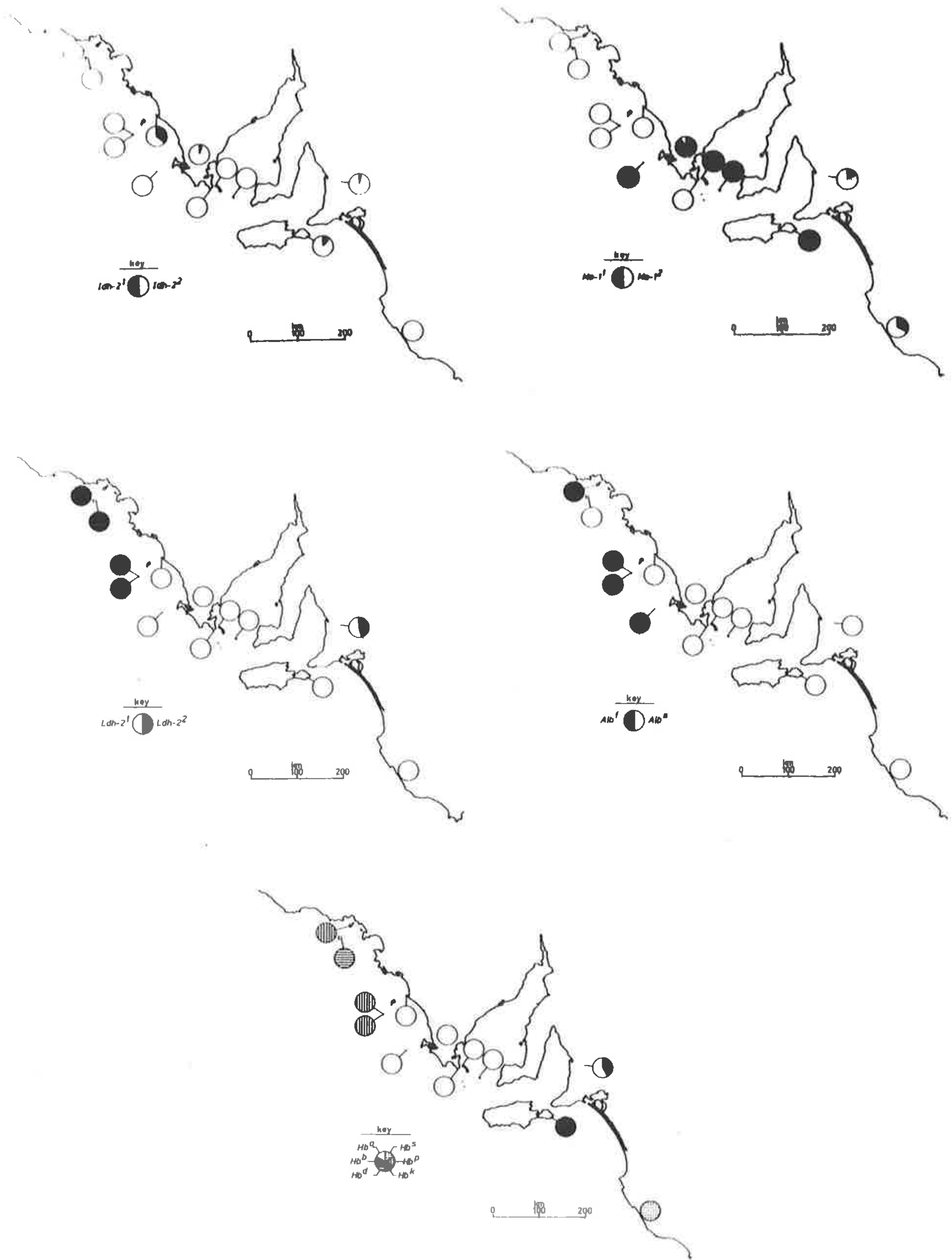


Figure 2 Geographic variation in the *Idh-2*, *Me-1*, *Ldh-2*, *Alb* and *Hb* gene frequencies in *R. f. greyii* populations.

for haemoglobin. Four of the six "alleles" are each restricted to one population of *R. f. greyii*. Hb^{P} is found in the populations on the Pearson Islands and Goat Island.

Variation in R. f. fuscipes, R. f. assimilis *and* R. f. coracius

Variants of four enzymes (GOT-2, LDH-1, ES and IDH-1), which were invariant in *R. f. greyii*, were found in either *R. f. fuscipes* or *R. f. coracius* (Table 2). Also, alleles which were not detected in *R. f. greyii* populations were encountered at the *Got-1*, *Hb*, and *Ldh-2* loci in the other sub-species. The variation in these sub-species will be considered more fully in the discussion.

Quantitative analysis

A genetic distance (Nei, 1972) was calculated for each pair of populations (Table 3). Using this measure and the unweighted pair-group method of cluster analysis using arithmetic averages (UPGMA) (Sneath and Sokal, 1973), a dendrogram was produced and is presented in Fig. 3. As a comparison, a correspondence analysis ("analyse factorielle des correspondances"), which is a modification of principal components for contingency tables (Benzecri, 1970; White, in preparation), was performed. This can be used to produce a dendrogram (Fig. 3b) by the UPGMA method and a two dimensional representation of the genetic relationship of all populations (Fig. 4). *R. f. fuscipes* and *R. f. coracius* have not been included in this latter analysis because their inclusion prevents a clear discrimination of the *R. f. greyii* populations. Because the samples of *R. f. assimilis* were from two reproductively isolated populations they were not included in any quantitative analysis.

INSERT TABLE 3. FIGURES 3 and 4

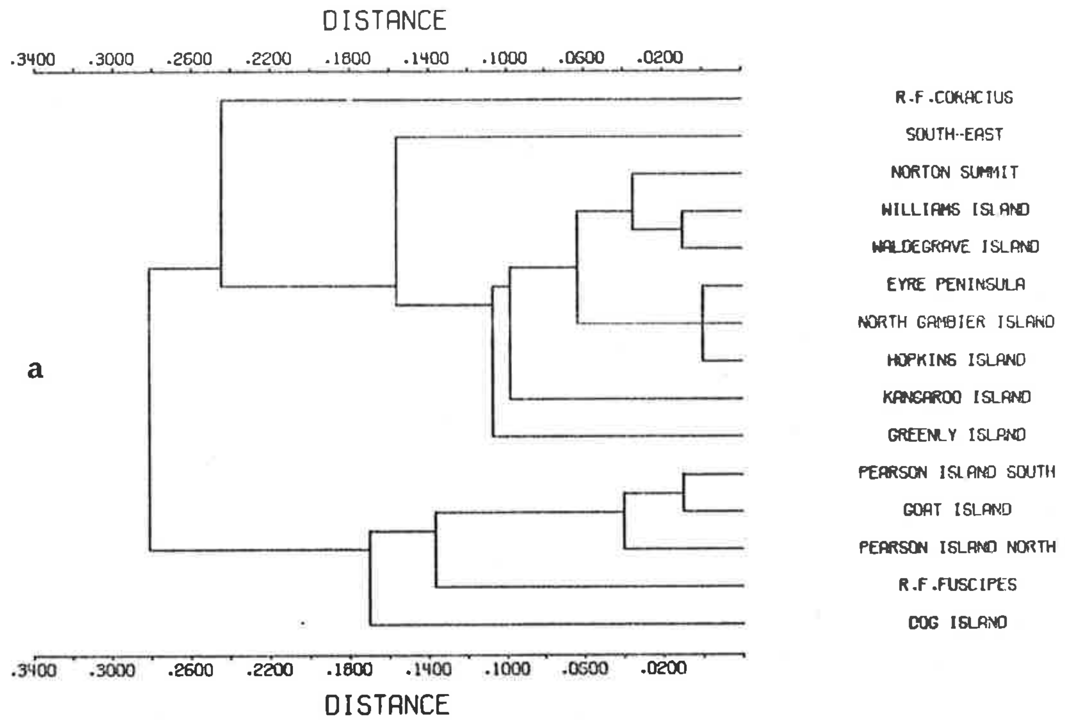
DISCUSSION

Variability estimates

On average, mammalian populations are polymorphic at about one-third of their electrophoretically detectable loci and an individual is heterozygous on the averag at about 5% of its loci (Harris and Hopkinson, 1972; Selander and Johnson, 1973; Lewontin, 1974; Powell, 1975). The *R.f.greyii* populations studied here, with

TABLE 3. *Genetic distances with standard errors, between populations of* R. fuscipes, *calculated according to Nei (1972).*

	RFC	SE	NS	KI	EP	NGI	HI	WMI	WGI	GRI	PIS	PIN	GOI	DI
SE	.18±.11													
NS	.27±.14	.12±.08												
KI	.22±.13	.16±.10	.11±.07											
EP	.22±.13	.15±.09	.06±.04	.07±.07										
NGI	.21±.13	.16±.10	.07±.05	.07±.07	.00±.00									
HI	.21±.13	.16±.10	.07±.05	.07±.07	.00±.00	.00±.00								
WMI	.29±.15	.13±.09	.03±.02	.13±.09	.05±.05	.06±.07	.06±.07							
WGI	.31±.15	.14±.09	.04±.02	.14±.10	.06±.05	.07±.07	.07±.07	.01±.01						
GRI	.29±.15	.23±.12	.14±.09	.14±.10	.07±.07	.06±.07	.06±.07	.13±.10	.15±.10					
PIS	.51±.21	.30±.15	.16±.10	.30±.15	.29±.15	.30±.15	.30±.15	.22±.13	.24±.13	.22±.13				
PIN	.59±.23	.38±.17	.23±.12	.38±.17	.36±.17	.37±.17	.37±.17	.29±.15	.30±.15	.29±.15	.02±.03			
GOI	.48±.20	.29±.14	.15±.09	.29±.15	.27±.14	.29±.15	.29±.15	.21±.12	.22±.13	.21±.12	.01±.01	.06±.07		
DI	.38±.18	.21±.12	.08±.06	.21±.12	.19±.12	.21±.12	.21±.12	.13±.10	.15±.10	.29±.15	.15±.10	.21±.12	.13±.10	
RFF	.37±.17	.32±.15	.19±.11	.36±.17	.30±.15	.32±.15	.32±.15	.23±.13	.25±.13	.23±.13	.12±.08	.18±.11	.11±.08	.19±.12



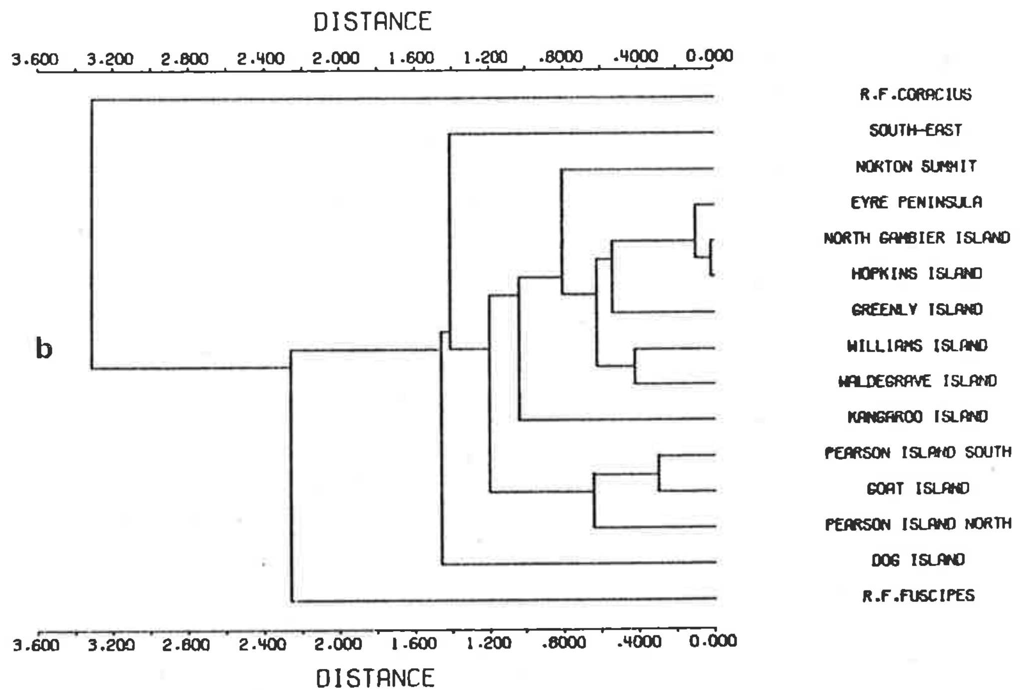


Figure 3 Cluster analysis of *R. fuscipes* populations.

a. The dendrogram is derived from Nei's (1972) genetic distance.

b. The dendrogram is derived from the distances between populations in the space defined by all the principal axes of the correspondence analysis.

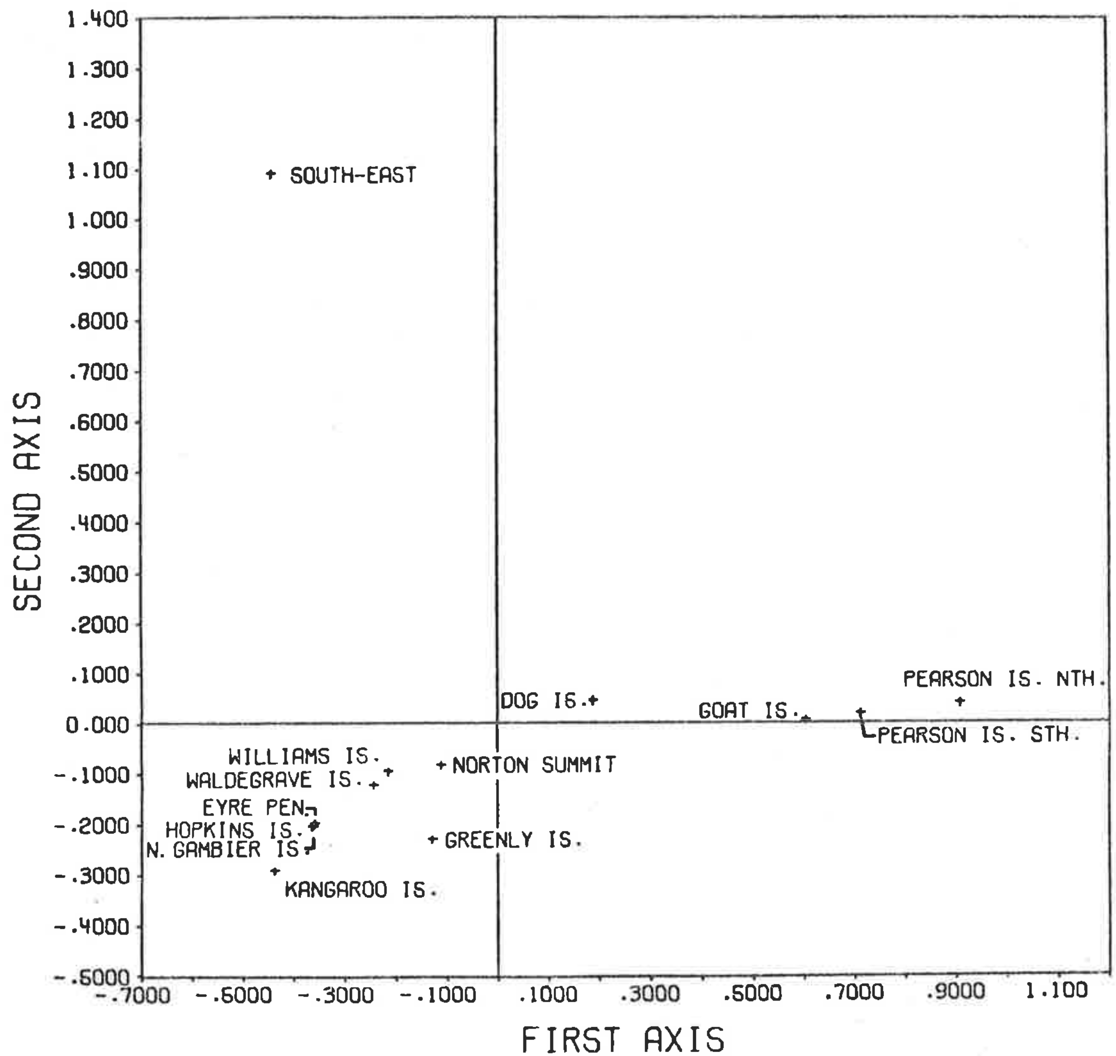


Figure 4 *R. f. greyii* populations plotted on the first and second principal axes of the correspondence analysis. This plane displays 54% of the variation in gene frequencies between populations.

the exception of Norton Summit, show much lower values for the proportion of polymorphic loci and mean heterozygosity than the average mammalian population (Table 2).

The results also indicate that large populations of *R. f. greyii* are more variable than small populations (Table 2). While there are studies which have found lower levels of genetic variability in island populations compared to mainland populations (e.g. Saura *et al.*, 1973; Soulé and Yang, 1973; Avise *et al.*, 1974; Gorman *et al.*, 1975), some have found no difference (e.g. Berry and Murphy, 1970; Ayala *et al.*, 1971; Johnson, 1971; Berry and Peters, 1975). Reduced genetic variation has also been found in populations which resemble island populations, with respect to low effective population size, a recent bottleneck or small habitat area (Avise and Selander, 1972; Bonnell and Selander, 1974). The paucity of genetic variation in the small populations of *R. f. greyii* can be attributed to one or more of three causes. First, genetic drift will have a more marked effect in these populations because of small population size. Second, a catastrophic event such as fire, famine, etc., is likely to affect the whole population on a small island, while it may affect only a part of a large population. Loss of variability in the affected part of a large population can be replenished by migration from adjacent areas but this is unlikely to happen in a small population. Third, on the small islands, decreased genetic variability may be a response to decreased environmental variability.

Geographic variation

The patterns of geographic variation at individual loci can be interprete as follows:

1. For the *Pgm* locus, it is likely that the Pgm^2 allele is a mutant, which arose after the isolation of Kangaroo Island from the other populations. If the Pgm^2 allele was even moderately widespread before the isolation of Kangaroo Island, it might be expected to be present in some of the nearby

populations. Similarly, it can be argued that the *Got-1*^{b} allele, although present in two populations, probably arose by mutation after the isolation of the Pearson Islands (Schmitt, 1975). The two Pearson Islands are separated by a narrow channel, a few metres wide and at very low spring tides a narrow, dry sand-bar connects the two islands.

As all specimens of *R. f. assimilis* and *R. f. coracius* are homozygous *6-Pgd*^{2}/*6-Pgd*^{2} it is possible that the *6-Pgd*^{2} allele which is present only in the South-East population of *R. f. greyii* was introduced as a result of introgression of that population with *R. f. assimilis*. Alternatively, it may be a relic from the time when the two sub-species were sympatric.

2. At the *Idh-2* locus, the *Idh-2*^{2} allele is more frequent than the *Idh-2*^{1} allele in all populations. In the Waldegrave Island population a significant excess of heterozygotes at the *Idh-2* locus was detected, and it has been suggested that selection may be maintaining this polymorphism (Schmitt, 1977). Because both alleles are found in several *R. f. greyii* populations there is a good case for arguing that the two alleles were present in the ancestral population. If drift has played an important part in the loss of genetic variability on the small islands, the probability of fixation of a particular allele on an island will be proportional to its frequency when the island was isolated. All islands except Waldegrave Island, where the polymorphism has been maintained, are monomorphic for the *Idh-2*^{2} allele. This suggests that the *Idh-2*^{2} allele was the common allele in the ancestral population as it is in the large contemporary populations.

3. The distribution of variation at the *Alb*, *Ldh-2* and *Me-1* loci suggests that the alleles found at these loci were present before fragmentation of the ancestral population. The north-western populations are monomorphic for the *Me-1*^{2} allele while most of the islands towards the south-east are monomorphic for *Me-1*^{1}. These observations suggest that there may have been a cline in allele frequencies in the ancestral population, the *Me-1*^{2} allele being the most frequent allele in the north-west, with *Me-1*^{1} increasing in frequency to the south-east. The fact that all *R. f. fuscipes* individuals

are homozygous *Me-1*^{2}/*Me-1*^{2}, while *R. f. assimilis* and *R. f. coracius* individuals are homozygous *Me-1*^{1}/*Me-1*^{1}, gives added support to this suggestion. Similarly, the contemporary distribution of alleles at the *Alb* and *Ldh-2* loci suggest the existence of clines for these genes in the ancestral population. In both cases, the alleles most frequent towards the south-eastern end of the distribution of *R. f. greyii* are present in *R. f. assimilis* and *R. f. coracius*, while the allele most common towards the north-western end of *R. f. greyii* is also present in *R. f. fuscipes*.

4. The pattern of haemoglobin variation also throws some light on the possible composition of the ancestral population and on which alleles are probably recent mutants. Three alleles, *Hb*^{b}, *Hb*^{d} and *Hb*^{k} are each found separately in only one population and may be considered to be recent mutants, like *Pgm*^{2} and *Got-1*^{b}. *Hb*^{a}, *Hb*^{p} and *Hb*^{s} are widespread, the first two being found in more than one population of *R. f. greyii* and all three are found in at least one other sub-species. *Hb*^{s}, like *6-Pgd*^{2} appears from consideration of populations of *R. f. greyii* only, to be a recent mutant.

From these patterns in gene frequencies, it seems likely that the ancestral population was polymorphic at at least five loci (*Alb*, *Hb*, *Idh-2*, *Ldh-2* and *Me-1*) and therefore had a level of genetic variability similar to most modern mammalian populations.

Quantitative analysis

The general relationships between populations as seen in the genetic distance matrix, dendrograms and correspondence analysis scattergram are in agreement with the locus by locus trends just discussed. Within *R. f. greyii* two groups are evident, one consisting of Goat Island, Pearson Island South and Pearson Island North and a second consisting of all other populations, except Dog Island and South-East. The latter two are apparently not closely associated with either group, and this genetic distinctiveness is concomitant with marginal geographic positions. The first principal axis of the

correspondence analysis, separates the two main groups within *R. f. greyii* while axes two and three separate the South-East and Dog Island populations respectively, from the other *R. f. greyii* populations. The position of the Dog Island population in Fig. 4, which shows only the first and second axes, is therefore somewhat misleading.

Ignoring selective forces, it would seem that the genetic distance between any two populations will be related to the time of their isolation from one another and the geographic distance between them. For example, it is expected that Waldegrave Island will show a greater affinity to the Eyre Peninsula population than to the Pearson Islands, despite being about equidistant from both. This is because Waldegrave Island has been isolated from Eyre Peninsula for about 6,500 years, while the Pearson Islands have been isolated from Waldegrave Island and Eyre Peninsula for some 14,000 years. Goat Island on the other hand, has been isolated from Eyre Peninsula and the Pearson Islands for periods similar to Waldegrave Island, yet shows greater genetic similarity to the Pearson Islands than to Eyre Peninsula. A possible explanation is that the suggested clines in gene frequency led to alleles with a high frequency in the north-west being fixed on Goat Island and the Pearson Islands but not in the Eyre Peninsula population.

The genetic distances which involve *R. f. fuscipes* or *R. f. coracius* must be taken with a good deal of caution, each being based on the genes of only six individuals. The dendrograms derived from the correspondence analysis and Nei's genetic distance differ in their placement of *R. f. fuscipes* and *R. f. coracius* with respect to *R. f. greyii*. The former, from a consideration of the current taxonomy of the species, gives a more satisfactory arrangement, placing the *R. f. greyii* populations in one group before adding the other sub-species. However, the latter illustrates the relationship between *R. f. fuscipes* and the north-western populations of *R. f. greyii*, and between *R. f. coracius* and the south-eastern *R. f. greyii* populations. This relationship implies that *R. f. greyii* has, in the past, been a link

between the other sub-species and may have been part of a large, roughly linear population, stretching from south-western Australia along the southern coast to north-eastern Australia. Whether this population was continuous at any one time is problematical. However, climatic conditions and the sea level have been more favourable than at present for such a connection across southern Australia to exist (e.g. Jennings, 1971). Parsons (1969) and others have discussed a possible late Pleistocene migration route across southern Australia, on coastal lowland which is now under the sea, to explain disjunction in the distribution of plant species in Australia.

Taxonomic relationships

A comparison of the genetic distances between the populations studied here and the distances of comparable taxonomic groups of mammals was made using Nei's genetic distance and from data presented by Nei (1975) (Table 4).

INSERT TABLE 4

It shows that the genetic distances between races and sub-species of *R. fuscipes* are, in general, greater than the distances between similar groups. It should be emphasised that these genetic distance estimates for *R. fuscipes* are based on about sixteen genetic loci and have standard errors of the order of one half or more of the actual estimate. One of the factors contributing to these larger than normal estimates of genetic distance would be genetic drift in the small populations. As succinctly pointed out by Lewontin (1975), forces which decrease genetic variability within populations will increase variability between populations.

Genetic distance and time of separation

The differences between populations of *R. f. greyii* have evolved within the last 14,000 years and the data presented here represent one of the few examples where an estimate of the time over which genetic differentiation has occurred, can be made.

Table 4. *A comparison of the genetic distance between races of* R. f. greyii *and sub-species of* R. fuscipes *and similar taxonomic groups of mammals (from Nei, 1975).*

Species	Genetic distance between races	Genetic distance between sub-species
Man	0.011 - 0.019	-
Mus musculus	0.010 - 0.024	0.194
Kangaroo rats	0.000 - 0.058	-
Pocket gophers	-	0.004 - 0.262
Gophers	-	0.009 - 0.054
R. f. greyii	0.00 - 0.38	-
R. fuscipes	-	0.11 - 0.59

Sarich (1977) calibrated an "electrophoretic clock", equating one unit of Nei's genetic distance to approximately 20 million years (MY) separation. On this basis the average genetic distance between *R. f. greyii* populations, which is 0.17, would correspond to an average time of separation of about 2MY (after an adjustment is made for the proportion of "slow" and "fast" evolving proteins used to estimate genetic distance). This leads, in the case of *R. f. greyii*, to a discrepancy of about two orders of magnitude between Sarich's clock and the biogeographic information (Table 1). Another method for equating genetic distance and time of separation (Nei, 1971) predicts that the *R. f. greyii* populations have on average been separated for about 125,000 years, which again is considerably in excess of the values in Table 1.

Two factors may be contributing to these two overestimates of the time of separation. First, genetic drift may have accelerated the genetic differentiation of *R. f. greyii* populations to a greater extent than for populations of other species. Second, the calibration of Sarich's clock was based on an albumin immunological clock, empirically calibrated using populations separated for periods of the order of 20MY and more. It is possible that this calibration does not hold for populations isolated for much shorter periods of time.

Evolutionary forces

Studies of genetic variation in natural populations are always faced with the problem of determining the nature and relative roles of selective and non-selective forces in maintaining the variation. There are very few examples in which these have been determined. In order to enhance the possibility of making this distinction, populations with special features are studied. This study was undertaken in the hope that the unusual distributional characteristics of *R. f. greyii* would increase the

probability that data, relevant to this problem, would be obtained. While it is difficult to assess the roles of selection and drift in determining the genetic structure of *R. f. greyii* populations, it seems likely that drift has been a major influence affecting the gene frequencies on the small islands. Drift seems the most likely explanation for the existence of different allelic genes fixed on islands only a short distance apart and with no obvious geographic or biogeographic differences (e.g. *Got-1* on the Pearson Islands and *Me-1* on Hopkins and Williams Islands). The low level of genetic variability in these small island populations is also consistent with genetic drift. Whilst the author was impressed with the similarity of most islands with respect to their physical features and flora, there is little or no recorded information of this type for most of these islands. It is certainly possible that selection was important in determining the fixation of particular alleles on certain islands, and it may well have had an important role in producing or maintaining the suggested clines in the ancestral population.

SUMMARY

A study has been made of sixteen genetic loci in thirteen populations of *Rattus fuscipes greyii* . These populations have been reproductively isolated from one another for varying times, by geographical barriers. Seven small island populations are monomorphic for all loci and two other island populations are each polymorphic at one locus only. The mean heterozygosity for all small island populations is 0.007. These populations are genetically less variable than most mammalian populations. Three mainland populations and a large island population are polymorphic at two to four genetic loci (mean heterozygosity = 0.042).

The geographic patterns of variation at several loci throw light on the composition of the gene pool of the ancestral population from which it is presumed the contemporary populations were ultimately derived. Some

genes, found only in one population, are apparently recent mutants, while others are more widespread, indicating that they were probably present in the ancestral population. For some loci it seems likely that clines in gene frequency were present in the ancestral population.

A modification of the method of principal components for contingency tables gives an excellent summary of the data.

Genetic drift appears to have played an important part in determining differences between these populations which have developed within the last 14,000 years.

The genotypes of a few individuals of three other sub-species support the general conclusions made from *R. f. greyii*. It is suggested that in the past, *R. f. greyii* has been a link between the eastern and western sub-species of *R. fuscipes*.

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Mitochondrial iso-citrate dehydrogenase variation in the Australian bush-rat, Rattus fuscipes greyii

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Summary

Four reproductively isolated populations of the Australian bush-rat, *Rattus fuscipes greyii*, are polymorphic for electrophoretic variants of the mitochondrial enzyme, NADP-dependent iso-citrate dehydrogenase (M-Idh). The electrophoretic patterns of M-Idh and a small amount of breeding data are in agreement with the hypothesis that the variation is controlled by two alleles at an autosomal locus, *Idh-2*. In three relatively large populations, the proportion of heterozygotes at the *Idh-2* locus ranges from 0.07 to 0.23, while on a small off-shore island it is 0.60. In this latter population there is an excess of heterozygotes which is on the borderline of statistical significance, suggesting that heterotic selection may be maintaining the polymorphism. Populations on eight other small islands are monomorphic for the allele which is the most frequent in the large populations. In most species M-Idh is less variable than enzymic proteins and the results presented here constitute one of the few published examples of an extensive M-Idh polymorphism.

Introduction

Summaries of studies of isozymic variation in natural populations indicate that, on average, populations are polymorphic for about one-third of all proteins and on average each individual is heterozygous at 5-15 % of its loci (Harris & Hopkinson, 1972; Selander & Johnson, 1973; Lewontin, 1974; Powell, 1975). Nevertheless, there is evidence for consistent differences between certain groups of organisms and enzymes. Vertebrates have a mean heterozygosity value of about 0.05, while for invertebrates it is about 0.15 (Selander & Kaufman, 1973). Also, Gillespie & Kojima (1968) and Kojima et al. (1970) found that in *Drosophila*, the enzymes of glycolysis are less variable than non-glycolytic enzymes. One of the least variable enzymes is mitochondrial NADP-dependent iso-citrate dehydrogenase (M-Idh). This enzyme catalyses the oxidative decarboxylation of iso-citrate to α-ketoglutarate. Selander & Johnson (1973) found that the mean heterozygosity per individual

at the M-Idh locus for 19 vertebrate species surveyed was 0.0031. When only those species considered to be polymorphic were considered, the mean heterozygosity was 0.0140. For all glucose metabolizing enzymes, the estimates were 0.0491 and 0.1043, respectively. Thus M-Idh is not only polymorphic in fewer populations than most other enzymes surveyed, but where it is polymorphic, the mean heterogosity is low. A priori there does not appear to be any reason for M-Idh and some other enzymes to be less variable than others. However, it has been suggested by van Heyningen et al. (1973) that there is a strong conservation of the charge properties of mitochondrial enzymes possibly because their charge is important for their localization.

This paper describes inherited M-Idh variation in four populations of the bush-rat *Rattus fuscipes greyii* in southern Australia. This subspecies is particularly interesting because it is separated into several reproductively isolated populations.

Materials and methods

Specimens of *R.f. greyii* were caught in 'Sherman' traps and transported alive to Adelaide, where liver, heart and kidney extracts were prepared by homogenizing in 1 ml H_2O per gram tissue. Electrophoresis was carried out with 12.5 % 'Electrostarch' and a 0.155 *M* tris-0.043 *M* citric acid (pH 7.0) continuous buffer (Shaw & Prasad, 1970) for three hours at approximately 18 V/cm. The staining solution consisted of 100 ml 0.05 *M* tris-HCl (pH 8.0) with 2.5 m*M* $MgCl{\cdot}6H_2O$, 20 mg DL-trisodium iso-citrate, 10 mg NADP, 10 mg MTT and 2 mg PMS. The method for subcellular localization of the isozymes was essentially the same as Henderson (1965). Heart or kidney extracts were used for phenotypic scoring of individuals and liver was used for the subcellular localization.

Animals were kept and bred in plastic house-mouse holding cages, 40 cm × 30 cm × 15 cm. Males were separated from females before litters were born.

Results

Two regions of Idh activity were seen on gels, one migrating towards the cathode, the other to the anode (Fig. 1). It is presumed by analogy with other vertebrates that the cathodal Idh is found mainly in the mitochondria (Henderson, 1965, 1968; van Heyningen, 1973). An attempt to verify this was only partially successful. Using liver extracts, the supernatant fraction contained only the anodal Idh, as expected. However, attempts to recover Idh activity from mitochondria were unsuccessful.

The anodal enzyme was invariant in all animals sampled from populations of *R.f. greyii*. However, the eastern Australian subspecies, *R.f. coracius* was variable (Fig. 1). The cathodal enzyme showed three distinct phenotypes, Idh-2 1, Idh-2 1-2 and Idh 2 2 (Fig. 1). It is proposed that the variation is under the control of an

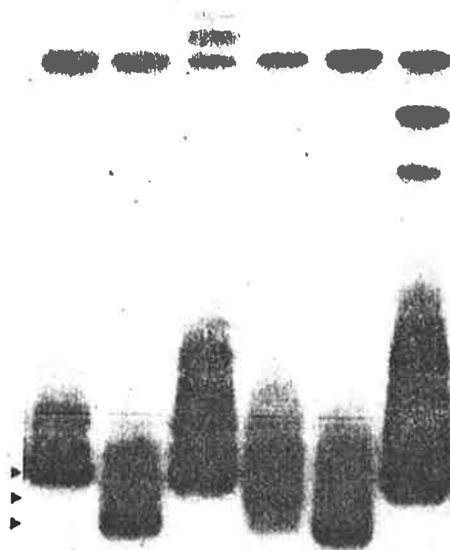

Fig. 1. Starch gel stained for Idh activity. The anode is at the top. From the left, samples 3 and 6 are *R.f. coracius*. Cathodal enzyme (M-Idh) phenotypes (from left) are: Idh-2B; Idh-2A; Idh-2B; Idh-2A-B; Idh-2A and Idh-2B. The positions of the three M-IDH bands are marked.

autosomal locus with two co-dominant alleles. The locus has been designated *Idh-2*, with alleles *Idh-2A* and *Idh-2B*. Family data presented in Table 1 are consistent with this mode of inheritance. The heterozygote has three bands, two corresponding to those in the homozygote as well as a hybrid band with intermediate mobility, suggesting that the active enzyme is a dimer. This pattern of variation is similar to that found for variants of soluble Idh in mouse (Henderson, 1968), man (Chen et al., 1972), *Rattus fuscipes coracius* (Fig. 1) and other vertebrates (Selander et al., 1971). The intensity of the band present in Idh-2A individuals was considerably less than that of Idh-2B individuals.

The population data are presented in Table 2 and Fig. 2. Four of the populations were found to be polymorphic for M-Idh. The only population from which Idh-2A individuals were sampled was Waldegrave Island. To test this population for agreement with genotypic expectations based on the Hardy-Weinberg principle, Fisher's exact test was used, yielding a probability of 0.045. For the other three populations, a F_{IS} (fixation index within a sample) value was calculated and tested for agreement

Table 1. Family data on the inheritance of mitochondrial iso-citrate dehydrogenase variation. The unknown male was a *R. f. greyii* from Eyre peninsula.

Parents' genotype	Number of matings	Genotype of offspring		
		AA	*AB*	*BB*
BB × *BB*	22	0	0	137
AB × *AB*	1	0	0	2
AB × unknown	1	1	3	1

Table 2. Iso-citrate dehydrogenase genotype numbers and gene frequencies in four polymorphic populations.

Population	Genotypes			Frequency *Idh-2A*	Mean heterozygosity
	AA	*AB*	*BB*		
Norton Summit	0	3	39	0.04	0.07
Kangaroo Island	0	11	37	0.11	0.23
Eyre Peninsula	0	2	17	0.05	0.11
Waldegrave Island	3	28	16	0.36	0.60

with zero (Kirby, 1975). Each population had a negative F_{IS}, with an overall mean of —0.08 ($P<0.1$) indicating a slight excess of heterozygotes. This result may be biased however, because no Idh-2A individuals were obtained from these populations.

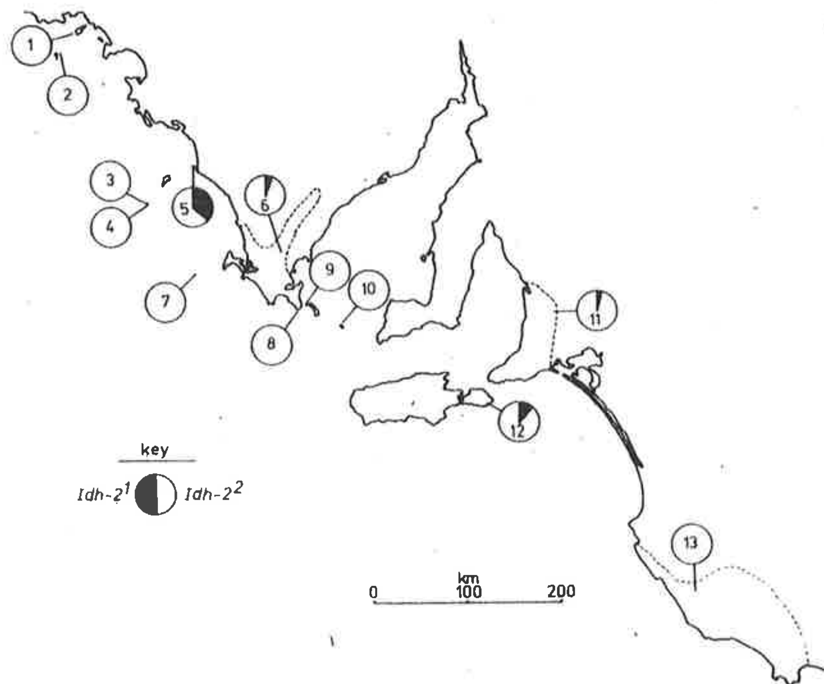

Fig. 2. Geographic distribution of *Idh-2* gene frequencies represented as pie-diagrams. The dotted lines incidate the extent of the mainland populations of *R.j. greyii*.
1 = Goat Is.; 2 = Dog Is.; 3 = Pearson Is. North; 4 = Pearson Is. South; 5 = Waldegrave Is.; 6 = Eyre Peninsula; 7 = Greenly Is.; 8 = Williams Is.; 9 = Hopkins Is.; 10 = North Gambier Is.; 11 = Norton Summit; 12 = Kangaroo Is.; 13 = South-east.

Discussion

During the last glacial period, *R.f. greyii* was probably distributed continuously across the southern part of South Australia (Schmitt, submitted for publication). It currently inhabits three isolated areas of the mainland as well as several off-shore islands (Fig. 2). The mainland populations each extend over an area of about 400 000 hectares, although within each area the actual amount of habitat suitable for the species is much less. The three mainland populations were probably isolated from one another within the last 10 000 years, by a climatic change which resulted in a reduction in the distribution of suitable habitat (Twidale, 1969). Kangaroo Island is similar in area to these mainland populations and the amount of genetic variation in the populations of *R.f. greyii* in these four relatively large areas is similar (Schmitt, submitted for publication).

The present off-shore islands of South Australia were isolated by the eustatic rise in sea level associated with the deglaciation which began about 18 000 years ago. The islands with populations of *R.f. greyii* were isolated from the mainland 6000-14 000 years ago.

This estimate is based on data on the mean sea level during the last 20 000 years (e.g. Fairbridge, 1960) and the present topography of the sea bed. There is some doubt however about the sea level, particularly during the last 6000 years and Waldegrave Island, which is separated from the mainland by a shallow strait, may have been connected more recently than 6000 years ago. Other than Kangaroo Island, all islands with populations of *R.f. greyii* are 100-300 ha, Waldegrave Island being about 300 ha.

Because the contemporary populations were all once part of a much larger population, it is likely that the *Idh-2* alleles in each population have a common origin in the ancestral population.

It is difficult to determine whether the two alleles are present because selective forces are maintaining them or they are selectively 'neutral', their frequencies being determined by stochastic processes. There appears to be a prima facie case for the polymorphism being maintained by heterotic selection. This is based on the observation of an excess of heteroxygotes, in the Waldegrave Island population. Furthermore, the difference in activity of the isozymes, determined by the two alleles may indicate an in vivo difference.

The apparent absence of the *Idh-2A* allele from all other populations studied is not surprising when other genetic data are considered. Animals from the nine small island populations were scored for sixteen genetic loci revealing only two cases of a polymorphism within a population, one for the enzyme glutamate oxaloacetate transaminase (Schmitt, 1975) and the M-Idh polymorphism on Waldegrave Island. The evidence suggests that random sampling drift has had a marked influence on the genetic structure of the small island populations. That the Waldegrave Island population has remained polymorphic for M-Idh, while most other small island

populations have become fixed at the sixteen genetic loci examined, may indicate selection has played some part in the maintenance of this polymorphism.

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Experientia: in press.

An electrophoretic investigation of the binding of $3-^{14}C$ coumarin to rat serum proteins.

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(3 January 1977)

Summary. The binding of coumarin to serum proteins of the rat has been demonstrated. Of the total bound coumarin (37% of injected dose), 36% was bound to the slow and fast α_1 globulins, 11% to the post albumins, 10% to globulin and 9% to albumin.

The binding of coumarin (5-6-benzo-α-pyrone) to purified serum and plasma albumins has been reported by Garten and Wosilait[1] and by O'Reilly[2]. The report of Garten and Wosilait[1] suggests ∿40% of coumarin binds to bovine serum albumin *in vitro*. Bauer-Staeb and Niebes[3] reported a range of binding for the related O-β-hydroxyethyl derivatives ranging from 5% for the tetra-hydroxyethyl rutoside to 71% for rutin. They used human serum in its unpurified form.

Piller[4] reported the binding of coumarin to rat serum proteins. *In vitro*, over a dose range of 3.3-50 μg/ml binding remained constant at ∿40% while *in vivo* over the same dose range ∿37% was in the bound form. This study did not include possible binding to protein fragments. There is no mention in the literature as to exactly what proteins coumarin can bind to. The only estimates available are those of Garten and Wosilait[1] who used a crystallized albumin preparation.

This experiment was designed to determine the binding of $3\text{-}^{14}C$ coumarin to normal rat serum proteins under *in vivo* conditions.

MATERIALS AND METHODS

$3\text{-}^{14}C$ coumarin, specific activity 5.5 mCi/m mol (37.5 μCi/mg was obtained from the Radiochemical Centre, Amersham, England). An analysis of radiochemical purity ascertained by thin layer chromatography on silica gel in a) benzene:ethanol (90:10), b) toluene:ethyl formate:formic acid (50:40:10) and c) benzene:chloroform 50:50 gave results of 99%, 99% and 98% purity respectively. An analysis of chemical purity showed the IR absorption spectrum to be identical to that of coumarin reference materials. (Information supplied by the Radiochemical Centre.)

$3\text{-}^{14}C$ coumarin at a dose level of 25 mg/kg in a 2% solution of A.R. ethanol in physiological saline was injected i.v. into each of 5 female albino rats of the S.P.F. strain (average weight 200 g). After 15 min, blood was removed by heart puncture and allowed to clot. Blood plasma

(8 µl) in duplicate was added to the slits of a 10-channel starch gel.

Separation of the plasma proteins was carried out by horizontal starch gel electrophoresis in a water cooled gel bed. The electrolyte buffer was 0.06 M lithium hydroxide, 0.229 M boric acid (pH 8.5), while the gel buffer was a mixture of the electrolyte buffer and a 0.079 M tris (hydroxymethyl) aminomethane, 0.007 M citric acid buffer in the ratio 1:5.4, (pH 8.5)[5]. Connaught hydrolysed starch was used at a concentration of 11.5%.

Electrophoresis was carried out for 4 hrs at 13 V/cm. The gel was then stained with a solution of amido black (3.7 g in 250 ml H_2O + 250 ml methanol + 50 ml glacial acetic acid). The gel was destained overnight in the same solution without amido black. An attempt was made to name the protein bands based on earlier results of starch gel electrophoresis of rat serum proteins[6].

For scintillation counting each of the duplicate gels were divided into 2-mm sections for 10 mm below the slot and for 60 mm above. This pattern was varied where necessary to accommodate the protein bands. Only the total activity of the whole bands are given. Each piece was finely razor-minced and placed in a plastic scintillation vial. To each, 1 ml Soluene (Packard) was added and this incubated at 60°C for 2 hrs or until the gel dissolved. 10 ml of Insta Gel (Packard) was added, followed by shaking and counting in a liquid scintillation counter. All sections with a count which was not significantly different from the overall gel background were ignored.

RESULTS AND DISCUSSION

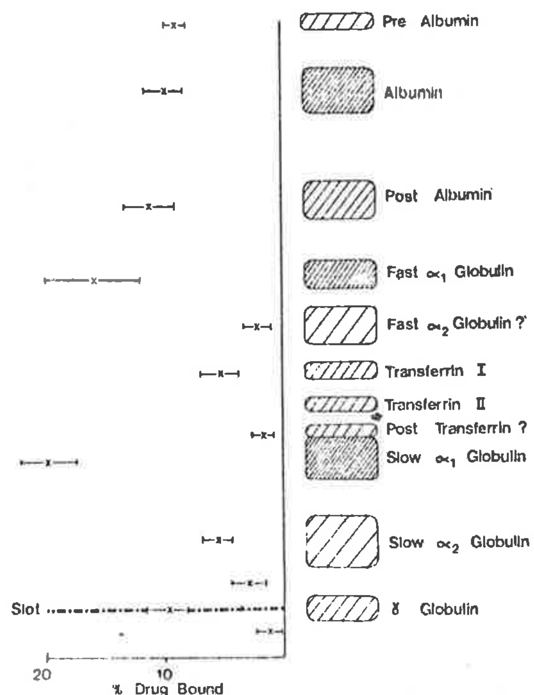
Previously[4], with i.v. administration of $3-^{14}C$ coumarin, approximately 37% of the injected dose bound to serum proteins. Garten and Wosilait[1] using purified bovine albumin presented evidence of a 40% bounding of coumarin. Contrary to this the figure shows that only 9% of the total

bound coumarin was bound to what was electrophoretically distinguishable as albumin. Since the total coumarin bound is approximately 37% of the injected dose this represents only 3.3% of the total coumarin. Such differences are, however, not surprising since the benzopyrones, in general, and coumarin, in particular, have been frequently reported to exhibit wide species variation in their actions and properties[7].

The figure shows a high percentage of the coumarin becomes bound to what is called the slow and fast α_1 globulins[6] (19.6% and 15.8% respectively). Although there is little decisive information available, some evidence suggests that these globulins are of considerably lower mol.wt than albumin[8]. Post albumins, to which 11% of the coumarin is bound have a mol.wt ∿100,000. γ globulin, to which 10% was bound has a mol.wt between 156,000 and 161,000. The only protein to which coumarin became bound (5.6%) which has a very high mol.wt is slow α_2 globulin[8]. Thus the greater proportion of coumarin which is bound, is bound to relatively low mol.wt proteins.

Renkin *et al.*[9] report the small pore system (radius 40 Å - c.f. albumin 35.5 Å) to permit the exchange of low mol.wt solutes (free coumarin has a mol.wt of 146), although the permeability rapidly declines as the molecular size nears that of the pores. There seem to be 2 systems by which the larger mol.wt molecules are transported[9]. About one half occurs via turnover of endothelial vesicles (radius 250 Å) and one half by ultrafiltration through large gaps or pores with a radius in excess of 1600 Å.

Certainly, the small pore system is important for the exchange of free coumarin and for that bound to the fast and slow α_1 globulins (mol.wt ∿45,000) and explains the rapid entry of coumarin into most tissues[10]. The endothelial vesicle and large pore system will allow the entry, albeit slower, of coumarin bound to the larger macromolecules like α_2 globulin.



Schematic representation of the results of starch gel electrophoresis together with the suggested designation of protein zones (nomenclature dervied from Beaton et al.[6]). The percentage of the total protein bound drug is shown for each band. Each estimation represents the mean of 5 duplicate determinations.

The mode of action of coumarin and related drugs is very complex[7,11] and while it seems that either a protein-coumarin type complex or just free coumarin could be responsible for macrophage activation[7,12-14], which results in increased protein lysis[15] through its intra- and extra-cellular digestion, we have yet to elucidate the exact importance of the free and bound coumarin.

It has frequently been reported[7] that in the initial 30 minutes after benzopyrone administration, there is the release of endogenous amines which result in the opening of additional numbers of endothelial junctions[7,16], and allow some extra protein (and protein bound coumarin) into the tissues. We must mention here that this effect is transient and the small additional protein inflow is more than compensated for by the later action, that of enhancing the lysis of all accumulated protein[7,17,18]. The effect of the drug in causing the opening of additional endothelial junctions does, however, allow extra protein bound drug into the tissues and into close proximity to the target cells.

Further work is currently in progress to ascertain the importance of drug protein binding in models of mild thermal oedema, acute and chronic lymphoedema and to relate this to the effectiveness of coumarin as an oedema reducing agent.

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ABBREVIATIONS

General

BP	before present
CA	correspondence analysis
CDA	canonical discriminant analysis
DNA	deoxyribonucleic acid
EC No.	Enzyme Commission number
EDTA	ethylenediamine tetraacetic acid
MTT	MTT tetrazolium
NAD	nicotinamide adenine dinucleotide
NADP	" " " phosphate
PMS	phenazine methosulphate
tris	tris(hydroxymethyl)aminomethane
UPGMA	unweighted pair-group method using arithmetic averages

Populations

DI	Dog Island
EI	Eyre Island
EP	Eyre Peninsula
GOI	Goat Island
GRI	Greenly Island
HI	Hopkins Island
KI	Kangaroo Island
NGI	North Gambier Island
NS	Norton Summit
PIN	Pearson Island North
PIS	Pearson Island South
RFA	*Rattus fuscipes assimilis*
RFC	*Rattus fuscipes coracius*

RFF	*Rattus fuscipes fuscipes*
SE	South-East
WGI	Waldegrave Island
WMI	Williams Island

<u>Proteins</u>

ACP	acid phosphatase
ALB	albumin
ES	esterase
G6PD	glucose-6-phosphate dehydrogenase
GOT	glutamate oxaloacetate transaminase
HB	haemoglobin
ICD	isocitrate dehydrogenase
LDH	lactate dehydrogenase
MDH	malate dehydrogenase
ME	malic enzyme
PGM	phosphoglucomutase
6PGD	6-phosphogluconate dehydrogenase
SOD	superoxide dismutase

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