

STUDIES ON THE ECOLOGICAL GENETICS OF  
THE FIELDMOUSE APODEMUS SYLVATICUS L.

Thesis submitted for the degree of Doctor  
of Philosophy at the University of Leicester

by

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December 1976



## SUMMARY

The results of many years work on the ecology of the fieldmouse (Apodemus sylvaticus) present a sound background to a study of its ecological genetics. Gel electrophoresis and orbital bleeding techniques were used to pursue this. A preliminary estimation of the degree of genetic variability in this species was made through studies on ten loci in 250 animals from eight populations. Similar levels of heterozygosity were observed to those found in the house mouse and two widespread polymorphisms were discovered at the lactate dehydrogenase regulator (Ldr) and phosphoglucomutase (PGM<sub>2</sub>) loci. The eight populations were similar in gene frequencies at both.

To discover the degree of change which occurs in a population over a period of time, two sites were visited three times at six-monthly intervals. They showed great constancy in gene frequencies at both the PGM<sub>2</sub> and Ldr loci when large samples were available. A third site was sampled bimonthly for two years. No real change in gene frequency was observed at Ldr but at PGM<sub>2</sub> significant changes were found during one winter when numbers were high. As survival rates were low, these results suggest natural selection.

The most important single factor influencing survival in overwintering fieldmice is food supply. The central role that phosphoglucomutase plays in glycogen metabolism suggested that the enzyme itself might be subject to selection and experiments were designed to test this. Liver glycogen levels were measured in animals that had been starved overnight.

When analysed according to PGM<sub>2</sub> genotype a highly significant difference was found between the levels in aa animals and those in ac and cc genotypes. This pattern was the same as that found in the survival rates of a large overwintering natural population. It was concluded that these genotypes are not neutral with respect to selection.

## ACKNOWLEDGEMENTS

This project was supported by an S.R.C. Research Studentship.

The Leicestershire and Rutland Trust for Nature Conservation Ltd. gave permission for the work at Charnwood Lodge.

The work at Wytham was made possible by the kind cooperation of Dr. H.N. Southern and John Flowerdew has contributed greatly through his help at Madingley, Wicken and Ely.

I have had much assistance from members of the Department of Genetics at the Royal Free Hospital School of Medicine and I am particularly grateful to Jo Peters for her suggestions on starch-gel techniques and Steve Jones for his valuable criticism and encouragement.

I have also been helped by various members of the Department of Genetics at Leicester who have introduced me to new fields in a relatively painless manner.

I should like to thank Robert Semeonoff for supervising the project and Sheila and Jim Mackley for their assistance with the typing and photography.

Finally, I should like to thank Anne, for putting up with so much.



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# CHAPTER 1

## INTRODUCTION

In order to improve our understanding of the forces affecting the dynamics of genes in populations it is necessary at some point to make direct observations on the behaviour of alleles at specific loci in natural populations. The main impetus for such an approach is a desire to detect and quantify the action of natural selection. Dobzhansky (1971) has written that it is only fairly recently that scientists have come to realise that selection can be detected by such a direct approach. It was considerably more recently that techniques whereby the products of more than a handful of genes could be identified specifically were developed and applied to natural populations (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Harris, 1966). A controversy has since arisen over the extent to which the newly discovered molecular variation is affected by natural selection. Although this controversy continues (King, 1976), the study of natural selection for its own sake remains a worthwhile subject of considerable importance in the field of evolutionary science.

A major problem in the study of natural selection is the identification of the agent which causes it. With regard to environmental selection, this has only usually been achieved when the selective agent is a feature which is distributed with the same physical pattern as the character being selected. Thus, the melanic form of the moth Biston betularia was found to be distributed in association



with high levels of industrial pollution (Kettlewell, 1973). Certain types of malaria were found to be especially frequent in the areas where the HbS gene has a high frequency (Allison, 1955), and the selection of snail shell morphs by thrushes was originally indicated by the association of darker morphs with woodland and lighter ones with grassland habitats (Cain and Sheppard, 1950, 1954). There is a good reason why selection has rarely been detected when its mode of action is less obvious. For many of the organisms studied, our knowledge of the relative importance of different environmental factors in their ecology is almost non-existent. As natural selection acts through the greater survival and/or more successful reproduction of one component of the population than another, the environmental factors which have the greatest influence on mortality and fecundity will offer the best opportunities for it to act. In many cases these factors have been virtually unknown when attempts have been made to study selection, the large volume of work on natural populations of Drosophila typifying this approach (e.g. Dobzhansky, 1971). When this study was begun it was hoped that by choosing an organism whose ecology has been studied in depth over many years, some problems encountered in other cases would be avoided. It is considered that, to some extent at least, this view has been vindicated.

The long-tailed fieldmouse (Apodemus sylvaticus L.) is generally distributed throughout Britain. It is most common in the vicinity of woods and in nature woodland is often the most common small mammal (Southern, 1964). In

woods, the numbers of fieldmice fluctuate annually.

Densities are high over winter but drop sharply in spring to a relatively constant, low level over the summer. At some period in the autumn, as breeding comes to an end, numbers increase to a level usually similar to that found in the previous winter. (Evans, 1942; Miller, 1958; Kikkawa, 1964; Bergstedt, 1966; Crawley, 1970). Such changes also appear to take place in open field habitats (Jefferies et al. 1973; Flowerdew and Green, personal communication).

These changes in numbers are a reflection of changes in survival rates and detailed studies of the factors which influence these have been made over several years in Wytham Woods, near Oxford. Watts (1966, 1969) summarised these and concluded that the factors that were most important in summer were different from those in winter. In winter, the survival rate depends heavily on the success of the acorn crop in the previous autumn, as nuts are known to constitute almost the entire diet at this time of the year (Watts, 1968). However, the spring decrease in numbers is associated with the onset of reproductive activity. While the survival rate of the few adults in the population is relatively constant over the summer, that of juveniles is very low. It is only towards the end of the breeding season that juvenile survival increases and this causes the increase in numbers observed in autumn. Watts (1969) therefore made the suggestion that it was social processes, particularly intra-specific aggression on the part of adult males, that were causing the low survival rates during the summer. Flowerdew (1972, 1974) attempted to test some



of Watts (1969) conclusions through field experiments. By supplying food on one grid in his study area, he was able to show that this did increase survival relative to that found on a control site, but that this was not the only factor affecting survival in summer populations. On a third area he showed that during the breeding season the removal of adult males led to an immediate increase in the survival of juveniles. The knowledge gained by these experiments must make Apodemus sylvaticus one of the most thoroughly understood species of small mammals, in ecological terms.

Intensive studies have also been carried out on the population dynamics of microtine rodent (Chitty, 1952, 1954; Krebs, 1964, 1966), but it could not be claimed that a similar degree of knowledge has been attained. Although the fluctuations in numbers that characterise such species have a period of 3-5 years, they have many features in common with the annual fluctuations in Apodemus (Watts, 1969). This is of particular interest because recent work on voles has concentrated on investigating an hypothesis developed by Chitty (1967) whereby the 'cycles' are caused by genetic changes in the populations. The development of the technique of gel electrophoresis as applied to natural populations has permitted several studies to be made on the behaviour of alleles at certain polymorphic loci during the 'cycle' (Semeonoff and Robertson, 1968; Tamarin and Krebs, 1969; Gaines and Krebs, 1971). These studies have shown changes in gene frequency associated with changes in population density at three different loci, in different species. The way in which these changes are related to the

'cycle' is not yet clear (Gaines and Krebs, 1971; Charlesworth and Giesel, 1972). While it appears that animals which are caught colonising a depopulated area are not a random sample of the parent population in terms of gene frequency at such loci (Myers and Krebs, 1974; Krebs et al. 1976), a recent attempt to influence demographic processes by altering the genetic constitution has not been successful (Leduc and Krebs, 1975). Certainly, there is good evidence from these studies that selection is occurring in vole populations (Birdsall, 1974), but the results would not appear to be directly applicable to Apodemus, particularly as little dispersal occurs naturally in fieldmouse populations (Watts, 1970; Flowerdew, 1972).

Studies which may have a more direct bearing on genetical processes in the field mouse have been made on two populations of wild-living house mice, one on the island of Skokholm off the Welsh coast (Berry and Murphy, 1970) and the one in California (Myers, 1974). Myer's study was inconclusive with respect to the genetical results. While changes in the genetic constitution at the Hbb locus of the populations on the study grids appeared to occur, they bore no relationship to any environmental factor or to each other. However, Berry and Murphy (1970) took samples from the Skokholm population at six-monthly intervals for several years and one of their collecting periods was shortly after the period of heavy mortality that occurs in late winter. They found similar changes in the frequency of Hbb heterozygotes over several successive winters. Although



subsequent work led to a reconsideration of the original interpretation (Bellamy et al. 1973; Berry and Jakobson, 1975a), the changes strongly suggest the action of natural selection.

The interacting factors that determine the probability of survival of a small mammal over a given time period are highly complex (Berry, Jakobson and Triggs, 1973; Berry and Jakobson, 1975b). Nevertheless, the studies that have been made on populations of voles and house mice have shown that it is possible to detect the action of natural selection using currently available techniques of live-trapping, orbital bleeding and gel electrophoresis. It was therefore considered worthwhile initiating a study on the fieldmouse where a strong background of ecological knowledge was available, even though little previous work had been done on its genetics. One additional aspect of interest concerns the annual repetition of periods of low numbers. It was considered that if this effect is real (cf. Tanton, 1965, 1969), it should allow for the stochastic influence of the founder effect to play some role in determining the genetic constitution of the population. This would be detectable by comparing gene frequencies between winter populations at the same site. The results presented in this thesis therefore fall into three main categories. The first is primarily concerned with a preliminary description of genetic variation in Apodemus populations (Chapter III). The second contains the results of the temporal studies made on three populations (Chapter IV), and the third category is a description of an investigation made into the action of natural selection at the physiological level (Chapter V).

## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1 STUDY SITE

The site chosen for the main part of this project was an area of mixed deciduous woodland at the north western end of Collier Hill in Charnwood Forest, Leicestershire (Figure 1). It forms part of Charnwood Lodge Nature Reserve, owned by the Leicestershire and Rutland Trust for Nature Conservation Ltd.

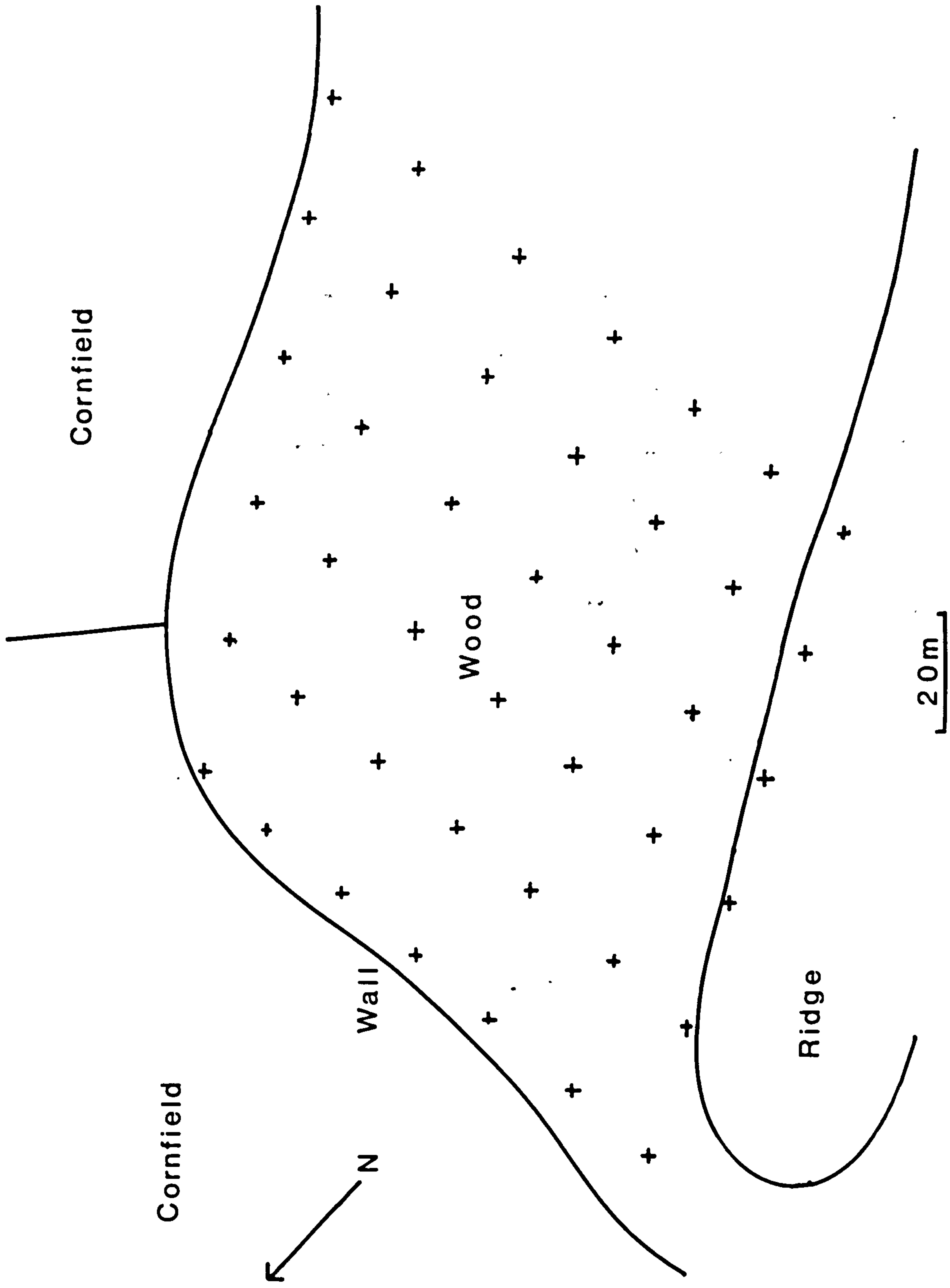
The area slopes away from a rock outcrop and is generally damp with a deep floor of leaf litter. The canopy consists mainly of oak (Quercus sp.) and elm (Ulmus glabra) with occasional beech (Fagus sylvaticus). Young sycamore (Acer pseudoplatanus) and hawthorn (Crataegus monogyna) covers about a quarter of the site. Ground cover is sparse. Bracken (Pteridium aquilinum) grows densely in one corner and blue-bells (Endymion nonscriptus) extend over a large area in spring. Otherwise fallen branches and trees provide the main cover, with clumps of bramble (Rubus fruticosus) and ferns (Dryopteris sp.) sparsely distributed. The site is bordered on two sides by arable land which was planted with winter wheat in 1974.

#### 2.2 TRAPPING METHODS

The Charnwood site was trapped every two months from February 1974 to January 1976 with 84 Longworth live traps, used without prebaiting. These were arranged in a grid of 42 stations spaced at 17-20 metre intervals. The total area of the grid was approximately 1 hectare. Apodemus sylvaticus

Figure 1. The study site at Charnwood

Positions of trap stations are indicated (+).  
Two traps were placed at each point for four consecutive nights in each trapping period. The grid was bounded by a dry stone wall and a ridge of rock outcrops. The total area was approximately 1 ha.





was the predominant small mammal. Only in summer did the density of the bank vole (Clethrionomys glareolus) reach a similar level. In addition, common and pygmy shrews (Sorex araneus and S. minutus) were caught infrequently.

The traps were left in position for four consecutive nights in each trapping period and visited every morning. After capture all animals were removed to the laboratory. There a blood sample was taken from the new individuals, which were also marked by toe clipping. When the traps were lifted at the end of the trapping period, all animals were released in the centre of the site. Such treatment did not appear too disturbing as recaptures usually occurred close to the original point of capture.

Population samples from the Madingley and Wytham sites were obtained through the cooperation of Dr. J.R. Flowerdew and Dr. H.N. Southern respectively, who allowed the author to take blood samples from animals caught in their trapping programmes. At most of the remaining localities the author worked unassisted. At all sites other than Charnwood animals were anaesthetised in the field, being dropped into a polythene bag containing ether-soaked cotton wool. After bleeding they were returned to the trap and allowed to recuperate for 15 minutes before release.

### 2.3 MAINTAINANCE IN CAPTIVITY

For studies on the inheritance of polymorphisms, a breeding colony of field mice was set up. Mated pairs were kept in mouse breeding cages of dimensions 44.5cm. x 28cm. Non-breeding animals were kept in similar cages with raised tops, as used for rats, in densities of up to six animals

of one sex to a cage. Hay and peat litter were provided and all animals were fed on Oxoid rat and mouse breeding diet. Under an 18 hour light cycle at about 22°C. and with minimum disturbance, breeding was maintained all year round. While most matings were successful, not all were and this is reflected to an extent in the breeding results.

#### 2.4 PREPARATION OF BLOOD SAMPLES

The animal to be bled was anaesthetised with ether for approximately 2 minutes before the sample was taken. A heparinised fine bore pipette was then inserted into the preorbital sinus and about 0.1ml blood allowed to flow up the pipette, (Riley, 1960). The sample was then blown into a 3ml. conical centrifuge tube. In the field the sample was most conveniently taken with the animal laid on the side of the trap. The bleeding pipettes were obtained from Bilbate Ltd., Daventry, Northants, already heparinised.

Separation of plasma and erythrocytes was performed up to 6 hours after taking the sample, without ill effect. 10 minutes centrifugation at 3000 rpm. was sufficient for this and could be performed in an MSE bench model or a portable field centrifuge supplied by Luckhams Ltd. of Burgess Hill, Sussex. The plasma was subsequently removed and frozen, and the erythrocytes washed once in 15x vol. 0.9% saline. They were then lysed in an equal volume of distilled water and frozen. Samples could be kept at -18°C. for up to four months without significant deterioration. Samples taken on a field trip were placed in a vacuum flask and frozen with dry ice. The dry ice was produced in the form of a cake by a Carbo-neige device mounted on a small CO<sub>2</sub> cylinder (Carbo-neige device supplied by Distillers Co. Ltd. CO<sub>2</sub> division).



## 2.5 GEL PREPARATION AND RUNNING

Starch gels were prepared in the standard way, exactly as described by Smithies (1955). Electrostarch (Strand Scientific Co. Nottingham) proved easier to handle than Connaught and was used almost exclusively at a concentration of 11%. 300ml. buffer were used for each gel, which was poured in a perspex mould 15cm. x 20cm. x 6mm. with a removable end. After pouring the gel was chilled.

Samples were inserted on 4mm. sections of Whatman 3MM chromatography paper strip 1.0cm. wide, in a slot cut by a razor blade. The volume of sample varied according to the system and will be given later. The gel was then placed in a horizontal box with separate anodal and cathodal buffer compartments. Connection was effected with 4 thicknesses of Postlip grey filter paper, supplied by Med Lab. Ltd., Derby. Gels were covered with cling film and run overnight in a cold room at 4°C. or under an ice pack for 2-3 hours, according to the voltage being used.

## 2.6 BUFFER SYSTEMS FOR ELECTROPHORESIS

Four enzymes for which most samples were screened were run on one buffer system. Lactate dehydrogenase, 6-phosphogluconate dehydrogenase, acid phosphatase and erythrocyte esterase were all run in a sodium phosphate buffer made by mixing 0.15M  $\text{NaH}_2\text{PO}_4$  and 0.15M  $\text{Na}_2\text{HPO}_4$  in the ratio 8.1 parts acid phosphate to 1.9 parts basic phosphate (pH 6.2). The buffer is used undiluted in the trays and diluted tenfold in the gel (Spencer, Hopkinson and Harris, 1968). The gels were run at a voltage of 3.0 V/cm. gel length, as read between the wicks, for 18 hours in a cold room. A volume of 10µl haemolysate was placed on each insert.

Phosphoglucomutase was run in the Tris, EDTA, Maleic acid, Magnesium chloride (TEMM) buffer system developed by Spencer, Hopkinson and Harris (1964). The concentrations were as follows:

Tris:	0.1M
EDTA:	0.01M
Maleic acid:	0.1M
MgCl <sub>2</sub> :	0.01M

The buffer was taken to pH 7.0 with NaOH and was used neat in the trays and diluted tenfold in the gel. Gels were run at 5.3 V/cm. for 18 hours in a cold room. Sample volume was 25µl on a double thickness insert.

For electrophoresis of plasma proteins the Poulik discontinuous buffer system was used (Poulik, 1957) in which the gel is made in 0.076M Tris-HCl pH 8.6 and the tray buffer is 0.3M boric acid taken to pH 8.9 with NaOH. For running plasma esterases the same gel buffer was used at pH 7.8 but 0.15M sodium phosphate was used in the trays at pH 7.8. All plasma gels were run at 15 V/cm. for 2-3 hours under ice packs, with sample volumes of 5µl on each insert.

## 2.7 STAIN METHODS

All gels were sliced horizontally with a taut wire after the run and the internal surfaces were used for staining.

Plasma proteins were stained for by immersing the gel in a saturated solution of naphthalene black for 5 minutes.

Plasma esterases were stained for specifically by dissolving 100mg. Fast Blue B dye in 100ml. 0.15M sodium phosphate buffer pH 6.5. Four millilitres of 1% w/v stock solution of  $\alpha$ -naphthyl acetate were then added and the



mixture poured over the cut gel surface. Staining took approximately 10 minutes at room temperature. All gels were destained in a mixture of methanol, distilled water and glacial acetic acid in the ratio 5parts:5parts:1part respectively.

Staining for four of the erythrocytic enzymes studied involved the precipitation of a formazan dye from the soluble tetrazolium salt MTT in the presence of phenazine methosulphate (PMS) and reduced NAD or NADP. Fresh stock solutions of these compounds were made up in distilled water on each occasion. The concentrations of MTT, PMS and NADP used were 5mg./ml., while that of NAD was 10mg./ml. Stain mixtures were dissolved in a 0.2M Tris-HCl buffer at pH 8.0 and the total volume of the mixture was made up to 80ml. in all cases, to cover the sliced gel. The recipes used were as follows:

#### 6-phosphogluconate dehydrogenase.

10mg. 6-phosphogluconate dissolved in

10ml. 0.2M Tris buffer and

10ml. 0.1M  $MgCl_2$

1ml. of each of the NADP, MTT and PMS stock solutions.

Incubated in the dark for one hour at 37°C.

(Fildes and Parr, 1963).

#### Lactate dehydrogenase.

0.7ml. 70% sodium lactate solution, dissolved in

10ml. 0.2M Tris buffer

1ml. NAD, MTT and PMS stock solutions

Incubated in the dark for 1½ hours at 37°C.

(Shows and Ruddle, 1968).

Malate dehydrogenase (NAD-dependent).

350mg. L-malic acid dissolved in  
25ml. 0.2M Tris buffer readjusted to pH 8.0 with NaOH  
1ml. NAD, MTT and PMS stock solutions  
Incubated in the dark for  $2\frac{1}{2}$  hours at  $37^{\circ}\text{C}$ .  
(Selander and Yang, 1969).

Phosphoglucumutase.

100mg. glucose 1-phosphate (containing 1% glucose 1,6-  
diphosphate) in  
5ml. 0.2M Tris buffer  
5ml. 0.1M  $\text{MgCl}_2$   
10ul. glucose 6-phosphate dehydrogenase  
1ml. NADP, MTT and PMS stock solutions  
Incubated in the dark for  $2\frac{1}{2}$  hours at  $37^{\circ}\text{C}$ .  
(Spencer et al. 1964).

Gels were photographed on a Kodak coldlight light box with a dark yellow filter. The film used was Kodak Pan X at F11 for  $1/8$  second, which was developed in Kodak D19 developer for 5 minutes at  $20^{\circ}\text{C}$ .

The demonstration of phosphopentomutase activity was performed by the labile phosphate method exactly as given by Quick, Fisher and Harris (1972), having prepared ribose 1-phosphate from inosine as they describe. This method relies on the differential rates of hydrolysis of ribose 1-phosphate and ribose 5-phosphate. An agar layer containing the prepared ribose 1-phosphate is poured over the cut gel and incubated. After removal of this layer, ribose 1-phosphate is stained for with an ammonium molybdate/sulphuric acid mixture which releases the phosphate group by acid hydrolysis. The subsequent reaction of the phosphate with

molybdate forms the dye prussian blue. As ribose 5-phosphate is more stable to acid than ribose 1-phosphate, pale areas in a blue background indicate phosphopentomutase activity.

Acid phosphatase and erythrocyte esterase were stained for using the relevant derivatives of 4-methyl umbelliferone, a fluorescent dye (Hopkinson, Mestriner, Cortner and Harris, 1975; Peters and Nash, 1976). 10mg. of the phosphate and the butyrate respectively were used in 0.05M acetate buffer at pH 5.2, though the butyrate requires dissolving in a few mls. of acetone first. A sheet of Whatman No.3 filter paper was soaked in this solution and placed over the cut gel. After 10 minutes it was removed and the gel surface examined under U.V. light. No photographs of these patterns could be obtained. However, gels screened in this manner could then also be stained for another system with a tetrazolium stain.

## 2.8 ESTIMATION OF LIVER GLYCOGEN

Animals used in the experiments on carbohydrate metabolism were all killed at a similar time, 8-9 hours into their 18 hour daytime period. They were etherised, their necks were broken and the liver was immediately removed and frozen in a dry ice/ethanol freezing mixture. Shortly before assaying, the liver was thawed. A sample of about 100mg. wet weight was then placed in a weighed conical centrifuge tube and the tube was reweighed to the nearest 0.1mg.

Glycogen content was estimated by the method of Hassid and Abraham (1957). In this method, proteins, fats and reducing sugars are destroyed by boiling the sample in 30% KOH. Glycogen is then precipitated by the addition of ethanol



to a concentration of 60%. Van Handel (1965) showed that the addition of a small volume of saturated sodium sulphate solution at this stage allows the quantitative recovery of small amounts of glycogen as it precipitates at the same concentration of ethanol. The precipitate is then dissolved in water and glycogen concentration assayed colorimetrically with the anthrone reagent. The presence of sodium sulphate does not affect this reaction (Van Handel, 1965).

The tissue sample was incubated in 1ml. 30% KOH for 15 minutes in a boiling water bath. After cooling, 0.1ml. saturated  $\text{Na}_2\text{SO}_4$  solution and 1.25ml 95% ethanol were added. The contents of the tube were mixed with a glass rod. Any precipitate adhering to the rod was washed back into the tube with 60% ethanol. The suspension was centrifuged for 15 minutes at 3000rpm. in a Sorvall H-S 4 swing out rotor. The supernatant was decanted off and the precipitate allowed to drain. The precipitate was then dissolved in exactly 4ml. distilled water in a microdisperser and diluted to a concentration of 3-30  $\mu\text{g}/\text{ml}$ . Samples from control animals were diluted x100 while those from starved animals were diluted x25.

The diluted liver glycogen solutions were assayed with a freshly prepared 0.2% solution of anthrone in 95%  $\text{H}_2\text{SO}_4$ . Four millilitres from each sample were placed in an optically clean tube in a cold water bath. In addition, two more tubes were prepared with each batch, one with 4mls. of a sterile standard glucose solution at 20  $\mu\text{g}/\text{ml}$ . and the other with 4mls. distilled water as a blank. Eight millilitres of the anthrone reagent were added rapidly to each tube and the contents were mixed by swirling. The tubes were then



placed in a boiling water bath for 10 minutes. After cooling, they were read in a Spectronic colorimeter which had been zeroed with the blank.

The amount of glycogen present in each sample after dilution was then calculated from the equation:

$$\% \text{ of glycogen in aliquot} = U \times 80 / 1.11 \times S$$

where U is the optical density of the sample, S is the optical density of the 80% glucose standard and 1.11 is a conversion factor from glucose to glycogen for this reagent (Hassid and Abraham, 1957).

## CHAPTER 3

### MOLECULAR VARIATION IN APODEMUS SYLVATICUS

#### 3.1 INTRODUCTION

In this chapter information on variation in A.sylvaticus gained through the study of eight electrophoretic systems will be presented. These are: plasma esterase, transferrin (Tf), erythrocytic esterase, acid phosphatase (ACP), phosphoglucomutase (PGM), lactate dehydrogenase (LDH), 6-phosphogluconate dehydrogenase (PGD), and NAD dependent malate dehydrogenase (NAD-MDH). Two more systems, haemoglobin and glucose 6-phosphate dehydrogenase, were also investigated but no data are presented because of unsolved technical problems.

Two of these systems have been studied in this species by earlier workers. Engel, Kreutz and Wolf (1972) reported a polymorphism at a lactate dehydrogenase regulator locus, and Arnason and Pantelouris (1966) described individual variation in plasma esterase bands. While variation was found in both these systems in the present study, only the former proved useful for the surveys of natural populations. In these studies, the following ten loci whose genetic basis is known or could be reliably inferred were used : two phosphoglucomutases,  $PGM_1$  and  $PGM_2$  of which  $PGM_2$  showed a previously undescribed polymorphism; two structural loci for lactate dehydrogenase subunits, LDH A and LDH B and the regulator locus Ldr; erythrocytic esterase; PGD; Tf; ACP and NAD-MDH.

Population surveys were carried out at eight sites in England and Wales (See Figure 6, Section 3.6). The aim of this work was firstly, to provide a preliminary estimate of the degree of genetic variability in this species. Secondly, the information gained on geographic differences in allele frequencies was required as a background to the data on temporal changes in frequency at three of the sites which will be presented in the next chapter. Finally, the pattern of genetic similarity between populations will be discussed. Berry (1973) suggested that the fieldmouse populations of Britain represented two distinct groups, and a comparison will be made between his work and the results presented here.

### 3.2 PLASMA ESTERASES

The clearest resolution of the plasma esterase bands was obtained in an adaptation of the Poulik (1957) buffer system described in the previous chapter. The pattern developed after staining with  $\alpha$ -naphthyl acetate is shown in Figure 2a. There are three main zones, of which the slowest migrating comprises a single band which does not vary. Migrating rather faster is a complex series of heavily staining bands which show much variation and are resistant to inhibition by phenylmethanesulphonylfluoride (PMSF) at a concentration of 0.017% w/v (Fahrney and Gold, 1963). The most anodal zones are close to these, slightly fainter and are inhibited by PMSF at this concentration. These zones will be referred to as esterase-1, -2, -3 according to their speed of migration, the esterase-1 system being fastest.



Figure 2. Plasma esterases in Apodemus sylvaticus

2b. Gel stained after running in the Tris-Citrate-borate pH8.6 buffer of Arnason and Pantelouris (1966).

The samples are the same, and are arranged in the same order.

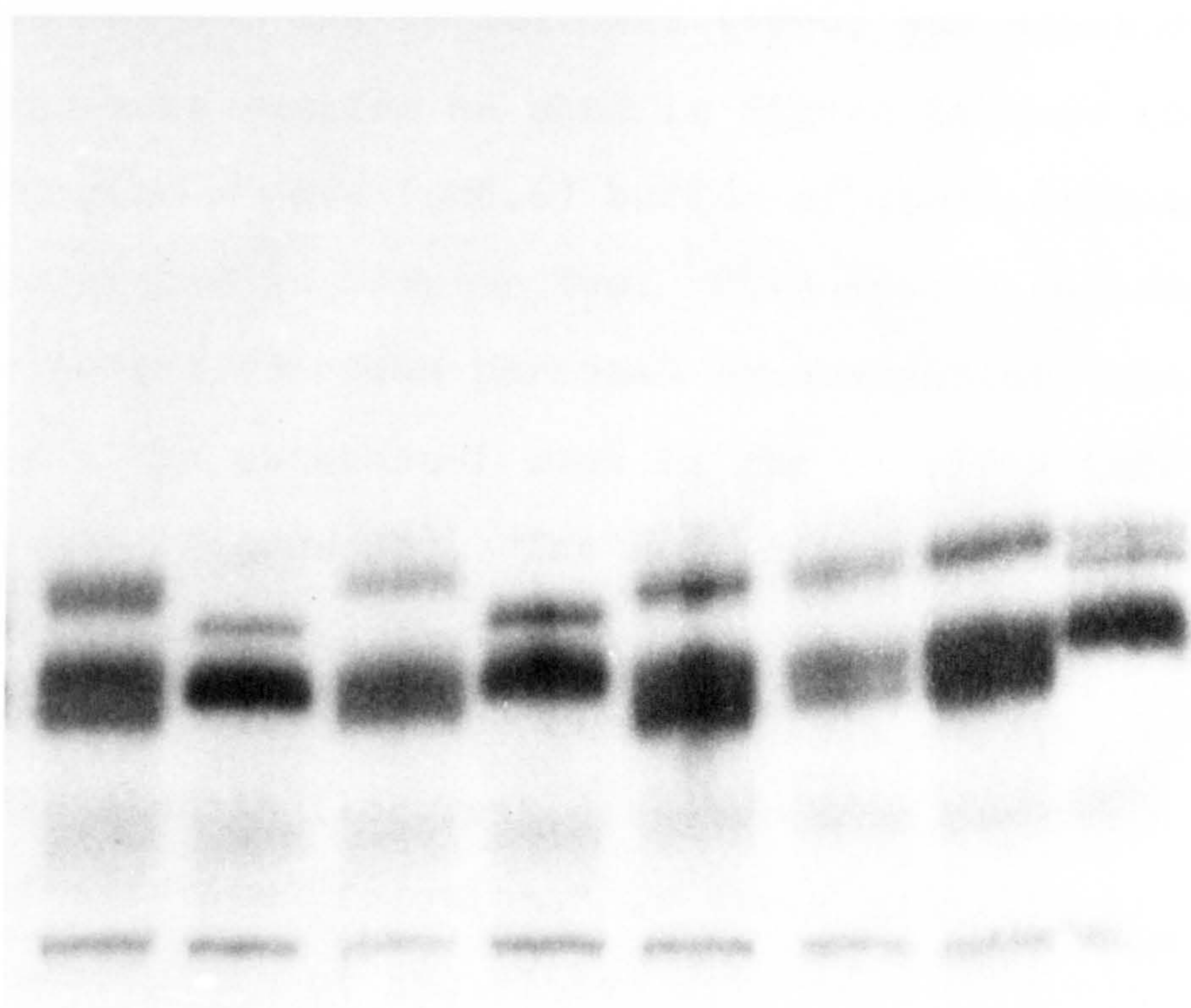
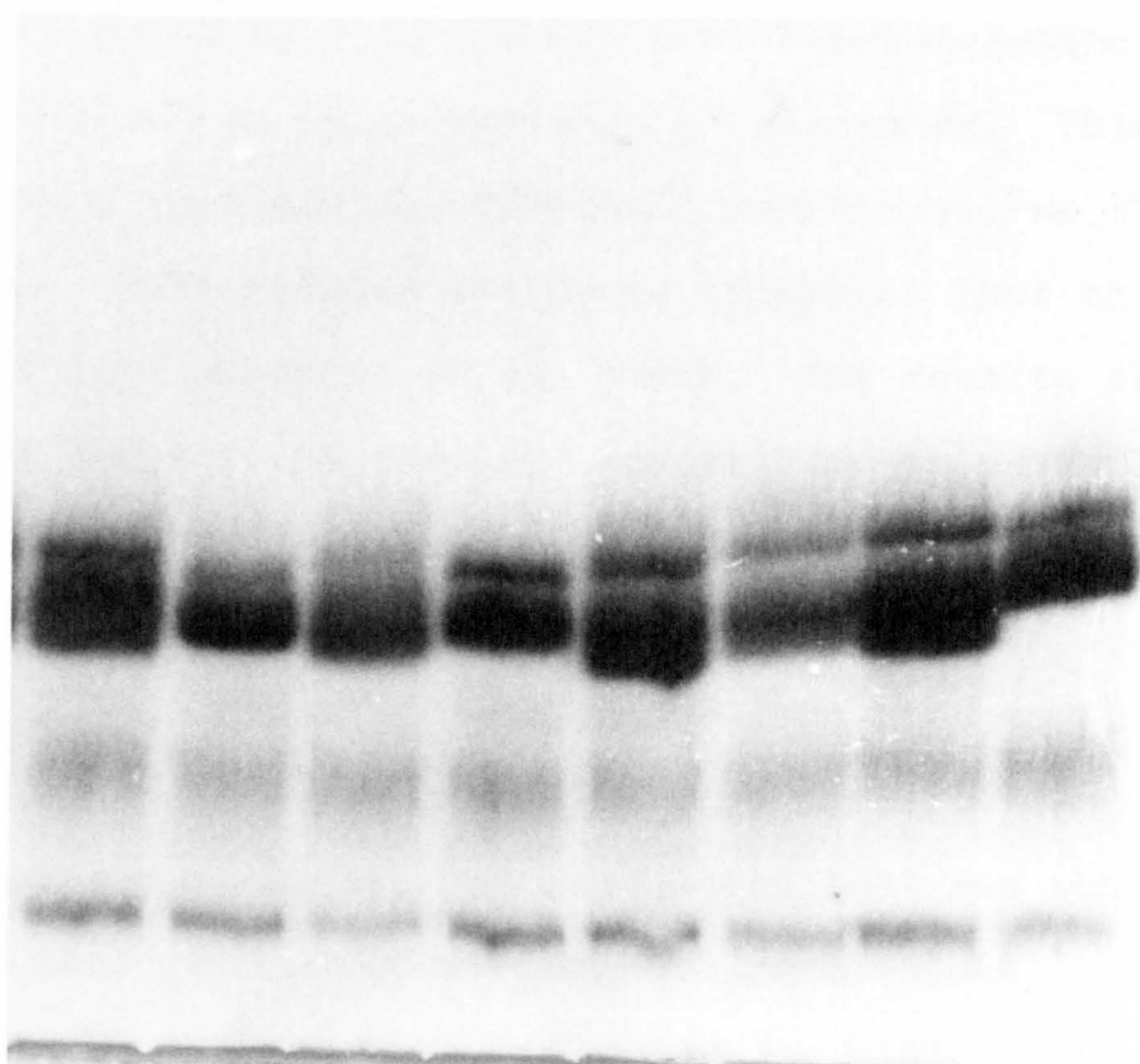
Zone	Sample	1	2	3	4	5	6	7
Esterase-1		NO VARIATION						
Esterase-2		Complex pattern						
Esterase-3		Single band Invariant						

2a. Gel stained with  $\alpha$ -naphthyl acetate after running in the Tris-HCl/Phosphate pH7.0 buffer described in the text.

Zone	Sample	1	2	3	4	5	6	7
Esterase-1		D	F	F	F	S	F	S
Esterase-2		Complex multiband patterns						
Esterase-3		Single band Invariant						

F = Fast; D = Double; S = Slow

The origin is at the base of the gel in both figures and the anode is towards the top.





A simple pattern of variation in the esterase-1 zone was found in some animals from Leicestershire which were obtained at an early stage in the study. This consisted of fast, slow and double-banded phenotypes, as shown in Figure 2a. Matings were set up to establish that these were the allelic products of one locus. The results are presented in Table 1 and indicate inheritance on a simple mendelian basis. When this work was extended to natural populations all the study sites were found to be fixed for the fast allele. A series of attempts were made to elucidate the mode of inheritance of the very variable bands of the esterase-2 zone. The resolution that was obtained in this region was not good enough to permit this, however, and it was considered that field data on bands which represented an unknown number of loci would be of dubious significance.

In view of the technical problems experienced, the work of Arnason and Pantelouris (1966) was examined closely. The same samples as used in Figure 2a were run in the Tris-Citrate-Borate (pH8.6) buffer of these authors, which was described in Chapter Two. This system was apparently their clearest (Arnason personal communication) but it can be seen that the esterase-1 zone is not resolved into its component bands (Figure 2b). The most likely explanation for this discrepancy is that it is due to the difference in pH between the two buffers (See Section 2.3). Although Arnason and Pantelouris (loc. cit.) appeared to resolve a large number of esterase "fractions" in their gels, it is clear that a description of the plasma esterases of Apodemus which relied entirely on their system would not be completely accurate.



TABLE 1

Breeding data on plasma esterase in Apodemus sylvaticus

Class of Mating	Number	Progeny		
		FF	FS	SS
FF x FF	2	26		
SS x SS	3			34
FS x FS	4	13	21	12

TABLE 2

Breeding data on PGM<sub>2</sub> in A.sylvaticus

Class of Mating	Number	Progeny		
		aa	ac	cc
cc x cc	3			29
cc x ac	2		14	13
ac x ac	3	11	19	8
ac x aa	2	8	17	

No significant deviations from expected Mendelian ratios for codominant alleles at a single locus are observed in either case.

### 3.3 PHOSPHOGLUCOMUTASE

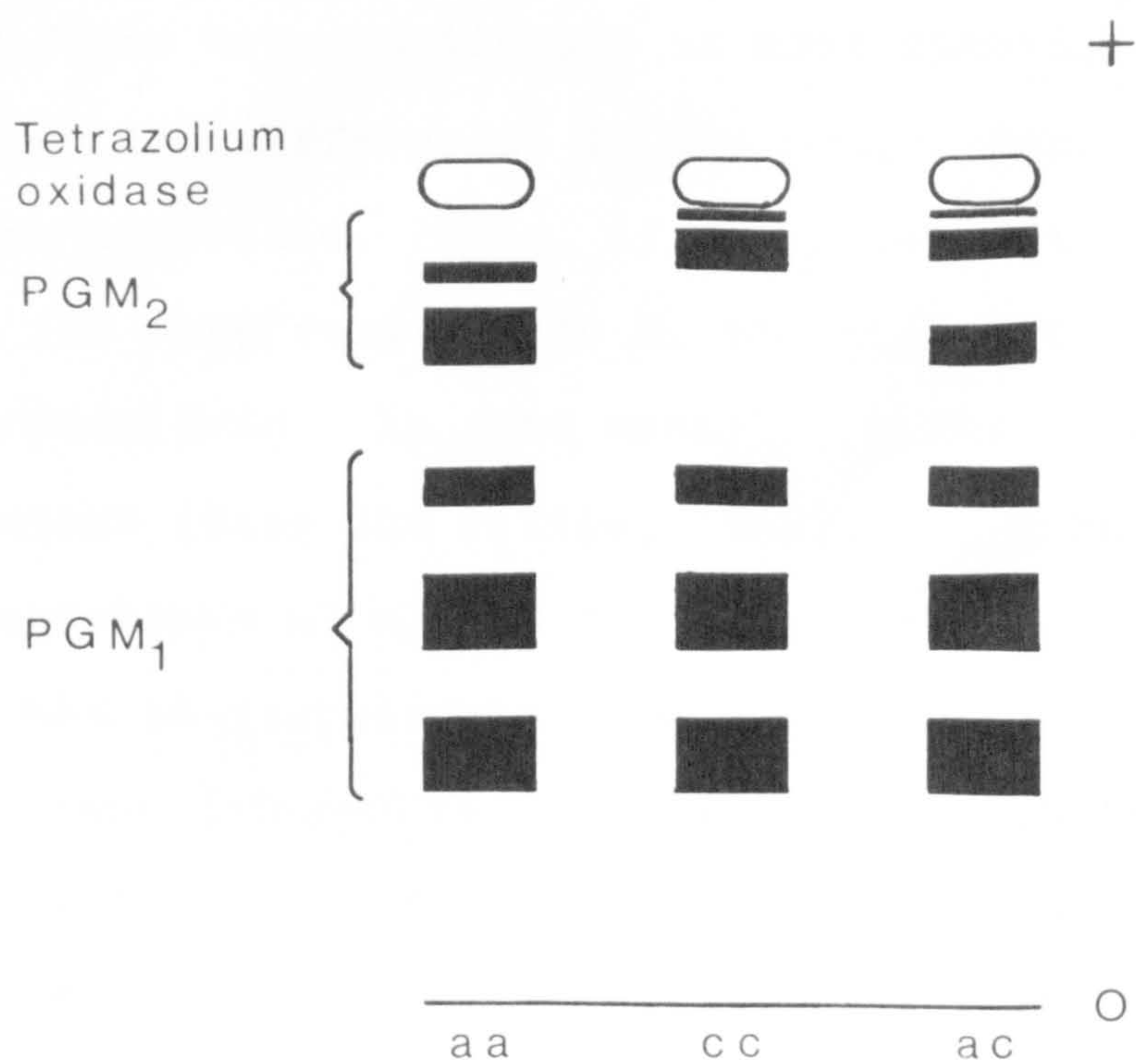
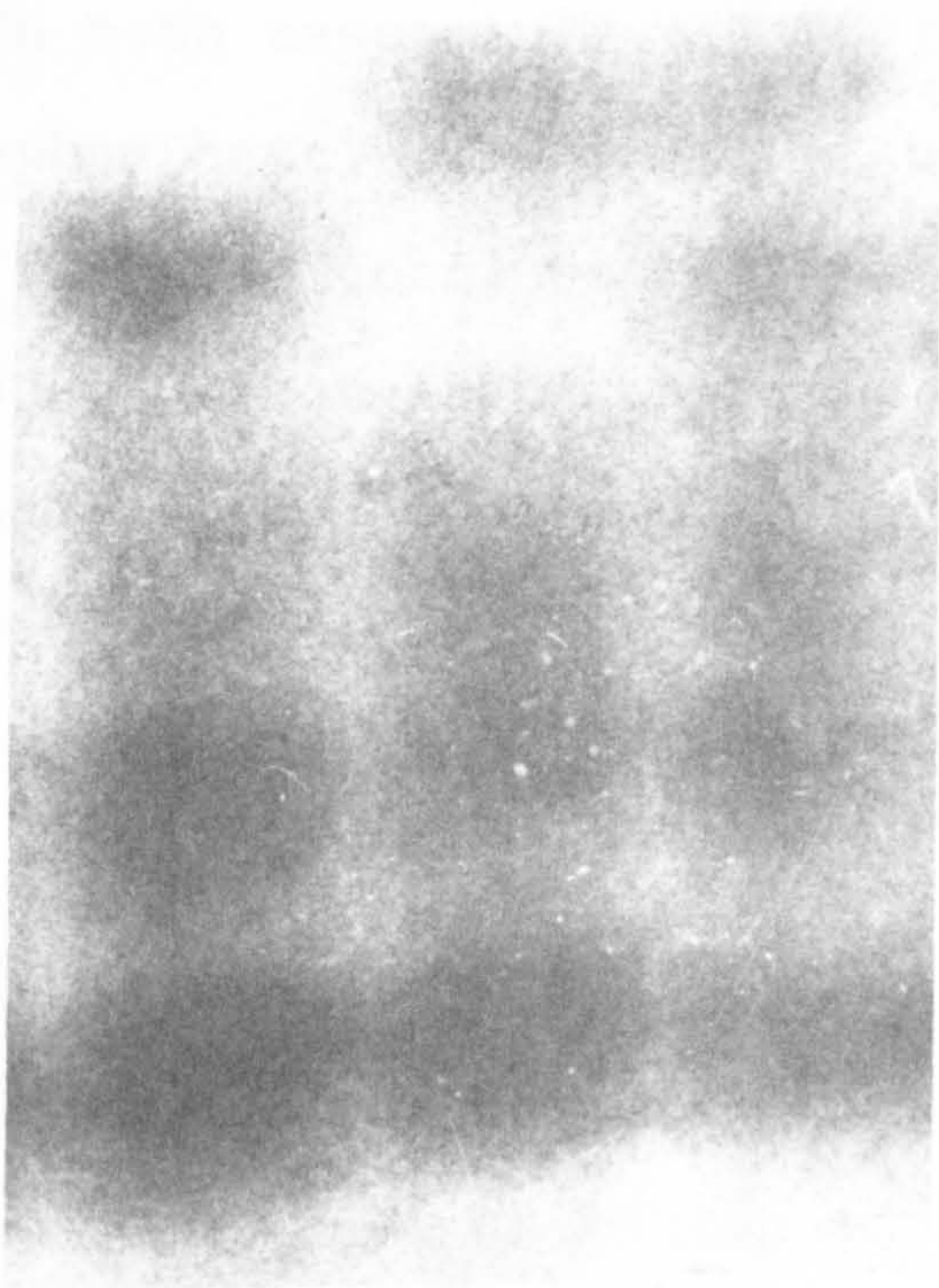
In the fieldmouse, phosphoglucumutase stains as a complex series of isozymes after electrophoresis by the method given in Section 2.6. Figure 3 shows that the red cell pattern can be clearly distinguished into a slower and a faster series of bands. The faster series is polymorphic but one variant was discovered in the slower region, indicating separate genetic control. Three alleles have been discovered in the fast region. They have been designated a, b and c according to the relative mobility of their major band with a being the slowest migrating allele. Of these three, the a and c alleles are common and a programme of matings has demonstrated the Mendelian basis of the polymorphism (Table 2).

The terminology applied to phosphoglucumutase isozymes differs between man and Mus. In human erythrocytes the least anodal series is termed  $PGM_1$  and the faster series  $PGM_2$  (Spencer et al. 1964). In Mus the two series are rather different in appearance and the faster one is generally termed Pgm-1 (Shows, Ruddle and Roderick, 1968), although Selander and Yang (1969) refer to it as Pgm-2. In Apodemus the pattern of PGM isozymes resembles that of man more closely than that of Mus in the TEMM buffer system (Miner and Wolfe, 1972). It is known that the products of the two loci found in human erythrocyte differ in molecular weight, stability to heat, and in their activity as phosphopentomutases (McAlpine, Hopkinson and Harris, 1970a,b; Quick, Fisher and Harris, 1972). This last property has been employed to compare the Apodemus isozymes with the human ones using the

Figure 3. Phosphoglucomutase isozymes in erythrocytes of  
A.sylvaticus

Photograph and diagram of the three common PGM<sub>2</sub>  
phenotypes. The minor bands in both loci show considerable  
variation in expression.





labile phosphate method (see Section 2.7) to detect PPM activity. In both cases only the faster series is active on ribose 1-phosphate. The level of activity in Apodemus is low, but the same conclusion has been reached by other workers (Fisher personal communication). Accordingly the PGM isozymes in Apodemus have been labelled in the same way as in man, with the locus controlling the least anodal series designated  $\text{PGM}_1$  and  $\text{PGM}_2$  being the polymorphic locus.

### 3.4 LACTATE DEHYDROGENASE

In mammals, lactate dehydrogenase is a tetrameric molecule formed by the association of two electrophoretically distinct subunits, A and B. These subunits associate at random so that after electrophoresis five isozymes can be detected, corresponding to the five possible combinations of the subunits : AAAA, AAAB, AABBB, ABDBB and BBBBB (Shows and Ruddle, 1968). The relative intensity of the isozymes differs between tissues in most mammals indicating tissue-specific differences in the proportions of each subunit that are available. Thus, if the A subunit is only produced at a low rate relative to B, the high B tetramers will predominate. In some mammals, particularly some species of rodent (Baur and Pattie, 1968), the B subunit is not detectable at all in erythrocytes. In the house mouse this trait is polymorphic. Expression of the B subunit is found in some laboratory strains but not others. Although this variation is affected by the genetic background to some extent, it has been shown to be inherited in a Mendelian fashion through inter-strain crosses (Shows and Ruddle, 1968),



with the  $B^-$  phenotype recessive to  $B^+$ . This polymorphism has also been found in wild populations of house mice (Selander and Yang, 1969). In A.sylvaticus the polymorphism was first discovered by Engel et al. (1972). Figure 4 shows that the gel phenotypes found in this study were similar to theirs, although the full five-banded pattern was rare. By a series of matings these workers established the genetic basis of the polymorphism in Apodemus and again showed the  $B^-$  phenotype to be recessive.

The lactate dehydrogenase polymorphism shown by these rodents cannot be due simply to an inactive or "null" allele at the structural locus for the B subunit. The gel phenotypes for other tissues show that an active form of the subunit can be synthesised by the organism but, in  $B^-$  individuals, it is simply not being expressed in erythrocytes. This argues strongly for the presence of an independent, regulatory locus (Shows and Ruddle, 1968). However it has not yet been shown that the regulator and structural genes map separately. The manner in which the product of the regulator, Ldr, locus controls the synthesis of the B subunit is not known. However a mechanism can be proposed on this basis: If the product of the Ldr gene were to act as a repressor, then a mutation which destroyed its activity would permit synthesis of the B subunit in erythrocytes. The homozygous wild type would have an erythrocyte LDH phenotype which was  $B^-$ . The homozygous mutant would be  $B^+$ . The heterozygote would possess one active dose of repressor protein. It is likely that this would be quite sufficient to repress synthesis and the erythrocytes would therefore

Figure 4. Lactate dehydrogenase isozymes in erythrocytes  
of A.sylvaticus

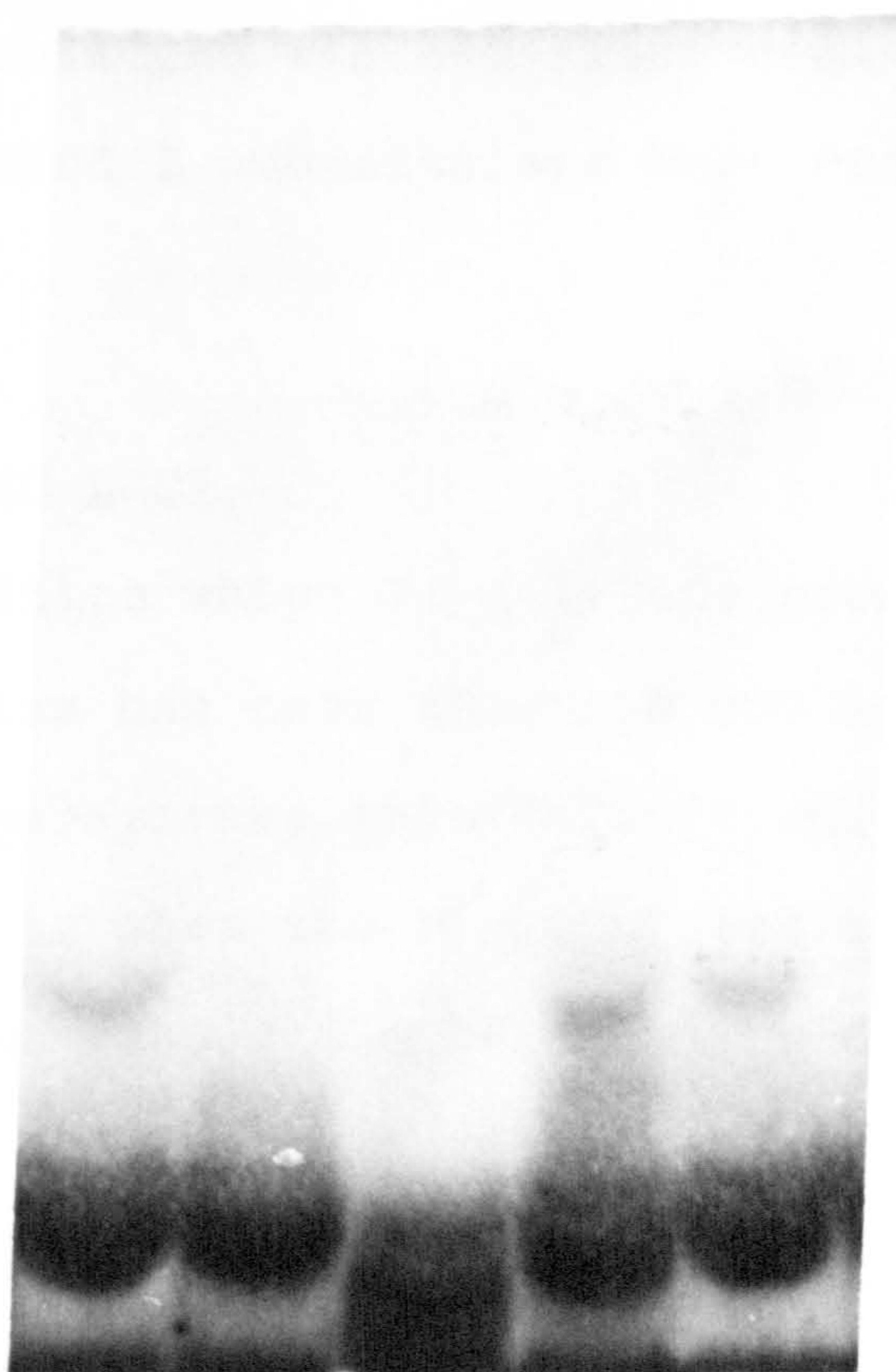
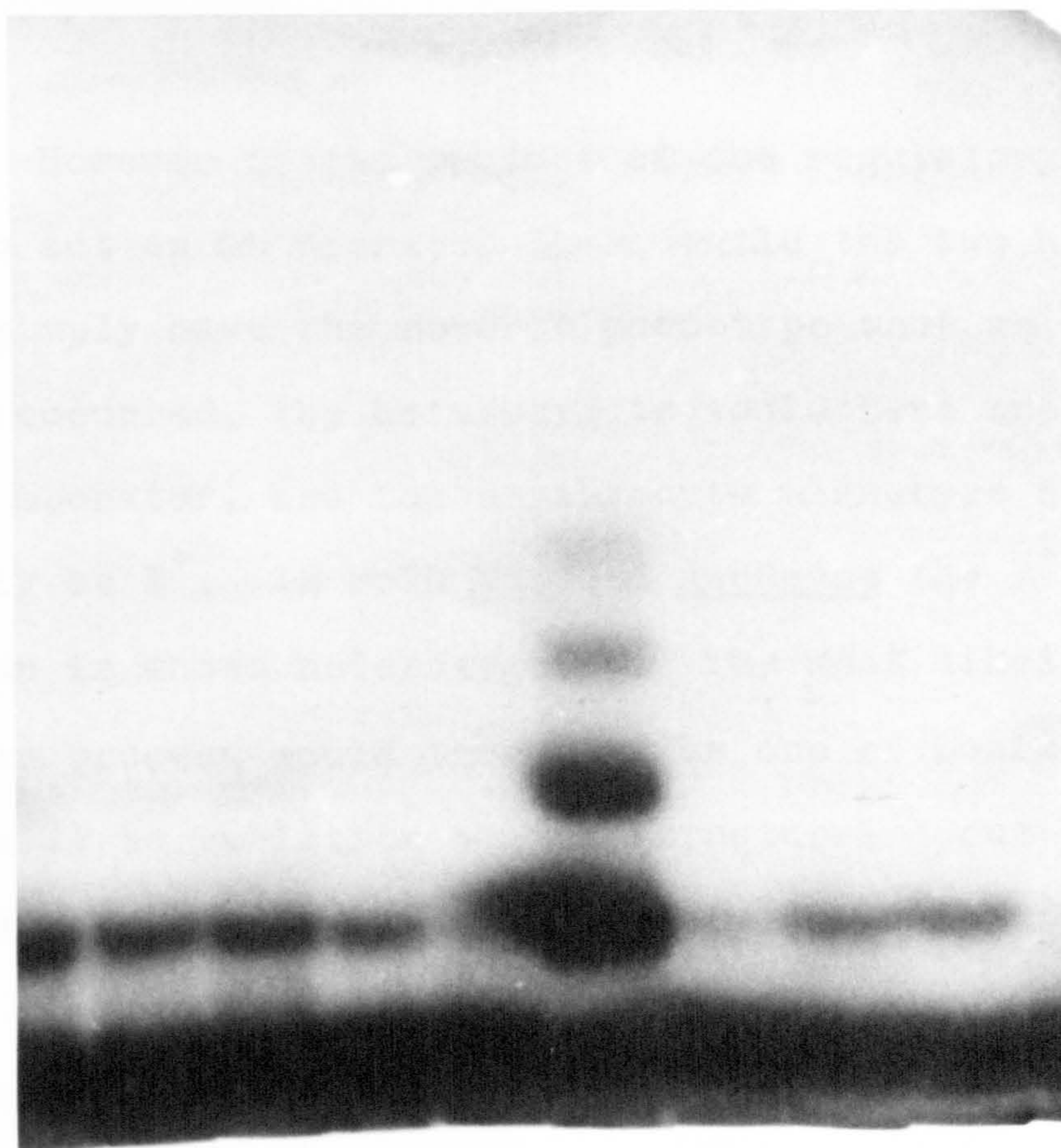
4a.	Sample	1	2	3	4	5	6	7	8	9	10
Ldr	phenotype	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>+</sup>	b <sup>-</sup>

Commonly, only the two slowest bands were expressed in b<sup>+</sup>  
phenotypes.

4b. A rare slow variant of the structural gene for the A  
subunit.

In both figures the origin is at the base of the gel and the  
anode is towards the top.





be  $B^-$ . However if the product of the regulatory locus were to act as an operator then, while the two homozygotes would simply have the reverse phenotype when an inactive mutant occurred, the heterozygote would have one dose of active operator, and the erythrocyte phenotype would probably be  $B^+$ . In both Mus and Apodemus the  $B^+$  phenotype is shown in known heterozygotes. The most likely mechanism for this process would therefore be one of positive control.

While no variation at the structural locus for the B subunit was found in this study, two variants were found in the A subunit. Both were of the type shown in Figure 4b, with the normal A band and one migrating rather more slowly. These individuals came from the same population, at Madingley near Cambridge (Figure 6) in different years. However this variation was not discovered at any other site and both the A and B subunits are here regarded as monomorphic.

### 3.5 MONOMORPHIC LOCI

The definition which Ford (1940) proposed for the term polymorphism has been the main one in use until quite recently. To paraphrase this definition, a locus is said to be polymorphic when the rarer of two alleles is present at a higher rate than could be maintained by recurrent mutation. Since the advent of the widespread use of gel electrophoresis, the discovery of a very high level of variation in natural populations has led some authors, particularly in the U.S.A., to use a more arbitrary definition for the term. Ayala and Tracey (1974) give two



common criteria used in this approach. The first is that a locus is regarded as polymorphic when the frequency of the most common allele is less than 0.95. The second criterion requires that the frequency of the second most common allele exceeds 0.01. In a two allele polymorphism these definitions amount to a minimum frequency of 5% or 1% respectively for the rarer allele.

In the present study, sample sizes were generally small. Although some variation was found at other loci, only for Ldr and PGM<sub>2</sub> could populations be compared in terms of gene frequency. It has therefore been convenient to adopt an arbitrary definition of a polymorphic locus. This section will be concerned with those loci where the most common allele has a frequency of 95% or greater.

In addition to the LDH B subunit (Section 3.4), two more of the ten loci studied showed no variation at all. After running in the phosphate buffer system (Section 2.6), acid phosphatase stains as one main and one, slightly faster, subsidiary band migrating approximately 4cm anodally. Over three hundred samples were screened from several populations but no variant was found. Similarly, malate dehydrogenase (NAD dependent) stains as a single band migrating slowly towards the anode and no variant was found in over two hundred individuals.

Three hundred and fifty plasma samples were stained for general protein after electrophoresis in the Poulik (1957) buffer system. The fast running proteins did not show good resolution but a clear double band of the transferrin pattern was obtained behind them. Two animals were found



on or near the Charnwood site which were heterozygous for a slower allele. Three bands of equal intensity were found in these individuals, in contrast to the faint fast and heavy slower band of the homozygotes. The variants were found at different times during 1974, but the allele did not reappear in the grid population subsequently.

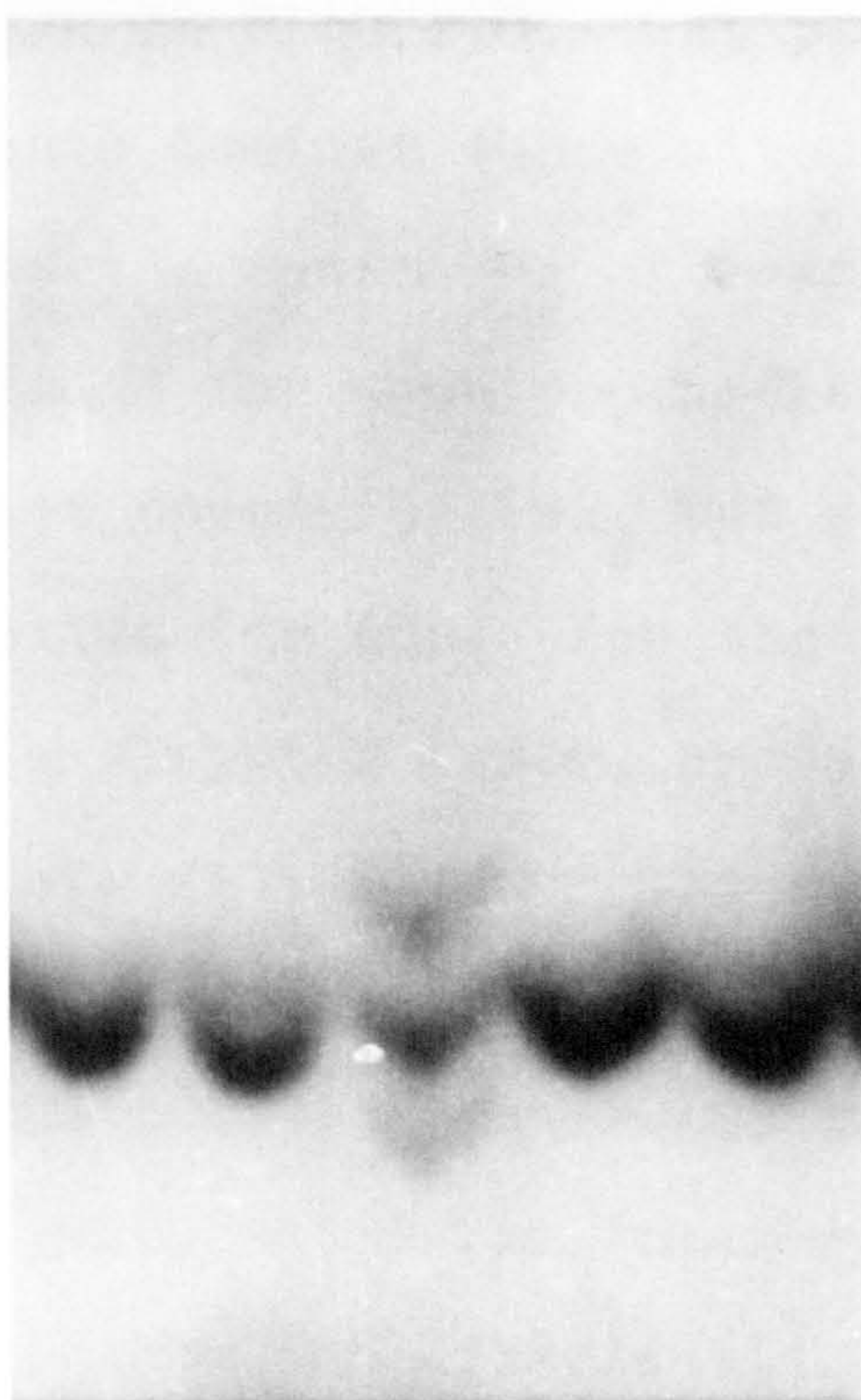
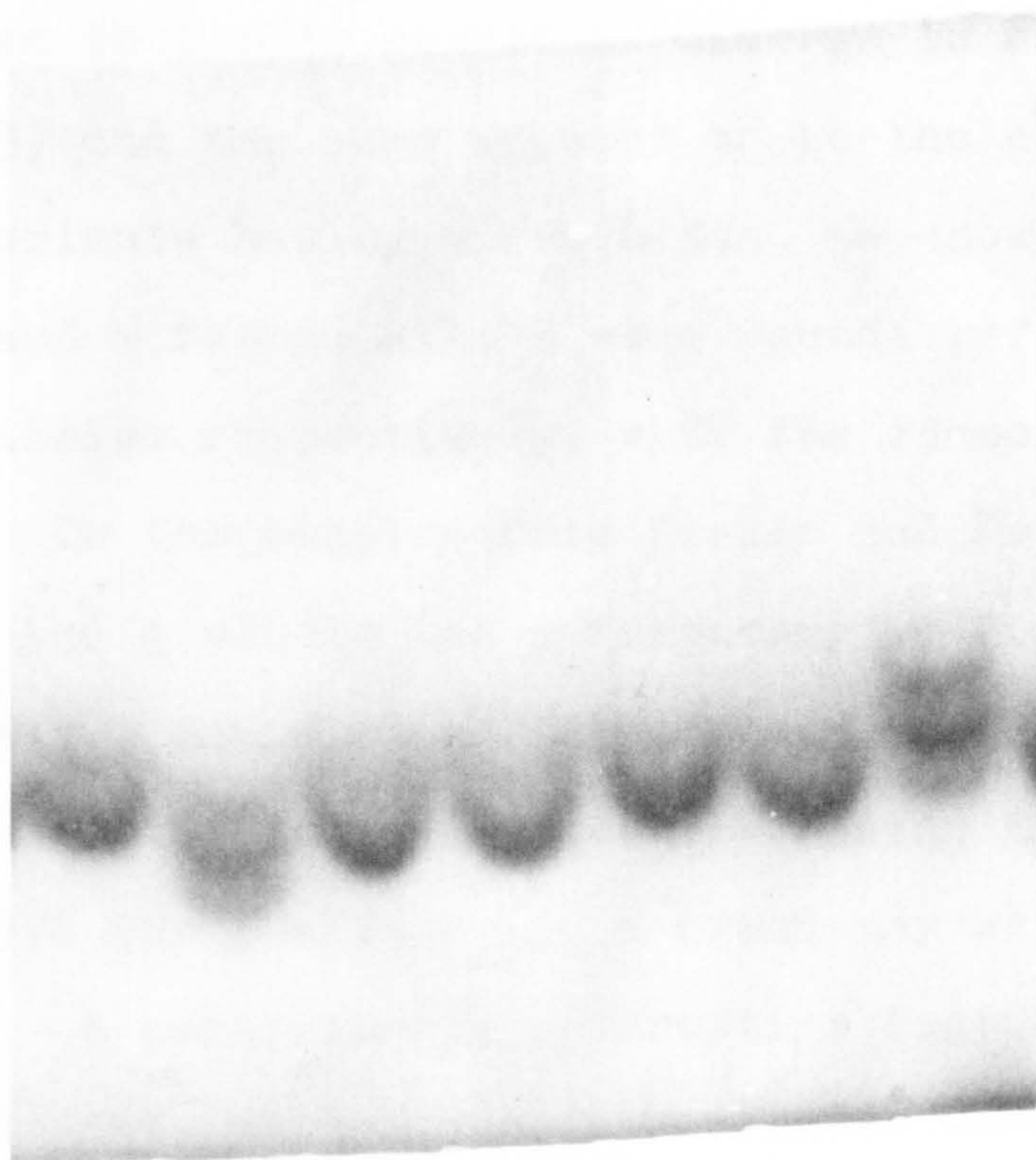
The last two loci to be considered here showed variation in more than one population. Erythrocytic esterase activity could only be detected using the fluorescent stain method described in Section 2.7. A single band was normally observed, in a similar region of the gel as acid phosphatase. In view of the substrate specificity the enzyme appears to have a similar function to Esterase-D in man and esterase 10 in Mus (Hopkinson et al., 1973; Peters and Nash, 1976). Four variants were discovered in a total of three hundred animals screened. All showed a similar phenotype, with staining in the usual region and in two bands below it. They therefore appear to be heterozygous for a slower allele. A triple banded phenotype is often found in heterozygotes and indicates a dimeric protein. The heterozygotes found in man and Mus at the equivalent loci show a similar pattern. Three of the four variant animals came from collections at Wytham Wood near Oxford. The slow allele attained a frequency of 0.04 in the December 1975 sample. It is conceivable that this allele could be maintained at a low level in this population.

Under the conditions used, 6-phosphogluconate dehydrogenase migrated slowly towards the anode. A total of seven variant individuals were discovered at this locus.

Figure 5. 6-phosphogluconate dehydrogenase variants in red  
cells of A.sylvaticus

a.	Sample:	1	2	3	4	5	6	7
	Pgd phenctype	bb	ab	bb	bb	bb	bb	bc

b.	Sample:	8	9	10
	phenotype	bb	ac	bb





It is known that this enzyme is dimeric in man (Fildes and Parr, 1963) and the same appears to be the case in Apodemus, all the variants having three bands, as shown in Figure 5. A slower and a faster allele were found, referred to as the a and c alleles respectively, with the common form being termed b. In the samples from Wicken and Prickwillow Farm, near Ely, the a allele had a frequency of 0.01 and 0.04 respectively. The site at which these variants were most abundant was also in Cambridgeshire, being Madingley. In January 1976 the a allele had a frequency of 0.04 and the c of 0.03. A particularly interesting feature of this sample was that, although no homozygotes were found, one animal was heterozygous for the a and c alleles.

### 3.6 GENE FREQUENCIES IN NATURAL POPULATIONS

The eight locations for which population data were obtained are shown in Figure 6. Gel phenotype numbers for the two polymorphic loci at these sites are shown in Table 3. Table 4 gives the estimates of mean heterozygosity per locus per individual for each population. These were obtained by direct count for PGM<sub>2</sub> but estimated from Hardy-Weinberg equilibrium for Ldr. For the smaller samples, mean heterozygosity is slightly lower, probably due to sampling error. The overall estimate for the species from this study is 9.88%.

Table 5 gives allele frequencies for both loci at each site. The data were tested for inter-site heterogeneity in allele frequency. For PGM<sub>2</sub> the total  $\chi^2$  value was 19.93 with 7 degrees of freedom ( $P < 0.01$ ). For Ldr the total  $\chi^2$  was

Figure 6. The geographic location of the study sites

Scale: 1cm : 40 km.

The eight populations sampled are indicated as follows:

- C Charnwood, Leicestershire. M.R. 470158 Alt. 178m.  
Sampled bimonthly 1974/6.
- Eg Egham, Surrey. M.R. 992703 Alt. 66m.  
Sampled January 1975.
- E Ely, Cambridgeshire. M.R. 600810 Alt. 0m.  
Sampled November 1975.
- M Madingley, Cambridgeshire. M.R. 399595 Alt. 61m.  
Sampled January 1975; June 1975; January 1976.
- R Rogate, Hampshire. M.R. 807238 Alt. 65m.  
Sampled February 1975.
- T Talsarnau, Gwynedd. M.R. 629363 Alt. 152m.  
Sampled April 1975.
- W Wicken, Cambridgeshire. M.R. 560705 Alt. 0m.  
Sampled October/November 1974.
- Wy Wytham, Oxfordshire. M.R. 461086 Alt. 140m.  
Sampled December 1974; May 1975; December 1975.

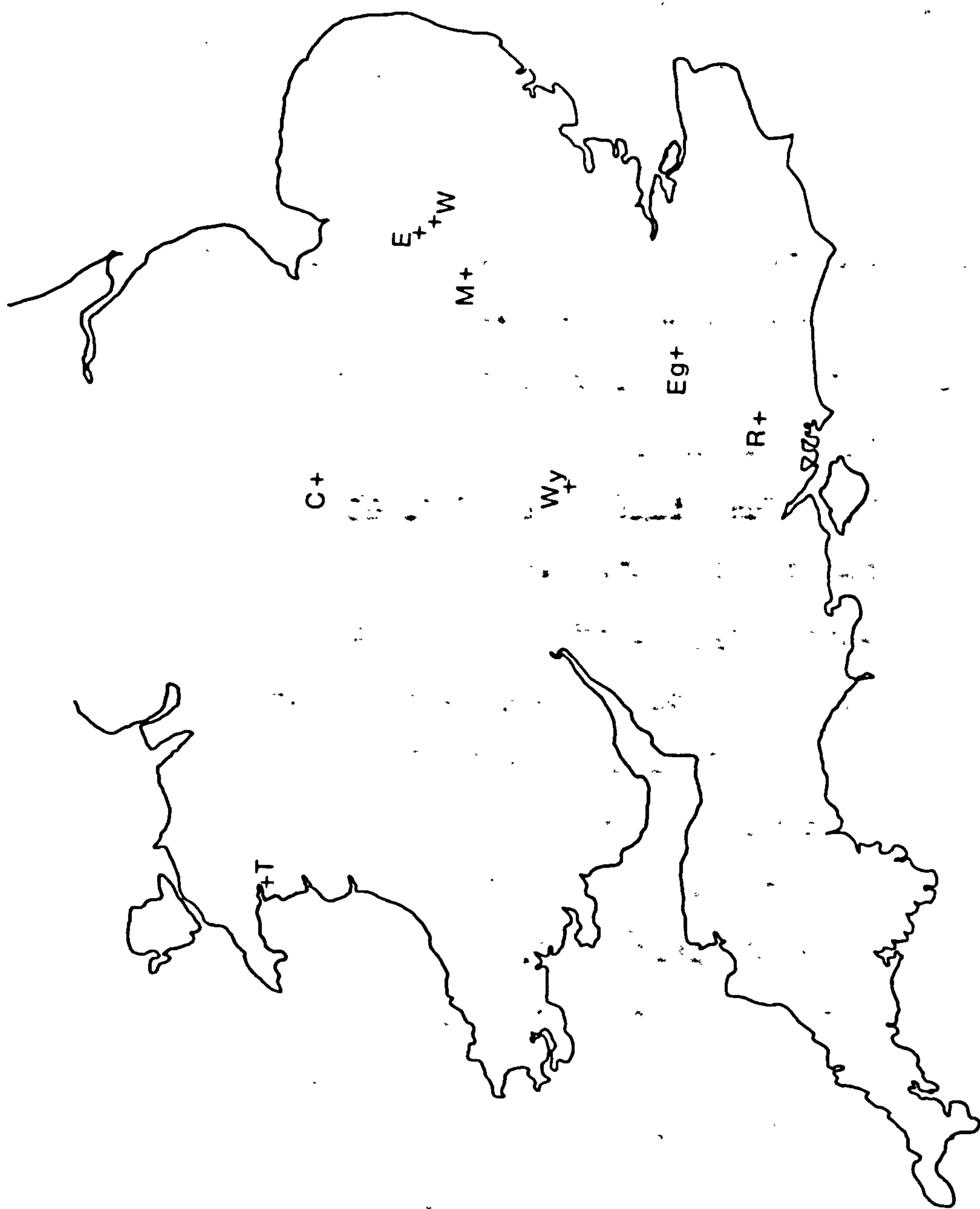




TABLE 3

PGM<sub>2</sub> and Ldr phenotype numbers for eight sampling areas

	PGM <sub>2</sub>			Ldr	
	<u>aa</u>	<u>ac</u>	<u>cc</u>	B <sup>-</sup>	B <sup>+</sup>
Wicken	13	21	2	3	32
Egham <sup>1</sup>	8	10	10	7	26
Rogate	7	6	5	3	14
Talsarnau	6	19	17	5	37
Ely	8	7	9	9	15
Wytham <sup>2,3</sup>	9	18	5	5	29
Madingley <sup>3</sup>	12	17	4	7	25
Charnwood <sup>3</sup>	15	27	4	5	41

<sup>1</sup> + 1 x ab, 1 x bb, 3 x bc<sup>2</sup> + 2 x bb<sup>3</sup> Data given for the December 1974/January 1975 collections.

TABLE 4

Mean per locus heterozygosity and sample size

	H%	N
Wicken	10.4	36
Egham	9.2	33
Rogate	8.2	18
Talsarnau	9.0	42
Ely	8.8	24
Wytham	10.7	34
Madingley	11.4	33
Charnwood	10.3	46

TABLE 5  
Allele frequencies

	PGM <sub>2</sub>			Ldr	
	<u>a</u>	<u>b</u>	<u>c</u>	<u>b</u> <sup>-</sup>	<u>b</u> <sup>+</sup>
Wicken	0.65	0	0.35	0.28	0.72
Egham	0.41	0.09	0.50	0.46	0.54
Rogate	0.56	0	0.44	0.42	0.58
Talsarnau	0.37	0	0.63	0.35	0.65
Ely	0.48	0	0.52	0.62	0.38
Wytham	0.53	0.06	0.41	0.38	0.62
Madingley	0.62	0	0.38	0.47	0.53
Charnwood	0.62	0	0.38	0.33	0.67



12.11 with the same number of degrees of freedom ( $0.1 > P < 0.05$  n.s.). The calculation of pairwise  $\chi^2$  values between sites for both loci enabled the main sources of this heterogeneity to be identified. For  $PGM_2$  the  $\chi^2$  values which are significant or nearly so are given in Table 6. These are restricted to comparisons involving the Talsarnau site. For the Egham population one comparison closely approached significance. For Ldr, however, only one significant value was obtained although the range of frequencies was slightly greater. This was between the Wicken and Ely sites and the  $\chi^2$  value was 5.67 ( $P < 0.05$ ).

There is no clear relationship between the observed differences in gene frequency and geography. The Welsh site is well removed from the others and shows several significant differences in  $PGM_2$  but not Ldr frequencies. In addition, the closest two sites, Wicken and Ely, are the only ones to show a significant difference at the Ldr locus. There is no clinal effect at either locus, but the distribution of the variants at the PGD locus shows some clumping (see Section 3.5). However, the samples are too small to reliably estimate the frequency of rare alleles.

Finally, while two loci are not sufficient to make a meaningful multivariate analysis, the Figure 7 indicates the similarity between the populations. Again, distance between sites bears no relation to distance on the diagram. It can be seen, however that the spread of the points is not great, populations tending to have similar frequencies at both loci.

TABLE 6

Differentiation in allele frequency at the PGM<sub>2</sub> locus

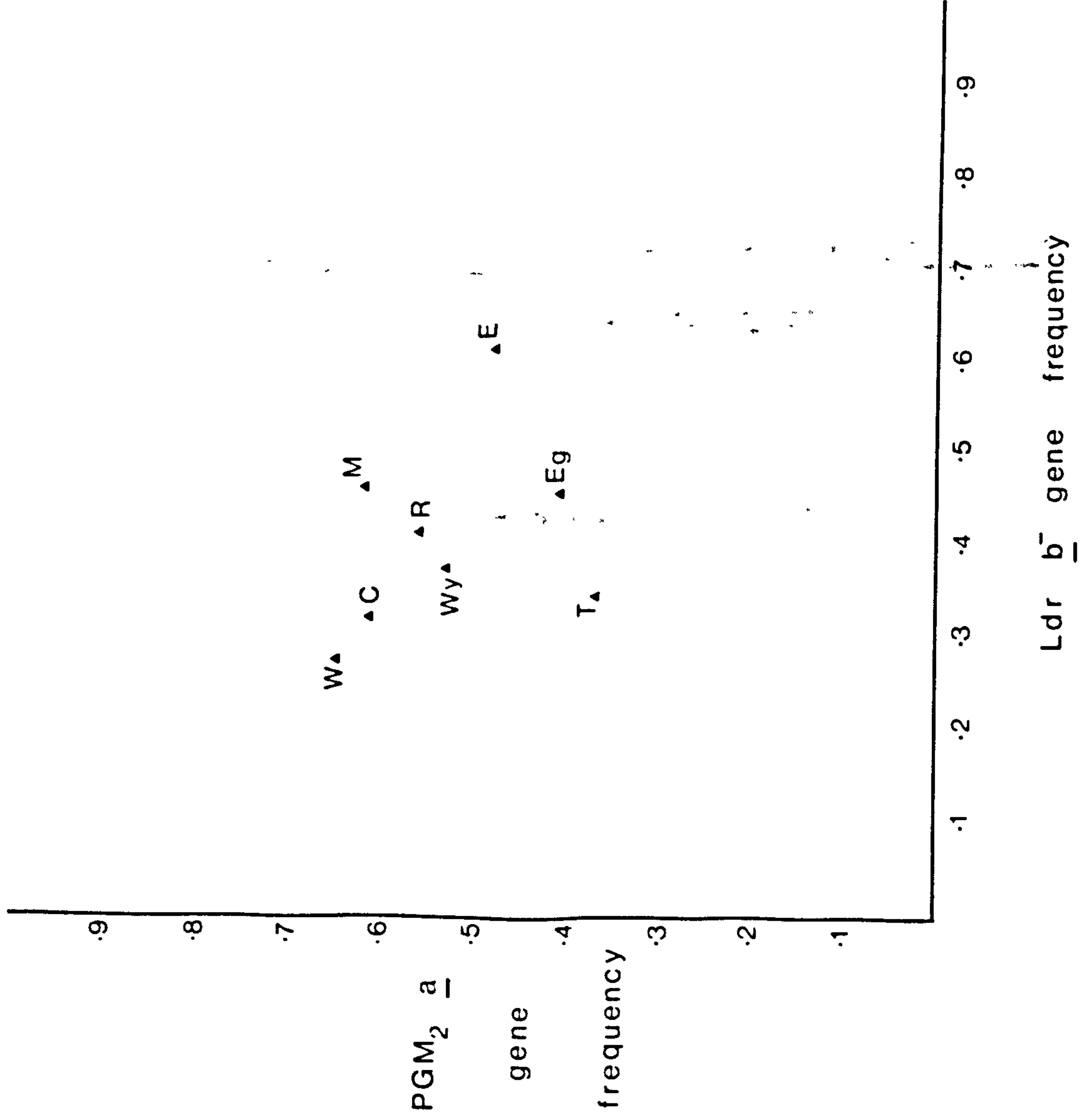
	Rogate	Wytham	Charnwood	Madingley	Wicken
Talsarnau	2.86	4.75	10.04	8.43	11.38
	P < 0.1 n.s	P < 0.05	P < 0.01	P < 0.01	P < 0.01
Egham	n.s	ns	2.81	2.41	3.83
			P < 0.1 n.s.	P < 0.1 n.s	P < 0.1 n.s

For each comparison the value of  $\chi^2$  with one degree of freedom and the probability of such a result occurring by chance is shown

Figure 7. A two dimensional genetical comparison of the  
study populations

Symbols as for Figure 6.





### 3.7 DISCUSSION

The first attempts to estimate the degree of genetic variability in natural populations using electrophoretic techniques met with considerable success (Lewontin and Hubby, 1966; Harris, 1966). The strengths and limitations of the technique for providing such an estimate were discussed by Hubby and Lewontin (1966) and this trio of papers has stimulated a vast quantity of work on a great range of species since that time. In reviewing the work done up to 1972, Lewontin (1974) emphasised the importance of adhering to certain criteria when attempting to provide an estimate of variability which can be reliably compared with other species. Of the five criteria he lists, three are adequately met by the present study. These are that the choice of loci is random with respect to known variation, that large enough numbers of individuals are screened at each locus and that they are obtained from the wild, not an old laboratory stock. The fourth criterion is that the choice of loci is random with respect to the genome. This is not met by the data given in this chapter. All the proteins studied are soluble, most were enzymes, only one regulatory locus and no structural proteins were included. However, it can be said that this technique can only be applied to soluble proteins and that enzymes dominate the list of loci in most surveys. Indeed, the inclusion of one regulatory locus is an advance over many.

The most important of Lewontin's (1974) criteria in which this study does not match many similar ones is in the size of the sample of loci screened. An indication of the

importance of a large sample is given by comparing the results obtained by O'Brien and Macintyre (1969) and Berger (1970) with those of Kojima, Gillespie and Tobari (1970) who worked on the same species of Drosophila, D.melanogaster and D.simulans. From surveys of ten and six loci respectively, the first two studies gave figures of 0.07 and 0.00 for mean heterozygosity in D.simulans. From a sample of 18 loci, Kojima et al. (1970) obtained a value of 0.160 which was more similar to that found for D.melanogaster in all three studies. It must be emphasised therefore that the estimate of the degree of variability presented here for A.sylvaticus is only a preliminary one, based on data collected for other purposes (see Section 3.1).

To consider first the proportion of loci that are polymorphic in this species, two out of the ten loci studied were found to be polymorphic in all populations. At some sites, and only under the 0.99 criterion for maximum gene frequency of the most common allele (see Section 3.5), two more loci could be classed as polymorphic. The proportion of loci polymorphic in this species can therefore be regarded as 20% or 40% accordingly. The small sample of loci screened renders this expression of variability liable to large errors. Discussion of these results will therefore be restricted to the estimate of mean heterozygosity which is less sensitive to such low frequency variants, although they are included in its calculation.

Many extensive surveys of variability have been made on various species of rodents. Comparison of the estimate of mean per locus heterozygosity of 9.88% given here for



Apodemus sylvaticus, with other work puts it among the more variable species. Similar figures have been obtained for two subspecies of Mus musculus, 11.0 and 9.91% although for a third it was rather lower (5.6%) (Selander and Yang, 1969; Selander, Hunt and Yang, 1969). Four species of Peromyscus have been studied and estimates range from 5.1 to 7.0% (Selander, Smith, Yang, Johnson and Gentry, 1971; Smith, Selander and Johnson, 1973). Several other species have shown rather less variability: cotton rats (Sigmodon spp.) have heterozygosity levels of from 1 to 3% (Johnson, Selander, Smith and Kim, 1972); kangaroo rats (Dipodomys spp.) give similar figures (Johnson and Selander, 1971); pocket gophers (Thomomys spp.) vary from 3.3 to 7.0% (Patton, Selander and Smith, 1972; Nevo et al. 1974) and mole rats (Spalax ehrenbergi) show an overall mean heterozygosity of 3.9% (Nevo and Shaw, 1972). Thus, among mammals, Apodemus appears fairly variable. While reptiles and fish are similar, amphibians are rather more variable than mammals (Powell, 1975). Invertebrates are generally much more variable, with values for mean heterozygosity in the Drosophila willistoni group averaging out at 17.7% (Ayala et al. 1974) while for D.pseudoobscura it is 12.8% (Prakash, Lewontin and Hubby, 1969). Even the phylogenetic 'relict' Limulus has a mean heterozygosity of 6.4% (Selander, Yang, Lewontin and Johnson, 1970). Although the estimate given for Apodemus is based on a small scale study, it falls in well with the established pattern.

In most surveys of allozymic variation a major feature of the results has been the similarity of the genetic

constitutions of local populations, despite a high level of individual variation (Lewontin, 1974). They are hardly ever fixed for different alleles, and although multiallelic polymorphisms are common, the same alleles are usually most frequent in all populations. Ayala et al. (1974) used Nei's (1972) measure of genetic distance to combine data from many loci and showed that in their extensive survey of the D. willistoni complex hardly any allozymic differentiation occurs between local populations. While the data for Apodemus are too limited to allow for such an approach, the basic pattern is similar. No populations are fixed for different alleles and the gene frequencies given in Table 5 vary within a restricted range from between 0.3 to a little over 0.6 at both loci. Some populations are distinct (Table 6) but only one sample differs significantly from any other, in both cases. When the data for both loci are combined (Figure 6), it can be seen that all the populations fall into a single cluster.

The differentiation that is observed between populations does not show any clear pattern. Comparison of the position of sites on the diagram in Figure 6 with their location on Figure 1 reveals that the change in gene frequency at neither locus is clinal and that it bears no relation to geographic distance. While the four significant comparisons at the  $PGM_2$  locus all involve distant sites, three similar ones are non significant. The single significant comparison at Ldr is found over a distance of less than ten miles.

The influence of climatic factors can be assessed on the basis of the geographic data. Rainfall varies markedly



across Britain from west to east. At first sight this might appear to have some relevance to the distinctiveness of the Talsarnau population at PGM<sub>2</sub>. Only one of the two fenland sites is significantly different from it however, although both are situated in the driest part of the country. Temperature, mean and range, probably does not vary very much over the study sites as even the Welsh one was close to the coast. There would not appear to be any environmental factor which replicates the pattern of differentiation shown by these eight sites in allele frequency. Selander, Yang and Hunt (1969) discovered a similar range of allele frequencies in the populations of house mice that they surveyed for haemoglobin and several esterase loci. Some clinal change was found and these workers were able to obtain significant correlation of allele frequency at one esterase locus with two environmental variables, latitude and rainfall, out of eight tried.

When some degree of isolation is present between populations, differentiation may be more pronounced. This was shown by Selander et al. (1971), in a study of allozymic variation in Peromyscus polionotus. Populations across the southern states of the U.S.A. were all very similar, while the populations of some small beach islands off the Florida panhandle were found to be very different. Levels of heterozygosity were greatly reduced, and allele frequencies often differed widely, even between neighbouring islands. A similar effect has been found by F.M. Johnson in a study of variation in D.ananassae (Johnson, 1971), on islands across the southern Pacific Ocean. Considerable



heterogeneity was observed between populations, but it was always most marked when a sea barrier intervened. For example, two samples separated by 800 miles of land surface did not differ greatly at one esterase locus, while two others, separated by a smaller distance of water, were very different.

It could be argued that populations of A.sylvaticus are, isolated from each other and that this is how the observed differentiation arises. However, there is some information which indicated that there is some degree of genetic continuity between the Wicken and Ely sites. These populations are both segregating for the same low frequency allele at the Pgd locus, which is only found at one other site (Section 3.5). In addition, it was discovered when analysing the genetics of a black coat colour variant found in the Ely population (see Appendix), that the same gene is carried by descendants of some Wicken animals. The occurrence of two such rare alleles in samples taken less than 10 miles apart is regarded as evidence that they are genetically continuous. However, they show a difference in allele frequency at the Ldr locus that is significant at the 5% level. The presence of such sharp discontinuities make these results somewhat unusual. Rasmussen (1970) has reported the discovery of significant genetic differences between populations of Peromyscus maniculatus inhabiting the same continuous forest only two miles apart. There was also great similarity between isolated populations. He interprets these results as indicating social subdivision of the populations but this argument would not apply to Apodemus for the above reason.

From an analysis of a large number of mice from several sites, Berry (1973) concluded that the British fieldmouse population could be divided into two "races". Data were collected on the frequency of twenty non-metric skeletal variants, the genetic basis of which is fairly well understood (Berry, 1968a). Coefficients of similarity were calculated between populations which were found to fall into an eastern and western group. Within "race" values were of the order of 10-20, while between "race" figures were over 30 and typically around 40. The boundary of the two distributions passed down the eastern side of the country. Three of the sites trapped in the present study would fall in the western category: Talsarnau, Charnwood and Wytham. The eastern sites are less easily allocated. Wicken, Ely and Madingley are all close and, with Egham, would fall into this group, Rogate cannot be allocated, and may be overlooked.

Comparison of the allozyme frequencies for populations within and between the eastern and western groups gives no clear pattern at either locus. The single significant difference at the Ldr locus is between two neighbouring populations which must both fall into the eastern group. At PGM<sub>2</sub>, there are as many pairwise comparisons within "races" that give significant  $\chi^2$  values as there are between them. The Talsarnau site differs significantly from Madingley and Wicken but also from Charnwood and Wytham. It is not significantly different to the Egham site, from which one of Berry's (1973) original eastern samples came. Thus the available data on allozyme



frequencies do not give support to his subdivision of the species.

Ayala (1975) has reviewed the large volume of work which has been done on the allozymic similarity between taxa, both by his own group on D.willistoni and by others. His general conclusion is that little change detectable by electrophoresis occurs prior to the first appearance of reproductive isolation. Even at a low level, as between the subspecies of D.willistoni, this permits noticeable genetic divergence. Results obtained by Selander, Hunt and Yang (1969) on two subspecies of Mus musculus show a similar pattern. There is no external differentiation between the "races" of Apodemus, as found in the latter case, and no reproductive isolation as animals from both have been used in crosses (Sections 3.2 and 3.3). That they should not show allozymic divergence would be expected from Ayala's (1975) conclusions.

Some cases have been reported in the literature in which data on allozyme frequencies does not reflect divisions below the species level. Selander, Yang and Hunt (1969) were unable to find any evidence in their studies of house mice in the U.S.A. that supported old subspecies. Similarly, in snails of the genus Partula, area effects in shell characters are not reflected by electrophoretic data (Murray, personal communication), although this is not the case in Cepaea (Johnson, 1976). More dramatically, electrophoretic comparison of man and the chimpanzee has revealed a high degree of genetic identity between these species (King and Wilson, 1975), whose phylogenetic lines diverged at least



12 million years ago (Simons, 1972).

It has been suggested that the genetic changes detectable by electrophoresis and by morphological studies are of different types. While the first are the result of amino-acid substitutions at structural loci, the second may be due more to changes in gene regulation (Wilson, Sarich and Maxson, 1974). These authors emphasise that morphological divergence is accentuated between mammals that may show only moderate protein differentiation, as appears to be the case in the "races" of Apodemus. However, there are alternative explanations for these phenomena. Ayala (1975) points out that morphological features may be controlled by only a small part of the genome, or that there may be different classes of structural genes.

It is well known that standard methods of electrophoresis detect only those amino-acid substitutions that result in a net charge change and that this is a considerable underestimate of the total (King, 1974). Recently developed techniques extending the proportion of detectable substitutions (Bernstein, Throckmorton and Hubby, 1973; Singh, Hubby and Throckmorton, 1975) have indicated that the old methods may present a rather misleading picture at times. Singh, Lewontin and Felton (1976) have studied the Bogota population of Drosophila pseudoobscura using similar new techniques and have found that whereas it had been regarded as much less variable than the northern populations, it in fact possesses considerable variability and has several unique alleles. The old interpretation (Lewontin, 1974) that this population has arisen from a

recent isolate has had to be completely reviewed. Much more work will have to be done along these lines before it can be said whether standard electrophoretic alleles are fully representative of all types of variation at the molecular level.

## CHAPTER 4

GENETIC CHANGES IN POPULATIONS OF APODEMUS SYLVATICUS

## 4.1 INTRODUCTION

Of the eight sites discussed in the last chapter (see Figure 6) three, Madingley, Wytham and Charnwood, were used for studies of temporal changes in gene frequency. Information is available on allele frequency at the two polymorphic loci, Ldr and PGM<sub>2</sub>, and on rare variants (see Section 3.5), from samples taken at six-monthly intervals. In addition, one site, Charnwood, was trapped at two-monthly intervals for two years from February 1974 to January 1976 (see Leigh Brown, in press).

Only one similar study has previously been made on A.sylvaticus. Flowerdew (1971) reports a personal communication from P. Handford, who found changes in frequencies of certain esterases between winter 1968 and winter 1969. In view of the uncertainty surrounding the genetics of esterases in Apodemus (Section 3.2), the significance of this report is hard to establish. There are, however several studies on other rodents (Semeonoff and Robertson, 1968; Tamarin and Krebs, 1969; Berry and Murphy, 1970; Gaines and Krebs, 1971; Myers and Krebs, 1971; Krebs et al. 1973; Myers, 1974; Berry and Jakobson, 1975).

In Wytham Great Wood, oak (Quercus robur) and sycamore (Acer pseudo-platanus) are the dominant tree species, with a field layer mainly consisting of Dog's mercury (Mercurialis perennis) and some bracken (Pteridium aquilinum) and bramble (Rubus fruticosus) (Elton, 1966; Southern and Lowe, 1968). Madingley Wood falls in to the 'boulder clay' wood-



land category of Rackham (1968). This is similar to that found at Wytham with the addition of (Fraxinus excelsior) and a hazel (Corylus avellana) understorey. The field layer is also similar (Flowerdew, 1976). The site at Charnwood has already been described (Section 2.1).

#### 4.2 GENETICAL STUDIES AT MADINGLEY AND WYTHAM

In a relatively large and longlived organism such as the fieldmouse, temporal studies of natural populations depend on the assumption that the method of collecting data does not interfere with natural processes, for example by increasing mortality or reducing trappability. In this study, the collection of a blood sample might seem likely to have such an effect. While it was not possible to study the effect of bleeding on mortality, its effect on trappability might be expected to be at least as great and could readily be determined. This was done at Wytham where two grids,  $\frac{1}{4}$  mile apart, were trapped for two consecutive nights in each period. The animals caught on one were bled on the first day and the proportion recaptured is shown in Table 7, along with that found on the other, control, grid. Clearly, bleeding has very little effect on recapture rate.

Data on genotype frequencies, gene frequencies and population sizes at the Wytham and Madingley sites are presented in Tables 8 and 9. The full data for Charnwood will be given in the next section and discussed at more length. There was no significant divergence from Hardy-Weinberg equilibrium at the  $PGM_2$  locus in any of the samples. The Ldr frequencies are estimated, as before, assuming Hardy-

TABLE 7

Effect of bleeding on recapture rate in A. sylvaticus  
 (Data from Wytham Woods December 1974 collection)

	Grid A	Grid B
14.12.74	16 marked, bled and released	18 marked and released
15.12.74	13 recaptures	16 recaptures
Recapture rate	81%	89%

Difference is not significant

TABLE 8

Genotype and Allele frequencies at three sites over 12 months at PGM<sub>2</sub>

<u>Wytham</u>	<u>aa</u>	<u>ac</u>	<u>cc</u>	<u>p<sup>a</sup></u>	<u>N</u>	<u>Pop size</u>
December 1974	0.27 <sup>1</sup>	0.53	0.15	0.53	34	35
May 1975	0.32	0.34	0.34	0.48	29	31
December 1975	0.32 <sup>2</sup>	0.32	0.29	0.52	28	33
<u>Madingley</u>						
January 1975	0.36	0.52	0.12	0.62	33	62
June 1975	0.40	0.40	0.20	0.60	5	7
January 1976	0.36	0.52	0.11	0.63	36	69
<u>Charnwood</u>						
December 1974	0.32	0.59	0.09	0.62	46	48
June 1975	0.40	0.60	0.0	0.70	5	5
January 1976	0.30	0.61	0.09	0.61	23	23

<sup>1</sup> +bb frequency 0.06

<sup>2</sup> +ab frequency 0.07



TABLE 9

Genotype and Allele frequencies at three sites for Ldr  
over 12 months

<u>Wytham</u>	<u>B<sup>+</sup></u>	<u>B<sup>-</sup></u>	<u>p<sup>b<sup>-</sup></sup></u>
December 1974	0.85	0.15	0.38
May 1975	0.86	0.14	0.37
December 1975	0.86	0.14	0.37
<u>Madingley</u>			
January 1975	0.78	0.22	0.47
May 1975	0.80	0.20	0.45
January 1976	0.76	0.24	0.49
<u>Charnwood</u>			
December 1974	0.89	0.11	0.33
June 1975	0.40	0.60	0.77
January 1976	0.86	0.14	0.37

Weinberg equilibrium (Section 3.6). Large fluctuations in numbers are observed at Charnwood and Madingley, although the Wytham numbers were unusually stable (H.N. Southern, personal communication) in 1975. The Charnwood and Madingley sites are similar in that they border arable land; it will be seen that this may well be important (Section 4.3).

The most obvious feature of the data is the constancy with which the initial frequency at any site is maintained. The greatest change observed between samples of reasonable size occurred in the Wytham  $PGM_2$  frequencies between December 1974 and May 1975, but this is not significant. The lack of change at Ldr is even more striking. It should be noted that even in lowland Britain, Apodemus sylvaticus does not average much more than two generations a year (Jewell, 1966) and little genetic change in a population would be envisaged in such a short time.

#### 4.3 GENETICAL AND ECOLOGICAL STUDIES AT CHARNWOOD

The fluctuations in fieldmouse numbers on the Charnwood grid over the study period are shown in Figure 8. The periods during which animals were found in breeding conditions are indicated and it can be seen that there is close correspondence between the onset of reproductive activity and the summer drop in numbers in both years.

Numbers of animals trapped are given in Table 10, with the minimum survival rate for each period (Flowerdew, 1972). This is taken as the proportion of the total caught in any trapping period that were subsequently recaptured.

Figure 8. Charnwood population numbers 1974/6.

The figures shown are the actual numbers trapped in each period.

Animals were in reproductive condition during the periods indicated.



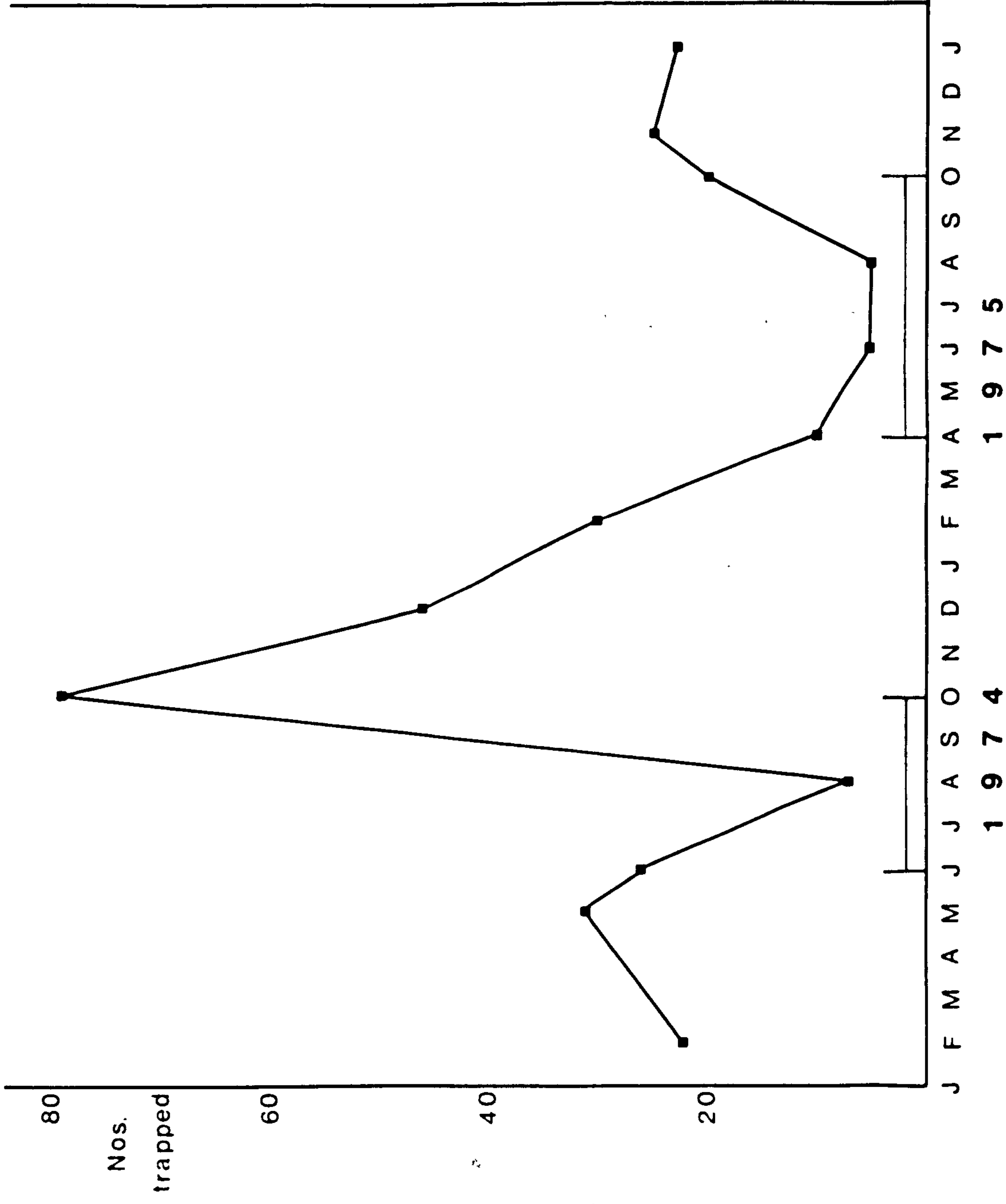


TABLE 10

Minimum survival in relation to overall numbers -  
Charnwood 1974/75

	Overall numbers	Minimum survival rate (Fraction later recaptured)
February	19	0.58
May	31	0.42
June	26	0.15
1974 August	7	0.29
October	79	0.19
December	46	0.35
February	29	0.26
April	10	0.20
June	5	0.60
1975 August	5	0.60
October	26	0.38
November	25	0.52

Comparison of Table 10 with Figure 8 reveals that the large peak in numbers in October 1974 did not coincide with an increase in survival rate. In a population of nearly eighty, only two were recaptures. The increase was much larger than the reproductive potential of the earlier residents and must therefore have been due to large-scale immigration. This large population subsequently showed a considerably poorer survival rate than was found in the winter of 1975/6.

Each animal caught on the Charnwood grid was typed for  $PGM_2$  and Ldr phenotype. The data obtained are given in Tables 11 and 12. Figures 9 and 10 show the changes in frequency with time. For clarity, only three confidence intervals are shown in each diagram. These are representative of three different sizes of sample and it can be seen that only in the winter are numbers high enough to give reliable estimates of gene frequency. During these months the frequencies at the Ldr locus did not show any consistent pattern of change. In the last two winter periods, the frequencies at this locus were very similar. In Figure 10 a rather different picture is given for  $PGM_2$ . Both these winters showed clear patterns of change in the frequency of the a allele. From the confidence intervals these appear to be real, with a steady increase occurring in 1974/5 and a decrease in 1975/6. From the genotypic frequencies given in Table 11 it can be seen that the first change is due to a steady loss of cc animals from the population, as shown in Figure 11, while the second appears to be due to a decrease in aa numbers. These trends were analysed by the method of Yates (1948, in Snedecor and Cochran, 1967) for



TABLE 11

Numbers of each phenotype and estimated gene frequency at the Ldr locus:

Trapping period	Charnwood 1974/76 Phenotype numbers		Gene frequency $b^-$ allele	No. scored
	$b^-$	$b^+$		
February	2	7	0.47	9
May	5	13	0.53	18
June	8	10	0.67	18
August	1	5	0.41	6
October	15	64	0.44	79
December	5	41	0.33	46
February	6	24	0.45	30
April	4	5	0.67	9
June	3	2	0.77	5
August	2	3	0.63	5
October	3	15	0.41	18
November	3	20	0.36	23
January	3	19	0.37	22

TABLE 12

Gene frequencies and numbers of each genotype at the  
 PGM<sub>2</sub> locus, Charnwood 1974/76

Trapping period		Gene frequency <u>a</u> allele	Genotype numbers			Total scored
			<u>aa</u>	<u>ac</u>	<u>cc</u>	
	February	0.67	3	6	0	9
	May	0.68	6	11	0	17
	June	0.69	7	11	0	18
1974	August	0.75	3	3	0	6
	October	0.54	25	36	18	79
	December	0.62	15	27	4	46
	February	0.69	12	16	1	29
	April	0.67	3	6	0	9
1975	June	0.70	2	3	0	5
	August	0.60	1	4	0	5
	October	0.74	10	8	1	19
	November	0.67	9	14	1	24
1976	January	0.59	6	14	2	22

Figure 9. Ldr gene frequency at Charnwood 1974/6

Estimated assuming Hardy-Weinberg equilibrium.

The Central figures are the sample sizes for each period.

Figure 10. PGM<sub>2</sub> gene frequency at Charnwood 1974/6

95% confidence intervals are given for each of three representative sample sizes.

Confidence intervals taken from Sokal and Rohlf (1973).



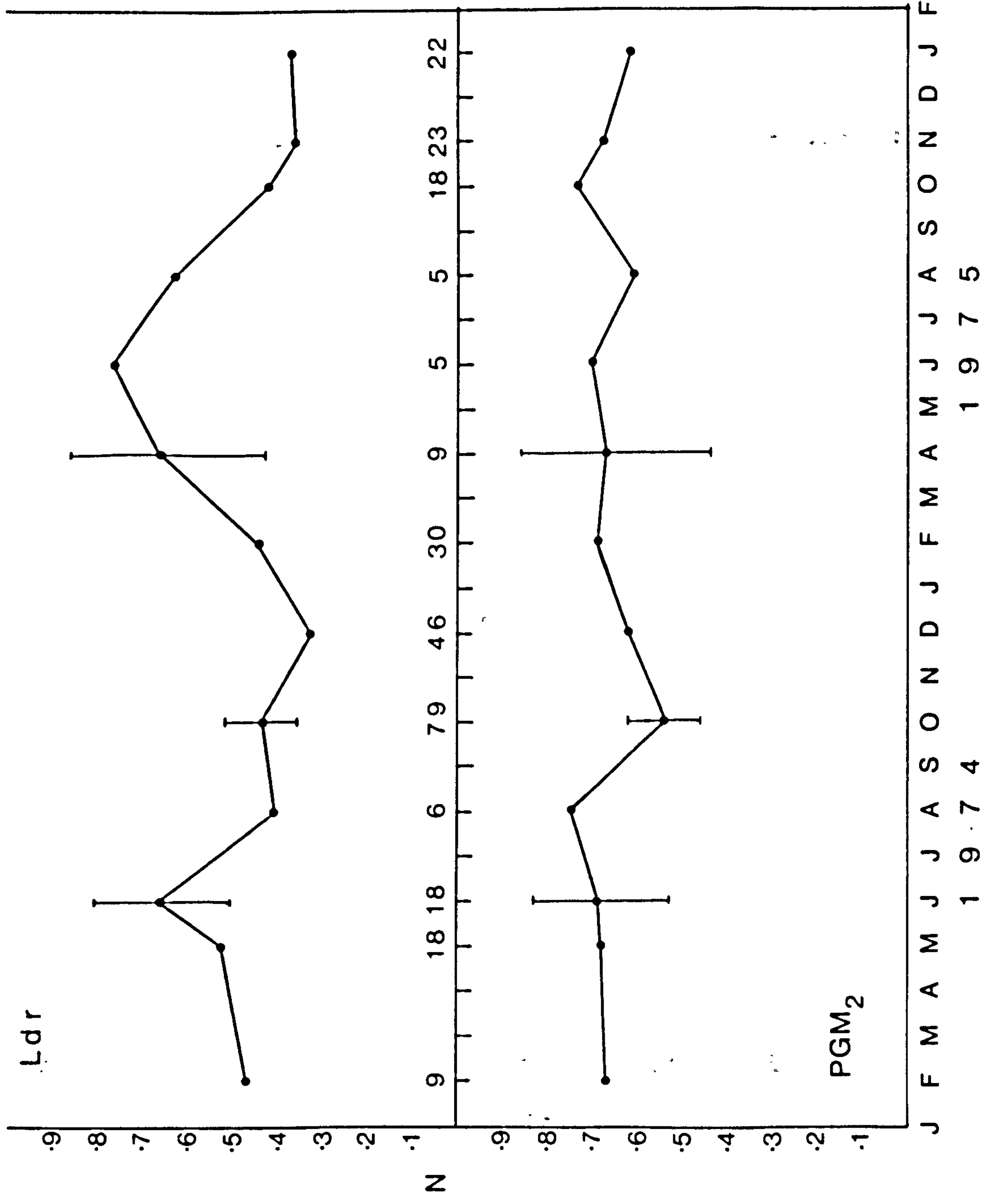
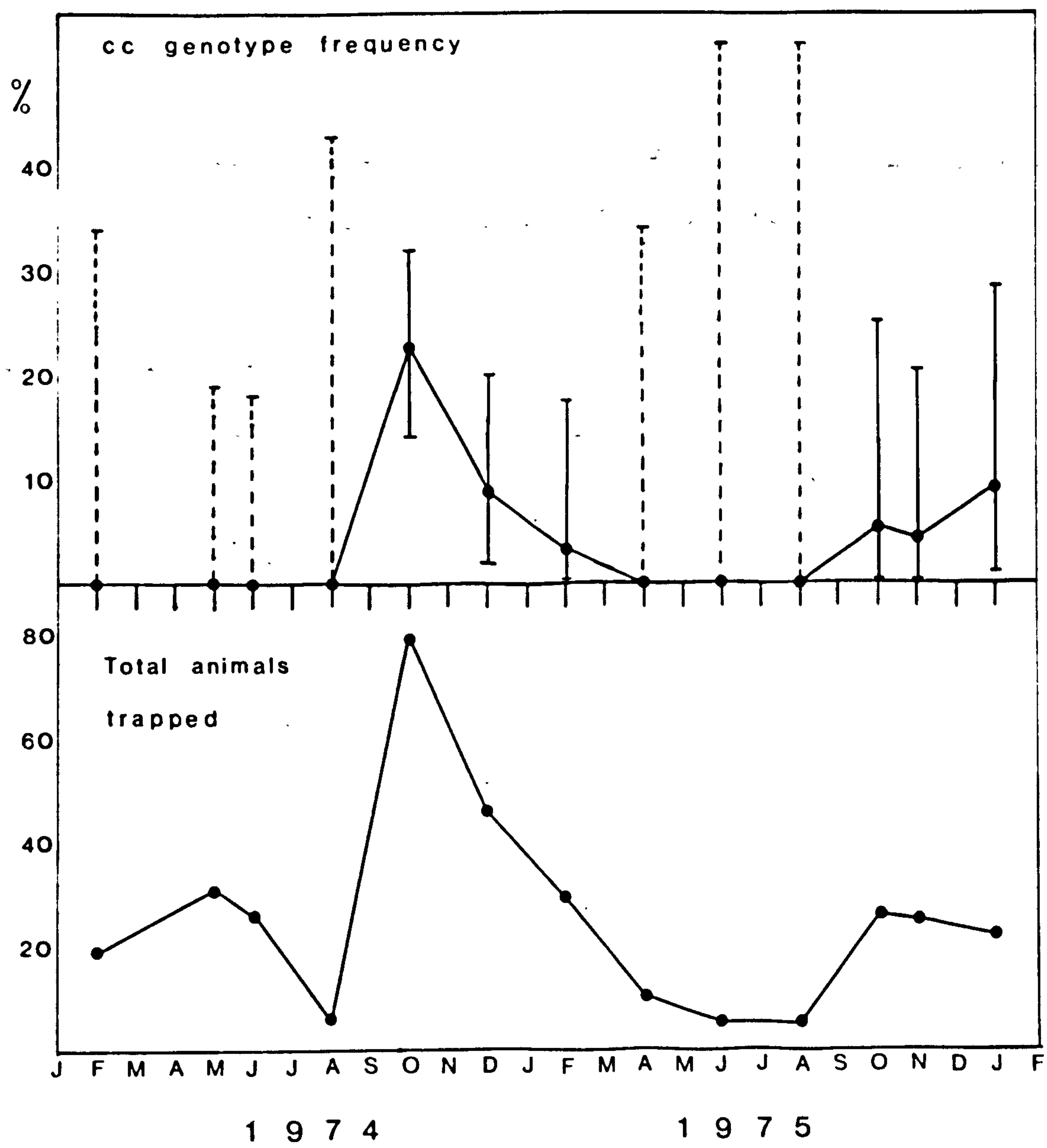


Figure 11. PGM<sub>2</sub> cc genotype frequency at Charnwood 1974/6  
in relation to population size

The sudden appearance of this genotype in October 1974  
is probably an artefact of sample size, as indicated  
by the 95% confidence intervals.

Confidence intervals taken from Sokal and Rohlf (1973).





testing a linear trend in proportions. For the change over winter 1974/5, the result was significant at the 2% level. For the following winter the result was not significant.

A closer analysis of the data for the period October 1974 to February 1975 reveals a slightly different pattern of differences between genotypes. An estimate of minimum survival was made from the recapture rate for each genotype in the October population (Table 13). Overall, aa homozygotes showed a much higher recapture rate than the other two genotypes ( $\chi^2_1 = 7.32, p < 0.01$ ). However there is also a sex difference in favour of males ( $\chi^2_1 = 5.49, P < 0.05$ ). The heterozygote class had a rather high proportion of females in October and a bias could result from their lower survival. The figures were therefore recalculated for males alone and the same trend was found although the  $\chi^2_1$  value of 2.75 was not significant ( $P < 0.1$ ). This was the only trapping period for which the sample size was large enough to permit such an approach.

An apparent contradiction between Table 12 and Table 13 should be clarified. In Table 12 the ac heterozygotes show similar proportions in the population to aa homozygotes. In Table 13 they show a survival rate similar to that of the cc animals. This came about because there was much heavier recruitment of ac animals through immigration in December than of other genotypes. This observation will be returned to in the next section.

TABLE 13

Rate of recapture of animals first caught in October 1974

## 1) By sex

	Male	Female
Recaptured	17	3
(Proportion)	(0.37)	(0.10)
Not recaptured	29	27
(Proportion)	(0.63)	(0.90)

$$\chi^2_1 = 5.49; P < 0.05$$

2) By PGM<sub>2</sub> genotype, both sexes

	<u>aa</u>	<u>ac</u>	<u>cc</u>
Recaptured	11	7	2
	(0.50)	(0.19)	(0.11)
Not recaptured	11	29	16
	(0.50)	(0.81)	(0.89)

cc group combined with ac

$$\chi^2_1 = 7.32 \quad P < 0.01$$

3) By PGM<sub>2</sub> genotype, males only

	<u>aa</u>	<u>ac</u>	<u>cc</u>
Recaptured	9	6	2
	(0.56)	(0.33)	(0.17)
Not recaptured	7	12	10
	(0.44)	(0.67)	(0.83)

cc group combined with ac

$$\chi^2_1 = 2.75 \quad P < 0.1 \text{ n.s}$$

#### 4.4 DISCUSSION - MADINGLEY AND WYTHAM

It has been pointed out (Myers, 1974) that in continuous studies of populations where it is likely that most of the animals on a site are being caught, statistical methods which require the use of random, independent sampling procedures are not strictly applicable. This is not regarded as a major problem in this study because of the time lapse between samples. The significant changes of winter 1974/5 occurred over a period of 4 months, during which replacement of individuals had been almost complete. Contingency tests are only used to compare classes within a sample and not to compare adjacent samples. The methods of analysis are therefore valid in the specific circumstances in which they are used. Further, the trapping procedure at Charnwood (Section 2.2) involved the removal of animals over a period of 4 nights. Such methods will result in animals whose home ranges approach, but do not normally include, the grid, extending their movements onto it as it becomes vacant (Stickel, 1946). Therefore the animals caught can be regarded as a sample of the entire area in which the grid is situated. This view is permissible as the distribution of fieldmice is not restricted by vegetation type, or cover (Evans, 1942; Southern and Lowe, 1968).

In the data collected at six-monthly intervals, no statistical problem would arise because such a period is long enough to render the samples effectively independent. Little change was observed in allele frequency between samples collected at this interval (Tables 8 and 9), but



the study period spanned very few generations. Further, although one of the reasons given for making a short term continuous study of Apodemus populations was that they normally show very low densities in summer (see Introduction), numbers of fieldmice at Wytham stayed unusually stable in the year studied (H.N. Southern, personal communication). At the other two sites, the fluctuations that were observed may be more apparent than real, for reasons which will be discussed shortly. There would therefore be no grounds to expect genetic change at these sites arising through the action of the founder effect.

Two temporal studies of feral house mouse populations have taken a genetical approach, one on the Skokholm island population, with samples taken in spring and autumn (Berry and Murphy, 1970) and the other on two neighbouring grass-land sites sampled monthly in California (Myers, 1974). In the Skokholm population, spring and autumn samples could be differentiated in terms of degree of heterozygous excess and allele frequency. If figures for six month intervals are extracted from Myers' data, virtually no change is observed between them on either of her grids. Within these six month intervals considerable changes occur, a situation which is paralleled by the data on  $PGM_2$  frequency at Charnwood (Tables 8 and 11). So, in the present study and that of Myers, six month intervals were not close enough to detect short term genetic changes in the populations. In the Skokholm study they were. The exact reason why this should be is not clear. It is known that mice living on islands are subject to considerable environmental stress

(Berry, 1968b) and that the breeding season is curtailed (Jewell, 1966). It may therefore be suggested that the environment of the Skokholm mice, though maritime, shows a stronger seasonal element than that of northern California or the Midlands.

Seasonal changes in the frequencies of chromosomal inversions have been known in some populations of Drosophila pseudoobscura for many years (Dobzhansky, 1971). These changes are of a size that can only be attributed to the action of natural selection, and in some cases can be correlated with changes in important environmental factors such as rainfall and temperature (Strickberger and Wills, 1966). There is some suggestion that similar changes may occur in allozyme frequencies as well (Dobzhansky and Ayala, 1973) although Berger (1971) found that out of five studied only one locus showed any temporal change in gene frequency. As natural populations of Drosophila go through several generations in one year, seasonal changes in selection pressures will affect all stages of the life cycle. Even though the alleles at a locus are not selectively neutral, differing responses at each stage may diminish the net effect observed in the adult population. However in the Skokholm mouse population in early spring, when much winter mortality occurs, the whole population will consist of non-reproductive adults (Berry, 1968b). This is an ecologically simplified situation and the chances of detecting changes at individual loci may therefore have been increased. This argument could also be applied to the changes detected at Charnwood (Section 4.3).



#### 4.5 CHARNWOOD POPULATION ECOLOGY

Much work has been done on the ecology of fieldmice in Britain (see Watts, 1969; and Corke, 1974 for reviews). The changes in population numbers at Charnwood parallel the results of several other studies. The most obvious feature in Figure 8 is the very large increase in numbers found in October 1974 which occurred through large-scale immigration (Section 4.3). This coincided with the harvesting of the cornfields which are adjacent to the grid (Section 2.1) and other workers have described influxes in similar circumstances (Miller, 1958; Bergstedt, 1965). In two studies, traps were laid in both field and wood habitats and marked animals which had been caught first in the cornfields were subsequently trapped in the woodland (Kikkawa, 1964; Corke, 1974).

The similarities between previous studies on fieldmice near woodland edges and this one are so great that it seems reasonable to draw a parallel with the pattern of movements they discovered before harvesting, as well. At this time the overall tendency was for animals to move out of the woods, where numbers dropped to a very low level. If the same pattern of movements occurred at Charnwood, as seems likely, it follows that the observed numbers on the grid in summer are only a part of the population to which they belong. With the autumn influx moving animals back onto the grid, it becomes a sampling site for a much larger area. The dramatic fluctuations in numbers at the Madingley site may have a similar explanation, as it adjoins arable land. Such an interpretation of demographic events has important



genetical implications. If the observed changes in numbers on the grid are not reflected in the whole population it follows that there will be no grounds for genetic change through the action of the founder effect, which might arise from low summer populations. A lack of any genetic bottleneck is supported at both sites by the observed constancy of gene frequencies between winters (Tables 8 and 9).

Genetic changes were observed in the Charnwood population between October 1974 and February 1975. In Table 10 it is shown that the period over which this change took place was one of rather low survival rate by comparison with the following winter. It has been clearly demonstrated from many years work at Wytham Woods that over-winter survival rate in Apodemus is strongly correlated with the autumn acorn crop, which forms the major food supply at this time of year (Watts, 1969; Flowerdew, 1972, 1974). To apply these conclusions to the Charnwood data it would appear that in 1975/6 the food supply was adequate for the numbers present but in 1974/5 it was not. Although no measurements were made on the acorn crop (cf. Flowerdew, 1976) at Charnwood, personal observation suggests that it was similar in both years. The difference in survival rate must therefore have arisen from the difference in the numbers on the grid in each autumn. One major difficulty with the apparent control of winter numbers by food supply has been raised by Watts (1969) who points out that in years of poor food supply and low survival, the weight of surviving individuals increases as rapidly as in good years. Similar observations were made at Charnwood over the two winters.

A possible explanation for this is that the poor survival rate is the result of competition, whereby the least successful individuals are eliminated by starvation, but the survivors are scarcely affected (Watts, 1969).

#### 4.6 CHARNWOOD POPULATION GENETICS

The small sample sizes found in summer at Charnwood were associated with large variations in the estimates of gene frequency at the Ldr locus (Figure 9). These are not significant. The six estimates derived from samples taken during the winter periods lie in the region 0.35-0.45 and the smaller differences between them again are not significant. It is concluded that the gene frequency at this locus did not change in this population over the study period, but the low numbers would make such change difficult to detect. Similarly, Berger (1971) found no change at four out of five loci in two populations of Drosophila melanogaster over twenty two weeks. In view of his results, the constancy observed at the Madingley and Wytham sites (Section 4.2) and the similarity in gene frequency between widely separated populations (Section 3.7), there would be little cause to expect significant changes during the study period at Charnwood.

In this study only during the winter periods of high density could any fluctuations in gene frequency be shown to be real. In contrast to the Ldr locus, PGM<sub>2</sub> gene frequencies did change in winter and statistical analysis showed that for one period these were significant. This period commenced with the large influx of animals onto the



grid in October 1974. Discussion is simplified by the complete cessation of reproductive activity by this time so that the genetic change can be ascribed mainly to the differential rate of recapture of animals of different genotypes, and not to any differences in fecundity. It cannot however be immediately assumed that differences in recapture rate are caused by differences in mortality, as dispersal would also cause a low recapture rate.

Considerable attention has been paid to the causes of the cyclical changes in density observed in several vole species (Chitty, 1967; Tamarin and Krebs, 1969; Gaines and Krebs, 1971; Krebs, Keller and Tamarin, 1969). Krebs et al. (1973) recently summarised the studies which have been made on genetical changes which occur in parallel with the demographic changes. One major ecological conclusion was that dispersal is a very important process as fenced populations reached abnormally high densities and caused considerable habitat destruction (Krebs, Keller and Tamarin, 1969). More detailed study of dispersal led to the discovery of anomalous frequencies of certain transferrin genotypes among dispersing animals (Myers and Krebs, 1971). The conclusion of Krebs et al. (1973) was that changes in the pattern of movements were giving rise to the changes in population numbers and to the genetic changes because of this link. Most recently, an attempt to influence demographic events by controlling the genetical constitution of a population has not succeeded (Leduc and Krebs, 1975). However, it cannot be assumed that dispersal will act randomly with respect to genotype and so it is necessary to



indicate whether, at Charnwood it is more likely that the genetic changes are being caused by differential mortality than differential dispersal.

Dispersal in small mammal populations is most readily detected as immigration into a depopulated area (Andrzejewski and Wroclawek, 1962; Myers and Krebs, 1971), although Stickel (1946) showed that many such 'immigrants' will simply be animals extending adjacent home ranges. Watts (1966, 1970) trapped a wide area of Wytham woods to study the extent of movement in undisturbed populations. He found very little long-range movement in field mice but rather more in bank voles. Only in studies such as these can the extent of dispersal be estimated. Field data on recapture rates alone cannot distinguish dispersal from mortality. No estimates of the contributions made by these two processes to differences in recapture rate can therefore be made on the data presented here. However, several authors have suggested that the animals which will be most likely to disperse, particularly from a favourable and densely populated area, will be those lowest in the social hierarchy (Watson and Jenkins, 1968; Watts, 1969; Christian, 1970). These animals are forced to disperse because of their inability to compete successfully with their neighbours. They will probably only survive if a suitable unoccupied habitat is available. If this is not the case, and pilot trapping at Charnwood indicated that the study site is in the most favourable area for small mammals, they will die. Under these circumstances mortality becomes a consequence of dispersal. These arguments are speculative,

however, and the strongest evidence that the change in PGM<sub>2</sub> gene frequency occurred through mortality is presented in the next chapter, when the existence of physiological differences between PGM<sub>2</sub> genotypes that can be directly related to fitness, will be demonstrated.

If the low recapture rate shown by the cc homozygotes is indeed due to differential mortality then this study has demonstrated the action of natural selection at PGM<sub>2</sub>, or some locus closely linked to it. Food supply is the most likely agent of selection, mediated through individual competition induced by an unusually high population density. There have been several cases reported of changes in gene frequency being associated with changes in population density. The Skokholm mouse population shows a roughly 10-fold drop in numbers over winter, the exact size of which depends mainly on the late winter temperature (Berry, 1968b). Over this period, genotype frequencies at the Hbb locus have been observed to change from a considerable heterozygote excess almost to the frequency expected on the Hardy-Weinberg equilibrium (Berry and Murphy, 1970; Berry and Jakobson, 1975a). Again, no reproduction occurs during this period and the change occurs solely through mortality, no emigration being possible from the island.

The interest in vole population processes has uncovered several instances of genetic changes, at various loci, occurring in association with changes in density (Semeonoff and Robertson, 1968; Tamarin and Krebs, 1969; Gaines and Krebs, 1971). These have been interpreted by Krebs et al.



(1973) as evidence for the existence of a genetic basis to the cycles as proposed by Chitty (1967). However it cannot yet be claimed that genetic changes are causing the cycles. Indeed, as Gaines and Krebs (1971) point out, certain aspects of their data would favour the alternative; that the changes in reproduction and mortality in the population are producing genetic side effects (e.g. Charlesworth and Giesel, 1972). It is hardly possible to manipulate populations of rodents to demonstrate density-dependence, but this has been done for chromosome inversions in Drosophila pseudobscura by Birch (1955). He showed that the CH arrangement had a higher fitness in relation to ST when it was reared under conditions of low larval density, than under the normal conditions of larval crowding. This result fits well with field observations (Dobzhansky, 1971) that the frequency of the CH arrangement increases during spring, when numbers are low and decreases during the summer, when numbers of flies are higher.

The phenomena which have been discussed in this section are therefore not without precedent. In the data collected at Charnwood, changes in gene frequency can only be detected when density is high. Similar changes could occur at times of low density but they would be difficult to demonstrate. The events described would rapidly lead to fixation for the a allele, but the polymorphism is widely distributed in this country (Section 3.6). Further, in the data from Madingley and Wytham the frequencies at this locus were shown to be stable between winters. Some balancing effect must therefore operate. Heterosis does



not seem an adequate mechanism in this case as the heterozygotes show a recapture rate intermediate between those of the homozygotes (Table 13). Density dependence could be involved but its action is not likely to be as straightforward as in the case studied by Birch (1955). If the c allele is less fit under conditions of high density, such as characterise Apodemus populations over winter (Miller, 1958; Kikkawa, 1964; Crawley, 1970) then the alternative conditions of low density which might favour it are found during the summer. As this is the reproductive period any advantage held by the cc genotype may be involved with differences in fecundity, as in the case discussed by Charlesworth and Giesel (1972). The annual repetition of high density conditions suggests the possibility of cyclical changes in fitness. The maintenance of polymorphisms by such a mechanism has been the subject of investigations by Haldane and Jayakar (1963) and Hoekstra (1975). These workers have shown that the conditions required for this are rather specific and that usually such situations will be unstable. Although Gillespie (1973) showed that a polymorphism could occur in a varying environment he was concerned with random fluctuations and he concluded that this would only happen if the geometric mean fitness of the heterozygote was greater than that of either homozygote. This appears to be a form of heterosis, and for the reasons given above, it is unlikely to apply to these results.

The final possibility is a mechanism for maintaining the  $PGM_2$  polymorphism is that the alleles show differences

in fitness in response to different sets of environmental conditions that are spatially distributed. Levene (1953) and Maynard Smith (1966) were the first to demonstrate that a polymorphism could be maintained in such a way through differential fitness in different niches. Recently, the concept of environmental heterogeneity has received much attention, particularly with regard to various attempts which have been made to explain differences in levels of variability between large animals and smaller ones (Selander and Kaufmann, 1973). Experimental studies have shown that varying the environment of a Drosophila population both temporally and spatially, leads to an increase in the level of heterozygosity, (Powell, 1971; McDonald and Ayala, 1974). Gillespie and Langley (1974) have given a theoretical basis to the expectation that levels of variation will be higher in variable environments. While this principle has been well established, no single enzyme polymorphism has been shown to be maintained through temporal or spatial variation in the environment. There are few species whose ecology is so simple to render such an approach possible. A. sylvaticus is certainly not one and it must be concluded that, on the evidence presented in this Chapter, the polymorphism at the PGM<sub>2</sub> locus is probably maintained by selection, but the way in which this is done is not known.



## CHAPTER 5

## PHYSIOLOGICAL STUDIES ON THE PHOSPHOGLUCOMUTASE

POLYMORPHISM IN APODEMUS SYLVATICUS

## 5.1 INTRODUCTION

The field data presented in Chapter 4 strongly suggest the action of natural selection. These were only observations however, and experimental evidence was required to support this interpretation. One technique often used in genetical studies on Drosophila is that of the population cage, such as Birch (1955) used in his study of the CH and ST inversions previously discussed. There are obvious practical limitations to the use of this approach with small mammals but experimental manipulation of rodent populations by removal, feeding or fencing has proved illuminating on several occasions (Andrzejewski and Wroclawek, 1962; Myers and Krebs, 1971; Flowerdew, 1972, 1974, 1976; Tamarin and Krebs, 1969). The genetic manipulation of a small mammal population has been attempted only once (Leduc and Krebs, 1975) and the experiment was not successful. There has been considerable speculation on the way in which social and behavioural differences which have been observed between individuals in the laboratory (Defries and McClearn, 1973) are reflected in the behaviour of a small mammal population as a whole (Brown, 1968; Anderson, 1970; Christian, 1970; Selander, 1970; Berry and Jakobson, 1974). Our present knowledge of movements and social interactions in free-living mouse populations has been obtained almost entirely through live-trapping techniques. Randolph (1973) has shown that



the trap-revealed home range may be an underestimate. As home range is closely related to the social position of the individual our view of social processes in rodent populations may therefore be oversimplified. It would be difficult to devise a meaningful experiment involving the manipulation of small mammal populations without a better understanding of these social processes than we have now.

There is some reason to believe that mammals and other vertebrates differ qualitatively from many invertebrates in terms of their population genetics. The higher levels of heterozygosity found in invertebrates have been interpreted as arising from a requirement for flexibility which is met through high levels of intrapopulation variability (Selander and Kaufman, 1973). These authors suggest that, in mammals, each individual possesses a higher degree of adaptability arising from the homeostatic control of their internal environments, and the requirement for intra-population variability is lessened. The importance of the interaction between genetic and physiological factors has been demonstrated by the correlations observed by Bellamy et al. (1973) between physiological variables and genotype at the Hbb and Esterase-2 loci in Skokholm house mice. An animal with the level of homeostatic control found in mammals can respond to a given environmental situation in a variety of ways. The probability of a mouse surviving a winter on Skokholm is a product of several factors including age, social status, biochemical genotype, and thermoregulatory physiology (Berry, Jakobson and Triggs, 1973). Thus, an animal that does not possess

the optimum biochemical genotype may still be able to survive by virtue of its fitness in the other features. Berry and Jakobson (1975a, 1975b) found that, although natural selection causes pronounced short term changes in mouse populations, the effects of the same selective agent varies between populations and between years. While real selective differences appear to exist at loci such as Hbb (Berry and Murphy, 1970), in Mus and PGM<sub>2</sub> in Apodemus (section 4.6) it may well be only rarely that they are expressed unambiguously and override the various other factors which affect survival. Too little is known of these factors in Apodemus for experiments at the population level to be a useful approach in the investigation of possible selective differences at the PGM<sub>2</sub> locus.

If differences can be demonstrated at the molecular level between the products of different alleles at an enzyme locus in terms of activity, stability or Michaelis constant it has often been suggested that they would not be selectively neutral in a natural population (Clarke, 1975; Harris, 1976). Gibson (1970) has carried out experiments of this nature on alcohol dehydrogenase in Drosophila melanogaster. He showed that in populations reared on alcohol supplemented medium the F allele has a higher frequency than in control populations, and that this allele shows a higher enzyme activity in homozygotes. These assays were conducted on crude extract but work on purified enzyme has shown that the ADH F enzyme is more active but less stable than that formed by the S allele (Vigue and Johnson, 1973; Day, Hillier and Clarke, 1974a). However, these



authors have stated that the difference in activity is not due to a difference in  $K_m$  or enzyme quantity but to differences in "catalytic efficiency" (Day, Hillier and Clarke, 1974b). Despite the obvious relevance of such differences to an organism that subsists on rotting fruit, their full significance is not known, especially in view of the recent discovery of modifiers of ADH activity which map on other chromosomes (Ward, 1975). Similarly, temperature-dependent differences in the activity of esterase alleles in catostomid fish have been found where the 'cold-adapted' enzyme shows a higher frequency in northern latitudes and the heterozygote is the most active genotype at intermediate temperatures (Koehn, 1969). In this case the interpretation of these differences is complicated by the fact that the in vivo substrate of the enzyme is unknown. Merrit (1972) discovered similar differences in lactate dehydrogenase alleles in another fish species and Harris (1976) has reported that at 18 out of 30 enzymes that are polymorphic in man there are differences in activity detectable in vitro.

Hochachka and Somero (1973) describe the properties of pyruvate kinase in arctic and temperate species of fish and show how enzymes do adapt to fit the metabolic requirements of the organism. They have shown differences in the response of  $K_m$  (phosphoenolpyruvate) to temperature between the two species, of a type that would allow the pathway to function in a similar manner although the environmental temperature was very different. Such a link between the in vitro biochemical properties of an enzyme and its metabolic significance is very important and has not yet



been fully established for any polymorphism. Only in the case of sickle-cell haemoglobin in man (Allison, 1955) has this depth of knowledge been attained. Miller et al. (1975) have reported differences between genotypes in some properties of  $\alpha$  glycerophosphate dehydrogenase but not others. This example pinpoints the difficulties encountered in the interpretation of purely kinetic data as it is impossible to determine which of these properties will be most important in vivo and on that will hinge the neutrality or otherwise of the alleles.

Although inspection of gel staining patterns suggested some differences in activity between the PGM<sub>2</sub> a and c enzymes, there were technical reasons why a study of molecular differences was not attempted. The most convenient way of separating the products of the two major PGM loci is by electrophoresis (McAlpine et al. 1970a). Only after this initial separation could studies on the various genotypes at PGM<sub>2</sub> be attempted. In view of the small quantities of enzyme that would be yielded by such methods it was considered worthwhile adopting a rather different approach, but one where a positive result would tie in closely with the role of PGM in vivo and involve the selective agent suggested by the field data (Section 4.6). Accordingly, the experiment described in this chapter is physiological rather than biochemical in orientation. Its design and interpretation is dependent on what is known of the properties and metabolic role of PGM, which is therefore summarised in the following two sections.

## 5.2 THE PHOSPHOGLUCOMUTASE REACTION

Phosphoglucomutase catalyses the conversion of glucose 1-phosphate to glucose 6-phosphate, a reaction which is reversible in vitro and has an equilibrium constant,  $K_{eq}$ , of 17. Thus the concentrations of an in vitro system at equilibrium will be approximately 5% glucose 1-phosphate and 95% glucose 6-phosphate (Ray and Peck, 1972). In vivo concentrations rather different to these have been observed in resting heart tissue (Williamson, 1966). The two monophosphates are not the only compounds involved in the reaction as glucose 1,6-diphosphate is required as a co-factor. The enzyme can exist in both phosphorylated and dephosphorylated form and the reaction is believed to proceed by the following simplified mechanism:

Glucose 1-phosphate	+ phosphoenzyme
1↓	
Glucose 1,6-diphosphate	+ dephosphoenzyme
1↓	
Glucose 6-phosphate	+ phosphoenzyme (Smith, Taylor

and Whelan, 1968). The cofactor requirement is usually very small. In bacteria the mechanism differs as the enzyme is not phosphorylated and is consequently more dependent on diphosphate concentration (Ray and Peck, 1972).

In addition to phosphate, PGM has a requirement for a divalent cation of which the two most usually found are  $Mg^{2+}$  and  $Zn^{2+}$ , (Peck and Ray, 1971). Of these the magnesium bound form is active, the zinc form essentially inactive. Assay techniques for PGM activity therefore involve the use of a chelating agent such as EDTA or imidazole, and excess  $Mg^{2+}$  (Spencer et al. 1964; Harshmann et al. 1966). PGM is



inhibited by sugar monophosphates other than glucose 1-phosphate, and by 1,3-diphosphoglycerate (Ray and Peck, 1972; Alpers, 1968). Other metabolites which have a similar effect are ATP and citrate. Glucose 1,6-diphosphate has been shown to release PGM from inhibition by these compounds (Beitner et al. 1975) but 2,3-diphosphoglycerate, inhibits PGM by competing with the glucose diphosphate (Quick et al. 1974).

The two major human phosphoglucomutase fractions, the products of the  $PGM_1$  and  $PGM_2$  loci have been shown to differ in several respects. The molecular weight of the  $PGM_2$  isozymes as determined by gel filtration is about 60,000 while that of the  $PGM_1$  enzyme is about 51,000. The  $PGM_2$  isozymes are more stable and the relative activities due to each locus differs between tissues.  $PGM_2$  contributes up to 10 to 15% of the total PGM activity in most tissues but in erythrocytes the relative contributions of the two loci are approximately equal (McAlpine et al. 1970a,b,c). In addition, kinetic differences have been found between the two sets of isozymes. The ability of the  $PGM_2$  enzyme alone to catalyse a ribomutase reaction in both man and Apodemus has been mentioned (Section 3.3). The extent to which this, rather than the glucomutase reaction is favoured depends on the concentration of glucose 1,6-diphosphate. At low levels of cofactor, the ribomutase reaction is quite important but at higher levels the glucomutase activity of the isozymes increases so much that their activity on ribose 1-phosphate becomes almost negligible (Quick et al. 1974). These authors have shown that this occurs through a lowering in the  $K_m$  of



these isozymes for glucose 1-phosphate as the levels of diphosphate increase, a property not shared by the PGM<sub>1</sub> isozymes.

### 5.3 THE METABOLIC ROLE OF PHOSPHOGLUCOMUTASE

The conversion of glucose 1-phosphate to glucose 6-phosphate is the reaction which links the two major pathways of glycogenolysis and glycolysis. In muscle, adrenalin induced activity stimulates glycogen breakdown and the glucose 1-phosphate produced passes directly into glycolysis via PGM and phosphoglucose isomerase. In liver, glycogenolysis occurs in response to various stimuli and is mainly involved with the correction of blood hypoglycaemia. Thus, the glucose 6-phosphate produced by PGM is largely converted to free glucose by glucose 6-phosphatase and released into the blood.

It has been assumed by some workers that PGM is as active in the synthesis as in the degradation of glycogen because the reaction can be reversed in vitro (Najjar, 1962; Williamson, 1966). However there is some evidence that this is not the case, notably from observations made by Beloff-Chain et al. (1964) that the rate of incorporation into glycogen decreases in the order glucose 1-phosphate > glucose > glucose 6-phosphate, and also from labelling studies by Figueroa et al. (1962). A pathway has therefore been suggested which bypasses the PGM step in an anabolic direction (Smith et al. 1968). In addition, enzymes are known which can break down glycogen by hydrolysis rather than phosphorolysis and the product of these reactions is

free glucose. The interactions of the pathways relevant to the PGM step are summarised in Figure 12 although the relative importance of some of the pathways is not well known.

The possible existence of bypass reactions is significant in view of the handful of cases of PGM deficiency in man which have been reported, (Thompson et al. 1963; Brown and Brown, 1968; Fiedler and Pettenkofer, 1968; Brinkmann et al. 1972). The extent of the clinical symptoms associated with this deficiency varies. Brinkmann et al. (1972) reported a partial, and Fiedler and Pettenkofer (1968) a complete, deficiency of activity of the PGM<sub>1</sub> isozymes and neither case showed any clinical symptoms. However Brown and Brown (1968) reported a case of hepatomegaly in a 17 month old boy whose liver contained 16% glycogen instead of the normal 5%. This was due to his possessing less than 15% of normal PGM activity in the liver and being consequently unable to metabolise glucose 1-phosphate. While in this case muscle glycogen levels were normal, in the case described by Thompson et al. (1963) muscle glycogen was as much as 5 x normal levels and there was consequent myopathy. There was a normal level of phosphorylase activity but an inability to metabolise glucose 1-phosphate again indicated PGM deficiency although there were additional defects in glycolysis. It appears that a complete deficiency of PGM<sub>1</sub> can be compensated for under some circumstances but the last two cases suggest that though there are bypass pathways (Figure 12) these cannot always be relied on.

Flux through the glycogenolytic pathway is normally

Figure 12. Pathways of Glycogen Metabolism in Liver

The main pathways are indicated with solid arrows.  
Subsidiary and postulated reactions carry hollow arrows.  
Simplified, after Smith, Taylor and Whelan (1968).

The numbers apply to steps catalysed by single enzymes.

- |   |   |                               |
|---|---|-------------------------------|
| 1 | : | Glycogen phosphorylase        |
| 2 | : | Phosphohexose isomerase       |
| 3 | : | Hexokinase                    |
| 4 | : | Glucose 6-phosphatase         |
| 5 | : | $\alpha$ -amylases            |
| 6 | : | $\alpha$ -glucosidases        |
| 7 | : | $\alpha$ -glucosidases        |
| 8 | : | Glucose 1-phosphate kinase    |
| 9 | : | Glucose 1-phosphate dismutase |



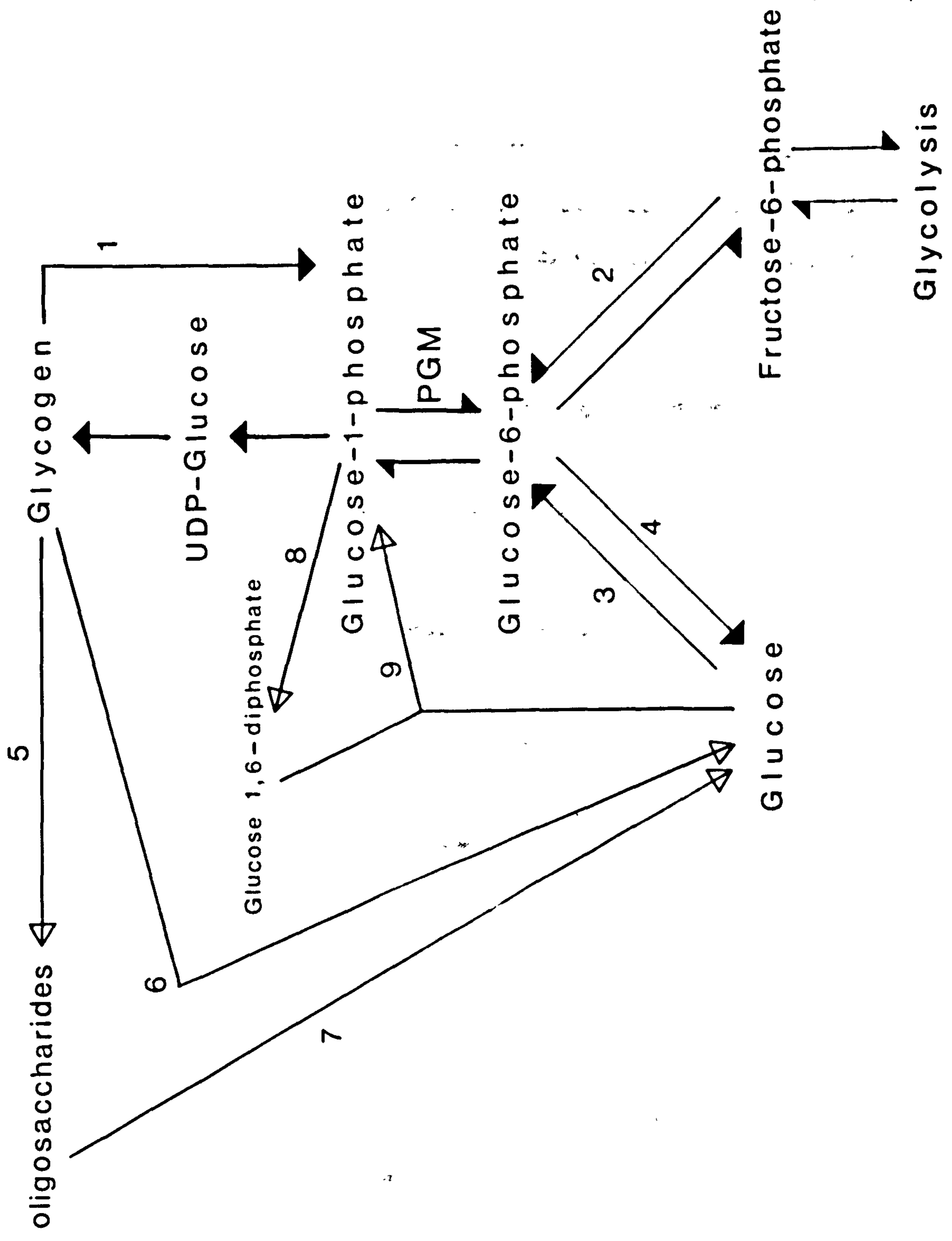
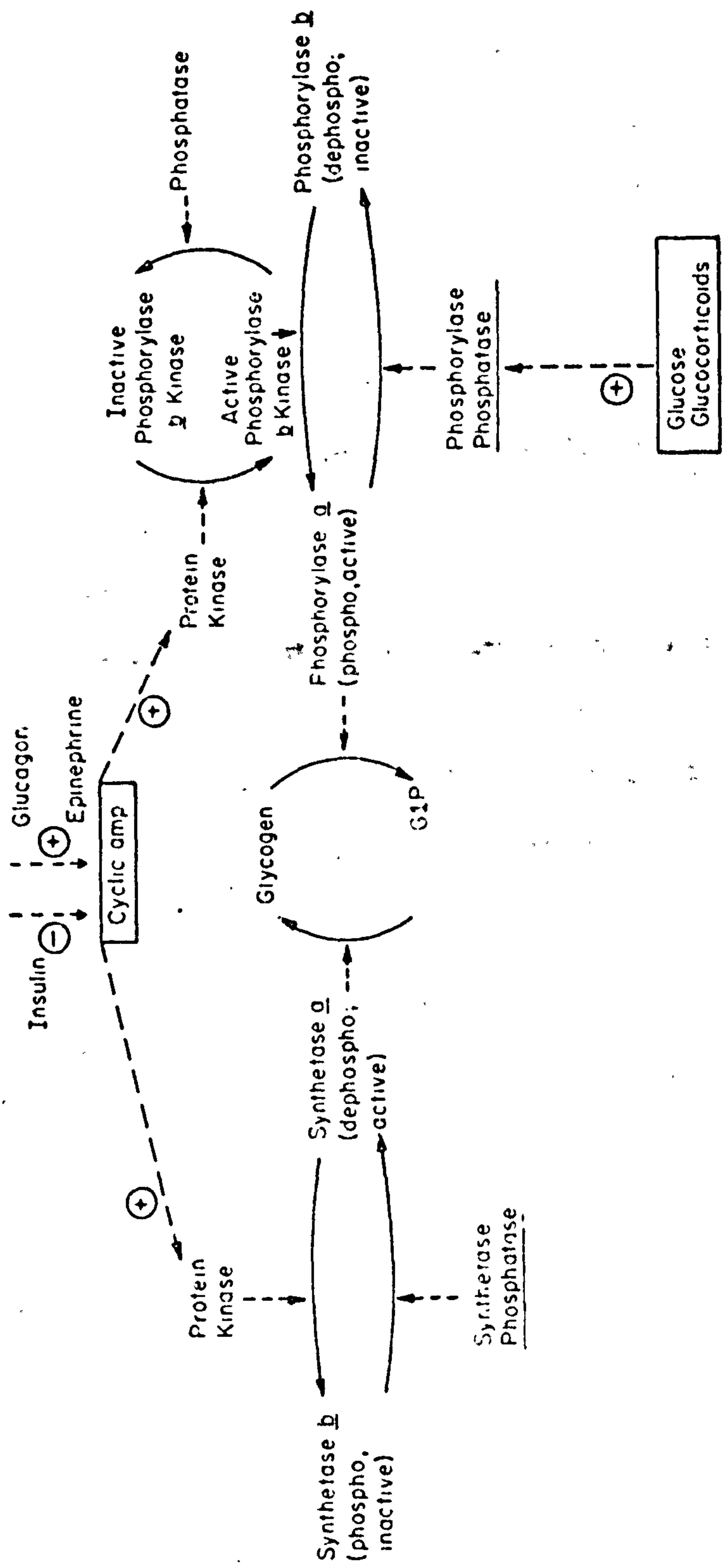


Figure 13. Control of Glycogen Metabolism in Liver

After, Alstzuler and Finegold (1974).

The main sites of hormonal regulation are shown.





closely regulated by the concerted action of several hormones, as indicated in Figure 13. Insulin, adrenalin and glucagon are known to modulate the activity of adenyl cyclase and thus control the levels of cyclic AMP which activates protein kinase (Soderling and Park, 1974). Glucocorticoids and glucose itself, affect the activity of phosphorylase phosphatase and synthetase phosphatase (Altszuler and Finegold, 1974) and so both the synthesis and degradation of the enzymes which act on glycogen itself are controlled. There is also evidence for regulation of some steps by metabolite concentrations, particularly glucose 6-phosphate in muscle and in liver, UDP, phosphate and adenylates appear to have allosteric affects on both glycogen synthetase and phosphorylase. This brief summary of the many and varied control systems whose mechanisms are known emphasises the importance to an animal of efficient coordination between the processes of glycogen synthesis and breakdown and the metabolic requirements of its tissues. There are already several mechanisms known controlling flux through this pathway, but this does not rule out the existence of others.

#### 5.4 CARBOHYDRATE PHYSIOLOGY IN APODEMUS - RESULTS

Having concluded that a physiological approach would be a suitable, and feasible, way of investigating possible differences between PGM<sub>2</sub> genotypes, there remained many ways in which this might be done. The most relevant experiment would be one that replicated the environmental conditions that were presumed to have caused the gene frequency changes. In Section 4.6 it was suggested that

because winter survival rates in fieldmice are closely connected with food supply (Watts, 1969; Flowerdew, 1972), this may have been the cause of selection. Although in the Skokholm house mouse population temperature in late spring is known to affect survival rates (Berry, 1968b), the importance of food supply in Apodemus is emphasised by the possibility of a direct link via the role PGM plays in carbohydrate metabolism. There is therefore some reason to suspect that this may have been the selective agent.

Any effect that PGM genotype has on an animals carbohydrate metabolism must ultimately be reflected in stored glycogen levels, by affecting the rate at which they change. Such change may be brought about in two ways, the first of which is through increasing the amount of work done by an animal. This primarily affects muscle glycogen and has been much studied particularly in heart muscle (Poland and Blount, 1966; Bockman et al. 1971; Terjung et al. 1974). Segel et al. (1975) emphasise the importance of the exact conditions under which the heart is removed in such studies, and they froze the heart in situ while beating. With such an experiment in mind, heart tissue was included in a preliminary study of glycogen levels in field mouse tissues. As only one animal was used, the results are not tabulated but levels of approximately 4%, 0.1%, and 0.05% were recorded for liver, brain and heart tissue respectively. The very low levels of glycogen found in heart muscle (0.5% is normal in rat heart (Segel et al. 1975)), were tentatively ascribed to the trauma associated with removing field mice from the cage. The possibility of detecting differences



between genotypes after exercise was therefore considered remote.

The second approach depends on the close association between the liver glycogen levels and the nutritional state of the animal. While this does not normally exceed 5% even with excess food, it is rapidly reduced by fasting. Glycogen in the liver is not as labile as in heart tissue and it was considered that the induction of glycogenolysis by fasting would be the most likely way to detect any differences between PGM<sub>2</sub> genotypes, as differences in the level of glycogen remaining after a given period. This approach was therefore adopted and the null hypothesis for the experiment was that the genotypes do not differ in the level of liver glycogen remaining after a short period of fasting.

There are undoubtedly many other factors which could contribute to differences in the rate of mobilisation of glycogen besides phosphoglucomutase genotype. It was therefore particularly important that the genetic background of the experimental animals be similar. This was achieved by using only F<sub>1</sub> animals from crosses between a strain derived from Cambridgeshire and one from Leicestershire. Both a and c alleles were contributed by each strain to the crosses. There is little reason to suspect a major difference in genetic background as these populations are very similar in gene frequency (Figure 7). While it had been hoped to analyse for glycogen variation between groups of sibs as well as between genotypes, this was not possible. The contribution of each mating to each genotype was very



different as there were two ac x ac crosses, one cc x ac and one aa x aa. The method used to assay for glycogen levels has been described in detail in Section 2.8. The recovery rate of glycogen standards by this technique was 92%, at an initial concentration of 5%.

The experiment was conducted in two stages of which the first was designed to provide an estimate of the mean resting glycogen level in Apodemus liver. This was obtained from 28 mice of very different ages and backgrounds. The mean and standard error of the results are given in Table 14. This was not, unfortunately a sufficient sample to test for differences in resting levels between PGM<sub>2</sub> genotypes but differences at rest would be more difficult to link with genotype at this locus than differences in the rate of mobilisation.

The second stage in this experiment used animals of a similar genetic and environmental background, as described above and in Section 2.3. The experiments were conducted over a period of ten days by withholding food, which had been provided ad lib., from the start of the night period. An overnight fast in A.sylvaticus will amount to a 24 hour one in a laboratory mouse as these animals are strongly nocturnal even in a laboratory colony. A total of 59 animals were so fasted and their liver glycogen levels assayed as before. The results are also given in Table 14 and a comparison of the two means by a t-test indicates that the difference is highly significant. Not surprisingly, such a period of starvation causes a marked reduction in the mean level, from almost 3% to 1.3% by weight.

Table 14

Glycogen content of liver in control and fasted  
A.sylvaticus. (mg/g wet weight)

	<u>Control</u>	<u>Fasted</u>
Mean	28.9	13.4
Se	2.41	1.58
N	28	59

Student's  $t = 5.409$       D of F = 85  
 $P < 0.001$

Table 15

Glycogen content of liver in fasted A.sylvaticus according to PGM<sub>2</sub> genotype. (mg/g wet weight)\*

	<u>aa</u>	<u>ac</u>	<u>cc</u>
Mean	6.3	16.5	15.2
Se	1.66	4.02	2.25
N	14	18	16

Testing for overall heterogeneity.

Kruskal Wallis  $H = 6.353$   $P < 0.05$

Combining groups ac and cc and comparing the mean with the mean for the aa group

(All allowing for the difference in sample size).

Student's  $t = 3.324$  D of F = 46

$P < 0.01$

\*A similar analysis of the small number of control (unfasted) animals whose PGM<sub>2</sub> genotype was known gave the following result:

	<u>aa</u>	<u>ac</u>
Mean	26.1	22.2
Se	3.8	3.7
N	8	8

This indicates that the difference between the genotypes is only revealed by fasting.



Of the 59 fasted animals, 48 had been typed for genotype at the  $\text{PGM}_2$  locus. The data are given in Table 15. There is a clear difference between the aa group and the other two in mean liver glycogen levels. Testing the data for overall heterogeneity by the Kruskal Wallis test indicated that the result was significant. The aa group was then compared with the combined data for the other two groups. Allowing for the difference in sample size, a t-test on the means gave a level of significance of 1%. The null hypothesis is therefore rejected as differences do exist between  $\text{PGM}_2$  genotypes in liver glycogen level after fasting.

As has been mentioned (Section 5.2) the  $\text{PGM}_2$  isozymes contribute less than 10% of the total PGM activity in human liver. Electrophoresis of Apodemus liver homogenates confirmed that there was much less activity in the region of the  $\text{PGM}_2$  isozymes than found in red cells. It must however, be emphasised that the starch gel technique is not appropriate to the quantitative comparison of different samples.

## 5.5 PHOSPHOGLUCOMUTASE: A POSSIBLE SITE OF REGULATION

The results presented in the previous section are of considerable importance in several respects. The demonstration of differences between  $\text{PGM}_2$  genotypes in their carbohydrate metabolism is of immediate relevance to their fitness in nature. However, the manner in which such differences could come about is not immediately obvious in view of the regulatory mechanisms controlling glycogen

metabolism, briefly summarised in Section 5.3. It is rather more difficult to ascribe these physiological results to effects of a linked gene than it was for the field data. In this case it is necessary to postulate a gene linked to PGM<sub>2</sub> which is involved in the control of glycogen metabolism. This has now become a rather contrived alternative and it is worth giving serious consideration to possible ways in which structural differences at the PGM<sub>2</sub> locus itself could bring about such results. This approach has the advantage that its conclusions could be readily tested, but it rests on the assumption that PGM can act as a regulatory step in glycogen metabolism. The evidence for this assumption will now be reviewed.

Although Johnson (1974) classified PGM as a regulatory enzyme, the grounds he stated were that the substrate/product ratio were removed from equilibrium in vivo. This approach cannot be justified. Thermodynamic equilibrium is only relevant to the closed, in vitro, system. In the cell, both substrate and product are involved in several alternative pathways as well as the one under consideration and the kinetics of the various reactions involved are more important than simple thermodynamics.

The possibility that PGM can have a regulatory role cannot be dismissed lightly however. It is immediately suggested by the results of several authors who have found differences in the activity of the enzyme in different physiological states. Hashimoto et al. (1967) injected mice with insulin and adrenalin and compared the liver phosphoglucomutase activity with control animals. They found that



insulin increased PGM activity considerably and adrenalin decreased it. They also found that if the homogenate was incubated with histidine and  $Mg^{2+}$  there was no difference between hormone-treated animals and controls. Thus, the differences in activity of the enzyme after hormone injection appears to result not from direct activation but from the changes made in the internal environment, particularly with regard to  $Mg^{2+}$  concentration. Peck and Ray (1971), in a more thorough study, showed how the PGM activity of an homogenate depends on the ratio of enzyme molecules in the inactive  $Zn^{2+}$  bound form to those in the active  $Mg^{2+}$  form, and that this ratio was altered by insulin injection. They were able to establish that the total catalytic potential did not change under these conditions.

The work of Joshi et al. (1967) is particularly interesting as they isolated PGM by DEAE Sephadex chromatography, and showed two main peaks in liver extracts. In addition, under some circumstances they detected another minor peak which they were able to identify as dephosphorylated enzyme. Although there are some technical problems associated with chromatographic purification of PGM (Ray and Peck, 1972), the proportions of total activity in human liver attributed to Peak I and Peak II by Joshi et al. (1967) are fairly similar to the relative activities of the  $PGM_1$  and  $PGM_2$  loci determined by McAlpine et al. (1970b). This analogy is supported by differences in amino-acid composition and in peptide fingerprint map patterns (Joshi et al. 1967). These authors found changes in PGM activity with hormone treatment, but as in the work of Peck



and Ray (1971), only in fasted animals. In contrast to the results of Hashimoto et al. (1967), both insulin and adrenalin were found to increase PGM activity but the question of activation by imidazole is not dealt with in detail. It can be concluded from these studies that PGM activity is affected by in vivo hormone treatment and that this is at least partly due to its dependence on  $Mg^{2+}$ .

Other factors have been shown to affect PGM activity in ways which also imply possible mechanisms for control. Beitner et al. (1975) investigated the interactions of ATP,  $Mg^{2+}$ , citrate, Glucose 1,6-diphosphate and PGM. Unfortunately, these workers used cellogel electrophoresis in citrate/phosphate buffer, conditions which make it very difficult to draw analogies between their isozymes and those separated on starch gels. Nevertheless, they showed that ATP and citrate, which are both well known inhibitors of phosphofructokinase, are potent inhibitors of PGM. The inhibition could be reversed by the addition of glucose 1,6-diphosphate or  $Mg^{2+}$ . The inhibition did not appear to be entirely due to the removal of  $Mg^{2+}$  ions as one PGM isozyme was isolated which was  $Mg^{2+}$  independent and this showed the same response to glucose 1,6-diphosphate as the others. Beitner et al. (1975) suggest that the activation they observed might occur through the formation of a diphosphate  $Mg^{2+}$  complex. The kinetic studies of Quick et al. (1974) showed that only the  $PGM_2$  isozymes responded to increases in the concentration of diphosphate, as has already been mentioned (Section 5.2). If this compound does play a regulatory role as seems likely, there must be some circumstances when the  $PGM_2$

isozymes are of greater significance than their normal activity suggests. Beitner et al. (1975) also indicated a possible mechanism by which the synthesis of the diphosphate cofactor could be controlled. If the main source of diphosphate other than the PGM reaction is via glucose 1-phosphate kinase, Eyer and Pette (1967) suggest that this is the same as phosphofructokinase, which itself is activated by AMP and is closely controlled. However, the way in which glucose 1,6-diphosphate is produced is a subject of some controversy (Rose et al. 1975).

However many mechanisms might exist whereby PGM activity could be controlled, they will have no bearing on cellular metabolism if it never becomes a rate-limiting step. The main step which is rate-limiting in glycolysis is that catalysed by phosphofructokinase and PGM does not appear to limit flux under steady state conditions. Results obtained by Williamson (1966) indicate that this may not be the case all the time. He showed that the substrate/product ratio was removed from equilibrium in resting heart tissue. As has been mentioned this is of little significance in itself. However, on administration of insulin, the ratio of glucose 1-phosphate to glucose 6-phosphate rose from about 1 : 97 to 1 : 10. Such a change in relative concentrations implies that under some circumstances PGM may limit flux through this pathway.

Although the evidence presented in this section has been for the most part indirect, there are several indications that the activity of PGM responds to the in vivo hormonal state and to the concentrations of several metabolites.



Combined with the above evidence that this step may be rate-limiting in some physiological conditions, this evidence strongly suggests that PGM is indeed tied in with in glycolytic regulatory processes as Beitner et al (1975) suggest. The position has therefore been reached where it can be postulated that structural differences between PGM molecules, particularly at the PGM<sub>2</sub> locus, could influence metabolic processes as found in the results presented in the previous section.

#### 5.6 DISCUSSION - FASTING AND PHOSPHOGLUCOMUTASE

A full explanation of the results presented in Section 5.4 requires not only that PGM has a regulatory function under some circumstances, but also that the physiological changes induced by the experiment in some way involved PGM.

Many physiological changes occur as a mammal adapts to the fasting state. Processes of glycogenolysis, lipolysis and gluconeogenesis that are designed to maintain a constant if reduced blood glucose level, are orchestrated by changes in the levels of circulating hormones. In man it has been shown that, while blood glucose drops by 25%, insulin levels fall to about half that found normally, adrenalin increases by a half, glucocorticoids increase and glucagon shows the most dramatic change of all, reaching a level twice that found in the fed state by the third day of fasting, (Narahara and Cori, 1968; Marliss et al. 1970; Misbin et al. 1971; Walter et al. 1974). A full description of the metabolic effects of such changes is not possible here but an identification of the major aspects will be



attempted. The way that insulin and adrenalin act on glycogen metabolism through cyclic AMP is indicated in Figure 13. Glucagon appears from this diagram to have a similar effect as adrenalin but there are some differences. Glucocorticoids such as cortisone have been shown to have a special role in promoting lipolysis in adipose tissue, in conjunction with growth hormone, and gluconeogenesis from amino-acids in muscle (Narahara and Cori, 1968). Similarly, the primary role of glucagon in long term response to starvation is indicated by a comparison of the degree of increase in cyclic AMP concentration caused by a given dose of adrenalin and glucagon. While adrenalin causes a three fold increase, glucagon causes a fifty fold rise in cyclic AMP levels (Makman and Sutherland, 1964). This difference suggests that glucagon is primarily associated with gluconeogenesis, a process which requires large concentrations of cyclic AMP (Robison et al. 1967). It appears therefore that while all these hormones have some effect, the one with a specifically short term role in increasing liver glycogenolysis in a fasting animal is adrenalin (Misbin et al. 1971; Alstzuler and Finegold, 1974) with the decrease in insulin levels contributing as well. Thus if these two hormones can be shown to influence the levels of some of the inhibitors of PGM that have been mentioned (Section 5.2) then the relevance of the starvation experiments will have been established.

Evidence of hormonal influences on PGM activity has already been outlined (Section 5.5). The effects that Hashimoto et al. (1967) and Peck and Ray (1971) observed

being attributable to activation by  $Mg^{2+}$ . The importance of  $Mg^{2+}$  levels in tissues undergoing glycogenolysis is emphasised by its close link with adenylyl cyclase activity (Drummond and Duncan, 1970) and thus with cyclic AMP concentration. While this suggests that PGM activity may be coordinated with that of other enzymes in the pathway, it does not yet indicate the relevance of structural differences at the  $PGM_2$  locus. However, the results of four independent studies have indicated how this could arise. One of the experiments that Joshi et al. (1967) performed involved estimating PGM activity after fasting. As in the above cases they had previously observed that hormone induced decreases in activity could be reversed by incubation of the liver homogenate with an imidazole/ $Mg^{2+}$  activating mixture. However, after starvation they found that there was a residual difference from controls even after activation. Fractionation of the liver homogenate from these animals by DEAE Sephadex chromatography revealed that the difference was entirely due to a marked decrease in the Peak I fraction. As these fractions appear to correspond directly to the  $PGM_1$  and  $PGM_2$  loci (Section 5.5), this suggests that on starvation, the relative activity of the  $PGM_2$  isozymes increases. Efforts were made to examine this possibility in Apodemus. There appeared to be no change in gel pattern but a more quantitative technique would be required for a conclusive result. Such a change in the relative activity of the  $PGM_1$  and  $PGM_2$  isozymes would suggest that they may have rather different roles in vivo. This possibility is supported by the differences in properties



summarised in Section 5.2, and by the suggestion of Dully et al. (1969) that there may be more than one pool of glucose 6-phosphate in the cell, only one of which is accessible to exogenous glucose phosphate. The possibility of such compartmentation of these metabolic processes would remove the difficulty associated with the relatively low in vitro activity of PGM<sub>2</sub> in liver. This aspect requires further investigation.

If an increase in the relative activity of PGM<sub>2</sub> isozymes on fasting is found in Apodemus, then the genotype at this locus becomes very relevant. It has been shown that the level of citrate in the tissues increases during starvation (Garland and Randle, 1964). Beitner et al. (1975) have shown how glucose 1,6-diphosphate acts as a deinhibitor on citrate and ATP-inhibited PGM, and, as mentioned before, Quick et al. (1974) have shown that the PGM<sub>2</sub> isozymes are much more sensitive to concentration of the diphosphate. Thus, differences in tertiary structure caused by the amino-acid substitution that distinguished the a and c allozymes in A.sylvaticus could give rise to differences in response to deinhibition by diphosphate and thus to differences in the rate at which glycogenolysis occurs during starvation.

It should be emphasised that the above formulation of events occurring during fasting and how they impinge on phosphoglucomutase is speculative. Its verification would require a close study of the PGM step in vivo and in particular, of the roles played by each of the two major loci. The greater dependance of PGM<sub>2</sub> on glucose 1,6-



diphosphate suggests, by analogy with bacterial enzyme (Section 5.2) that the mechanisms may differ so that the diphosphate is involved in initiating the conversion by this enzyme. This simple suggestion could be readily tested with the use of labelling techniques. After running a gel to separate the isozymes, labelled diphosphate would be used in the staining mixture. If the  $\text{PGM}_2$  reaction does involve the diphosphate in the same way as in the bacterial enzyme, the labelled 6-phosphogluconate would build up in the  $\text{PGM}_2$  band.

The most useful tissue in which to investigate differences between the two major enzymes would undoubtedly be the erythrocyte. Here the relative activities are almost equal and McAlpine et al. (1970b) suggested that this was due to the greater stability of  $\text{PGM}_2$  isozymes as erythrocytes are rather long-lived cells. This is not a complete explanation, however, as even by comparing the gel patterns of liver and red cells it can be seen that these isozymes stain much more heavily in absolute terms in erythrocytes. An alternative explanation can be proffered on the basis of their differing response to glucose 1,6-diphosphate. PGM plays a rather different role in erythrocytes than other cells (Beutler, 1975). There is little glycogen in the normal red cell (Sidbury et al. 1961). Glucose enters the cell from the plasma and is phosphorylated by hexokinase to glucose 6-phosphate. The only way that glucose 1-phosphate is formed is from galactose, which has to be converted to glucose before it can be metabolised, through a well known series of reactions (Ng, 1971; Paniker and Iyer, 1971), which

end with the formation of glucose 1-phosphate. Galactose levels in the plasma are not subject to as rigorous control as is glucose, because galactose cannot be stored. The presence of a large proportion of the total PGM activity as the PGM<sub>2</sub> enzyme means that the flux through this pathway can be controlled through changes in the levels of glucose 1,6-diphosphate. In addition, it has been mentioned that red cell phosphoribomutase has been identified as PGM<sub>2</sub> in both man and Apodemus (Sections 3.2, 5.3). Inosine can also be used as an energy source in red cells (Bentler, 1975) and is metabolised via a phosphoribomutase step. It is difficult to see how this can occur under normal circumstances as red cells have a very high concentration of glucose 1,6-diphosphate which indicates that PGM<sub>2</sub> will be functioning almost entirely as a glucomutase (Quick et al. 1974). Problems such as this point the need for a detailed study of the role of PGM in this tissue.

Ultimately, the kinetic differences between different PGM<sub>2</sub> genotypes in Apodemus which have been postulated to explain the results of Section 5.4 will only be identified by isolation and analysis in the manner that Quick et al. (1974) used to compare the products of different loci. Their approach could be extended to include differences in the response to inhibition as well as activation. Finally, the basic experiment performed in this study needs to be repeated. A.sylvaticus has often not proved easy to breed in captivity and so a colony of mice of similar numbers to those used here takes some time to build up. If possible, the repeat experiment should be performed with replicates



using animals from different strains to indicate the effect differences in the genetic background may have on this character.

While explanation of the results given in Section 5.4 has proved lengthy and involved, it has been of considerable use in pointing out areas of ignorance in a field that has been thought of as well understood (Ryman and Whelan, 1971). It remains to make some suggestions as to how differences in the rate of glycogen mobilisation could affect fitness in the wild. It may be noted that the grouping of genotypes by mean glycogen level after starvation is the same as that of the survival rates observed in the field at Charnwood (Tables 13 and 15). The animals with the highest minimum survival rate are those with the lowest mean glycogen level after starvation. Although this may appear strange at first sight, it should be emphasised that glycogen is only a short term store and that the glycogen in human liver may virtually disappear after only a few hours fasting, although survival is possible for several weeks. Berry and Jakobson (1975b) observed that a major feature of the ways in which house mice adapt to an adverse environment was by an increase in the ability to mobilise body resources. Although they were referring to brown fat, it could be that the same principle applies to glycogen metabolism. Lipolysis and gluconeogenesis from amino-acid are the processes involved in long-term adaptation to food shortage. While these processes are initiated a wild mouse will need to retain its peak activity. The rapid mobilisation and use of stored glycogen may therefore be of advantage at times



when food is scarce and more energy than usual is required to find the available food resources. The animals that maintain a high level of activity through rapid glycogen utilisation will find the dispersed food packets most efficiently and thus deprive the less active animals still further. The conditions prevalent in the Charnwood Apodemus population from October 1974 to February 1975 may well have been similar to those summarised here.

Without doubt, the polymorphism that is most thoroughly understood in all aspects is that of human haemoglobin S. (Allison, 1955). Its population genetics, ecology and biochemistry are all well known. Of the multitude of other polymorphisms that have been discovered with the aid of electrophoresis, only a handful have been investigated in anything like such detail (Section 5.1) and the most thorough work has been done on the Drosophila melanogaster ADH locus (Gibson, 1970; Vigue and Johnson, 1973; Day, Hillier and Clarke, 1974a,b). These studies have detected differences in enzyme kinetics, as have been found for many human enzyme polymorphisms (Harris, 1971, 1976) but little is known as to their metabolic and physiological relevance for a free-living organism. In this study, this last step has been made, but kinetic differences between the a and c enzymes have not yet been demonstrated. When this is done and the differences suggested here are quantified, the Apodemus PGM<sub>2</sub> polymorphism should provide a major contribution to our knowledge of the metabolic differences that may exist between individuals and the role such differences play in evolutionary adaptation.

## CHAPTER 6

### GENERAL DISCUSSION AND CONCLUSIONS

This project was initiated to investigate the ecological genetics of the fieldmouse Apodemus sylvaticus. In particular it was hoped that the annual fluctuations in numbers found in this species (Miller, 1958; Crawley, 1970) would allow the interaction of stochastic and deterministic processes in the genetics of the populations to be studied. As has been indicated (Chapter 4), there is probably less change in effective (using the word in both a genetical and an ecological sense) population size than was originally believed. In addition, the degree of movement found suggests that populations may be continuous over wide areas (Section 3.7). There was no genetic evidence for bottlenecks in numbers in the populations studied, (Section 4.2) and while these results may be due to unusual circumstances prevailing at the three sites studied (Section 4.6), they suggest that similar work in different areas may throw fresh light on population processes in Apodemus. However, it was the bimonthly sampling at Charnwood that yielded the most interesting results. The discovery of genetic changes in the population over a short time period strongly suggested the action of natural selection.

The question of how much genetic variation is maintained by selection is undoubtedly the major current problem in the field of population genetics. Despite its seemingly perennial recurrence, it has served a useful function in stimulating new ideas and fresh lines of research. The case put forward by the "neutralist" school is based on arguments



developed from the observation of apparent constancy in the rate of amino-acid substitution during the evolution of protein molecules (Kimura, 1968; King and Jukes, 1969; Kimura and Ohta, 1971a). While the nature of the original argument has recently been questioned (Fitch and Langley, 1976), the proponents of this view claim that what is known of the behaviour of mutations, both in evolving amino-acid sequences and as detected in electrophoretic surveys of natural populations, is best explained by the hypothesis that the majority are effectively neutral with respect to selection. It is instructive to consider how the data presented here bears on the contention between this view and that held by the "balance" school, in Dobzhansky's (1955) terminology, that most of the variation in natural populations is maintained by the action of selection.

The data presented on gene frequencies in natural populations (Section 3.6), indicated that when both polymorphic loci are taken together (Figure 7) the eight populations are rather similar. This fits well with the much more extensive data available on other species where considerable similarity between local populations has been found, a phenomenon usually attributed to the action of selection (Prakash, Lewontin and Hubby, 1969; Selander and Yang, 1969; Ayala, Powell and Dobzhansky, 1971; Ayala, Powell and Tracey, 1972; Ayala et al. 1974). However, the neutral mutation school has shown that a relatively small amount of migration is required between populations to overcome the random divergence that would otherwise result if no selective forces act on the alleles (Kimura and Ohta,



1971b). An important feature of this argument is that the level of migration necessary does not depend on the population size and so a small number of migrants, in absolute terms, would maintain considerable similarity in gene frequencies between populations. Much of the data presented in Chapter 4 is similarly inconclusive. Although this aspect of the study was designed to look for random changes in gene frequency, the failure to detect such changes proves little, for the reasons given above. Further, while the latter part of this chapter, and all of Chapter 5, describes selective differences at the  $PGM_2$  locus which were first detected as gene frequency changes in the field, this is of little relevance to the problem. To claim otherwise would indeed be to "erect a neutralist strawman" to quote Lewontin (1974). The proponents of the neutral theory do not pretend that natural selection does not exist (Kimura and Ohta, 1971b). These authors merely claim that, the number of alleles materially affected by selection is low relative to the number segregating in a population. Thus there is, little data given here that can be regarded as of relevance to this question.

The conclusions of this study raise the question of what the field of ecological genetics as a whole has to offer towards a solution of the selectionist neutralist controversy. Essentially, ecological geneticists work on the promise that selection does maintain most polymorphisms and hope to justify this view simply by demonstrating cases where this has been found. They are indeed the 'naive pan-selectionists' of Kimura and Ohta (1971a). The manner in which they hope

to do this is through the establishment of a series of polymorphisms where detailed biochemical, physiological and ecological study has demonstrated the action of natural selection beyond doubt (Clarke, 1975). Such a demonstration would indeed be convincing if the number of cases established approached the number of polymorphisms known in a given species. However, because the failure to demonstrate selection would not necessarily mean that selection never acts on that locus, this approach is not a particularly rigorous one. In pointing this out, Lewontin (1972, 1974) comments that while there have been unqualified successes, and he cites the studies on mimicry in Papilio (Clarke and Sheppard, 1960, 1971) and the demonstration of protective melanism in Biston (Kettlewell, 1973), there have also been glaring failures, particularly with the human blood groups. Although the list of successes may be extended to include sickle-cell haemoglobin in man (Allison, 1955), shell colour in Cepaea (Cain and Sheppard, 1954), the work on the ADH locus in D.melanogaster (Vigue and Johnson, 1973; Day, Hillier and Clarke, 1974a,b; Morgan, 1976) and the less detailed studies on American fish species by Koehn (1969) and Merrit (1972), this does not counter the impression that despite the discovery of vast numbers of polymorphisms since the advent of the widespread use of gel electrophoresis, there have been very few which have been clearly shown to be maintained by natural selection. A possible reason for this could be that many ecological geneticists are still preoccupied with "primroses, snails, ladybirds and the Pale Brindled Beauty Moth" (Lewontin 1972). While such organisms were the most



useful tools for some of the early successes mentioned above, this is no longer the case generally, except for specific purposes such as the physiological aspects of shell colour in snails, discussed by Jones (1973). Lewontin (1972, 1974) suggests that ecological genetics will never be able to provide a clear answer to the selectionist/neutralist controversy. He is almost certainly right.

The effort involved in demonstrating the action of natural selection is usually considerable. It would require immense resources for a thorough investigation of many of the polymorphisms of only one species, particularly in the light of the discovery of the extent of molecular variation that is not detected by standard electrophoretic techniques (Bernstein et al. 1973; Singh et al. 1975). Harris (1976) has demonstrated quantitative in vitro differences in activity between allelic forms of polymorphic human enzymes in 18 out of 30 cases. This has been quoted as evidence that these polymorphisms will be subject to selection (King, 1976). However, the work on human inborn errors of metabolism suggests that although the heterozygotes may have enzyme activities in the region of 50% normal, they show few, if any, clinical symptoms (Harris, 1975, p.236). This would suggest that the buffering capacity of many metabolic pathways is such that the much smaller differences in activity found between electrophoretic variants might be of little physiological significance. Similarly it is not sufficient simply to quote environmental associations as evidence for selection, for example the shell colour polymorphisms in species of Cepaea (Cain and Sheppard, 1950, 1954), as this



does not indicate by what balancing mechanism the polymorphism is maintained. In the case of the PGM<sub>2</sub> polymorphism in A.sylvaticus the extensive background of ecological knowledge allowed the identification of a possible factor that caused the low rate of survival observed at the time of the selective changes in gene frequency (Section 4.6). On the assumption that there was a direct link between food supply and PGM<sub>2</sub> gene frequency, physiological experiments were carried out to investigate the nature of the differences between the genotypes (Chapter 5). The results upheld the assumption and strongly suggest that the alleles at the PGM<sub>2</sub> locus are not neutral with respect to selection. However, as in the above cases, it has not been possible to identify the mechanism that maintains the polymorphism. This may partly be due to the low numbers that characterised the field studies but probably also to the nature of the organism being studied. Berry et al. (1973) have indicated that even over a period when selection is known to be affecting a particular locus in a particular way, the probability of an individual's survival depends not only on its genotype at that locus, and on various environmental factors but also on several other aspects of its physiology. These interactions would suggest that it is only rarely that conditions arise to highlight the selective differences at a single locus such as PGM<sub>2</sub>, in the manner found here (Section 4.7) and that normally the least fit individuals would be a very heterogeneous group genetically. It would therefore be very difficult to suggest a rationale for determining the conditions under which the

c allele is favoured, and thus the mechanism that balances the polymorphism.

One of the more significant aspects of the studies on the ADH locus in D.melanogaster has been the indication and recent confirmation that the polymorphism is balanced by the action of frequency-dependent selection favouring the S allele (Kojima and Tobari, 1969; Morgan, 1976). It is all the more unfortunate that the application of these results to natural populations depends on the acceptance of vague statements to the effect that D.melanogaster lives on fermenting fruit where the alcohol concentration can reach toxic levels and that larvae may be found in rotting fallen fruits where the temperature may reach 40°C (Vigue and Johnson, 1973; Clarke, 1975). The studies of Koehn (1969) and Merrit (1972) are no better in this respect because in neither is the presumptive environmental selective agent known to cause mortality, or affect fecundity. It can be fairly claimed that in the case of PGM<sub>2</sub> in Apodemus, while the details of the enzymatic differences remain to be worked out, the environmental agent of selection has been identified and confirmed by experiment. In addition, the results of this experiment have indicated the way in which some animals can respond to conditions of low food resources. It has not previously been suggested that at such times it may be of advantage to an animal to remain active and use the slender reserves it still possesses, than to conserve them. In a species where food supply regularly appears to have a direct effect on population size (Watts, 1969), the ability of some



individuals to respond to such pressure may be important. Small mammals usually form a multi-species community, although this is hardly the case at Charnwood. It is known that voles are able to exclude fieldmice when ground cover is dense (Evans, 1942; Southern and Lowe, 1968). This suggests that in winters when the nutritional conditions for Apodemus are poor relative to those for voles, the continued presence of fieldmice in the habitat could depend on the ability of some individuals to remain active and fully exploit what resources are available. Thus, although this study may be of little relevance to currently important topics in population genetics, it does have some significance for the wider field of evolutionary genetics.

If the conclusions arising from this study are generally correct, and ecological geneticists will never be able to demonstrate that most polymorphisms are maintained by balancing selection, what direction can they take? Some of the early successes in the field such as the studies on mimicry in Papilio (Clarke and Sheppard, 1960, 1971) were notable because they matched in sophistication the contemporary state of other aspects of population genetics (sensu lato). In recent years the advances made in the molecular aspects of population genetics have been matched by a considerable expansion of the theoretical, but not of the ecological side. The position is far from irredeemable however, as the work on the ADH locus in Drosophila melanogaster has indicated. If ecological genetics is the experimental study of genetical processes in natural populations (Ford, 1971), then a major part of the field



must be the identification and measurement of the specific factors in the environment that affect each gene studied. Thus, in addition to the analysis of the biochemical and physiological aspects of a molecular polymorphism the relevant physical and biotic features of the environment should be quantified. The specification of which environmental factors are relevant can be accomplished by an analysis of the major components of mortality using methods such as those of Varley and Gradwell (1970). While factors affecting fertility may be more difficult to identify in the field, these may be more readily dealt with by laboratory studies. In this way the sophistication of the techniques whereby the differences between allelic gene products can now be detected and measured will be matched by the depth and detail of our knowledge of the environmental selective agents. Ecological genetics will then rapidly advance in its most constructive direction, that of quantifying the mechanisms involved in the process of evolutionary adaptation.

## APPENDIX

Preliminary data on the genetics of the Black coat colour variant in Apodemus sylvaticus

One of the populations that were sampled in this study, from Prickwillow near Ely (Figure 6), was unusual in that black individuals were a regular, if rare, occurrence (Flowerdew and Green, personal communication). Such a phenotype has only been reported from one other locality, in Surrey, and was only recently observed there (Gurnell personal communication). The hairs in this form are generally completely black but there are some scattered white ones, especially near the ears and in the region of the normal pectoral stripe. In addition, the hairs on the feet are usually white (for a full description, see Green, in press). One black male was obtained with the help of Dr J.R. Flowerdew to investigate the genetic basis of this variant.

The black male was mated with two wild type females and the classes of the progeny are shown in Table 16a. There were no intermediate forms and both black and wild type offspring were produced in both males and females. It therefore appeared that the variant gene might be an autosomal dominant, as the ratio of black to wild type was not significantly different to 1:1. To test this matings were set up between the offspring. There were insufficient mature animals to do reciprocal crosses, but between the three crosses made, 29 offspring were scored. It was expected that the black x black mating would yield a 3:1

Table 16a

Results of Mating male (Black) x females 234, 236 (wild type)

	Progeny	
	Black	Wild Type
Female	5	5
Male	<u>3</u>	<u>7</u>
Total	8	12

Table 16b

Results of Test crosses between progeny of cross a

1) Male 269 (Black) x Female 270 (Black)

	Black	Wild Type
Female	6	0
Male	<u>3</u>	<u>0</u>
Total	9	0

2) Male 267 (wild type) x Female 269 (wild type)

Male 268 (wild type) x Female 268 (wild type)

Female 267 (wild type)

	Black	Wild Type
Female	3	10
Male	<u>1</u>	<u>16</u>
Total	4	26



ratio of blacks to wild types but that the two wild type x wild type crosses would yield all wild type progeny. The results (Table 16b) did not support this hypothesis but are in good agreement with those expected for an autosomal recessive gene. All black animals were obtained in the black x black cross and a close approximation to a 3:1 ratio of wild type to black in the matings between the  $F_1$  wild type animals.

If the black allele is recessive, the simplest explanation for the results shown in Table 16a is that either or both the 'wild type' females were heterozygotes. From records, these females, which had only been chosen for being in breeding condition, were found to be  $F_1$  progeny of a cross between a Leicestershire male and a female from the Wicken population (Figure 6). The Leicestershire strain had been used to study the inheritance of plasma esterases (Table 1), and no black offspring had been observed in over 100 individuals. Only one cross had been made between Wicken animals and though no blacks had been observed, there were only 11 progeny. The Wicken strain would therefore appear to have been the most likely source of the allele as Wicken Fen is less than 8 miles from the Ely site. Further studies are being initiated to investigate the possibility that the black allele is present in this population (Flowerdew and Green, personal communication). Remating the original black male to a female of the Leicester strain yielded 11 wild type offspring and no blacks.

Black individuals have been discovered in a variety of species of wild rodents (Searle, 1968), but the persistence of the form in a population is unusual. However, as the author has not been involved in the field studies, no further data is included here. Similarly, while the data given here strongly suggest that the character is controlled by a recessive allele at an autosomal locus, no formal symbol is suggested as complementary work is still being undertaken in other laboratories. Although it is not easy to draw homologies with other species on the basis of one mutant allele, in view of its complete recessivity to wild type and the presence of some white hairs in the homozygote, this black form appears to be very similar to non-agouti in Mus. Homologies with black variants in more distantly related species of rodents cannot be suggested as some of these show a degree of intermediary in the heterozygote (Searle, 1968; Smeonoff, 1972). The occurrence of mutant coat colour genes in wild populations of Mus has been investigated systematically by Petras (1967). He tested 169 individuals thoroughly at 7 loci but found no mutant alleles. This suggests that the frequency of such variants in populations of Mus must be very low, as is probably the case in most populations of Apodemus. The discovery of a population such as the Ely one is all the more interesting, and strongly invites further study.



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