A STUDY ON THE EFFECTS OF TEMPERATURE ON THE CORRELATED RESPONSES BETWEEN BODY WEIGHT AND TAIL LENGTH IN MICE

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INTRODUCTION

Fisher and Wright in the early 1920's were largely responsible for the mathematical theory on which present day population genetics is based. Lush (1945) applied this theory to animal breeding problems.

Mendelian theory formed the basis for the genetic theories of populations just as it did for individuals. But now many genes were considered to be associated with the productive characters that were studied and a continuous rather than a discontinuous scale of measurement was used.

Up to now a large proportion of animal breeding research has been devoted to the estimation of phenotypic and genetic parameters based on the assumption of additive genetic variance. But many laboratory selection experiments have demonstrated the importance of a wide variety of gene and chromosome interactions which would suggest a more careful evaluation of the additive nature of gene action is needed. Selection theory would suggest that the estimated genetic parameters may not adequately predict selection response over a long term experiment.

The mathematical theory of population genetics has always tended to outstrip both verification of these theories with laboratory species and practical application of these theories to domestic species.

For example the nature of genetic variation of metric characters, moulded by natural selection during evolutionary history, and the relationship between genetic variation of metric traits and 'fitness' are questions which need considerably more study (Robertson 1955, 1958, 1963).

Because population genetics deals essentially with genes and final genotypes there still remains a vast developmental gap between the two (Rae 1958). Thus although population genetics theory, as applied to animal breeding problems, has succeeded in bringing about significant genetic changes in population, it could still be said that this approach has to some degree only defined the problems, rather than actually solved them.

Physiological genetics is the study of developmental and other processes through which genetic differences come to expression as phenotypic differences.

Wright (1941) discussed in some detail the physiology of the gene as it was then understood with particular reference to his work on the interactions of genes and substrate to produce melanin pigment in the guinea pig. More recent work in this field has confirmed and expanded Wright's theories. Thus although genes were first postulated from their end effects expressed in different phenotypes, the biochemical discoveries of deoxyribose nucleic acid (DNA) as the genetic material and the rapid advances in knowledge of the nature of gene action, now provides a definite basis for further advances in developmental genetics. A few steps in the chemical pathways of pigment production in mice, drosophila and some plants have been discovered, but the complexity of higher organisms has discouraged extensive experimentation in this field. Because fewer steps intervene between the gene and the phenotypic character in relatively simple organisms such as protozoa, yeast, bacteria and viruses, most investigations have been concentrated on these organisms.

Another approach which has been developed is that of canalisation - the tendency for a developmental process to produce a definite end result in spite of the genetic and environmental forces tending to produce variability (Waddington 1957). The disruption of canalization (or the uncovering of genetic variability in an invariant character) by either genetic or environmental means has been demonstrated in laboratory species.

Cockrem (1962) has discussed a possible approach for the selection of domestic animals on physiological traits. As Cockrem pointed out, the knowledge of domestic animals' physiology, biochemistry, endocrinology and other fundamental developmental processes has increased markedly, as have the techniques available to study these processes in more detail. This physiological approach has been used for the analysis of the effects of the N gene in Romney sheep (Cockrem 1956) and to study face cover relationships in Romney sheep (Cockrem 1967).

Although population and physiological genetics have to some extent developed along separate paths, there is obviously a fundamental and important connection between the two. Thus a number of population geneticists (Rae 1958, Robertson 1958, 1963, Cockrem 1962, Turner 1964 and Freedeen 1966) have suggested that the future of animal breeding research lies in a closer liaison between the geneticist and the other biological disciplines. If there was more knowledge of the physiological and other processes governing the variability in the characters which are being selected, the efficiency of selection may be increased by selecting nearer and nearer to the gene action (Cockrem 1962). Also the mechanisms by which some breeds, or individuals within a breed, adapt to certain environments better than others may be studied at a more fundamental level. Another way to use this approach is to select two or more lines from a common base population and then study possible physiological differences between these lines. Work of this type has had some success in laboratory species, especially mice (Roberts 1965).

This thesis study uses known and postulated physiological functions of the tail as a basis to study the genetic correlation between tail length and body weight in the mouse.

Selection has been carried out for body weight in the mouse in three temperature environments. The correlated response of tail length and other characters were also observed. While tail length is temperature labile (greater tail length growth in a hot environment than a cold environment), there is also a fairly strong genetic correlation between body weight and tail length (Falconer 1954). It has also been suggested that the functional reason for the increased tail length in a hot environment is that it acts as a 'heat radiator' (Harrison et al., 1959), while this suggestion has been refuted by other workers (Cockrem 1963, Barnett 1965b).

Cockrem (1959) showed that it was possible to produce lines of mice with opposite relationships to those predicted by the genetic correlation between body weight and tail length, by using an appropriate selection technique in a temperate environment. In this study the possibility that a similar result may be obtained by selecting for body weight in hot and cold environments is also being investigated.

In conclusion it could be said that this experiment is studying three major factors:

1. Selection response and associated correlated responses in three temperature environments.
2. Possible genotype-environment interactions between the selected lines and the three temperatures and possible changes of genetic correlations in different environments.
3. The relationship between body weight and tail length in the mouse with special reference to the function of the tail as a thermoregulatory organ.

Chapter I

REVIEW OF LITERATURE

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INTRODUCTION

This thesis topic involves the major fields of genetic and of temperature effects on body weight growth in the mouse as well as the appropriate correlated aspects.

The relevant literature is therefore considered in these two major sections with a third smaller section reviewing maternal effects and fertility in the mouse.

A. GENETIC ASPECTS OF GROWTH

Review articles by Chapman (1951, 1961) and Roberts (1965) cover most of the relevant material on selection for body growth in the laboratory mouse and rat. The theory and analysis of selection experiments, using results from the laboratory mouse, are also covered in the book by Falconer (1960a).

1. Selection for body weight

Several experiments on selection for body weight in mice have been described and these are summarised in Table 1.

TABLE 1

Summary of selection experiments for body weight in mice

Reference	Base Population	Type of Selection	Realised h^2	Selection Limit	Remarks
Goodale (1938, 1941)	5 male and 11 female albino mice.	Progeny test. One male mated with about five females. Selected for body weight at 60 days of age.	Not available. Response = 0.6 gm/generation.	Limit reached after about 28 generations. Males then averaged about 43 gms c.f. the starting point of about 25 gms.	No control line kept
MacArthur (1944a, 1949)	Six inbred strains crossed.	Selection for high and low 60-day weight. Combination of mass and sib selection with some progeny testing. Mating at random, each male with several females.	Realised h^2 declined from 25% to 10% over 21 generations. (Response measured as divergence between high and low lines). 60% of total change in first 7 generations.	Limit after about 23 generations. Large line males then about 38 gms, small line males averaged about 12 gms. Divergence of 21 times the original genetic standard deviation.	Two-way selection also provided in-built control. Asymmetry of response - greater response upwards. Due to change of variance with the mean. Number of correlated responses noted.
Falconer (1953, 1955)	Four inbred strains crossed (N-strain).	Selection for high and low six week weight. Within litter selection - one male and one female from each family. Six pairs mated per generation at random with no sib mating. Litter size standardised to eight mice.	Realised h^2 20% for upward selection and 50% for downward. Realised h^2 calculated from the divergence was 35%.	Response ceased in both lines after about 20 generations. Large line about 28 gm and small line 12 gm. Divergence of 16 times the original genetic standard deviation.	Correlated responses noted. Causes of asymmetrical response discussed. Further discussed by Falconer (1960a)

TABLE 1 Contd...

Reference	Base Population	Type of Selection	Realised h^2	Selection Limit	Remarks
Rahnefeld *et al.* (1963)	Reciprocal cross of two inbred strains.	Mass selection for growth between 3 and 6 weeks of age.	Realised h^2 about 18%. Heritability estimates varied from 22% to 26%.	Progress still being made after 17 generations. Change in mean growth 4 to 5 gms - six times the original genetic standard deviation.	Highly inbred line as a control population.
Sutherland *et al.* (1965) (abstract only).	Three selection lines with diverse origins plus a control line.	Mass selection for gaining ability between 4 and 11 weeks of age.	Realised heritabilities were 24%, 27% and 30% in the three lines.	Only ten generations of selection reported. Limit not yet reached.	Generations 10 to 14 selection for efficiency food consumption and gain, respectively.

In other experiments large and small strains of mice have been crossed to examine further inheritance of size in the mouse.

Butler (1952) made five crosses between mice of different body size, which included MacArthur's and inbred strains. In all five crosses the F_1 and F_2 means were intermediate between the parents. The backcross means were half-way between the F_1 and the respective parent. Butler concluded that on a gram scale at least part of the factors which affect body size are proportionate rather than additive.

Chai (1956a, 1956b) used the same approach and came to similar conclusions, although using completely different strains in his crosses.

Lewis and Warwick (1953) also utilized MacArthur's lines in an experiment where the large and small lines which had reached a limit, were crossed with an unselected control line from the same strain. Selection for large and small 60 day weight was effective over five generations in both inbred and outbred populations derived from common parent stocks. The realized heritability was only slightly lower in the inbred line (42% and 37% respectively). Presumably an infusion of genes from the base population was responsible for the renewed response. This was also found by Falconer and King (1953) who crossed MacArthur's and Goodale's lines which had reached a limit and obtained a renewed response to selection for body weight.

These selection experiments reviewed here, although employing different methods of selection, different base populations and different management procedures, have shown that selection for body weight is feasible and that a realized heritability in the range of 20% to 40% is usually obtained.

Falconer (1955, 1960a) has estimated that many genes of approximately equal effects contribute to the additive genetic variation of body weight in mice. Asymmetry of response is a fairly regular occurrence in two-way selection experiments, but it is often difficult to differentiate between various possible causes of asymmetry of response (Falconer 1960a).

Heritabilities are a function of a particular population and a particular environment (Lush 1945, Falconer 1960a). Thus responses to selection should not be expected to be the same in different selection experiments. However, allowing for the many differences outlined above, the responses to selection for body weight in different experiments have been remarkably similar.

2. Genotype-environment interaction involving weight

A genotype-environment interaction occurs when the ranking of a number of genotypes changes when the environment changes.

The existence of genotype environment interaction may mean that the best genotype in one environment is not the best in another environment. This is of importance in animal breeding, since the prevalence and intensity of genotype-environment interactions will determine the degree to which animals will need to be selected in the environment in which they are to be used.

Hammond (1947) suggested that animals should be bred in an optimum environment for the maximum expression of their genotypes. He further postulated that animals so selected would still be superior when transferred to poorer environments. At this time there was no objective evidence to judge the validity of Hammond's premises.

Falconer (1952), in a theoretical study of the problems of environment and selection, pointed out that, if only two different environments are considered, then genotype-environment interaction may be expressed as a genetic correlation. Thus performance in the two different environments can be regarded as two different characters which are genetically correlated. The theoretical conclusion was that the expectation of a great increase in heritability would be the only justification for favouring selection in an environment other than the one in which the improved breed was required to live.

Falconer (1952) only considered two different environments, but Robertson (1959) and Dickerson (1962) have extended the theory to the case of more than two environments. It is now more convenient to estimate the average degree of genetic correlation from the ordinary components of variance for genotypes and for interaction. The errors involved in this method and the optimal structure for analysis are discussed by these authors.

McBride (1958), who has reviewed genotype-environment interactions as they affect animal breeding, has noted that two main experimental approaches have been used to study genotype-environment interactions, which he called the static and dynamic approaches.

The static approach screens the normal range of genotypes found in a population in two or more environments.

However the importance of the environment in selection studies can only be answered conclusively by actually selecting in different environmental conditions, that is, a dynamic approach.

(i) Dynamic approach

The different selection experiments for body weight in different environments also lend themselves to tabulation (Table 2).

The general conclusion is that animals should be selected in the environment in which they are to live, which is in agreement with Falconer's theoretical conclusion. But the asymmetrical correlated response, found in both Falconer's experiments, was not expected. Falconer (1960b) found that over the first few generations the results were in reasonably good agreement with the theory and suggested that the discrepancies probably arose from changes of the genetic parameters which took place during the course of selection.

Bohren, Hill and Robertson (1966) have also studied this problem using both algebraic treatment and computer simulations. They suggest that any symmetry found in correlated responses is perhaps more surprising than asymmetry.

(ii) Static approach

As well as the dynamic approach, a number of workers have used the static approach.

TABLE 2

Summary of selection experiments for body weight in different environments

Reference	Base Population	Type of Selection	Environments	Realised h^2	Change of Environments	Remarks
Falconer and Latyszewski (1952)	Four inbred strains crossed (N-strain)	Selection for high six week weight. Selection within litters. Mating at random with no sib mating. Six single pair matings per generation. One male and one female from each family. Litters standardised to eight mice.	One strain fed ad lib. while the other restricted to about 75% of normal intake, from weaning at 3 weeks until 6 weeks of age. Restricted diet reduced 6 week weight by about 10%.	29% on restricted diet and 20% on full diet. Selection over eight generations.	Made after 5, 7 and 8 generations of selection. When reared on full diet the full diet strain were just superior to the restricted diet strain. On the restricted diet the restricted diet strain were superior, the full diet strain showing no improvement over the unselected level.	Conclusion was that should select animals in the environment in which they are to live. But asymmetrical correlated response not explained. Also some correlated responses noted.
Falconer (1960b)	C stock - constructed from crosses between Bateman's high lactation line, MacArthur's and Goodale's large lines and the C57BL/Fa. inbred strain.	Two-way selection for growth of mice between 3 and 6 weeks of age. Within litter selection as above. 12 single pair matings in each line.	One line fed normal diet ad lib. Other line fed normal diet ad lib. Diluted with 50% digestable fibre. Low diet fed from 3 to 6 weeks of age - reduced growth by about 20%.	Upward selection - highplane 26%, low plane 31%. Downward selection - high plane 42%, low plane 25%. Calculated up to generation seven.	Thirteen generations of selection - each generation growth of all four lines measured on high and low planes. Selection for increased growth - best 'all-round' performance from selection on low plane as before. Selection for decreased growth reduced growth on high plane only by selection on high plane, while growth on low plane was reduced equally by selection on high and low planes.	General result - select in the environment in which animals are to live. If good performance under a variety of conditions is desired, then selection should be made under the conditions least favourable to the desired expression of the character. Asymmetry of response between up and down lines.

TABLE 2 Contd...

Reference	Base Population	Type of Selection	Environments	Realised h^2	Change of Environments	Remarks
Park et al. (1966)	Four highly inbred lines of albino rats crossed.	Mass selection for post weaning gain (3-9) weeks of age). 20-30 single pair matings per line. Litter size standardised to six. Also random bred control line.	Three feeding regimes - (1) Ad lib. feeding of standard diet (full feeding - F.F.) (2) Restriction of feed intake to ¾ or less of F.F. (low feeding - L.F.). (3) Ad lib. feeding of diet with only 14% protein (low protein - L.P.)	F.F. - 10.5% L.F. - 5.7% L.P. - 11.9%	Selection for 17 generations. Exchange of environments in generations 4, 5, 8, 10, 11, 13 and 17. When compared in each regime, the selection line developed in that regime tended to give a larger response than those developed in other regimes.	Some evidence that F.F. line more adaptability in other environments than L.F. of L.P. lines. L.P. line only selected from generations 1-6 and generations 11-17.
Dalton and Bywater (1963)	4 inbred strains crossed.	Selection for litter size and litter weight at weaning (25 days of age). Largest whole litters selected and whole litters who were heaviest at weaning. Mating at random - no sib mating. Also random bred control line in each diet.	Normal diet and diluted diet (30% cellulose added) fed ad lib.	Litter size - 6% normal diet -2% diluted diet. Litter weight 6% normal diet -2% diluted diet. High correlation between litter size and litter weight.	No significant response was obtained to selection for litter size or litter weight. The diets used did not bring about a differential response to selection for the traits and the response to the dietary switch was small and temporary in its effect.	Due to strong maternal effects and low h^2, no selection response for litter size or litter weight at weaning in these mice.
Korkman (1961)	Wild agouti-type mice originally captured prior to 1957 and selected for large and small sex difference. These two strains crossed.	Within litter selection for 40-day body weight. 15 pairs mated per generation. Least related mice mated.	Normal diet - mice bread and wheat germ ad lib. Low plane-feeding every alternate day. Very low plane of nutrition.	High plane - 22%. Low plane - 7%. Low plane mice averaged 12 gms at 40 days of age and high plane mice 19 gms.	Selection for 18 generations. Changes of environment in 7th, 10th, 13th and 16th generations. Mice selected in their environment superior to mice selected in other environment and tested in new environment.	Conclusion - performance best improved by selection in that plane of nutrition in which the performance is subsequently to be measured.

Young (1953) studied genotype-environment interactions in the growth rate and reproductive performance of three strains of mice when kept in two food and two temperature environments arranged in a factorial design. The results were largely negative, but three positive interactions were found.

Bakels (1963) tested four sire progeny groups of albino mice on two diets varying in protein content. The 15 day litter weight was measured and then corrected for weight of dam immediately after parturition and litter size. Analysis of variance using the corrected litter weights revealed that there were no significant interactions between sire groups and diets.

Barnett and Scott (1963) showed that inbred strains of mice A and A2G, born and reared at an environmental temperature of -3°C, were lighter at the ages from 3 to 16 weeks than were the controls at 21°C. But the body weight of mice of strain C57BL was unaffected by the low temperature. Also the growth of two F_1 strains of mice was not depressed by the cold.

Barnett and Coleman (1960) revealed an enhancement of heterosis in the cold environment. F_1 mice obtained by crossing A2G and C57BL mice were found to be more fertile than either parent at 21°C and -3°C. But the difference was much greater at -3°C where about five times as many mice were reared by the F_1 mice, as by the inbred lines, while at 21°C only twice as many mice were weaned by the F_1 parents.

Harrison (1963) showed that growth was depressed in a hot environment (32°C) in two inbred strains and the F_1 hybrid.

But the magnitude of this depressing effect differed in the different strains, the F_1 hybrid being least affected. The difference in the growth of the hybrids and inbreds also showed that, at least for the inbreds, a high temperature was favourable for growth in young animals, but unfavourable for mature mice.

Cockrem (1963) exposed two strains of mice differing in body weight-tail length ratio to three temperatures (7, 21 and 32°C). For both strains and sexes of mice, the cold (but not the hot) environment depressed body weight growth. There were no interactions and the effect of the temperatures was proportional in both strains of mice.

Bigham (1965) studied the responses of four strains of mice in the same three temperatures that Cockrem used. Eight different characters of the mice were studied, which included body weight and tail length and the relationship between these characters. Rate of body weight growth (3 to 6 weeks) differed between the strains in each temperature environment. It was concluded that differences were present between the strains in the way in which they adapt to high and low environmental temperatures.

To obtain a general picture of the significance of genotype-environment interactions would require very many strains and environments to be tested as Young (1953) has noted. The conclusion from these static approach experiments would seem to be that, unless a severe modification in the environment is encountered, genotype environment interactions for body weight in mice are not very important.

Park et al., (1966) have also noted that any generality in deductions made from different selection experiments investigating selection in different environments are limited by the specific conditions of each experiment.

Table 2 reveals that to date selection has only been studied in different nutritional environments. As in practice different environments are as much due to differing climate, as well as differing nutritional levels, any results of selection in different temperature environments could be interesting.

3. Correlated responses to selection for body weight

Falconer (1960a) and Lerner (1950, 1958) have discussed the theoretical treatment of correlated responses and phenotypic and genetic correlations. Lerner (1958) has pointed that there is a large volume of literature noting that correlated responses have occurred, but very often these studies have been in a form that could not be critically examined.

Falconer (1954) used a selection experiment with mice to check on the validity of the theory of genetic correlation. Two-way selection was made for weight at six weeks in one pair of lines and for tail length at the same age in another pair. The responses and correlated responses of both characters were observed in both pairs of lines. Selection was made within litters and response (measured as the divergence between the upward and downward lines) was observed over six generations.

Estimates of the genetic correlation between body weight and tail length, calculated separately for the two pairs of lines, were 0.62 and 0.57. Falconer therefore concluded that this good agreement showed that the theoretical treatment of genetic correlations adequately accounted for the correlated responses to selection actually observed.

Cockrem (1959) also used body weight and tail length in mice in a selection experiment to test whether strains of mice could be produced with opposite relationships to those predicted by the genetic correlation. Genetic correlation estimates in Cockrem's base population were 0.66 for males and from 0.77 to 0.92 for females which were in reasonable agreement with Falconer's estimates. Selection was then made on the deviations of the actual body weight at six weeks from the expected values. The expected body weight was estimated from the observed length of the tail by use of the phenotypic regression of body weight on tail length. Lines for positive and negative deviations were selected and reported after six generations. A definite response was observed and two lines of mice, L.C.A. (high body weight - short tail) and L.C.B. (low body weight - long tail) were formed. It was concluded that the presence of a genetic correlation does not preclude the formation by selection of different combinations of correlated characters.

Cockrem's results could be interpreted as being due to either pleiotropy or linkage of the tail length and body weight genes. Linkage is less likely to occur between any two genes in mice than drosophila, as mice have a greater number of chromosomes. However, Roberts (1967) concluded that limits to artificial selection for body weight in the mouse may be due to linkage in the lines he studied.

But pleiotropy, the condition in which a single gene influences more than one character, is more likely to explain Cockrem's results. If indeed a proportion of the genes are pleiotropic for tail length and body weight then further selection for tail length alone should give the correlated response of body weight which would not occur if linkage was the cause of the correlation. Experiments to test this hypothesis have not yet been analysed. There is also the possibility that pleiotropic genes may be influencing the two characters in the opposite direction. Thus, although overall there is a positive correlation, there may still be some genes contributing a negative covariance.

Most of the selection experiments already reviewed have noted correlated responses associated with selection for body weight. The selection lines once formed have also provided ideal material in which to study these correlated responses in more detail. These correlated responses are summarised in Table 3.

A large variety of correlated responses to selection for body weight have been noted and the detailed study of some of these correlated responses (Fowler 1958, 1962, Fowler and Edwards 1960, and Edwards 1962) has demonstrated that several physiological mechanisms have been affected during the course of selection for large or small body size.

As well as Hull's (1960) and Fowler's (1958) results on carcass composition showing that differences can occur in selected lines in fat percentage, Fenton (1956) has also obtained a similar result. He studied two strains that both laid down fat at a similar rate until 6 weeks of age. Then the C57BL/FN strain increased its percentage of fat in the carcase

TABLE 3

Summary of correlated responses to selection for body weight in mice

Reference	Selection Experiment	Correlated Responses
MacArthur (1944b)	Two-way selection experiment	Large line was more docile and inactive than the small, had comparatively shorter ears, feet and tail, were more economical in gains from food eaten, developed faster, bred a little earlier, had a higher ovulation rate and produced more young per litter. Falconer and King (1953) noted that while Goodale's mice were large-bodied but not very fat, MacArthur's large line were smaller in linear dimensions, but were very fat.
Falconer (1953, 1955)	Two-way selection for 6 week weight.	Compared with the small line, the large line had longer tails, had higher twelve-day and three-week weights, had a larger litter size and were less active. The number of fertile matings and post-natal viability fell in both lines.
Falconer and Latyszewski (1952)	Selection for 6 week weight on high and low planes of nutrition.	Fertility increased in both strains. 12-day litter weight (milk yield) remained steady in the first lactations, but in the second lactations it declined in both strains. The fat content of the mice of the full diet strain was about 24% greater than the restricted diet mice after 8 generations of selection, when both reared on the full diet.
Falconer (1960b)	Two-way selection for post-weaning growth on high and low planes of nutrition.	The mice produced by selection for increased growth on the low plane, but later reared on the high plane, were compared with those produced by selection on the high plane. Their growth was the same, but they were heavier, had less fat and more protein, and were better mothers.
Fowler (1958)	Studied growth and carcass composition of Falconer's large and small N and C strains.	Large lines fatter at 6 weeks of age than the small lines and this difference increased with age. For similar weights or ages the amount of fat was lower in the C strain than in the N strain. Cause of this strain difference may be due to different genes present in the foundation population and the slightly different criteria of selection.
Roberts (1961)	Studied the lifetime growth and reproduction in some of Falconer's large and small strains. 6 lines studied.	The two large strains both attained similar mature weights, but these were attained at different ages, one at six months the other at one year. Thus genetic differences may occur in the shape of the growth curve. Mean life span of small strains exceeded that of large strains by about 6 months. The large strains had a shorter reproductive life, producing on average only 4½ litters, against approximately 11 in the small strains. Thus small strains eventually weaned almost twice as many offspring as the large strain.
Fowler and Edwards (1960)	Studied fertility of mice in large and small N and C strains.	Confirmed that large mice had a higher ovulation rate, about twice that of the small mice. Egg number correlated with body protein rather than total body weight. Considerable infertility in both large and small N strains, while fertility of C strains were not affected by selection. Large N strain sterility due to low libido of the males and not to female infertility. In small N line sterility probably due to hypo-functioning of the anterior pituitary of some females.

TABLE 3 Contd...

Reference	Selection Experiment	Correlated Responses
Edwards (1962)	Studied size and endocrine activity of the pituitary in large and small N strain and mice related to strain C.	The pituitaries of large mice were larger than those of small mice at all ages. But the weight of the pituitary per unit of body weight was identical in both large and small mice. No differences were detected in the unit potency (endocrine activity per unit weight of pituitary tissue) of gonadotrophins in the pituitaries of large and small mice. Also no difference in the rate of thyroid secretion.
Fowler (1962)	Studied the efficiency of food utilization, digestability of foodstuffs and energy expenditure of mice in large and small N strain.	Large mice consumed more food and utilized it more efficiently during the period of most rapid growth than small mice. Large mice absorbed a greater proportion of protein but this did not account for the weight difference between the large and small lines. Energy expenditure of large mice was greater than small mice at all ages, but similar for the same body weights.
Hull (1960)	Three lines of mice were selected for five generations for high body weight at 3, $4\frac{1}{2}$ or 6 weeks of age.	The proportion of fat in the carcasses of the selected animals increased markedly in the line selected for high 3 week weight, while in the other two lines the proportion remained the same as that in the control line. Not expected result from theoretical deductions. Not yet fully explained.
Rahnefeld et al. (1962)	Selection for post weaning growth in mice.	Estimated the genetic correlation between body weight and litter size:- 0.153 (not significantly different from zero). Actual genetic change in litter size during 13 generations of selection was 0.082 ± 0.035 mice per generation.
Sutherland et al. (1965)	Selection for gaining ability in mice from 4-11 weeks of age.	Over ten generations of selection total feed consumed increased by about 10%, so that grams of feed required per gram of gain was reduced by about 50%.

quite markedly, while the I/FN strain showed no such increase.

Dickerson and Gowen (1947) studied hereditary obesity and efficient food utilisation in the mouse and their results emphasized that a distinction needed to be made between - "the hereditary association of increased fat deposition with lower feed requirements per unit gain in weight, and the developmental association of increased fat deposition with higher food requirements."

Although not selecting directly for body weight in mice, Barnett (1961, 1965) selected a mixed stock of mice for fertility for twelve generations at -3°C. A control line at 21°C was not subject to any deliberate selection. A response to selection was obtained and was accompanied by a possible genetically determined increase in body weight, but a decline in abdominal adipose tissue.

Thus amongst other correlated responses observed selection for an increase in body weight in mice results in an increase in tail length and fertility and a change in carcass composition. There is some evidence that correlated responses observed in different environments (Falconer and Latyszewki 1952, Falconer 1960b, Barnett 1965) may be different from those observed in standard experimental environments and that changes may have occurred at the physiological level.

B. FERTILITY AND MATERNAL EFFECTS IN THE MOUSE

1. Maternal effects

Analysis and interpretation of selection experiments may be profoundly affected by maternal effects. A brief summary of this non-genetic source of variation as it affects both body weight and litter size is given here. It has already been noted that Falconer and his associates have used a 'within-litter' method of selection to avoid the complications of maternal effects.

Growth of young mice may be affected by both prenatal (uterine environment) and post-natal (mothering ability) maternal effects. Butler and Metrakos (1950) demonstrated the importance of post-natal maternal effects in a cross-fostering experiment with three strains of mice. Chai (1956a) estimated the maternal effect on body size in several F_1 hybrids to contribute more than a quarter of the total variation in body weight at 60 days, which was a larger source of variation than was the genetic constitution of the hybrids. Brumby (1960) and Bateman (1954) concluded that the prenatal maternal effect is greater than the post-natal maternal effect. Brumby found that maternal effects in body weight persisted until about 3 months of age.

Bateman (1963) mass selected mice for high and low five week body weight for ten generations. He reasoned that family selection, and to a lesser extent individual selection, could give qualitatively different results from within family selection by procuring heritable maternal effects. The responses of the selected lines could be accounted for if one quarter of the maternal effect stemmed from maternal five week weight and maternal genotype (selected through family differences which constituted two-thirds of the superiority of selected individuals) determined the remainder.

It has been a fairly common observation in selection experiments with mice (Falconer (1953), MacArthur (1949)), that large mice usually produce large litters and vice versa. From this association a maternal effect on litter size can arise. Thus when the large mice produce large litters, the weights of the offspring in the litter are depressed. When the daughters from this large litter are mated they tend to produce smaller litters because of their lower body weight, but now the offspring in their litters will tend to be larger. This cyclic phenomena has been studied by Falconer (1955, 1960c, 1964) who has calculated estimates of the standardized partial regression coefficients relating mothers litter size, daughters six week weight and daughters litter size.

Monteiro and Falconer (1966) have further investigated maternal effects in mice in relation to compensatory growth.

Even from this brief examination of maternal effects it is plain that they may be an important factor in selection experiments with mice, and may overshadow the true genetic situation.

2. Fertility in the mouse

The usefulness of selection experiments is limited if fertility is impaired, as the intensity of selection is affected by the reproductive rate.

Roberts (1965) has reviewed the literature dealing with the genetic analysis of litter size and fertility in mice. He notes that fertility data is a by-product of most experiments with mice. The correlated responses of various aspects of fertility on selection for body weight in mice have already been mentioned (Table 3).

The effect of different temperatures on fertility in the mouse must also be considered.

Laurie (1946) found colonies of mice were able to live and reproduce in meat cold stores kept at temperatures of -10°C to -20°C.

Barnett (1965) has reviewed reproductive adaption to cold in mice and concluded that cold evokes a general slowing of the processes of reproduction in female mice. Barnett's inbred strains could be maintained as breeding stocks at -3°C (except for strain GFF). The main effects on reproduction were the delay in the onset of breeding, high infant mortality, a greater variation of performance, longer oestrous cycles, the mean interval between parturitions was increased, litter size was sometimes reduced and in some strains there was a longer reproductive life (Barnett and Manly (1956, 1959), Barnett (1956), Barnett and Coleman (1959)). These parameters of reproduction were influenced in different ways in different strains. Reproductive performance at 10°C was similar to that at 21°C for all strains (Barnett and Manly (1956)). Barnett (1965) also found a progressive decline in nestling mortality in the cold which he suggested was the result of a cumulative maternal effect.

Biggers et al., (1958) exposed TO mice to temperatures of 5, 21 and 28°C when they were pregnant (approximately 12 days of pregnancy). The average litter size at birth (live + dead) were 7.04, 7.25 and 8.06 for the hot, cold and temperate mice respectively. The litter sizes in the hot and cold rooms were more variable than those in the temperate room, significantly so in the case of the cold mice. Post-natal mortality was greater in the extreme environments than in the temperate and greater in the cold than the hot.

Ogle (1934) found that white mice subjected to a warm humid environment (32°C) showed a low percentage of matings that resulted in pregnancy, small litter size and low viability of offspring compared to mice at 18°C. Sundstroem (1922) found that albino mice kept at 31°C and 21°C had similar litter sizes but the conception rate was slowed down in the hot environment. Sumner (1909) subjected white mice to 26°C and 7°C and found that mortality was higher in the lower temperature. However, fertility in this line of mice was very low at both temperatures.

Thus extreme temperatures (both hot and cold) may have a deleterious effect on reproductive performance in the mouse.

C. PHYSIOLOGICAL ASPECTS OF ACCLIMATION TO HEAT AND COLD

1. Terminology

The following definitions of terms relating to thermal experience are used in this review and thesis. They are among those suggested by Hart (1957) and endorsed by Eagan (1963) to avoid confusion arising from their variable meanings in the past.

The term acclimation is used to describe the resulting alterations in the animal when temperature is the only variable.

Acclimitization is the term used when modifications induced by climate are considered. There are now multiple factors to be considered including season, latitude, temperature, humidity etc.

Adaptation is reserved to designate racial or species differences in relation to climate and inherited differences over many generations may be involved.

2. Acclimation responses

Although the principle interest in this review is the effects of selection in various environments, acclimation will also be of importance in the interpretation of the results of experiments of this kind. Also of interest are the relationships between body weight and tail length in the mouse with special reference to the possible function of the tail as a thermoregulatory organ. Therefore a summary has been made of the acclimation responses of small homeotherms to both heat and cold.

The changes taking place during cold acclimation have been reviewed many times (Hart 1952, 1958, 1961, 1964, Smith 1960, and Heroux 1961a). Barnett (1965) and Bigham (1965) have reviewed acclimation with regard only to small homeotherms, that is rats and mice. While Barnett (1965) only reviewed acclimation to cold, Bigham (1965) reviewed acclimation to heat and cold and notes that less information is available dealing with acclimation to heat. Schmidt-Nielsen (1964) discusses the life of desert animals and the different morphological and physiological adaptions developed by animals in hot, desert environments to maintain water and heat balances. In 1963 an International Symposium on Temperature Acclimation was held (Smith (1963)), which followed the International Symposium on Cold Acclimation (Smith (1960)), already mentioned. In 1966 an International Symposium on Metabolic Adaptions to Temperature and Altitude (Smith (1966)) was held.

It would be quite impossible to even attempt to summarise these numerous and extremely comprehensive reviews. However, they contain essential background information for this review and the following comments are pertinent.

It is now evident that the climatic adjustments observed are not identical in every respect with those induced in the laboratory by uninterrupted exposure to a constant temperature (Heroux 1961, 1963, Hart 1961, 1964). Cold acclimation of mice and rats in the laboratory would appear to be purely metabolic, with little change in thermal insulation. When exposed outdoors to the natural fluctuating environment however, both metabolic and insulative changes are observed.

Bigham's (1965) review of acclimation in small homeotherms showed that the adjustments that occurred during cold acclimation were: a high metabolic rate was maintained, a large increase in food consumption, a reduction in body growth, total deposition of body fat and tail length growth, a possible increase in the weight of hair grown, but no change in the weight of the pelt, and little change in the insulative value of the pelt and hair.

The evidence for the responses of rats and mice to heat appear contradictory and Bigham concluded that basal metabolic rate may be decreased, possibly as a result of a general reduction in voluntary activity. Other associated changes may be: a change in the growth rate but results are contradictory, an increase in tail length growth, little or no effect on the amount of body fat deposition or hair weight growth, while food consumption may be decreased.

As well as studies on metabolic and insulative changes, extensive work has been done studying acclimation responses at the physiological, endocrine and cellular levels (Depocas 1961, Potter 1958, Smith and Hoijer 1962).

3. Body weight - tail length relationships in the mouse

There is ample evidence that both body weight and tail length growth are affected by temperature. There is also a fairly strong positive genetic correlation between body weight and tail length in the mouse (Falconer, 1954).

The changes that occur in body weight and tail length on exposure to extremes of temperature have been postulated to have adaptive value.

The climatic rules of Bergmann (1847) and Allen (1906) (cited by Scholander 1955) suggested that animals in cold environments should tend towards large spheres with short extremities, while in hot environments smaller size and larger extremities should be advantageous. The reasoning behind these deductions was that in the cold it would be desirable to reduce the surface area relative to weight to conserve heat in order to maintain a constant internal body temperature, and vice versa in a hot environment. Scholander (1955) reviewed the evolution of climatic adaption in homeotherms and concluded that there was no physiological evidence in animals or man that Bergmann's and Allen's rules reflected phylogenetic pathways of heat-conserving adaptation. Johansen (1962a) who discussed the evolution of temperature regulation in mammals, also pointed out that body temperature itself may have adaptive value as is the case in some primitive mammals and for the camel (Schmidt-Nielsen, 1964). Thus a large activity range of body temperature may express a specialisation rather than a primitive condition as has often been thought.

Scholander (1955) admits that conservation of heat is the prime factor in adaptation to cold, but he has shown that the importance of the size of the surface area may be outweighed by underlying, less noticeable physiological factors, such as the insulation and exposure of the surface, its vascularization and vaso-motor tone, and its ability to tolerate extremes of temperature.

A similar type of reasoning to that of Scholander (1955) can also be applied to the acclimation of small homeotherms in the laboratory. However, it is important to remember that results observed in the laboratory where standard conditions apply and often inbred animals are used, may have limited generality. Theoretically, the adaptive change in total body weight in the cold would be an increase, but it is usually found that rats and mice acclimated to cold at first lose weight and then grow at slower rates than controls at warmer temperatures (Heroux 1961, Barnett 1965). Insulative adaptions are not of importance in acclimation of small homeotherms to cold, where increased metabolic rate and behavioural responses (such as nest-building) are the important responses observed (Hart 1961). Thus it would seem that any change observed in body weight on acclimation of mice to cold would have little adaptive value.

Similarly it is found that acclimation to high temperatures in the mouse influences body weight growth, (Bigham 1965, Harrison 1963, Harrison et al., 1959). Usually high temperatures reduce the growth rate, but as was found in the cold this varies with age and with genotype, and is not always the case. Again any change in body weight is probably of no adaptive significance.

Tail length is also temperature labile (Bigham 1965, Barnett 1965, Harrison 1963, Harrison et al., 1959, Chevillard et al., 1963). Thus a longer tail is observed in hot environments and shorter tails in cold environments.

Harrison (1958) showed that mice whose tails had been removed were less heat tolerant than controls. After their detailed study of tail growth in several strains of mice, Harrison et al., (1959), concluded that the longer tail of the heat reared mice, with their large surface area, absence of hair, rich vascular supply and multiple arteriovenous anastomoses, functions as an important heat regulatory structure.

Barnett (1965b) confirmed that low environmental temperature diminished tail growth in both inbred and hybrid strains. However, he also found that a mixed stock of mice (derived from 4 inbred strains) which was selected to produce large litters at -3°C over 17 generations, showed an increase in tail length, both absolute and relative and nearly reached the relative length of the controls at 21°C. This result did not comply with Allen's 'rule' and in this case a superior acclimation to cold, due at least partly to selection, was not accompanied by the expected change in tail length. Barnett also cites an experiment by Wilber and Robinson (1961), who found no effect of removing the tail on the thermoregulation of rats, which is in direct contrast to Harrison's (1958) results. Barnett (1965b) concluded that these observations do not provide any evidence that the tails of mice have a thermoregulatory function in a cold environment.

Cockrem (1963) exposed his high body weight - short tail and low body weight - long tail strains of mice to 7, 21 and 32°C from 3 to 6 weeks of age. He found that in the hot the short tailed strain showed less tail length response than the long tailed strain which was opposite to what would be expected if a long tail had adaptive value in the heat. In the cold the long and short tailed strains showed a similar increase in tail length while the long tailed strain should have grown less, if a short tail had adaptive value in the cold.

Thus we have contradictory interpretations of the observation that tail length is temperature labile.

Cockrem (1963) suggested that discussions on acclimation to various ambient temperatures should be based on more fundamental characters than solely anatomical features such as tail length and body weight. The efficiency of the tail as a 'heat radiator' will depend on its surface area, its blood supply and its insulation. Periphereal tissue growth and periphereal tissue adaptation to extremes of temperature may also be important factors to consider (Le Blanc 1963, Heroux 1959a, 1959b, 1960, 1961b, 1961c). For example mitotic rates of periphereal tissues are decreased in cold acclimated rats compared to warm acclimated rats, due mainly to a large increase in the mitotic duration (Heroux 1960). In addition the animal's ability to adapt to a particular ambient temperature will depend on its ability to change its metabolism, the temperature at which it vaso constricts periphereal blood vessels and perhaps its fur and fat insulation.

Recent work by several Canadian workers have studied this very aspect in rats.

In several homeothermic animals the extremities (body appendages) have been shown to play an important role in heat loss and its regulation. Johansen (1962b) showed that subjecting muskrats to a positive heat load resulted in a marked increase in tail temperature while tail blood flow increased concommitantly by a factor of more than 400. Immersing the tail in ice caused vasocontriction and a drop in the temperature of the tail to about 0°C. Occlusion of the blood flow to the tail during a positive heat load led to severe hyperthermia and death from heat apoplexia. Due to this vasomotor tone it was concluded that the muskrat tail is an indispensible heat exchanger. Longo and Luck (1953) noted an increased blood flow in the tail of a monkey at 40°C of about 10% of the cardiac output, about twice the flow at room temperature.

Heat loss in rodents does not seem to be due to panting or sweating as in most other homeotherms. There have been very few functional sweat glands found histologically in rodents (Grant 1963, Terrent 1946, Ring and Randall 1947) and respiratory exchange is probably of minor importance. Thus vasomotor control of skin temperatures is a very likely means of heat loss and regulation in rodents.

Rand <u>et al.</u>, (1965) found that vasodilation in the rat tail occurred at ambient temperatures of 27 to 30°C, with blood flow increasing by a factor of about fifteen. Measurement of heat loss using a gradient calorimeter on the tail confirmed this vasodilation. After vasodilation it was calculated that the tail could lose up to 20% of the total heat production of the rat although its surface area is only 4 to 6% of the total body surface. Rats were also acclimated to 11, 20 and 30°C for four weeks. Acclimation to cold caused a decrease in the critical vasodilation temperature and the maximum heat loss of the tail was increased. After acclimation to 30°C no sudden vasodilation occurred up to ambient temperatures of 33°C.

This experiment established quantitatively the importance of the tail in the control of heat loss in the rat.

Rand et al., (1965) measured, both in vivo and in vitro, the effective thermal conductivity of the skin of rats. There was no significant change in the conductivity of the skin, in the fur covered areas, during acclimation to cold, which suggested that the rat does not use vasomotor control of the skin circulation to the fur covered areas in its temperature regulation.

Thompson and Stevenson (1965) confirmed Rand's observation that vasodilation does occur when rats are subjected to a positive heat load (exercise on a tread mill in this case compared to rising ambient temperature used by Rand). They also found that foot skin showed vasodilation but not skin on the back under the fur. Increasing or decreasing the speed of exercise resulted in more or less vasodilation respectively. Thompson and Stevenson (1966) also found that cold acclimated rats had a higher tail skin temperature during rest and greater vasodilation during exercise than unacclimated controls.

In concluding this section, and this review of literature, it is now clear that heat balance in rodents is most probably due to vasomotor control of the extremities and any morphological changes, such as changes in body weight and tail length, are likely to be of only minor importance. Apart from Barnett's (1965) experiment to select for increased fertility at -3°C, no other experiment is known where selection has occurred in different temperature environments. The literature reviewed here would suggest that selection response for body weight in mice is readily available. Furthermore hot and cold environments will have different effects on growth of the body and the tail in mice, two characters which are genetically correlated. If genetically different strains are established in different temperature environments, any difference in body weight - tail length relationships could most logically be explained at the physiological level.

Chapter II

MATERIALS AND METHODS

Chapter II

MATERIALS AND METHODS

A. EXPERIMENTAL ANIMALS

The mice used in this study were a sample from the LC lines established by Cockrem (1959). These lines originated from the three inbred lines:- CBA/FaMac, C57BL/FaMac and 101/FaMac.

Selection had been continued in these lines, but at the 25th generation of selection, both the LCA and the LCB lines were outcrossed to the CBA strain. At the 35th generation of selection the LCA and LCB lines were crossed reciprocally (March, 1965) and then selected for six-week body weight for two generations. Selection was then ceased and the population was random bred for a further three generations, at which stage (March 1966), they were taken over for this study.

Thus the foundation population used in this experiment was formed from a cross between the LCA and LCB lines and was a good heterogeneous stock from which to start selection.

B. HOUSING AND FEEDING

Mice were kept in three temperature environments. The mice kept in the temperate (or standard) environment were housed in a room in the Small Animal Research Unit, where the temperature was controlled at 21°C ± 2°C. As no cooling system was available, in mid-summer temperatures would rise for short periods to about 26°C. Lighting was by natural daylight and humidity was not controlled. Mice were kept in plastic boxes (12" x 6" x 4½") which were kept on wall racks.

The hot environment was provided by putting the plastic cages on four hot plates kept in the room controlled at 21°C. The hot plates were controlled at 31°C ± 3°C by a thermostat fitted into the side of a tin cage (i.e. cage temperature was controlled). Each hot plate could hold four boxes and the position of each box was changed daily to average out the variation in temperature both within and between hot plates.

The cold environment was provided by temperature controlled rooms in the Animal Physiology Unit. One of these rooms was kept at 7°C ± 1°C and the mice were kept in this room for most of the experiment. This room was completely enclosed and normal daylight hours were provided by lighting controlled from an outside photo-electric cell. Humidity was not controlled and was higher than in the other two treatments. After the experiment had been in progress for about a year (just after the fourth generation were mated) the mice were moved to a smaller room with a temperature control of 7°C ± 2°C and the daylight cycle was still provided by the photo-electric cell control. Mice in the cold environment were also kept in plastic cages and no special nesting material additional to the wood shavings bedding was provided.

In all environments food and water were provided ad libitum.

A standard pelleted feed was used and gross energy determinations of this feed, using a bomb calorimeter, gave a value of about four kilo-calories per gram.

Disease did not cause any major problems in this experiment. Infantile diarrhoea or a similar disease did occur spasmodically but this could be treated. Although infected mice usually did not die, by the time they recovered they were often quite emaciated, and these mice were not included in the generation average and were thus not available for selection. It was observed that the incidence of disease was much lower in the cold environment.

C. EXPERIMENTAL DESIGN

1. Experiment I. - Selection Experiment

Three selection lines and two control lines were established in the three temperature environments. A calendar of events is shown in Table 4. The Cold Control line was not formed until the S_2 generation and was a random sample from the Control S_1 offspring. Both control lines were maintained by random selection and then the eight males and females mated at random with the restriction of no sib mating and with the proviso that each family be represented in the next generation by at least one individual.

Selection for six-week body weight was carried out in the three selection lines. In each line the eight heaviest males and females respectively were selected and then mated at random avoiding sib mating.

In all lines two additional matings were made in each generation, which were used if deaths or an infertile mating occurred.

Male and female pairs were kept together permanently and two litters were produced from each pair, although selection was carried out on the first litters only. Litters were not standardised to a constant number at birth.

First litters were weaned at three weeks of age, ear marked, the body weight and tail length recorded and then stored with four to six mice in a cage, with males and females separate. At six weeks of age body weight and tail length were again recorded. In the cold environment weaned mice from each family were divided between different storage cages to avoid any selection for huddling.

It was attempted to keep all lines contemporary, but fertility problems were encountered in the Hot line which made this ideal impractical (Table 4). The reduction in the hot temperature from 32°C to 31°C in the S_2 generation was made to increase the fertility. In the S_o generation the Hot line was mated at 32°C. But in the S_1 generation mice were mated at 21°C and then the females only, moved back to the hot plates when they had been pregnant for about two weeks. Females were mated again at 21°C when they had weaned their first litter.

As the result of a trial held in the S_2 generation to test the difference in six week body weights of the offspring produced by female mice exposed to 31°C both before and after parturition, the management procedure in the Hot line was further altered. Thus from the S_3 generation on, female mice only were put on the hot plates four days after parturition and the offspring taken off the hot plates at six weeks of age.

2. Experiment II. - Change of environments

The design of this experiment is set out in Table 5. An extra two or three mice were randomised into each group and these were used as replacements if deaths occurred. Four to six mice were kept in each cage and where necessary numbers were made up to this with spare mice.

Ideally all fifteen subgroups should have been established at the same time. However, owing to the different times at which second litters were produced, this was not possible. Not all the second litters born were needed for this cross-over experiment (the number varying with the litter size) so mice were randomised into groups in the order they were born until the thirty mice plus spares had been reached. The Hot line did not produce any S_4 second litters until about a month after the other lines.

The identification of each subgroup (HH, HM etc.) in Table 5 will be the way in which these subgroups will be referred to in the rest of this thesis.

D. MEASUREMENTS

1. Body weight

For most of this experiment body weights were taken using two similar Avery balances, read to 0.2 of a gram. This gave an accuracy of about ± 0.4 grams. All the fourth generation mice and the cross-over mice were measured on a beam balance which read to an accuracy of about ± 0.2 grams.

2. Tail length

Tail length was measured by restraining the mouse in a specially made container, and then drawing their tails out along a ruler graduated in centimetres. Care was taken not to stretch the tails. Tails were read to an accuracy of ± 0.2 centimetres.

3. Fertility

Litter size at birth (live + dead) were recorded for both the first and second litters. Mice about to litter were looked at daily. Mice were not sexed until weaning at three weeks of age.

4. Fat analysis

The mice were killed, skinned and the fur was clipped off the pelt. The gut was removed, the food expelled and then the gut was returned to the carcass. The whole carcass and pelt was then cut into small pieces, weighed and freeze dried. Once dry the carcass was again weighed and then the ether soluble material was extracted in a soxhlet extractor using di-ethyl ether. After evaporation of the ether the residue was weighed and this ether extract was then taken to represent 'carcass fat'.

Six males and six females were analysed from each line at six weeks of age. The Hot line mice were from the S_4 generation, while the samples from all other lines were from the S_5 generation.

E. STATISTICAL ANALYSIS

1. Selection response

Selection response has been expressed in terms of the realized heritability (Falconer 1953, 1960a).

$$R = h^2S$$

Linear regression lines were fitted to the observed generation means (response -R) and the cumulated, weighted selection differentials (S). The regression coefficients and standard errors thus calculated are estimates of the realized heritability and usual tests of significance with the regression coefficients can be carried out (Snedecor 1956).

To take into account the differences in fertility of the selected parents (which means that some parents contribute more offspring to the next generation than others) the deviations of the parents from the mean were weighted according to the number of offspring they produced. The unweighted selection differential can also be calculated in the normal manner (i.e. the mean phenotypic value of the individuals selected as parents expressed as a deviation from the population mean) and a comparison of this value and the weighted value will show whether natural selection is an important factor (Falconer 1960a). The expected selection differentials can also be calculated from the table of Scores for Ordinal Data (Fisher and Yates, 1948, Table XX).

The regression coefficients calculated may be influenced by inbreeding and maternal effects and thus may not be accurate estimates of heritability. However, irrespective of its accuracy as a heritability estimate the realized heritability is probably the best empirical description of the effectiveness of selection (Falconer 1960a).

2. Inbreeding

The restriction of population size, as occurs in most laboratory experiments where space is limited, leads to an increase in homozygosis within the population.

Wright (1922) first introduced the concept of the coefficient of inbreeding (F), which he defined in terms of the correlation between uniting gametes. Wright (1931) showed that under random mating within a closed population the rate at which inbreeding increased (ΔF) is $\frac{1}{8}M + \frac{1}{8}F$ (with M males and F females respectively).

But this relationship assumes that the parents have equal chances of contributing offspring to the next generation.

However, in practice, under artificial selection, this is not the case and to allow for the fact that the parents may not have an equal probability of leaving offspring and that the distribution of family size may not be Poisson the effective population size (Ne) must be considered and not the actual population size (N) (Falconer 1960a, Robertson 1961).

The formula used by Gowe, Robertson and Latter (1959) to calculate Ne has been used in this study.

$$\frac{1}{2Ne} = \frac{1}{2\Sigma n} + \frac{N\sigma^2}{2(\Sigma n)^2}$$

N is the potential parents from which is sampled n gametes and σ_n^2 is the variance of n. In applying this formula all four different samplings of gametes which take place in reproduction must be considered (male to male, male to female, female to male, and female to female).

The rate of inbreeding can then be calculated from the Ne value.

$$\Delta F = \frac{1}{2Ne}$$

So as to be able to compare the rate of inbreeding calculated in this study with that quoted by Falconer (1953) in a similar study, one inbreeding coefficient was also calculated by the 'coancestry' method (Cruden 1949).

3. Analysis of variance and covariance

As well as looking at selection response with time it is also possible to look at differences between mean phenotypic values at one point of time using analysis of variance.

Also at this one point of time analyses of covariance can be carried out both to adjust for previous differences and look at the relationships between certain variables.

(a) Experiment I

All analyses were carried out at the fourth generation. Because the experimental design was not orthogonal two sets of analysis of variance were carried out.

Analyses of variance of tail lengths and body weights was carried out between the three selection lines. The model used was:-

$$X_{ij} = \mu + a_i + e_{ij}$$

where - X_{ij} = the individual observation.

μ = the general mean.

a_i = the three temperatures (that is fixed effects).

e_{ij} = the error term (assumed to be normally and independantly distributed with zero mean and constant variance).

i = 1 to 3.

j = 1 to n_i (that is unequal subclass numbers)

Analyses of covariance were also carried out between the three selection lines. The model used was:-

$$Y_{ij} = \mu + a_i + \beta X_{ij} + e_{ij}$$

where - Y_{ij} = the individual observation of the dependant variable.

μ = the general mean.

a_i = the three temperature effects.

β = the regression coefficient of y on x within the classes.

X_{ij} = the deviation of the independant variable from the total mean.

i = 1 to 3.

j = 1 to n_i.

To include the two control lines in the calculations, analysis of variance was carried out within a 2 x 2 table, that is two lines (selected and control) and two environments (standard and cold). The model used was:-

$$X_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where - a_1 and a_2 are the selected and control lines.

b_1 and b_2 are the standard and cold environments.

$(ab)_{ij}$ are the interactions.

$k = 1$ to a_i.

Because of unequal subclass numbers a weighted means analysis was carried out (Snedecor 1956).

The presence of interactions eliminates the additive nature of the main effects (a_i and b_j) acting together. In the presence of an interaction care must be taken in interpretation of the mean squares for main effects.

The final interpretation of any of these analyses is dependant on establishing which means differ from which. Thus providing there was a significant F test differences between main effect means were tested using Duncan's Multiple Range Test (Duncan 1955).

(b) Experiment II

Analyses were simplified in this experiment by the presence of equal subclass numbers. However, again the design was not orthogonal and all means could not be tested in one analysis. Variables in the three selection lines were analysed factorially in a 3 x 3 table.

The model used was:-

$$X_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

where - a_i are the three selected lines.

b_j are the three environment (hot, standard, cold).

$(ab)_{ij}$ are the interactions.

$k = 1$ to 5.

To include the control lines in the analysis the above model was modified to a 2 x 3 table to test each pair of selected and control lines in the three temperature environments and also modified to a 2 x 2 table (as in Experiment I) to analyse the two pairs of selected and control lines within each environment.

Covariance analysis was also carried out to adjust seven week differences for differences at four weeks (that is the time when the mice were randomised on to their respective treatments).
The model used was:-

where - $Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \beta X_{ij} + e_{ijk}$

$\beta =$ the regression coefficient of y on x within the classes.

$X_{ij} =$ the deviation of the independant variable from the total mean.

i and j varied with the particular factorial design being used.

$k = 1$ to 5.

In all tables of results where analyses are presented the probability of the differences being the result of the chances of sampling are shown as:

NS Not significant at the 5% probability level.

* Probability greater than 5% but less than 1%.

** Probability greater than 1%.

Chapter III

RESULTS FROM EXPERIMENT I

Chapter III

RESULTS FROM EXPERIMENT I

A. FERTILITY TRIAL OF MICE AT 31°C

As the first parturition was delayed by the hot treatment the mating procedure was altered during the experiment in an attempt to prevent this and thus keep the lines reasonably contemporary.

A small trial was carried out to test whether the change in management technique may have affected the six week body weight of the mice reared under this new management. In the S_2 generation mice which had been mated at 21°C were returned to the hot environment when about two weeks pregnant and also at varying periods after parturition. The offspring born were then weighed as usual at six weeks of age, and the average body weights for each group are shown in Table 6.

It was thought that the mice that were exposed to the hot environment several days after parturition, may have had a better chance of establishing lactation and perhaps lactate better than mice exposed to the hot environment prior to parturition. This would then be expressed as a maternal effect in the six week body weights of the offspring. However, as is shown in Table 6, this did not prove to be the case, for the average body weights of the offspring reared by the mothers that had been exposed to the heat after parturition were actually slightly lower than the average body weights of offspring reared by mothers exposed to the heat when pregnant.

These results, plus similar observation by Pennycuik (1966a, 1966b), seemed sufficient justification to alter the management procedure in the Hot line. Thus from the S_3 generation, female mice from the Hot line littered at 21°C and were then exposed to the hot environment four days after parturition.

B. RESPONSE TO SELECTION

1. Body weight response

Figures 1, 2 and 3 show that a response to selection for body weight has been obtained in all selection lines. The body weight changes were similar in males and females (Figures 1 and 2) so weighted means of the sexes are presented in Figure 3.

There was very little difference in the rate of response between the Cold and Medium lines where males increased in weight at about .98 grams per generation and females at about .74 grams per generation. The hot environment had a marked effect on body weight, depressing it by about two grams compared to mice in the Medium line. But although selection started from a lower level in the Hot line, this line actually increased at a slightly faster rate than the other two selection lines (1.2 grams per generation for males and .85 grams per generation for females). However, absolute body weights were higher in the Medium and Cold lines throughout the experiment and it was not until the fourth generation that the Hot line body weights

Figure 1. Response to selection for six weeks body weight. Mean body weights of male mice in each generation plotted against time.

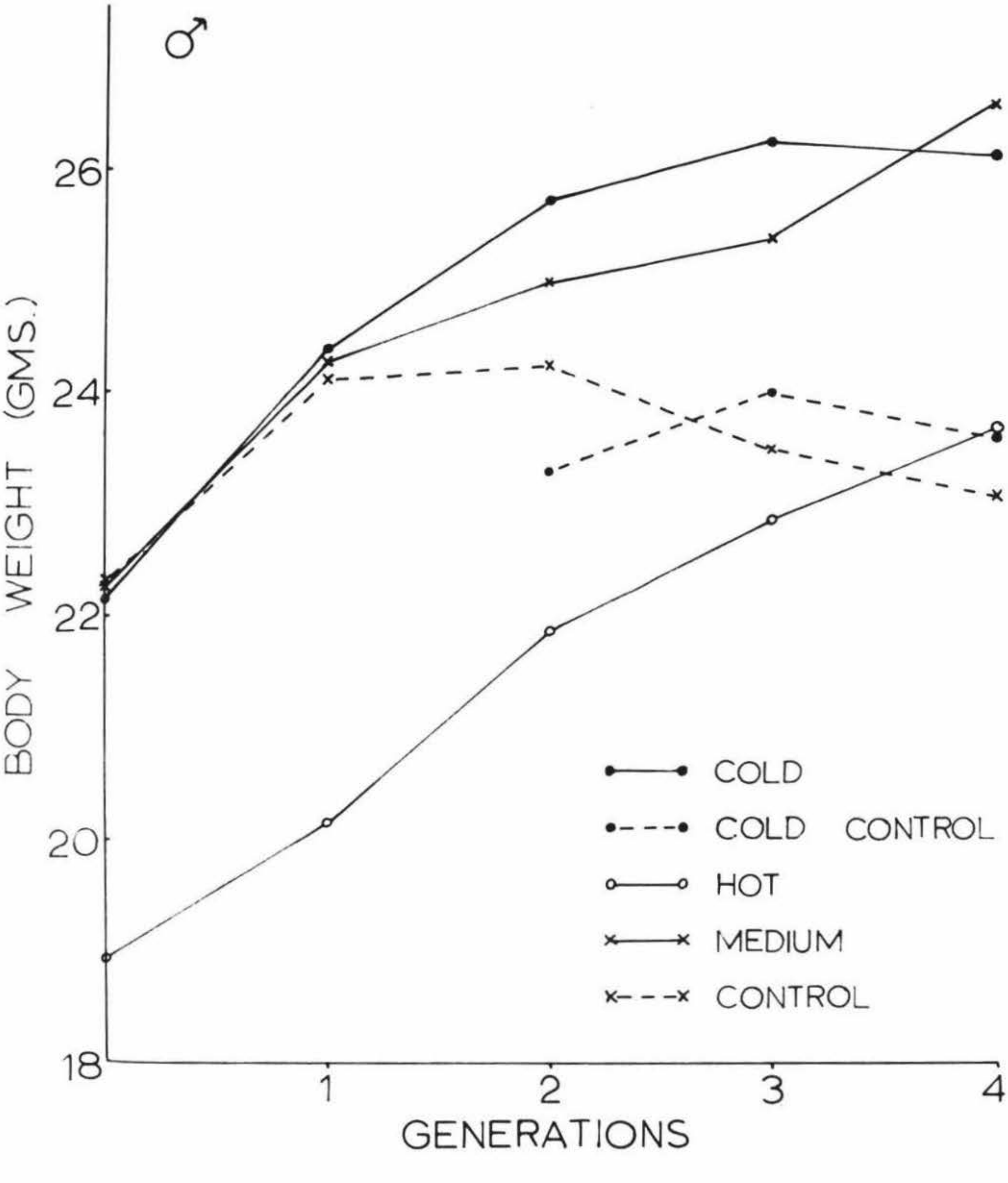


Figure 2. Response to selection for six week body weight. Mean body weights of female mice in each generation plotted against time.

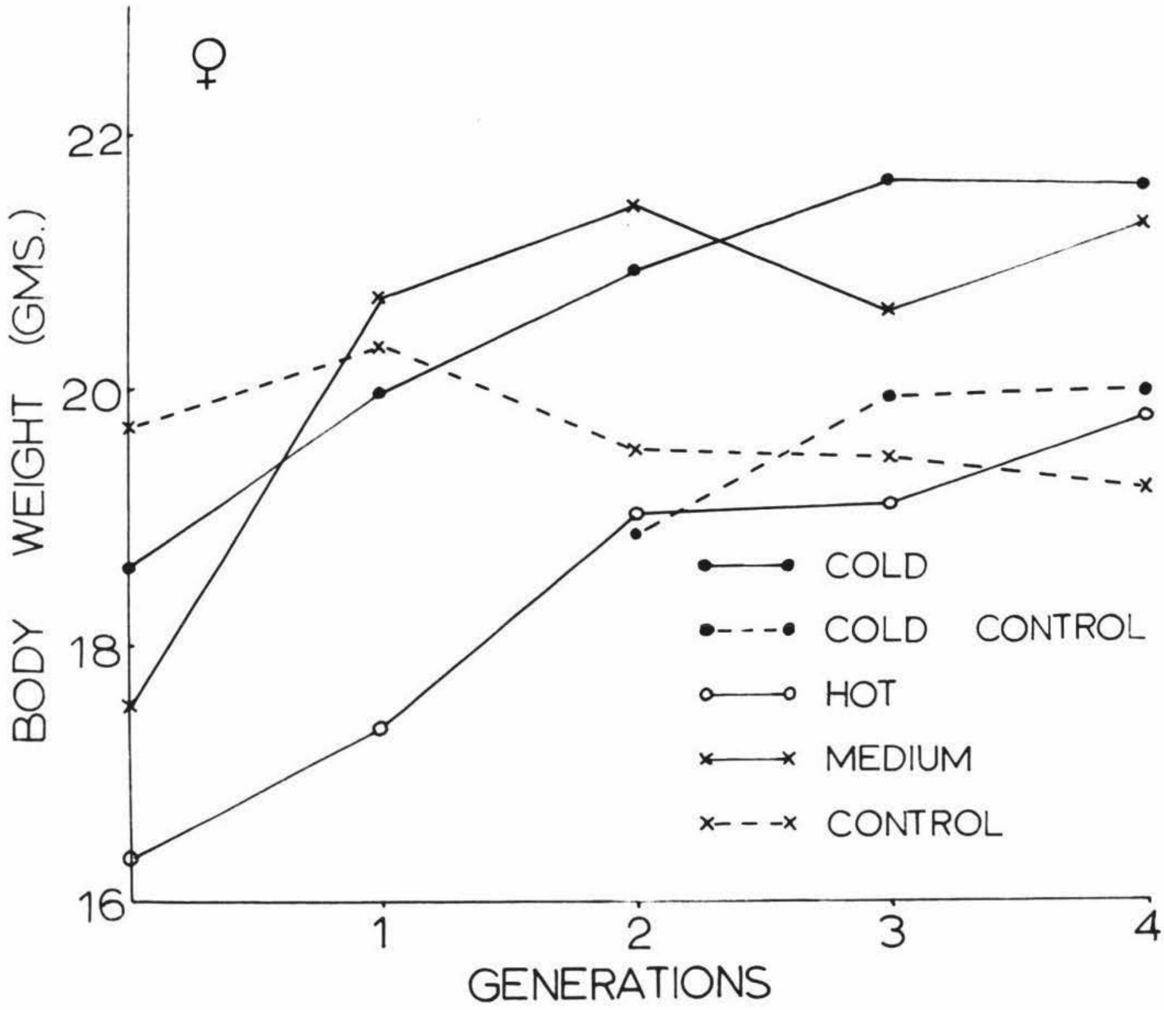
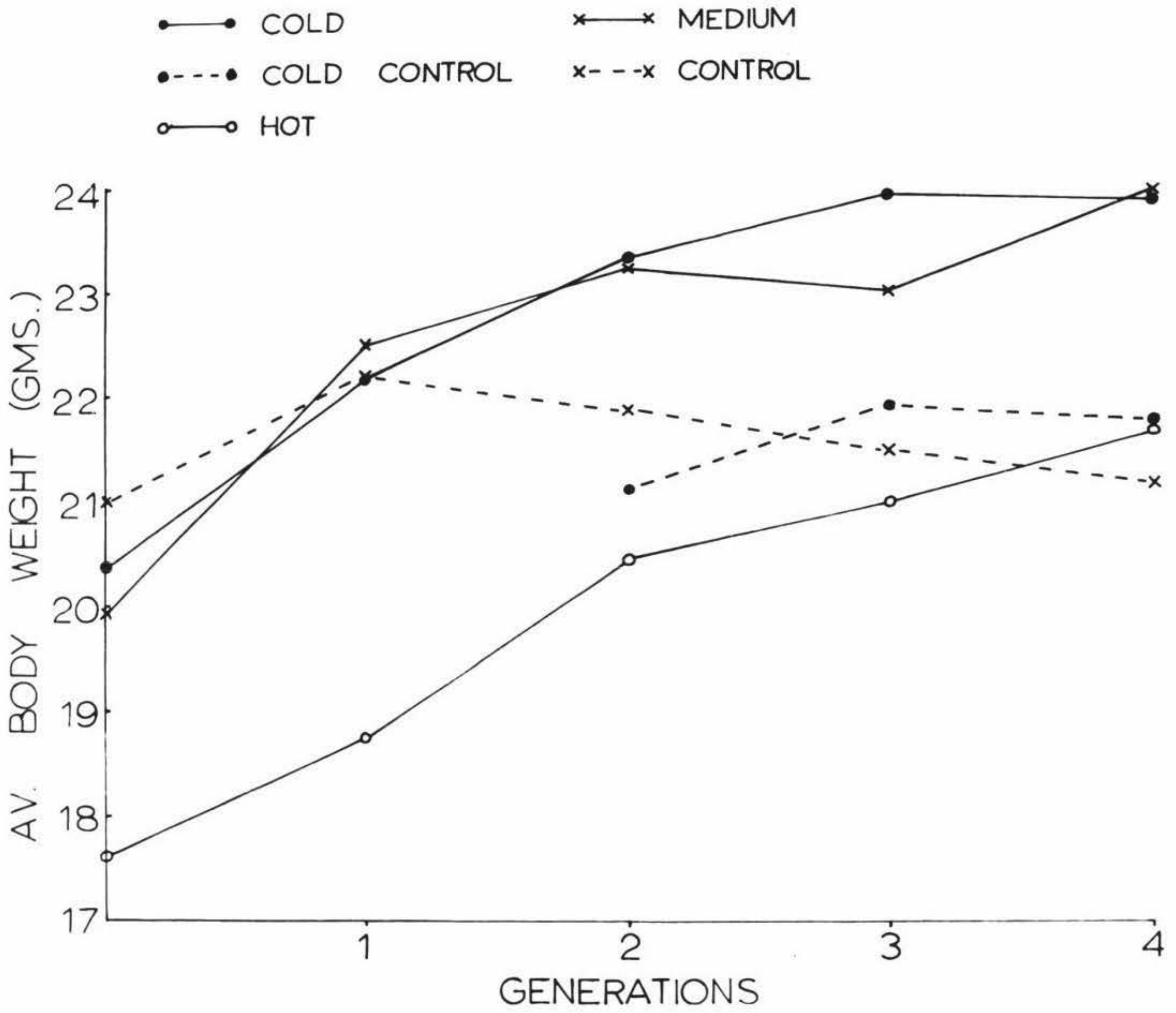


Figure 3. Response to selection for six week body weight. Mean of the sex means (weighted mean) plotted against time.



equalled those of the two control lines.

Control line body weights fluctuated but by the fourth generation had diverged from their respective selection lines.

2. Selection differentials

Response to selection depends mainly on the selection pressure applied to each line and this can be expressed in terms of the selection differential (see Chapter II, section E (1)).

The selection differentials and the resulting response are shown separately for males and females in Tables 7 and 8.

In all lines the expected and actual selection differentials (expressed in standard deviation units (σ)) are in good agreement which indicates that the distribution of weights in each line conform fairly closely to a normal distribution.

The weighted and unweighted selection differentials (expressed in grams) also agree reasonably so that natural selection, acting against artificial selection, was of no importance in any of the selection lines in this experiment.

The total selection differential applied to the Medium line was about two grams higher than in the Cold or Hot lines.

3. Realized heritabilities

By fitting a regression to the weighted, cumulated selection differentials and the body weight response (shown in Table 7 and 8) estimates of the realized heritabilities may be obtained. These are shown in Table 9 and the error variance about these regression lines

TABLE 7

Male selection differentials and selection response

			Actual			
Selection line	Generation of offspring	Expected σ^1	Unweighted σ^2	Unweighted g_3	Weighted g_4	Response g
Medium	0					22.2
	1	1.15	0.95	3.07	3.06	24.25
	2	1.29	1.18	2.80	2.87	25.01
	3	0.96	0.91	2.29	1.94	25.34
	4	1.20	1.25	1.84	1.96	26.58
	Total	4.60	4.29	10.00	9.83	
	Mean	1.15	1.07	2.50	2.46	
Cold	0					22.13
	1	0.85	0.92	2.35	2.34	24.35
	2	1.13	1.05	1.95	2.02	25.70
	3	0.96	0.95	1.33	1.39	26.21
	4	1.15	1.04	1.70	1.81	26.10
	Total	4.09	3.96	7.56	7.56	
	Mean	1.02	0.99	1.89	1.89	
Hot	0					18.93
	1	1.05	1.01	2.95	2.93	20.13
	2	1.13	1.09	1.65	1.64	21.84
	3	1.25	0.92	1.46	1.24	22.84
	4	1.18	0.96	1.49	1.65	23.63
	Total	4.61	3.98	7.55	7.46	
	Mean	1.15	0.99	1.89	1.86	

1. Calculated from the Table of Scores for Ordinal Data (Fisher and Yates, 1948, Table XX).

2. $\frac{\text{Selection differential in gms}}{\text{Standard deviation for body weight}}$

3. The deviation of the mean phenotypic value of the individuals selected as parents from the generation mean.

4. The selection differentials of the selected parents weighted according to the number of offspring they produce.

TABLE 8

Female selection differentials and selection response

Selection line	Generation of offspring	Expected σ^1	Actual Unweighted σ^2	g_3	Weighted g_4	Response g
Medium	0					17.53
	1	0.96	0.89	1.22	1.25	20.72
	2	1.13	1.10	2.11	2.18	21.42
	3	1.05	1.22	2.23	2.30	20.59
	4	1.18	1.29	1.91	2.00	21.26
	Total	4.32	4.50	7.47	7.73	
	Mean	1.08	1.13	1.87	1.93	
Cold	0					18.61
	1	1.24	1.18	1.87	1.89	19.97
	2	1.25	1.22	1.91	1.83	20.92
	3	0.81	0.85	1.06	1.22	21.60
	4	1.20	1.31	1.33	1.30	21.55
	Total	4.50	4.56	6.17	6.24	
	Mean	1.13	1.14	1.54	1.56	
Hot	0					16.34
	1	1.03	0.96	1.96	2.09	17.36
	2	1.08	1.02	1.34	1.33	19.01
	3	1.13	0.69	0.92	1.08	19.08
	4	1.15	1.01	1.40	1.42	19.75
	Total	4.39	3.68	5.62	5.92	
	Mean	1.10	0.92	1.40	1.48	

1. Calculated from the Table of Scores for Ordinal Data (Fisher and Yates, 1948, Table XX).

2. $$\frac{\text{Selection differential in gms}}{\text{Standard deviation for body weight}}$$

3. The deviation of the mean phenotypic value of the individuals selected as parents from the generation mean.

4. The selection differentials of the selected parents weighted according to the number of offspring they produce.

indicated by the standard errors. Values for males and females were calculated separately and all but two of these were significantly different from zero. In both males and females realized heritabilities were greater in the Hot and Cold lines than in the Medium line, Hot line values being greater than in the Cold line. In all three lines realized heritability estimates were greater for males than females. In the Medium line it was also possible to estimate realized heritabilities from the regression of the divergence between the Medium line and its Control line plotted against the cumulated, weighted selection differential. If the Control line is not changing genetically through random drift this should then give a more accurate heritability estimate by removing the random environmental fluctuations which will have occurred over the period of selection. As shown in Table 9 this resulted in a higher realized heritability in the males and a lower nonsignificant heritability in the females. As the Cold Control line was not formed until the second generation of selection no meaningful result was available from a divergence estimate of heritability because of the short period in which this line was functioning.

It is also possible to test if the difference between these realized heritability estimates are significant (Table 9a). Analysing male and female estimates separately no significant difference was obtained. However, as the differences between estimates were similar in both males and females the estimates were pooled by combining the sexes (Table 9b) and the F test was significant at the 5% level.

Although pooling the regression values in this manner does show a real difference between lines in response to selection, it should be noted

TABLE 9a

Analysis of differences between the realised heritabilities

						Deviations from regression		
Selection Line	d.f.	Σx^2	Σxy	Σy^2	b	d.f.	Residual	M.S.
MALES								
Medium	4	60.623	24.619	10.490	.41	3	.492	
Cold	4	34.621	18.846	11.772	.54	3	1.513	
Hot	4	32.599	21.755	14.927	.67	3	.408	
Within						9	2.413	0.268
Reg. Coeff.						2	1.514	0.757
Common	12	127.843	65.220	37.189	.51	11	3.927	.357
			F = 2.825(NS)					
FEMALES								
Medium	4	40.101	13.377	10.109	.33	3	5.647	
Cold	4	24.371	12.043	6.337	.49	3	.386	
Hot	4	20.608	12.430	7.940	.60	3	.442	
Within						9	6.475	.719
Reg. Coeff.						2	1.072	.536
Common	12	85.080	37.850	24.386	.44	11	7.547	.686
			F = 0.724(NS)					

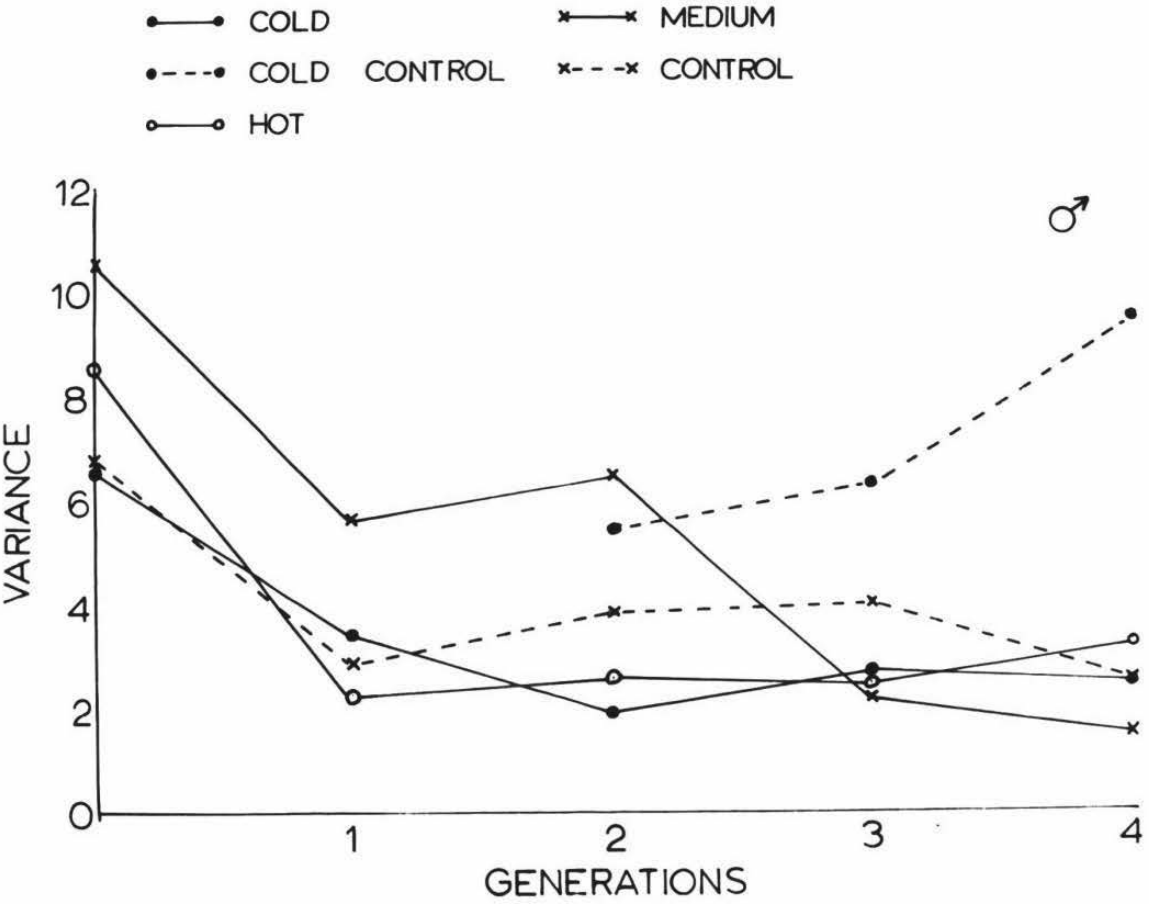
TABLE 9b

Analysis of differences between realised heritabilities with sexes pooled

Selection Line	d.f.	Σx^2	Σxy	Σy^2	b	d.f.	Residual	M.S.
Medium	8	100.724	37.996	20.599	.38	7	6.161	
Cold	8	58.992	30.889	18.109	.52	7	2.047	
Hot	8	53.207	34.185	22.867	.64	7	0.989	
Within						21	9.197	0.438
Reg. Coeff.						2	3.004	1.502
Common	24	212.923	103.07	61.575	.48	23	12.101	0.526

F = 3.43 (P 5%)

Figure 4. Total phenotypic variance of six week body weight in male mice plotted over all generations for all lines.



that this does not give a true heritability value for the sexes combined. To get this the weighted selection differential of each mated pair must be plotted against the mean body weight response (the weighted mean of the sexes). However, as the difference between the response in the three selection lines is the main interest in this experiment and not the most accurate heritability estimate, this seems to be a valid approach.

4. Change in body weight variance during selection

The graph in Figure 4 shows that as body weight increased in the three selected lines the total phenotypic variance declined. Variance was lower in the Hot and Cold lines than in the Medium line until generation three when the Medium line variance decreased to a similar level as the other two selection lines.

The variance in the Cold Control line was higher than in the Cold line, but until generation three the Control line variance was lower than in the Medium line.

As a similar picture was also found in the females, only the male values have been graphed.

5. Inbreeding

The effective number of breeding individuals (see Chapter II, section E (2)) shown separately for males and females for all lines in Table 10 may be compared to the actual number, that is eight. Eight pairs of mice were mated in all lines in each generation, thus giving a total actual number of breeding individuals per generation of sixteen. In the two control lines the effective number of parents in each generation was

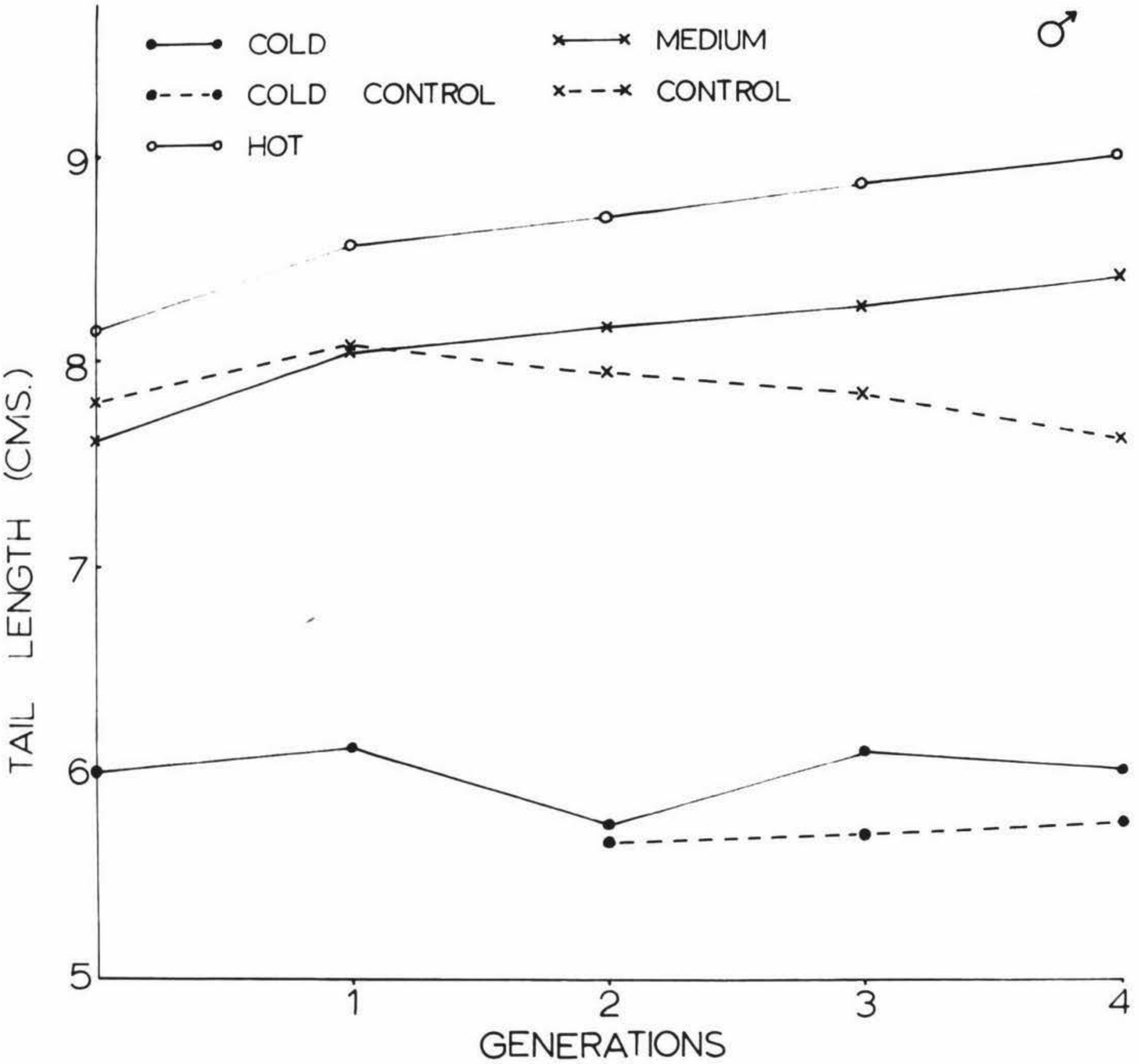
TABLE 10

Table showing the effective number of breeding (Ne) in each generation and the rate of increase in inbreeding (ΔF) per generation for all lines

	S_o	S_1	S_2	S_3	S_4	Harmonic Mean	Total	ΔF per Generation
Cold								
Males	3.30	3.73	2.95	2.67	5.10	3.38	6.93	7.28%
Females	4.30	2.67	4.30	2.95	4.30	3.55		
Cold Control								
Males			5.10	4.30	5.10	4.82	10.27	4.89%
Females			4.30	6.22	6.22	5.45		
Medium								
Males	1.14	3.30	2.95	2.95	5.10	2.44	5.66	8.87%
Females	4.30	3.73	1.70	4.30	4.30	3.22		
							Coancestry	3.42%
Control								
Males	4.30	4.30	5.10	5.10	5.10	4.78	10.08	4.99%
Females	4.30	6.22	5.10	6.22	5.10	5.30		
Hot								
Males	2.43	4.30	2.67	4.30		3.21	7.21	6.95%
Females	3.30	5.10	4.30	3.73		4.00		

(See Chapter II, Section E (2) for formulae and method of calculating these results)

Figure 5. Correlated response of six week tail length in males.



about ten which resulted in an inbreeding increase of about 5% per generation. In the three selection lines, on the other hand, the effective number of parents was less (between five and seven) which resulted in a higher inbreeding rate of about 8% per generation. The harmonic means shown in Table 10 must be used, for the generations with the lowest effective number of parents have the most effect on inbreeding over a number of generations. Thus "an expansion in numbers does not affect the previous inbreeding; it merely reduces the amount of new inbreeding", Falconer (1960a).

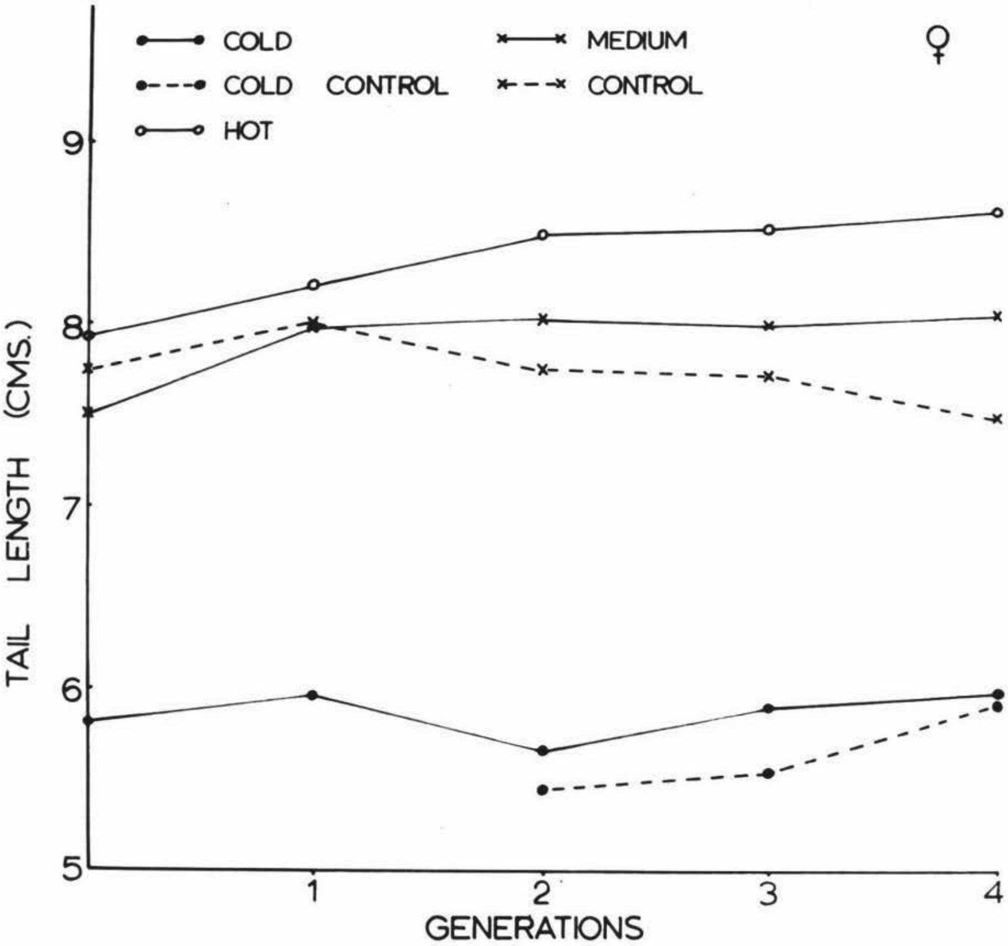
Also shown in Table 10 is a ΔF value calculated for the Medium line by the coancestry method (Cruden 1949). As Falconer (1960a) noted this is just a pedigree relationship and it does not necessarily correspond to inbreeding coefficients calculated from the effective number of parents, specially in selection lines.

C. CORRELATED RESPONSES

1. Tail length

Mean tail lengths in each generation have been plotted separately for males and females (Figures 5 and 6). There has been a gradual increase in tail length in the Hot and Medium lines over the four generations of selection for body weight. The rate of increase was slightly higher in the Hot line (.19 cms and .17 cms per generation for males and females respectively) than in the Medium line (.17 cms and .10 cms per generation for males and females respectively). In the Cold and Cold Control lines

Figure 6. Correlated response of tail length in the female.



however, there was no significant increase in tail length at all. The Cold Control tail lengths were slightly lower than those in the Cold line. The gradual decrease in tail length in the Control line after generation one, corresponds with the decrease in body weight in this line. After generation one the tail length in the Medium line was greater than that in the Control line.

Throughout the experiment the absolute tail lengths were longest in the Hot line and shortest in the Cold and Cold Control lines. The Hot line tail lengths were about one half a centimetre longer than those in the Medium line, while the Cold line tail lengths were about two centimetres shorter than those in the Medium line.

The total phenotypic variance for tail length did not behave in the same way as for weight (Figure 4). Tail length variance is plotted in Figure 7 while coefficients of variation for tail length are shown in Figure 8. The coefficient of variation (the ratio of the standard deviation over the mean, usually presented as a percentage) eliminates any relationship between the mean and the variance and thus demonstrates more clearly variance changes and variance differences between lines which have quite different mean tail lengths. With body weight variance the coefficient of variation was not used as the variance decreased as the mean increased. Where there is a positive association between the mean and variance (as Falconer (1953) found) the coefficients of variation can be informative if used in association with the original data. Figure 8 shows that tail length variance is greater in the Cold, Cold Control and Hot lines than in the Medium or Control lines. Table 11 presents mean standard deviations for both body weight and tail length.

Figure 7. Total phenotypic variance of six week tail length plotted over all generations for all lines.

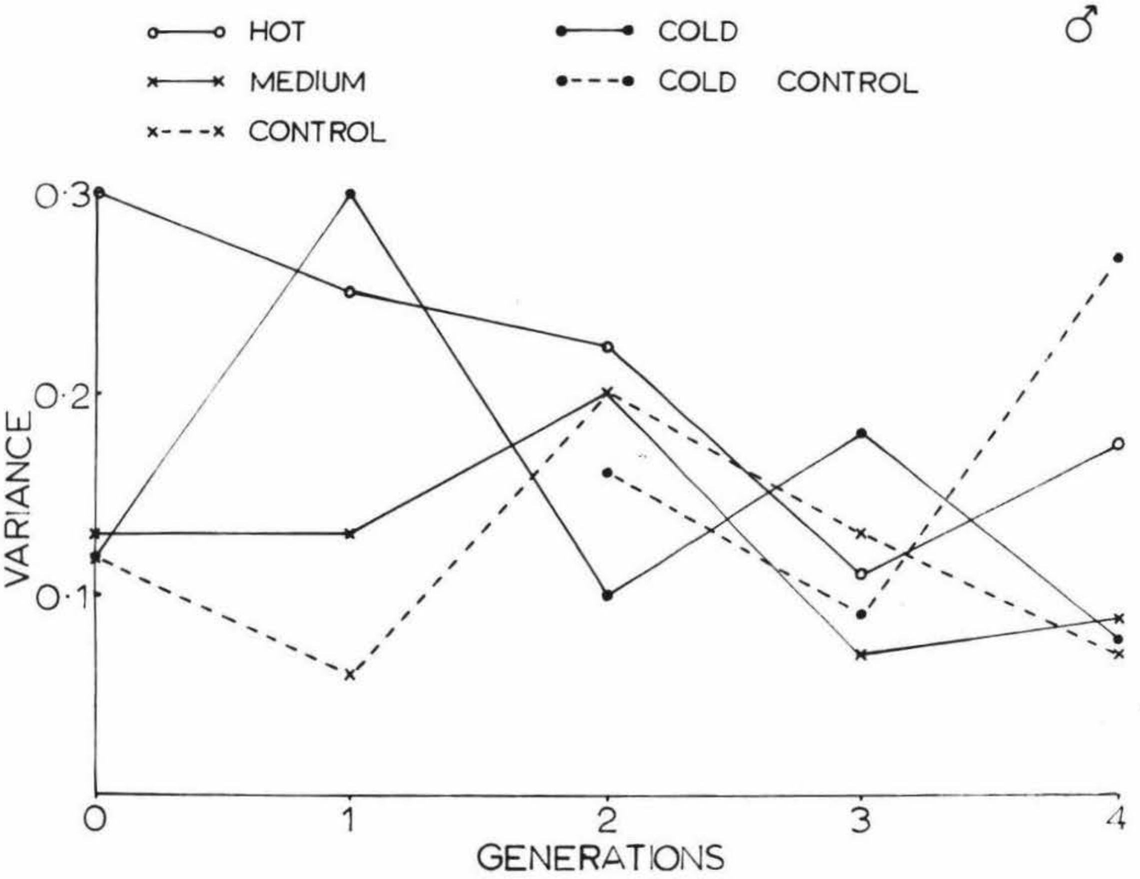


Figure 8. Coefficient of variation of six week tail length for all lines over all generations.

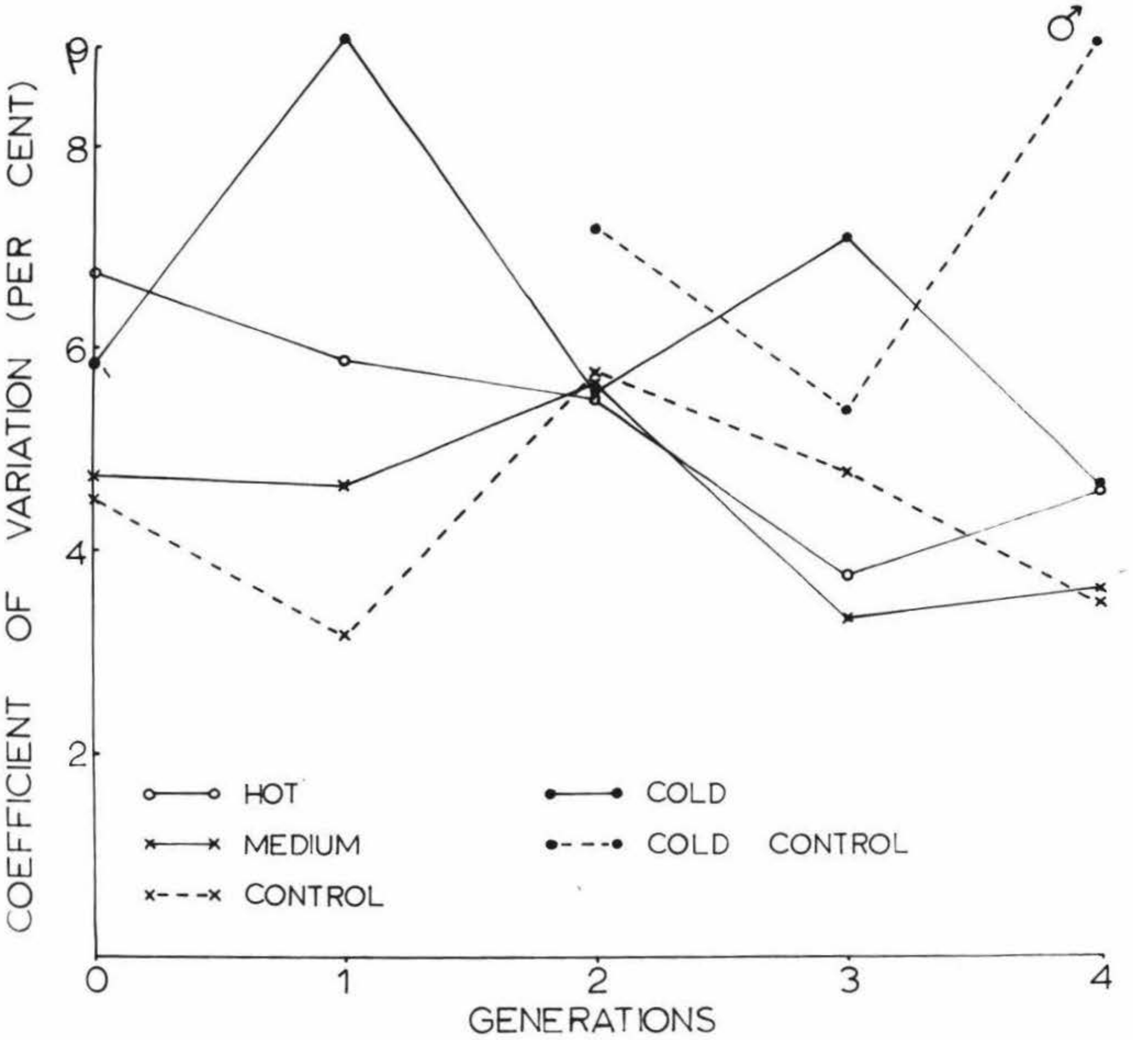


Figure 9. Male mean body weight at three weeks (weaning weight).

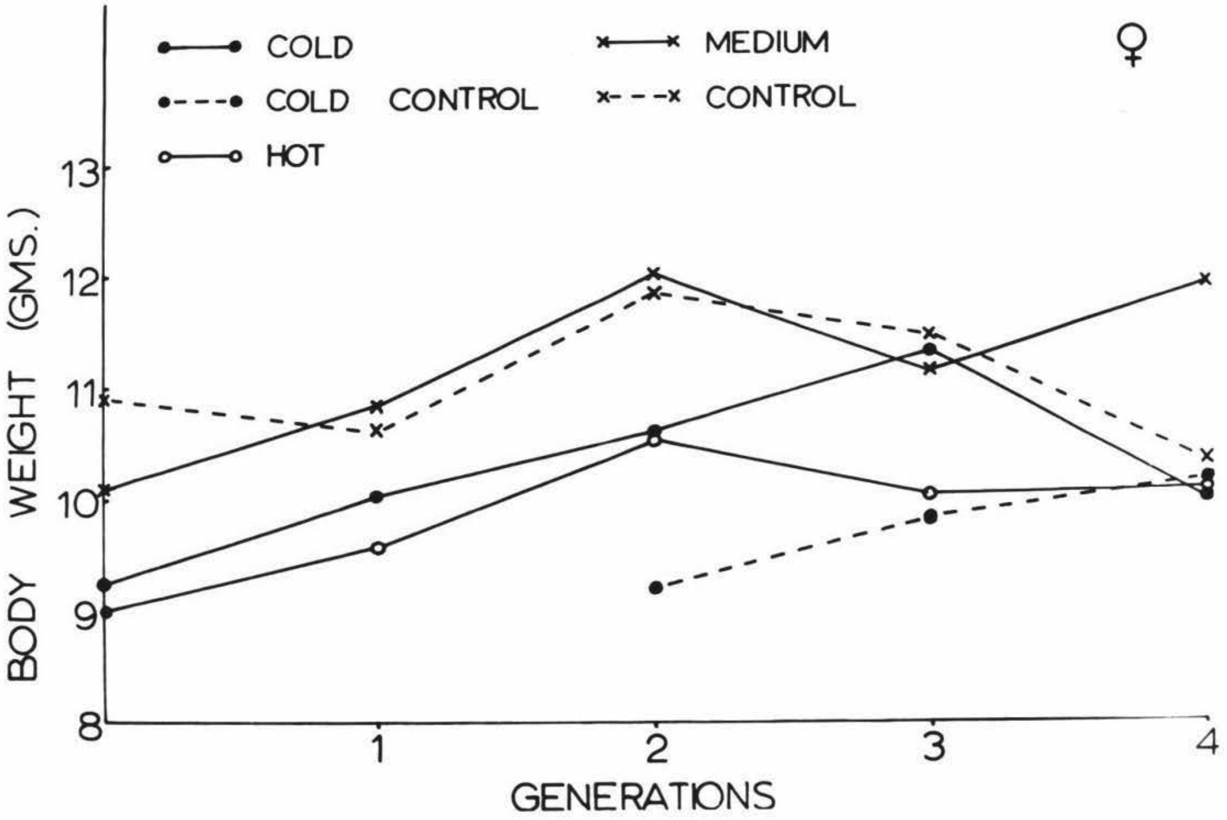


TABLE 11

Average standard deviations within generations

(Standard deviations calculated separately for each sex within generations and sex means averaged over all generations)

	Hot	Medium	Control	Cold	Cold Control
σ_p (Weight)	1.72	1.89	1.73	1.60	2.15
σ_p (Tail length)	0.43	.33	.31	.38	.41

This table shows clearly that while body weight variance in the standard environment (21°C) is slightly higher than in the two extreme environments, tail length variance is higher in the extreme environments (the exception being the body weight variance in the Cold Control line which is higher than in all other lines).

3. Weaning weight

Figures 9 and 10 show that weaning weight increased in the three selection lines over the period of selection for six-week body weight. In generation S_0 the Hot and Cold line weaning weights were lower than in the Medium or Control lines and the Hot line weaning weights remained depressed compared to the other two selection lines. Cold line weaning weights increased up to generation S_3 at which stage there was a sharp decline, the reason for this decline being unknown.

The Control line weaning weights were about equal to those in the Medium line until generation S_4, at which stage a divergence between

Figure 10. Female mean body weights at three weeks (weaning weight).

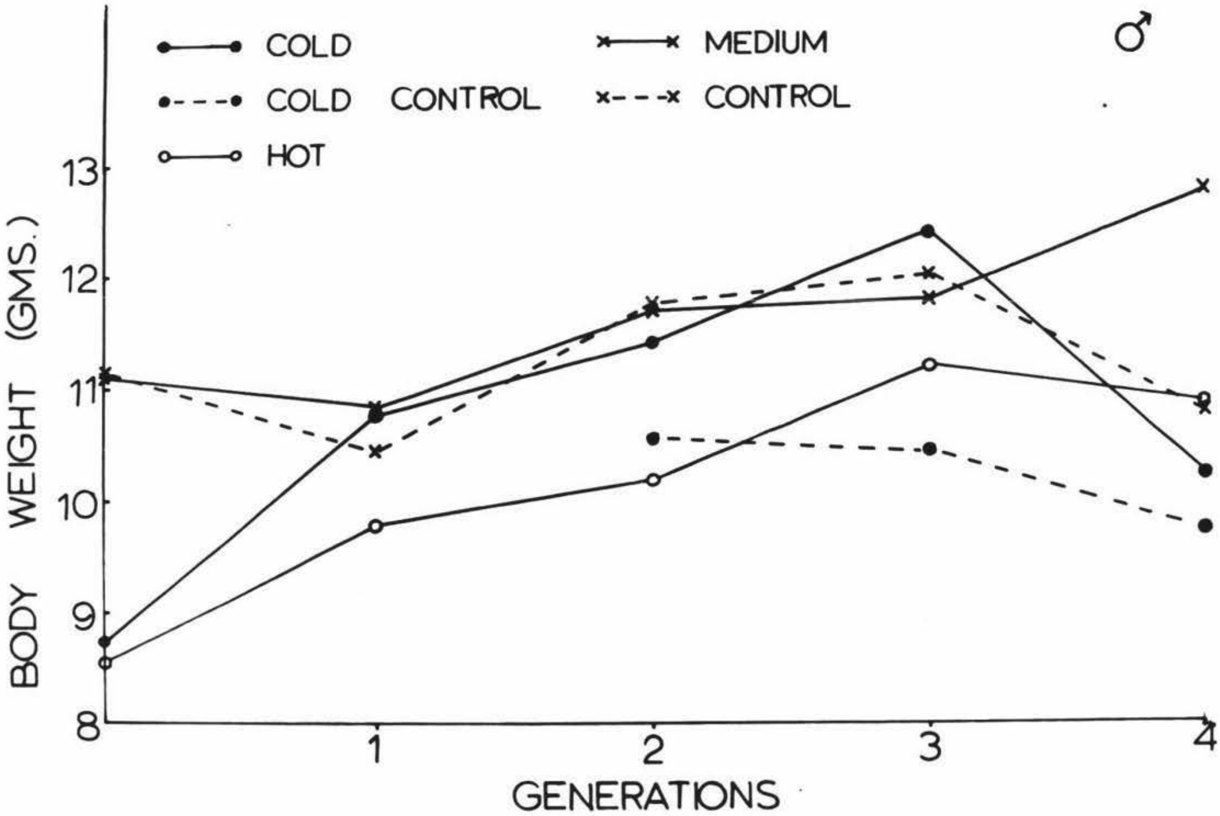


TABLE 12

Means and variances for body weight and ether extract (fat) from the six males and females sampled from all lines for fat analysis

Lines	Live weight	Carcass weight	Carcass weight variance	Dry weight	Dry non-fat	Fat	Fat%*	Fat variance
				MALES				
Hot	24.55	21.84	3.61	7.72	5.251	2.469	11.3	.66
Medium	25.13	22.02	1.94	7.41	5.666	1.744	7.9	.29
Control	25.83	21.49	1.02	7.16	5.576	1.584	7.4	.031
Cold Control	23.55	19.75	3.88	6.41	4.979	1.431	7.2	.21
Cold	25.17	21.29	0.69	7.24	5.400	1.840	8.6	.084
				FEMALES				
Hot	19.47	17.55	1.66	6.47	4.121	2.349	13.4	.67
Medium	20.80	18.36	1.09	6.58	4.710	1.870	10.2	.20
Control	19.55	16.62	2.10	5.56	4.282	1.278	7.7	.066
Cold Control	18.85	15.60	1.59	5.34	4.008	1.332	8.5	.084
Cold	21.28	17.91	1.82	6.18	4.428	1.752	9.8	.15

* $\frac{\text{Fat extract in gms}}{\text{Carcass weight in gms}}$

All weights in grams

these lines developed. The Cold Control weaning weights were on average slightly lower than the Cold line values.

3. Fat in the carcass

The method of extracting fat from the mouse carcass is described in Chapter II (Section 1) (4)). Six males and six females were sampled from each line. The body weights at various stages of analysis and the final fat weight extracted are shown in Table 12.

The means presented in Table 12 show that in absolute values there is more fat in mice from the Hot line than the other two selection lines while in the two Control lines mean values were less than those in their respective selected line.

Analysis of variance between all lines for fat extract is shown in Table 13. There was a significant difference between lines in both males and females, and the ranking of the means is also presented. Significant differences between individual means were tested using Duncan's multiple range test and the underscoring shown indicates means comprising a group that are not significantly different. In the males the Hot line had significantly more fat in the carcass than all other lines but there was no significant difference in fat extract between the four other lines. In the females the difference between the fat extract in the Hot and Medium lines was not significant but the Hot line was significantly fatter than the other three lines while the Medium line was not.

As can be seen in Table 12 there is variation in body weight between lines. Thus in Table 13 the covariance analysis of fat extract

TABLE 13

Analysis of variance between all lines for fat extracted from the carcass

		Mean Squares	
	d.f.	Males	Females
Between	4	0.977	1.148
Within	25	0.249	0.234
F		3.92*	4.91**

Hot Cold Medium Control Cold Control

Hot Medium Cold Cold Control Control

Analysis of covariance of fat extract adjusted for carcass weight

	Regression Coefficients						Residual Mean Squares				Adjusted Means				
	Hot	Medium	Control	Cold Control	Cold	Within	Pooled Regression	Difference between Regressions	Within	Adjusted Means	Hot	Cold	Medium	Cold Control	Control
MALES															
d.f.	5	5	5	5	5	25	20	4	24	4					
	.36 ± .11*	.087 ± .18	-0.008 ± .08	.063 ± .11	.13 ± .16	.16 ± .06*	0.183	0.281(NS)	0.199	0.679*	2.358	1.824	1.625	1.679	1.550
FEMALES															
	.15 ± .31	.17 ± .19	-0.047 ± .09	.11 ± .10	.25 ± .08*	.12 ± .07	0.241	0.116(NS)	0.22	0.726*	2.310	1.670	1.735	1.520	1.209
MALES AND FEMALES POOLED															
d.f.	11	11	11	11	11	55	50	4	54	4					
	.296 ± .13*	.12 ± .12	-0.035 ± .06	.077 ± .07	.214 ± .07*	.14 ± .04**	0.18	0.277(NS)	0.187	1.994**					

* $P < 0.05$

** $P < 0.01$

adjusted for carcass weight is presented. Carcass weight is the live weight minus the gut content, the whole carcass (head, pelt and carcass) then being extracted for fat.

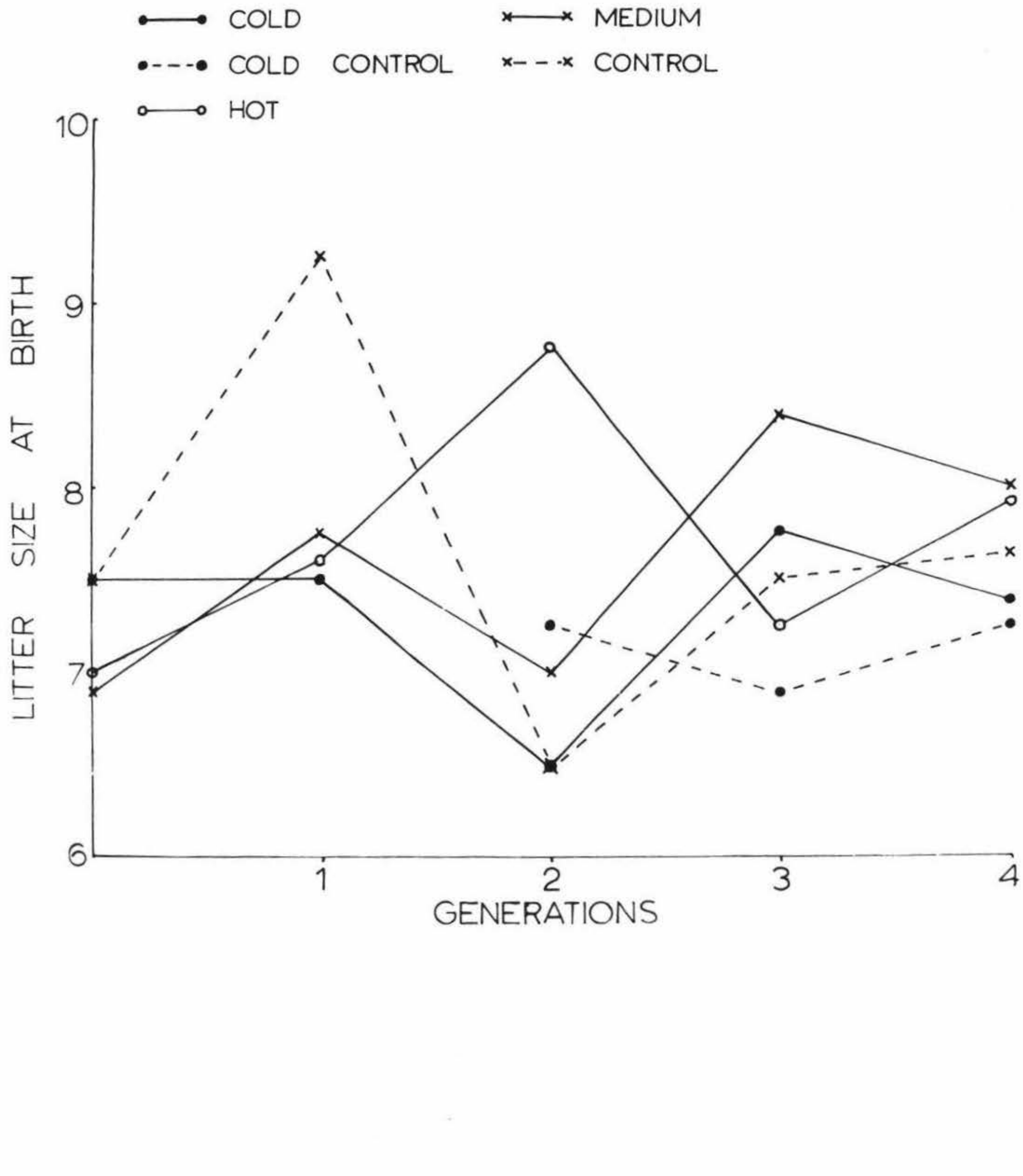
Only two of the regression coefficients presented in Table 13 were significantly different from zero and although the regression coefficients were higher in the Cold and Hot lines there was no significant difference between regressions in either males or females. For both males and females there was a significant difference between adjusted means. The adjusted means are presented and the rankings are the same as for the unadjusted mean. In both males and females the Hot line is significantly fatter than all other lines, the difference between these four other lines being not significant.

When males and females were pooled and then covariance analysis carried out it is found that the regression coefficients in the Cold and Hot lines were significantly different from zero while those in other three lines were not. There is now a highly significant difference between adjusted means.

The values for dry non-fat extract shown in Table 12 illustrates the fact that the Hot line mice laid down more fat at the expense of protein deposition compared to the other two selection lines (Dry non-fat will also contain skeletal material, but as this will be fairly constant this value gives an approximate estimate of carcass protein).

Also shown in Table 12 are fat percentages (fat as a percentage of carcass weight) which also shows that in these terms the Hot line was fatter than all other lines while the two selected lines were slightly

Figure 11. Correlated response of litter size. (Live plus dead mice born). Mean litter size of first litters only.



fatter than their respective control lines. The covariance analysis in Table 13 in association with these fat percentages illustrates very well the care that must be taken in presentation of percentages alone and anomalies that might arise by making interpretations from percentages. Thus although these percentages do in this case show the general picture they do not reveal the low relationship between fat extract and carcass weight in the Medium, Control and Cold Control lines compared to the significant relationship in the Hot and Cold lines (Table 13 - Regression coefficients).

4. Fertility

Both the selection applied to the lines and the temperature in which mice were gestating and rearing their young could have affected the reproductive performance of the lines being studied.

The main correlated response which might have occurred was changes in litter size and this data is plotted for all lines in Figure 11. The large fluctuations in litter size in all lines make the interpretation of this data difficult. When regression lines were fitted to the points shown in Figure 11 the regression coefficients were .15 ± 0.23 in the Hot line, .29 ± .18 in the Medium line, -0.135 ± .16 in the Cold line and -0.15 ± 0.35 in the Control line. None of these regression coefficients were significant.

It is also of interest to look at possible temperature effects on various aspects of fertility and this is done in Table 14 by averaging fertility data over all generations. Where appropriate standard errors have been calculated for the means, and this provides a measure of the

TABLE 14

Fertility data averaged over all generations

	Average Litter Size First Litter1/	Average Second Litter Size	Average Litter Size for both Litters	Average Interval between Mating & First Litters (Days)	Average Interval between Parturitions (Days)	Average Pre-weaning Mortality2/	Average Post-weaning Mortality3/	Average Total Mortality4/ (0-6 weeks)	Infertile Matings5/
Medium (21°C)	7.81 ± 0.32	8.15 ± 0.48	7.95 ± 0.27	23.83 ± 1.06	33.12 ± 1.78	3.74%	1.38%	5.12%	0%
Control (21°C)	7.75 ± 0.28	8.06 ± 0.52	7.87 ± 0.26	22.83 ± 0.50	30.39 ± 1.84	3.26%	1.16%	4.42%	0%
Cold (7°C)	7.13 ± 0.26	7.22 ± 0.36	7.17 ± 0.21	27.04 ± 1.10	32.72 ± 1.67	9.60%	2.48%	12.08%	4%
Cold Control (7°C)	7.38 ± 0.38	7.50 ± 0.58	7.43 ± 0.34	25.47 ± 1.17	37.43 ± 2.01	5.80%	5.3%	11.10%	0%
Hot (31°C)	7.73 ± 0.31	6.80 ± 0.43	7.33 ± 0.26	30.72 ± 1.77	42.39 ± 2.21	8.66%	1.68%	10.34%	3.5%

1. All litter sizes live plus dead mice born.

2. $\dfrac{\text{Number of mice dying between birth and weaning.}}{\text{Total number of mice born (live + dead)}}$

3. $\dfrac{\text{Number of mice dying between weaning and six weeks.}}{\text{Total number of mice born (live + dead)}}$

4. $\dfrac{\text{Number of mice dying between birth and six weeks.}}{\text{Total number of mice born (live + dead)}}$

5. $\dfrac{\text{Number of infertile matings.}}{\text{Total number of pairs mated}}$

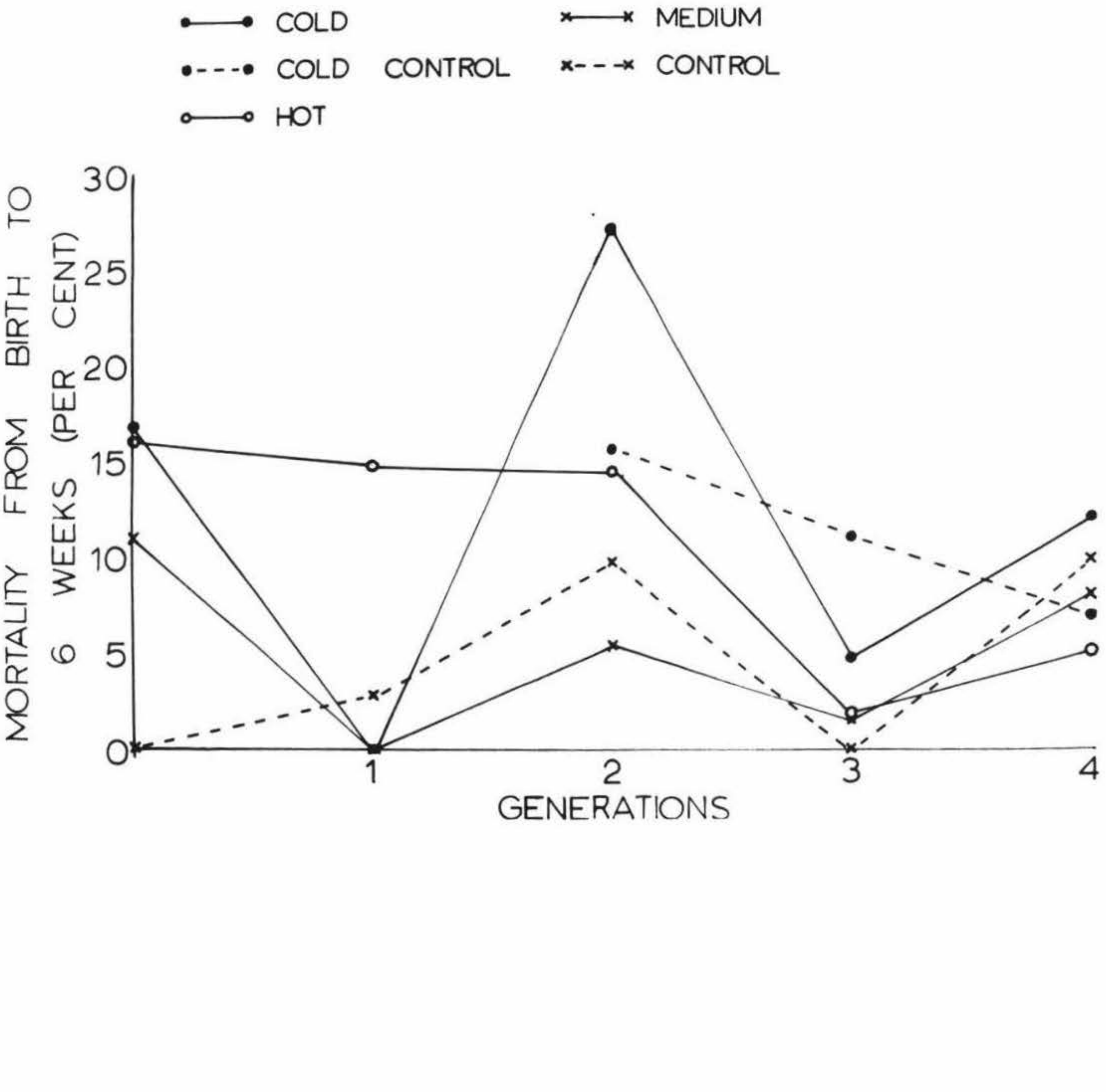
variance of these various characters in each line.

First litter sizes were about equal in the Hot, Medium and Control lines and smaller in the Cold and Cold Control lines. In all lines except the Hot line, second litters were slightly larger than first litters. Total litter size (mean of the first and second litters) was larger in the lines in the standard environment than the lines in the two extreme environments. There was very little difference between either of the control lines and their respective selection line in litter size.

As well as the number of offspring born, the time period before offspring were produced is also of interest. This data is presented in Table 14 as the average interval between mating and the birth of the first litters and the average interval between first and second parturitions. Except in the Hot line males and females were left together all the time. The gestation period for mice is about 21 days and the oestrous cycle about 5 days in standard conditions (21°C). Mice in the Medium and Control lines averaged about 22 or 23 days between pairing and producing their first litters, while the mice in the extreme environments, specially in the Hot environment, took longer to produce their first litters. Similarly second litters were produced later in the two extreme environments.

The final number of mice available at six weeks of age with which to carry out selection on, is a function both of the original number of mice born and the number dying before six weeks of age. Total, pre-weaning and post-weaning mortality percentages are shown in Table 14. Total mortality was about 5% in the standard environment, but about twice this (10-12%) in the lines in the two extreme environments. The fluctuations in mortality

Figure 12. Total number of mice dying between birth and six weeks of age as percentage of mice born (live and dead).



from generation to generation are shown in Figure 12. In all lines but the Cold Control line pre-weaning mortality was higher than post-weaning mortality.

The standard errors (or the variance) of these fertility characters might be expected to vary in the different temperature environments. However, standard errors are very similar in all lines for each fertility character and no effect of temperature on variance can be seen in this data.

The percentage of infertile matings was negligible.

D. ANALYSIS OF VARIANCE AND COVARIANCE FOR BODY WEIGHT AND TAIL LENGTH

The linear models used for the analyses shown here are presented in Chapter II, Section E (3).

The analysis has been carried out at the fourth generation, that is the last generation which is presented in this thesis. The means and variances of body weight and tail length at three and six weeks of age are presented separately for males and females in Tables 15a and 15b. Any differences between lines revealed by these analyses must of course be interpreted in association with the graphs of response with time already presented.

Because the experimental design was not orthogonal (that is there are three selection lines but only two control lines) analyses were carried out between the three selection lines and then between the selected

TABLE 15a

The means and variances of S_4 body weights (gms) and tail length (cms) at three and six weeks of age for all lines at the fourth generation

MALES		Hot	Medium	Control	Cold	Cold Control
Weight (gms)						
3 weeks	No. of mice	27	26	23	30	31
	Mean	10.94	12.79	10.78	10.24	9.74
	Variance	2.439	2.091	0.912	1.542	3.957
6 weeks	No. of mice	25	25	23	30	29
	Mean	23.63	26.58	23.05	26.10	23.57
	Variance	3.219		2.445	2.450	9.397
Tail length (cms)						
3 weeks	No. of mice	27	26	22	30	31
	Mean	6.75	6.63	6.01	4.85	4.90
	Variance	.276	0.098	0.036	0.052	0.210
6 weeks	No. of mice	25	25	22	30	29
	Mean	8.98	8.38	7.60	6.01	5.76
	Variance	0.174	0.089	0.069	0.077	0.268

TABLE 15b

The means and variances of S_4 body weights (gms) and tail length (cms) at three and six weeks of age for all lines at the fourth generation

FEMALES

		Hot	Medium	Control	Cold	Cold Control
Weight (gms)						
3 weeks	No. of mice	26	33	32	24	25
	Mean	10.15	11.93	10.33	10.02	10.26
	Variance	2.344	1.318	1.532	1.602	1.681
6 weeks	No. of mice	26	33	32	22	25
	Mean	19.75	21.26	19.18	21.55	19.95
	Variance	2.978	2.149	1.513	2.447	2.063
Tail length (cms)						
3 weeks	No. of mice	26	33	32	24	25
	Mean	6.63	6.45	6.01	4.90	5.07
	Variance	0.355	0.091	0.152	0.073	0.223
6 weeks	No. of mice	26	33	32	22	25
	Mean	8.60	8.03	7.47	5.97	5.90
	Variance	0.192	0.062	0.112	0.111	0.255

TABLE 16

Results of the analyses of variance carried out at the fourth generation for all characters in the three selection lines

	d.f.	Six week body weight	Six week tail length	Three week body weight	Three week tail length
Males					
Between lines	2	63.408**	69.321**	48.125**	32.497**
Within lines	77	2.399	0.111	2.005	0.126
		Cold = Medium Hot	Hot Medium Cold	Medium Hot Cold	Hot = Medium Cold
Females					
Between lines	2	23.941**	45.194**	30.558**	21.27**
Within lines	78	2.495	0.117	1.708	0.172
		Cold = Medium > Hot	Hot > Medium > Cold	Medium > Hot = Cold	Hot = Medium > Cold

and control lines in the standard and cold environments so as to test for interaction between the control and selected lines in these environments.

1. Analyses of variance between the three selected lines

The results of these analyses are presented in Table 16. For both body weight and tail length at three and six weeks of age there was a highly significant difference between the three lines in both males and females.

Also shown in Table 16 are the rankings of the means for the four variables as determined by Duncan's multiple range test.

Hot line six week body weights were lower than Medium or Cold line weights.

Hot line six week tail lengths were higher than in the Medium line and tail lengths in both these lines were higher than the Cold line tail lengths.

Male and female weaning weights were ranked in the same order (Medium > Hot > Cold) but in the females the difference between Hot and Cold line values was not significant. These weaning weight rankings did not hold in the previous three generations (Figures 9 and 10) where the Cold line values were about equal to those in the Medium line.

Mean three week tail lengths were ranked in the same order as at six weeks (Hot > Medium > Cold) but the difference between the Hot and Medium line values was not significant at this age.

TABLE 17

Weighted means analysis of variance between the control and the selected lines in the medium and cold environments in a 2 x 2 table

	Means				Mean Squares			
	Selection Lines		Control Lines					
Variable	Medium	Cold	Medium	Cold	Interaction	Within	Treatments	Lines
Males								
d.f.	24	29	21	28	1	102	1	1
Six week body weight (gms)	26.576	26.96	23.123	23.566	6.922(NS)	4.14	.1988(NS)	229.04**
Six week tail length (cms)	8.38	6.01	7.60	5.76	1.82**	0.131		
Three week body weight (gms)	12.85	10.24	10.83	9.97	19.98**	2.04		
Three week tail length (cms)	6.62	4.85	6.01	4.93	3.11**	0.104		
Females								
d.f.	32	21	31	24	1	108	1	1
Six week body weight (gms)	21.26	21.55	19.18	19.95	1.055(NS)	2.005	0.885	91.88**
Six week tail length (cms)	8.03	5.97	7.47	5.90	1.64**	0.125		
Three week body weight (gms)	11.93	10.16	10.33	10.26	19.847**	1.501		
Three week tail length (cms)	6.45	4.91	6.01	5.07	2.458**	0.135		

2. Analyses of variance between the control and the selected lines

As there were two lines (a selected and a control) in the standard and cold environments, differences between these means were analysed factorially in a 2 x 2 table. Because of uneven subclass numbers a weighted means analysis was carried out (Snedecor 1956). This analysis gives an interaction term between lines and environments. As Snedecor (1956) notes "the existence and interpretation of an interaction usually ends the investigation."

The means and mean squares for these analyses are shown in Table 17. Where there is a significant interaction term, mean squares for treatments and lines are not shown as the interaction is then the main interpretation of interest.

For six week body weight there was no interaction for either males or females and a significant difference between lines but not treatments.

In the other three characters there was a highly significant interaction term.

For both six and three week tail lengths this can be interpreted by looking at the means which reveals that there is a significant difference between the mean tail lengths in the Medium and Control lines but not between the Cold and Cold Control lines. This can also be seen in Figures 5 and 6.

Mean three week weights show a similar interaction, there being a larger difference between the lines in the standard environment than in the cold environment. Again this is the situation only in this

TABLE 18

Results of covariance analyses between different characters in the three selection lines at the fourth generation

Variable		Regression Coefficients			Residual Mean Squares				Adjusted Means		
Y	X	Hot b_{yx} ± s.e.	Medium b_{yx} ± s.e.	Cold b_{yx} ± s.e.	Pooled regression	Difference between regression coefficients	Within lines	Adjusted means	Hot	Medium	Cold
MALES											
d.f.		24	24	29	74	2	76	2			
Six week tail length	Six week body weight	.19 ± .028**	.012 ± .051(NS)	.079 ± .031*	.072	.461**	.082	66.293**	9.19	8.26	5.93
Six week tail length	Three week tail length	.72 ± .096**	.82 ± .098**	.82 ± .17**	.0402	.01245(NS)	.0394	2.8908**	8.42	7.91	6.89
Six week body weight	Three week body weight	.85 ± .16**	.02 ± .18(NS)	.78 ± .19**	1.5404	10.9339**	1.788	57.17**	23.83	25.74	26.69
Three week tail length	Three week body weight	.28 ± .091**	.13 ± .036**	.10 ± .029**	.0507	.4953**	.0624	21.5743**	6.81	6.36	5.04
FEMALES											
d.f.		25	32	21	75	2	77	2			
Six week tail length	Six week body weight	.19 ± .033**	.088 ± .026**	.043 ± .047(NS)	.077	.371*	.085	45.148**	8.73	7.98	5.89
Six week tail length	Three week tail length	.60 ± .086**	.57 ± .11**	.92 ± .18**	.0485	.0768(NS)	.0493	4.125**	8.26	7.80	6.72
Six week body weight	Three week body weight	.69 ± .18**	.73 ± .19**	.76 ± .23**	1.670	.0967(NS)	1.63	21.804**	20.28	20.50	22.17
Three week tail length	Three week body weight	.36 ± .095**	.14 ± .041**	.15 ± .036**	.0567	.7746**	.0754	17.8161**	6.80	6.20	5.11

particular generation and the relative differences between means in the two environments was actually reversed in the third generation (Figures 9 and 10).

3. Covariance analyses

As was observed in Table 16 there were significant differences between the three selection lines for all four characters. It is thus pertinent to question whether differences at six weeks were due to differences already established at three weeks or whether there are still differences in tail lengths between lines when adjusted to the overall mean body weight at three weeks.

The covariance analyses are shown in Table 18, males and females being analysed separately. The ultimate difference between the adjusted means (adjusted to the total mean of X by the within subclass regression coefficients) is based on the assumption of parallel regression lines in the different populations. In Table 18 the regression coefficients and standard errors are presented within each line (with probability values testing these regression coefficients against zero) and then a Residual mean square calculated for the difference between regression coefficients. When the regression coefficients are significantly different this could end the analysis and care must be taken in the subsequent interpretation of the difference between Adjusted means. Values for adjusted means are also presented in Table 18 and these may be compared with the actual means presented in Tables 15a and 15b.

Covariance of six week tail length adjusted for six week body weight showed that there was a significant difference between regressions in both males and females. The Hot line regression coefficients were higher than those in the Cold or Medium lines.

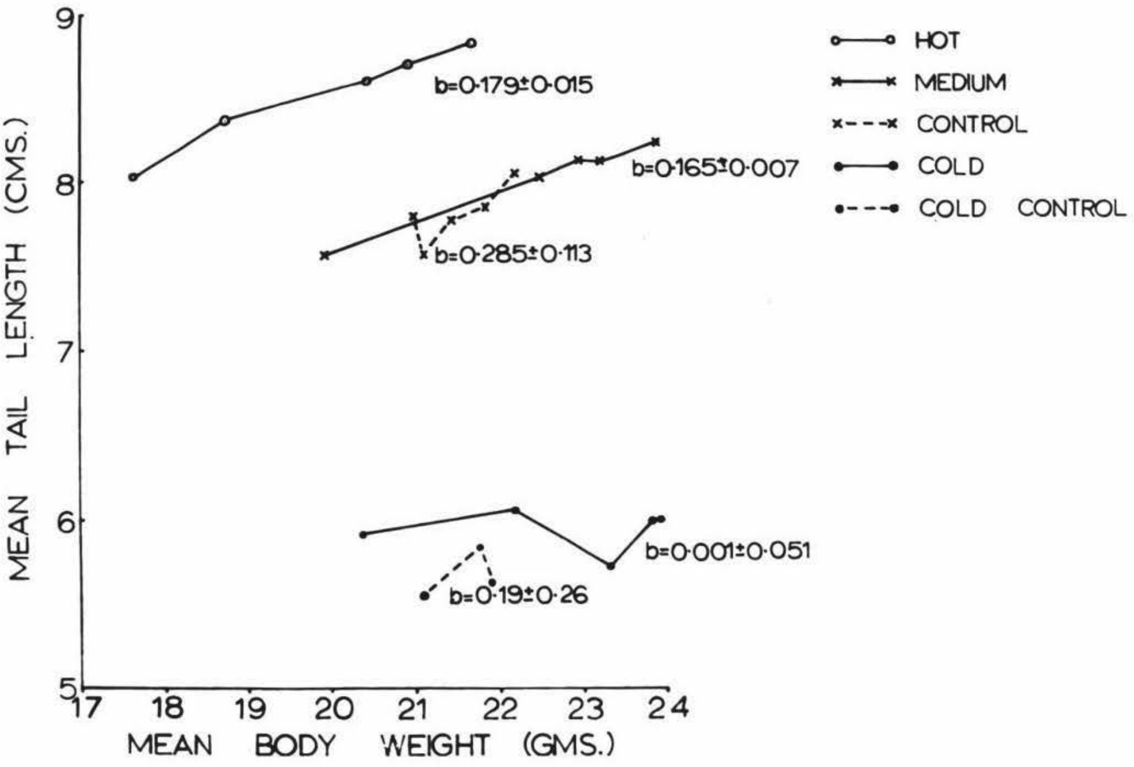
Although there is a significant difference between regression coefficients, these differences are not large, and it is concluded that the highly significant difference between adjusted tail length means is a real difference. This can also be seen from the values for the adjusted means which are presented.

A similar interpretation can be applied to three week tail lengths adjusted for three week body weight. Hot line regression coefficients are still higher than those in the Cold or Medium lines and all regression values between body weight and tail length were higher at three weeks than six weeks. The adjusted means for three week tail length are now all significantly different (Hot > Medium > Cold) whereas the difference between the original Hot and Medium lines means was not significant.

Covariance of six week tail length adjusted for three week tail length needs no interpretation, there being no significant difference between regressions and a highly significant difference between adjusted means in both males and females. Differences between the three adjusted mean tail lengths were reduced compared to the original differences, but all differences were still significant.

When female six week body weights were adjusted for three week body weights there was no difference between regressions but a significant difference between adjusted means. Adjusted mean values were still ranked in the same order (Cold > Medium > Hot) but there was now a significant

Figure 13. Mean six week tail lengths plotted against mean six week body weights (sexes averaged) and regression coefficients (± standard errors) shown for each line.



difference between the Cold and Medium lines but the difference between the Medium and Hot lines was not significant. This means that the adjusted gain in weight between weaning and six weeks was greater in the Cold line than in the other two lines in this generation. This covariance analysis is equivalent to adjusting weight gains for the initial weight (Cockrem 1956).

Covariance of male six week body weight adjusted for three week weight is complicated by a small non-significant regression coefficient in the Medium line. It seems as though this relationship is peculiar to this generation, and as the females were not affected it is probably not a litter size effect. The same regression coefficient (that is between six and three week weight) had a value of 0.35 in the Control line in the same generation while this regression for the Medium line in the S_0 generation was 1.08 and in the base population 0.52. The reason for this low regression in the fourth generation is not clear but as the regression values in the Hot and Cold lines were very similar the adjusted means analysis has still been presented. As in the females it was found that the Cold line has a higher gain from three to six weeks when adjusted to the overall mean weaning weight.

4. Phenotypic body weight - tail length relationships

The phenotypic relationships between body weight and tail length over the period of this experiment are shown in Figure 13. Means for six week body weight and tail length (male and female means averaged) have been plotted for all lines and regression coefficients fitted to these points.

While body weight has been increased by selection in the Cold line there has been no correlated increase in tail length. Tail length increase per unit body weight increase was slightly greater in the Hot line than in the Medium line, both of these regression coefficients being significantly different from zero. The Control line body weights fluctuated over the course of the experiment and tail lengths changed concurrently but the regression coefficient was not significant. The regression for the Cold Control line was also not significant, but only three points were available to fit this regression, as this line was established two generations after the other lines.

As is shown in Table 18 the phenotypic regressions of tail length on body weight in the fourth generation are very small in the Medium and Cold lines (about 0.05) and slightly larger in the Hot line (0.19). The three week regressions are larger than those at six weeks with the Hot line regression still being greater than the Cold and Medium line values. The phenotypic correlations between body weight and tail length quoted by Falconer (1954) are between 0.4 and 0.5. If the regressions shown in Table 18 are expressed as correlations it is found that the approximate values are:-

	Hot	Medium	Cold
Males	0.8	0.048	0.44
Females	0.74	0.52	0.2

The only really low correlation is that for males in the Medium line. If this value is calculated on a within litter basis (as used by Falconer (1954)) instead of over the whole population it is then found to be about 0.1. This low value was probably peculiar to this particular

TABLE 19

Genetic correlations between body weight and tail length
calculated from the response and correlated
response to selection

	Males	Females
Hot line	0.73	0.76
Medium line	0.75	0.41
Cold line	-0.03	0.081

Genetic correlations calculated by the formula
presented by Falconer (1954)

$$r_G = \frac{\text{Correlated response of tail length}}{\text{Response of body weight}} \times \frac{h^2 \text{ of body weight}}{h^2 \text{ of tail length}} \times$$

$$\frac{\text{Standard deviation of body weight}}{\text{Standard deviation of tail length}}$$

generation as it did not occur in the base population or the S_0 generation where the phenotypic correlations were 0.68 and 0.58 respectively.

5. Genetic correlations between body weight and tail length

These have been calculated from the response and correlated response of body weight and tail length in the three selection lines as discussed by Falconer (1954) and are presented in Table 19. The response and correlated response over the four generations of selection were found by fitting a regression to the appropriate body weights and tail lengths plotted against generation number (Figures 1, 2, 5 and 6) and the heritabilities and standard deviations for each line were also available. However a realized heritability for tail length was not available from this experiment so that the value quoted by Falconer (1954) of 0.6 was used in all calculations (formula shown in Table 19).

For both males and females in the Hot and Medium lines the realised genetic correlation was between 0.41 and 0.76. In the Cold line on the other hand the realised genetic correlations were virtually zero (-0.03 and 0.081 for males and females respectively).

6. Body weight - litter size relationships

It has often been noted that the more mice that are born the smaller they are at birth and the lighter they are when weighed at subsequent ages (this maternal effect persisting until three months of age, Brumby (1960)).

As the difference between average litter size for all lines was not large in this study (Table 14 and Figure 11) it was thought that litter size would not appreciably bias the body weight changes or differences.

TABLE 20

Regressions of six and three week mean body weights for each family on litter size at weaning in all lines in the fourth generation

	Six Weeks		Three Weeks	
	Males	Females	Males	Females
Hot	-0.32 ± 0.28	-0.75 ± 0.27	-0.69 ± 0.19*	-0.71 ± 0.22*
Medium	-0.15 ± 0.097	-0.57 ± 0.63	-1.28 ± 5.2	-0.19 ± 0.7
Control	-0.20 ± 0.19	-0.32 ± 0.087*	-0.39 ± 0.18	-0.22 ± 0.26
Cold	-0.43 ± 0.14*	-0.49 ± 0.15*	-0.46 ± 0.22	-0.39 ± 0.22
Cold Control	-0.23 ± 0.60	0.19 ± 0.35	-0.20 ± 0.4	-0.07 ± 0.34

The validity of this assumption was checked by fitting regressions to mean family body weights and litter size at three weeks. These regressions are presented in Table 20 and were calculated for males and females separately for three and six week weights in the fourth generation.

All but one of these regressions was negative, that is the higher the litter size, the lower the body weights and vice versa. However, only five of the twenty regressions calculated were significantly different from zero, four of these being in the Cold and Hot lines. Regressions were higher in the Hot and Cold lines (but not the Cold Control line) than in the other lines, but because of the large error variance no meaningful result can be obtained. This may be due to the small numbers of litters analysed (eight per line). But even if these regressions were significant the average regression coefficient over all lines would be about -0.5, that is a decrease of about 0.5 gms in mean body weight for a unit increase in litter size.

Although the litter size at birth was very similar in all lines, there could possibly have been a greater differential between three week litter sizes due to the higher mortality in the extreme environments. Mean three week litter sizes averaged over all generations and in generation four are shown below for all lines.

	Hot	Medium	Control	Cold	Cold Control
Average	7.38	7.38	6.88	6.75	7.13
S_4	7.00	7.33	7.43	6.63	6.74

The maximum difference in litter size at three weeks is about 0.6 which would mean that the mean body weight in the Medium line may be about 0.3 gm less than an adjusted estimate (0.6 x -0.5). This bias would not effect any of the body weight differences shown in Figures 1, 2 and 3.

Summary of Experiment I

1. A response to selection for body weight was obtained in the three temperature environments.
2. By the fourth generation of selection mean absolute body weights were greater in the Cold and Medium lines than in the Hot line (Cold = Medium > Hot = Control = Cold Control).
3. Realized heritabilities were greater in the Hot line than in Cold line and greater in the Cold line than the Medium line (Hot > Cold > Medium). This ranking held for both male and female estimates while in all three lines male heritability estimates were greater than those for females.
4. Total phenotypic variance for body weight declined in the three selection lines over the period of the experiment. Body weight variance was lower in the Hot and Cold lines than in the Medium line.
5. Inbreeding in the three selection lines increased at a rate of about 8% per generation.
6. There was an increase in tail length in the Hot and Medium lines over the four generations of selection for body weight but no

increase in the Cold line. By generation four the absolute tail length means were ranked in the order - Hot > Medium > Cold.

7. Total phenotypic tail length variance was greater in the lines in the extreme environments (31°C and 7°C) than in the lines in the standard environment (21°C).

8. Weaning weights increased in the three selection lines. Hot line weaning weights were lower than those in the Cold or Medium lines.

9. The mice in the Hot line were significantly fatter than the mice in all the other lines. There was no significant difference between the four other lines for fat extract but the Cold and Medium lines had higher values than their respective Control lines. There was a significant relationship between fat and body weight in the Hot and Cold lines but not in the other three lines.

10. There was no increase in litter size over the period of selection in any of the lines. Litter size at birth was lower in the Cold lines than in the other three lines. The time between mating and production of first litters was longer in the mice in the two extreme environments, specially in the Hot environment. Mortality between birth and six weeks of age was greater in the two extreme environment (about 10%) than in the standard environment (about 5%).

11. The genetic correlation between body weight and tail length in the Hot and Medium lines was about 0.7 but there was no genetic

correlation in the Cold line.

12. Body weight differences were not appreciably biased by litter size differences.

Chapter IV

RESULTS FROM EXPERIMENT II

TABLE 21

Mean body weights and tail lengths of mice from all lines at the start (4 weeks) and the finish (7 weeks) of exposure to all environments (five mice in each subgroup)

			Lines									
			Males					Females				
Environments			Hot	Medium	Control	Cold	Cold Control	Hot	Medium	Control	Cold	Cold Control
Hot Environment 31°C	Body Weight	4 weeks	16.65	18.06	17.96	18.20	15.66	14.50	16.74	16.06	15.88	16.44
		7 weeks	24.18	25.04	23.76	25.26	23.38	20.20	20.82	19.54	20.50	20.94
	Tail Length	4 weeks	7.96	6.80	6.62	5.04	5.02	7.40	6.98	6.74	5.10	5.20
		7 weeks	9.16	8.58	8.32	7.62	7.70	8.46	8.46	8.14	7.36	7.54
Standard Environment 21°C	Body Weight	4 weeks	17.64	18.64	18.30	17.56	16.56	14.18	16.34	15.20	16.30	13.96
		7 weeks	28.62	26.54	25.88	25.94	25.68	21.3	22.12	20.16	23.06	21.78
	Tail Length	4 weeks	7.94	6.94	6.70	4.96	4.98	7.4	6.96	6.64	5.12	5.18
		7 weeks	8.52	8.16	7.84	6.78	6.66	8.14	8.00	7.64	6.76	6.80
Cold Environment 7°C	Body Weight	4 weeks	17.64	18.70	18.50	18.76	18.28	15.84	16.14	15.84	15.98	15.08
		7 weeks	28.04	27.32	27.50	27.80	25.88	22.86	22.36	21.18	22.4	22.16
	Tail Length	4 weeks	7.80	6.94	6.70	5.08	5.32	7.76	6.90	6.80	5.10	5.12
		7 weeks	8.22	7.74	7.28	5.98	6.12	8.12	7.54	7.36	6.06	6.06

Chapter IV

RESULTS FROM EXPERIMENT II

A selection response for body weight and the differing correlated responses for tail length having been obtained it was of interest to study the effects of the alternative environments on any particular response. The experimental design for this experiment has been presented in Chapter II.

Body weight and tail length means at four and seven weeks for all subgroups (five mice in each subgroup) are presented separately for males and females in Table 21.

1. Analyses of the three selection lines (3 x 3 table)

Results of the analyses of these lines are presented in Table 22 and 23.

(a) Body weights

Four week body weights were lower in the Hot line than in the other two lines, this difference being significant in the females only.

A significant difference for seven week body weight was found between environments but not lines in both males and females. All lines had lower body weights in the hot environment than in the standard and cold environments, where body weights were very similar. Mean body weight of the HH group

TABLE 22

Analyses of variance between the three selection lines in the three environments (3 x 3 table)

		Mean Squares			
Source of variation	d.f.	7 week body weight	4 week body weight	7 week tail length	4 week tail length
MALES					
Environments	2	34.28**	2.03	13.69**	0.00065
Lines	2	1.99	5.46	4.89**	31.38**
L x E	4	5.10	1.03	0.252*	0.045
Within	36	2.55	2.69	0.0701	0.077
FEMALES					
Environments	2	17.53**	0.58	2.73**	0.043
Lines	2	1.07	10.13**	9.92**	23.925**
L x E	4	1.83	2.50	0.31(P 10%)	.052
Within	36	2.42	2.23	0.128	0.13

TABLE 23

Analyses of covariance between the three selection lines in all environments (3 x 3 table)

Variable		Within subgroups regression coefficient	Residual Mean Squares			
Y	X		Within subgroups	Lines	Environments	L x E
MALES						
d.f.		36	35	2	2	4
7 week tail length	4 week tail length	0.71 ± 0.11**	0.032	0.136*	4.94**	0.288**
7 week tail length	7 week body weight	0.073 ± .021**	0.0583	13.93**	4.071**	0.271**
7 week body weight	4 week body weight	0.67 ± 0.12**	1.39	7.87**	24.01**	2.94(P>10%)
FEMALES						
d.f.		36	35	2	2	4
7 week body weight	4 week body weight	0.78 ± 0.12**	1.11	2.52(NS)	15.74**	0.662(NS)
7 week tail length	4 week tail length	0.89 ± 0.076**	0.027	0.226**	3.23**	0.585**
7 week tail length	7 week body weight	0.091 ± .036*	0.111	10.26**	2.97**	0.38*

(terminology as in Chapter II) was lower than all other subgroups but the HM and HC groups were just as heavy or heavier than the other two lines in the cold and standard environments.

Covariance of seven week body weight adjusting for four week body weight showed a difference between environments and lines in the males. This was the result of the lower Hot line four week weights leading to significantly higher adjusted seven week weights. This was also reflected in an interaction term which was significant at the 10% probability level. A similar effect was found in the females but neither the line or interaction residual mean square was significant.

(b) Tail lengths

At four weeks, tail lengths were already significantly different between lines. This line difference may be a result either of selection or of the original environment in which each line was reared (that is, Hot line > Medium > Cold).

Analysis of seven week tail lengths showed that there were highly significant differences between lines and environments in both males and females. An interaction between lines and environments was significant at the 5% probability level in the males and the 10% level in the females. Within all lines tail lengths were longer in the hot and shorter in the cold environment. The Hot line had the longest and the Cold line the shortest tail lengths within any particular environment.

Covariance analysis to adjust seven week tail lengths for four week tail lengths resulted in significant line, environment and interaction residual mean squares for both males and females.

The nature of the interaction term between lines and environments for tail length will be discussed after the Control line analyses have been presented. The seven week tail lengths adjusted for four week tail lengths are presented below for males only (a similar result being found in the females), with the unadjusted means in parenthesis.

		Lines		
		Hot	Medium	Cold
Environments	31°C	8.20(9.16)	8.45(8.58)	8.73(7.62)
	21°C	7.58(8.52)	7.93(8.16)	7.95(6.78)
	7°C	7.38(8.22)	7.51(7.74)	7.07(5.98)

These means show that there has been a compensatory increased growth effect in the hot environment by both the Medium and Cold lines. Thus adjusted means in the hot environment are greater in the Medium and Cold lines than in the Hot line.

(c) Tail length - body weight

Covariance to adjust seven week tail lengths for seven week body weights resulted in significant line, environment and interaction residual mean squares for both males and females. The male adjusted means are shown below.

TABLE 24

Analyses of variance between the Medium and Control lines
in all three environments (3 x 2 table)

		Mean Squares			
Source of variation	d.f.	7 week body weight	4 week body weight	7 week tail length	4 week tail length
MALES					
Lines	1	2.58	.341	.90**	.363
Environments	2	22.96**	.96	2.21**	.036
L x E	2	1.34	.038	.025	.008
Within	24	1.471	1.827	.112	.096
FEMALES					
Lines	1	16.28*	3.74	0.61**	0.36*
Environments	2	6.41	1.02	1.61**	0.01
L x E	2	0.45	0.44	0.025	0.035
Within	24	2.63	1.592	0.066	0.066

	Hot	Medium	Cold
31°C	9.33	8.69	7.71
21°C	8.37	8.16	6.82
7°C	8.11	7.68	5.89

These adjusted means are very little different from the original means, but the HH group mean tail length was increased due to the lower body weights in this group.

This analysis corroborates the fact that there are both line and environment differences for seven week tail length and that the interaction term is one of degree rather than ranking. That is, there is a greater response to the warmer environments by the Medium and Cold lines than the Hot line.

2. Analyses of the Medium and Control lines (3 x 2 table)

(a) Body weights

These analyses are presented in Tables 24 and 25. There were no significant differences for body weights at four weeks. But in the females Medium line weights were higher than the Control line weights.

Male seven week weights showed a difference between environments (Cold > Medium > Hot) but no difference between lines. As differences in four week weights were very small covariance was not carried out.

Female seven week weights were significantly different between lines but not environments. After covariance had

TABLE 25

Analyses of covariance between the Medium and Control lines in all three environments (3 x 2 table)

Variable		Within subgroups regression coefficient	Residual Mean Squares			
Y	X		Within subgroups	Lines	Environments	L x E
MALES						
d.f.		24	23	1	2	2
7 week tail length	7 week body weight	0.0498 ± 0.056(NS)	0.1126	0.71*	1.31**	0.035(NS)
7 week tail length	4 week tail length	0.96 ± 0.10**	0.0239	1.12*	1.16**	0.02(NS)
FEMALES						
7 week body weight	4 week body weight	0.88 ± 0.19**	1.456	4.94(NS)	10.10**	0.065(NS)
7 week tail length	4 week tail length	0.903 ± 0.089**	0.0126	0.04(NS)	1.76**	0.005(NS)
7 week tail length	7 week body weight	-0.0138 ± 0.033(NS)	0.0683	0.55**	1.43**	0.025(NS)
d.f.		24	23	1	2	2

adjusted for four week weight differences there was a significant difference between environments only.

Thus in the females the line difference at seven weeks is due to a line difference already established at four weeks, so that the adjusted gain in body weight between four and seven weeks was similar in the Cold and Cold Control line.

(b) Tail lengths

At four weeks tail lengths were already longer in the Medium line than the Control line but only significantly so in the females.

Analysis of seven week tail lengths showed a highly significant difference between lines and environments and no significant interaction. Thus tail lengths were longer in the Medium than the Control line in all environments and both lines had the usual ranking in the environments (Hot > Medium > Cold).

After covariance had adjusted seven week tail lengths for four week tail lengths there were still differences between environments but no significant difference between lines in the females and a difference significant at the 5% probability level in the males.

Thus, especially in the females and to a lesser extent in the males, genetic differences between the Medium and Control tail lengths were largely established by four weeks.

TABLE 26

Analyses of variance between the Cold and Cold Control lines in all three environments (3 x 2 table)

Source of variation	d.f.	7 week body weight	4 week body weight	7 week tail length	4 week tail length
		Mean Squares			
MALES					
Lines	1	13.73**	13.46	0.012	.048
Environments	2	16.05**	7.79	6.626**	0.142
L x E	2	2.14	2.87	0.048	0.049
Within	24	1.215	5.943	0.081	0.065
FEMALES					
Lines	1	0.97	5.99	0.04	.027
Environments	2	8.90*	2.69	4.83**	.005
L x E	2	4.38	0.25	0.02	.004
Within	24	1.806	2.845	0.148	0.112

(c) Tail length - body weight

Covariance of seven week tail lengths for seven week body weights still resulted in significant differences between both lines and environments. However, this analysis has limited meaning as the within subgroup regression coefficient was very small and non-significant.

3. Analyses of the Cold and Cold Control lines (3 x 2 table)

These analyses are presented in Tables 26 and 27.

(a) Body weights

In both males and females four week body weights were higher in the Cold line than the Cold Control line, but not significantly so.

Analysis of male seven week body weights showed highly significant differences for lines and environments. Covariance analysis adjusting for four week weights did not alter this situation.

Female seven week weights were only significantly different between environments. Covariance for four week body weight did not alter this.

The seven week adjusted body weight means (with the unadjusted means in parenthesis) are shown below.

	31°C	21°C	7°C
Cold	20.4(20.5)	22.7(23.06)	22.2(22.4)
Cold Control	20.6(20.94)	22.4(21.78)	22.3(22.16)

TABLE 27

Analyses of covariance between the Cold and Cold Control lines
in all three environments (3 x 2 table)

Variable		Within subgroups regression coefficient	Residual Mean Squares			
Y	X		Within subgroups	Lines	Environments	L x E
MALES						
d.f.		24	23	1	2	2
7 week tail length	7 week body weight	0.065 ± 0.052(NS)	.0786	.085(NS)	3.80**	0.074(NS)
7 week body weight	4 week body weight	0.23 ± 0.082*	0.952	7.604**	11.39**	1.86(NS)
FEMALES						
d.f.		24	23	1	2	2
7 week body weight	4 week body weight	0.42 ± 0.14**	1.372	0.01(NS)	7.32*	1.57(NS)
7 week tail length	7 week body weight	0.16 ± 0.05**	.107	0.13(NS)	5.27**	0.075(NS)

These adjusted means illustrate that there was no difference between lines for weight gains over the 4-7 week period and that any differences in unadjusted means (although not significant) were the result of differences already present at four weeks.

(b) Tail lengths

There was no difference between tail lengths at four weeks.

Analysis of seven week tail lengths showed that in both males and females that there was a highly significant difference between environments but no difference between lines. Thus both the Cold and Cold Control lines increased tail length when moved to warmer environments. But the fact that there was no significant difference between lines shows that no genetic differences in tail length were revealed by these warmer environments.

There was no need to carry out covariance of seven week tail length adjusting for four week tail lengths, as the differences in tail length at four weeks were very small.

(c) Tail length - body weight

It was found that in five out of six cases (both males and females considered) seven week tail lengths were actually slightly longer in the Cold Control line. Since the Cold Control line had lower seven week body weights than the Cold line, covariance analysis of seven week tail length adjusting for seven week body weight was carried out. There was still differences between environments but no significant differences between lines.

The female adjusted means (with unadjusted means in parenthesis) are shown below.

	31°C	21°C	7°C
Cold	7.57(7.36)	6.56(6.76)	5.97(6.06)
Cold Control	7.68(7.54)	6.81(6.80)	6.01(6.06)

Although there was no significant difference between lines the differences between the adjusted means were greater than the unadjusted differences.

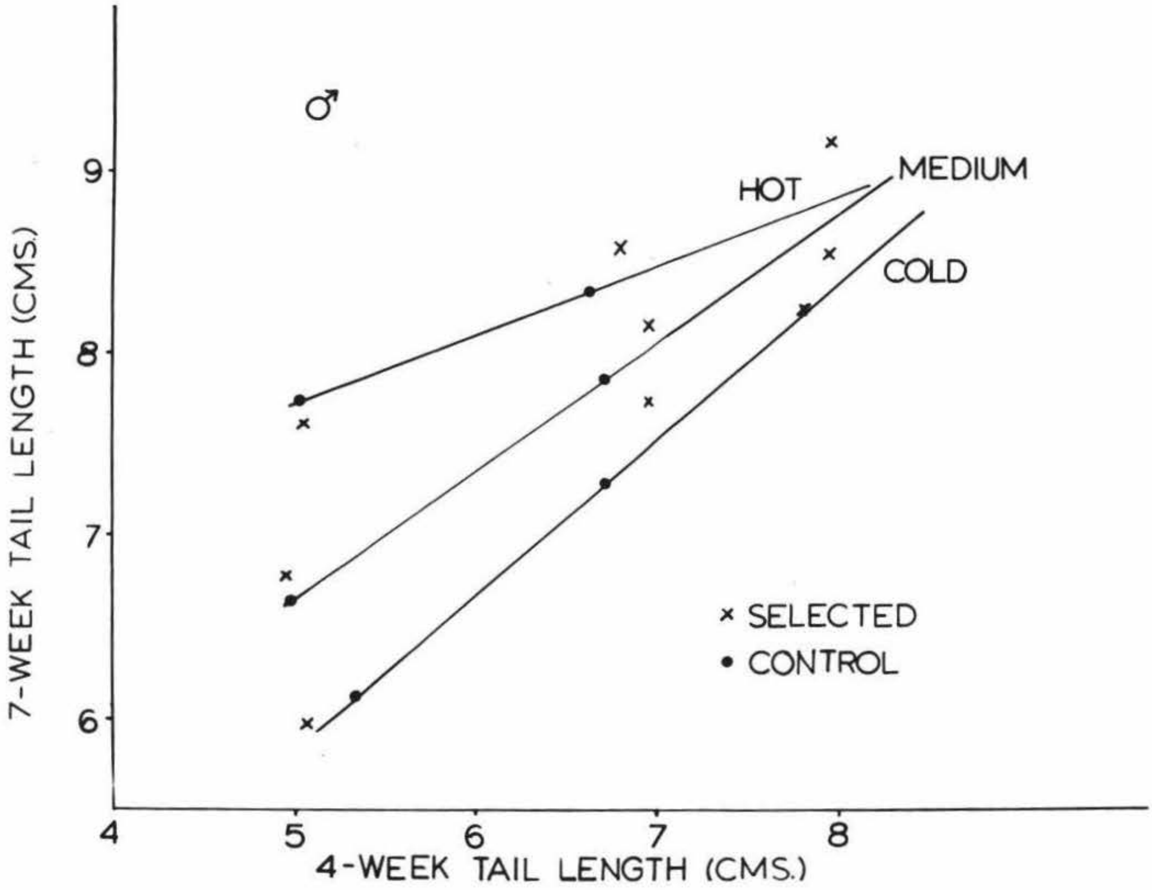
• Body weight and tail length analyses in all lines

(a) Body weight

Body weight analyses need very little additional discussion. However, the lack of difference between the selected and control lines, specially obvious in the males in the standard environment and the females in the cold environment needs some interpretation.

The most logical explanation would seem to be one of sampling, especially as there were only five mice in each subgroup. In the first litters in generation four and again in generation five there were fairly large (significant) differences between the selected and control lines for six week body weights. In generation five these six week differences were still maintained at

Figure 14. Mean 7 week tail length plotted against mean 4 week tail length for all male subgroups (see explanation in text).



seven weeks. Another possible explanation could be a litter size effect. However, as was shown also in the previous chapter, for the first litters, differences between the size of the second litters used in this crossover experiment were not large. Even assuming a significant regression of body weight on litter size the maximum bias of body weights between the selected and control lines would be about 0.5 gms.

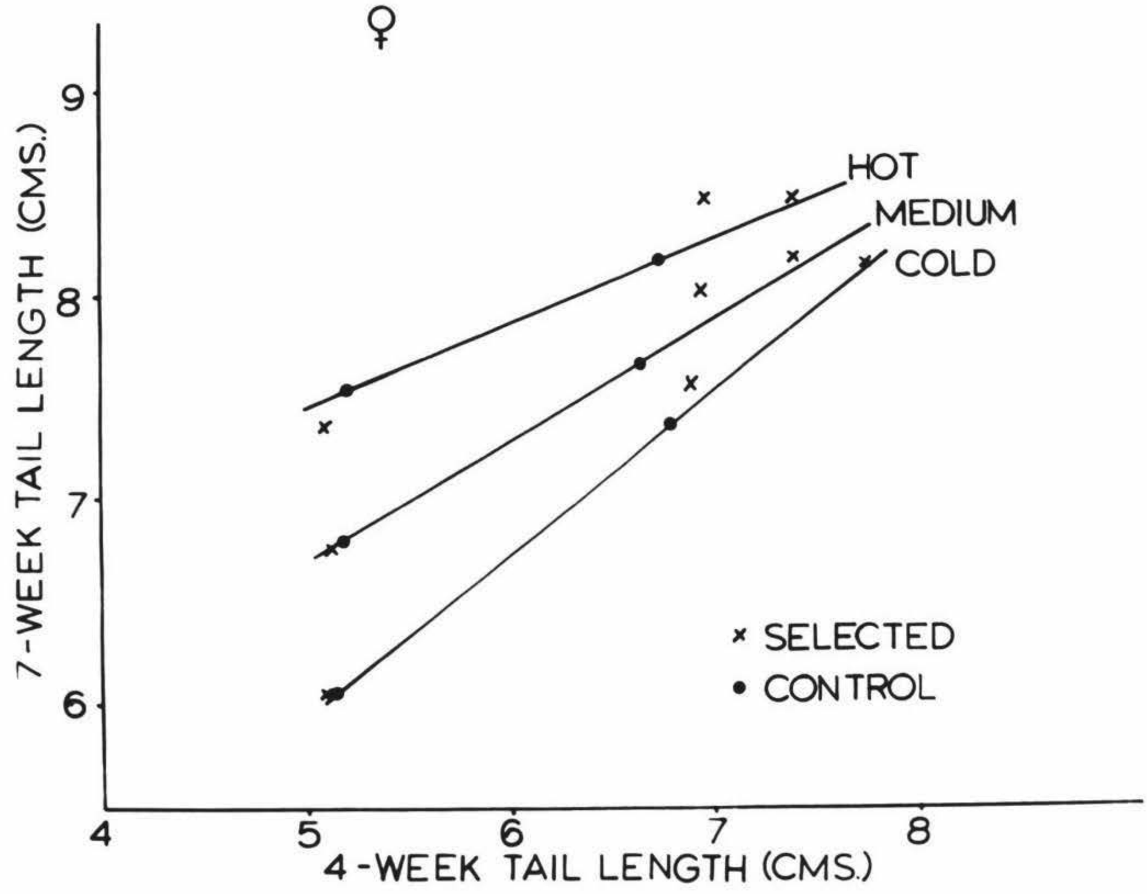
Thus it would seem that a sampling effect has occurred.

Also, in several cases, the genetic differences between the selected and control lines were already established at four weeks. In these cases covariance adjusting for four week weights results in no adjusted differences between lines from four to seven weeks. Thus covariance is actually correcting out differences already established. This does not alter the final interpretation of results, as long as it is clear that this is what has occurred.

(b) Tail length

In Figures 14 and 15 mean seven week tail length has been plotted against mean four week tail length for both males and females. All subgroups within all lines are shown on these graphs with the control and selected points being differentiated by different symbols. The three lines on each graph are the result of joining the Cold Control and Control line points within each environment. Each selection line has not been differentiated as it was considered that this would complicate these figures too much. Tail lengths are ranked at four weeks

Figure 15. Mean 7 week tail length plotted against mean 4 week tail length for all female subgroups (see explanation in text).



as Hot line > Medium line > Control line > Cold line = Cold Control line.

From studying these graphs, interpretation of the previous tail length analyses are made much simpler. The interaction between environments and the three selection lines (3 x 3 Analyses) is shown by the different gradients of the three lines. Thus there is a greater difference between tail lengths in the two extreme temperatures in the Cold (and the Cold Control) line than the Hot line. This is the compensatory growth effect by the shorter tailed mice already mentioned.

But there was no significant difference between the Cold and Cold Control line tail lengths (at either four or seven weeks) in any environment (2 x 3 Analyses, Section 3).

However in all cases the seven week tail lengths were lower in the Control line than the Medium line at both four and seven weeks in all environments. Covariance analyses adjusting for four week tail lengths confirmed that these differences shown in Figures 14 and 15 were significant for males only (2 x 3 Analyses, Section 2).

Thus these analyses confirm the conclusion arrived at in Experiment I that there was no correlated response of tail length in the Cold line (revealed by any of the three environments) but there was in the Medium and Hot lines.

Thus in spite of the increase in tail length by the Cold lines in warmer environments, the fact that there is no genetic change in tail length in these environments (gauged by the difference between the Cold and Cold Control lines) is the factor of importance. On the other hand the Medium line did show a genetic increase in tail length in all environments.

The situation is complicated for the Hot line as there was no control line in this environment. Thus it is difficult to ascertain how much of higher four week tail length in the Hot line is due to genetic or environmental causes.

SUMMARY OF EXPERIMENT II

1. Body weight

(a) In all lines (selected and control) seven week body weights were lower in the hot environment.

(b) In the three selected lines there was very little difference between seven week body weights in the cold and standard environments. When seven week weights were adjusted for four week weights the Hot line male weights were significantly heavier than the other two selection lines in the cold and standard environments. This effect was also found in the females but was not significant.

(c) The difference between the Medium and Control lines for seven week body weights were smaller than expected, specially in the males in the standard and cold environments. This seems most likely to be a sampling effect. In the females the genetic difference in body weight between the Medium and Control lines was already established at four weeks and there was no difference between these lines for adjusted body weight gains from four to seven weeks.

(d) Seven week body weights were higher in the Cold than in the Cold Control lines in both males and females in nearly all environments. In the males this difference in weight was partly established at four weeks but there was also a higher adjusted gain in body weight between four and seven weeks in the Cold line than in the Cold Control line.

But in the females the higher weights in the Cold line than in the Cold Control line were due to differences already established at four weeks so that there was no difference between lines in adjusted gains from four to seven weeks.

2. Tail length

(a) Within all lines (selected and control) there was a significant difference between environments for seven week tail lengths. That is tail lengths were longer in the hot environment and shorter in the cold environment than the tail lengths in the standard environment.

(b) Line differences between the three selected lines for seven week tail lengths are complicated by differences in tail length between these lines, which were already established at four weeks.

(c) Analyses between the three selected lines for seven week tail length revealed an interaction between lines and environments. This was due to a compensatory growth effect in the hot environment by the Medium and Cold lines. Thus the Cold line mice (which had the shortest tails at four weeks) showed the greatest response between four and seven weeks to the hot environment.

(d) The Cold Control line mice (which had similar tail lengths to those in the Cold line in the cold environment) showed similar increases in tail length to the Cold line when moved to the standard and hot environments.

(e) In all environments the Control line tail lengths were lower than the Medium line tail lengths. After seven week tail lengths had been adjusted for four week tail lengths this line difference was significant in the males but not the females.

(f) It is concluded that there was no genetic increase in tail length in the Cold line which would show only in warmer environments. But there was a genetic increase in tail length in the Medium line showing in all environments and the same was probably true for the Hot line.

Chapter V

DISCUSSION OF EXPERIMENT I

A. RESPONSE TO SELECTION

1. Body weight response

A response to selection was obtained in all three selection lines, the increase in body weight per generation being slightly greater in the Hot line than in the Medium and Cold lines.

The response obtained in the Medium line of about 0.8 gms/generation is higher than that found in other similar selection studies for body weight in mice (Table 1). The response in this experiment was expected to be higher than that found in Falconer's studies (Table 1 and 2) as in all these experiments within litter selection was carried out, so that only one half of the total genetic variance was available for selection. This within-litter selection method eliminates the complications of maternal effects and reduces the rate of inbreeding. But the increased rate of progress available from mass selection was of more importance in this study as only a limited amount of time was available. In this study no measure of asymmetry of response was available (that is there was no line selected for decreased body weight).

The response calculated over only four generations of selection would be expected to be biased upwards as most selection studies have shown the greates response over the first few generations and then a gradual decline. Thus MacArthur (1949) obtained 60% of his total response in the first seven generations of selection (response ceasing after about 23 generations).

Although no other selection experiments for body weight in different temperature environments are known, it is of interest to compare the response in the Hot and Cold lines in this study with the response found in experiments selecting on low nutritional diets (Table 2). In this study the response was just as good or better in the two lines in the adverse environments (that is the hot and cold environments) than in the standard environment.

Both Falconer and Latyszewski (1952) and Korkman (1961) found lower body weight responses on their low diets. This was specially marked in Korkman's study where the low diet level was very low. It would be expected that after the nutritional level had been reduced below some minimum level which is needed for growth that body weight response must then cease. This level was probably exceeded in Korkman's experiment. But Falconer (1960b), who measured his response from the divergence between his upward and downward selected lines, found no difference in response between the lines on his high and low diets over 13 generations of selection and a slightly higher response in the low diet line over the first four generations of selection.

Allowing for the fact that the response in all three lines in this experiment would probably be lower if selection had been continued longer, the similarity in response between all selected lines is of interest.

It might have been expected that the two extreme temperatures may have reduced response, but this was not found. This emphasises the point that little is known about what constitutes a 'normal' or optimum environmental temperature for selection.

However, although there was no marked difference in body weight response between selection lines, absolute body weights were lower in the Hot line, while there was no significant difference in weights between the Medium and Cold lines. Both the Medium and Cold lines were significantly heavier than their respective Control lines by generation four so that a genetic response had been obtained. There was no Control line in the hot environment.

Acclimation studies with mice and rats in hot and cold environments (Barnett 1965, Harrison 1963, Bigham 1965) have shown that rearing mice in these extreme temperatures usually results in lower mature body weights. This was found in the hot environment in this study but not the cold environment, neither Cold line or the Cold Control line being affected (that is the body weights in the Control and Cold Control line were not significantly different). These results can be directly compared to the acclimation study of Bigham (1965) who exposed LCA and LCB strain mice to the same hot and cold temperatures as used in this study. Both his hot and cold groups of mice had lower body weights than the controls at 21°C and in this case hot group mice were heavier than cold group mice (also found by Biggers et al. 1958).

2. Selection differentials and realized heritabilities

The weighted selection differentials were higher in the Medium line than in the other two lines and selection differentials for males were higher than those for females.

Falconer (1953) tabulates selection differentials for each generation of selection for both large and small lines. Mean weighted selection differentials up to generation four were 1.76 gms/generation in the large line and 1.39 grams per generation in the small line. Falconer calculated his selection differentials from each mated pair and selection differentials were calculated separately for males and females in this study. But Falconer's values will be roughly comparable to the average of the two weighted selection differentials in this study. These were 2.19, 1.72 and 1.67 gms/generation in the Medium, Cold and Hot lines respectively. The larger value of 2.19 in the standard environment compared to Falconer's value is probably mainly due to the fact that mass selection was being used in this study compared to the within family selection method employed by Falconer.

The lower selection differentials in the Hot and Cold lines resulted in higher realised heritabilities in these two lines than in the Medium line. Realised heritabilities were calculated separately for males and females and the means of these estimates were 0.37, 0.51 and 0.63 for the Medium, Cold and Hot lines respectively. All these values were higher than most of those found in a number of other body weight selection experiments with mice (Tables 1 and 2).

The best estimate of heritability for body weight in mice in a standard environment would be that quoted by Falconer (1953, 1955) of 0.35. This was calculated from the divergence of his large and small lines and thus eliminated errors due to asymmetry of response. In a standard environment the asymmetry of response for body weight has nearly always been due to a greater response in the small line than the large line (Falconer 1953, 1955, 1960a, 1960b). Falconer (1960b) found that the realized heritability for upward selection was about 70% and 20% for downward selection over the first five generations. However, after this response changed and was about 12% upwards and 45% downwards.

The realized heritabilities calculated in this study could be biased estimates of heritability if asymmetry of response was important. The only check of the validity of these estimates is the realised heritability calculated from the difference in response between the Medium line and the Control line. This gave a value of 0.33 (average of the two sex values), which although lower than the Medium line value of 0.37, is still higher than most other estimates of heritability found in selection studies for large size in mice (Table 1 and 2).

However, regardless of what the true heritability might be, the difference in heritabilities between lines (which was significant when the sexes were pooled) is itself of interest.

There were higher heritabilities in the two extreme temperature environments in this study. Higher realized heritabilities were also found in low nutritional environments (Falconer and Latyszewski 1952, and Falconer 1960b), but in neither of these experiments were the differences significant. On the other hand Park <u>et al</u>. (1966), Korkman (1961) and Dalton and Bywater (1963) all found lower (although not always significantly lower) heritabilities on low planes of nutrition when selecting upwards. This is most likely due to the low feed level limiting response as was discussed previously.

3. Body weight variance

Total phenotypic variance (calculated over the whole population as against between or within litters) for body weight was lower in the Hot and Cold lines than in the lines in the standard environment. The exception was the variance in the Cold Control line which was higher than in all other lines. However none of the differences between phenotypic variances were very large (Table 11).

The genetic variance for body weight (calculated by multiplying the phenotypic variance by the realised heritabilities) was higher in the Hot and Cold lines than in the Medium line (values of 1.08, 0.82 and 0.70 respectively). Thus the environmental variance was reduced in the extreme temperatures in this study but the genetic variance was not, when compared to the variances in the standard environment.

Both Falconer and Latyszewski (1952) and Falconer (1960b) found that phenotypic variance of body weight (calculated within litters), was lower in their restricted diet lines. Falconer and Latyszewski (1952) found that both the genetic and environmental variances were reduced by the restriction of diet, but the environmental variance more than the genetic.

It has been suggested that adverse environmental conditions may increase phenotypic variability (Waddington 1942, Michie 1955, McLaren and Michie 1956).

The phenotypic variances for body weight in this study would support this 'environmental destabilisation' theory. Similar results have also been found by Ashoub et al. (1958), using temperature environments and Searle (1954) and Honeyman (1957) using nutritional environments.

Contrary results were found in Falconer's selection studies and in acclimation studies by Harrison et al. (1959) and Barnett and Scott (1963).

4. Inbreeding

Inbreeding occurs when individuals which are more closely related than average are mated together, thus increasing the degree of homozygosity in the population. Increasing the level of inbreeding usually produces a decline in characters associated with the fitness of an animal (e.g. fertility, variability, growth etc). Inbreeding depression may be due to the fixation of undesirable recessive genes, but is more likely to be due to the fact that the heterozygote is superior in fitness to the homozygote (Robertson 1955, 1956, Lerner 1954, Falconer 1960a).

The relationship between inbreeding and selection has been discussed by Robertson (1960, 1961, 1962). It was shown that selection within a closed population results in an increase in the inbreeding rate, both because the population is closed and thus the numbers are restricted, and because of the effects of selection.

In this study the rate of inbreeding increase was calculated from the effective population size which takes into account the effects of the restricted population size and selection. As was expected this gives values of ΔF that are considerably higher than those quoted in other selection studies.

Falconer (1953) calculated his increase in inbreeding (Δ F) by the 'coancestry' method (Cruden 1949) which does not take into account the effects of selection and quotes an inbreeding rate of 1.7% per generation compared to the rate in this study of about 8% per generation in all selection lines.

The Δ F value calculated by the coancestry method in this experiment for the Medium line only, resulted in a value of 3.42% per generation. This is about twice that found by Falconer due to the mass selection as compared to the within family selection method.

Falconer (1953) discusses the magnitude of decline of heritability and response which may be accounted for by random fixation due to inbreeding. This would probably have been of importance in this study if selection had been continued.

B. CORRELATED RESPONSES

1. Tail length

The longer tail length found in the hot environment and the shorter tail length found in the cold environment compared to the tail length in the standard environment (21°C) is in agreement with several other studies which have exposed mice and rats to hot and cold temperatures (Harrison *et al.* 1959, Harrison 1963, Chevillard *et al.* 1963, Cockrem 1963, Bigham 1965, Barnett 1965).

It has also been observed that on selection for body weight in mice in a standard environment there is a correlated increase in tail length (Falconer 1953, 1954, 1955). This was found in this experiment in both the Hot and Medium lines but not in the Cold line. The rate of increase in tail length per generation was greater in the Hot line than the Medium line.

Tail length variance did not behave in the same way as body weight variance. Total phenotypic variance for tail length was now higher in the lines in the extreme temperatures than in the lines in the standard environment (Table 11).

Harrison *et al*. (1959) and Harrison (1963) found that the variability in tail length of heat reared mice was significantly lower than that of control reared mice, due mainly to the shorter tailed individuals making the greater response to heat. But Barnett (1965b), although not finding any really consistent effect of the cold environment on variance in tail length, did find in some strains a suggestion that low temperature increased variance.

The higher phenotypic variance for tail length in the extreme environments would support the hypothesis of environmental destabilisation (Ashoub *et al*. 1958) discussed in Section 3 of this Chapter.

(a) Body weight - tail length relationships

One of the most interesting aspects of Experiment I is the observation that there is no realised genetic correlation between body weight and tail length in the Cold line, while the genetic correlations in the Medium and Hot lines were in good agreement with other estimates (Falconer 1954, Cockrem 1959).

It was found in the review of literature that there was ample evidence of both body weight and tail length being influenced by the environmental temperature at which mice and rats are reared. These acclimation studies have been extended in this experiment by selecting for body weight in three temperature environments.

As has already been discussed a body weight response was found in all selection lines and an increase in tail length in the Hot and Medium lines only. But absolute weights were lower in the Hot line mice. Thus by generation four three lines of mice had been produced which differed in body weight - tail length relationships. The mice in the hot environment had a low body weight and a long tail, those in the cold environment had a high body weight and a short tail, while those in the standard environment had a high body weight and a tail intermediate in length between the tail lengths in the two extreme environments. Thus the mice produced in the hot and cold environment have similar body weight - tail length proportions to the LCA and LCB lines produced by Cockrem (1959) by actually selecting for these relationships in a standard temperature environment.

In neither the selected or the Control line in the cold environment was there any significant increase in tail length over the four generations of selection. Throughout the experiment the Cold Control line mice were lighter than the

Cold line mice but their tail length was only slightly shorter. There was also a sex difference of about two or three grams in body weight in the two Cold lines, but no significant difference between the male and female tail lengths.

Thus in all the mice in the cold environment there does not seem to be any association between body weight and tail length. However from these results in Experiment I it is not possible to say whether the lack of a tail length response in the cold is the result of an environmental suppression. This is tested in Experiment II by exposing all lines to the other environments.

2. Weaning weight

Associated with the increase of six week body weight there was an increase in weaning weight (three week weight) in the three selection lines. Weaning weights were lower in the Hot line than in the other two selection lines while the Control line weaning weights did not show a consistent pattern. However, the increase in weaning weights were not large and the Cold line weaning weights declined sharply in generation four in both males and females probably due to some unknown environmental effect.

Falconer (1953) also noted a small increase in weaning weight (about $\frac{1}{2}$ a gram) over eleven generations of selection for six week weight. In his small line however weaning weight over the same period decreased by about $2\frac{1}{2}$ grams so that the asymmetry of response was actually greater at this stage than at six weeks of age.

Weaning weight is largely determined by the mother while the three to six week growth is probably mainly determined by the individual. In Falconer's experiments weaning weight was not subject to any direct selection, since there was no selection between families. Bateman (1963), on the other hand, mass selected mice for body weight and found that maternal genotype, selected through family differences, constituted two-thirds of the superiority of selected individuals.

Likewise in this experiment it could be possible that selection for a heritable maternal effect may be having an effect on the weaning weights, and some of this effect would still be reflected in six week weights. The regression of six week weights on three week weights was about 0.6 (males and females averaged). Falconer (1955) discusses mothering ability (that is the maternal effect) in terms of two components, one related to anatomical development and the other to physiological efficiency. The anatomical component it is suggested is directly related to body size while the physiological component is not directly related to body size, but is a component of natural fitness and will thus show overdominance as postulated by Lerner (1954).

Although maternal effects could be affecting selection response in this study it is not possible to partition out this component. However, this is not really necessary as a heritable maternal effect, although complicating genetic interpretation, may actually be a desirable feature (Bateman 1963).

Harrison <u>et al</u>. (1959) and Harrison (1963) found that the weight of young heat-reared mice (3-4 weeks of age) usually increased more rapidly than the controls (at 21°C). This was not found in this study, probably because the Hot line mice were suckled on the heat, while Harrison did not expose his mice to the heat until the mice were three weeks of age. Pennycuik (1966a, 1966b) found that lactation was diminished in mice in a hot environment.

Barnett (1965) found that cold reared mice were lighter at three weeks than controls (at 21°C). In most generations in this study (except generation S_o and S_4) this did not occur, but this probably just reflects the colder temperature (-3°C) at which Barnett's mice were kept (c.f. 7°C in this study).

3. Fat in the carcass

While the Hot line mice were significantly fatter than all other lines, there was no significant difference in fat extract between the other lines (this situation was not altered by covariance analysis adjusting for body weight differences).

However, there was a suggestion that the Cold and Medium lines were fatter than their respective Control lines, that is selection for body weight in these two lines may have caused a correlated increase in fatness. But this difference between selected and Control lines was reduced when covariance analysis adjusted for the lower body weights in the Control lines.

In the Cold and Medium lines the increase in body weight resulted from an increase in fat and protein while in the Hot line the large increase in fat was at the expense of protein deposition.

Fowler (1958) analysed Falconer's N strain which had been selected for large and small size at six weeks and found that total amounts of protein, water and fat in the carcass had been altered as well as the percentage composition of the carcass. Thus the large line mice were fatter than those in the small line.

Selection had probably not been carried out for long enough in this study to get a significant difference between the selected and the Control lines. As there was no Control line in the hot environment it could not be ascertained whether the fatness of these mice was due to selection or to the environment.

A temperature effect on fat metabolism was shown by Bigham (1965), who found a significantly greater amount of body fat in his high body weight strains of mice (but not his low body weight strains) in a hot environment. Bigham (1965) also found no difference in fatness between his medium and cold groups of mice. The hot environment may be comparable to a restricted diet environment, for it is known that food intake is reduced by heat (Bigham 1965). But Falconer and Latyszewski (1952) and Falconer (1960b) found that mice reared on restricted diets had less fat than mice reared on a full diet when both lines were reared on the high plane. The fact that the Hot line mice were fatter than mice in the other lines would suggest that temperature is the more important factor in this study. A reduction of heat production may occur in a hot environment (Bigham 1965). This general reduction of voluntary activity in the hot environment may mean that any excess food requirements are laid down in fat, even though food intake is reduced.

Barnett (1965) concluded that exposure of laboratory mammals to cold generally results in a reduction of fat, due to the high rate of heat production which must be maintained. This was not found in either the Cold or Cold Control lines in this study. Heroux (1963) found an increase in abdominal and subcutaneous fat in wild rats in the winter which emphasises the point that generalisations from laboratory animals and laboratory conditions must be made with care.

4. Fertility

The fertility data collected during this study (presented in Table 14) is really only a by-product of this experiment and thus will be discussed very briefly.

No significant correlated response of litter size was found in any of the selection lines. This is probably just due to the fact that selection in this study had not been continued for long enough.

The effect of temperature on reproductive performance would support most other studies reviewed in Chapter I which found a general slowing and reduction of reproductive performance in hot and cold environments. The exception was the size of the first litters in the hot environment which were not very different from those in the standard environment. However this was largely offset by the longer time interval between mating and first parturition found in mice in the hot environment, compared to those in both the standard and cold environments.

5. Body weight - litter size relationships

Regression of body weight on litter size were negative as was expected (that is the higher the litter size the lower the body weights of the offspring and vice versa), but only a few of these regressions were significantly different from zero. Most of the regressions in the Hot and Cold lines (but not the Cold Control line) had higher values than those in the standard environment.

This effect was also shown by Biggers et al. (1958), but in this case nearly all regressions were significant (probably due to the larger number of litters analysed).

As was discussed in Chapter 3, Section D (6), even if the regressions in this study had been significant, this effect would not have significantly biased any of the body weight differences.

Chapter VI

DISCUSSION OF EXPERIMENT II

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DISCUSSION OF EXPERIMENT II

1. Body weight

(a) The environment and selection

Since Falconer (1952) first discussed the problems of selection response in differing environments a number of studies have been published which have looked at this question (Table 2).

The general conclusion from all these studies is that selection should take place in the environment in which the animals are to live. But Falconer (1960b) would also suggest that if good performance under a variety of conditions is desired, then selection should be made under the conditions least favourable to the desired expression of the character.

The second part of the present selection study provides some additional experimental evidence on the response to environments other than those in which selection was made.

In the Hot line, although body weight was low in the hot environment, there was a marked increase in body weight when these mice were moved to the two cooler environments. In the males only this resulted in the Hot line mean weights in the standard and cold environments being significantly heavier than the Cold and Medium line weights in these environments. As the hot environment was the least favourable environment for growth, these results support Falconer's suggestion that selection should occur in the least favourable environment.

It is also possible to look at the performance of all lines in each environment.

In the hot environment body weight growth was depressed in all lines. After covariance had adjusted the Hot line weights for their low weaning weights there was very little difference in weights between the three selected lines.

Similarly in the standard and cold environments, apart from the heavier Hot line weights in the males, there was very little difference between selection line weights.

Thus although selection in the hot environment was better for performance in all the environments selection in medium or cold environments gave similar results for either environment subsequently.

(b) Temperature effect on growth

The effects found in this crossover experiment confirm the effects found in Experiment I.

The hot environment depressed body weight growth in all lines (selected and control) and was a less favourable environment for growth than the cold environment.

But within all lines (selected and controls) body weights in the standard and cold environments were very similar, with some suggestion of increased growth in the cold environment.

These results are directly comparable to similar studies in this laboratory by Cockrem (1963) and Bigham (1965).

Cockrem (1963) found exactly opposite temperature effects than those found here using the same temperature treatments. Thus for both strains (LCA and LCB) and sexes of mice, the cold (but not the hot) treatment depressed body weight growth. Bigham (1965), again using the same temperatures and four strains of mice, found that both his hot and cold groups of mice had lower body weights than the control at 21°C.

But in agreement with Cockrem (1963) and Biggers et al. (1963), Bigham (1965) found hot group mice were heavier than cold group mice.

However, Barnett (1965) has found that the body weights of certain strains of mice and especially F_1 strains of mice have not been depressed by cold temperatures (-3°C). Thus the results in this study may to some extent be a strain effect and may be connected with the amount of heterozygosis. The base population was a heterogeneous stock and four generations of selection will not have changed this very much.

2. Tail length

(a) Compensatory growth

On first sight the compensatory growth effect by the Medium and Cold lines in the hot environment does not seem to support the conclusion reached in Experiment I that there is no genetic correlation between body weight and tail length in the Cold line.

However, once the Control lines were also included in the analyses it was found that there was actually no genetic increase in tail length by the Cold line in the warmer environments.

However, the compensatory growth effect by the short tailed strains (both Cold and Cold Control) is itself of interest.

Harrison et al. (1959) found that the shorter the tail length when exposed to the heat (at 3 weeks of age) the greater the increase in tail growth. This is in agreement with the results in this study. But Harrison et al. (1963) also concluded that this phenomenon accounts for the smaller within genotype variability and the smaller phenotypic variance for all genotypes reared in the hot (c.f. reared at 21°C). But in the present study phenotypic variance was actually higher in the hot environment than in the standard environment.

Bigham (1965) also found that within his long tailed strains, mice of the shorter initial tail length showed a greater tail length increase than did mice of longer initial tail length, which was in agreement with Harrison's results. On the other hand Bigham (1965) also found that his short tailed strains (these being genetically short tailed) did not show this greater tail length increase in the hot environment. A similar result was shown by Cockrem (1963) who found that the LCA (short tailed) strain did not show as great an increased tail length growth under a hot environment, as the LCB (long tailed) strain did. Again the difference in tail length between these strains was a genetic one.

A problem in the interpretation of the tail length results in this study is found when the differences between tail lengths at four weeks are considered. This complication was mentioned when discussing Figures 14 and 15 but needs some further discussion.

Compensatory growth is a description of an effect and not a theory of how this effect works.

However, following Dickinson (1960) it could be considered that there is a certain genetic potential for tail length growth and that its realisation depends on the environment. In the first environment (birth to 4 weeks of age) the realised growth will depend on the environment and the potential from the correlated response to body weight selection in this environment. The former is by far the larger effect. The lower realised growth in the adverse environment (cold) leaves a greater potential available when the animal is moved to a better second environment (e.g. hot) for the 4-7 week period. The subsequent realisation of this potential could show as the compensatory growth.

Because the genetic differences in tail length resulting from possible correlated responses are small compared with these compensatory growth effects, they can only be detected by using the Control lines.

When this was done it was clear that selection in the cold environment had not changed the genetic potential, that is the compensatory growth of tail length was similar in Cold and Cold Control lines and thus the genetic potential was similar. However, compensatory growth was less in the Control line at both four and seven weeks in all temperatures than in the Medium (selected) line. This difference still held even though the four week tail lengths were smaller in the Control line, so that a greater compensatory growth might have been expected. But the results show that the genetic potential of the Control line was less than that of the Medium line.

3. Experimental design

The differences between lines for four week tail lengths (and in some cases for four weeks body weight) has complicated both the analysis and the interpretation of results. An experimental design which crossed mice between environments at a younger age (either soon after birth, or when the mother was pregnant) would have solved this problem. However, although this type of design was considered, the management problems associated with this type of experiment and the large populations that would be needed were considered to make this type of experiment an impractical proposition. Even if these management problems had been overcome, Bigham (1965) found that his mortality in the cold environment was due to deaths of the lower body weight mice. If the lower body weight or less fit mice had died in the extreme temperatures this could have seriously biased results.

Crossing over mice at four weeks of age resulted in very few deaths in any of the environments.

4. Body weight - tail length relationships

It is now possible to consider body weight and tail length relationships in terms of acclimation to different environments and the function of the tail as a thermoregulatory organ.

If indeed a long tail in a hot environment and a short tail in a cold environment are of functional significance, this should be reflected in the seven week body weights for all groups. But when the Hot line mice were moved to cooler environments their long tails did not seem to be any disadvantage, as body weights were just as good or better than those of the mice reared in these environments (these mice having shorter tails).

Although both the Cold and Medium lines (with shorter tails) actually showed a lower growth rate when moved to the hot environment, this depression of growth was similar to the Hot line already in this environment with a longer tail length. Thus here again tail length does not seem to be of direct adaptive value in terms of body weight growth.

When Hot line mice were moved to cooler environments the tail growth almost stopped and tail growth between 4 and 7 weeks was very small. On the other hand both the Medium and Cold lines showed compensatory growth in the hot environment.

Since these tail length changes were mostly independant of body weight changes it would seem that both a direct effect of temperature on tail length growth in the hot environment and reduction in tail length growth in the cold environment due to vasoconstriction, could provide possible answers for these results. These hypotheses will be considered further in the next chapter in relation to the experiment as a whole.

These conclusions are very similar to those of Cockrem (1963), who found that genetic differences in tail lengths and body weights were not associated with differences in the ability of these two strains to adapt to different temperatures.

Chapter VII

FINAL DISCUSSION

Chapter VII

FINAL DISCUSSION

1. Selection for body weight in different temperature environments

No other experiments are known where selection for body weight has been compared in different temperature environments.

However, Barnett (1965b) reports an experiment in which a genetically heterogeneous stock of mice was selected for ability to produce large litters in a cold environment (-3°C). The fertility and the 16 week body weight of these mice increased during twelve generations of selection. By this stage the mice in the cold were heavier than the controls (at 21°C) despite a smaller amount of abdominal adipose tissue (Barnett 1961). But associated with this increase in body weight there was an increase in tail length which ceased after about fifteen generations of selection. Although Barnett's mice were kept at the fairly severe cold temperature of -3°C it is worth noting that cotton wool was provided as a nesting material. No special nesting material was provided in the cold environment (7°C) in this study.

Barnett's result is in direct contrast to the Cold line in this study where there was an increase in body weight over four generations of selection, but no increase in tail length. In Barnett's study it can not be ascertained whether the increase in body weight is a genetic or a long term acclimation effect. Barnett and Widdowson (1965) found that a highly inbred strain of mice bred at -3°C for fourteen generations did increase in body weight but did not show any increase in tail length. This increase in body weight is most likely due to acclimation, and in this case the effect of the cold on the growth of the tail was independant of body weight growth.

The increase in body weight in this present experiment in the Cold line was at least partly genetic for this line was significantly heavier than the Cold Control line. Although not set up until the S_2 generation, the Cold Control line body weights did not increase significantly over the three generations that this line functioned, nor did the tail lengths increase in this line.

The lack of genetic correlation between body weight and tail length was confirmed in Experiment II.

In Experiment II the performances of all lines were studied in all temperature environments. This type of experiment has been carried out using different nutritional environments, but as far as is known this is the first experiment to report on the performances of selected lines in different temperature environments.

There is no evidence from this study that would support Hammond's thesis that selection should occur in the optimum environment. In fact in this study it is difficult to say what the optimum environment is, the performance by all lines in the standard and cold environments being very similar. However, the hot environment was the most severe environment, judging by the depressed growth rates found in all lines in this environment. There is some suggestion that this adverse environment is the best environment in which to select. Thus the Hot line body weights were increased (so that the males were significantly heavier than those in the other two selection lines) when moved to the two cooler environments. It was also found in Experiment I that the realized heritability in the Hot line was higher than in the selected lines in the other two temperatures. This higher realized heritability in the Hot line, plus the results from Experiment II would suggest that although absolute weights are depressed in a hot environment, genetic progress is not. But before advocating selection in a hot environment, two undesirable correlated responses must be noted. Fertility was adversely affected by the hot environment and the longer period between mating and first parturition means that the generation interval is increased and thus the rate of genetic progress would be reduced. Also the Hot line was fatter than all other lines, this increased fat anabolism being at the expense of protein deposition. If results from mice have any generality (and obviously application of these sort of conclusions to domestic species must be made with care) then this change in carcass composition would certainly be undesirable.

As has already been mentioned the performance of the Cold and Medium lines in each environment was very similar. However, there was still a higher realized heritability in the Cold than in the Medium line, although, unlike the Hot line, this was not reflected in the cross-over results.

Thus the best conclusion from all these results would seem to be that selection should take place in the environment in which the animals are to live.

2. Tail length responses

In both Experiment I and II it was found that tail length was temperature labile. Thus tail lengths were longer in the hot environment and shorter in the cold environment, as most other workers have observed.

But the results from Experiment II do not confirm that the tail of the mouse is necessarily an important organ for heat regulation.

To at least some extent body weight should reflect the over-all fitness of an animal. The fairly small body weight differences between lines and environments (apart from the lower weights in the hot environment), associated with large differences in tail length would suggest that tail length per se has not been of critical adaptive importance. Although the mechanism of this increased tail growth in the hot environment is not known a possible theory can be suggested. It would seem that the increased tail length growth is independant of the body weight growth in the hot environment (that is body weight growth is depressed by the hot environment). Thus a direct temperature effect on the mitotic rate of the tail tissues and a vasodilation effect are possible causes of an increase in tail growth in a hot environment. These effects are most likely related, with vasodilation probably being the primary effect.

Rand et al. (1965) found that there was an abrupt vasodilation in the tail of the rat at ambient temperatures between 27 and 20°C. This resulted in blood flow in the tail increasing from less than 5 ml. to about 40 ml. per 100 ml. tissue per minute. Chevillard et al. (1963) concluded that the greater tail growth in a hot environment was due to a greater blood flow after vasodilation and thus a higher level of cellular oxygenation. However, as Cockrem and Wickham (1961) have pointed out, the association between cutaneous blood flow and growth (wool growth in this case), may be due to other factors besides the increased supply of nutrients supplied by the increased blood flow. Diffusion rates from the capillaries to the cellular mass usually increase at higher temperatures. Also temperature affects the speed of a chemical reaction. Thus increased rates of biochemical processes may be just as important as an increased supply of nutrients. Heroux (1960) also found an association between tissue temperature and mitotic rate. Thus the higher the tissue temperature, the higher was the induction rate (rate at which cells start division) and the shorter was the mitotic duration. It was found, for example that a 7°C difference in tissue temperature resulted in a seven-fold difference in mitotic duration.

Thus it can be seen that there are a number of possible explanations for the increased tail growth in the hot environment. Probably all of these have at least some part in the final result, as would be expected in a complex process such as growth.

Rand et al. (1965) also found that there was a decrease in the critical vasodilation temperature after cold acclimation and also a greater vasodilation when these cold acclimated rats were moved to warmer environments. This even greater increase in blood flow could account for the compensatory growth effect in tail length when the Cold line was moved to warmer environments. These observations by Rand et al. (1965) have been confirmed by Thompson and Stevenson (1965, 1966). This increased blood flow could also be facilitated by an increase in the number of capillaries found in the peripheral tissue of cold acclimitized rats (Le Blanc 1963).

The opposite effects to those postulated to occur in the hot environment could be occurring in the cold environment, that is a decreased mitotic rate and vasoconstriction of the blood vessels in the tail. The vasoconstriction of periphereal vessels in cold acclimated mice could be associated with the increased level of catecholamines found in cold acclimated animals (Chevillard et al. 1963, Evonak and Hannan 1963, Sellers and Schonbaum 1963). Heroux (1960) found that in rats acclimated to 6°C for 118 days the mitotic activity in the ear and tail tissues was very slow. Heroux concludes that the adaptation that has taken place in the cold is one that allows the cells to overcome the mitotic blocking effects of cold (Heroux 1959a) but which then only allows mitotosis to continue at a much slower rate.

It is worth noting that these possible explanations for increased and decreased tail growth in hot and cold temperatures respectively are factors that can be fairly easily measured. Thus the validity of this hypothesis could at some later stage be checked.

3. The genetic correlation between body weight and tail length

Probably the most interesting aspect of this study has been the observation that the genetic correlation between body weight and tail length is not realised in the cold environment but is in the standard and probably the hot environments.

It is realised that this study suffers from some experimental limitations, so that the results must to some extent be considered as preliminary ones. Thus the small subgroup numbers in Experiment II resulted in some sampling variation and as some of the differences found were not very large, random drift can not be entirely discounted. However, the results, being positive rather than negative, do give encouragement for looking at this problem further. One possible way of doing this would be to replicate this experiment, using larger populations, selecting for longer, using larger numbers when mice are changed between environments, and exchanging mice between environments more often. Another obvious, but interesting possibility, would be to reverse the selection. That is select in the same three temperature environments for increased tail length and study the correlated response in body weight.

The reason for the lack of genetic correlation between body weight and tail length in the cold environment is not obvious.

This study was originally set up based on two major considerations. Firstly it had been shown by Cockrem (1959) that it was possible to select combinations of body weight and tail length genes which were not contributing to the genetic correlation between these characters. Also on physiological grounds, it might have been expected that, if a short tail was indeed of adaptive significance, then natural selection might have been preventing any increase in tail length, even though body weight was increasing.

Falconer (1960b) has discussed the genetical problems of genetic correlations due to pleiotropy, when two quantitative characters are being considered. Three sets of genes are postulated - those that effect each character separately, plus genes that affect both characters. Because interaction of all these genes seems inevitable, the genetical situation is a complex one.

The conclusion from Experiment II was that tail length was not of adaptive significance when all lines were tested in all environments from four to seven weeks of age. Thus the lines with longer tails which were moved into the cold environment probably vasoconstricted their tails so that their length was of no disadvantage to them. But the Cold lines which were kept permanently in the cold environment may have had enough time (six weeks) in which to have become acclimated to the cold environment with concurrent changes in critical vasodilation temperatures (Rand <u>et al</u>. 1965). In this case the tail could have adaptive value in these particular circumstances. The lack of correlated response in the cold environment could then be explained as a negative selective pressure.

Alternatively a correlated response may only occur when there is a positive selection pressure and as this was only available in the hot and standard environment, this could be a possible explanation for the correlated response in these environments. But in the cold environment there was no positive selective pressure available and hence the lack of response observed.

It can only be concluded that it is not really known what has occurred, and that the above explanations are some possible theories that can be advanced.

Some final implications

Falconer (1960a) concluded that there had not been enough studies on correlated responses over reasonably long time periods (that is generations). Falconer (1960b) also concluded that - 'a wider empirical knowledge of the responses to selection in different environments and of correlated responses in general is needed'.

It has been realised for some time (Lush 1945) that heritability estimates are a function of a particular population and environment. However, although the same reasoning would seem to hold for other genetic parameters, this does not seem to have been considered in any detail until recently.

Bohren <u>et al</u>. (1966), used computer simulation to study the changes in genetic variances and covariances as selection proceeded. The conclusions reached were.

"It has been accepted in quantitative genetic theory that predictions of direct response have only short term validity because of the necessary changes that selection would bring about in the genetic variance. It appears from the results that the genetic covariance between two characters may be even more sensitive to changes in gene frequency brought about by selection and presumably also due to changes due to random sampling when the population size is small.

The validity of existing theory for the prediction of correlated responses is likely to be much poorer than for the prediction of direct responses."

In the light of these conclusions it would seem reasonable to conclude that similar reasoning could be extended to genetic covariance estimates made in different environments.

Thus any genetic covariance estimates may not only change with time, but also may not hold in different environments.

The results of this and other similar experiments would suggest that accurate prediction and decisions based on genetic parameters estimated within any particular environment or population may not apply to other environments or populations. It would seem that the parameters may define problems, such as low heritabilities or undesirable correlations but that the solution of these problems lies in examining the physiological basis of these parameters or correlations.

It could also be suggested that examining the physiological basis of any genetic situation prior to carrying out time consuming and possibly expensive calculation of parameters, may reveal possible outcomes of experiments at more fundamental levels. If indeed genetic parameters have limited generalility as has been suggested, then it may be more profitable to set up selection experiments even without predicting the likely outcome and then, if and when problems occur, look for possible explanations at the physiological level.

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