ANALYSIS AND INFERENCES FROM LONG-TERM QUANTITATIVE GENETIC SELECTION EXPERIMENTS

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DECLARATION

I declare that this thesis is my own composition and that the research described in it is my own work, except where otherwise stated.

Said H. Mbaga
December, 1996

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To the memory of Daudi Liana (1905-1996)

ABSTRACT

This study was aimed at understanding the genetics of growth in mouse lines divergently selected for body weight for 50 generations and to investigate the linearity or non-linearity of offspring-parent regression obtained from unselected, random bred lines. The relationship between offspring and parents was investigated using a large body of data comprising 1090 offspring(family mean)-parent pairs. Linear and polynomial models were fitted for the regression of 6-week body weight in offspring on one or both parents. Regression analysis of offspring mean on mid-parent showed that heritability of 6-week body weight was similar to the estimates from simultaneously regressing offspring on both parents provided that the records were standardised within parent-sex category. Regression of offspring family means (sons and daughters) on sire's weights were somewhat non-linear, while offspring-dam regressions were generally linear. Multivariate regression analyses combining both parents gave similar results compared to results of offspring-one parent regression.

The objectives of the second study was to quantify the responses and estimate base population parameters as well as their changes arising from selection from lines divergently selected for high or low body weight. This study used 10-week body weight data from 30 generations, from lines which were previously selected for 20 generations based on an index of lean mass (P-lines), and crossed to form the P6-lines. The mean divergence in 10-week body weight at generation 21 was 13.3g increasing to 32.2g at generation 50. The overall divergence relative to the base population (i.e the P-lines) was $6.3\sigma_p$. The low line (L) appeared to plateau at generation 40, and the plateau was probably due to natural selection favouring heterozygotes through the joint action of artificial and natural selection, but not due to exhaustion of additive genetic variance. When response was considered separately for each sex in the P6-lines, females of the high line (H) responded more to upward selection than the males, while males responded more than females in the L line. Sexual dimorphism (ratio of male/female mean) was consistently higher in the L line. The genetic correlation between male and female 10-week body weight estimated by Restricted Maximum Likelihood (REML) was 0.84±0.03.

Estimates of heritability and litter variance (expressed as a proportion of the phenotypic variance, i.e. c^2) inferred to generation 21 of the P6-lines assuming homogeneity of variances were 0.39±0.02 and 0.26±0.02 respectively. Using the likelihood ratio (LR) test it was found that heritability and c^2 estimates from the high and low line were not significantly different when model accounting for heterogeneity of additive and common environmental effect in the two lines was fitted. Selection for body weight was associated with a positive correlated response in litter size at birth in the P6-lines in the direction of selection in both lines. However, in the H line, litter size declined after generation 30 presumably due to inbreeding and unfavourable environment. Heritability estimates for litter size from bivariate analysis were 0.07±0.04 and 0.28±0.05 and the corresponding genetic correlation with 10-week body weight were quite asymmetrical being, -0.23±0.11 and 0.48±0.07 in the high and low line respectively,

Analyses of data from generations 21-30, 31-40 and 41-50 separately, but including full pedigree information to generation 21, showed a marked increase in genetic variance and heritability in later generations, but the estimates were confounded with an increase in the environmental variance. These changes could not be attributed to the effect of selection in an infinitesimal model and suggested that some changes in variance due to changes in gene frequency had occurred during the course of selection. Similarly, litter size tended to increase after generation 40, suggesting that there are still some genes with pleiotropic effects segregating in the population and the selection limit observed was not due to attenuation of additive genetic variance.

Chapter 1

GENERAL INTRODUCTION AND REVIEW

1.1 Introduction

Artificial selection is based mainly on an individual's phenotype or family information on the assumption that under similar environment, a difference in phenotype reflects the genetic superiority of a given individual. In explaining results from artificial selection, one is always interested in having a better knowledge of the character selected including among other things a) how long the rates of response found in early generations continue, b) to obtain a measure of selection limits, c) to estimate long term correlated responses, particularly in fitness (Hill, 1979,1980), d) to use selected lines to search for quantitative trait loci that have affected the selected and correlated traits (Eisen,1994). Inferences about the effect of long term selection will depend on the knowledge about the amount and kind of genetic variation present in the base population and at any point in the selection program.

Regarding progress in selection, the rates of response in most cases are reported either as changes per year or per generation, the former becoming important when we have overlapping generations. For the purpose of comparison, response in such programme can then be evaluated on a short or a long term basis. The necessity of considering long-term implications for the definition of the breeding goal is widely perceived. However, most breeders are forced to take the effect of competition into account, which implies emphasis on a short term response. Inefficient design and inadequate scale of experiment including confounding with environmental trends and inbreeding depression limits usage of large animals for long term selection experiments (Dickerson, 1974). Therefore, laboratory animals (e.g. mice, *Drosophila*, *Tribolium*) have often been used to validate quantitative genetic theories. Laboratory animals would help us learn more quickly about the expected changes in genetic trends in long term selection experiments. Where there are limits to selection, long term selection may help in elucidating the nature of those limits and in turn provide information on expected changes which are important in devising ways to overcome them (Eisen,1974).

Genetic parameters such as heritability and genetic correlations are required inputs for prediction of future responses and the assumption of linearity of the heritability is usually accepted by animal breeders. Estimation of heritability by offspring-parent regression assumes that the offspring-parent relation is multivariate normal distributed, implying a linear relation. However, such assumptions may lead to biased estimates of response if the relations deviates much from a linear expectation. Gimelfarb and Willis (1994) concluded that non-linear offspring-parent regression do not predict the same response as that to that expected when the relation is linear. Likewise, non-linearity in the relationships between offspring and parents has been attributed to be the cause of asymmetries in response observed in most bi-directional selection experiment (Frankham, 1990). Some recent studies, particularly that of Gimelfarb and Willis (1994) and Koerhuis (1996) have again focussed attention on what is an old, and generally ignored problem.

Most researchers also ignore the fact that heritability estimates may differ between males and females. When the differences are large, selection for increasing or decreasing value of a trait may result in different responses between the sexes which become more important if only one sex is selected, i.e. sex specific selection (Meagher, 1992). The divergence between sexes in the evolution of sexual dimorphism (defined as the ratio of male/female or female/male) also depends on the genetic correlation between a given trait in males and females (Yamada and Scheinberg,1976; Lande,1980; Meagher,1992). For example, if the genetic correlation between males and females is close to unity and the intensity of selection and heritabilities does not differ between sexes, then selection of one or both sexes will give similar response in both sexes. However, when the genetic correlation deviates much from unity, selection response is expected to differ in the two sexes. Two populations were mainly used in this study, the P-lines and the P6-lines derived from the former. In the P-lines, only males were selected based on an index of lean mass at 10-weeks of age and in later generations the lines were crossed and both sexes selected for 10-week body weight (the P6-lines). It is of interest then, to investigate whether the genetic correlation is sufficient to limit independent evolution of body weight in males and females and to ask what factors other than genetic correlation might contribute to difference in responses.

In mice, selection for body weight or growth rate has often been found to result in a positively correlated response in litter size in the direction of selection (Eisen *et al.*,1973; Brien *et al.*,1984; Beniwal *et al.*, 1992b). Because litter size is a major component of sow productivity it should be considered in defining selection objectives in a pig breeding programs and Eisen (1974) supports the view of many investigators who see growth in the laboratory mouse as an excellent model for growth in large animals, e.g. in pigs or of interest for itself. Broadly the objectives of this study are: a) to investigate aspects of the genetics of growth in mice lines divergently selected for 10-week body weight for 50 generations, b) to examine the correlated response in litter size at birth, c) to evaluate linearity or non-linearity of offspring-parent regression using 6-week body weight data obtained from unselected random bred control mice lines.

1.2 Literature review

1.2.1 Selection limits

Theoretically, the limit to selection will inevitably be met when all alleles affecting the trait have been fixed in the population or line (Roberts, 1966a; Bulmer, 1980) or when overdominant alleles have reached an equilibrium state (Eklund and Bradford, 1977). Experimental evidence from laboratory mice suggests that the limit to selection for body weight or growth rate will be determined largely by exhaustion of genetic variance (Roberts, 1966b). Plateaux are commonly described in the literature on selection experiments and many factors can affect rate of response and the time needed to reach the selection limit (Roberts,1967; Eisen,1974; Roberts,1974a,b). These may be categorised into environmental factors, genetic properties of the trait and how selection was conducted. However, among long term selection experiments it appears to be a common phenomenon for plateaux to be reached without exhaustion of additive genetic variance (Al-Murrani and Roberts, 1974; Bünger *et al.*, 1990; Beniwal, 1992a). The possible reasons for failure to respond when genetic variance is present have been summarised and discussed by Falconer (1989). Briefly, the factors include a) the limit being intrinsic imposed by the nature of the character or the way in which it is measured, b) Reduction in fertility in selected lines, and selection differential in later generations, c) Favourable alleles being dominant, d) Artificial selection being opposed by natural selection and, e) natural selection may favour heterozygotes.

The presence of genetic variance can be detected by reversing the direction of selection, or by suspending selection so that natural selection alone operate (Al-Murrani and Roberts, 1974a; Eisen, 1972; Falconer, 1989). Attempts to predict the limit to selection and possible ways of overcoming it have also received much attention (Eisen, 1974; Vangen, 1993; Bünger,*et al.,* 1990). Eisen(1974) reviewed a number of laboratory experiments in mice (1965-1974) and concluded that response tends to plateau after 10-30 generations of selection depending on the genetic strain and direction of selection. However, few experiments have shown a positive response in the direction of selection for more than 50 generations without reaching a plateau. These include the famous divergent selection for oil content of maize seed (Dudley, 1977), selection for abdominal bristle number in *Drosophila* (Yoo, 1980), selection for 6-week weight in mice (Bünger *et al.*,1990), and selection for pupa weight in *Tribolium* (Enfield, 1976). The results of these experiments showed that in later part of the experiment heritability declined substantially e.g. in Bünger *et al.*(1990), h^2 declined from 0.59 at the start to 0.09 after 50 generations of selection, whereas, Dudley (1976) reported a decline in h^2 from 0.20 and 0.18 in two maize protein strains (IHP and ILP) to 0.15 and 0.07 respectively after 76 generations. These reductions were also associated with a gradual decline in cumulative selection differentials. Understanding the nature of selection limits would be very relevant to exploration of methods of overcoming the limit and making further progress.

1.2.2 Heritability estimate

Since the main use of heritability estimates lies in their ability to predict response to selection in plant and animal breeding improvement programs, it is useful to have reliable estimates for predicting the effect of selection. While realised heritability measures accurately the response to selection, it is only available 'after the fact' (Falconer, 1989), whereas the expected estimates like those derived from degree of resemblance between relatives can be obtained in advance of a selection program but are only of practical value if they reflect the realised heritabilities. Sheridan (1988) reviewed experiments in both laboratory and farm animals in which resemblance between relatives and realised heritability estimates were available. Of 198 comparisons, 54% differed by more than 30% of the realised heritability and 38% differed by more than 50%. Realised heritabilities differed substantially with direction of selection in about half of the bi-directional experiments and concluded that there was a general lack of good agreement between estimated and realised genetic parameters.

James (1990) and Hill and Caballero (1992) suggested that the disagreements found in Sheridan's survey could result from improper sampling errors of estimates for comparison, distribution of gene effects and segregation of genes of large effect in long term experiments. In addition, deviations even from well controlled experiments can arise from genetic asymmetry (Frankham, 1990), response plateaux without gene fixation, maternal effects, G × E interactions, physiological ceilings and major gene mutations (Bell, 1974). These deviations have been identified many times, however, by definition they are unpredictable, hence they cannot be meaningfully incorporated into a selection index, but hopefully their early detection may facilitate appropriate modification of breeding programs or interpretation of resulting responses. Further, Thompson and Atkins (1994) argue that simple comparison of base population and realised heritability estimates are 'naive' even when sampling variances have been estimated appropriately, since the two estimates are not necessarily equivalent in expectation. Likewise, if directional selection is practised, different estimates of realised heritability may be obtained in the two directions, giving a valid description of responses, but both cannot be a valid estimate of heritability in the base population (Falconer,1989). Simianer(1994) concludes that all predictions are conditional on the model used being correct. A critical point is that selection lines should be replicated because inbreeding effects and chance fixation of genes will cause different expected responses in lines of different size affecting the mean response in long term selection (Hill,1972; Falconer,1973; Yoo,1980). Likewise if the primary objective of the selection experiment is to estimate correlated responses, replication is required if the sampling variance of correlated response is to be computed (Hill, 1980).

Estimates of heritabilities may also be biased upward or downward depending on the degree of assortative mating. Reeve (1961) has shown that when genetic variance is entirely additive, there is no bias from assortative mating in computing heritability from the regression of offspring on mid-parent. However, more serious bias is likely to arise due to correlation between sibs when non-random mating is used. The genetic consequence of assortative mating by the phenotypic value is to increase the additive variance and consequently heritability through the correlation between the breeding values of mated pairs. In theory, estimates of heritability derived from offspring-one parent regression would tend to be biased upward by a factor $1+r$ where, r is the correlation between mated pairs (Crow and Kimura, 1970).

Mixed model methodology

For balanced data, it has been common practice to estimate the variance components by equating the mean squares in the analysis of variance (ANOVA) to their expectations. However, in recent decades quantitative analyses of animal breeding data have moved from the conventional ANOVA to the more optimal Maximum Likelihood methods, e.g. Restricted Maximum Likelihood (REML). Because of its statistical properties, REML is now considered the optimal procedure for a wide range of animal breeding data analyses. The REML algorithm estimates the treatment effects and variance components in a linear mixed model: that is, a linear model with both fixed and random effects (Meyer, 1989,1991a). Like regression, REML can be used to analyze unbalanced data sets; but, unlike regression, it can also account for more than one source of variation in the data and provide an estimate of variance components associated with the random terms in the model. Assuming polygenic additive genetic inheritance, the REML method offers the best use of all available data as it accounts for all known relationships among relatives in the pedigree (Thompson, 1977b; Patterson and Thompson, 1971; Searle, 1989).

When the additive covariance structure between individuals is modelled by the numerator relationship matrix **A**, the estimation and prediction procedures are known as the 'animal model'(AM). By inclusion of the covariance matrix of the random effects in the model, the numerator relationship matrix accounts for a decrease in genetic variance due to selection, inbreeding and assortative mating (Kennedy *et al.*, 1988), provided all the data on which selection is practised are included (Van der Werf and de Boer,1990). In the context of a mixed model, the drift variance and the covariance among generation means appear as the average relationship or coacentry among individuals in the same or different generations (Sorensen and Kennedy, 1993). The mean covariance of performance of collateral individuals is $2FV_A \sim tV_A/N_e$, where F is the inbreeding coefficient and t is generation number (Hill, 1990).

Regarding the effect of changes in environment Thompson (1986) suggested the use of across generation relationships in selected line(s) and mixed model analysis to disentangle genetic and environmental trends, without recourse to the control line or assumptions of symmetric response, by using measures of divergence between lines. However, efficiency of computation and time for convergence has been limited, especially when large data sets are analyzed and if many parameters are to be estimated particularly with the AM models. A derivative free method (DFREML) has been suggested (Graser *et al.*,1987; Meyer, 1989) as an efficient computing method which allows animal models comprising thousands of effects to be handled computationally. DFREML attempts to locate the optimum of the likelihood function without knowledge of its derivative, hence the name DFREML.

In analysis of animal breeding data using mixed model methodologies it is often assumed that the variances for fixed and random effects involved in the stratification of data are homogeneous. In the United States for example, across-herd evaluations for swine assume that all records come from the same population with constant genetic and residual variances (See, 1994). In dairy cattle, heterogeneity of genetic and residual variances for yield traits are also widely documented (Brotherstone and Hill,1986; Dong and Mao,1990; Boldman and Freeman,1990). Hill (1984) considered methods of accounting for heterogeneous variance when constructing selection criteria and suggested that observations should be scaled using estimated standard deviations. Alternatively, a logarithmic transformation to improve normality and to adjust for the relationship between mean and variance which may be a source of heterogeneity can be used (Falconer,1989). However, increased computer power and development of more efficient algorithms in recent decades have made it possible to partition and identify meaningful sources of variation as well as assess their magnitude. This becomes important when the population can be stratified with respect to some criterion e.g. herd, line, and tests can be performed to verify whether the variances between strata are homogeneous or not (Beniwal, 1992a,b; Heath *et al.*,1995). If the source of heterogeneity is due solely to differences in environmental variance, the use of unscaled data may lead to loss of efficiency as the tendency will be to select animals from the more variable groups especially if selection is intense (Hill, 1984). Hence, accounting for heterogeneity in variances arizing both from environmental and that due to genetical differences among groups in selection programs is expected to maximize genetic progress (Hill, 1984; Gianola,1986; See,1994).

Maternal effects

Maternal influences are an environmental effect with respect to the individual's offspring, but when considered as a trait of the dam they can be partitioned into genetic and environmental components. Maternal effects are important to animal breeders who would like to eliminate the influence of maternal effects so that selection is for direct genetic merit. The expression of maternal ability in offspring is confounded with the expression of genes for growth, half of which were received from the dam (Willham, 1980; Thompson, 1976). Interest in maternal effects also exists for improving maternal performance (Roehe and Kennedy, 1993a,b). Roehe and Kennedy(1993a,b) evaluated the presence of maternal effects on selection response and used animal model evaluation in a simulated pig population in which maternal effects were included or excluded from the model. They concluded that; a) when maternal effects were included in the model, genetic progress for the direct effect was little affected by their presence; b) when maternal effects were present but were ignored, response could be further reduced, but the effect was often not great. Lande and Kirkpatrick (1990) concluded from numerical studies that accounting for maternal effects can lead to a considerable increase in the efficiency of artificial selection. If maternal effects are large enough, they can be exploited by breeding specific dam lines (Roehe and Kennedy, 1993b), for example, to improve mothering ability, birth weight and other traits considered as traits of the dam. However, the response obtained will depend on the extent to which the trait is genetically influenced by the dam.

In mice maternal effects are commonly studied by cross-fostering pups in order to separate prenatal and post-natal maternal effects. Eisen and Bandy(1986) used a crossfostering design to estimate postnatal maternal correlated response in lines selected for 6-week body weight, litter size and on an index of (large) litter size and small body weight. They found that the correlated response due to prenatal maternal genetic effects was much larger than that from postnatal maternal effects and correlated responses in reproductive and maternal traits were little affected by the size of the litter in which the dam was reared. Rutledge *et al.*(1972) found that in the mouse, growth from 0-7 days of age is due primarily to postnatal maternal differences rather than direct genetic differences. About the time of weaning (21 days of age) the importance of postnatal maternal effect begins to decline while direct genetic effect became more important. From day 6 to day 49 the variance due to dams genotype varied from about 10-15% of the phenotypic variance. Similarly, the conclusion regarding postweaning decline in maternal contribution in mammalian species to total phenotypic variance has been documented (Hanrahan and Eisen,1973; Bell,1974; Gotz *et al.,* 1991; Slawinski,1982). There is also considerable evidence indicating that maternal effects can influence body weight traits measured postweaning e.g. in beef cattle (Meyer, 1992), in Merino sheep (Swan and Hickson, 1994) and for litter size in mice and pigs (Falconer,1965; Van der Steen, 1985). Maternal effects acting in this manner have been argued to result from genes being more active (or more expressed) in later parts of growth, whereas, in early stage of growth their effects are masked by the maternal environment (Riska *et al.,*1985).

1.2.3 Linear vs. non-linear offspring-parent regression

Assuming that the joint distribution of genotypic (or phenotypic) values among relatives is multivariate normal and the number of loci is very large, Bulmer(1980) showed that the expectation of an offspring phenotypic value conditional on the parental value depends on the offspring genotype and the offspring environment conditional on the parent phenotype *i.e.* $E(Y_O \mid Y_S) = E(G_O + E_O \mid Y_S)$ where subscript O and S stand for offspring and sire or dam respectively. Since offspring environment is assumed to be distributed independent of parental environment, then the expectation of offspring phenotype is equal to the expectation of offspring genotype conditional on the parental phenotype *i.e* $E(G_O \mid Y_S)$. When both parents are considered, the joint regression of the offspring's genotypic value, G_O, on the genotypic values of both parent, G_S and G_D has the expectation, $E(G_O|G_S, G_D)$ $=\frac{1}{2}(G_S + G_D)$. This derivation assumes the contributions from the two parents are independent, additive and mating is at random and the joint regression of the offspring on one or both parents is linear.

In prediction of response the assumption of linearity of the regression function of offspring on parent is usually accepted by animal breeders in situations where non-linearity is moderate. However, there is experimental evidence indicating that the regression of offspring on one or both parents can be non-linear, for example in mice (Nishida,1972) and in *Drosophila* (Robertson,1977; Gimelfarb and Willis,1994; Mäki-Tanila,1982). In theory, non-linear offspring parent regression is expected for characters showing genetic asymmetry due to directional dominance (i.e. some loci are dominant in one direction more than in the other, $\sum d \neq 0$) and/or asymmetrical gene frequencies or skewness in the distribution of environmental effects (Bulmer,1980; Mäki-Tanila, 1982; Ward,1994; Frankham,1990). This sort of non-linearity may become more important in reproductive characters which are known to have a large dominance component (Frankham, 1990).

Nishida (1972) investigated the linearity or non-linearity of offspring -parent regression in mice at different age intervals from 0 to 70 days, but his results were very inconsistent. He found that across the age groups, offspring-parent relations could be describe by linear, quadratic, cubic or quartic curves with different direction of concavity (downward or upward) depending on the age of the animals. A linear relation was observed only at birth and at 6 days of age, and there was no unifying feature to describe the different curves. Nishida and Abe (1974), attributed the non-linearity to skewness in the distribution of genotypes or environmental effects.

Further, they concluded that if the genotypic skewness is larger than the environmental, then the regression curve will be convex and, when less, concave. Similar conclusions on the differences in contribution of genotype and environment as the probable cause of non-linearity were arrived at by Curnow (1960), Bulmer,(1980), and Mäki-Tanila (1982).

In the study of non-linearity, exponential or ordinary polynomials have been used mainly to describe the possible relation between offspring and parents. The high level of non-linearity observed in some of the studies could be associated with some drawbacks of polynomial models especially when fitted terms are highly correlated. Nelder (1966) argued that ordinary polynomials are accompanied by some disadvantages. The most important being that the polynomials are 'unbounded' i.e. as any x (independent variable) is increased indefinitely any polynomial containing it eventually takes a value (either positive or negative) as large as we please. However, he argued that for biological organisms the response cannot be infinite, at some point a saturation or biological limit will be attained and response tends to zero. Recently, Gimelfarb and Willis (1994) attempted to model the regression surface for different measured characters in *Drosophila.* The characters measured were body weight and abdominal bristle score at 2 days and wing length, from 176 and 159 families respectively, from unselected random mated population. Polynomial regressions of up to $5th$ order were fitted to standardised offspring and parental observations. Significant non-linearity was found for all the characters investigated, but no biological reason was given for these deviations. Assuming that truncation selection is practiced and the actual offspring-parent regressions are polynomial, they concluded that if the relationship is non-linear, the response by the mean to selection is determined in such a case not only by the selection differential, but also by changes caused by selection in higher moments of the parental distributions. However, Kempthorne(1960) argued that if non-linearity is observed and we designate the parental value by x, then x^2, log x, $\sqrt{x}$ and indeed any function of x which can be defined, then in selecting for x, it implies that we are also selecting for x^2 or log x or against $\frac{1}{x}$ and so on, which is biologically difficult to interpret. However, if the purpose is not to model a specific biological mechanism, but only to see whether an actual (but unknown) regression surface for a particular trait can be approximated better by a curved surface rather than a plane, fitting of parental terms not higher than quadratic or cubic have been recommended (Robertson, 1977).

From the perspective of a biological model of gene expression, the offspring-parent regression coefficient represents the genetic contribution to the kinetics of biochemical metabolism occurring within an individual, and the offspring-parent regression coefficient is a function of the population means as well as variances of the genetic and environmental effects (Ward, 1994). Without going into the details of derivations, he showed that the genotype-phenotype mapping function for simple linear metabolic pathway is P = E/G, and the offspring-parent regression coefficient follows as:

$$\beta_{op} \cong \frac{Var(G)\frac{\mu^2(E)}{\mu^4(G)} - [\mu(P) - \frac{\mu(E)}{\mu(G)}]^2}{Var(P)} \tag{1.1}$$

where G,P=J and E are the genotype, phenotype and environmental components respectively. The equation illustrate explicitly that the offspring-parent regression coefficient for metabolic flux (J) through a simple linear pathway is a direct function of $\mu(E)$. On the basis of his model, Ward concluded that the offspring-parent regression coefficient can increase directly with the average value of the environmental contribution to the phenotype. From a biological standpoint he attributed the increase in regression coefficient in an extreme environment to the consequence of change in environment rather than the 'new genes' being expressed under extreme environments. He further pointed out that if the phenotypic value is the outcome of metabolic flux through a complex biochemical network, then the genotype-phenotype mapping function is always a non-linear function of an additive genetic component. It follows that if the regression coefficient can change due to change in environment, then for experiments spanning more than one generation, non-linearity in one generation versus several generations can be in part be attributed to GxE. However, to test this model requires that the genotypes (e.g. sibs) are reared in replicated environment.

Non-linearity can also be caused by extreme observations (outliers) which may arise from recording errors. Koerhuis (1996), investigated the effect of outliers as potential sources of curvilinearity on egg production traits. With Box-Cox power transformation (Box and Cox, 1964) of the data, the size and linearity of heritabilities and normality of errors improved considerably. Moreover, even after outlier eliminations and transforming the data, non-linearity was still present in some characters. The non-linearity obtained even after outlier elimination or transformation was attributed to the fact that traits like egg production are highly skewed in their distributions. In situations where the distribution of the trait is not normal other methods of data transformation have been suggested e.g. logarithmic transformation, scaling of observations by the group standard deviation and power transformation to improve homogeneity and normality (Hill,1984; Ibe and Hill,1988; Koerhuis, 1996).

Genetic changes

The analysis of animal breeding data and predictions from them generally assume the traits of individuals and their relatives to be multivariate normally distributed and implicit in this is the assumption of the infinitesimal model (Bulmer,1976;1980). The model assumes that a quantitative trait is determined by infinitely many unlinked additive genes each of small effect, and gene frequencies are assumed not to change measurably due to selection. Genetic variance of any trait under artificial selection could change due to a) a change in gene frequency, b) correlation of frequencies between pairs of loci induced by selection or linkage disequilibrium and, c) inbreeding, with the amount defined by the inbreeding coefficient. Under truncation selection on a normally distributed trait, selection reduces the additive genetic variance of the selected individuals by a proportion G = 1-kh^2 (Bulmer, 1980), where h^2 is the heritability of the trait, k is a factor depending on the intensity of selection. When selection is by truncation of a normal distribution, then $k = i(i - x)$, where i is the intensity of selection and x is the corresponding deviation of the point of truncation from the population mean. The additive genetic variance in any generation (t +1) can then be expressed in terms of that of the previous generation t by the recurrence equation:

$$V_{A(t+1)} = \frac{1}{2}(1 - h^2_{(t)}k)V_{A(t)} + \frac{1}{2}V_A,$$

where V_A is the additive variance in the base population.

Considering the effect of linkage disequilibrium, the additive genetic variance of the means of full-sib is half the additive variance of the selected parents, which is $\frac{1}{2}(1 - h^2)$ and is expected to be reduced by negative associations between loci induced by selection for an optimal value (Bulmer, 1989, Falconer, 1989). Based on the evaluation of the recurrence relations most of this reduction would occur in the first two or three generations after selection starts.

The above recurrence is based on theoretical expectations, however, its utility in analysing actual data collected from selection experiment is limited. Hill and Caballero (1992) suggested the use of Restricted Maximum Likelihood method (REML) to infer the trends of variance components by estimating base population parameters from data for different numbers of generations. A few experiments have used this approach and it was found that the genetic variance changed with increasing number of generations, implying that the infinitesimal assumption of constancy of genetic variance did not hold (Meyer and Hill, 1991; Beniwal *et al.*,1992a; Keightley *et al.*, 1994; Heath *et al.*, 1995). More recently Heath *et al.*(1995) attempted to fit models in which variance components were allowed to vary continuously (in effect regressing all variance components nested within line on generation number) using a method based on the animal model. The analysis of 6-week body weight mice data in lines selected for 20 generations suggested that there was an increase in phenotypic variance of about 50% in the Low selected lines over the course of the experiment which was attributed to increases in the environmental and additive variance components. Variance changes in the High selected lines were generally smaller than in the Low lines, although there was an estimated 20% increase in the environmental variance. Several candidate models were evaluated to explain this increase, and the dominance model was thought to be the most likely one, due to presence of some directional dominance despite the fact that the model did not predict well the observed pattern of variance changes.

Results so far available on selection limits suggest that models based on the exhaustion of the additive variance may not be sufficiently comprehensive to describe fully many of the situations derived in practice. Turelli and Barton (1990) questioned the genetic model based on the infinitesimal model, that is, with infinite loci the distribution of breeding value remains normal under selection. Using multilocus population genetic theory, they concluded that under most forms of selection the distribution of breeding values is systematically driven away from normality through generation of third and higher order linkage disequilibria. Despite the fact that experimental evidence indicates that genetic variance does not necessarily diminish, the infinitesimal model can be used to predict short term response to selection (Turelli and Barton, 1990; Hill, 1994). Other models require information on gene frequencies and gene effects and perhaps linkage associations, and such data are simply not available (James,1990).

In long term selection experiments, inferences regarding selection responses will depend, among other factors, on the type of gene action underlying responses to selection, for example the effect of sex-linked genes (Grossman and Eisen, 1989; Hasting and Veerkamp ,1993), genetic drift (Falconer, 1989), the founder effect and the effects of mutation (Frankham 1979; Hill,1982; Hill and Rasbash, 1986) and all sorts of variations which may arise at animal or molecular level (see review, Cunningham, 1990). Regarding mutation, Hill (1982) with additional experimental evidence, has shown theoretically that new variation from mutation can sustain an appreciable continuous selection response in large populations. When selection is practised immediately from an inbred base, the pattern of response is highly dependent on the size of mutant gene effects. If effects are small, variance accumulates approximately linearly and cumulative response quadratically. If mutant genes have large effects and are therefore rapidly fixed by selection, the cumulative response is expected to be nearly linear, but subject to large variation between generations and replicate lines. Lyon and Searle(1989) in their review, found as high as 25% of visible mutations in mice and in most cases the mutant had an effect on body size (mostly downward). Keightley and Hill (1990) using Restricted Maximum Likelihood approach (REML) found that the component for new variation due to mutation linked to selection (V_{mu}/V_e, i.e. the extra variation due to mutation per generation expressed relative to total environmental variance) could be as high as 0.01 per generation. Data on mammals are scanty for estimation of mutation effects and a general figure widely cited is 10^{-3} (Lynch, 1988).

Of importance to the transmission of a trait from one generation to the next and for predicting the short term response to selection is the narrow sense heritability, the ratio of additive variance to phenotypic variance ($\frac{V_a}{V_p}$). Dominance variance is generally not considered important as it does not predict the response to selection (Lynch, 1994). However, dominance variance can affect the heritability of traits following inbreeding particularly if the recessive alleles are rare (Robertson,1952; Falconer,1971; Al-Murrani and Roberts,1974a). Likewise, during bottleneck events, non-additive genetic variance (both dominance and epistatic) can be 'converted' into additive genetic variance and therefore become available for selection to act upon (Carson, 1990). The possibility that non-additive genetic variance, for example due to epistatic effects, can contribute to observed increases in total genetic variance within populations, particularly in small populations or populations undergoing 'bottleneck' or under stress, has been considered (Goodnight,1987,1988; Cockerham, 1984; Bryant *et al.,* 1986; Blows and Sokolowiski,1995).

1.2.4 Correlated response in litter size

Litter size as a reproductive trait has a major influence on the efficiency of animal production. Studies of litter size in mice have received increased interest because of the attention to this trait by the pig breeders. Improvement of this trait can be achieved from direct selection or indirectly by selecting for components of litter size

(Nielsen *et al.*,1990) or including it in selection index with other traits, e.g. growth traits. Regarding direct selection, a number of studies have been carried out on litter size, both in the short and long term (Falconer, 1971; Eklund and Bradford,1977; Buis,1988; Joakimsen and Baker,1977; Baker *et al.*, 1978; Vangen,1993). Nielsen (1994) reviewed experimental findings of selection for increasing or decreasing litter size in mice and concluded that the realised heritability for increasing litter size ranged from 0.10 to 0.20, while selection for decreasing litter size had higher realised heritability, but reached a selection limit in fewer generations. According to Eisen (1972), plateaux for litter size arise if there is a negative genetic correlation between direct and maternal effects, which would be expected to occur after many generations of selection. The negative covariance between direct additive and direct maternal effects were also found in the study of body weight traits at 3, 6 and 8 weeks and gain between 3-6 weeks in mice (Hanrahan and Eisen, 1973). They found a consistent antagonism between the two parameters in mice, tending to reduce progress in selection. This antagonism imply that the direct additive effects tend to promote large size, but the indirect (maternal) effect produce smaller offspring. In other words, increased litter size (direct effect) is associated with a reduction (indirect maternal) in piglet birth weight, which suggest a uterine constraint on pre-natal growth, due to increased competition among litter mates for uterine resources.

Evaluation of reproductive performance in Meishan and Large White pigs and their crosses revealed that increased prolificacy in Meishan pigs is controlled very largely by maternally acting genes. Both ovulation rate and pre-natal survival were enhanced in the Meishan and contributed to enhanced litter size (Lee and Haley, 1995). A negative covariance between direct additive and direct maternal effects were found. However, in practical animal breeding we are interested not only in the mode of inheritance of a particular trait but also in its relationship with other traits and correlated responses when selecting on the primary trait. Correlated characters are of interest in connection with the genetic cause of correlation through the pleiotropic action of genes arising from linkage disequilibrium i.e. a favourable allele for one trait that increases the frequency under selection may drag along with it an allele of another tightly linked gene that has a positive or detrimental effect on an unselected trait (Falconer, 1989). Generally, selection for larger mature size often increases litter size in mice (Eisen *et al.*,1973; Brien *et al.*,1984; Beniwal *et al.*, 1992b), but other experiments found no significant correlated response in litter size of mice selected for body weight (Falconer and King,1953; Barria and Bradford,1981; Bayon *et al.*,1987). Bayon *et al.*(1987) attributed the absence of response in litter size to fewer pleiotropic genes and probably fixation of these genes during the course of selection. The presence or absence of correlated response may depend to a large extent on the duration of the experiment and the genetic correlation between body weight and litter size. Table 1.1 summarises results of the genetic correlation between body weight and litter size. These results show estimates of genetic correlation that range from 0.00 to as high as 0.63. Standard errors of estimates were not given in the study of Joakimsen and Baker(1977).

Table 1.1: Estimate of the genetic correlation between body weight at various ages and litter size in mice.

r_g	No. Gen	Trait*	Reference
0.59 ^{a}	15	6-wk	Joakimsen and Baker (1977)
0.63 ^{b}		,,	
0.62 ^{c}		,,	
0.03 ± 0.07^a	15	6-wk	Bayon*et.al.* (1987)
0.42 ± 0.06^b			,,
0.21 ± 0.07^c		6-wk	,,
0.23±0.10		6-wk	de la Fuente *et.al.*, (1986)
0.23±0.08	12	3-wk	Eisen (1978)
0.27±0.10	20	Lean Mass	Beniwal (1992b)

* body weight. a,b and$c$ are estimates for high,low and divergence respectively.

Most long-term selection studies in mice where there is a reduction in response suggest that natural selection probably contributed to the selection limits for body weight (Barria and Bradford,1981; Berger *et al.*,1989). In mice selected for body weight, natural selection appears to act through reproductivity (Roberts,1974a; Wilson *et al.*,1971; Eisen,1973,1974; Beniwal *et al.*,1992). Bradford (1971) reported increased sterility and prenatal mortality in a line selected for large 6-week body weight, whereas Roberts (1967) attributed the lack of any response to natural selection acting on viability between conception and the time selection was carried out. Investigation of male reproductive capacity using mice with high genetic potential for post weaning growth, revealed a decrease in sperm production and sperm motility as well as absolute testes size (Berger *et al.*,1989). The reduction in fitness in the presence of antagonistic natural selection effects was attributed to a deviation from optimum phenotype for body size, inbreeding depression, pleiotropy or a combination of these factors (Roberts,1966b; Eisen, 1974).

1.2.5 Sexual dimorphism

Frankham (1968) defined sexual dimorphism (in the case of body weight in mice) as the ratio of male to female phenotypic means. Prediction of selection response and interpretation of selection experiments are generally based on the assumption that additive genetic variance is the only important parameter. However, there is evidence indicating that selection response may be influenced by the genetic variance in sexual dimorphism (Frankham, 1968). Eisen and Hanrahan(1972) and Hanrahan and Eisen (1973) concluded that if the genetic correlation between sexes is less than unity, a small response in sexual dimorphism in post-weaning growth rate in mice is possible and the presence of additive genetic variance of sex difference can be successfully exploited by means of artificial selection. In terms of expressivity, it is quite common for a character to be manifested to different degrees in males and females, e.g. body size, intensity of pigmentation and temperament. The increase in male body size in polygamous species has been attributed to male-male competition (Clutton-Brock *et al.*,1977). Likewise, Cue and Hayes(1990) reported a significantly large difference between sexes in parameter estimates of weight gain in mice, whereas, Hanrahan and Eisen (1973) attributed the differences in gain and absolute body weight to hormonal effects and the more rapid postweaning gain in males. Another body of theory assumes the existence of 'sexually antagonistic' genes (SA) i.e. those genes that are favoured by selection in one sex but disfavoured in the other (Rice,1992). A new SA mutation favouring females will be transmitted with equal frequency to son and daughters. When in females (males) it experiences a gain (loss) in gene frequency. To accumulate in the gene pool, gain must exceed loss, requiring the mutation to have a net advantage when averaged across the sexes.

Presence of dominant genes at sex linked loci have been hypothesised to play an important role in the evolution of sexual dimorphism as they are expected to make a disproportionate contribution to the responses in males and females (Griffing,1965; Charlesworth *et al.*,1987). Hastings and Veerkamp(1993), using maximum likelihood based segregation analysis, showed that 25% of the difference in mice body weight at 10-weeks was due to sex-linked genes at the X-chromosome and the remainder by autosomal additive genes. However, there is scanty evidence in the literature addressing the effect of long term selection for body weight on the trends in sexual dimorphism in mice or other laboratory animals and most of the reported experiments were purposefully designed to alter sexual dimorphism.

Conclusions

Comparison of responses from selection experiments with theoretical predictions may not always show agreement because of lack of validity of the assumptions used in the analyses, particularly when results from short and long term experiments are compared. Long term selection experiments with mice provide an opportunity to evaluate genetic models underlying responses in selection experiments.

While we can accurately estimate the base population parameters using mixed model methodologies, prediction of the environmental trend would often be more difficult the longer the experiment. Experiments should also be replicated in order to compute variance of response, particularly if the objective of the experiment is to estimate correlated responses.

There is much theoretical work attempting to model offspring-parent relations mostly in short term experiments. Generally, the mean or response in offspring generation is a result of an intricate interplay between the distributions of environmental and genotypic components in the offspring and their parents. However, from the few published experiments, it appears that the difficulties in interpreting curvilinearity arises because the true relationships or the distributions of the genotypic and environmental components are often not well known.

Chapter 2

DESCRIPTION OF POPULATIONS AND RESPONSE TO SELECTION FOR 10-WEEK BODY WEIGHT

2.1 Introduction

Data for this study were obtained from a long term selection experiment in mice conducted at the University of Edinburgh (ICAPB). The study involved two populations of mice taken from the same base population referred to as the protein lines (P) and the fat lines (F) respectively. The protein lines consisted of three replicates each with high, low and unselected control lines. Selection was within families on an index of predicted lean mass for 20 generations, designated P-lines. At generation 20, three replicates within each selected line were crossed to form new replicates, designated P6-lines i.e. the three high lines were crossed to make the P6 high line and the three low lines similarly crossed to form the P6 low line. This was done by making all possible two way crosses of the three replicates. The P6-lines were not replicated and no contemporary control was subsequently maintained. After crossing, the criterion for selection in the P6-lines was changed to 10-week body weight and selection was continued for a further 30 generations making a total of 50 generations of selection in the protein line. Between generation 32 and 40 there was no response in the high line and a declining trend was evident. At generation 40 of the protein line, one subline of relaxed selection was taken from both the high and low directions of selection which ran parallel to the selected lines for a further 10 generations. The aim was to see whether these lines, particularly the high line had indeed reached a plateau and to characterise the nature of those limits. Further, a growth experiment was conducted at around generation 56 of the protein line to evaluate the dynamics of sexual dimorphism, defined as the ratio of male : female phenotypic means. The description of this experiment will be given in chapter 6.

In the fat (F) lines the ratio of gonadal fat pad weight to body weight in 10-week-old males was used as the selection criterion. These lines were divergently selected for increased or decreased body fat for 20 generations. The selection procedure and population structure was identical to that used in the P-lines i.e. three replicates each with high, low and unselected control. 6-week body weight records derived from the unselected control lines (P and F) were used to investigate linearity or non-linearity of offspring-parent regression.

The study is partly a continuation of an earlier study by Beniwal *et al.*(1992a,b), but their analyses were only up to generation 38 of the protein lines (20 generations of P and 18 generations of P6). Analysis of the P and P6-lines indicated a steady decline in additive genetic variance in the high and the low lines, even though allowance was made in the model for reduction in additive variance due to inbreeding and linkage disequilibrium. An apparent plateau was observed at around generation 38 in the high line, and in the later part of the experiment the number of mice born dead in the high line (P6) was observed to increase, but not in the low line. Litter size declined in the high line suggesting reduction in general fitness.

This chapter gives a general description of the materials used and their historical background. Simple analyses were also done to characterise the populations and the results obtained provided a basis for more sophisticated analyses. A brief discussion of selection response for 10-week body weight in terms of the pattern, duration and total amount of response is given at the end of this chapter. The methods of analysis used, particularly for estimation of genetic and phenotypic parameters, are deferred to their respective chapters.

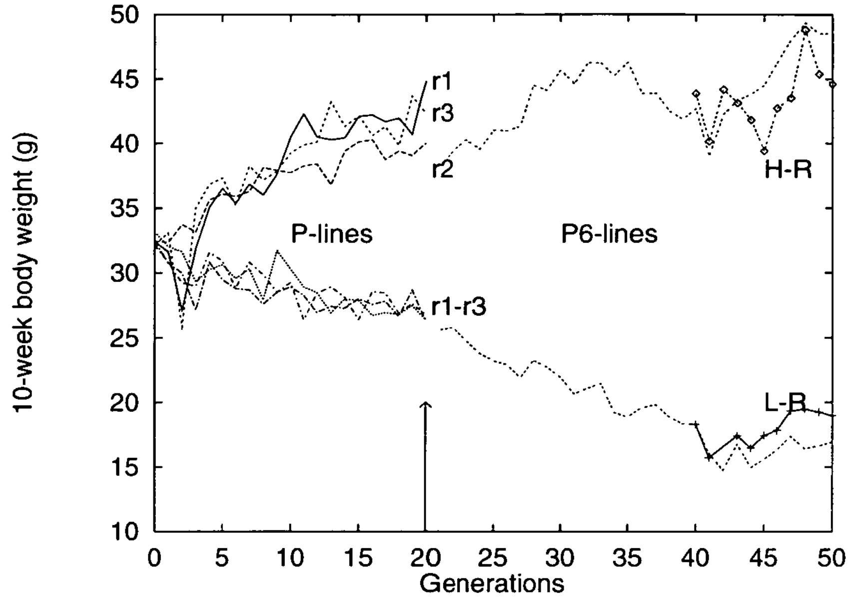
2.2 Development of lines

The lines used in this study were derived from the 'G' strain which was developed by crossing two inbred lines, JU and CBA and the F_1 crossed to an outbred strain, CFLP. The resulting crosses were randomly mated for one generation and the second cross formed generation zero of the selection experiment. The base population was divided into three lines referred to as high (H), low (L) and control (C) according to the selection criterion. Each line was further subdivided into three replicates each comprising sixteen full-sib families. This protocol was used for both the P-lines and the F-lines. Both the P and the F lines were maintained for 20 generations with full pedigree records available.

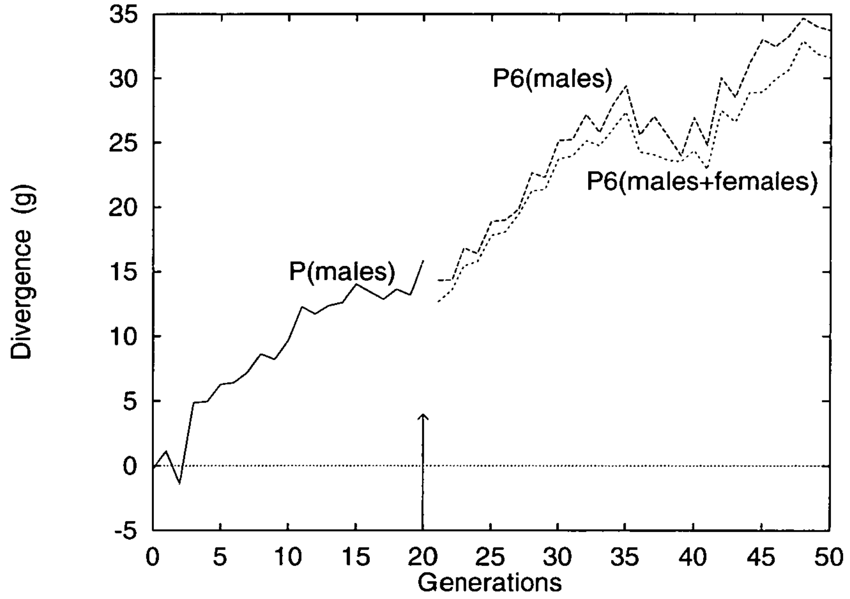
2.2.1 Selection lines (P and P6)

The P-lines were initially selected for lean mass growth on a simple phenotypic index of predicted lean mass, more strictly fat-free mass of males at 10-weeks of age: Lean Mass = body weight(g) - [8 × gonadal fat pad weight(g)]. As gonadal fat pads represent about one-eighth of the total fat in 10-week-old males, this index was used as a simple phenotypic predictor of fat-free mass, although it is very highly correlated (r=0.94) with 10-week weight (Sharp *et al.*,1984). Crossfostering was practised and where there was a mating failure, the second best individual from the family was used. In the first 7 generations of the P-lines, each replicate comprised 16 mating pairs (families) and from generation 8 to 20 they consisted of 8 matings pairs. The size of families was reduced possibly due to limited space, and nowhere it is stated why such decision was made. Up to generation 20, litter size was adjusted to between 6 and 12 pups at birth by culling and cross fostering, and weaning took place at the age of 3 weeks.

From generation 20 of the P-lines, the three replicates in each direction of selection were crossed using all possible two-way combinations to form a single replicate line for H and L line respectively, designated as the 'P6-lines'. Approximately 16-full-sib families for each direction of selection (high and low) were used to start the P6-lines. These lines were further selected for 30 generations, hence a total of 50 generations of selection. No control was maintained after the replicate lines were crossed for the remaining part of the experiment, possibly due to the reason explained above. After crossing, the criterion of selection was changed from lean mass to 10-week body weight. Selection was practised within families for both males and females and mating was by the maximum avoidance scheme of Falconer(1973). This scheme has the theoretical expectation that the inbreeding coefficients were the same for all families in a generation, and the rate of inbreeding was the same in all generations. In the P6-lines no crossfostering or family replacement to make up for losses through death or infertility was practised. Throughout the experiment, male and female offspring or members of each family were reared in separate cages after weaning at 21 days. In addition, a single replicate was formed at generation 40 from each of the P6 high and low lines, equivalent to generation 20 of P6-lines, by mating individuals at random for a further 10 generations, these lines are referred to as 'the relaxed lines'. The generation means and the divergence between H and L lines for 10-week body weight are shown in Fig. 2.1.



a. Generation means for 10-week body weight (H-lines above and L-lines below)



b. Divergence (H-L)

Figure 2.1: Generation means for 10-week body weight before and after the replicate lines were crossed (above) and the divergence between H and L lines (below). The means for P-lines are those of males, and those for P6 are averages of males and females. The arrow marks the generation number at which the P-lines replicate were crossed. Control P-lines are not shown. HR = relaxed high line, LR = relaxed low line.

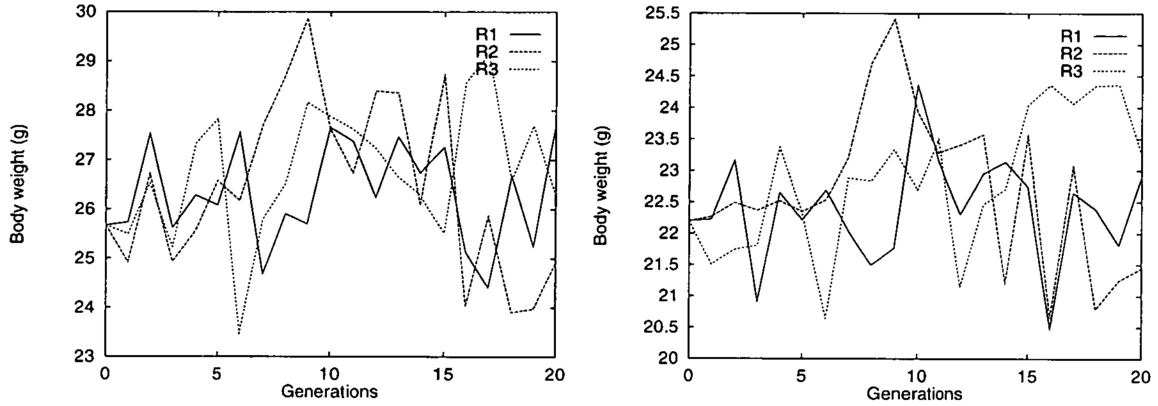
Records of 10-week (males only) and 6-week body weight (males and females) as well as litter size at birth (born alive or born dead) and litter size at weaning were available in the P-lines. In the P6-lines, 3-week body weight records instead of 6-week body weight measurements were made. However both males and females had 10-week body weight measurements, unlike the P-lines.

2.2.2 The control lines (P and F)

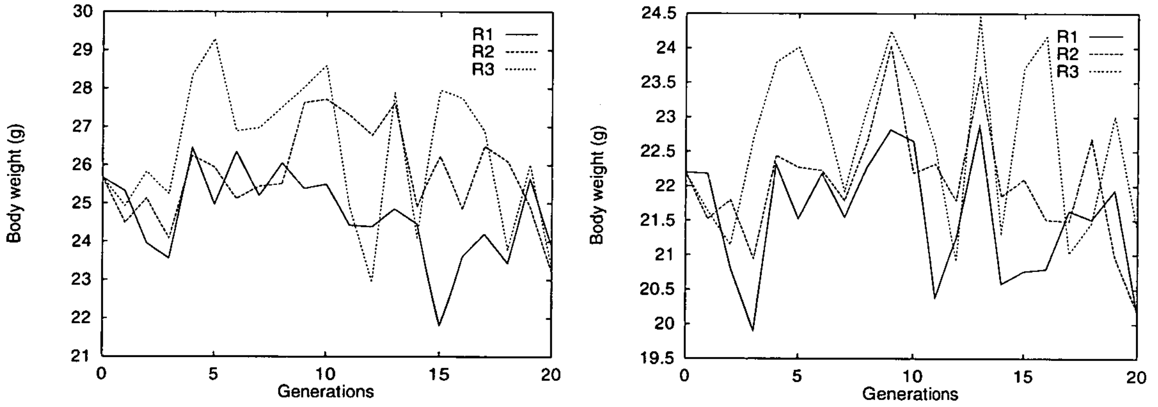
Records of 6-week body weight from unselected control lines (P and F) were used to study the linearity or non-linearity of offspring-parent relationship. 10-week body weight was not used because P-line and F-line females had no records on 10-week body weight. In this study we designate the control lines as PC and FC for protein line and fat line respectively. Initially each line was analyzed separately, while a third analysis was based on the combined records of both lines (PC+FC) designated as 'C-population/lines'. The generation means of six-week body weight in males and females displayed an increasing trend between generation 1 to 8 followed by a decreasing trend from generation 9 to 20 for both PC and FC lines (Fig. 2.2). This pattern coincided with changes in family size, the possible reason for this change is given in section 2.2.1. The change and possible causes of this trend will be examined in chapter 3.

The mean 6-week body weight, coefficient of variation and number of records for the two control lines are given in Table 2.1. The coefficients of variation were of nearly the same magnitude within sex in the two populations and males generally had higher variance for 6-week body weight than females. This difference in variance between males and females is important because it affects the estimation of heritability obtained by offspring-mid parent regression (Falconer, 1989).

Throughout the study, the following notation will be used for the lines, H = high selected, L = low selected, HR= high relaxed, LR = low relaxed, PC = protein control line, FC = fat control line, C = FC+PC control lines.



PC Males PC Females



FC Males FC Females

Figure 2.2: Generation means of 6 week weight for males and females in PC and FC individual replicates. R1,R2,R3= replicates.

Table 2.1: Means, standard deviations and % CV for parents and offspring: Control lines

Line	Individual	Family mean(g)±sd^a	% CV	N†
PC	Sire	26.16 ±3.40	12.9	557
	Sons	25.57 ±3.46	13.5	542
	Dam	22.29 ±2.52	11.3	557
	Daughters	22.04 ±2.43	11.0	550
FC	Sire	26.74 ±3.31	12.4	542
	Sons	26.45 ±3.37	12.7	535
	Dam	22.33 ±2.55	11.4	542
	Daughters	22.61 ±2.54	11.2	534
C	Sire	26.43 ±3.37	12.7	1099
	Sons	26.22 ±3.03	11.5	1078
	Dam	22.54 ±2.55	11.3	1099
	Daughters	22.42 ±2.18	9.6	1085

^{a} standard deviation of family means. † Number of sib families

2.3 Long term response to selection

Response in 10-week body weight

In the P-lines, mean body weight at generation 0 (base population) for males was 32.47±5.35 (Fig. 2.1). Both H and L lines showed an increased response in 10-week body weight and at generation 20 the mean (±*sd*) 10-week body weights averaged over replicates were 42.2 ±4.26g and 26.5±2.02g for the H and L lines males respectively, and the divergence was about 15.7g.

The P-lines have previously been analyzed by Beniwal *et al.* (1992b), and it is not the intention of this study to repeat the analysis. Instead, in the analysis of 10-week body weight data, focus is on the selected P6-lines. In all analyses involving the P6-lines, data and pedigree information prior to crossing of the P-line replicates were excluded, though by doing so the estimates will be somewhat biased by not including the pedigree information prior to crossing of the replicate lines. Likewise, there has been so many changes in the way the experiment was conducted prior and after the lines were crossed e.g. a change in trait (lean mass to body weight), absence of controls, and replicates, traits being recorded in both sex in the second half, but not in the first half, the effect of crossing of the lines which need to be accounted for etc. Hence analyses were restricted to data and pedigree information in generation

21 to 50. For simplicity it is then assumed that the genes present in the parental generation which formed the first generation of the P6-lines are independent, and by definition these animals have an inbreeding coefficient of zero. In this study we define 'generation 0' as the base population in the P line and generation 21 was an assumed base following crossing of the P-replicate lines.

The mean 10-week body weight after crossing of the replicate lines (P6-lines) and related statistics are summarised in Table 2.2 for each sex. At generation 21 of the P6-lines, the mean body weight in the two lines selected for 10-week body weight differed by 13.3g and the divergence increased to 32.21g at the end of the experiment (generation 50). There were no 10-week body weight records in the base population (generation 0) and up to generation 20 for females, hence only males are considered. The total response in males after 50 generations of selection were 20.35g and -13.90g in the H and L lines respectively, and the divergence was 33.46g ($= 6.25\sigma_p$).

Regression of 10-week mean body weight on generation number in the P6 high line gave an average response of 0.62g per generation (generation 21-35) and decreased to 0.33g between generation 35 and 50. The generation means in the L line decreased consistently in the direction of selection up to generation 40, then remained more or less constant until the end of the experiment. The average response was 0.40g per generation. Response measured as the divergence between H and L P6-lines was linear in the first 15 generations, but decreased in generation 35-40 by about 5g, then maintained a parallel relation with the initial direction of response (Fig. 2.1b).

Within lines, both males and females showed similar trends in generation means (not shown in Fig. 2.1). However, when response was considered separately for each sex in the P6-lines, females of H lines responded more to upward selection than males, the regression coefficients being 0.55g vs. 0.49g per generation respectively. In the L line the opposite effect was observed, females responded less to downward selection, the regression coefficients being 0.33g vs. 0.45g per generation for females and males respectively. The difference in response may be confounded with environmental changes; however no control line(s) was available to correct for environmental trends. The difference between males and females expressed as a ratio of male mean to female mean, i.e. 'sexual dimorphism' (Frankham, 1968) and the trends in sexual dimorphism during the period of selection will be further investigated in chapter 6.

Table 2.2: Number of records, means at the start and at the end of experiment, standard deviation and coefficient of variation for 10-week weight in the P6-lines

Line	Sex	N[a]	Mean (g)		Change (g)	Pop. mean[b] (g) ±*sd*	%CV
			Start (gen 21)	end (gen 50)			
High	Male	1608	42.89	52.82	9.93	47.33±5.50	11.60
	Female	1575	34.43	45.70	11.27	40.04±5.10	12.70
	Both	3184	38.66	49.26	10.60	43.63	
Low	Male	1294	27.95	18.57	-9.38	21.82±3.82	17.53
	Female	1264	22.77	15.53	-7.24	17.95±3.18	17.70
	Both	2558	25.36	17.05	-8.31	19.88	

[a]= Number of records. [b]= refers to population mean in the P6-lines computed as averages of males and females . The number of litter size record in the P6-lines were 420 and 434 for H and L line respectively

Selection differentials and realised heritabilities

Unweighted selection differentials (SD) were calculated as the difference in the actual body weight between selected individuals and the sex-litter mean. These selection differentials were calculated using 10-week body weights in the P6-lines. Parents which produced no offspring that survived to be weighed at 10-weeks were excluded in this computation. For comparison, the expected selection differentials were calculated on the assumption that the heaviest individuals in the family were selected to be parents in the next generation (or the lightest in the case of the L line).

The realised and expected SD in the H and L lines pooled over sex are illustrated in Fig 2.3. In the H line, there is a fairly good agreement between the realised and the expected SD at least in the first 10 generations, and in the remaining generations the SD were generally much lower than expected. In the L line, there was a good agreement between the realised and the expected selection differentials up to generation 40 followed by a gradual decline in the remaining 10 generations to about zero in generation 47-49. This decline could be attributed to reduction in fitness as a consequence of reduction in body weight or the antagonism between natural selection (favouring heterozygotes) and artificial selection.

Selected males and females in the H line were on average 2.14g and 2.41g heavier than the population mean in the period between generation 21 and 33 (mean 2.27g) and this declined to 1.66g and 1.35g (mean 1.50g) for males and females respectively in the remaining 16 generations. The overall means considering all generations (generation 21-50 of P6-lines) were much lower, i.e. 1.87g and 1.83g (mean 1.85g) in males and females respectively. In the L line, the SD for males and females were on average 0.85g and 0.73g (mean=0.79g), the means computed across all 30 generations.

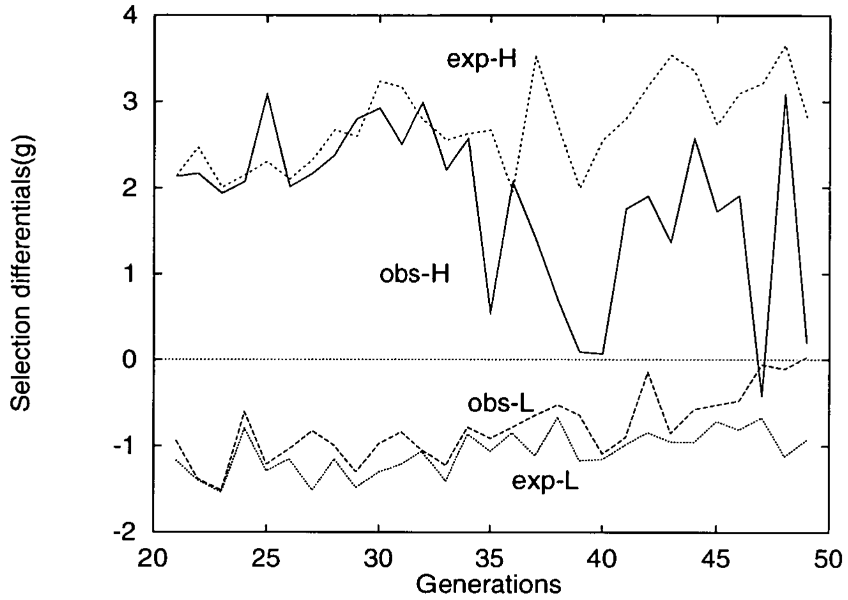


Figure 2.3: Realised (obs) and expected (exp) selection differentials in High and Low P6-lines.

Because there were no control lines, the realised heritabilities were estimated from the regression of generation mean on cumulated selection differential. However, this method is less accurate because generation means are subject to vary due to random genetic drift, sampling errors in estimating the generation means, differences in selection differential, and environmental factors (Falconer, 1989). The realised heritabilities were 0.31±0.03 for upward (generation 21-35) and 0.42±0.03 for downward selection (generation 21-50) respectively, and 0.34±0.03 for the divergence (generation 21-35). These are within-family heritabilities as selection was carried out within litters. The means in generations 21-35 were used because the regressions for the H line and for the divergence were linear over this period.

Phenotypic components of variance

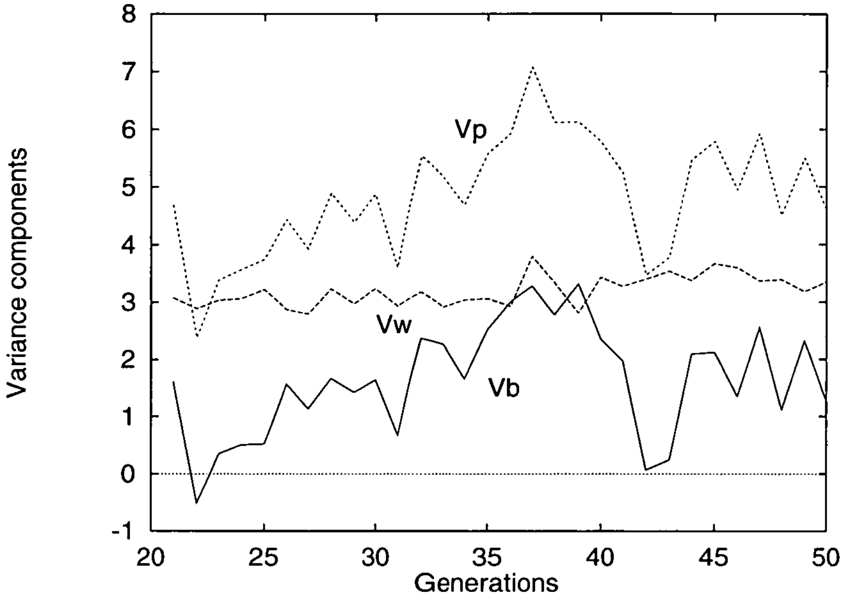
Both genetic and environmental sources of variance contribute to the covariance of relatives, the covariance of phenotypic values being the sum of the genetic and environmental covariances. A simple one-way analysis of variance (GLM procedure) was carried out to partition the environmental source of variation into the between (Vb) and within (Vp) component of variance. The aim of this analysis was to elucidate the nature of variation which may contribute to the variance between means of families, which may possibly shed light on the behavior of the H line in the period between generation 30 and 40, since selection differential was declining. Because males and females differed in their means and variance, as well as the difference between H and L line, partitioning were done on $\log_e$ transformed data for each generation and line separately. Pooled means within generations for each variance component were obtained and the means are plotted in Fig. 2.4.

In the high line the expected variance between line $E(V_b)$ was 1.9 expessed in log value i.e. $((1+(n-1)t)/n)Vp$, where n is the average family size (n=10), t the intraclass correlation *t=0.34* and V_p the total phenotypic variance (4.689) in generation 21. The expectation agrees well in the first 10 generations, but a large increase between generation 30 and 40 was observed. The total phenotypic variance increased in the first 20 generations with Vb contributing more to this increase. The within family component of variance remained fairly constant in the 30 generations of selection, suggesting that the source of this changes are not genetical. A large increase in total variance in generation 35-40 in the H line presumably suggests a temporary environmental change contributing to the difference between families but had no significant effect on the within component. It is unlikely that this effect is genetic in nature since after generation 40 the between component reverted more or less to its original level in generation 21. A contrasting picture is shown for the L line (lower figure) Vb remained fairly low and constant over the experimental period, fluctuating around zero. Vw showed a decreasing trend but tended to stabilise after generation 35, the decrease being a result of selection by increasing the degree of homozygosity.

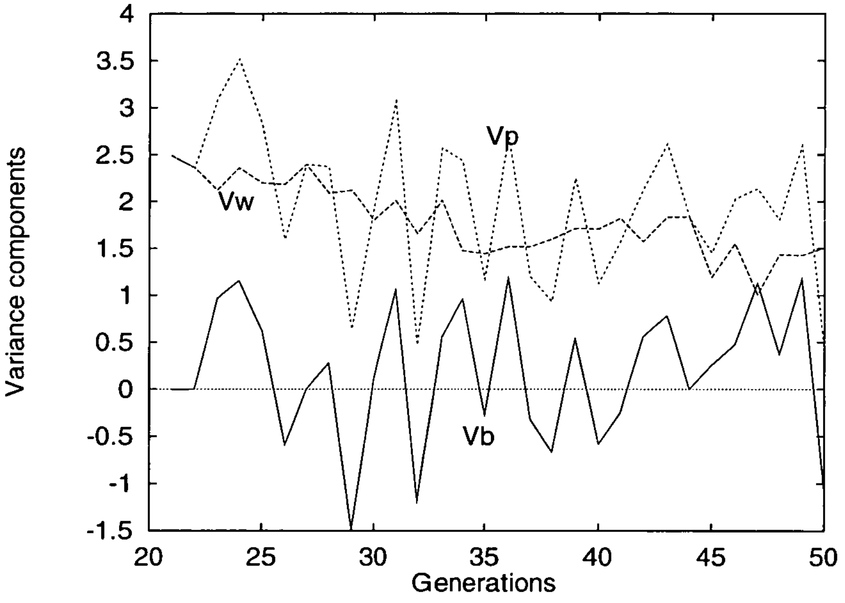
2.4 Discussion

Selection response for 10-week body weight in the P6-lines

The total responses combining both sexes expressed in units of phenotypic standard deviation or in percentage (in brackets) of the initial body weight (generation 21 of the P6-lines), were 1.7 (28.0%) and 2.2 (33.7%) in the H and L lines respectively.



a. High line



b. Low line

Figure 2.4: Development of variance components in the H and L P6-lines. Vb, Vw and Vp represent the between, within and total phenotypic variance respectively. The scale on the y axis is in log_e.

On both the logarithmic and normal scale, the L line showed a larger response to selection than the H line. Considering the total responses from generation 0 of the P-lines to the end of the experiment (generation 50, P+P6), the H line males showed an increase of 62.6% ($3.8\sigma_p$) vs. 42.8% ($2.6\sigma_p$) in the L line and the divergence of $6.25\sigma_p$. Eisen (1980) reviewing a number of long term selection experiments where the lines had plateaud, he found that response to selection in these lines ranged from 2.0 to 6.9 σ_p or 3.6 to $9.8\sigma_a$. The higher response in the H line agrees with Falconer's (1973) observations, where a 45% increase in 6-week body weight in the H line and a 38% decrease in the L line after 23 generations of selection were observed, values expressed as deviations from the control line. The responses observed in this study for individual lines may be biased downwards or upwards depending on the trends in environment which were not corrected for due to absence of control line. The reverse trend when only the P6-lines were considered could be explained by the reduction in response in the H line between generations 30-40. In this study P6-lines were not replicated due to resource constraints and some problems may arise in interpretation or generalising the results obtained in subsequent analyses. The importance of replication has been discussed elsewhere (Hill, 1972; Falconer, 1973; Yoo, 1980).

Previous analysis of these selected lines suggested that the upward selection line (H) seem to have attained a selection plateau after 38 generations of selection. This decline was attributed to reduction in selection differentials, but not to exhaustion of additive genetic variance (Beniwal *et al.*,1992b). The present experiment used part of the data used by Beniwal and in addition, data from 10 more generations of selection. Crossing of the lines was done at generation 20 of the P-lines, this may have provided a renewed source of genetic variation which maintained response to the current levels. This argument is in line with that of Roberts(1967) who suggested that if different lines selected in the same direction are not fixed for the same alleles at various loci affecting the trait then, when crossed they ought to contain some genetic variance and response to further selection from crosses may be expected. An interesting observation is that, while most laboratory experiments seem to reach their selection limit at about 30 generations (Eisen,1980), response in the present experiment (and in particular, the H line) seem to have surpassed this limit, especially if we consider response in the earlier 20 generations before replicate lines were crossed. A continued positive response for 50 or more generations has also been observed in some experimental studies, for example: selection for abdominal bristle number (Yoo,1980), selection for 6-week weight in mice (Bünger *et al.*,1990), and selection for pupa weight in *Tribolium* (Enfield,1976). However, these experiments are few in literature and a number of reasons were given to explain such a prolonged response including mutation, segregation of recessive genes at low frequencies etc.

Regarding selection differential, a large difference between observed and expected selection differential in males and females of the H line, particularly after generation 35 was evident. This reduction may partially explain the decline in response during this period (generation 35-40). Beniwal *et al.*(1992b) reported a correlated increase in number of mice born dead, particularly in the H line (P6) after the first 10 generations of selection, which persisted almost to the end of the experiment and suggests a major environmental effect during these periods, possibly a disease situation. All mice (H and L) were reared in the same environment throughout the course of the experiment, hence if the source of changes was environment then it is expected that both high and low line mice would be affected. In the L line, litter size generally declined with selection, but there was no evidence of increased mortality (born dead). However, the decline in selection differential to nearly zero near the end of selection experiment suggests a conflict between natural and artificial selection. The counteracting effect of natural selection can be deduced from the observed regression of response towards the population mean in the relaxed L line (Fig. 2.2).

The reduction in response in the H line between generation 30-40 was associated with a large and sharp increase in between family variance in the H line, and consequently the total environmental variance, particularly at about the period when the response in the H line was decreasing. If only few families were affected by this disease, then the between family component will be expected to increase, however the distribution of family means around these periods did not clearly show the affected families and it suggest that the prevailing environment developed gradually with advance in selection. It was also difficult to isolate the affected families due to the mating protocol used i.e. avoiding mating full-sib. Falconer(1990) stated that directional selection based on phenotypes is *synergistic* with respect to the unidentified environmental factors. As such, selected individuals have the genetic ability to perform relatively well or poorly in that environment. Consequently, sensitivity should be increased by directional selection, and the increased sensitivity will be seen in an increased environmental component of variance. Differences among families in variance for a trait could also occur due to genotype × environment interaction such that significant heterogeneity among families is an indication but not conclusive proof of the existence of G × E interaction. However, in this study the environment was not replicated and the between component could only increase if different genotypes have different sensitivity to the prevailing macro-environment. The within family component of variance in the H line remained constant despite selection and inbreeding, whereas in the L line, Vw and Vp declined as selection advanced.

Estimates of realised heritabilities for 10-week body weight in the P6-lines seem to fall within the range for body weight reported in other experiments. For example, Falconer (1973) obtained values of 0.40 and 0.33 for upward and downward respectively, and 0.37 for the divergence in lines of mice selected for 6-week body weight. The values varied across replicates: for example, realised heritability varied from 0.25 to 0.46 in the high line and from 0.16 to 0.50 in the low line replicates. Comparison of estimates of within family heritability (h_w^2) in this study and those of the P-lines in the H line (0.53) and L line (0.44) indicates that the within family heritability declined in the H line with advance in selection, but there was only a small change in the L line. The values in the P-lines were derived from estimates of additive (Va) and residual (Ve) variances obtained from analysis of 10-week body weight (Beniwal *et al.*,1992b), i.e. $h_w^2 = \frac{1}{2}Va/(\frac{1}{2}Va + Ve)$. The change in variances and heritability estimates as a result of selection will be investigated further in chapter 4. In conclusion, the results of this chapter shows that the L line has reached a selection plateau after 40 generations of selection for decreasing body weight, while H line mice continued to respond. The plateau in the L line could partly be due to the opposing forces of directional and natural selection, inbreeding, or exhaustion of additive genetic variance.

Chapter 3

LINEAR *VS* NON-LINEAR OFFSPRING-PARENT REGRESSION

3.1 Introduction

Reliable estimates of genetic variances, covariances as well as heritabilities are needed to formulate breeding plans, predict response to selection and estimate the genetic merit of animals. If the objective is only to estimate genetic parameters e.g. heritability, simple methods of estimation can be applied appropriately to experiments designed optimally for this purpose (Hill 1970; Hill and Nicholas 1974; Hill,1990). Such experiments are usually of short term and involve only parent and offspring generations. Heritability of a trait can then be estimated using simple methods based on parent-offspring regression or intraclass correlations between sibs. If records for a given trait are recorded on one or both parents and their offspring, then regression of offspring on one parent or on mid-parent values provides an unbiased estimate of heritability assuming no environmental covariance and no selection. Gimelfarb and Willis (1994) suggested that where both parental records are available, heritability can also be estimated by multiple regression of offspring values on both parents, since the mean in offspring is determined by the regression on the character in both parents.

In a short term experiment, the estimated heritability can be used in the classical equation of quantitative genetic namely $\Delta G = ih^2\sigma_p$ to predict the response in offspring generation, where ΔG is the expected genetic change produced by selection, i the standardised selection differential and σ_p the phenotypic standard deviation. The prediction equation assumes that the regression of offspring on parent is linear, which imply the genotypes have a multivariate normal distribution. However, the use of linear regression for estimating heritability or for prediction of change from selection is justified only when such linear relationships can be reasonably expected on genetic grounds. Both experimentally and theoretically, it has been demonstrated that the relationship between offspring and parent or among sibs can be non-linear within or across generations (Nishida,1972; Mäki-Tanila,1982; Gimelfarb, 1986). The implication of assuming that the offspring-parent relations is linear, while it is not, is that the response by a character to selection predicted by a non-linear offspring-parent regression fitted to family data can be quite different from the response predicted by the linear regression fitted to the same family data. Further, the non-linearity of offspring-parent regression may result in asymmetry of responses to positive and negative selection (Kempthorne, 1960; Frankham, 1990; Gimelfarb and Willis, 1994), which can not be predicted by a linear regression.

If the relationship between offspring and parent is linear, the three dimensional regression surface is planar. However, there may be many biological or environmental mechanisms which can make the regression surface curved. Evaluation of a curvilinear relation from regression on one or both parents could provide insight into the degree of bias in predictions by assuming a linear relationship between parent and offspring. The reason is that the mean among offspring will depend not only on parental means but also on higher moments of the parental distribution. To avoid complexity which may arise due to selection, unselected control populations of mice were used to investigate parent-offspring relations. Linear and non-linear regression models were employed to fit regression line/curve or regression surfaces using 6-week body weight data.

The objectives of this chapter are:

a. to evaluate and compare estimates of heritability based on regression of offspring family mean values on one/mid parent values with that based on simultaneously fitting both parents.

b. to investigate if the regression of offspring on individual parent or mid-parent is linear or essentially non-linear.

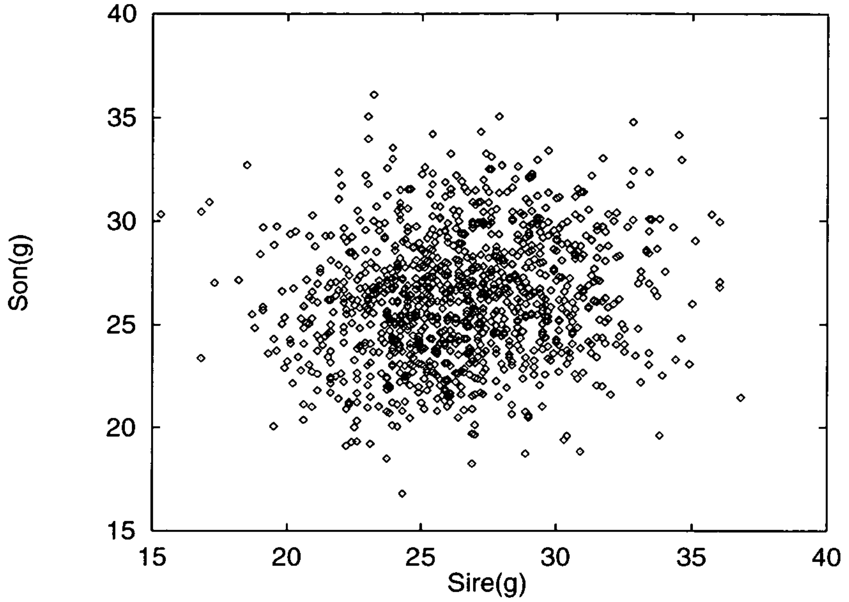
3.2 Methods of analysis

General regression model

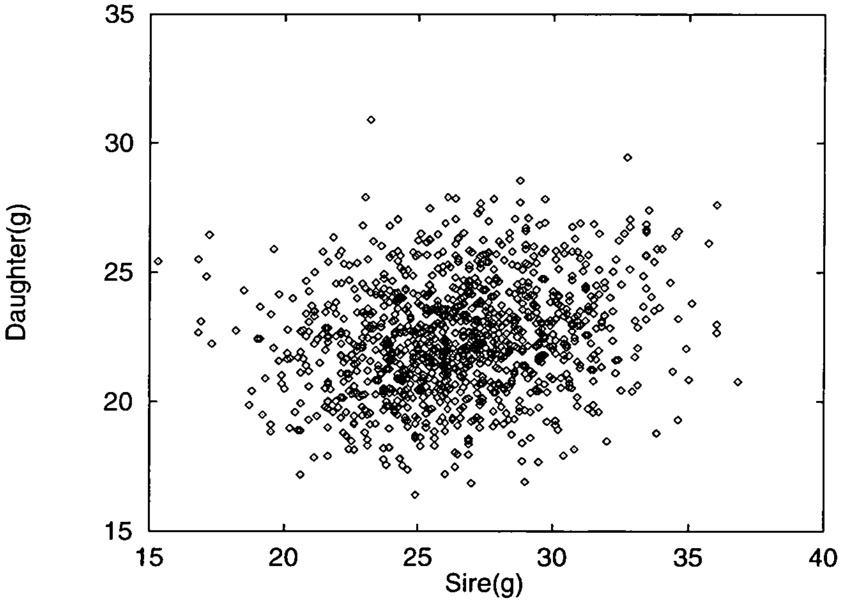
This study used 6-week body weight records, spanning 20 generations from unselected control lines of mice derived from the protein (PC) and fat (FC) lines described in chapter 2 (section 2.2.1). These lines were used since the amount of data was large and also avoiding complications introduced by selection. Likewise there was no information on 10-week body weight in females of P- and F- lines. The distribution of offspring family means plotted against parental values shows no distinct relation, except the sire-daughter and dam-daughter distribution which tends to linear (Fig. 3.1 and 3.2). Graphical assessment of 6-week weight records by sex or combined males and females did not show any evidence of non-normality in the distribution of phenotypic values.

Analysis of variance on parental and offspring family sex-means showed that effects of generation and replicate were significant ($P < 0.05$). Also most of the interactions between or among the three fixed effects were significant. These factors were included as fixed effects in the regression models to account for their effects i.e. group mean, on offspring and parent 6-week weight. Litter size ranged from 3 to 17 pups (mean=10.2). It was a general practice to standardise litter size down to 10 pups after weaning for those dams who littered more than 10 pups. It was anticipated that the effect of litter size of birth would have persisted up to 6 weeks, the period when body weight data were recorded. The linear regression coefficients of body weights on litter size after correcting for group effects were significant ($P < 0.001$). Thus, litter size at birth was included as a linear covariate to account for any systematic variation in body weights due to the number of pups born.

The regression models in which linear and non-linear terms were fitted for weight of male, female, mid-parent or both parents were all subsets of the same general regression model. Statistical analyses were performed using GENSTAT 5.3 (1993) and significance of non-linearity was evaluated from the change in residual mean squares when non-linear terms were introduced into the model. The general regression model of offspring family mean on parent performance can be written as:

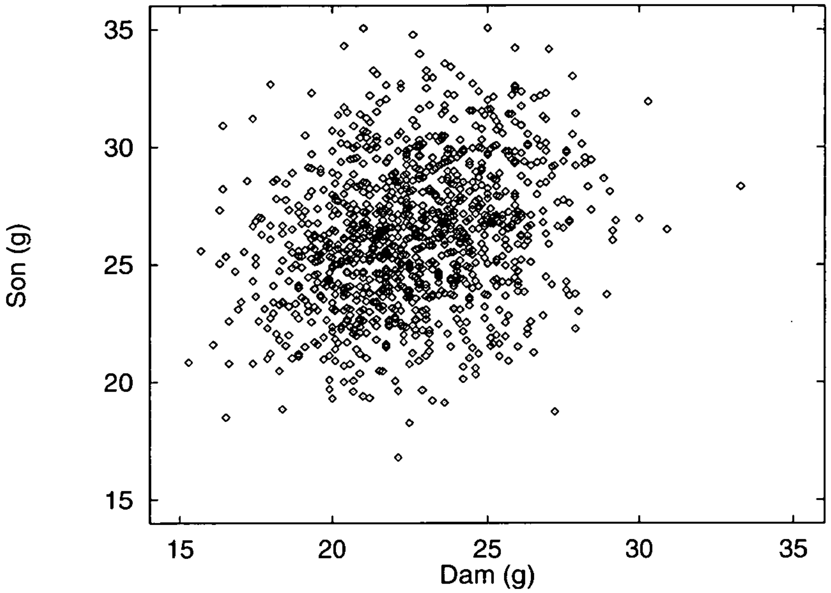


Sire-son

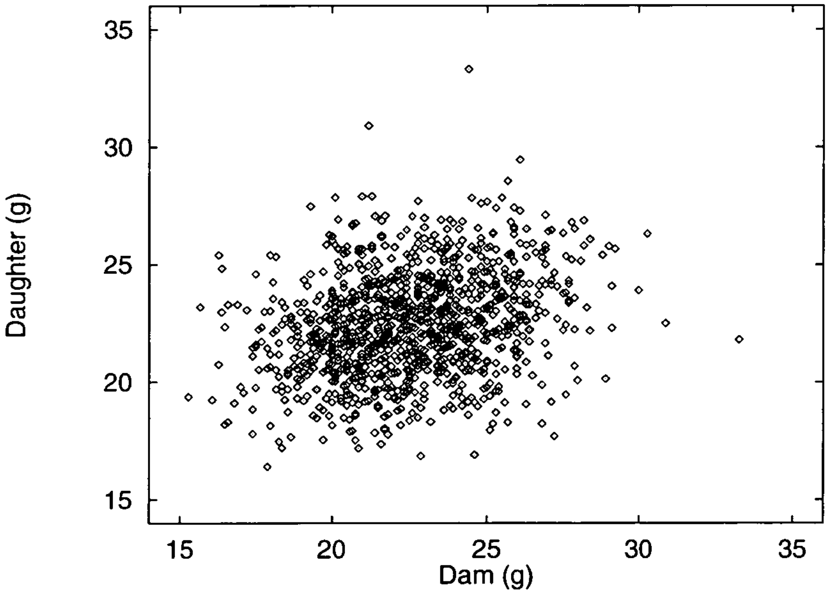


Sire-daughter

Figure 3.1: Distribution of 6-week body weights of sons and daughters family mean plotted against sire values (C-line)



Dam-son



Dam-daughter

Figure 3.2: Distribution 6-week body weights of sons and daughters family mean plotted against dams values (C-line)

$$Y_{ij} = U + A_i + \sum_{m=0}^{3}\sum_{n=0}^{3} b_{sd} S_{ij}^{m} D_{ij}^{n} + b_f F_{ij} + e_{ij}. \tag{3.1}$$

where

Y_{ij} is the offspring family mean in family j.

U is the overall mean.

A_i is the group mean (generation, replication, line and their interaction effects). Both parental and offspring observations were corrected for this effect, by including group effect in the model as fixed factors.

F_{ij} is the effect of litter of birth (covariate) and b_f the regression of weight on litter size.

e_{ij} is the residual error term.
S_{ij}^{m} and D_{ij}^{n} are performance of the sire (s) and dam (d) within i^{th} group and j^{th} litter respectively. b_{sd} is the corresponding partial regression coefficient for sire and/or dam.

Standardisation

Parental values within each sex category were transformed to standard deviates by subtracting the mean and dividing by the standard deviation so heritability could be estimated across sexes. The mid-parent values were obtained by averaging standardised sire's and dam's values. Hence, each sex category in the regression has a mean of zero and a unit variance.

Univariate analysis

Univariate regression was performed first on unstandardised and then on standardised records. For simple linear regression for, example, on sire performance, regression was such that $m = 1$ and $n = 0$. To estimate heritabilities, the regression coefficients from unstandardised were adjusted to account for unequal variances by multiplying the coefficients by the appropriate ratio of standard deviations (Falconer, 1989). With the standardised data, estimates of heritabilities and associated standard errors from individual parent regression were obtained by doubling the regression coefficients and the standard errors of estimate respectively.

The two populations FC and PC had a similar history and generations were nearly contemporaneous. Therefore, there is no reason to expect genetic differences in the two lines contributing to variation in 6-week body weight, except from genetic drift. The regression coefficients obtained for each line were compared assuming equal error structure, the null hypothesis being $\beta_1 = \beta_2$ in line 1 and 2. This hypothesis was tested using t-test after pooling the residual variance assuming that the error structure is equal in the two population for the regression pairs involved. In essence, the test is of whether the regression lines are parallel. None of the regression coefficients were significantly different from each other, hence the two lines were combined.

Bivariate regression

Regression of offspring on mid-parent phenotypic value provides a simple method of utilising all the observations on the parents for the trait expressed in both sexes. Rather than averaging the parental effects, we may partition the offspring record such that it consists of contributions from both sire and dam. In this regard a bi-parental (bivariate) analysis was done by simultaneously regressing standardised sons, daughters or their mean on both standardised parental values such that $m{=}1, n{=}0$ and $m{=}0, n{=}1$ (eq. 3.1).

When offspring (o) are regressed on both parents (s and d) simultaneously the coefficient b_{sd} in equation 3.1 can be split to represent two partial regression coefficients, b_s and b_d, for regression of son/daughter on sire plus dam respectively. Using standardised data, heritability estimated by regression on both parent was obtained as $h^2 = b_s + b_d$. (Gimelfarb and Willis , 1994).

Curvilinear regression

To describe the relationships between two variables it is suggested that a scatter plot of the observations be made before curve fitting. Because mice were randomly mated, the distribution of offspring values is also expected to be random owing to sampling in the transmission of gametes from one generation to the next. Fig. 3.1 and 3.2 shows that the relationships are not clear and one has to rely on statistical methods *i.e.* regression analysis fitting either linear or non-linear regression models and examining the extent to which residual are minimised.

However, in multiple regression analysis, the problem in determining which independent variables, X, are most important in explaining variation in the dependent variable,Y, is well known. When X's or variables derived from X are highly correlated it is not possible to define uniquely the fraction of the total variation attributable to a particular X variable. To circumvent this problem the linear parental terms (e.g. x and derived polynomials were fitted as orthogonals such that x, x^2, x^3 are assumed to be independent. Orthogonal polynomials up to 4^{th} order of individual parents were fitted using both unstandardised and standardised data. When offspring were regressed on both parents, the full model (equation 3.1) was fitted such that,

$$1 \leq m + n \leq 4. \tag{3.2}$$

The multivariate stepwise 'forward' selection procedure of GENSTAT 5.3 was used to select parental terms which significantly explained the variation among offspring. At its simplest, the method begins with the regressions of Y on $X_1 \ldots X_k$ (here, X representing sire and dam values including their products with different order) taken singly. The X-variable which gives the smallest residual mean square is selected. Suppose that X_1 is selected, next all k-1 regressions are worked out. The variable giving the greatest additional reduction in sum of squares of Y (the offspring) after X_1, is selected. The process stops when no X_i not yet selected gives a variance ratio exceeding the outratio, a 'boundary' value of 2 was used.

3.3 Results

3.3.1 Univariate

Regression coefficients and heritability estimates from univariate analyses are given in Table 3.1. The estimates of regression coefficients and hence, the heritabilities derived from offspring-dam regression were higher than those from offspring-sires regression in all lines. The large coefficients for offspring-dam regression may be attributed to maternal effects (genetic or environmental). Such differences are not uncommon in regression analysis involving offspring and dams in traits like body weight. After adjustment for the differences in variance between the sexes, heritability estimates differed depending on the pair involved in the regression analysis. Average estimates from sire were 0.32, 0.28 and 0.29 and from dams were 0.50, 0.71 and 0.60 in PC, FC and C-lines respectively. Regression of offspring on mid-parent yielded similar estimates of heritability (0.41) in all lines using unstandardized data.

On standardisation, the regression coefficients from similar sex or opposite sexes increased or decreased depending on the differences between the standard deviations of the regressed pairs. For standardised variables
$b^* = b\frac{\sigma_2}{\sigma_1}$,
where b and b* are the regression coefficients before and after standardisation, and σ_1 and σ_2 are the standard deviations of independent (sire or dam) and dependent (son or daughter) variables respectively. The difference in estimates obtained when regression was performed on the same sex after standardisation could be attributed to the difference in variances between sire and son or dam and daughters e.g. for son-sire regression
$0.11=0.10\times(3.368/3.034)$
and for daughter-dam regression
$0.34=0.29\times(2.546/2.176)$.
The standard deviations for each sex category are given in Table 2.1 (chapter 2). The standard deviations in the offspring are those of family means, hence they are expected to be lower than those based on individuals.

3.3.2 Bivariate

Regression coefficients and derived heritabilities from bi-parental regression analysis are also shown in Table 3.1. Estimates of heritabilities from regression of son, daughter and offspring means on both parents were 0.46, 0.59 and 0.52 respectively. These estimates were similar to those obtained from offspring mid-parent regression using standardised data and the residual mean square and R^* values were of similar magnitude. Bivariate analyses gave regression coefficients and heritabilities which were slightly higher than those obtained from univariate analyses, *cf.* C_{A^*} and C_{B^*} estimates. The lower estimates from univariate could arise from biases from assortative mating, the correlation between mated pairs was (r=-0.11). The variance of single parents is simply the phenotypic variance, but because of the correlation with unmeasured parent the regression of offspring on single parent is increased (decreased) by the factor (1+ r). The mean heritability estimate from regression of son

Table 3.1: Regression coefficients (b) from univariate offspring-parent regression and derived heritabilities (h^2) in the control lines

Line	Regression pair	b±*se*	r.m.s	R^*	$h^2 \pm se$
PC	Son-Sire	0.12 ±0.04	6.85	28.9	0.24 ±0.08
	Daughter-sire	0.13 ±0.03	3.45	29.1	0.40 ±0.09
	Son-Dam	0.29 ±0.05	6.56	33.5	0.47 ±0.08
	Daughter-Dam	0.26 ±0.04	3.26	33.0	0.52 ±0.08
	Off-Mid	0.41 ±0.05	3.72	39.6	0.41 ±0.05
FC	Son-Sire	0.08 ±0.04	6.69	20.6	0.20 ±0.08
	Daughter-Sire	0.12 ±0.03	3.51	20.8	0.36 ±0.09
	Son-Dam	0.43 ±0.05	5.75	31.8	0.75 ±0.09
	Daughter-Dam	0.32 ±0.04	3.11	29.8	0.68 ±0.08
	Off-Mid	0.41 ±0.05	3.66	32.5	0.41 ±0.05
C	Son-Sire	0.10 ±0.03	6.77	26.4	0.20 ±0.06
	Daughter-Sire	0.12 ±0.02	3.49	26.2	0.37 ±0.06
	Son-Dam	0.36 ±0.03	6.17	32.9	0.61 ±0.06
	Daughter-Dam	0.29 ±0.03	3.19	32.5	0.59 ±0.06
	Off-Mid	0.41 ±0.03	3.70	37.5	0.41 ±0.03
C_{A^*}	Son-Sire	0.11 ±0.03	0.74	26.4	0.22 ±0.06
	Daughter-Sire	0.19 ±0.03	0.74	26.2	0.37 ±0.06
	Son-Dam	0.30 ±0.03	0.67	32.9	0.61 ±0.06
	Daughter-Dam	0.34 ±0.03	0.67	32.6	0.68 ±0.06
	Son-Mid	0.45 ±0.04	0.66	33.1	0.45 ±0.04
	Daughter-Mid	0.58 ±0.04	0.64	35.9	0.57 ±0.04
	Off-Mid	0.51 ±0.04	0.53	39.2	0.51 ±0.04

Line	Off-Mid	Bivariate			r.m.s	R^*
		b_s	b_d			
C_{B^*}	Son	0.14±0.03	0.32±0.03	0.46±0.04	0.66	34.5
	Daughter	0.22±0.03	0.36±0.03	0.59±0.04	0.63	36.8
	Off	0.18±0.02	0.34±0.03	0.52±0.04	0.52	40.5

R^* is the percentage of variance accounted for, R^* = 100 x (1-(Residual.m.s.)/(Total m.s.)). Off & Mid denotes offspring mean and Mid-parent respectively. $_{A^*}$ and $_{B^*}$ regression based on standardised C lines data. b_s and b_d are the regression coefficients for sire and dam respectively using C lines data.

on sire and son on dam (univariate) was 0.42, and that for daughters 0.52. Hence, $0.42 = 2((\frac{1}{2}0.46)\times 0.89)$ for sons, and $0.52 = 2((\frac{1}{2}0.59) \times 0.89)$ for daughters.

3.3.3 Non-linear regression analysis

One parent

Table 3.2 shows the result obtained from multivariate (non-linear) regression performed on orthogonalised individual parent or mid-parent values (e.g s, s^2, s^3..) using standardised and unstandardised data (C-lines). Significant ($P < 0.05$) non-linearity was found for regression of offspring on sires. Both standardised and unstandardised data gave similar relationship despite the differences in size of the regression coefficients. A cubic relation with negative coefficient was observed for son-sire regression, whereas daughter-sire regression had a positive quadratic relationship. The quadratic and cubic terms in addition to the linear term were significant for the regression of mean of offspring (son and daughter) on sires. The offspring-dam regression gave no evidence of non-linearity, either with standardised or unstandardised data. The daughter- mid-parent regression also showed evidence of significant non-linearity, being quadratic, but was not significant for sons or mean of offspring. For clarity only significant terms to the nearest 3 decimals places are given.

The two dimension regression surfaces for pairs which showed non-linear relations are shown in Fig. 3.3 and 3.4. The observations are expressed as deviations from group (generation,replicate and line) mean in standard deviation units. The regression lines or surfaces were drawn including intermediate coefficients, where coefficients of higher order were significant, but not the intermediate one, e.g. cubic but not quadratic. The son-sire fitted curve showed a cubic relation whereas, daughter-sire relation was quadratic (concave upwards) (Fig. 3.3). Similar patterns were observed for offspring-sire, i.e. a cubic relation, and for daughter-mid-parent regression, a quadratic relation (concave upward) (Fig. 3.4).

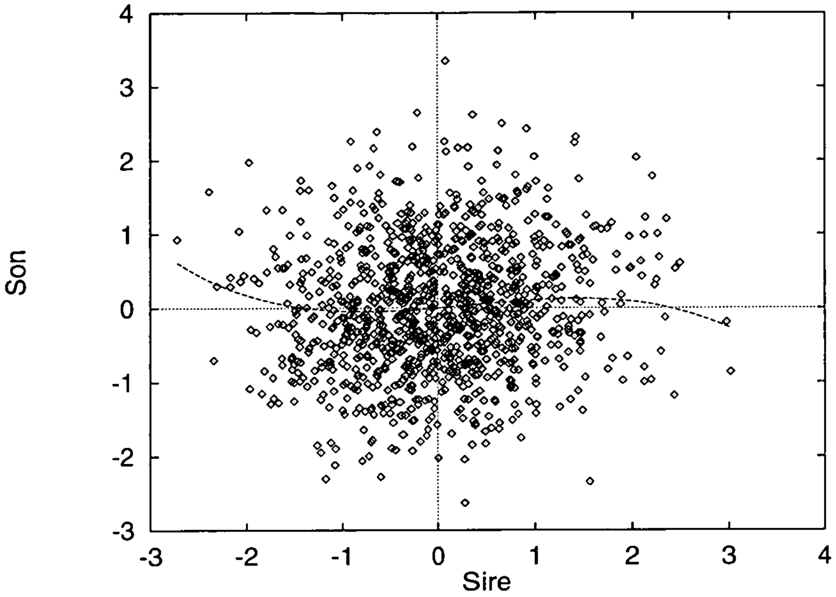
Both parents

Where both parents were involved, regression was done on standardised data only. The analyses were done based on non-orthogonal parental terms, hence the changes in the regression coefficients will depend to a large extent on the correlations among the fitted variables. Results of multivariate regression analysis fitting model 3.1, such that $1 \leq m + n \leq 4$ are given in Table 3.3. Only the cubic sire term (s^3)

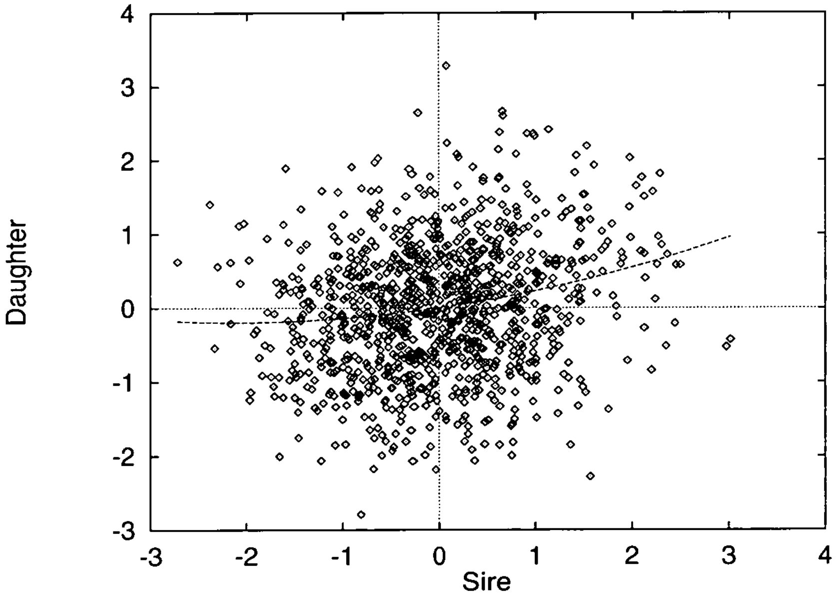
Table 3.2: Significance of regression coefficients from polynomial regression analyses on one parent and mid-parent.

		One parent		
	†	Sire(b_s)	Dam(b_d)	Mid-Parent (b_p)
Unstandardized				
Son	1	0.102±0.025	0.361±0.029	0.418±0.043
	2	ns	ns	ns
	3	-0.002±0.001	ns	ns
Daughter	1	0.116±0.018	0.293±0.029	0.391±0.029
	2	0.007±0.004	ns	0.020±0.008
	3	ns	ns	ns
Offspring	1	0.108±0.021	0.327±0.028	0.407±0.033
	2	0.009±0.004	ns	ns
	3	-0.002±0.001	ns	ns
Standardised				
Son*	1	0.111±0.029	0.302±0.029	0.449±0.042
	2	ns	ns	ns
	3	-0.033±0.012	ns	ns
Daughter*	1	0.186±0.029	0.342±0.029	0.579±0.042
	2	0.044±0.019	ns	0.072±0.036
	3	ns	ns	ns
Offspring	1	0.120±0.025	0.340±0.028	0.513±0.038
	2	0.040±0.018	ns	ns
	3	-0.026±0.011	ns	ns

1,2,3 are the linear, quadratic, cubic and quartic parental terms. The quartic term was not significant in all pairwise regressions. ns = not significant. Offspring = (son+daughter)/2.

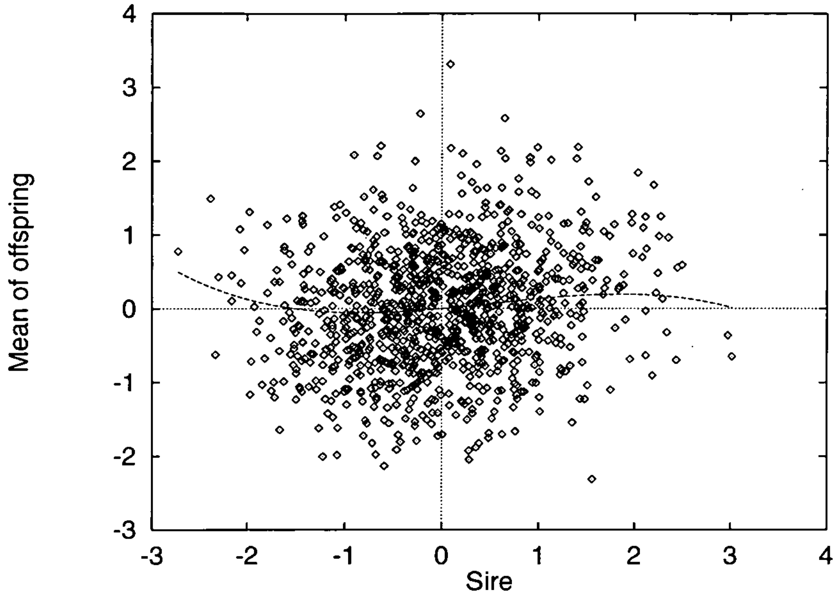


a. son-sire

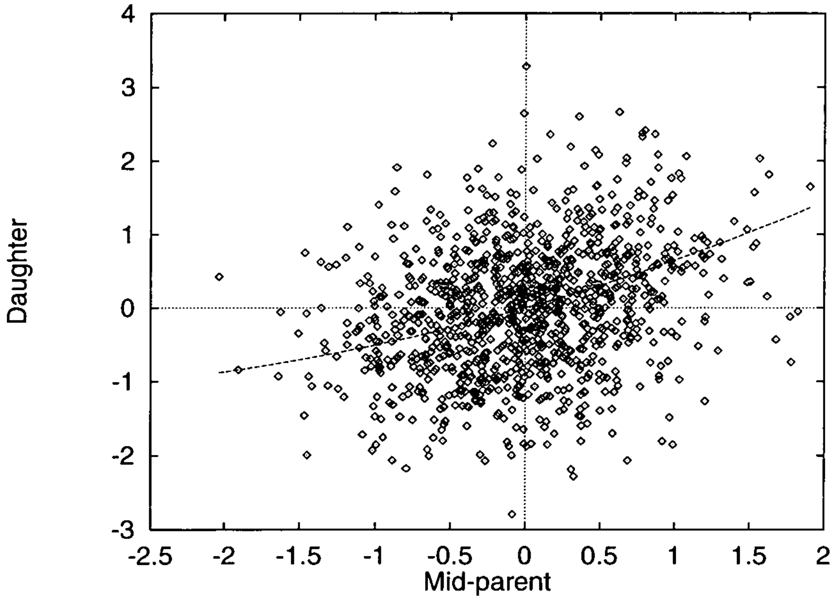


b. daughter-sire

Figure 3.3: Distribution of family mean in the C-population plotted against parental values and the fitted regression curves for sire-son and sire-daughter. Each point represents the deviation of family observation from group mean (generation-replicate-line). The scale on both axes is in standard deviations.



a. offspring-sire



b. daughter-mid-parent

Figure 3.4: Distribution of family mean in the C-population plotted against parental values and the fitted regression curves for sire-offspring mean and mid-parent-daughter. Each point represents the deviation of family observation from group mean (generation-replicate-line). The scale on both axes is in standard deviations.

in addition to the linear parental terms were significant for son-parent regression. Regression of daughters on parents (both) was essentially linear and none of the $s \times d$ terms were significant ($P > 0.05$), the s^3 term was only marginally significant (p=0.070). There were no significant improvement in the residual mean squares or the percentage of variance accounted for by the model when the values are compared with those obtained by a linear regression of offspring on both parents (Table 3.1).

Multivariate step-wise regression

Results of analysis using *step forward* multivariate regression, fitting sire and dam terms are shown in Table 3.4. The results shows that non-linearity was mainly arising from the sires, in both son and daughters- parent regressions. The linear, quadratic and cubic terms for sires were statistically significant ($p < 0.05$) for the regression of son on parent. The cubic term for daughter-sire regression was however, only marginally significant (p=0.068). It appears that dams do not contribute to the observed non-linear relationships.

The three dimensional plot of son and daughter on both parents shows that the son-parent regression surface is slightly more curved than the daughter-parent surface particularly at the extremes (Fig 3.5). The daughter-parent surface was nearly planar. In both figures the response surface was generally flat between ± 2 standard deviations of the parental values. However, the higher peaks at the extreme left of the two figures (-3:3) for sire's and dam's co-ordinate are difficult to comprehend, but suggests that if selection is intense, asymmetry in response is expected for upward and downward selection.

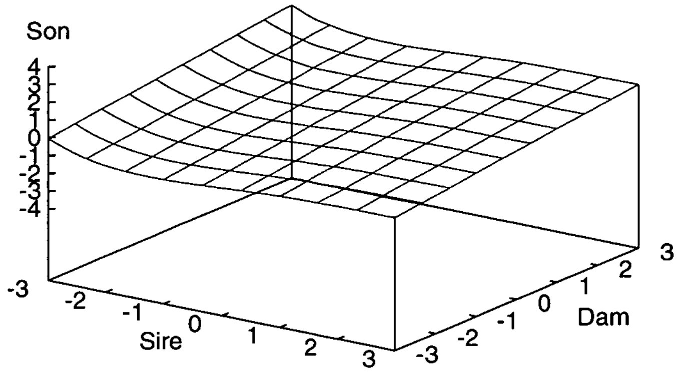
Change in distribution of means

In Fig. 2.2 (chapter 2) we observed a peculiar pattern of distribution in the means of control lines i.e. means tended to increase in generation 1-8 and decline in generation 9-20. This pattern was consistent in all lines and in both sexes and suggests a change in environment or decline in variance which could be confounded with an increase in inbreeding. The rate of inbreeding was estimated using a sub-function embedded in the DFREML (Derivative free Restricted Maximum Likelihood) package (Meyer, 1989). For the first 5 generations inbreeding was close to zero and thereafter increased to about 5% in generation 10 (Fig. 3.6). A more rapid increase was observed from generation 10 to 20 and at the end ΔF was about 20%. Using litter size at birth as a criterion, which is expected to be mostly affected by inbreeding,

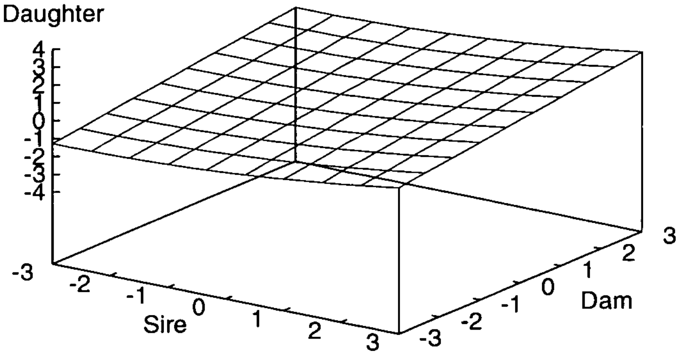
Table 3.3: Significance of regression coefficients from polynomial regression analyses on both parents: Full model.

Terms[a]	$b \pm s.e.$	t(950)	t pr.	
Son				
s	0.2299±0.0485	4.74	<0.001	***
d	0.3368±0.0513	6.57	<0.001	***
s^2	-0.0187±0.0455	-0.41	0.681	
d^2	-0.0594±0.0401	-1.48	0.139	
s^3	-0.0360±0.0121	-2.97	0.003	**
d^3	-0.0066±0.0133	-0.50	0.620	
s^4	0.0084±0.0068	1.22	0.221	
d^4	0.0036±0.0059	0.61	0.539	
sd	-0.0309±0.0565	-0.55	0.585	
s^2d	0.0059±0.0205	0.29	0.774	
sd^2	0.0103±0.0224	0.46	0.645	
s^2d^2	0.0092±0.0155	0.59	0.554	
s^3d	0.0179±0.0136	1.32	0.186	
sd^3	-0.0048±0.0132	-0.36	0.716	
Daughter				
	$b \pm s.e.$	t(957)	t pr.	
s	0.2796 ±0.0468	5.97	<0.001	***
d	0.3719 ±0.0502	7.40	<0.001	***
s^2	0.0354 ±0.0437	0.81	0.418	
d^2	-0.0129 ±0.0393	-0.33	0.742	
s^3	-0.0204 ±0.0113	-1.82	0.070	
d^3	-0.0071 ±0.0130	-0.54	0.586	
s^4	0.0034 ±0.0065	0.53	0.593	
d^4	-0.0000 ±0.0058	-0.01	0.992	
sd	-0.0103 ±0.0543	-0.19	0.849	
s^2d	0.0208 ±0.0183	1.14	0.256	
sd^2	-0.0013 ±0.0214	-0.06	0.952	
s^2d^2	-0.0047±0.0139	-0.34	0.734	
s^3d	0.0170 ±0.0117	1.46	0.144	
sd^3	-0.0047 ±0.0130	-0.36	0.716	

[a] s = sire and d = dam. The residual mean squares (r.m.s) and percentage of variance accounted by the fitted effects were 0.64, 0.63 and 35.3, 37.3 for the regression of sons and of daughters on both parents respectively. **, *** significant at the 1% and 0.1% level respectively.



a. Son: $f(o) = 0.242s + 0.042s^2 - 0.037s^3 + 0.326d$



b. Daughter: $f(o) = 0.279s + 0.051s^2 - 0.020s^3 + 0.389d$

Figure 3.5: Polynomial three dimensional regression surface of 6-week body weight in offspring on the 6-week body weight in mothers and fathers. The scale on x, y and z axes is in standard deviations.

Table 3.4: Significance of regression coefficients from polynomial regression analyses on both parents: Multivariate stepwise regression.

Terms	$b \pm s.e.$	t(957)	t pr.	
Son				
s	0.2419±0.0439	5.51	<0.001	***
d	0.3266±0.0410	7.96	<0.001	***
s^2	0.0417±0.0195	2.14	0.033	*
d^2	-0.0270±0.0198	-1.36	0.174	
s^3	-0.0376±0.0117	-3.22	0.001	**
d^3	0.0007±0.0097	0.07	0.942	
sd	0.0132±0.0270	0.49	0.624	
Daughter				
s	0.2791 ±0.0429	6.51	<0.001	***
d	0.3895 ±0.0402	9.68	<0.001	***
s^2	0.0514 ±0.0185	2.77	0.006	**
d^2	-0.0154 ±0.0194	-0.79	0.430	
s^3	-0.0202 ±0.0110	-1.83	0.068	
d^3	-0.0050 ±0.0095	-0.57	0.571	
sd	0.0280 ±0.0261	1.08	0.282	

[a] s = sire and d =dam. The residual mean squares (r.m.s) and percentage of variance accounted by the fitted effects were 0.65, 0.64 and 35.5, 37.4 for the regression of sons and of daughters on both parents. *,**,*** significant at the 5%, 1% and 0.1% level respectively.

a plot of litter size in the PC replicate lines (Fig 3.7) shows that it tended to follow a similar trend to that observed for 6-week body weight. In the period between generation 0 and 10 litter size tended to increase or decrease together in the three PC replicates, but a general decline in the remaining generations. The cyclic trend may suggest that the environment was not uniform. A similar trend was observed in the FC lines. However, on regressing litter size on generation number, 1-20, 1-9 or 10-20 for each replicate individually and combined, the trends were not significant but all the coefficients were negative.

To investigate the relationship between parents and offspring during these periods, data were split into two phases, generation 1-8 and generation 9-20. A univariate linear regression model was fitted to each set of data for each parent and offspring sex in turn. Table 3.5 shows that regression coefficients were consistently higher in set one (generation 1-8). All linear terms were significant except the son-sire regression coefficient in phase 2. The lower coefficient of sons and daughters in generation 9-20 could be attributed in part to an increase in inbreeding (Fig. 3.6). Fitting a non-linear regression to these subsets (using standardised data), the results showed that non-linearity in the relation between parents and offspring was more pronounced in the period between generation 1-8, for regression involving sires. The son-sire and daughter-sire non-linear terms were b_s^2=0.056±0.028 and b_s^3=-0.051±0.018 respectively. In the period between generation 9-20 the relationships were generally linear for all pairs.

Table 3.5: Linear univariate regression coefficients in generation 1-8 and 9-20.

		Unstandardised		Standardised	
Data		Sire	Dam	Sire	Dam
1-8	Son	0.165 ±0.035	0.413 ±0.046	0.166 ±0.041	0.356 ±0.039
	Daugghter-	0.139 ±0.026	0.377 ±0.033	0.228 ±0.041	0.421 ±0.038
9-20	Son	0.047 ±0.036	0.296 ±0.047	0.039 ±0.042	0.233 ±0.043
	Daugghter-	0.092 ±0.025	0.192 ±0.038	0.138 ±0.042	0.237 ±0.044

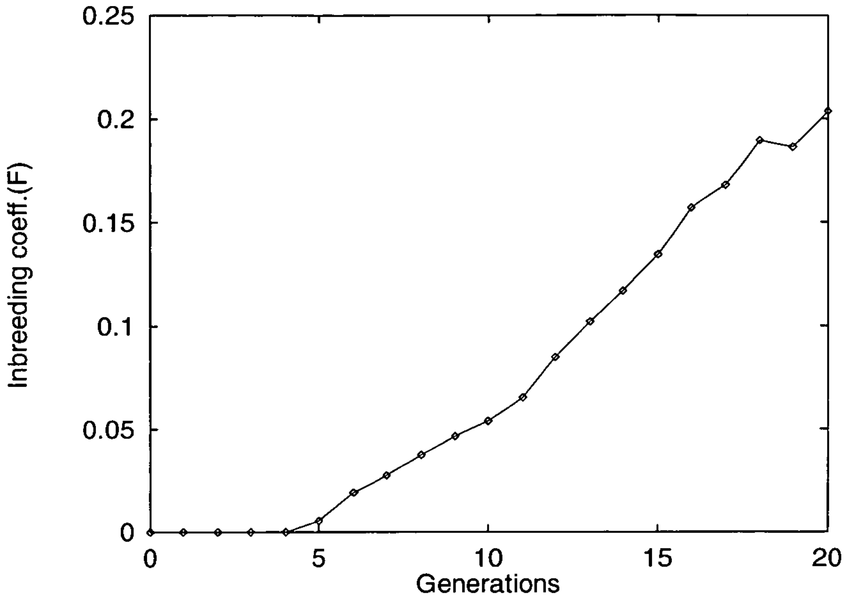


Figure 3.6: Development of inbreeding in the PC-line

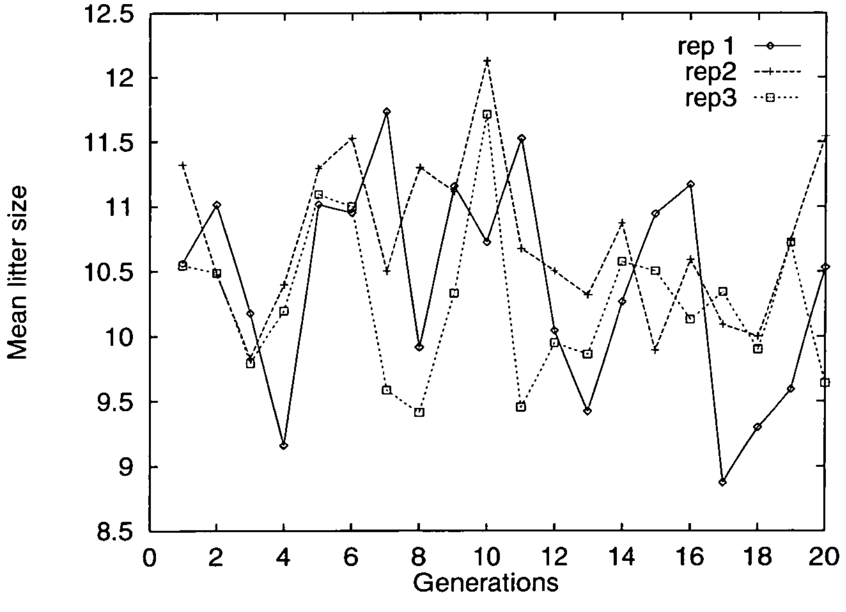


Figure 3.7: Mean litter size at birth plotted against generation number for each PC replicate lines.

3.4 Discussion

3.4.1 Linear estimate of heritability

It has been argued that if directional selection of parents is based on the character whose heritability is being estimated, it does not affect the regression of offspring on parents, either single or mid parent, but it reduces the precision of estimates. The reason is that selection reduces the variance of parents (Robertson, 1977; Falconer, 1989) and induces a change in gene frequencies (Bulmer, 1980). Similarly, if the relationship between offspring and parents is not linear, the mean among offspring depends not only on the parental means but also on higher moments of parental distributions (Gimelfarb and Willis, 1994). To avoid complications which may be introduced by selection, analyses were done using randomly mated unselected control lines.

The average heritability from mid-parent regression was $h^2 = 0.41$ which is biased downward compared to that obtained from regression based on standardised data (h^2=0.51). Estimates of heritability obtained from standardised data and those from unstandardised data differed because the variances of family means are lower than those based on individuals. If regression was based on individuals rather than family means, the variance in parents and offspring will be the same, which is the expectation because parents in generation $t+1$ are a subset of offspring in generation t and $b^*= b$. Since males are bigger and more variable than females, standardisation increases the regression coefficient for daughter-sire and reduces that for son-dam. However, estimates of heritabilities from standardised data are not expected to differ much from those of unstandardised data if estimates from the latter are adjusted for the differences in variance between sexes. The advantage of standardising the data is that heritability estimated from mid-parent regression is unbiased by the difference in variance between sexes.

The variance of single parents is simply the phenotypic variance, but because of the correlation with the unmeasured parent the covariance and regression of offspring on single parent is increased (decreased) by the factor 1+ r (Crow and Kimura, 1970). In this study the correlation between pairs was r = -0.11, which shows that mating was not perfectly random. For bivariate analysis, the covariance between offspring and parent is also affected by assortative mating i.e. $Cov(O,P)=\frac{1}{2}V_A(1+r)$, but the regression coefficient is not. If data are standardised, heritability estimates derived from offspring-mid parent and those from bivariate analyses were similar. From results of this study it appears that there is no added advantage in considering the two parents separately. It is logical to expect these results if both parents have the same variance because the covariance between offspring and mid-parent is $\frac{1}{2}((Cov(O,S) + (Cov, O, D))$, whether regression is on mid-parent or both parent considered jointly.

3.4.2 Non-linear offspring-parent regression

As the true offspring-parent regression is unknown, the regression of offspring on parent was evaluated by fitting non-linear functions. Significant non-linearity in the relationship between offspring and sires as well as between daughters and mid-parent was found. However, the contribution of non-linear terms was relatively small. Theoretical considerations have indicated that non-linear heritability may arise due to directional dominance and asymmetrical gene frequencies (Frankham, 1990; Gimelfarb, 1986; Mäki-Tanila, 1982; Bulmer, 1980; and Robertson, 1977). These workers reported a large departure from linearity when dominance is incomplete and the recessive genes are at low or moderate frequencies. Unlike additive effects, the dominance effects are not inherited through individuals but through pairs of individuals. An individual's dominance effect (d_i) could be partitioned into a sire × dam combination effect, $f_{sire \times dam}$, and an individual Mendelian sampling deviation, m, from this combination effect such that $\mathrm{d}_i = f_{sire \times dam} + \delta_m$, where $f_{sire \times dam}$ represent the average dominance effect of many full sibs produced by sire (s) and dam (d), and m is the Mendelian sampling deviation of individual i from this average sire × dam combination effect (de Boer, 1994). In this study however, none of the offspring-sire × dam interactions were significant (Table 3.3 & 3.4). Previous analysis of the P- and F-lines used in this study showed evidence of dominance effects in expression of 6-week body weight, but the contribution of dominance was found to be not significant (Hastings and Veerkamp, 1993). However, failure to detect a significant dominance effect does not exclude dominance as one of the possible contributors to non-linearity observed in this study.

The lower estimates of regression coefficients in generation 9-20 relative to those in generation 0-8 (Table 3.5) suggests a large change in variance which could partly be attributed to inbreeding and genetic drift (Bulmer, 1976). The trends of litter size between generation 0 and 10 (Fig. 3.7) suggests a change in environment which may have affected the relations between parents and offspring. Non-linearity was found in this period between sire and offspring, but not in the period between generation

9 and 20. In this study, parents and offspring were kept in similar environments and it was expected that the distribution of the environmental values would be similar. By definition the mean of environmental deviations for a particular genotype is zero, and it is commonplace in quantitative genetics to assume that the distribution of environmental deviations will be the same for all genotypes. However, if mating is at random as was the case in this study, a situation may occur such that genotypes of higher values have also greater environmental variance (Robertson, 1977). A linear regression of genotype on phenotype requires skewness of the same sign and magnitude for both genotype and environment, though Mäki-Tanila (1982) further stated that this is true only when genetic and environmental components contribute equally to the phenotypic variation. When genotypic skewness is larger than environmental, the regression of offspring on parent will be convex and when less, concave, (Mäki-Tanila, 1982; Nishida and Abe,1974; and Curnow, 1960). If this is the case, the convex regression line of daughter-sire relation (Fig. 3.2) and daughter-mid-parent (Fig. 3.3) may suggest that the distribution of genotypic component is more skewed than that of environmental component (Nishida and Abe, 1974). However, this conclusion is purely theoretical and difficult to prove since the distributions (genotype and environment) of offspring and parents are not known in practice. In the presence of dominance, the regression of offspring on one parent is linear despite dominance, but regression on both parents the shape of the curve will be a hyperbola, tending to a strait line when the number of loci is large (Bulmer, 1980, Mäki-Tanila, 1982). As such the regression curves obtained in Fig. 3.3 and 3.4 do not offer a simple biological or biometrical explanation.

The non-linear curves and the three dimension plot (Fig. 3.5) suggests that if selection is practised and the offspring-parent relation is non-linear, asymmetry of response is expected. Asymmetry in responses will increase as the intensity of selection increase, similar to findings of Gimelfarb and Willis (1994). Extreme values (outliers) of the independent variable can also produce non-linearity of regression surfaces particularly if they are many (Koerhuis, 1996), but if they are few and do not deviate too much from the mean, their effect on the estimate of regression coefficient may be negligible. In the present study, exclusion of the outliers that were found did not change the general conclusions.

3.4.3 Conclusions

Estimates of heritability from bivariate regression were similar to those obtained from offspring-mid-parent regression if data are standardised.

Slight but significant non-linearity was found for regressions of sire on son and sires on daughter as well as that between daughters and mid-parent. Offspring-dam regression was essentially linear, however there was no immediate biological reason(s) to describe the observed relations.

With single or both parents regression, polynomials of higher order could be rather impracticable and may have no biological meaning. It is thus reasonable to suppose that the regression can be approximated by a linear, quadratic or cubic curve.

Chapter 4

UNIVARIATE ANALYSIS OF 10-WEEK BODY WEIGHT

4.1 Introduction

Results from chapter 2 show that the decline in response in both lines were associated with reduction of selection differentials. This was particularly vivid in the L line towards the end of experiment. In the H line, response was restored after the initial phase of depression (generation 30-40). There is no evidence to suggest that the high line had reached selection limit, since selection was suspended at generation 50. One of the aims of this study is to estimate base population parameters and response to divergent selection. With one line selected for high value of the trait and the other for low values, analysis of both lines eliminates the need for an unselected control line(s) (Hill, 1972,1980). In this regard the common environment effects are eliminated by measuring the difference between means of the two lines. A derivative free (DFREML) algorithm to maximise the likelihood directly has been suggested for estimation of base population parameters (Graser *et al.,* 1987; Meyer, 1989) and the effect of selection can then be predicted from the knowledge of base population parameters.

For two divergently selected lines interpretation of the results would depend on the assumptions regarding the variance components in the two lines. The simplest model is to assume that there is a homogeneity of additive genetic effect and residual error components between lines. Heterogeneity of phenotypic variance could be attributed to both genetic and environmental factors. Heterogeneity arising from environmental factors can be accounted for by including the fixed factors and covariates in the model, and if the source of heterogeneity is due to scale effect, then appropriate scaling e.g. by expressing the observation as deviations relative to the estimate of standard deviation from the group or logarithmic transformation have been suggested (Hill, 1984, Falconer, 1989). However, if heterogeneity is due to both fixed and random factors, then mixed model analysis can be used to simultaneously estimate the variances in separate blocks (Beniwal *et al.,* 1992a,b; Heath *et al.*,1995).

Apart from estimation of base population parameters, mixed methodologies in selection experiments may be used to find out how parameters change as a consequence of selection (Beniwal *et al.,* 1992a, Hill and Caballero, 1994; Heath *et al.*,1995). Assuming an infinitesimal model, inclusion of the numerator relationship matrix (**A**) for additive animal effects will account for genetic drift, decline in genetic variance due to selection (Sorensen and Kennedy, 1993), inbreeding and assortative mating (Kennedy *et al.,* 1988), provided that all data on which selection are based are included (Van der Werf and de Boer, 1990). However, in the situation where response has ceased or response continues for a longer period than expected (see review by Eisen, 1980) one may wish to find an explanation for absence or presence of such limits. Selection limits are often attributed to attenuation of additive genetic variance, though several other factors may explain the reason for failure to respond when genetic variance is present (Falconer, 1989). However, in the context of mixed model methodologies, there is so far no efficient way of estimating the trends in variance components in real data. The common practice used to infer the genetic trend is to estimate variance components from different generations. If full pedigree information is incorporated in generation blocks, the estimates from the different generations are those of the base population (Sorensen and Kennedy, 1986; Meyer and Hill, 1991).

Broadly, the objectives of this chapter are to provide further analyses and attempt to infer the probable genetic mechanism behind the pattern of responses observed. Specific objectives are:

a. to estimate base population parameters and investigate the effect of heterogeneity of variance in the P6-lines divergently selected body weight.

b. to investigate the long term effects of selection on the trends of variance components.

4.2 Methods for estimation of variance components

This chapter uses data from the P6-lines divergently selected for 10-week body weight described in chapter 2. It should be recalled that no control line was maintained after the P-lines were crossed and only a single replicate was maintained in each direction of selection. In all analyses we ignored data and pedigree information from the P-lines. Hence we assume the parents in the first generation of P6-lines (generation 21) are not inbred and have an inbreeding coefficient of zero.

10-week body weight data were initially subjected to simple linear regression analysis to evaluate the significance of the following fixed effects: generation, line, sex and sex by line interactions. From analysis of variance (ANOVA), all the main effects were found to be highly significant ($P<0.001$), whereas interaction effects were only significant at the $P< 0.05$ level. For estimation of base population parameters, a univariate animal model using a derivative free restricted maximum likelihood (DFREML) package (Meyer,1989) with some modifications to the model to account for heterogeneous variances or maternal effects was used. Analyses were done taking each line individually or in combination. A line effect was included in the model because in generation one, the H and L line had already diverged by 13.3g due to previous selection for lean mass and the fixed effect of generation was included to account for environmental trends between generations. A coded common litter effect was fitted as an additional random effect (an effect which contributes to resemblance between full sibs). Litter size of birth (live young) was found to be highly significant ($P<0.001$) in the two lines and was incorporated in the model as a linear covariate in the analyses to account for systematic variation in body weight due to litter size of birth.

4.2.1 Additive genetic and common environmental effects

Homogeneous variances

The following linear model was fitted to untransformed or log transformed 10-week body weight data when individual lines or combined lines were analyzed together. When both lines were analyzed together it was initially assumed that the variance components in the two lines were homogeneous. Transformation was done to reduce the scale effect due to differences in the means and variances in the two lines. In matrix notation the model may be written as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e} \tag{4.1}$$

where:

$\mathbf{y}$ = vector of untransformed or transformed 10-week body weights

$\mathbf{b}$ = vector of fixed effects of generation,line,sex and sex × line interactions. Litter size at birth was included as a linear covariable

$\mathbf{a}$ = vector of random animal effects

$\mathbf{c}$ = vector of random litter effects

$\mathbf{e}$ = vector of random residual effects.

$\mathbf{X}$ is the incidence matrix, for the fixed effects of generation, line, sex and line by sex interaction. $\mathbf{Z}$ is the incidence matrices for the random effects i.e. animal effect and residual variance and $\mathbf{W}$ is the incidence matrix for the common environmental effect.

It is assumed that common environmental and residual effects are independently distributed with mean of zero and variance σ_c^2 and σ_e^2 respectively. Thus var($\mathbf{c}$)= $\mathbf{I}\sigma_c^2$, var($\mathbf{e}$)= $\mathbf{I}\sigma_e^2$ and var($\mathbf{a}$)= $\mathbf{A}\sigma_a^2$, where $\mathbf{A}$ is the numerator relationship matrix, σ_a^2 is the variance of additive genetic effects, σ_c^2 is the variance of common environmental effects and σ_e^2 is the residual variance.

The above model assumes homogeneity of additive, common environmental and residual variances. Fully pedigreed information was included (excluding the P-lines). The analysis assumes the trait to be controlled by an infinite number of genes with small additive effect, and no interactions among the gene effects i.e. an infinitesimal model.

Solution according to model 4.1 were obtained from the following mixed model equations

$$\begin{pmatrix} \mathbf{X'X} & \mathbf{X'Z} & \mathbf{X'W} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\mathbf{A}^{-1}\alpha_1 & \mathbf{Z'W} \\ \mathbf{W'X} & \mathbf{W'Z} & \mathbf{W'W}+\mathbf{I}\alpha_2 \end{pmatrix} \begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \\ \hat{\mathbf{c}} \end{pmatrix} = \begin{pmatrix} \mathbf{X'Y} \\ \mathbf{Z'Y} \\ \mathbf{W'Y} \end{pmatrix} \tag{4.2}$$

where $\alpha_1 = \sigma_e^2/\sigma_a^2$ and $\alpha_2 = \sigma_e^2/\sigma_c^2$

In all analyses convergence was assumed when the variance of the log likelihood was smaller than 10^{-8}. The standard errors of estimates for heritability (h^2) and common environmental effect (c^2) were obtained through approximating the likelihood curve by a quadratic approximation to the log likelihood using fixed point estimates of the parameter around the point of maximum likelihood and taking the second differential with respect to parameter of interest. The log likelihood at the maximum θ was obtained at convergence when all parameters (t) were fitted. In order to compute the standard errors associated with the estimates several points were fitted on either side and around the point of maximum and estimates obtained by maximising the remaining t-1 parameters given θ. The quadratic coefficient is a measure of information of estimates (Nelder and Mead, 1965) and the inverse of this coefficient is the estimated sampling variance of the estimate (Smith and Graser,1986; Meyer and Hill,1991; Thompson and Atkins, 1994).

Heterogeneity of additive genetic variance

An approach suggested by Visscher and Thompson (1990) and Beniwal *et al.*(1992) was used where the diagonal elements for animal and fixed effects are manipulated during evaluation. This entailed fitting separate variance components to blocks of animals. Using this approach the animals in a numerator relationship matrix were partitioned into two groups (in this case High=1 and Low=2).

The relationship matrix **A** is written as the product of a lower triangular, diagonal and upper triangular matrix (Thompson,1977a).

Thus:

$$\mathbf{A} = \mathbf{TDT}',$$

where $\mathbf{T}$ is lower triangular and describes the transfer of genes from one generation to the next, and $\mathbf{D}$ is the diagonal and represents the variance-covariance matrix of Mendelian sampling terms in the two lines.

By substituting $\mathbf{TDT}'$ for $\mathbf{A}$ in equation (4.2):

$\mathbf{Va} = \mathbf{TDT}'\sigma_a^2$

The diagonal matrix was partitioned into two parts, one corresponding to the high and the other to the low line respectively.

$$\mathbf{Va} = \mathbf{T}\begin{pmatrix} D_1 & 0 \\ 0 & D_2 \end{pmatrix}\mathbf{T}'\sigma_{\mathbf{a}}^{\mathbf{2}}, \tag{4.3}$$

$$= \mathbf{T}\begin{pmatrix} \mathbf{D_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{pmatrix}\mathbf{T}'\sigma_{\mathbf{a1}}^{\mathbf{2}} + \begin{pmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{D_2} \end{pmatrix}\mathbf{T}'\sigma_{\mathbf{a2}}^{\mathbf{2}} \tag{4.4}$$

$=\mathbf{A_1}\sigma_{a1}^2 + \mathbf{A_2}\sigma_{a2}^2$

where σ_{a1}^2 and σ_{a2}^2 are the additive genetic variance in the high and low line respectively.

The $\mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\alpha$ in the mixed model equation (equation 4.2) can be rewritten as:

$\mathbf{Z}'\mathbf{Z} + \mathbf{A_1^{-1}}\alpha_{11} + \mathbf{A_2^{-1}}\alpha_{12}$

where $\alpha_{11} = \sigma_e^2/\sigma_{a_1}^2$ and $\alpha_{12} = \sigma_e^2/\sigma_{a_2}^2$

The corresponding phenotypic variance and heritabilities in the two lines were estimated as:

$Vp_H = V_{AH}$ + Vc + Ve.
and
$h_H^2 = V_{AH}/Vp_H$ for the H line.
and
$Vp_L = V_{AL}$ + Vc + Ve.
and $h_L^2 = V_{AL}/Vp_L$ for the L line.

Heterogeneity of common environmental variance

In the analysis with heterogeneous additive and common environmental effects a similar approach was used by splitting the data into two blocks taking into account the differences in common environmental variances.

Let Vc = $\mathbf{W}\ \sigma_c^2$, where $\mathbf{W}$ is the incidence matrix for the common environmental effect and σ_c^2 the common environmental variance. The $\mathbf{W}$ matrix can be decomposed and written as:

$$Vc = \mathbf{W}\sigma_c^2 = \begin{pmatrix} \mathbf{W_1} & 0 \\ 0 & 0 \end{pmatrix} \sigma_{c1}^2 + \begin{pmatrix} 0 & 0 \\ 0 & \mathbf{W_2} \end{pmatrix} \sigma_{c2}^2 \tag{4.5}$$

$=\mathbf{I}_1\sigma_{c1}^2 + \mathbf{I}_2\sigma_{c2}^2$

where $\mathbf{I}_1$ and $\mathbf{I}_2$ are not identity matrices, but matrices with appropriate ones and zeros for the high and low line respectively.

In the mixed model equation (4.2), the $\mathbf{W}'\mathbf{W} + \mathbf{I}\alpha_2$ can be re-written as:

$\mathbf{W}'\mathbf{W} + \mathbf{I}_1\alpha_{2_1} + \mathbf{I}_2\alpha_{2_2}$

Where,

$\alpha_{2_1} = \sigma_e^2/\sigma_{c_1}^2$

and,

$\alpha_{2_2} = \sigma_e^2/\sigma_{c_2}^2$

Fitting both heterogeneous additive and common environmental variance, the analysis provide separate estimates of Va and Vc, and allowed separate estimation of heritabilities and variance of litter size (c^2) in the two lines.

4.2.2 Maternal effects

The ability of the dam to provide a suitable environment for the expression of traits such as body weight in her progeny is partly genetic and partly environmental. To account for the likely impact of maternal genetic variation on total phenotypic variance and partition the two effects (maternal and common environmental) two models were fitted to the data;

- a A model which does not account for covariances between maternal and direct breeding values, cov(**a**, **m**)=0, but includes a random effect of individual's litter of birth.
- b A model which allows for a covariance between the two random effects (direct additive and indirect maternal genetic effect) for each animal.

The animal model (AM) for maternally influenced traits, in matrix notation, is

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Um} + \mathbf{Sc} + \mathbf{e} \tag{4.6}$$

where

a,**b**,**e** are defined in equation 4.1 and **m** is the vector of random maternal (indirect) genetic effects

c = vector of permanent (common) environmental effects. These include the permanent environmental influence of dam's mothering ability and maternal non-additive genetic effects of the dam.

X,**Z**,**U** and **S** are incidence matrices relating record to fixed, animal, maternal genetic and permanent environmental effects respectively. The matrices **U** and **S** relate records through the dam to their effects. Estimates of maternal effect are for all animals in the analysis while estimates of common environmental effects are only for dams of progeny with records.

The general model (model b) assuming covariance between direct additive and maternal additive genetic effect assumes that:

$$Var\begin{pmatrix} \mathbf{a} \\ \mathbf{m} \\ \mathbf{c} \\ \mathbf{e} \end{pmatrix} = \begin{pmatrix} g_{11}\mathbf{A} & g_{12}\mathbf{A} & \mathbf{0} & \mathbf{0} \\ g_{21}\mathbf{A} & g_{22}A & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_c^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2 \end{pmatrix} \tag{4.7}$$

where

A is the numerator relationship matrix among all animals with or without measured phenotype and **I** is the identity matrix.

$g_{11}\mathbf{A}$ = additive genetic variance for direct effects

$g_{22}\mathbf{A}$ = additive genetic variance for maternal effects

$g_{12}\mathbf{A}$ = additive genetic covariance between direct and maternal effects

σ_c^2 = variance due to permanent (common) environmental effects

σ_e^2 = residual error variance.

For the model ignoring the covariance between additive and maternal genetic effect (model a), the terms g_{12} and g_{21} in equations 4.7-4.10 should be replaced with zero.

The variance of **y** (the observed phenotype), is

$$Var(\mathbf{y}) = \begin{pmatrix} \mathbf{Z} & \mathbf{U} \end{pmatrix} \begin{pmatrix} g_{11}\mathbf{A} & g_{12}\mathbf{A} \\ g_{21}\mathbf{A} & g_{22}\mathbf{A} \end{pmatrix} = \begin{pmatrix} \mathbf{Z}' \\ \mathbf{U}' \end{pmatrix} \mathbf{S}\mathbf{I}\sigma_c^2\mathbf{S}' + \mathbf{I}\sigma_e^2 \tag{4.8}$$

Solution according to model 4.6 were obtained by solving the following mixed model equations (MME)

$$\begin{pmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} & \mathbf{X}'\mathbf{U} & \mathbf{X}'\mathbf{S} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z}+\mathbf{A}^{-1}\alpha_1 & \mathbf{Z}'\mathbf{U}+\mathbf{A}^{-1}\alpha_2 & \mathbf{Z}'\mathbf{S} \\ \mathbf{U}'\mathbf{X} & \mathbf{U}'\mathbf{Z}+\mathbf{A}^{-1}\alpha_2 & \mathbf{U}'\mathbf{U}+\mathbf{A}^{-1}\alpha_3 & \mathbf{U}'\mathbf{S} \\ \mathbf{S}'\mathbf{X} & \mathbf{S}'\mathbf{Z} & \mathbf{S}'\mathbf{U} & \mathbf{S}'\mathbf{S}+\mathbf{I}\alpha_4 \end{pmatrix} \begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \\ \hat{\mathbf{m}} \\ \hat{\mathbf{c}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \\ \mathbf{U}'\mathbf{y} \\ \mathbf{S}'\mathbf{y} \end{pmatrix} \tag{4.9}$$

with

$$\mathbf{G} = \begin{pmatrix} g_{11} & g_{12} \\ g_{21} & g_{22} \end{pmatrix}; \mathbf{G}^{-1} = \begin{pmatrix} g^{11} & g^{12} \\ g^{21} & g^{22} \end{pmatrix} and \begin{pmatrix} \alpha_1 & \alpha_2 \\ \alpha_2 & \alpha_3 \end{pmatrix} = \sigma_e^2 \begin{pmatrix} g^{11} & g^{12} \\ g^{21} & g^{22} \end{pmatrix} \tag{4.10}$$

where **G** is the additive direct-maternal variance-covariance matrix and

$\alpha_4 = \sigma_e^2/\sigma_c^2$.

The models considered in this study are summarised in Table 4.1. All models assume that the residual variance is homogeneous and constant in the two lines.

Table 4.1: Summary of fitted effects and variances estimated for 10 -week body weight using different models.

				Model assumptions		
Model	Fitted effects			Va	Vc	Ve
1a†	**a**	**c**				
2a	**a**	**c**		hom	hom	hom
2b	**a**	**c**		het	hom	hom
2c	**a**	**c**		het	het	hom
3a[a]	**a**	**c**	**m**	hom	hom	hom
3b[b]	**a**	**c**	**m**	hom	hom	hom

Model 1a = individual line analysis. Model 2a-2c and 3a-3b= analysis of both lines. hom = homogeneous variance; het = heterogeneous variance. **a**,**c** and **m** are the direct additive, common environmental and maternal genetic effects respectively. *a*= **a** and **m** uncorrelated; *b* = **a** and **m** correlated.

4.3 Results

4.3.1 Univariate analysis

Results of individual lines and combined line analyses fitting model 2a-2d described in Table 4.1 are given in Table 4.2 for untransformed data. Individual line analyses show a distinct difference in the estimated variance components between the two lines. Using untransformed data and excluding generation effects in the model, the heritability estimates from individual line analyses were 0.33 for H line and 0.45 for L line. c^2 values were high, 0.30 and 0.27 for the H and L lines respectively. The H line had higher estimates of all the variance components; for example, phenotypic variance was four times larger than in the L line. Estimates using model 2a including generation effect were much lower, i.e. 0.19 and 0.20 for heritabilities and 0.32 and 0.24 for c^2 in the H and L line respectively. The lower estimates of heritabilities when generation effect was included in the model suggests a confounding between response and generations effect. The combined estimates of heritability and c-square obtained from H+L line analysis (model 2a) were 0.38 and 0.25 respectively.

Results of analyses with heterogeneous variances using untransformed data (model 2b and 2c) shows that both models grossly overestimated Va and consequently the heritability values in the H line. The opposite was true for the L line. The difference in variance components suggests a large scale effect, because at generation 21 the lines had already diverged substantially and could be treated as two distinct populations. Hence the remaining analyses were performed using log-transformed data to minimise this effect particularly when H and L line were analyzed together fitting heterogeneous variances. Table 4.3 present the results of analyses based on log transformed data. Excluding generation effect in the model still gave relatively higher estimates of variance components and h^2 estimates in both lines. In both analyses, heritability values were higher in the L line, and the opposite was observed in the case of c^2. Heritability estimates for individual line analyses or from the model assuming homogeneity of variances did not differ between transformed and untransformed data.

Results of analyses with heterogeneous additive and common environment show that h^2 was slightly higher in the L line, but the differences were not significant ($P > 0.05$). Significance tests for heritability and variance estimates were carried out using the likelihood ratio (LR) test (see. Mood *et al.*, 1973), for which $2\times(L_2 - L_1)$ is assumed to follow a Chi-square distribution where L_1 and L_2 are the maximum log-likelihoods for different sets of parameters and the parameters in L_1 are subsets of those in L_2. Comparisons of the models fitted in terms of the differences in log-likelihoods are given in Table 4.4. Estimates from model 2b and 2c did not differ significantly, the heritability estimates and c-square effect were 0.39, 0.26 and 0.42, 0.25 for the H and L line respectively.

Using model 3a and 3b, the combined line analyses gave direct heritability (h_d^2), maternal heritability (h_m^2) and c-square effect estimates of 0.32, 0.05 and 0.24 respectively (Table 4.3). Comparison of model 3a and model 2a indicated significant maternal effect in the expression of 10-week body weight of mice. However, model 3b and 2a were surprisingly not significant different ($P > 0.05$). The results show a small but negative estimate of covariance between direct additive genetic effect and

Table 4.2: Estimates of variance components and genetic parameters for 10-week body weight using untransformed data with the inclusion or exclusion of a generation effect in the model

		Variance components						
Line	Model	Va	Vc	Ve	Vp	h^2	c^2	Log L
H	1a†	6.91	6.51	7.70	21.11	0.33	0.30	
L	1a†	2.35	1.46	1.40	5.21	0.45	0.27	
H	1a‡	3.50	9.37	6.04	18.91	0.19	0.31	
L	1a‡	0.76	2.16	0.94	3.86	0.20	0.24	
H+L	2a†	5.56	3.72	5.41	14.69	0.38	0.25	-6285.36
H+L	2a‡	5.37	3.25	4.89	13.53	0.39	0.24	-9205.62
H	2b‡	18.02	3.05	2.69	23.76	0.75	0.13	-8649.96
L		0.03	3.05	2.69	5.77	0.01	0.52	
H	2c‡	17.81	7.77	2.46	28.04	0.63	0.27	-8575.65
L		0.55	0.99	2.46	4.00	0.14	0.25	

† excluding generation.‡ including generation. Model abbreviation see, table 4.1.

direct additive maternal effect (cov_{am}= - 0.023).

In order to compare predicted generation means from the analysis with the observed means, predicted divergence for each generation and line were calculated as the sum of estimates of line, generation effects and the mean breeding values of animals. The observed and predicted divergence are given in Fig. 4.1, and the predictions agreed well with the observed divergence, suggesting that the model of analysis used fitted the data reasonably well.

4.4 Change in variances

4.4.1 10-week body weight

Inferences about the genetic trend can be obtained by estimating base population parameters from data for different numbers of generations. Analyses were carried out to see if the additive variance changes as a consequence of selection in the two lines. Using 10-week body weight data, four types of analysis utilising different blocks of data were done, with or without including full pedigree information as

Table 4.3: Estimates of variance components and genetic parameters for 10-week body weight fitting different models in the H and L line ‡. Log transformed data.

		Variance components x 10^{-2}								
Line	Model	Va	Vm	σ_{AoAm}	Vc	Ve	Vp	h^2	m_a^2	c^2
H	1a†	0.401			0.360	0.424	1.118	0.34 (0.03)		0.30 (0.02)
L	1a†	0.662			0.421	0.370	1.439	0.46 (0.04)		0.28 (0.02)
H	1a‡	0.161			0.410	0.530	1.101	0.15 (0.04)		0.37 (0.02)
L	1a‡	0.212			0.310	0.550	1.072	0.19 (0.02)		0.28 (0.02)
H+L	2a	0.477			0.307	0.415	1.198	0.39 (0.02)		0.26 (0.02)
H	2b	0.464			0.307	0.414	1.185	0.39 (0.02)		0.26 (0.01)
L	2b	0.499			0.307	0.414	1.220	0.42 (0.03)		0.25 (0.01)
H	2c	0.462			0.331	0.414	1.206	0.39 (0.03)		0.27 (0.05)
L	2c	0.502			0.282	0.414	1.199	0.43 (0.03)		0.24 (0.03)
H+L	3a	0.351	0.055		0.263	0.445	1.115	0.321 (0.02)	0.05 (0.02)	0.24 (0.02)
H+L	3b	0.388	0.070	-0.023	0.256	0.442	1.111	0.35 (0.02)	0.06 (0.01)	0.23 (0.02)

† excluding generation effect. ‡ including generation effect. The remaining models, 2a-2c and 3a-3b included generation effect as well as the effects of sex, line and line× sex interactions. Values in bracket are standard errors of estimates.

Table 4.4: Comparison of differences in log likelihoods fitting different model. Analysis of log transformed data.

Models			$2log(L_2/L_1)$	Extra df†.
2_a	*vs*	2_b	0.454	1 ns
2_a	*vs*	2_c	1.236	2 ns
2_b	*vs*	2_c	0.782	1 ns
3_a	*vs*	2_a	5.502	1 *
3_b	*vs*	2_a	5.626	2 ns
3_a	*vs*	3_b	0.124	1 ns

: † $\chi^2_{1,0.05}$ =3.84. $\chi^2_{2,0.05}$ = 5.99.

ns = not significant ($P > 0.05$). * = significant ($P < 0.05$).

described below. All analyses were restricted to the P6-lines (generation 21-50).

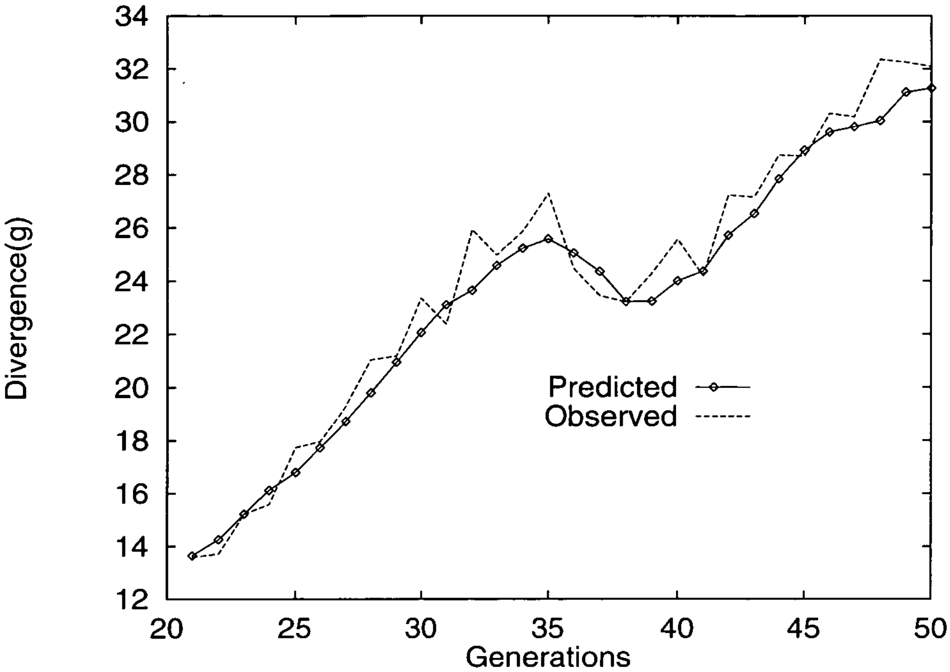


Figure 4.1: Mean predicted value and phenotypic mean, expressed as divergence (H-L) for each generation, for 10-wk body weight

A. The analyses comprised the combined H and L line data split into three 10 generation periods, i.e. generations 21-30, 31-40 and 41 to 50 with full pedigree fitted to generation 21 in all three blocks.

B. The data sets comprised records and pedigree information from generations 21-40 of H+L and generations 41-50 of selected and relaxed lines ((H+HR) + (L+LR)). Full pedigree information to generation 21 was used.

C. Records from generations 21-40 of selected lines (H+L) and data set 41-50 of selected and relaxed lines (H+HR + L+LR) were used. The pedigree information was restricted to individual blocks.

D The analysis used only the L line data grouping the observations into three blocks of generations, i.e. 21-30, 31-40 and 41-50 including full pedigree information in all blocks. This was thought necessary to reduce bias which may arise from observed increased in Vp and possibly Ve in the H line in the period between generation 31-40.

When full pedigree information was fitted, a group effect was fitted as an additional fixed effect in the model, the other fixed effects being generation, sex, line and sex × line interactions. Approach C is such that the variances in generation 21 are estimated from records of generation 21-40 and those for generation 41 are estimated from record of generation 41-50. It is assumed that animals in the first generation of each block are unrelated and non-inbred. Estimates in generation 21 are then compared to those in generation 41.

Results

Table 4.5 gives the estimates of variance components derived from univariate analysis of generation blocks of data. It should be noted that when a full pedigree information is included (Method A,B and D), the estimates are those of generation 21, i.e. base values. Further, the difference in estimates can arise due to unequal number of observations between the blocks of data, and the assumption that the residual variance is the same in the two lines within the blocks. The results can be summarised as follows.

a. Using method A, the results shows that estimates from the three blocks differed appreciably. In the H line, the additive genetic (Va) and common environmental (Vc) variances estimates from generation data 31-40 were more than double those obtained using data from generations 21-30. The number of observations was much less during this period (generations 31-40), but the h^2 estimate was of a similar magnitude. A large increase in the total phenotypic variance was observed during this period in the H line similar to results of Fig. 2.4 (chapter 2). The estimate of Va in using data from generations 41-50 was higher than the value obtained using data from generations 21-30.

   In the L line all estimates showed a consistent tendency to increase. The increase in all variance components from analysis of data in generations 41-50 compared to estimates derived from generations 31-40 were more than two-fold. The estimates in the two blocks are much more comparable in terms of number of records since the number of observations used for estimation were nearly equal.

b. Results of method B show that estimates of additive genetic variance from generations 21-40 were similar in the H and L line. Comparison of these estimates with those obtained by analysis of selected lines and relaxed lines indicates a decrease in Va in the H line, but a very large increase in Va in the L line. Estimates of Va and Vc from analysis of H+HR using data from

Table 4.5: REML estimates of variance components fitting heterogeneous Va and Vc to blocks of generations

			Variance components x 10^{-2}						
†	Line	Generation	N*	Va	Vc	Ve	Vp	h^2	c^2
A	H	21-30	1166	0.308	0.158	0.383	0.851	0.36±0.05	0.17±0.03
		31-40	895	0.649	0.695	0.382	1.728	0.37±0.06	0.40±0.06
		41-50	1190	0.617	0.268	0.382	1.269	0.48±0.04	0.21±0.03
	L	21-30	854	0.346	0.176	0.382	0.905	0.38±0.04	0.19±0.04
		31-40	924	0.433	0.188	0.382	1.001	0.43±0.04	0.19±0.05
		41-50	780	1.054	0.523	0.382	1.961	0.54±0.05	0.27±0.05
B	H	21-40	2061	0.331	0.414	0.419	1.164	0.28±0.03	0.36±0.02
	L		1778	0.337	0.181	0.419	0.937	0.36±0.03	0.19±0.04
	H	41-50	1190	0.273	0.204	0.558	1.035	0.26±0.03	0.20±0.06
	HR		427	0.282	0.370	0.558	1.209	0.23±0.04	0.31±0.02
	L	41-50	780	1.074	0.359	0.409	1.842	0.58±0.06	0.19±0.04
	LR		320	0.711	0.184	0.409	1.304	0.54±0.05	0.14±0.05
C	H	41-50	1190	0.256	0.203	0.554	1.033	0.25±0.06	0.19±0.04
	HR		427	0.275	0.373	0.554	1.203	0.24±0.07	0.31±0.04
	L	41-50	780	0.877	0.289	0.392	1.559	0.56±0.06	0.20±0.05
	LR		320	0.581	0.178	0.392	1.151	0.52±0.05	0.16±0.05
D	L	21-30		0.292	0.219	0.422	0.933	0.31±0.06	0.23±0.05
		31-40		0.407	0.296	0.422	1.125	0.36±0.05	0.26±0.04
		41-50		0.950	0.549	0.422	1.922	0.49±0.07	0.29±0.05

† A,B & D the estimates refers back to generation 21. C estimates refers to generation from which the generation block begins. N* = Number of records.

generations 41-50 were lower compared to those from H+L line analysis. This difference however, was not found in the L line.

c. Comparing Va estimates from generation data 21-40 and 41-50, the results show a decrease in Va in the H line, whereas in the L line the Va estimate was more than double. This analysis ignores the reduction of variance due to inbreeding and selection prior to generation 41, hence the estimates are biased. At generation 41, inbreeding coefficient was estimated using a subroutine embedded in DFREML (Meyer, 1989,1991a) to be about 20% in both lines.

d. Analysis of the L line shows that all components of variance (except Ve) increased.

The analyses were based on the assumption that if the infinitesimal model holds, the estimates in generation 21 (i.e. the assumed base) derived from blocks of data would be of a similar magnitude. These results show the different blocks do not contain the same amount of information and suggest that the genetic variance have changed in the course of the experiment.

4.4.2 Simulation of body weight data

A stochastic simulation (data and pedigree) was used to check the adequacy of the univariate models used in analysis of 10-week body weight data in terms of base parameter estimation. Likewise the simulated normal deviates were employed to investigate the pattern of changes of the additive genetic variance due to inbreeding and selection expected under an infinitesimal model. Ten replicates were simulated assuming an infinitesimal model for the upward selection. In the model, base animals were unrelated and non-inbred and had neither pedigree information nor phenotypic records. To asses the importance of selection, simulation was carried out by selecting the best in each family to mimic the selection strategy in the experiment. 12 individuals per family (6 male, 6 female) and a total of sixteen families per generation were simulated. One male and one female was selected in each family and mated. Selection was done within family avoiding full-sib mating; in essence the selected individual in litter l was mated with individual in litter $l + 1$ or *vice versa.* Each pair selected was programmed to produce 12 offspring. With this mating structure a total of 192 individuals per generation and 5760 mice per replicate in total were generated for subsequent REML analysis.

The model used for simulation was

$$Y_i = \mu + a_i + c_i + \epsilon_i. \tag{4.11}$$

where,

Y_i is the phenotypic value of body weight for the i^{th} individual, a_i is its breeding value. c is the common environmental effect due to litter and ϵ_i the residual variance. The μ is the phenotypic mean in the base population.

Initial heritability of 0.39 and c^2= 0.19 values were used. Generations were non-overlapping. Random normal deviates were drawn from a normally and independently distributed population with mean zero and variance σ_e^2 . The breeding value of individuals were taken from a **NID**(0, σ_a^2) population, with variance (1-F)σ_{Ao}^2, where σ_{Ao}^2 is the variance in the base population and F the mean inbreeding coefficient of parents. In subsequent generation the breeding values of offspring were calculated as

$$a_i = \frac{1}{2}(a_{si} + a_{di} + m_i), \tag{4.12}$$

and

$$m_i = a_i - (\frac{a_{si}}{2} + \frac{a_{di}}{2}). \tag{4.13}$$

where a_{si} and a_{di} are breeding values of male and female parents respectively. m_i is the deviation of breeding value of animal i from the average breeding value of both parents, that is, the deviation due to Mendelian sampling. The residual genetic value for each m_i was drawn from a normal distribution with mean zero and variance equal to,

$$Var(m_i) = \left(\frac{1}{4}(1 - F_{si}) + \frac{1}{4}(1 - F_{di})\right)\sigma_a^2 \tag{4.14}$$

where F_{si} and F_{di} are the inbreeding coefficients of the sire and dam of individual i.

Each replicate from simulated data was analyzed using DFREML program (Meyer, 1989, 1991a). In the model sex and generation were fitted as a fixed effect, and animal additive effect and common environmental effect were fitted as random effects. Litter size at birth was omitted in the model since in the analysis all families had equal numbers of observations. From the results of 10 replicates empirical standard errors were computed for each component. Results of analyses are shown in Table 4.6. There is good agreement in estimates of from simulated data and those obtained from analysis of actual 10-week body weight data in terms of h^2 estimates. The c^2 value (c^2=0.18) was slightly lower since we assumed an equal family size in each generation; however, the standard errors of estimates (h^2 and c^2) were nearly the same (though slightly lower) compared to those obtained from actual data.

Table 4.6: Estimates of variance components and genetic parameters for simulated body weight data.

	Variance components					
Rep.	Va	Vc	Ve	Vp	h^2	c^2
1	0.377	0.141	0.409	0.928	0.406	0.152
2	0.396	0.174	0.401	0.973	0.407	0.179
3	0.378	0.170	0.419	0.968	0.391	0.175
4	0.388	0.159	0.417	0.965	0.402	0.165
5	0.398	0.191	0.427	1.017	0.391	0.188
6	0.413	0.196	0.410	1.020	0.405	0.192
7	0.382	0.188	0.403	0.974	0.392	0.193
8	0.427	0.230	0.401	1.059	0.403	0.217
9	0.433	0.197	0.398	1.029	0.420	0.192
10	0.404	0.158	0.414	0.976	0.413	0.162
Mean	0.400	0.180	0.410	0.991	0.403	0.181
s.e†	0.019	0.025	0.009	0.039	0.010	0.018

†= empirical standard error. Rep= replicate.

Records from simulated data were subdivided into generation blocks of equal numbers of generations and the different variance component estimated using model 2c, fitting heterogeneous Va and Vc among blocks and including fully pedigrees for the base parents. Hence, the estimates refers to the base population. The residual variance was assumed to be similar in all blocks. The sub-divided data gave similar estimates of variances and genetic parameters in the base population (Table 4.7). The results of simulation by splitting the data into generation blocks differ from those obtained from the analysis of actual 10-week body weight data. The conclusion from this simulation is that, assuming an infinitesimal model and including full pedigree information, the base population parameters will be of similar magnitude from the subgroups.

Table 4.7: REML estimates of variance components, h^2 and c^2 from subdivided simulated data.

	Variance components †					
Data set	Va	Vc	Ve	Vp	h^2	c^2
0-10	0.390	0.157	0.418	0.966	0.404±0.021	0.163±0.022
11-20	0.411	0.204	0.418	1.033	0.398±0.027	0.198±0.021
21-30	0.379	0.186	0.418	0.983	0.386±0.024	0.189±0.023

† = Base population estimate, pooled over 10 replicates. All estimates refers to base generation.

4.5 Discussion

Analyses of individual lines using untransformed data indicates a significant heterogeneity of variances in the two lines (Table 4.2). The genetic variance in the H line was much higher than in the L line, but heritability estimate was lower. Estimates of heritabilities were likely to be biased because of not accounting for the environmental trends. However inclusion of a generation effect biased downward the parameters due to being confounded with response to selection. Analysis of both H+L lines provides estimates which are unbiased by environmental trends even when generation is included in the model since the response is derived from the divergence in phenotypic means of the two lines (Hill, 1980; Thompson and Atkins, 1994). The combined heritability estimate obtained by analysis of H+L assuming homogeneity of variance was 0.39 ±0.02. For comparison with estimates of heritability obtained by regressing response on cumulative selection differential, the individual heritability was computed using the formula $h^2 = h_w^2(1-t)/(1-r)$ where h_w^2 is the within family heritability (from regression of response on selection differential), r is the relationship between family members ($r = 0.5$) and t the intraclass correlation ($t = 0.5h^2 + c^2$). The value of c^2 was obtained from REML analysis. Using this conversion the h^2 was 0.36, a value closer to that obtained by regressing selection differential on cumulative response (h^2=0.34, chapter 2).

Results of fitting heterogeneous additive variance (model 2b) or heterogeneous additive and common environmental variance (model 2c) using untransformed data grossly overestimated the variance components as well as h^2 in the H line. Similarly, the variance components and h^2 in the L line were underestimated. The bias in estimated parameters are as a result of large heterogeneity of phenotypic variances which introduces the so called 'scale effect'. To account for this effect, data were log transformed and the analyses repeated fitting similar terms in respective models.

Based on the likelihood ratio test, results indicated that on transformed scale the additive as well as the common environmental effects were not significantly different in the two lines ($P > 0.05$). However, there was a tendency for Va to be higher in the L line which is opposite to the estimates obtained from analyses of untransformed data.

In interpreting results from this study, for simplicity we assumed the animals in generation 21 of the P6-lines were unselected with an inbreeding coefficient of zero. The two lines (H and L lines) have been selected previously before the replicates in each line were crossed. Results from a previous analysis of the P-lines (chapter 2) showed that the H and L line differed by 13.3g in generation 20 of the P-lines before the lines were crossed to form the P6-lines. Based on analyses of log transformed data, it appears that despite this difference in means, the estimates of base population parameters were not significantly different in the two lines. Similar estimates were obtained by Beniwal (1991) who used part of the data used in this study (generation 0-20 of P-lines and 21-38 of P6-lines). The estimates of heritability were 0.39 and 0.41 in the H and L lines respectively. These estimates refer to generation 0 of the P-lines. The estimates of heritabilities obtained in this study are within the range reported in the literature for body weight in mice, for example, heritability value of 0.37 for divergence in 6-week weight in mice (Falconer,1973), 0.34-0.42 for growth traits (Eisen and Prasetyo, 1988), and 0.42 for 6-week body weight (Meyer and Hill, 1991).

When both lines were analyzed together on the assumption that all variance components are homogeneous, the additive maternal effect was estimated as 0.056 ± 0.02 which was significant ($P<0.05$). However, there is no reason to expect the maternal effect to be similar in the H and L line. Individual line analysis showed that the maternal additive genetic effect in the H line was not significantly different from zero, i.e. $m^2 = 0.042 \pm 0.035$, but contributed significantly to progeny phenotype in the L line i.e. $m^2=0.093 \pm 0.041$ (not shown in Table 4.3). The significance of maternal effects is that the phenotypic value of an offspring is composed of a direct maternal genetic and maternal environmental effects. Apart from maternal environmental contribution to offspring phenotype, genetic differences among dams for the maternal effect may be expressed in the phenotypic values of their offspring as well (Willham, 1972). Hastings and Veerkamp (1993) found this component to be not significantly different from zero in mice. Estimates of maternal effects are usually confounded with the c^2 estimate (c^2=0.26), which includes the permanent environment and all non-additive genetic effects. Keightley and Hill (1992) found that maternal environmental effects accounted for about 10% of the variance when body weight of dam and offspring were included in analysis of 6-week weight of mice. Estimates of maternal effects generally differ from one study to the other depending on whether litter size is standardised or not. Differences may also occur depending on whether the estimates include prenatal and/or postnatal maternal effects.

Model 3b indicated a small but negative covariance between direct additive and additive maternal genetic effect. Despite the the estimate of the covariance between direct additive and maternal direct effect being small, the sign suggests an antagonistic pleiotropic effect of alleles on animal's own performance and their effect on maternal performance. In other words the direct effects promote large size (in case of upward selection), but indirectly this has a negative feed-back limited by the size of the uterus resulting in many but smaller offspring.

Change in variances

In the H line all variance components increased. If indeed genetic variance was increasing, then we would have expected the H line to show a positive response between generations 35 and 40. These changes could not be attributed to the effect of selection in an infinitesimal model. The decline in response in the period between generation 35 and 40 seem to be mainly environmental and could have resulted from a gross reduction in selection differentials. The number of observations declined by about 20% in the period between generation 21-40 relative to the period between generation 21 and 30. Similarly the number of breeding individuals declined in the period between generation 34 and 38. The number of breeding pairs during this period were 13,10,7 and 9 for generation 14,15,16 and 17 respectively. These values deviate from the planned 16 pair mating and the decline could result because no family replacement was practiced after the lines were crossed.

The increase in genetic variance obtained in this study contrasts strongly with the decrease in additive genetic variance that would be predicted based solely on the additive genetic variance in the base population (see results of simulation). However, Bryant *et al.*, (1986) found that when experimental populations of houseflies were subjected to population bottlenecks, both the additive variance and the heritability increased for several traits. They also found that an increase in additive variance were accompanied with an increase in total phenotypic variance, as observed in this study. The results were inconsistent with models of additive effect of alleles within loci or of additive effect among loci. Examination of several plausible models revealed that the multiplicative epistatic model could have explained the increase in additive genetic variance and h^2. This model yielded greatest additive genetic variance for intermediate level of heterozygozity. Likewise under stressful conditions an increase in environmental stress has been associated with an increase in heritability in many cases, although the opposite trend has also been found (Meyer and Hill, 1991; reviewed in Hoffmann and Parsons, 1991; Heath *et al.*, 1995). The possibility that non-additive genetic variance can be expressed as additive genetic variance has been statistically deduced by many other workers, e.g. Goodnight (1987,1988) and Cockerham (1984) and for a review see Carson, (1990). Because the analysis reported in this study hinges on the infinitesimal model, non-additive variance if present will be lumped with the direct additive component. The effect may have an appreciable contribution since the population bottleneck extended for about 5 generations.

Linkage disequilibrium can also cause an increase in genetic variance. The size and duration of any increase are dependent on the degree of of linkage; if the genes are tightly linked the small, gradual increase will result. In this case selection for favourable alleles will increase the frequencies of favourable and unfavourable alleles at different loci. At other loci where the frequency of unfavourable alleles is high, favourable alleles might nevertheless appear in coupling on rare gametes of high genetic value, due to recombination (Hospital and Chevalet, 1996, Heath *et al.*, 1995). These gametes are then likely to be selected for, so that the frequency of favourable alleles will increase at such loci, leading to a release of genetic variance. However, this model is less likely to explain the increase in additive variance observed in this study. Bulmer (1989) argued that a typical pair of loci chosen at random from the mouse genome are likely to be either on different chromosomes or loosely linked if they are on the same chromosome. To explain the increase in genetic variance, the model requires that the adjacent genes must be in repulsion association (having opposing effect). He further suggest that linkage diequilibrium can be ignored in discussing the variability maintained by the balance between selection and mutation.

Analysis of the L line give a strong indication that additive genetic variance has increased. The increase in Va was also associated with the increase in Vp which could be attributed to an increase in homozygosity as homozygotes are more sensitive to the environment than heterozygotes. However, the trend in the response suggests that the L line appeared to have reached a plateau (Fig. 2.1a chapter 2). The estimate of Va in generation 21 suggests that the lack of response is not due to exhaustion of additive variance which is also supported by the observed divergence in the low relaxed line. The increase in Va may be explained by the joint effect of natural and artificial selection, in the presence of disruptive selection (Bulmer,1976; Falconer, 1989). If the loci have an overdominant effect on fitness, then natural selection favours heterozygotes. The consequence of selection acting this way would be maintenance of the genetic variability of the character. This argument may be supported by the regression of litter size towards the base value, i.e. increase rather than decrease (Fig. 5.2, chapter 5). The increase in litter size in the L line near the end of the experiment suggests that on average selection tended to favour larger mice, probably heterozygotes. Moreover, due to small number of observations the magnitude of estimate of Va in generation 41, which suggests a threefold increase, is likely to be biased. In addition, fitting full pedigree information inbreeding is accounted for, but not for the response accruing from selection which may bias the estimates. Beniwal *et al.* (1992a) reported a decline in additive genetic variance in the P-lines (both H and L line) selected for lean mass. Their results indicated a consistent decline in additive genetic variance in both H and L lines contrary to conclusions of this study. However a bias in estimates of variances and genetic parameters in generation 21 in this study could have been biased by not including pedigree information and data from which selection was based, i.e. generations 0-20 (Van-der Werf and De Boer, 1990; Meyer and Hill, 1991). Likewise, this approach has some disadvantages, in that information content increases with number of generations.

With the inbreeding coefficient rising to more than 25% for both lines, (Fig. 5.4, chapter 5) some decrease in genetic variance might be expected. That this did not occur suggests that not all genes influencing body weight were fixed despite selection for more than 50 generations, or decline in variance was offset by new mutations. However, the simplistic assumption that the residual variance is the same across generations and between lines is certainly not true. To accommodate an additional random effect would require modification of the model assumptions and fit separate estimate of residual variances i.e. $Ve = \mathbf{I}_1\sigma^2{}_{e1} + \mathbf{I}_2\sigma^2{}_{e2}$. In this regard seven parameters need to be estimated viz. σ^2_{a1}, σ^2_{a2}, σ^2_{c1}, σ^2_{c2}, σ^2_{e1}, σ^2_{e2}, σ^2_{e}. In practice this partitioning is possible, but may be computationally very demanding particularly with an animal model and individual parameters may not be well estimated as there might be little information on each of them.

4.6 Conclusions

Evidence of heterogeneity in phenotypic variances in the high and low line was found. Logarithmic transformation of records reduced scale effects when models with heterogeneous variances were fitted. Following this transformation, estimates of additive and common environmental variances from models fitting heterogeneity of variances did not differ significantly from that of assuming homogeneity of variances.

The analysis shows that the infinitesimal model assumption of constant variance does not hold even if reduction of variance due to selection and inbreeding is considered. The additive variance and phenotypic variance increased in both lines.

Selection limit observed in the L line may not be due to attenuation of additive variance, but due to a negative association between further reduction in body weight and fitness.

Chapter 5

BIVARIATE ANALYSIS OF BODY WEIGHT AND LITTER SIZE

5.1 Introduction

Studies of litter size in mice have received increasing interest partly because of the attention to litter size by the pig breeding industry. It is well known that an individual's weight is influenced by the size of the litter in which it was born and reared, i.e. its mother's litter size, and, depending on the genetic correlation, an individual's weight influences the size of the litter it subsequently produces (Falconer, 1973). As such, litter size as a measure of reproductive performance has been found to change in the direction of selection for body weight (Brien *et al.*, 1984). However, long term selection for growth traits has been found to be associated with reproductive problems (Roberts,1974a; Eisen,1974; Barria and Bradford,1981) and inconsistent results in terms of response and estimates of genetic parameters are found in literature.

Beniwal (1992b) observed that litter size in lines selected for lean mass (P-lines) and later for increased body weight (P6-lines) tended to increase for the first 30 generations and later declined. There was also an increase in percentage of mice born dead in the high lines, but not in the low lines. Selection for body weight has been continued for 10 more generations and it is of interest to re-evaluate the trends in 10-week body weight and correlated response in litter size. Likewise, estimates of the genetic correlation between body weight and litter size are liable to change as a result of selection due to change in gene frequencies (Bohren *et al.*, 1966). However, univariate analysis of litter size may give biased estimate of base population parameters because it assumes zero correlation with trait under selection (Johansson and Sorenson, 1990 and Meyer, 1991b). As animal models are becoming the standard for genetic evaluation, analysis including continuous (e.g. body weight) and discrete (e.g. litter size) traits becomes feasible and easy to implement by assuming that the discrete trait has an underlying continuous distribution.

The objectives of this chapter are to:

a. examine the effect of long term divergent selection for 10-week body weight on litter size at birth;

b. estimate the genetic parameters of litter size at birth by univariate and bivariate Maximum Likelihood methods using an animal model.

5.2 Statistical analysis

The analyses described in this chapter used only data from the P6-lines, generations 21-50. However, reference to the P-lines will be made where appropriate because the P6-lines were derived from the P-lines. Analysis of litter size in the P-lines and part of P6-lines were described earlier by Beniwal (1992b). It should be recalled that only one female was selected in each family and allowed to produce only a single litter. Table 5.1 gives the number of records used for univariate and bivariate analyses of litter size and 10-week body weight along with the means, standard deviations and coefficients of variation. For bivariate analysis all 10-week body weight records (males and females) were included and the number of animals with litter size records comprised approximately 14% of the total records. Because we had reasonably large number of litter size classes, the distribution was close to normal (Fig. 5.1).

Table 5.1: Data structure used in the analysis of litter size and body weight

	Records		Litter size	
Line	Litter size	Body weight	Mean±*sd*	%cv
H	393	3184	12.4 ±3.6	29
L	414	2558	6.9 ±2.1	29
H+L	807	5742	9.7	

Other statistics for body weight are given in Table 2.2, chapter 2.

Sometimes a distinction is made between estimates for litter size recorded as the total born or total born alive. Few estimates of the genetic correlation between the two appear in the literature, but those available are very close to one, and indicate that the two measures are genetically very similar. For example, Young *et al.* (1978) and Lobke *et al.* (1983) reported genetic correlation estimates of 0.96±0.33 and

0.99±0.01 respectively. The higher value reported by Lobke *et al.*,(1983), suggests that the lines experienced no mortality at all, or the effect of natural selection was neglibly small. In the present study the genetic correlation between number born and number born alive was lower than the values above (r_g= 0.79±0.08). Litter size was defined in this study as number born alive and was considered as the trait of the dam. The model in this case assumes litter size to be a dam's trait (ovulation rate, uterine capacity, embryonic loss etc.) and males have no direct contribution to variation in litter size.

For estimation of base population parameters, a univariate and a bivariate animal model (IAM) using a PEST program for multivariate restricted maximum likelihood of Groeneveld *et al.* (1996) was used. Analyses were performed using individual lines or combining both the high and low line (H+L). This package was used because of its flexibility when fitting unequal design matrices and because standard errors of estimates for (co)variances and ratios are computed in one run, whereas DFREML requires re-evaluation of likelihoods for a number of fixed points of the parameters around their maximum likelihood estimate.

Full pedigree information to the base parents (generation 21) was included in all analyses. Two models were evaluated in both univariate and bivariate analyses, a) including generation effect in the model and b) excluding generation effect. The aim was to evaluate the extent to which the estimates of base population parameters will be biased if there is any environmental trend.

5.2.1 Univariate analysis

Consider the linear mixed model used for univariate analysis

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Ie} \qquad (5.1)$$

where

$\mathbf{y}$ = vector of litter size observations

$\mathbf{b}$ = vector of fixed effects (line, generations)

$\mathbf{a}$ = vector of random animal effects

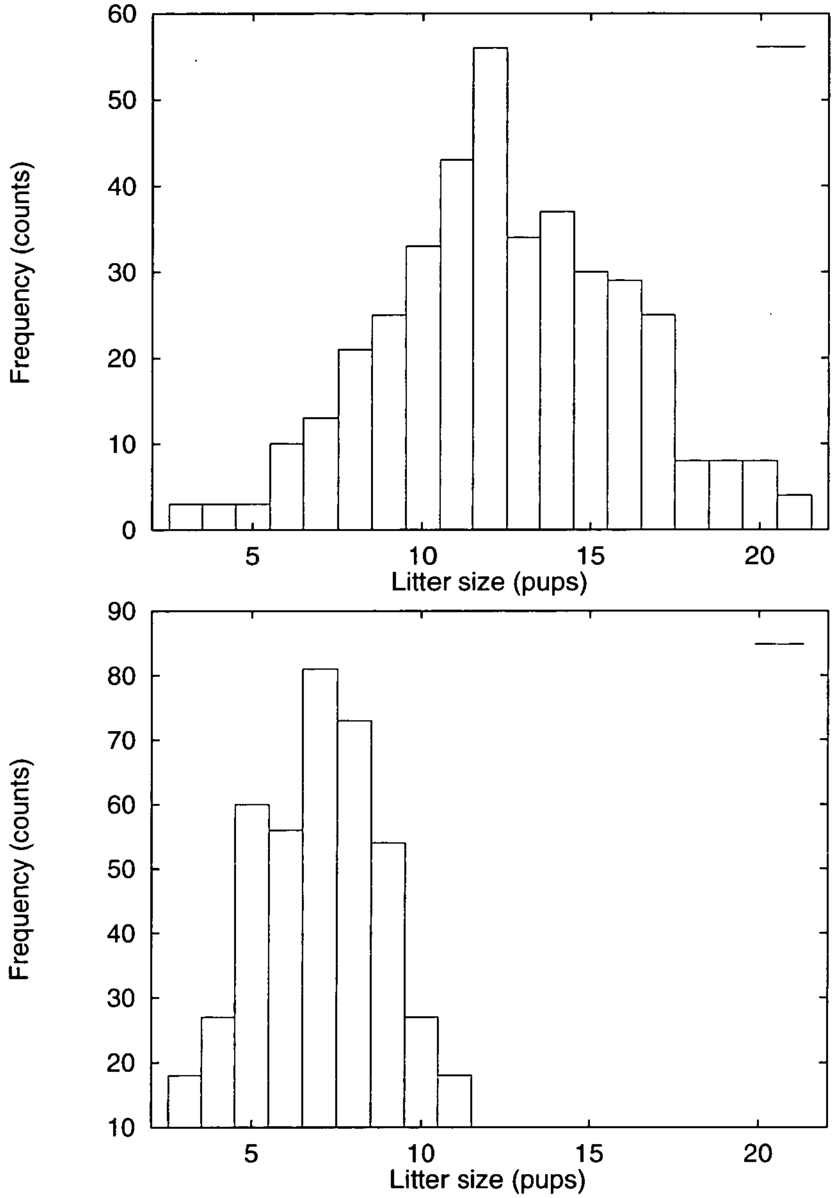


Figure 5.1: Frequency distribution of litter size in the high line (top figure) and low line (bottom figure). Means and coefficients of variation are given in Table 5.1.

$\mathbf{e}$ = vector of random residual effects

$\mathbf{X}$,$\mathbf{Z}$ and $\mathbf{I}$ are known design or incidence matrices for fixed effects, random animal effect and residual effect respectively. It is assumed that the residual effects are independently distributed with mean of zero and variance σ_e^2. var($\mathbf{e}$)= $\mathbf{I}\sigma_e^2$ and var($\mathbf{a}$)= $\mathbf{A}\sigma_a^2$, where, $\mathbf{A}$ is the numerator relationship matrix and σ_a^2 is the variance of additive genetic effects.

5.2.2 Bivariate analysis

In animal breeding, situations exists where not all animals have measurements on all traits. For example, growth traits can be measured on males and females, but reproductive traits (e.g. litter size) can be measured only on the females. In this regard the analysis described in this study used all 10-week body weight data for males and females, but litter size information only from dams (selected females). The fixed and random terms for body weight are as described for the univariate analysis (chapter 4, section 4.2). A line effect was included only when both lines (H+L) were analyzed together. No covariate or a common litter environmental effect was fitted for litter size. A common litter effect was not fitted for litter size because only one litter per female was usually available and only one female per family was used as parent in the next generation. As such the design or incidence matrices may not be equal for each trait i.e. 'unequal design matrices'.

The bivariate model for body weight and litter size may be written as:

$$\begin{pmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{pmatrix} = \begin{pmatrix} X_1 & 0 \\ 0 & X_2 \end{pmatrix} \begin{pmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{pmatrix} + \begin{pmatrix} Z_1 & 0 \\ 0 & Z_2 \end{pmatrix} \begin{pmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{pmatrix} + \begin{pmatrix} W_1 & 0 \\ 0 & 0 \end{pmatrix} \begin{pmatrix} \mathbf{c}_1 \\ \mathbf{0} \end{pmatrix} + \begin{pmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{pmatrix} \tag{5.2}$$

where:

$\mathbf{y}_1$ and $\mathbf{y}_2$ are vectors of observations body weight and litter size respectively.

$\mathbf{b}_1$ and $\mathbf{b}_2$ are vectors of fixed effects ($\mathbf{b}_1$ = generation, line, sex, sex × line interactions, and litter size at birth (covariate) and $\mathbf{b}_2$ = generation, line).

$\mathbf{a}_1$ and $\mathbf{a}_2$ are vectors of random animal effects for body weight and litter size respectively.

$\mathbf{c}_1$ is the vector of random litter effects for body weight

$\mathbf{e}_1$ and $\mathbf{e}_2$ are vectors of random residual effects for body weight and litter size respectively.

X_i, Z_i, W_i are incidence matrices relating records of the ith trait (i=1,2) to fixed and random animal effects respectively. X_i is the incidence matrix for the fixed effects, Z_i is the incidence matrix for the random animal effect and W_i is the incidence matrix for the random litter effect.

It is assumed that

$$var \begin{pmatrix} a_1 \\ a_2 \\ c_1 \\ e_1 \\ e_2 \end{pmatrix} = \begin{pmatrix} g_{11}\mathbf{A} & g_{12}\mathbf{A} & 0 & 0 & 0 \\ g_{21}\mathbf{A} & g_{22}\mathbf{A} & 0 & 0 & 0 \\ 0 & 0 & c_{11}\mathbf{I} & 0 & 0 \\ 0 & 0 & 0 & r_{11}\mathbf{I} & r_{12}\mathbf{I} \\ 0 & 0 & 0 & r_{21}\mathbf{I} & r_{22}\mathbf{I} \end{pmatrix} \tag{5.3}$$

where

A, **I** are the numerator relationship and identity matrices respectively. g_{ii} are elements of **G**, the additive genetic variance and covariance matrix for animal effects. c_{ii} are elements of the common environmental variance for body weight. r_{ii} are the elements of **R**, variance and covariance matrix for residual effects.

In the analyses of combined lines (H+L) it was assumed that the variance and covariance structure between the lines for the two traits are homogeneous. The distribution of untransformed litter size was more normal than the distribution based on log values. Probably due to the fact that litter size is a discrete trait. Similarly, there was no evidence indicating a relationship between amount of variation and the mean for litter size within line. Logarithmic transformation of body weight data and analysis of H+L lines with an assumption of homogeneous variance did not affect heritability and c^2 estimates (chapter 4, section 4.3), hence analyses were done using untransformed data for both body weight and litter size.

5.3 Results

5.3.1 Response in litter size

The mean correlated responses in litter size in individual lines and for divergence before and after the replicate lines were crossed are shown in Fig. 5.2 and 5.3. In the P-lines litter size increased or decreased in the direction of selection for body weight. Crossing of the P-line replicates resulted in an increase of mean litter size by 1.7 and 1.4 pups in the high and low P6-lines respectively. After crossing of the replicate lines, litter size in the P6 H line showed a tendency to increase in the period between generation 21 and 30, but declined thereafter to the end of the experiment. There is a slight tendency for mean litter size to increase after generation 40, but a full recovery was not realised (unlike body weight, Fig. 2.2, chapter 2). In the L line, mean litter size decreased consistently in the direction of selection, but there was an increase in litter size in the later part of the experiment.

To estimate the response in litter size, correlated responses were expressed as a) change per generation or b) change per unit increase in body weight. Regression of litter size on generation number showed that the rates of response in the H and L lines were fairly similar before the lines were crossed (Table 5.2). In the P6 H line litter size was decreasing at the same rate to that of the L line, whereas the combined (P+P6) regression analysis shows a positive, but lower rate of response in the H line compared to that in the L line. The regression of individual's 10-week weight on mother's litter size was -0.14g per pup and -0.315g per pup in the H and L P6-lines respectively. The lower and negative coefficient in the H line was unexpected, but could be explained by the reduction in mean body weight as observed in Fig. 2.1.

The correlated response measured as the divergence in litter size between H and L was positive and showed an increasing trend when both P and P6-lines were considered (Fig. 5.3). This divergence was about 9 pups between generation zero and generation 30. It appears that response in litter size measured as a divergence between H and L line has reached a plateau after 30 generations of selection. The divergence in the P6-lines (generation 21-50) was about 5.4 pups on average and remained fairly constant to the end of the experiment. This trend was not significantly different from zero (b = -0.017±0.028) and was influenced much by the decline in litter size in the H line. However, this measure is misleading since the two lines differed in body weight at the start of generation 21. The appropriate approach is maybe to describe the correlated response in litter for each line individually. Litter size in the high relaxed line regressed towards the population mean, except in the last 2 generations where a sudden increase in mean litter size was observed, probably due to sampling. In the low relaxed line, the response after relaxation was more rapid and litter size regressed towards the population mean (Fig. 5.2).

Table 5.2: Linear regression of litter size on generation number fitted in the High, Low and Control P and P6-lines

Line		Regression coefficient†	t-ratio
	High	0.069±0.025	2.77*
P	Low	-0.082±0.020	-4.05**
	Control	-0.018±0.019	-0.95 ns
P6	High	-0.096±0.025	-3.78***
	Low	-0.096±0.017	-5.65***
P+P6	High	0.066±0.017	3.87***
	Low	-0.121±0.010	-11.51***
	H-L	0.188 ±0.023	8.13 ***

† pup/generation.

, **, *** Significant at the 5%,1% and 0.1% level respectively. ns not significant.

5.3.2 Univariate and bivariate estimates

Univariate analysis

To estimate the base population parameters (generation 21) a univariate and bivariate analyses were done on litter size. Estimates for body weight obtained in chapter 4 are given along with those of litter size (Table 5.3). These estimates were obtained using only the pedigree and data information in the P6-lines (generations 21-50). Heritability estimates for litter size from individual line analyses excluding generation effect were 0.16±0.06, 0.25±0.06 and 0.18 ± 0.03 for the H, L and combined H+L lines respectively. The inclusion of a generation effect in the litter size model gave estimates of Va and h^2 which were not significantly different from zero. The corresponding heritability estimates from univariate analyses of body weight data were 0.32±0.02, 0.45±0.02 and 0.39±0.01 for the H, L (excluding generation) and combined H+L (including generation) lines respectively.

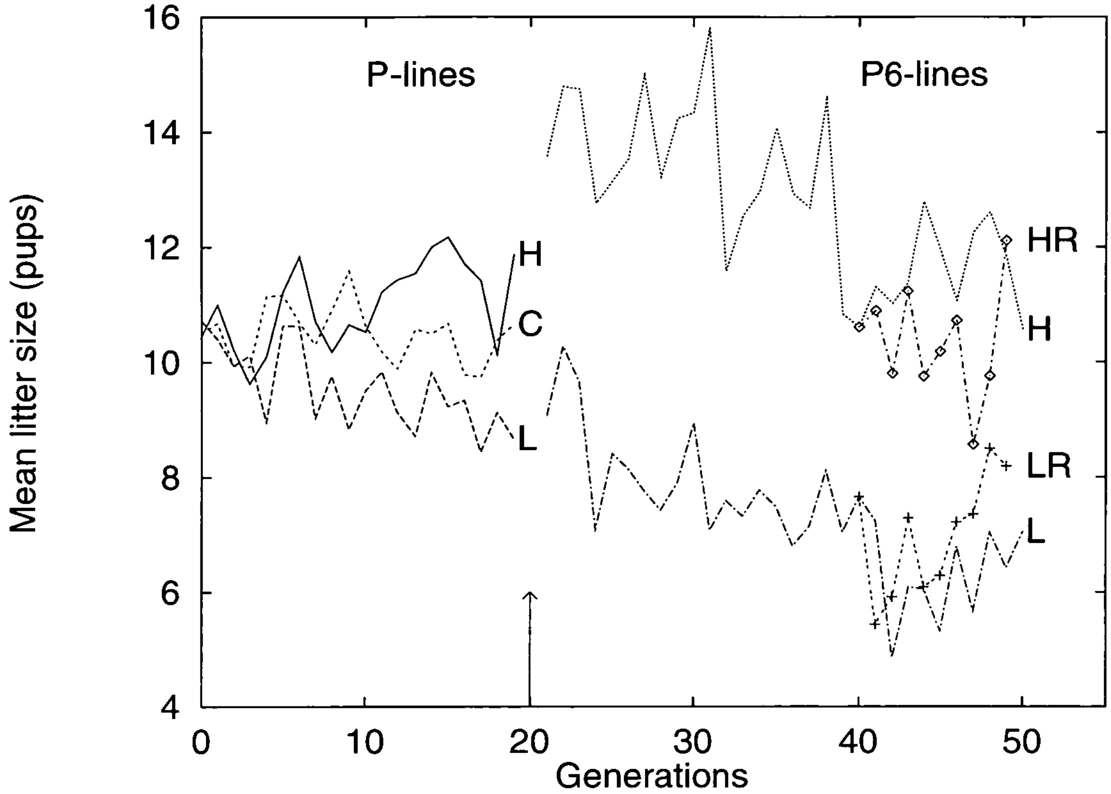


Figure 5.2: Correlated response in different lines for litter size. In the P-lines values are averaged over replicates. The arrow indicates the generation number when the replicate lines were crossed to form the P6-lines.

Bivariate analysis

Estimates of (co)variances, phenotypic and genetic parameters derived from a bivariate analysis excluding or including generation effect in the litter size sub-model are shown in Table 5.4 and 5.5. Individual line heritability estimates for body weight from bivariate analyses were slightly lower than those from the univariate analyses, but the difference was not significant. The estimate of h^2 for litter size in the L line was 0.28, the value being four times higher than that obtained in the H line. The residual and total phenotypic variances were of the same magnitude using either the univariate or bivariate approach, however the latter gave much lower standard error of estimate.

Excluding the generation effect in the model, the estimates of genetic correlations (r_g) differed in magnitude and sign in the H and L line. In the H line r_g was negative (-0.23), but had a relatively large standard error. However, the sign of r_g conforms to the trend (decline) of litter size observed in the H line (Fig. 5.2). In the L line the r_g estimate was moderately high (0.48) and positive. The genetic covariance

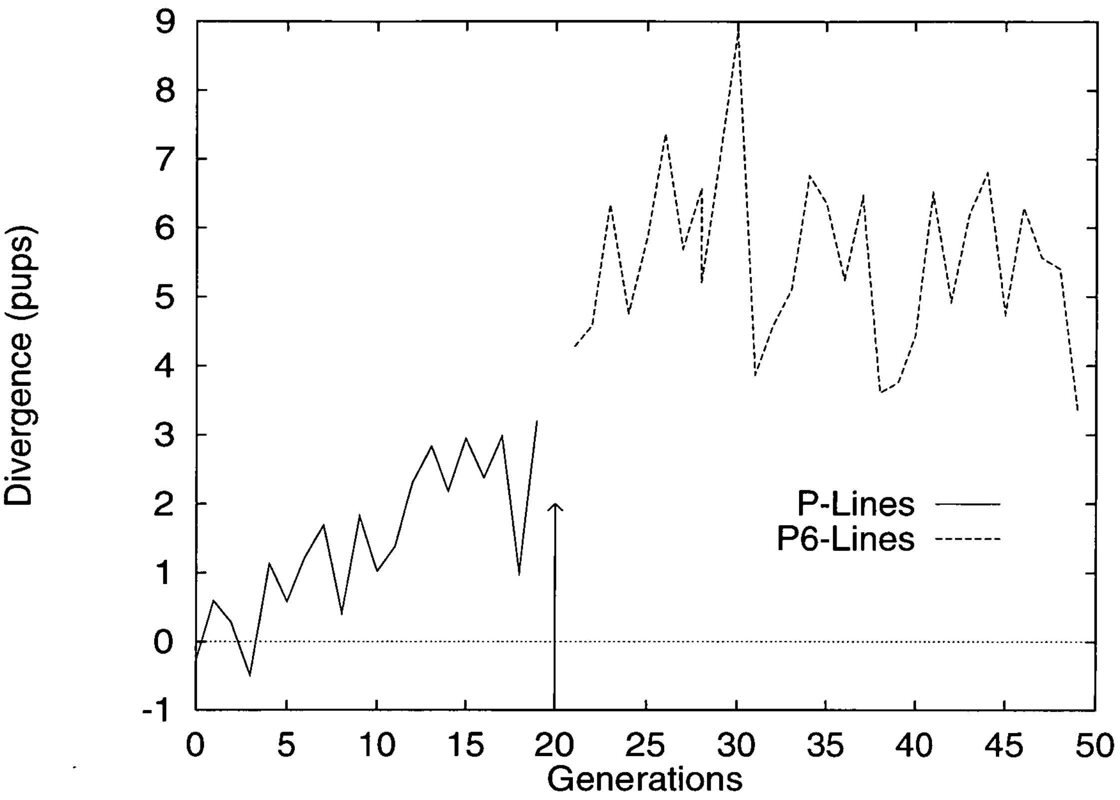


Figure 5.3: Correlated divergence in litter size expressed as the difference in litter size between High and Low lines in both P-lines and P6-lines.

Table 5.3: Univariate estimate of variance components and genetic parameters for litter size and 10-week body weight assuming homogeneity of variances.

		Fixed	Variance		components		
Trait	Line	Effects	Va	Vc	Ve	Vp	h^2
Litter size	H		2.070		10.406	12.476	0.16±0.06
	L		1.024		2.982	4.006	0.25±0.06
	H+L	line	1.463		6.566	8.029	0.18±0.03
	H	gen	0.033		11.899	11.931	0.00±0.01
	L	gen	0.000		3.128	3.128	0.00±0.00
	H+L	line,gen	0.000		7.296	7.296	0.00±0.00
Body weight	H	sex	6.908	6.506	7.701	21.11	0.33±0.02
	L	sex	2.348	1.457	1.402	5.207	0.45±0.02
	H+L	gen,line × sex	5.378	3.251	4.897	13.526	0.39±0.01

Table 5.4: Bivariate estimates of (Co)variance components and genetic parameters for litter size and 10-week body weight excluding generation effect in litter size sub-model.

Variance	Traits			Correlations	
Components	Body weight	Litter size	Covariance	r_g	r_p
H line					
Va	6.929±0.525	0.914±0.497	-0.571±0.288		
Vc	6.662±0.471	-	-		
Ve	7.782±0.289	11.462±0.784	2.932±0.495		
Vp	21.33	12.376	2.361		
h^2	0.324±0.020	0.074±0.038		-0.227±0.111	0.145
c^2	0.312±0.015				
L line					
Va	2.284±0.136	1.144±0.221	0.769±0.108		
Vc	1.448±0.117	-	-		
Ve	1.454±0.069	2.882±0.206	0.569±0.126		
Vp	5.186	4.026	1.338		
h^2	0.441±0.022	0.284±0.048		0.475±0.066	0.293
c^2	0.279±0.018				
H+L lines					
Va	5.351±0.293	1.769±0.262	-0.246±0.206		
Vc	3.413±0.198	-	-		
Ve	5.551±0.163	6.532±0.387	1.986±0.258		
Vp	14.315	8.301	1.961		
h^2	0.374±0.012	0.213±0.029		-0.080±0.067	0.179
c^2	0.238±0.014				

r_g and r_p are additive genetic and phenotypic correlations.

Table 5.5: Bivariate estimates of (Co)variance components and genetic parameters for litter size and 10-week body weight including generation effect in litter size submodel.

Variance	Traits			Correlations	
Components	Body weight	Litter size	Covariance	r_g	r_p
H line					
Va	6.681±0.497	0.000±0.002	0.008±0.657		
Vc	6.675±0.459	-	-		
Ve	7.923±0.275	11.504±0.735	1.979±0.545		
Vp	21.278	11.504	1.987		
h^2	0.314±0.021	0.000±0.000		0.998±24.19	0.113
c^2	0.313±0.016				
L line					
Va	2.295±0.148	0.116±0.181	-0.057±0.232		
Vc	1.443±0.117	-	-		
Ve	1.307±0.074	3.166±0.246	1.112±0.159		
Vp	5.706	3.282	1.054		
h^2	0.518±0.021	0.035±0.055		-0.097±0.417	0.195
c^2	0.279±0.018				
H+L lines					
Va	5.342±0.310	0.004±0.007	0.148±0.132		
Vc	3.418±0.207	-	-		
Ve	5.558±0.164	7.433±0.351	1.886±0.244		
Vp	14.318	7.437	2.034		
h^2	0.381±0.013	0.007±0.040		0.901±2.810	0.404
c^2	0.272±0.021				

r_g additive genetic correlation

r_p phenotyipc correlations.

between the two traits when the two lines were analyzed together was associated with a large error of estimates and the corresponding genetic correlation did not differ significantly from zero (r_g = -0.08±0.07). Transformation of body weight, but not litter size or both traits (results not presented) still gave a poor convergence and the estimates were generally poor. Heritabilities were 0.38±0.01 and 0.20±0.03 for body weight and litter size, similar to values obtained from analysis of untransformed data. When generation effect was included, the estimates of variance components, h^2 and c^2, for body weight were of similar magnitude to those obtained from a model excluding generation effect, except h^2 was slightly higher in the L line (Table 5.5). However, for litter size, estimates of additive genetic variances were not significantly different from zero in both individual and combined line analyses, consequently the heritabilities were zero. The estimates of genetic correlations were in all cases overestimated or poorly estimated, based on their size and the magnitude of the standard errors.

5.4 Discussion

Correlated response

If we consider the overall trend in litter size combining the P-lines and the P6-lines it appears as if litter size reached a plateau in the H line after 50 generations of selection for lean mass and subsequently on 10-week body weight. The total divergence in litter size was 9 pups at generation 30, but was reduced to 5 pups if all generations are considered. This reduction could be attributed to the low response in body weight, the period between generation 35 and 40. The apparent plateau in litter size appears to be caused by the unhealthy state of mice during this period (see discussion chapter 2). For the first 30 generations of the P+ P6 lines, the trend in litter size in both lines responded in the expected direction i.e., increasing (decreasing) with an increase (decrease) in body size. Experiments on body weight or weight gain have shown that litter size tends to increase or decrease more or less in the direction of selection (e.g. Nagai *et al.*, 1978; Eisen,1978; de la Fuente *et al.,* 1986). The expected positively correlated response in the direction of selection is often attributed to associated correlated responses in anatomical structures connected with reproduction (Wirth-Dzieciolowska, 1992).

The initial rapid increase in litter size especially in the H line after the P-lines were crossed could be attributed partly to heterosis and partly to change in the age of mating; from 9 weeks in the P-lines to 12 weeks in the P6-lines. The reason(s) for this change has not been given, even in the paper by Sharp *et al.,*(1984) where description of the base populations and selection procedures were given. The mating of animals at later age when they are heavier may have contributed to an increase in litter size. In the latter part of the experiment there was a tendency for litter size to increase following recovery of the mice from the unfavourable environment they were experiencing in the period prior to generation 40, albeit this increase was slow. The tendency for litter size to increase in the L line in generation 41 through generation 50 is probably due to relatively larger mice being selected since extremely small mice are often less fit. This is evidenced by the tendency for mean body weight to increase during this period despite selection for reduced body size.

In the P6-lines, the correlated responses were derived by regressing litter size on body weight, and it was observed that the increase in litter size per unit increase in body weight was higher in the L line. On regressing dam's litter size of birth on subsequent dam's litter size, the coefficients were 0.044±0.05 and 0.118±0.043 in the H and L lines respectively, which shows that mice born from large dams often produce smaller litters. The lower regression coefficients in the high line relative to those in the low line were unexpected, but a similar explanation i.e. the decline in mean body weight in the high line could explain these differences. This decline could be associated with changes in ovulation rate or decrease in pre-natal survival in the period between generation 30-40. An increase in mortality (dead born) was reported in previous analysis (Beniwal,1992b). Depending on the length of the experiment, selection for body weight may not necessarily produce a correlated response in litter size. For example, Falconer and King (1953) and Barria and Bradford (1981) attributed the absence of correlated response to few pleiotropic genes which became fixed during early stages of selection.

Litter size in both the high selected and relaxed lines tended to decrease, suggesting that a high proportion of polymorphic genes influencing litter size were either fixed or lost. Body weight recovered after a period of depression, but litter size did not respond much. It is possible that increased level of homozygosity at various loci contributed to decline in fitness presumably resulting in greater embryonic loss. The sudden increase in litter size between generation 47 and 50 of the relaxed H line could arise due to sampling. The converse was observed in L line, where litter size tended to regress towards the initial population mean. This difference suggests that natural selection was important in the L line, but had a weaker effect in the H line. The reduction in litter size in both lines with advance in selection could also be partially attributed to inbreeding depression. The trend in inbreeding coefficients estimated from REML analysis using the pedigree information in the P6-lines are shown in Fig. 5.4.

Inbreeding level was generally higher in the L line but, the level converged after 20 generations of selection. Regression of inbreeding coefficient (F) on generation number gave an average increase in inbreeding coefficient per generation of 1.03% and 0.96 % in the H and L line respectively. The regression of litter size on inbreeding levels was -0.107±0.019 and -0.118±0.022 pups per 1% inbreeding in the H and L line respectively, corresponding to an overall reduction of about 2.6 pups at the end of experiment, where the F value were about 0.25%. Inbreeding has a consequency of reducing the mean phenotypic value, more markedly in characters connected with reproductive capacity e.g litter size, the phenomenon known as 'inbreeding depression'. The actual coefficients of inbreeding could be higher, as we ignored earlier inbreeding in the P-lines. The undesirable effect of inbreeding on reproductive

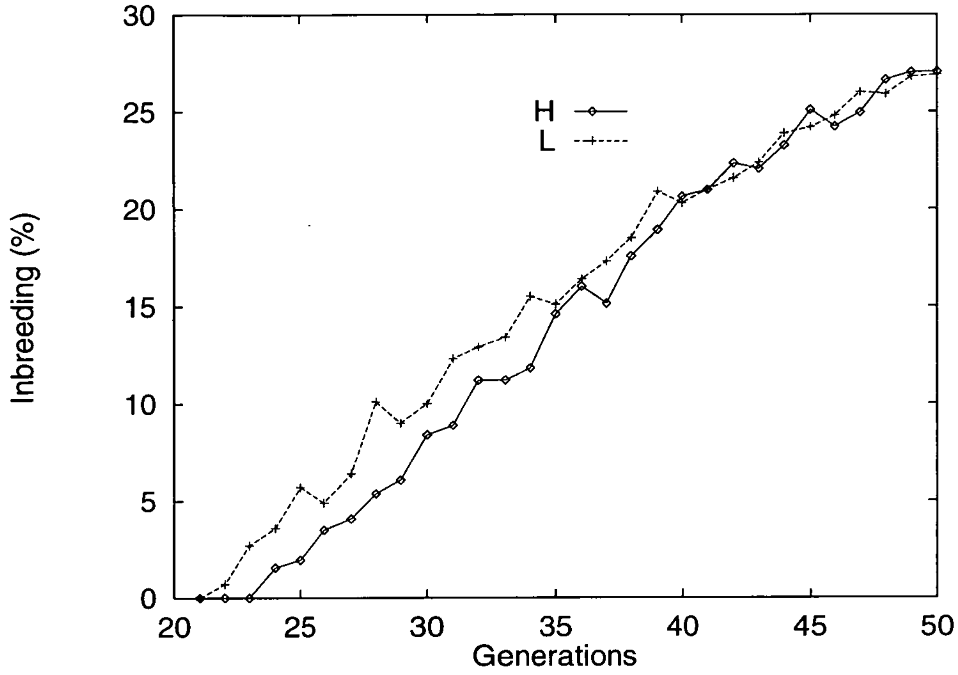


Figure 5.4: Development of inbreeding in H and L P6-line estimated from the numerator relationship matrix.

traits (fertility) in selection experiments for body weight has been observed in mice (e.g. Falconer,1973; Eisen *et al.,* 1973). Further, the reduced fitness has been attributed to deviation from optimum phenotype for body size, pleiotropy or a combination of these factors (Eisen,1974).

Genetic parameters

In estimating (co)variance and genetic parameters, the models fitted assumed that gene action was additive and that a maternal effect was accounted for by the random litter effect and systematic difference due to litter size. However, a litter effect was not fitted for the litter size sub-model, hence the additive and maternal effects will be confounded. The estimates of heritability from the univariate or bivariate analyses of litter size in the L line excluding a generation effect were generally higher than those of the H line. The higher heritability in the low fitness direction (L line) is in agreement with conclusions of Frankham (1990). Combined line analyses (univariate or bivariate) gave heritability estimates which are within ranges reported in other studies, ranging from 0.10 to 0.20 (see review by Nielsen, 1994). These values are typical for litter size in multiparous animals. Low estimates of h^2 for litter size are not uncommon especially when litter size is not the primary trait in selection programs. Beniwal (1991) found heritability values to range from 0.00 to as high as 0.21, and heritability estimates in the L line did not differ from zero despite the presence of a correlated response. The reason for the low estimate was not evident even after a simulation study. In this study, biased estimates were obtained when generation effect was included in the model probably due to confounding effect of generation and response.

The estimates of heritabilities for body weight from bivariate analysis (h^2 = 0.37) was only slight lower than that obtained from univariate analysis combining the two lines (h^2 = 0.39). For litter size, a lower heritability estimate for the H line was obtained from bivariate analysis than that from a univariate analysis i.e. 0.07 *vs.* 0.16 respectively. Slightly higher heritability values for the L and H+L lines i.e. 0.28 *vs.* 0.25 and 0.21 *vs.* 0.18 were obtained from bivariate and univariate analyses respectively. However, estimates from bivariate had much lower standard error than those from univariate suggesting that the estimates from univariate analyses were biased by assuming zero correlation body weight. In bivariate analysis an increase in the accuracy of estimation results from better connections in the data, and in addition we account for residual covariance between the traits (Thompson and Meyer, 1986).

Estimates of genetic correlation between 10-week body weight and litter size were quite asymmetrical. For the H line the genetic correlation was negative (r_g =-0.22±0.11), whereas in the L line the estimated genetic correlation was moderately higher (r_g= 0.48±0.07) and positive. However, the sign of the coefficients were in the direction of observed response, despite the large standard error of estimate. The assymetry observed in this study is similar to that reported by Bayon *et al.* (1987) for the genetic correlation of 6-week body weight and litter size, the values being 0.03±0.07 (not significant different from zero) and 0.42±0.06 in the high and low line respectively. Moderately low to high genetic correlations have been found by other authors with values ranging from 0.00 to 0.63 (Table 1.1, chapter 1).

The model fitted when both lines were analyzed together assumes that the two lines have a common base and pattern of inheritance, i.e. changes of gene frequency or maternal effects are the same in both directions. Similarly we assume that the variances are homogeneous in the two lines. These assumptions may not be true and some degree of bias in estimated parameters is expected if the variances are not homogeneous. A bias may also arise in the estimation process if litter size, being a discrete trait, is not normally distributed in the underlying scale. However, Banks *et al.* (1985) have demonstrated that REML analysis seem to be robust in terms of expectation to skewed distributions in observed scale in estimating variance components. Although divergent analysis give estimates of base population parameters which are unbiased by the environmental trend, pooling of estimates of genetic correlation (i.e. analysis of H+L) particularly when the values differ in magnitude as well as the sign, in the H and L line might not be statistically appropriate. Estimates of heritabilities were not biased too much, but the genetic correlation were heavily biased downward.

Results of univariate analysis including a generation effect in the model were highly biased downward. Estimates of additive variance and h^2 were zero. Possible reason is that litter size was not a selected trait and generations were not contemporaneous with that of body weight (selected trait). Similarly, the unexpected results of a genetic correlation and heritability estimate of zero for litter size when generation effect was included in the litter size sub-model, conflict with the observed response in litter size. The large difference in estimates of variances and covariances for the two traits when generation effect was included could have resulted from lack of information for estimating the genetic components because there was no divergence. The estimates were generally poor even in individual line analysis with unacceptable standard errors. Likewise, in using either of the line as a control (divergent analysis) to eliminate any environmental trends, symmetry of response is assumed in the two direction, which is reasonable assumption in the short, if not the long, term (Hill, 1980). We also assume that the environmental trend is common in the two direction of selection, a wrong assumption given the behaviour of the two lines (Fig. 2.1).

In long term selection experiments, estimates of genetic parameters are liable to change due to change in gene frequencies. However, the results of this study particularly the negative correlation between body weight and litter size in the H line and in the combined H+L analyses contrast with the results of Beniwal*et al.* (1992b) who reported a positive genetic correlation (r_g = 0.27±0.10) between litter size and lean mass (P-lines). The change in sign and magnitude of correlation could probably be explained by negative correlation between direct and maternal genetic effect in the later part of experiment, as evidenced by decline in litter size in both the high selected and relaxed lines. Selection for body weight is expected to increase the frequency of pleiotropic loci that act favourably by increasing body weight directly and indirectly through improved maternal environment and hence, litter size. When most of the pleiotropic genes are fixed, the remaining pleiotropic loci would then exert a negative relationship between direct and maternal effect, which may explain the negative genetic correlation between body weight and litter size in the H line. Alternatively, the change in genetic correlation could arise from segregation of loci connected with fitness (total number born and the quality of offspring weaned, Falconer,1989) much more affected by the change in environment.

Regarding the number of records, only selected females had complete records for the two traits and litter size records for other animals were treated as missing. In general, two types of missing data can be identified in this study. (i) In the first type, males have no records on litter size as litter size was considered as the trait of the dam. (ii) Females with no record on litter size because they were not selected. As a result of this structure, females with both body weight and litter records formed only 14% of the total records (n=807) and 86% of the animals had no litter record (n = 2032 females + 2901 males). However, since all data on which selection was based were included in the analysis, this sort of unbalance will have only a trivial effect on parameter estimates. In several studies it has been shown that culling (missing data) tends to affect (reduce) the estimated genetic correlations and when they are small the sign may change (Walter and Mao, 1985; Bertrand and Kriese, 1990; Mallinckrodt *et al.*,1995). Because the experiment was not replicated, only tentative conclusions may be drawn from this study regarding the observed responses and the resulting estimates of genetic parameters.

5.5 Conclusions

Asymetrical genetic correlation between body weight and litter size was in the anticipated direction for individual line analysis when generation effect was excluded from the litter size model. The low estimates of heritabilities and genetic correlations when generation effect was included in the litter size model were due to confounding between generation and response.

The decline in litter size and the negative estimate of genetic correlation between body weight and litter size could be due to reduction in fitness and due to negative genetic correlation between direct and maternal genetic effects which would be expected to occur after many generations of selection.

Chapter 6

EFFECT OF SELECTION ON SEXUAL DIMORPHISM

6.1 Introduction

The presence of sexual dimorphism (e.g. in body weight for most mammals and birds) suggests that natural selection for a sex difference may have occurred at some period during the evolution of species. Sexual dimorphism in mice is defined as the ratio of a male character to that of the same female character (M/F) in the scale of measurement (Frankham, 1968). Korman (1957) and Eisen and Hanrahan (1972) experimentally demonstrated that sexual dimorphism can be altered directly by mating for example, large males with small females (increased sexual dimorphism) or small males with large females (decreased sexual dimorphism). If selection intensity is equal among sexes, selection will produce a different response only if the genetic correlation between performance in the two sexes is different from unity (Korman,1957; Frankham, 1968; Eisen and Hanrahan, 1972).

Males of the P-lines (described in chapter 2) were selected based on an index of lean mass and females mates were randomly selected prior to crossing of the P-line replicates. As body weight and lean mass were found to be highly correlated ($r_g = 0.94$) one would expect that selection of males would not change sexual dimorphism if the genetic correlation (r_g) between sexes for lean mass or body weight is close to unity. However if the genetic correlation is much less than unity, selection of males (the heavier sex) would enhance the sexual dimorphism in the high line. The opposite would be expected for the low line because males would be less heavy than females. In contrast, analysis of the P6-lines showed that sexual dimorphism at 70 days (10 weeks) was relatively higher in the low line, and declined with advance in selection in both lines.

The initial hypothesis in this study was that selection for body weight (increase or decrease) would result in a shift in the growth curve at fixed age and that the rate at which the means changed in the two sexes differed. Regression of sexual dimorphism was done on generation number to quantify and characterise the trends in the high and low line using 6-week and 10-week body weight data from the P-lines and P6-lines respectively. A growth experiment was also set up to evaluate the dynamics of sexual dimorphism measured at different ages from birth to 100 days in the high and low line.

6.2 Materials and Methods

Selection experiment: 10-week body weight

The first analysis is based on 10-week body weight data from P6-lines selected for 30 generations for that trait after 20 previous generations of selection based on an index of lean mass. These lines will be referred to as 'selection lines'. Description of the lines and the summary statistics for high (H) and low (L) lines are given in chapter 2. After the initial 20 generations of selection for lean mass, males were on average 15% and 20% heavier in the H and L P6-lines respectively. Sexual dimorphism for 10-week and 6-week body weight were defined as the ratio of male to female generation means. Fig. 6.1 shows the trends in sexual dimorphism and the divergence between H and L lines in the P6-lines respectively. To reduce scale effects (males are heavier and more variable than females), the values are plotted using log transformed data. Both lines show a declining trend in sexual dimorphism from generation 21 to 35 and a reverse (increased), but erratic trend from generation 36 to 45. In the last 5 generations, sexual dimorphism declined in both lines. The overall means were 0.168±0.031 and 0.196±0.033 in log units in the H and L line respectively. The divergence in sexual dimorphism shows a rapid decline in the first 7 generations and thereafter fluctuated around -0.05. However, the overall trend did not differ significantly from zero.

Correlated divergence in sexual dimorphism: 6-week body weight

In the P-lines, there were no body weight records for females since selection was based on male performance only and females were chosen at random (refer, chapter

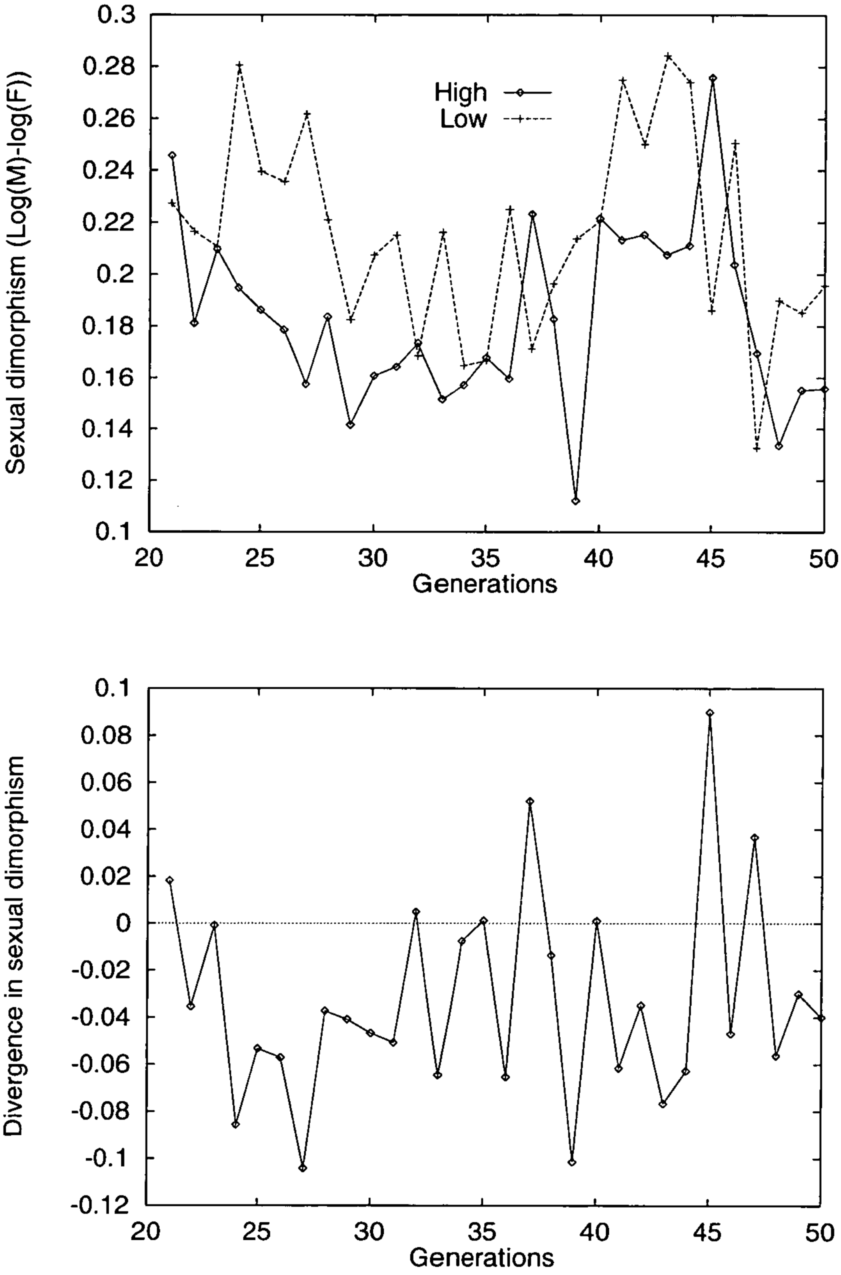


Figure 6.1: Pattern of sexual dimorphism for 10-week body weight in the H and L P6-lines (top) and the divergence between H and L lines (bottom). The scale on the y-axis is $\log_e$.

2). In order to have an idea on the previous trend in sexual dimorphism 6-week body weight data were used instead. The mean, standard deviation and coefficient of variation in males and females for 6-week body weight are given in Table 6.1.

Table 6.1: Number of records, means, standard deviations and coefficients of variation for 6-week body weight records in the P-line

Line	Sex	N	Mean (g)†	%CV
High	Male	3410	28.83 ±4.51	15.66
	Female	3408	25.01 ±3.55	14.19
	Both	6818		
Low	Male	2866	23.49 ±3.15	11.81
	Female	2783	19.94 ±2.45	12.31
	Both	5649		

† averages of 3 replicates in each line over generations.

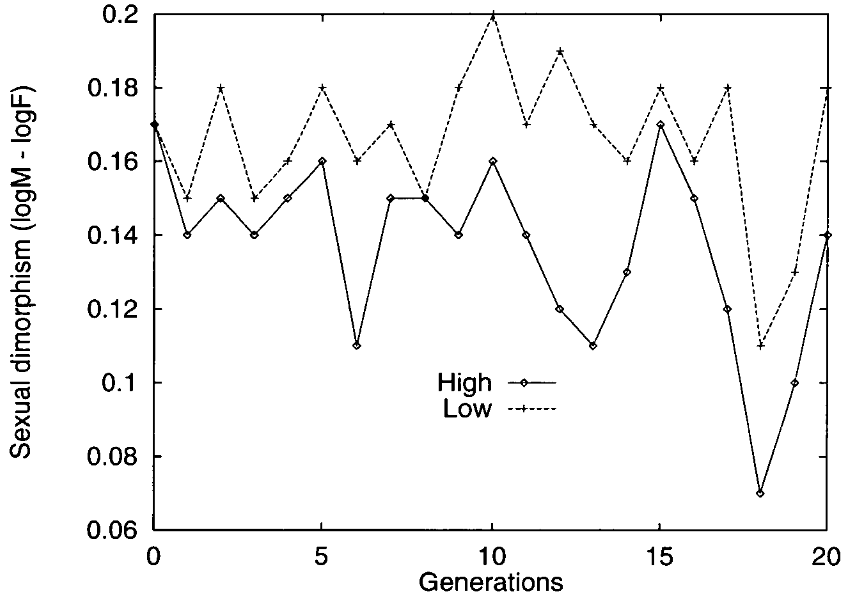


Figure 6.2: Pattern of sexual dimorphism for 6-week body weight in the H and L P-lines. Each point represent the mean of three replicates. The scale on the y-axis is $\log_e$.

Fig. 6.2 shows the pattern of sexual dimorphism for 6-week body weight in the P-lines (generation 0-20). Sexual dimorphism was higher in the Low (L) line (mean 0.166±0.021) from the beginning to the 20^{th} generation, but the trend was constant, except in the last three generations (18 to 20). In the High (H) line a declining trend was evident (mean 0.138±0.020).

Growth experiment

A growth experiment was established in generation 56 (of P+P6 lines) to evaluate the dynamics of sexual dimorphism measured at different ages and provide additional information regarding the rate of growth in males and females. From generation 50, selection for 10-week body weight continued for a further 3 generations, thereafter selection was suspended. At generation 56, 12 mating pairs were established in the H line and L P6-lines. In both lines body weights were recorded from birth (day 0) to day 100. After weaning, all mice were fed ad-libitum using standard mice diet. Records were taken at about 10.00 am on every day of measurement. Records of body weight were taken at day 0 and after every 3rd day up to weaning at 21 days (Phase I), thereafter, every week up to 70 days of age (Phase II) and then bi-weekly up to 100 days of age (Phase III). The 100th day was assumed to be the point of maturity though from other mice experiments evidence shows that mice continue to put on weight after 100 days, albeit at a very low rate. During phase I, total litter-sex weight was measured and the average weight calculated for each sex. Individual measurement were not taken at this stage owing to the difficulties in identification. Sexing was done by looking at the distance between the anus and penis/vagina, the distance being much wider for males. Mice from each litter were individually marked (ear notching) and weaned at 21 days. Thereafter, individual weights were taken according to the above schedule. No crossfostering was done. At weaning, litter size was adjusted to 10 pups for all families whose litter exceeded 10 pups. After weaning, males and females were placed into separate cages, mixing mice from different litters where the number from one litter was too small. Feeding was ad-libitum using the standard mice diet. Twelve matings were set initially and a total of 61 and 50 mice were recorded in the high and low line. To distinguish these lines from the selected lines they will be denoted by Hg and Lg for the high and low growth line respectively.

6.2.1 Statistical analysis

Significance of fixed effects

Regression analyses using the GLM procedure, MINITAB 9.1 (1985), were done on 10-week body weight (generation 21-50) and on body weight at different age intervals (i.e. the growth experiment) to evaluate the effects of different fixed factors on body weight. Least squares means for each sex were computed for each generation. For regression analyses, two models were used: Model 1, for the selection experiment, and Model 2, for the growth experiment.

Model 1.

$$Y_{ijlkl} = \mu + S_i + G_j + L_l + b_1 C_{kl} + \epsilon_{ijlk} \tag{6.1}$$

Model 2.

$$Y_{ijkl} = \mu + S_i + b_2 D_j + L_l + b_1 C_{kl} + \epsilon_{ijlk} \tag{6.2}$$

where

Y_{ijkl} is the individual body weight observation.

μ is the overall mean

S_i is the sex effect ($i = 1$ male, 2 female)

G_j is the generation effect ($j = 21...50$)

L_l is the line effect ($l = 1$ high, 2 low)

C_{kl} is the effect of litter size at birth (covariate) for the lth line and b_1 is the regression coefficient.

D_j is the effect of day (coded as period 0 or 1) and b_2 is the regression coefficient.

ϵ is the residual effect.

In the growth experiment, day effect was fitted as a linear covariate, the reason being that not all female mice littered on the same day and some of the mice were recorded on day 1 rather than day 0.

The response in 10-week body weight and the trend in sexual dimorphism were evaluated using a regression approach. Further, exponential and logistic growth functions were used to assess the growth curves for each sex separately in the growth experiment.

6.2.2 Estimation of variance components

Bivariate analysis

A bivariate analysis was done using 10-week body weight data (from the P6-lines) and 6-week body weight data (from the P-lines) to estimate the genetic correlation between performance of males and females. In the P-lines, there were no 10-week body weight records for females since selection was based on male performance only (refer, chapter 2). Therefore, 6-week body weights were used instead, on the assumption that sexual dimorphism at the 6-weeks of age is highly correlated with sexual dimorphism at 10-weeks. For estimation of base population parameters, a bivariate animal model (IAM) using a multivariate restricted maximum likelihood (REML) package of Groeneveld *et al.* (1996) was used. Analyses were done for individual lines separately or on combined (H+L) lines. In the mixed model analysis of combined H+L lines, generation, replicate and line effects were fitted as fixed factors for 6-week body weight, while generation and line effect were considered as fixed factors in the analyses of 10-week body weights (no replicates). Litter size at birth (live young) was incorporated in the model as a linear covariate in all the analyses. Animal and common litter effects were fitted as random factors in addition to the residual terms. The performance of males and that of females were considered as two different traits. A bivariate model for the two traits (1 = male; 2 = female) could be written as:

$$\begin{pmatrix} \mathbf{y_1} \\ \mathbf{y_2} \end{pmatrix} = \begin{pmatrix} X_1 & 0 \\ 0 & X_2 \end{pmatrix} \begin{pmatrix} \mathbf{b_1} \\ \mathbf{b_2} \end{pmatrix} + \begin{pmatrix} Z_1 & 0 \\ 0 & Z_2 \end{pmatrix} \begin{pmatrix} \mathbf{a_1} \\ \mathbf{a_2} \end{pmatrix} + \begin{pmatrix} W_1 & 0 \\ 0 & W_2 \end{pmatrix} \begin{pmatrix} \mathbf{c_1} \\ \mathbf{c_2} \end{pmatrix} + \begin{pmatrix} \mathbf{e_1} \\ \mathbf{e_2} \end{pmatrix} \tag{6.3}$$

where

$\mathbf{y}_i$ = vector of observations for the ith trait (1=male; 2=female)

$\mathbf{b}_i$ = vector of fixed effects for the ith trait, (generation, line and covariate i.e. litter size at birth). In the analysis of 6-week body weight replicate (r) effect was included as an additional fixed effect (r=3)

$\mathbf{a}_i$ = vector of random animal effects for the ith trait

$\mathbf{c}_i$ = vector of random litter effect

e_i = vector of random residual effects for the ith trait.

X,**Z** and **W** are incidence matrices relating records of the ith trait to fixed and random animal effects respectively.

It is assumed that

$$var\begin{pmatrix}\mathbf{a}_1\\\mathbf{a}_2\\\mathbf{c}_1\\\mathbf{c}_2\\\mathbf{e}_1\\\mathbf{e}_2\end{pmatrix}=\begin{pmatrix}g_{11}\mathbf{A} & g_{12}\mathbf{A} & 0 & 0 & 0 & 0\\ g_{21}\mathbf{A} & g_{22}\mathbf{A} & 0 & 0 & 0 & 0\\ 0 & 0 & c_{11}\mathbf{I} & c_{12}\mathbf{I} & 0 & 0\\ 0 & 0 & c_{21}\mathbf{I} & c_{22}\mathbf{I} & 0 & 0\\ 0 & 0 & 0 & 0 & r_{11}\mathbf{I} & 0\\ 0 & 0 & 0 & 0 & 0 & r_{22}\mathbf{I}\end{pmatrix} \tag{6.4}$$

where

A is the numerator relationship matrix; **I** is the identity matrix; g_{ii} are elements of **G**, the additive genetic variance and covariance matrix for animal effects
c_{ii} are the variances and covariances due to common litter environment and
r_{ii} are the variances and covariances due to common litter environment. The elements are defined below

g_{11} = additive genetic variance for direct effects for males

g_{12}=g_{21} = additive genetic covariance between body weight in males and in females

g_{22} = additive genetic variance for direct effects for females

c_{11} = common environmental effect for males

$c_{12} = c_{21}$ = covariance between the common environmental effects in males and females

c_{22} = common environmental effect for females

r_{ii} = are the elements of **R**, variance and covariance matrix for residual effects.

6.3 Results

6.3.1 Selection experiment: 10-week body weight

Regression coefficients of male and female average 10-week body weight on generation number are shown in Table 6.2. Additionally, the regression of the ratio of male mean to female mean body weight was computed and the trend in sexual dimorphism compared in the two lines. The result indicate that females of the H line responded at a faster rate than males, whereas in the L line females responded less to selection for decreasing weight than males. Because the lines were not replicated, the standard errors of regression are biased down. The reason being that regression model assumes the errors have equal variance and are uncorrelated, whereas in fact the errors increase with generation number and are highly correlated (Hill, 1980).

Table 6.2: Linear regression of sex mean and sex dimorphism ($\frac{Male}{Female}$) on generation number: 10-week body weight in generation 21-36.

Line	Sex	Regression coefficient†	t-ratio
H	Male	0.497 ±0.072	6.81**
	Female	0.546 ±0.071	7.68**
L	Male	-0.446 ±0.027	-16.26**
	Female	-0.331 ±0.025	-12.81**
H	$\frac{Male}{Female}$	-0.0032±0.0008	-3.63**
L	$\frac{Male}{Female}$	-0.0029±0.0013	-2.23*

† regression fitted using data in generation 21-36. *, ** Significant at the 5% and 1% level.

Bivariate analyses

Results of bivariate analyses for 6-week body weight and 10-week body weight are shown in Table 6.3. Results of combined H+L line analyses of 6-week body weight (generation 0-20) based on log transformed data indicate a large difference in h^2 and c^2 estimates between the two sexes. The heritability estimate was significantly higher in females than in males, 0.39 vs. 0.28 and the genetic correlation was r_g=0.97±0.01.

In the High P6-line, the estimates of additive genetic variances derived from data and pedigree information from generation 21 to 50 were similar in males and females,

Table 6.3: Bivariate estimates of (Co)variance components and genetic parameters for male and female 10-week and 6-week body weights.

	Variance components			Correlations	
	Sex				
	Males	Females	Covariance	r_g	r_p
H+L^a					
6-week P-lines‡					
Va	0.054±0.004	0.056±0.003	0.053±0.002		
Vc	0.091±0.003	0.050±0.002	0.056±0.001		
Ve	0.051±0.002	0.035±0.0002	-		
Vp	0.196	0.141	0.109		
h^2	0.28±0.01	0.39±0.01		0.97±0.01	0.65
c^2	0.47±0.02	0.36±0.02			
10-week: P6-lines†					
H line					
Va	7.008±0.615	6.517±0.583	6.397±0.344		
Vc	8.218±0.690	7.102±0.604	5.689±0.403		
Ve	7.704±0.407	5.934±0.324	-		
Vp	22.931	19.553	12.086		
h^2	0.31±0.03	0.33±0.03		0.95±0.04	0.57
c^2	0.36±0.02	0.36±0.04			
L line					
Va	3.258±0.186	2.076±0.148	2.176±0.110		
Vc	1.605±0.144	1.364±0.211	1.410±0.078		
Ve	1.008±0.103	1.115±0.092	-		
Vp	5.871	4.555	3.586		
h^2	0.55±0.03	0.46±0.03		0.84±0.04	0.41
c^2	0.28±0.02	0.29±0.03			
H+L^b					
Va	0.047±0.002	0.051±0.003	0.042±0.002		
Vc	0.040±0.002	0.046±0.004	0.035±0.002		
Ve	0.029±0.002	0.036±0.002	-		
Vp	0.116	0.133	0.077		
h^2	0.40±0.02	0.38±0.02		0.84±0.03	0.61
c^2	0.34±0.01	0.34±0.02			

r_g and r_p are additive genetic and phenotypic correlations. ‡ generation 0-20 ‡ generation 21-50. a,b Variance components × 10^{-1}.

consequently, similar values of heritability and c-square estimates were obtained. In the L line h^2 was higher in males than in females, whereas the estimates of heritabilities from combined (H+L) lines analysis using log transformed data did not differ between the two sexes. The average estimate of heritability from univariate analysis combining both sexes was 0.39±0.02 (chapter 4). Estimates of genetic correlation in the H line was closer to unity (r_g = 0.95±0.04), whereas in the L line it was much less (r_g = 0.84±0.04). Combined line analysis yielded r_g estimate of 0.84±0.03 which was lower than that obtained for 6-week body weight. The phenotypic correlations between males and females were moderately high in both lines.

6.3.2 Growth experiment

Growth curves

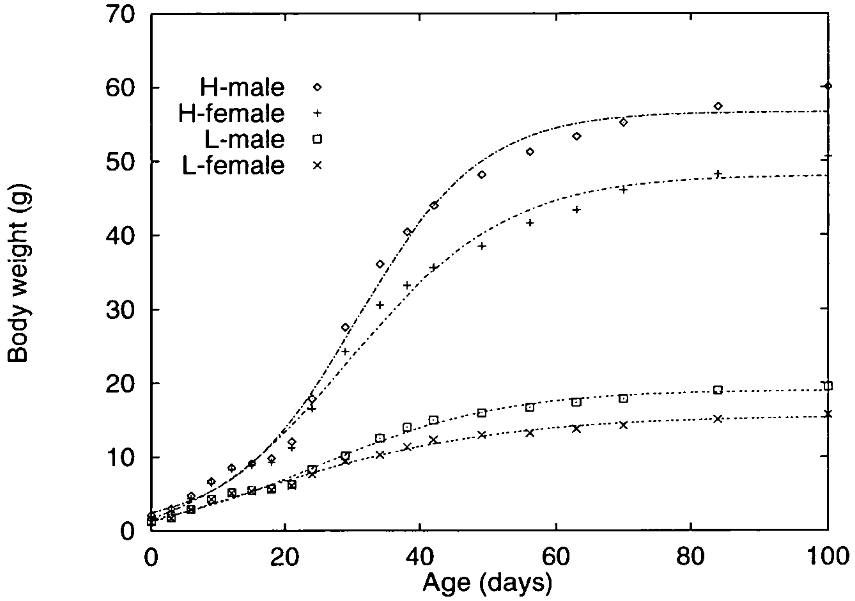
The observed growth curves for males and females are as shown in Fig. 6.3a for the high line (Hg) and the low line (Lg) respectively on the observed scale, and Fig. 6.3b on a log scale. On the natural scale males of the Hg line were much heavier than females, a difference of about 9g, whereas males of the Lg line differed by about 4g at 100 days of age. However, when the values were log transformed the difference between males and females, particularly after weaning, was larger in the Lg line than in the Hg line and showed a tendency of increasing with age. In the Hg line, there seems to be a constancy in relative growth of males and females after 42 days of age.

Logistic and exponential functions were fitted to the observed growth curves, the former on untransformed data and the latter on transformed data. The functions have essential features that they provide estimates of parameters that are biologically interpretable, such as age at point of inflection and the asymptotic weight. The two functions are described below.

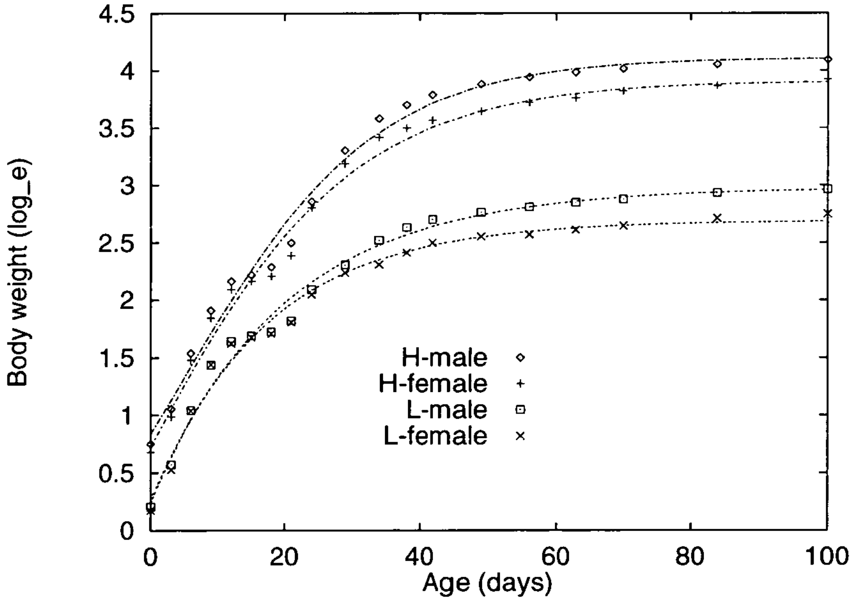
a) Logistic growth function:

$$y_i = \alpha + \frac{\gamma}{1 + exp(-\beta(x_i - \mu))} + \epsilon_i \tag{6.5}$$

where
α is the lower asymptote



a. Logistic Model (Normal scale)



b. Exponential Model (Log scale)

Figure 6.3: Observed and fitted growth curves for untransformed (a) and log transformed (b) body weights in males and females of Hg and Lg lines.

μ is the point of inflection for the explanatory variable (day)
β is the slope parameter
$\alpha + \gamma$ is the upper asymptote
ϵ_i is the random term.

b) Exponential regression function:

$$y_i = \alpha + \beta\rho^{x_i} + \epsilon_i \tag{6.6}$$

where

ρ defines the rate of exponential increase
α is the asymptote
β is the range of the curve between the value X=0 and the asymptote.
x is the observed body weight
ϵ_i is the random term.

The coefficients of determination (R^2) for males and females were slightly higher using the logistic function (R^2= 99.05) than those obtained using the exponential function (R^2= 98.32), the values in bracket being the average for males and females in both lines. However, both functions gave poor predictions between day 9 and day 20. The reduced rate of growth is common in mice a short period before weaning and may be attributed to competition for dam's milk. This reduction is followed by a rapid recovery once the mice start eating solid food.

Table 6.4 shows parameters of the growth curve predicted using the logistic function (equation 6.5). Males seem to have reached their maximum growth at about 84 days, which is within the period covered by the experiment. The age at inflection was not different in males and females of Hg line about 30 days, but differed quite appreciably in the Lg line. The early age at inflection for females of the Lg line agrees with results of Timon and Eisen (1970) who observed that females had a significantly faster rate of growth as measured by the age at point of inflection on the growth curve. Males of the Hg line reached the upper asymptotic weight much earlier than their female counterparts, whereas the predicted asymptotic weight in the females of the Hg line and both males and females of the Lg line were beyond 100 days of age.

An alternative way of presenting the difference in growth between males and females

Table 6.4: Growth parameters using the logistic growth function

Sex	Line	Upper Asymptote (g)	D	Age (d) at inflection
Male	Hg	56.61	84	30.67
Female	Hg	48.06	100	29.49
Male	Lg	19.01	100	25.20
Female	Lg	15.54	100	13.92

D = days at asymptotic weight.

is to express body weight at one age relative to body weight at another age. Table 6.5 show the relative growth rate computed from untransformed data, expressing the weight increase as the proportional increase over various periods from day 21 to day 100, for example the gain in generation 20 is expressed as $\frac{W_{20}-W_0}{W_0}$, where W_0 and W_{20} are body weights at generation 0 and 20 respectively. Before weaning there was no difference in relative growth between males and females in both lines. After weaning, males of both lines grew relatively faster than their female counterparts up to 42 days, but after 42 days the opposite was evident in the Hg line where females had higher gain than males. This change was not observed in the Lg line. Between 56 and 100 days of age females of both lines seem to gain relatively more than males, the ratio of female:male gain being 1.25 and 1.13 in the Hg and Lg line respectively.

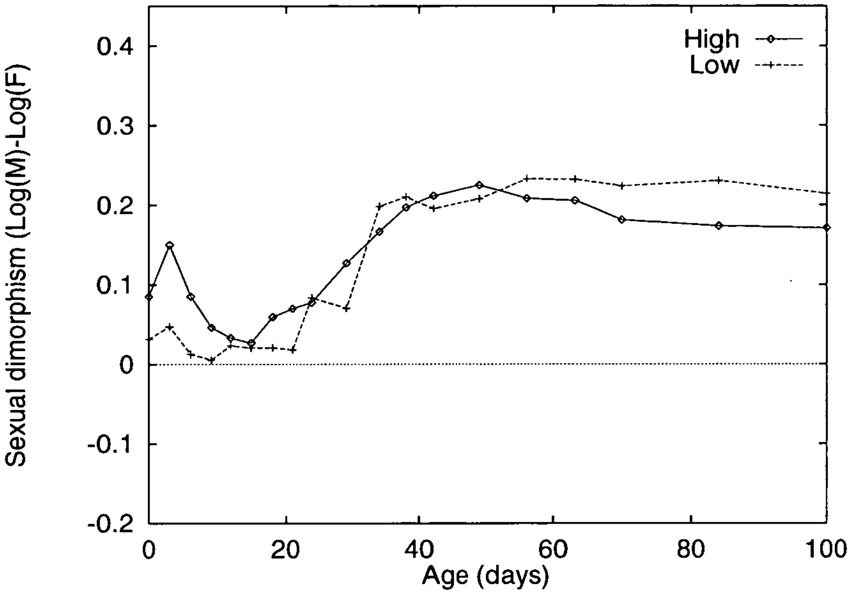
Table 6.5: Relative growth of Hg and Lg mice over the periods between 0 and 100 days

	Proportional gain in weight			
	Hg		Lg	
Period†	Male	Female	Male	Female
0-21	4.590	4.676	4.060	4.129
21-42	2.639	2.158	1.400	1.009
42-70	**0.255**	**0.293**	**0.189**	**0.156**
0-70	24.54	22.195	13.453	10.923
0-100	26.805	22.195	14.812	12.171
21-100	3.973	3.086	2.125	1.567
56-100	0.173	0.217	0.169	0.191
70-100	0.088	0.099	0.094	0.104

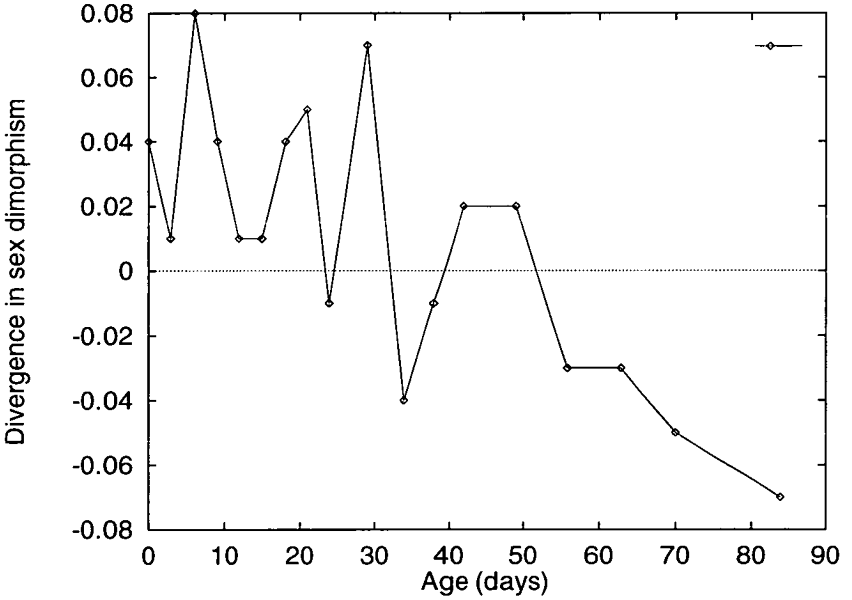
† The proportion gains are expressed relative to age at the left hand side in the row heading period. The period between 42 days and 70 days is emphasised as it correspond to the period in the selection experiment (10-weeks).

Sexual dimorphism

Sexual dimorphism in the growth experiment was defined as the ratio of male to female means ($\frac{M}{F}$), the means computed within day of measurement. The observed trends of sexual dimorphism in the growth experiment for the Hg and Lg lines based on log transformed data and the divergence are depicted in Fig. 6.4. The trends did not differ from sexual dimorphism derived from untransformed data. This observation agrees with Frankham (1968) who stated that, because sexual dimorphism is expressed as a ratio of male mean to female mean, it should then be free of scale effects. Sexual dimorphism was generally high in the Hg line between day 0 and 42 days. Thereafter, a switch over took place and the dimorphism was higher in the Lg line. The mean dimorphism measured from day 42-100 was 0.19±0.01 and 0.22±0.02 in the Hg and the Lg lines respectively. The divergence in sexual dimorphism shows a consistently decreasing trend from day 0 to the end of the experiment. At 70 days (10-weeks) the divergence between sexual dimorphism in the Hg line and Lg line was -0.05, a value similar to that obtained in the selection experiment for 10-week body weight.



Individual lines



Divergence

Figure 6.4: Sexual dimorphism in the growth experiment for the Hg and Lg line and the divergence between Hg and Lg. The scale on the y-axis is $\log_e$.

6.4 Discussion

In Fig. 6.1 we observed that sexual dimorphism decreased with increase in generation number when mice were divergently selected for high or low 10-week body weight. The increase in sexual dimorphism and its erratic nature after generation 36 could be due to change in environment as discussed in chapter 2 for the H line. Given the possible change in environment, the increased trend in sexual dimorphism in the H line implies that females were relatively more affected than males, whereas Korman (1957) observed that in a poor environment male mice performed less well than females. Divergence in sexual dimorphism did not display a clear trend and response in divergence fluctuated around -0.05. In contrast, in the analysis of 6-week body weight, the decline in sexual dimorphism was only evident in the high line. For 6-week and 10-week body weight, sexual dimorphism was higher in the L line. Similar patterns were observed in the growth experiment at 10-weeks (70-days) though the trend in sexual dimorphism in the growth experiment can not be directly compared to that from the selection experiment because the former is based on a single generation. It appears that previous selection for males only in the P-lines did not enhance sexual dimorphism in the H line (both in the P and P6 lines) contrary to the initial expectation. The higher sexual dimorphism in the L line could probably be attributed to slow growth of females because fertility in females may depend more strongly on body size than it does in males due to higher physiological costs of reproduction in females. This phenomenon can also explain the increase in sexual dimorphism in the L line between generation 35 and 45.

In the P6-lines (generation 21-50), females of the H line responded relatively more than males as selection advanced. The opposite was observed in the L line, where females grew at a slower rate than males. The intensities of selection (computed as average across the 30 generations) were 1.039 and 0.845 in the H and L lines respectively, and were approximately equal in both sexes. Thus, differential selection intensities can not be attributed as the cause of the observed differences in responses. Several plausible factors may explain the observed pattern of sexual dimorphism. These included (a) selection operating on loci (autosomal) whose effects are 'sex-influenced'. In this regard, the autosomal genes controlling body weight may be expressed differently depending on whether the genes are expressed in males or in females; (b) the effect of sex-linked genes; and, (c) physiological (genetical) differences in terms of differential rates to which the two sexes attain sexual maturity, and the rate to which the two sexes approach a selection limit.

The difference in response between males and females can be statistically expressed in terms of the differences in the estimated genetic parameters and most researchers do not explicitly acknowledge that heritability of a trait may differ between sexes. Response to selection for sexual dimorphism in body weight or body weight gain would generally depend on the difference between heritabilities in the two sexes (Hanrahan and Eisen, 1973) and the genetic correlation between males and females for the same trait. In this study analysis of the L line (P6) indicated that h^2 was higher in males (0.55) than in females (0.46). Likewise, the genetic correlation between males and females in the L line was much lower than in the H line, in the latter being closer to unity. The difference in h^2 and the genetic correlation would explain the observed difference in responses, i.e. males of the L line responded more to selection than females. Similar conclusions were obtained when genetic correlations deviated much from unity (Hanrahan and Eisen,1973; Yamada and Scheinberg,1976; Meagher,1992,1994). For 6-week body weight heritabilities differed between males and females (0.28 vs.0.39), but the genetic correlation was very high (0.97) which explains the non significant trend in sexual dimorphism observed.

However, it is not evident in this study whether the genetic correlation between males and females in the base population (P-lines) for 10-week body weight will be similar to that obtained using 6-week body weight. The results also show a reduction in the genetic correlation between males and females when the genetic correlation obtained in generation 0 (r_g=0.97) using 6-week body weight was compared to the value obtained using 10-week body weight (r_g=0.84, generation 21). From the growth experiment the differences in body weight between males and females showed an increased trend with advance in age particularly after sexual maturity. If we assume that the proportional gain between 6-week and 10-week is the same in both sexes and the genetic correlation between sexual dimorphism in 6-week and in 10-week body weight is high and closer to unity, then we may tentatively conclude that long term selection at fixed age after attaining sexual maturity (from 6-weeks onward) would result in reduction in sexual dimorphism for the upward selection, but an increase may occur in the downward selection because of the antagonism between growth and reproduction.

The difference in response between males and females in this study may also be confounded with that due to sex-linked inheritance (Griffing, 1965,1966; Sheridan *et al.*, 1968). Sex-linked genes are hemizygous in the heterogametic sex, thus increased selection pressure on these alleles may account for asymmetry of response. The consequence of this, is a proportionally larger contribution of the sex-linked genes to response in the females. If such genotypes are present in the base population, it is then expected that selection of males only in the P-lines for 10-week body weight will result in increased in sexual dimorphism. The contrary was observed, for example, the correlated sexual dimorphism measured using 6-week body weight was declining in the H line. This may suggest that the effect of sex-linked genes in females may still be strong enough to allow greater response in females. The presence of sex-linked genes in the lines used in this study was confirmed by Hastings and Veerkamp (1993) and recently by Rance (1996, unpub.). The former reported a large additive sex-linked effect in crosses derived from P6-lines, which accounted for about 25% of the divergence.

Regarding the difference in responses after sexual maturity, we may imagine an optimum body weight which may differ between sex, and males approaching this optimum at a faster rate than females. If body weights in either of the two sexes have not reached the maximum possible limit which can be attained by selection, then repeated cycles of selection will result in a shift in the growth curves, particularly if selection is practiced at a fixed age. Because of the differential rate of advance to these limits, selection will result in convergence of mean body weight of males and females at a fixed age, which is reflected as a decline in sexual dimorphism over time. This hypothesis can be used to explain the declining trend in sexual dimorphism observed in Fig. 6.1. However, to validate this hypothesis, two or more cycles of growth experiments need to be conducted and the change in growth curves at a fixed age evaluated for both sexes.

The growth experiment showed that sexual dimorphism was higher in Hg line before 42 days, but a switch over took place after 56 days. Synenki *et al.* (1972) using divergent selected lines for 6-week body weight, observed that females of small lines reached maturation about 4 days later and at lower body weight compared with large lines. Unfortunately, the onset of sexual maturity in the growth experiment was not monitored. Although possible, the monitoring of this trait is a tedious exercise. However, differential rates of maturity between sexes can contribute to decline in sexual dimorphism measured at a fixed age.

6.5 Conclusions

Males grow faster in early age, but approach their genetic limit for body weight much earlier than females. The long term effect of selection on sexual dimorphism will depend on the relative distance for the two sexes in reaching the maximum (minimum) possible body weight i.e. the genetic limits. In the L line, females would tend to respond relatively less because fertility in females possibly depend more strongly on body size.

Selection after mice have attained sexual maturity will result in shift of asymptotic weight for weight measured at a fixed age. However, the rate and direction to which the asymptotic weight shift will depend on the genetic correlation between the same trait measured in males and females at the same age.

This study was not designed to change sexual dimorphism but rather to quantify and characterize the trends in sexual dimorphism resulting from long term selection. However, the practicability of changing the sex difference by selection would depend on the differences in heritabilities, the genetic correlation between the two sexes, the age at which the changes are desired and the likely economic returns from changing the sex difference.

Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

In describing the variation within population, identification and estimation of all possible sources of variation underlying a given quantitative trait(s) is generally sought. However, this aim is difficult to achieve because of the complexity of the variation and limitations of the analytical procedures. The true biological situations in a polygenic trait with regard to the number of genes and their effects (distributions) are generally not known. In simulation studies, the loci are often assumed to have an equal additive effect and the effect of natural selection, particularly with long term selection, is ignored which at times give problems in merging theory and results from actual experiments. Because of these limitations, a balance between the most desirable ('true') but unknown model and a model which is practical to implement is generally sought. The statistical model often currently used to model biological processes is the 'infinitesimal model' which assumes a large number of unlinked genes each with infinitesimally small additive effects (Bulmer, 1976,1980).

The main theme of this thesis has been the study of the effect of long-term selection on a trait of growth, i.e. body weight in mice, and some of the reasons for this have already been given. Initially the relationships between offspring and parents were examined, and in subsequent chapters the changes in body weight and correlated response in litter size brought about by selection for body weight at 10-weeks for 50 generations were investigated.

Offspring-parent relation. In chapter 3, investigation of linear or non-linear parent relations showed that the offspring-sire regressions were somewhat non-linear, but not the regression of offspring on dams. Based on earlier theoretical studies (Bulmer,1980; Mäki-Tanila,1982; Nishida and Ibe,1974) it was concluded that the non-linear relations could have arisen probably from the skewed distributions of the genotypic component relative to the environmental component. Non-linearity can also be caused by heterogeneity of variance among regression pairs (parent-offspring) and presence of outliers; however, elimination of outliers and standardisation did not affect the observed relations. Most experiments reported in the literature are based on one or a few generations and the sample sizes are generally small. The study reported in this thesis had a relatively large sample size (N = 11814) from which 1099 observations for offspring-parent pairs spanning 20 generations were derived. The large number of data could have contributed to the near normality in the distributions of effects (genotype and environment) hence reducing the size of the non-linearity. However, due to the size of the non-linear coefficients, non-linearity observed will have only a trivial effect on predictions, particularly at low intensity of selection. Under these circumstances heritabilities can be estimated by regression of offspring on mid-parent or simultaneously on both parents provided that the data are standardised and the distribution of the data is normal or nearly normal.

When the relation between offspring and parent(s) performance is very curved, interpretations and predictions of effect of selection derived from such estimates may differ from those obtained by assuming a linear relation (Kempthorne,1960; Robertson, 1977; Gimelfarb and Willis, 1994; Koerhuis,1996). Koerhuis(1996) compared heritability estimates derived from offspring-parent regression, regression of half-sib average on individual, and Restricted Maximum Likelihood (REML) in the presence of non-linearity for eight overlapping generations in broiler-type female lines. He found that the regression of individual values on sib-means for hen housed production (HHP) and hen day percentage (HD%) were very curvilinear, for regression based on untransformed data. Transformation reduced non-linearity substantially, but his results were still complicated by the nature of the traits studied, i.e. egg production traits, which are known to be highly skewed. In chapter 4 we observed that variances changed in the course of experiment. These changes may be interpreted to indicate that regression of offspring on parent is non-linear across generations which imply that heritability is not constant. In many modern likelihood or variance component based method, linearity is a fundamental assumption, and analyses are usual done ignoring this aspect. In methods like REML, ignoring non-linearity could have little consequency in estimation of base population paramters since the information is derived from not only from offspring-parent relation, but from other sources of relatives, e.g. grandsire(dam), sibs etc.

Selection experiment. Results of 50 generations of selection for lean mass and subsequently on 10-week body weight showed that the low line had attained a selection limit after 40 generations whereas the high line continued to respond (Chapter 2). A reduction in mean body weight between generations 30 and 40 observed in the high P6 line was perhaps due to the reduction in selection differentials. Increased level of disease could have caused the reduction in selection differentials, although this effect was not evident in the low line. The P-lines were not replicated and no control line was maintained after the replicate lines were crossed. The disease situation and absence of replication and/or a control line have some implications on the parameter estimates obtained in chapters 4,5 and 6 as well as on the interpretations of the results. A similar situation may occur in the field where unpredictable changes in environment could occur in the breeding herd despite attempt to standardised the environment i.e. management, within and between herds used in animal breeding evaluations. In short or long term experiments, replication is emphasised for statistical reasons e.g. in estimating sampling error of estimate since genetic drift play a significant role. A statistical analysis comparing response of two lines at some generation uses only the within line variability as the source of error and inferences are being drawn about these two lines and not the population from which they were sampled (Hill, 1980).

The estimates of heritabilities obtained using a univariate model (chapter 4) or a bivariate model (chapter 6) for body weight were within the range reported for body weight in other studies. However, analysis of high and low lines individually without a control line is an inefficient procedure. An estimate of the actual responses, or the difference in response as a proportion of the total does require elimination of the environmental trend (Hill, 1980). However, the use of control line(s) requires extra facilities. In the absence of a control line, this bias could be reduced by divergent selection, with one line selected for high value of the trait with another contemporaneous line selected for low values. In the lines used in this study, the initial assumption that the variances between lines were homogeneous, the analyses furnishes combined estimates for the different parameters. This required elimination of line effect, by including line as an additional fixed factor in the model, since the lines did not have a common base (i.e. at generation 21). In the analysis it was assumed that responses were symmetrical, which may be a reasonable assumption in the short term, but not in the long term.

Using REML, it was possible to partition and estimate the variance components and genetic parameters for the high and low line at the same time accounting for environmental changes by taking the divergence between the two lines. At generation 21 the two lines had already diverged by 13.3g, where the base parameters were inferred and information prior to generation 21 was not included in the analyses. In this regard the two lines could be regarded as two distinct lines (no common base) though results from the model fitting heterogeneous additive and/or common environmental variances did not differ from that assuming the variances to be homogeneous. However, in practise, the breeder might only be interested in unidirectional response e.g. improving body weight at a given age (upward selection). Moreover, bi-directional selection can give useful information regarding the genetic of the trait, or correlated response in other traits e.g. body weight and fatness. The method of partitioning fixed and random effects has implications in practical animal breeding when the genetic and/or environmental variances between herds/flocks may be heterogeneous. However, in the presence of a scale effect, for example in the analysis of high and low lines together as exemplified in this study require that the data be corrected for this effect, e.g. by logarithmic transformation. Then the procedures described in section 4.2.1 (chapter 4) to partition the variances can be applied in subsequent REML analyses. Ignoring heterogeneity may lead to the tendency of selecting animals from the more variable groups, especially if selection is intense (Hill, 1984).

In predicting selection response and interpreting selection experiments involving quantitative traits, it is generally assumed that the direct additive variance is the only genetic parameter of importance. In this study it was observed that sexual dimorphism generally declined with increasing number of generations and was higher in the low line (Chapter 6). Further, the difference between males and females with regard to body weight appears at about the time of sexual maturity. Consequently, the decline in sexual dimorphism resulted from a shift in the growth curve at fixed age due to relatively higher response to selection in females than in males after sexual maturity. The higher sexual dimorphism in the low line was presumably due to relatively higher physiological cost of reproduction in the females of the low line than those of high line. Consequently low line females will respond proportionally less to selection, despite the fact that small mice also produce small litters.

Because the additive genetic variances and/or heritabilities differed between males and females (Table 6.3) and the genetic correlation was less than unity (r_g=0.84) including sexual dimorphism in the prediction models may give an additional response above that predicted by averaging the sexes. In this regard the quantitative trait in males and females can be treated as two separate traits and justify a bivariate analysis. In such an analysis the genetic correlation between the two sexes is accounted for rather than assuming that it is unity. Further, it appears from the bivariate analyses of 6-week and 10-week body weight that the size of the genetic correlation is more important in determining the response in sexual dimorphism than the differences in the heritabilities. However, in this discussion a distinction between increasing the accuracy of predictions and changing sexual dimorphism should be made. If the latter is desired the practicability will depend on the species, the trait, the age at which the changes are desired and whether the changes will have any additional economic advantage in the long run.

The heritabilities obtained by offspring-dam regressions were biased upward by the maternal environmental covariance (Table 3.1). Using REML, maternal effects can be disentangled by partitioning into direct additive maternal (for all animals) and environmental maternal effects (only for dams of progeny with records). A significant maternal genetic effect was detected for 10-week body weight (Vm/Vp = 5%), the effect contributing about 13% of the total additive variance. Nevertheless, the assumption that the maternal effect is homogeneous in the two lines may not be correct. From the individual line analysis, maternal heritabilities differed in the two lines, i.e. estimates of maternal heritability (h^2_m) were 0.04±0.03 and 0.09±0.04 in the high and low line respectively. It is also expected that maternal environmental effects contribute significantly to the observed phenotype and its value may differ within families because litter size was not standardised. Similarly, maternal environment may differ between the high and low line mice because of the differences in body weights and the size of litter produced. However, direct estimates of heritabilities and maternal heritabilities from individual lines without control may be biased for the reason explained earlier. Though maternal effects were found to be significant for 10-week body weight, there was no direct evidence to suggest that the effects would contribute to non-linearity of offspring-parent regression. This conclusion is derived from the absence of non-linear relations between offspring and dams (individual or simultaneously on both parents). Likewise, the trends in sexual dimorphism did not suggest maternal contributions since significant sexual dimorphism was observed after sexual maturity, the period when maternal effects are expected to be diminishing.

Trends in variance components. The expectation for this study from the analyses of blocks of generations separately was that the genetic parameters would be similar to those in the base population (generation 21 of the P6-lines) if infinitesimal assumptions hold. There was no evidence from this study that selection has caused a decrease in the genetic variance of 10-week body weight in the P6-lines. With selection and inbreeding coefficients rising to more than 25% for both lines (estimated from generations 21-50), some decrease in additive genetic variance and possibly heritability might have been expected. The divergence between selected lines and relaxed lines derived from generation 40 of the P6-lines suggests that there are still an appreciable amount of genetic variance present in the population. The results of chapter 4 contravene the assumption of the infinitesimal model of constant variance, because the additive variance appears to have increased in both lines despite selection for 50 generations. However, estimates of variance components from separate blocks assumed that the error variance (Ve) was homogeneous in the two lines. The assumption that Ve's are similar between the high and low line within each block might not be true given the large difference in phenotypic variances between the two lines (Fig. 2.4).

Given the limitation of the model (i.e. the residual variance fitted to have the same value in each line) it was concluded that an increase in the additive variance from analyses of generation blocks in the high line was presumably confounded with an increase in phenotypic variance (environmental and additive variance components) associated with stress between generation 30 and 40. In the low line an increase in the additive variance was attributed to possibly disruptive selection tending to favouring heterozygotes. This hypothesis is supported by an increase in body weight as well as litter size after generation 40. It is difficult to ascertain whether there is any potential contribution from mutation and the data do not give any evidence to suggest so. However, Frankham (1979) has shown that mutation may be playing a role in long-term selection, and the figure widely cited expressed as Vmu/Ve is 10^{-3} (Lynch, 1988). The critical point is that the favourable mutant genes should have an effect in the heterozygotes, and if they do not, they contribute little to response (Hill, 1982) and the overall effect will depend on the number and distribution of effects of mutants (Hill and Rasbash, 1986).

Comparisons of the additive genetic variance (Va) and heritability (h^2) estimates inferred to generation 21 (in this study) and those reported by Beniwal(1991) fitting heterogeneous additive and common environment variances are given in Table 7.1. Beniwal's estimates show that both Va and h^2 decreased in both lines, whereas comparison of the estimates from this study (generation 21-50) and those of generation 0 show a decline in Va and h^2 in the high line, but only in h^2 in the low line. No standard errors for the Va's were given in either analyses so no strict conclusion can be made regarding these comparisons. It was expected that the additive variances in generation 21 should be smaller relative to base values, however and to the contrary the estimates of additive variances were higher. The higher values possibly being contributed by the effect of crossing of the replicate lines in generation 20. Of course some bias is also expected because the estimates inferred in generation 21 did not account for selection and the reduction in additive genetic variance due to inbreeding prior to generation 21.

Table 7.1: Estimate of additive variances and heritabilities in the P and P6-lines based on log transformed 10-week body weight

		Line		
Generations		high	low	
	Va	0.577	0.439	Beniwal (1991)
0-20[a]	h^2	0.50	0.47	
0-38[b]	Va	0.437	0.383	
	h^2	0.38	0.41	
	Va	0.462	0.501	This study
21-50[c]	h^2	0.39	0.43	

a P-lines, *b* P+P6 lines, *c* P6 lines (inferences to generation 21). Va= additive variance. h^2 =heritability.

Correlated response. The results given in chapter 6 show that selection for lean mass and later for 10-week body weight resulted in a positive correlated response in litter size at birth in the first 30 generations of the high line, and 40 generations of the low line (P+P6). The total divergence was 9 pups at generation 30 but declined to 5 pups if all generations were considered. The positive response agrees with results of Nagai *et al.* (1978), Eisen (1978) and de la Fuente and San Primitivo (1985). In the P6-lines a positive divergence was significant only in the first 10 generations, and the means remained constant in the remaining 20 generations. The poor response in the high line suggested a poor maternal environment arising from the decline in fitness during the period when the mice were experiencing an unfavourable environment. However, the low line also showed a rapid decline in litter size the trend (both up or both down) suggesting fluctuation in environment. In the low line it appears that the line has attained a selection limit for body weight and litter size was subjected to inbreeding depression, resulting from reduction in fitness. However, a small positive response after generation 40 for both traits suggests that there are still some genes segregating with pleiotropic effects for litter size and body weight in the population.

The estimates of heritabilities for litter size and genetic correlations between litter size and body weight from bivariate analyses were quite asymmetrical, i.e. 0.07,0.28 for the h^2's and -0.22,0.48 for the r_g's in the high and low line respectively. Estimates from combined (H+L) lines analyses was 0.21 and -0.08 for h^2 and r_g respectively. The estimates derived from combining both lines are unbiased because line effect was included in the model. The low value of genetic correlation reflects inadequacy of information for estimating the covariances since all the information comes from the within line covariance, as the response from the divergence was essential zero. Litter size as a correlated trait is subject to environmental differences e.g. maternal, which ought to differ in the high and low line. A common litter effect was not fitted in the litter size sub-model because only one observation was available for dam's litter size, hence the estimates of the genetic parameters for litter size are confounded with some maternal environmental effects. Considering that the estimated r_g values differed in magnitude as well as the sign, the combined estimate of r_g becomes less meaningful in practical terms.

At the centre of the argument for not including litter size as a selection objective has been the low heritability of litter size and the fact that it is expressed only in females. However, and to the contrary, the result of this study shows that despite the fact that improvement of litter size was not among the selection objectives a positive response can still be achieved in long term selection programs due to its moderately high heritability and coefficient of variation (cv = 29%). Inconsistencies in the results of selecting for litter size directly or indirectly or estimation of heritability and genetic correlation between litter size and growth traits are common in the literature. Such inconsistency lies in the fact that litter size is a component of fitness and depends on fertility and maternal performance (see, Falconer,1989 pp. 337). In long-term selection the interpretation of the correlated response or parameter estimates will then depend on how and to what extent the various components of fitness are affected.

Implications

(a) The analyses of selected lines have shown that the infinitesimal model assumption did not hold. Hence, the genetic models currently used for prediction of responses to selection, particularly in long-term selection may need more realistic assumptions with respect to changes in gene frequencies, gene number and gene action affecting quantitative characters. Mixed inheritance models combining genotypes and marker frequencies or models allowing for non-additive gene interactions could provide additional information regarding the changes of variances.

(b) Pigs and poultry have been selected continuously but not exclusively for body weight over many generations. However, results from long-term selection and prediction of response may need continuous evaluation because of changes in genetic parameters due to selection or to unrelated animals being introduced from outside the breeding herd.

(c)Experiments with a single replicate may not allow accurate calculation of the sampling variance of the selected or correlated traits, much more important in long term selection experiments. Therefore, it is worthwhile to maintain at least a few replicates per line. Control lines could also be useful in unidirectional selection experiment to disentangle response from environmental trends.

(d)In the bivariate analyses of litter size and body weight most of the information were obtained from body weight data, and in females only the selected dams contributed to the body weight-litter size covariances. This could have resulted in problems reducing the accuracy of estimated parameters. In practical situations, if the aim is to improve both body weight and litter size, the amount of information can be increased by including as much family information as possible. In this respect, records of litter size should be taken from all female candidates (selected and unselected) so as to have full family information. This might be possible by breeding unselected females in a commercial herd, however, there is always a cost aspect to be considered. Moreover, the net contribution will depend on whether the importance of litter size is evaluated in specialised lines or in general purpose lines.

(e)The results of long term selection for litter size directly or indirectly often are limited by reduction in fitness. However, in pig breeding industries crossbreeding of European pigs and prolific lines (e.g. Meishan) can improve litter size substantially at the same time maintaining an acceptable degree of leanness.

Chapter 8

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