GENETIC ANALYSIS OF LONG-TERM SELECTION EXPERIMENTS

BY

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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.

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ABSTRACT

A long-term selection experiment with mice for 38 generations was analysed. Genetic parameters were estimated for lean mass, body weight, litter size and other associated traits using univariate and multivariate REML analyses with an animal model fitting litters as an additional random effect. Different combinations of selected and control lines were analysed. The change in genetic parameters during the course of selection and infinitesimal model assumptions were examined.

Three replicates, each having high and low selected lines for lean mass at 10 weeks of age were maintained for 20 generations with unselected controls (P-Lines). This resulted in a divergence of 7 phenotypic standard deviation units (σ_p) between the high and low lean mass lines. After 20 generations replicates were crossed and the selection criterion was changed to 10 week body weight (P6-Lines) without maintaining the controls. At generation 38 the selected lines diverged by 23.2g (8.7 σ_p) for 10 week body weight. The estimates of heritability (h^2) and c^2 (common litter variance/ phenotypic variance ratio) for lean mass were 0.5 and 0.2 from the univariate REML analyses of both control lines alone and of pooled data of control+high+low lines. Estimates of c^2 were higher in the high lines and lower in the low lines, controls being intermediate. The estimates of genetic parameters for body weight were similar to the lean mass in the P-lines. Analysis of the selected lines indicated a steady decline in their additive genetic variance (Va) during the course

of selection for both lean mass and body weight, even though allowance was made in the infinitesimal model for reductions in Va due to inbreeding and linkage disequilibrium.

The multivariate REML estimates of h^2 and c^2 for lean mass and body weight were similar to those from the univariate analyses. The genetic (r_g) and phenotypic correlations (r_p) between lean mass and body weight were very high (>0.9) and positive. Lean mass also showed positive correlations (r_g and r_p) with gonadal fat pad weight, but when gonadal fat pad weight was expressed as a proportion of body weight it showed small negative correlations with lean mass.

Litter size showed a consistent positive correlated response to selection for lean mass and body weight except in the later generations of high P6-lines when there was an increase in the percentage of dead born. Estimates of h^2 for litter size were 0.18 and 0.17 from the control and pooled data of the P-lines, respectively. Estimates of h^2 for lean mass and litter size were lower from a bivariate analysis than from univariate analyses. The genetic correlation between lean mass and litter size was estimated to be 0.27.

The REML analyses of selection experiment data with complete pedigrees accounts for the change in Va due to inbreeding and linkage disequilibrium between unlinked loci. Therefore, under the infinitesimal model assumptions, the estimates of Va are expected not to change due to selection. The results showed a

decline in the estimates of Va in selected lines during the course of selection, suggesting that the infinitesimal model assumptions did not hold well for lean mass and body weight in mice. This may perhaps be due to the presence of some genes with large effect (major genes).

CHAPTER 1

GENERAL INTRODUCTION

Present day market trends indicate an increasing consumer demand for lean meat free of excessive fat, which is thought to cause undesirable health effects, due to saturated fatty acids (WHO, 1990). In addition, removal of excessive fat from carcasses increases the labour costs to the processor. Obese animals also have a lower biological efficiency because of relative inefficiency of fat growth compared to lean tissue growth (Webster,1977). These factors have led animal breeders to emphasize selection for faster lean tissue growth and reduce fat deposition in livestock.

Knowledge of the genetic control and inter-relationships of economic traits related to lean growth and their relationships with other traits, for example reproduction, is necessary for effective selection and genetic improvement of livestock. The use of livestock for experiments to obtain such knowledge is expensive, laborious and time consuming. Laboratory animals, especially mice, provide a very good alternative model for studying the genetic parameters for various reasons: firstly, they have a very short generation interval; secondly, they are less expensive and easy to handle; thirdly, they reach maturity very quickly; and, fourthly, their physiology is similar to commercially important livestock species (Prosser,1973).

Traditionally, heritability estimates in the base population from selection experiments have been obtained by the regression of response on the cumulated selection differential, to give an estimate of realized heritability (Falconer, 1953 and 1973; Sharp

et al., 1984; Eisen, 1987a; Hastings, 1989). A further review of the literature for different traits is given in the introduction of the respective chapters (3,4 and 6).

The realized heritabilty estimates are primarily the description of response and in some circumstances they may not be a valid estimate of heritabilty in the base population (Falconer,1989):

(i) The response of characters with high heritability is expected to reduce due to decline in the additive genetic variance as a consequence of selection; this may result in an underestimate of heritability in the base population.

(ii) The changes due to environmental trends or inbreeding depression are included in the response, although these may be corrected by the use of control lines.

(iii) Selection in two directions may give different realized heritability estimates, which are valid descriptions of responses but both can not be a valid estimate of heritability in the base population.

(iv) It ignores the information available from genetic covariances among the members of a family or group of relatives, e.g. half-sibs, full-sibs etc., within generations.

Among the alternative methods to analyse selection experiments, analysis of variance has also been used to partition variance into within and between litter variance. The data are assumed to be a random sample, which is not the case in selection experiments. It accounts for some degree of relationships, i.e.

covariance between full-sibs, but it does not take into account all the relationships between animals.

Until recently, estimation procedures for variance and covariance components used Handerson's (1953) methods for mixed models and unbalanced data. The method III of "fitting constants" has found extensive use in animal breeding and is supported by the computer program of Harvey (1977), which was designed to estimate genetic parameters.

Maximum likelihood (ML) methods are becoming the method of choice and preferred over ANOVA based methods for the analysis of selection experiment results. This is particularly so for animal breeding data which are usually not a random sample, as the animals are selected for a specific objective, and have a high degree of unbalance. The ML analysis assumes a distribution for the data, usually normal. Maximum likelihood estimation involves, by definition, locating a maximum of the likelihood for a given set of data, model of analysis and parameters to be estimated. The ML estimates are consistent and efficient (Harville, 1977), that is all the available information is utilized in an optimal way. A major drawback of ML estimation is that the fixed effects are treated as if known, i.e. the loss in degrees of freedom due to fitting fixed effects is ignored. If the model comprises many fixed effects, as is invariably the case with animal breeding data, this can give biased estimates; for example the residual variance would be underestimated.

Patterson and Thompson (1971) suggested a modified ML procedure, known as Restricted Maximum Likelihood Method (REML), which overcomes the problem of loss in degrees of freedom due to fitting fixed effects, by maximizing only the part of likelihood which is independent of the fixed effects. This gives REML an advantage over the ML as it takes account of the loss of degrees of freedom used for the estimation of fixed effects and estimates of variance components are unbiased by the number of fixed effects. Because of these desirable properties, REML has now become an accepted method for the estimation of genetic parameters or variance components in the field of animal breeding research.

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 a very large number of loci governing the trait, each with a very small effect, i.e. the infinitesimal model (Hill and Meyer, 1988; Sorensen and Kennedy, 1984 and Kennedy 1990). The genetic variance of any trait under artificial selection could change due to: (a) a change in gene frequency. If however, the trait is determined by a very large number of loci, the change in gene frequency due to selection is so small that it does not contribute to change in the additive genetic variance (Bulmer, 1971). (b) correlation of frequencies between pairs of loci induced by selection (Bulmer effect, Bulmer, 1971), known as "linkage disequilibrium", (c) inbreeding, and (d) environmental factors.

Under the infinitesimal model, a decline due to linkage disequilibrium among unlinked loci and inbreeding in any selection experiment is predictable and can be accounted for when the pedigrees are known (Bulmer, 1980). Therefore, expectations of estimates of genetic variance do not change due to selection for any number of generations, provided the decline due to linkage disequilibrium and inbreeding are properly accounted for and the trait has been determined by a large number of loci. The long-term selection experiments with mice could provide an opportunity to verify the assumptions of infinitesimal model underlying the animal breeding analyses.

The ever increasing computational power allows use of the REML approach with even more complex mixed models, particularly animal models. This has further been helped by the 'DFREML' computing package devloped by Meyer (1988). In the animal model, each animal's breeding value is included in the model. The relationship among all the animals can also be included in these analyses in the form of the numerator relationship matrix, which is a matrix of the additive genetic relationships among all the individuals. Further, this gives an opportunity to include the animals without records, which provide information about the relationships. Sorensen and Kennedy (1984) have shown that including the complete pedigrees back to the base population, there is no bias due to selection and drift in the estimates of genetic parameters. There are a few reports on the use of REML with an animal model to analyse long-term selction experiments, especially with mice. Cue and Hayes (1990) analysed results from 15 generations of a mice

experiment on selection for 28 to 38 days and 48 to 58 days weight gain under different feeding regimes. Meyer and Hill (1991) analysed data from 23 generations of mice selected for increased and reduced appetite by REML fitting an animal model.

The present study was undertaken to investigate a long-term selection experiment with mice spanning over 38 generations of selection for lean mass at 10 weeks of age, using recently developed statistical methods for analysing selection experiment data. The genetic parameters are estimated using the Restricted Maximum Likelihood method with an individual animal model. All the estimates therefore refer back to the base population as the complete pedigrees were fitted in each analysis. Therefore, the selected lines of mice which originate from the same base population when analysed with an animal model should give similar estimates, as this method accounts for the change in variance components due to selection and inbreeding. However, the infinitesimal model (Bulmer, 1980) of many unlinked loci, each having a small effect, is assumed to govern each trait.

The objectives of this investigation are:

(a) to estimate the genetic parameters for lean growth and other related traits from the selected lines of mice by univariate REML analyses fitting an animal model,

(b) to assess the change in the genetic parameters during the course of selection,

(c) to estimate correlations of lean growth with other associated traits using multivariate Restricted Maximum Likelihood, and

(d) to investigate the effect of selection for lean growth on the litter size of mice.

CHAPTER 2

MATERIAL AND METHODS

The data for this study were taken from a long-term selection experiment with mice being undertaken at the Institute of Cell, Animal and Population Biology (formerly Institute of Animal Genetics), University of Edinburgh. The main objectives of this selection experiment are: (a) to alter the body composition of mice, and (b) to produce distinct lines of mice for lean growth, fatness and food intake. This study is limited to the selected lines for lean growth from the start of experiment (1980) to 1989.

2.1 DEVELOPMENT OF LINES

The "G" strain of mice was developed by crossing two inbred lines, JU and CBA, and the F_1 crossed to an outbred strain, CFLP. One generation of random mating followed the second cross, the next was designated as generation zero (base population) of the selection experiment.

The lines selected for lean growth were named as P-Lines. Sixteen full-sib families were established for each of the three replicates and, from each replicate, three selection treatments, high, low and control were taken at generation zero giving nine lines in total. Subsequently each line consisted of 16 pair-matings per generation up to generation 7, and from generation 8 to 20 they consisted of 8 pair-matings. The litter size was adjusted to between 6 and 12 pups at birth by culling and crossfostering (only upto generation 20), and mice were weaned at 21 days of age. Further details on these aspects are given by Sharp (1983) and Sharp *et al.* (1984).

At generation 20, three replicates within each selected line were crossed to form a new replicate (Replicate 6 or 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 three replicates. The P6-lines were developed by mating 16 pairs per generation in each direction for 17 generations. The P6-Lines were not replicated and control lines were discontinued.

Throughout the remaining text, the terms used for lines and replicates are:

(a) for P-lines (0 - 20 generations):

Replicate 1, 2 or 3 = control + high + low lines in replicate 1, 2 or 3. Control lines = control line of replicate 1, 2 and 3. High lines = high line of replicate 1, 2 and 3, and similarly low lines = low line of replicate 1, 2 and 3.

(b) for P6-lines (21 - 38 generations):

Replicate 6 = high P6 line + low P6 line.

2.2 SELECTION PROCEDURES

In the lean growth lines (P-Lines), body weight and gonadal fat pad weight were measured in the ten week old males upto generation 20. Within-family retrospective selection of males was practised. For each replicate in generation zero, four males from each of sixteen litters were pair mated to four females from another litter. After about two weeks, when males were ten weeks

old, they were killed and weighed. The gonadal fat pads were dissected out and weighed.

The body weight and gonadal fat pad weight were combined in a simple phenotypic index to predict the lean mass at 10 weeks of age: Lean Mass = Body Weight - 8 x Gonadal fat pad weight. This phenotypic index predicts fat free mass, as gonadal fat pad weight represents about one-eighth of the total fat in the mice of the base population. The offspring of males with the highest lean mass formed generation 1 of the high line, and the offspring of males with the lowest value of the lean mass formed generation 1 of the low line. The offspring of one of the remaining two males from each litter formed generation 1 of the control line.

In subsequent generations, up to 20, four males were mated from each litter and subsequently dissected at ten weeks of age in the high and low lines. In the control lines two males were mated from each family, chosen at random, so two males were dissected at ten weeks of age.

After generation 20, the selection criterion in P6 Lines was changed from an index on males to body weight in both sexes at ten weeks of age and mated at twelve weeks of age. The body weight and predicted lean mass are highly correlated (r=0.94, Sharp *et al.*,1984).

2.3 DATA STRUCTURE

Throughout the experiment, litter size, six week weight, ten week weight and gonadal fat pad weight were recorded in the lean growth lines. The measurement of gonadal fat pad weight was discontinued after generation 20. Three following traits are being considered in this investigation:

(a) Estimated Lean Mass (subsequently refered as Lean mass). The observations for this index trait were available for the males from base to 20 generations of P-Lines.

(b) Body Weight. The observations on body weight at ten weeks of age were available on the males of P-Lines and on both sexes of P6-Lines.

(c) Litter Size. The primiparous litter size at birth was considered as a trait of the dam.

Four and two observations per family were recorded in each of nine, selected and control P-lines, respectively for lean mass and body weight. The number of observations per family were inconsistent in P6-Lines. Usually one observation per family was available for litter size in all lines but in the cases of infertility or non-availabilty of females in some litters, more than one observation per family was present.

The actual number of records are less than expected from the above structure, presumably because of health and infertility problems. The detailed numbers of observations, animals and litters in different lines, replicates and their combinations used

Table 2.1: Data Structures used in the analyses of Lean Mass and Body Weight at 10 weeks of age.

Line / Replicate	Generations	Number of Records	Animals	Litters
P - LINES:				
Replicate 1 (C+H+L)	0 - 7	1081	1482	336
Replicate 2 (C+H+L)	0 - 7	1043	1437	329
Replicate 3 (C+H+L)	0 - 7	1024	1398	315
Overall (Rep 1+2+3)	0 - 7	3148	4317	980
High (Rep 1+2+3)	0 - 7	1370	1826	363
Control (Rep 1+2+3)	0 - 7	824	1271	347
Low (Rep 1+2+3)	0 - 7	1334	1792	366
Replicate 1 (C+H+L)	0 - 20	1964	2652	635
Replicate 2 (C+H+L)	0 - 20	1925	2597	624
Replicate 3 (C+H+L)	0 - 20	1903	2545	599
Overall (Rep 1+2+3)	0 - 20	5792	7794	1858
High (Rep 1+2+3)	0 - 20	2418	3156	664
Control (Rep 1+2+3)	0 - 20	1417	2134	622
Low (Rep 1+2+3)	0 - 20	2337	3076	668
Control + High	0 - 20	3645	5004	1239
Control + Low	0 - 20	3564	4924	1242
P6 - LINES:				
High	21 - 38	1808	3205	237
Low	21 - 38	1576	2912	247
High + Low	21 - 38	3384	6029	484
P + P6 LINES:				
High + Low	0 - 29	6320	7738	1527
High + Low	0 - 38	7949	9380	1768

1. C = Control, H = High, L = Low lines, Rep = Replicate
2. Generation 0 represent the base generation.
3. The numbers in all the replicates and lines include base animals/records.

Table 2.2: Data Structures used in the analyses of Litter Size at birth.

Line / Replicate	Generations	Number of Records	Number of Animals
P - LINES:			
Replicate 1 (C+H+L)	0 - 19	619	1300
Replicate 2 (C+H+L)	0 - 19	608	1273
Replicate 3 (C+H+L)	0 - 19	583	1210
Overall (Rep 1+2+3)	0 - 19	1810	3783
High (Rep 1+2+3)	0 - 19	616	1349
Control (Rep 1+2+3)	0 - 19	574	1277
Low (Rep 1+2+3)	0 - 19	620	1341
Control + High	0 - 19	1190	2534
Control + Low	0 - 19	1194	2526
High + Low	0 - 19	1236	2602
P + P6 LINES:			
High	0 - 37	898	1920
Low	0 - 37	910	1910
High + Low	0 - 37	1720	3559

1. C = Control, H = High, L = Low lines, Rep = Replicate
2. Generation 0 represent the base generation.
3. The numbers in all the replicates and lines include base animals/records. The control, high and low lines in each replicate have common base parents.

in the analyses are given in Table 2.1 for lean mass and body weight, and Table 2.2 for litter size. The number of animals indicate the actual number of animals in the pedigrees, including those without records but which appear as relatives.

2.4 STATISTICAL PROCEDURES

2.4.1 UNIVARIATE ANALYSIS

Univariate analysis was carried out for lean mass, body weight and litter size by Restricted Maximum Likelihood (Patterson and Thompson, 1971), using an individual animal model. The estimates were obtained by using the derivative-free REML package developed by Meyer (1988). The model of analysis included additive genetic effects of all animals as a random effect and incorporated information on genetic relationships between all animals including those without records. The common environment effect due to litters was fitted as an additional random effect. In the case of litter size, the common environmental effect was not fitted because usually only one female from each litter was used for mating. The models were as follows:

Model 1: For lean mass and body weight.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e} \quad \ldots . \quad (1)$$

Model 2: For litter size.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

Assumptions considered in these models are: $V(\mathbf{a}) = \mathbf{A}\sigma_a^2$, $V(\mathbf{c}) = \mathbf{I}\sigma_c^2$, $V(\mathbf{e}) = \mathbf{I}\sigma_e^2$, and $Cov(\mathbf{a},\mathbf{c}) = Cov(\mathbf{a},\mathbf{e}) = Cov(\mathbf{c},\mathbf{e}) = 0$

Where,

$\mathbf{y}$ = Vector of observations,

$\mathbf{b}$ = Vector of fixed effects,

$\mathbf{a}$ = Vector of random effects,

i.e. additive genetic values of individuals,

$\mathbf{c}$ = Vector of additional random effects (litters),

$\mathbf{e}$ = Vector of random environmental effects,

$\mathbf{X}$, $\mathbf{Z}$ and $\mathbf{W}$ = Incidence matrices for fixed and random effects,

$\mathbf{A}$ = Additive genetic relationship matrix among additive genetic values of individuals,

$\mathbf{I}$ = Identity matrix,

σ_a^2 = Additive genetic variance,

σ_c^2 = Common environment or litter variance, and

σ_e^2 = Error variance.

The mixed model equations for model 1 are :

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} & \mathbf{X'W} \\ \mathbf{Z'X} & \mathbf{Z'Z} + \mathbf{A}^{-1}\lambda & \mathbf{Z'W} \\ \mathbf{W'X} & \mathbf{W'Z} & \mathbf{W'W} + \mathbf{I}\gamma \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \\ \hat{\mathbf{c}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'Y} \\ \mathbf{Z'Y} \\ \mathbf{W'Y} \end{bmatrix}$$

Where

$$\lambda = \frac{\sigma_e^2}{\sigma_a^2} \quad \text{and} \quad \gamma = \frac{\sigma_e^2}{\sigma_c^2}$$

or $\lambda = \dfrac{1 - h^2 - c^2}{h^2}$ and $\gamma = \dfrac{1 - h^2 - c^2}{c^2}$, and

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2} \quad \text{and} \quad c^2 = \frac{\sigma_c^2}{\sigma_p^2} .$$

Where, h^2 represents the heritability of the trait, and c^2 is the ratio of common litter environment variance to phenotypic variance and this term is called "c - square" in the rest of the text. Similarly the mixed model equations can be written for model 2 by omitting the last row and column in the above equations.

Fixed effects considered were generations, replicates and litter size at birth as a covariable in the analyses of lean mass and body weight, whereas only generations and replicates were included in the analysis of litter size.

The data for P-lines were analysed for each replicate, line, and different combinations of lines, *viz.* control+high, control+low and high+low. The replicates were analysed with only generation as a fixed effect whereas for the analysis of lines, generations and replicates were considered as fixed effects, though litter size as a covariable was considered in every analysis. Lines were not fitted in the analysis of replicates because the three lines originated from the same base population and were connected through the common parents. Similar analyses were also carried out for P6-lines data and P + P6 data.

All the analyses were carried out by including complete pedigrees back to the base population. The pedigree files included all the parents with and without records, thus information about all known relationships was incorporated in the

analysis.

The iterations were stopped when the variance of the function values(-2 Log Likelihood) in an iteration was less than 10^{-6}. The standard errors of the estimates were obtained by a quadratic approximation to the log likelihood using a number of fixed point estimates of the parameters around their maximum likelihood estimates.

Natural log transformed data were also analysed to minimise the scale effects.

2.4.1.1 HETEROGENEITY OF ADDITIVE GENETIC VARIANCE

After the preliminary analysis, it was observed that the additive genetic variance (σ_a^2) differed between the lines *e.g.* high and low lines. When analysing control+high or control+low, the assumption of V(a) = $\mathbf{A}\sigma_a^2$ does not hold because the two lines differ in their σ_a^2 components. Visscher and Thompson (1990) have suggested partitioning the animals in a Numerator Relationship Matrix (**A**) into two groups if they have different variances e.g. animals from different groups being analysed together. The Relationship Matrix can be written as **A** = **TDT'**, the product of lower triangular matrix **T**, diagonal matrix **D** and **T'** as given by Thompson (1977).

Hence,

$$V(a) = \mathbf{A}\,\sigma_a^2 \quad \ldots\ldots (2)$$

$$V(a) = \mathbf{T}\,\mathbf{D}\,\mathbf{T'}\,\sigma_a^2 \quad \ldots\ldots (3)$$

T is a matrix that describes the transfer of genes from one generation to the next and the diagonal matrix **D** represent the variance -covariance matrix of the mendelian sampling terms (e_i) such that $\mathbf{D}\,\sigma^2_{\tilde{a}} = V(e_i)$. The diagonal matrix **D** can be partitioned into two parts, e.g. control and high, or control and low lines, each having different variances. Now the equation (3) can be rewritten as:

$$V(a) = \mathbf{T} \begin{bmatrix} \mathbf{D}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{D}_2 \end{bmatrix} \mathbf{T}'\, \sigma^2_{\tilde{a}} \qquad \ldots (4)$$

$$= \mathbf{T} \begin{bmatrix} \mathbf{D}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{bmatrix} \mathbf{T}'\, \sigma^2_{\tilde{a}1} + \mathbf{T} \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{D}_2 \end{bmatrix} \mathbf{T}'\, \sigma^2_{\tilde{a}2} \quad \ldots (5)$$

$$= \mathbf{A}_1\, \sigma^2_{\tilde{a}1} + \mathbf{A}_2\, \sigma^2_{\tilde{a}2} \qquad \ldots (6)$$

Where $\sigma^2_{\tilde{a}1}$ and $\sigma^2_{\tilde{a}2}$ are additive genetic variances in group 1 and 2. Now in the mixed model equations, the part $\mathbf{Z'Z} + \mathbf{A}^{-1}\,\lambda$ can be rewritten as:

$$= \mathbf{Z'Z} + \mathbf{A}_1^{-1}\,\lambda_1 + \mathbf{A}_2^{-1}\,\lambda_2 \qquad \ldots (7)$$

Where

$$\lambda_1 = \frac{\sigma^2_e}{\sigma^2_{\tilde{a}1}} \qquad \text{and} \qquad \lambda_2 = \frac{\sigma^2_e}{\sigma^2_{\tilde{a}2}}$$

An algorithm similar to Graser *et al.* (1987) was used to calculate the log likelihoods based on the mixed model equation given in equation 7. The log likelihood was maximised using a 'simplex' routine embeded into Meyer's DFREML package. As the selected and control lines originated from the same base population, they were connected by the common parents in the base

population. Hence, this approach (equation 7) was used to analyse the selected lines with controls to estimate additive genetic variance in each line when they were analysed together.

2.4.1.2 HETEROGENEITY OF COMMON LITTER ENVIRONMENT VARIANCE

The selected and control lines not only differed in σ_a^2 but also in the common litter environment variance (σ_c^2) component. A similar approach as given in section 2.4.1.1 was followed to account for the heterogeneous common litter environment variance.

$$V(c) = I\,\sigma_c^2$$

$$= \begin{bmatrix} D_3 & 0 \\ 0 & 0 \end{bmatrix} \sigma_{c1}^2 + \begin{bmatrix} 0 & 0 \\ 0 & D_4 \end{bmatrix} \sigma_{c2}^2 \quad \ldots \quad (8)$$

$$= I_1\,\sigma_{c1}^2 + I_2\,\sigma_{c2}^2$$

Where I_1 and I_2 are not identity matrices but with appropriate ones and zeros on the diagonals for the two groups. In the mixed model equations, $W'W + I\,\gamma$ can be rewritten as :

$$= W'W + I_1\gamma_1 + I_2\,\gamma_2 \quad \ldots \quad (9)$$

Where

$$\gamma_1 = \frac{\sigma_e^2}{\sigma_{c1}^2} \qquad \text{and} \qquad \gamma_2 = \frac{\sigma_e^2}{\sigma_{c2}^2}$$

This approach provides separate σ_c^2 for control and selected lines when they are analysed together. DRREML package (Meyer, 1988) was adjusted to calculate log likelihood based on the mixed model equation given in equation (9).

2.4.2 MULTIVARIATE ANALYSIS

Multivariate analysis of lean mass and body weight at 10 weeks of age was attempted because in the first 20 generations the selection was based on the predicted lean mass and thereafter on body weight. This would reduce the selection bias in estimates of correlated traits when selection is being practised on the other trait and therefore increase the precision of estimates. The univariate analyses do not take into account the covariances among the traits.

The multivariate analysis of two traits, each with three variance components (Va, Vc and Ve), requires a 9 dimensional search in the maximization of the likelihood. This is very demanding in terms of computational time and, in practice, it is very difficult to achieve convergence unless very good initial estimates of parameters are available for starting values. Recently, Thompson and Hill (1990) have suggested a method of univariate REML analyses for multivariate data with the animal model (T-H method). In the T-H method, the computations are considerably reduced because this employs a sequence of univariate analyses on canonical transformed traits and sums of pairs of transformed traits. Thompson and Hill (1990) have shown with a numerical example that the estimates of additive genetic variance are at least 97% efficient when this method is used for three variance components as compared with two components.

This approach was used to estimate the (co)variance components

for lean mass and body weight with model 1 (section 2.4.1). The computing programs were made available by R. Thompson and R. Crump (Institute of Animal Physiology and Genetics, Roslin). Lean mass and body weight traits are highly correlated, as the body weight is a component of the index to predict lean mass. Therefore, the analysis of these traits would require a large number of iterations to reduce the covariances between them close to zero. The number of iterations can be reduced by using less correlated traits. Hence the components of the index (body weight and gonadal fat pad weight) were analysed together by the T-H method. The (co)variances for lean mass (LM) and proportion of gonadal fat pad weight to body weight (FP) were derived from the (co)variances of body weight (BW) and gonadal fat pad weight (F) by simple arithmetic manipulations:

$$LM = BW - 8 \times F$$

$$V(LM) = V(BW) + 64 \times V(F) - 16 \times COV(BW,F)$$

$$COV(LM,BW) = [\ V(BW) + V(LM) - 64 \times V(F)\ ] / 2$$

$$COV(LM,F) = [\ V(BW) - V(LM) - 64 \times V(F)\ ] / 16$$

Using Taylor Series methods for the ratio traits, fat proportion, the following approximate estimates were also obtained:

$$COV(LM,FP) = (\bar{F}/\bar{BW})[(COV(BW,F)/\bar{F}) - (8xV(F)/\bar{F}) - (V(BW)/\bar{BW}) + (8xCOV(BW,F)/\bar{BW})]$$

$$V(FP) = (\bar{F}^2/\bar{BW}^2)[(V(F)/\bar{F}^2) + (V(BW)/\bar{BW}^2) - (2COV(BW,F) / \bar{F} \times \bar{BW})]$$

CAHPTER 3

GENETIC PARAMETERS FOR LEAN MASS USING UNIVARIATE ANALYSES

3.1 INTRODUCTION

Mice have been used extensively in experiments to alter their body lean mass content. They provide useful information about the consequences of long term selection which otherwise are very expensive and time consuming to obtain from domestic livestock species. The proportion of fat in the body could be reduced either by directly selecting against fat content, e.g. gonadal fat pad weight, or by increasing the body weight while keeping fat content constant. Both approaches have been used widely in selection experiments with mice. The results from such experiments have been reviewed for body weight or lean growth in mice by Roberts (1965), Eisen (1974) and McCarthy (1982); and for correlated response in body composition traits by Roberts (1979), McCarthy (1979,1982), Malik (1984) and Eisen (1989).

Different selection criteria have been used in the past to estimate the lean mass. Eisen (1987a) used hind carcass weight, expressed as a percentage of body weight (hind carcass percent), as an indirect selection criterion for lean tissue content at 12 weeks of age in a selection experiment for hind carcass percentage and epididymal fat pad percentage of body weight. The genetic correlation between hind carcass percentage and epididymal fat pad percentage was found to be negative (r_g = -0.67±0.04). This suggests that the selection for hind carcass percent could be an alternative selection criterion to reduce body fat content in mice. Total muscle in the body can be increased by selecting 12 week old male mice for the weight of the hind leg muscle system

(McLellan and Frahm, 1973). The genetic correlations of hind leg muscles weight with body weights at different ages and average daily gain between 21 to 42 days of age are positive and high. The gonadal fat pad weight accounts for about one-eighth of total body fat at that age. Sharp *et al.* (1984) estimated lean mass using an index: Body weight - (8 x gonadal fat pad weight) in 10 week old males. Selection based on estimated lean mass resulted in higher body weight at the age of measurement (Sharp *et al.*, 1984 and Hastings and Hill, 1989).

There are some reports about non-invasive methods for estimation of body composition. Bracco *et al.* (1983) have suggested a method of total body electrical conductivity in the rats and Lukaski *et al.* (1986) have given a bioelectrical impedance analysis method for estimation of fat free mass, though in humans. These methods are based on the fact that lean tissue, with its greater quantity of water and electrolytes, carry current more effectively than fat tissue. Walsberg (1988) reported that the technique of electrical coductivity to determine body lean mass and lipid stores in live animals is adequate for birds and mammels weighing 40 to 600g.

The realised heritability estimates for lean mass from some of the experiments have been summarised in Table 3.1. The realised heritabilty estimates for lean mass range from 0.24 to 0.70 with different selection criteria. The realized heritability estimates for hindleg muscle weight showed a great degree of asymmetry (McLellan and Frahm, 1973), because of sampling error as there

Table 3.1: Realized heritability estimates for Lean Mass

Selection Criteria	Age[1]	Gen[2]	Realized Heritabilities	References
Hindleg Muscle Weight				
High Line	12	7	0.24±0.06	McLellan and
Low Line	12	7	0.70±0.17	Frahm (1973)
Divergence	12	7	0.41±0.06	
Hind Carcass/Body Weight				
High Line	12	10	0.48±0.08	Eisen (1987a)
Low Line	12	10	0.33±0.08	
Divergence	12	10	0.40±0.04	
Body Weight-(8xGonadal Fat Pad Weight)				
High Line	10	11	0.56±0.075	Sharp *et al.*
Low Line	10	11	0.46±0.086	(1984)
Divergence	10	11	0.54±0.035	
High Line (0-11gen[2])	10	11	0.54±0.084	Hastings
High Line (12-20gen.)	10	9	0.31±0.082	(1989)
High Line (0-20gen.)	10	20	0.53±0.027	
Low Line (0-11gen.)	10	11	0.48±0.146	Hastings
Low Line (12-20gen.)	10	9	0.33±0.096	(1989)
Low Line (0-20gen.)	10	20	0.31±0.021	

1.Age = Age (weeks) of measurement of selection criteria.
2.gen.= generations of data set.

were no replicates in the experiment. If we exclude these extreme values the range becomes 0.31 to 0.56, which suggests that the realized heritability for lean growth is moderate to high. Hastings (1989) has observed a decline in realized heritabilty estimates in the later generations of the experiment in both the high and low lean mass lines, which is a consequence of decline in the response in later generations. Similar observations have also been made by Bunger *et al.*(1990) in lines selected for protein amount. The realized heritability estimate of protein amount in the beginning of the experiment was 0.146 and after 50 generations of selection it decreased to 0.048.

The reduction in the response of later generations in long term selection experiments and the decline in the genetic variance could be caused by linkage disequilibrium due to selection (Bulmer, 1976) and by inbreeding etc. In such circumstances it is difficult to obtain an unbiased estimate of heritability in the base population using the method of realised heritability, which is dependent on the response. Further, this method ignores the covariance structure among the animals.

The present study was undertaken to examine the lean mass data from the P-lines using REML with an animal model, which includes additive genetic effects for all individuals and accounts for all the variances and covariances among them. Parents without records can also be included in the analysis therby incorporating all known relationships which enables the correct correlation structure to be generated over many generations. Sorensen and

Kennedy (1984) observed that, by including all the relationships in the numerator relationship matrix, the animal model accounts for the change in genetic variance due to drift and selection, and provides unbiased estimates of genetic parameters in the base population. However, the infinitesimal model (Bulmer, 1980) is assumed in such analyses. Hence lines selected in different directions, which have originated from the same base population, should result in similar estimates of genetic parameters.

There are a few reports on the analysis of long term selection experiments with mice using the animal model, for example Meyer and Hill (1991) analysed a selection experiment for food intake, and Cue and Hayes (1990) for 28 to 38 day and 48 to 58 day weight gains of mice in different feeding regimes.

The objectives of this study were: (i) to use the univariate Restricted Maximum Likelihood method with an animal model for the estimation of genetic parameters from the lines of mice selected for lean mass, and (ii) to assess the change in the genetic parameters during the course of selection.

3.2 MATERIAL AND METHODS

The P-lines data for predicted lean mass for males (Body Weight - 8 x Gonadal fat weight at 10 weeks of age) were used from generation 0 to 20 in this analysis. The details of number of records and litters in the data files and number of animals in the pedigree files used for the analysis of different lines/replicates

are given in Table 2.1. The analyses were carried out in two parts, 0 - 7 generations and 0 - 20 generations. The data set of 0 - 7 generations comprised approximately half of the total number of records because, from generation 8 onward, the number of families in each generation was halved from 16 to 8. This analysis provided good starting values of genetic parameters for analysis of the complete data set which therefore require less computational time.

The univariate analyses were carried out by Restricted Maximun Likelihood method fitting an individual animal model with infinitesimal assumptions. The details of the model used is given in chapter 2. In brief, the fixed effects included in the model were generations, replicates and litter size as a covariate; the random effects were animals, common litter environment and residual environment. Each analysis included the complete pedigrees back to the base population. The fixed point estimates of the log likelihood were used to plot figures of the log likelihood curves. The analyses of control and selected lines were also carried out by taking account of the heterogeneous additive genetic and common litter environment variances (see (sections 2.4.1.1 and 2.4.1.2)

3.3 RESULTS

There were 5792 records and 7794 animals, spread over a period of 20 generations of selection for lean mass at 10 weeks of age, in three replicates with high, control and low lines in each

replicate (Table 2.1). Table 3.2 gives the summary statistics of the lean mass data used in the analyses. The average lean mass of different generations are plotted in Figures 3.1 and 3.2 for different lines within replicates, and lines averaged over three replicates, respectively. The average lean mass in the three replicates was similar, the overall estimate being 29.4g. However, in the 20^{th} generation, replicate 2 had a slightly lower lean mass (28.58g) than replicates 1 and 3. The selected lines in each of the three replicates showed substantial divergence from the control (Figure 3.1). In the 20^{th} generation, the high lines averaged 38.05g, which is about 5 standard deviations units (σ_p) higher than the controls, whereas the low lines averaged 23.04g, 2 σ_p below the controls. Similar average figures for lean mass at generation 20 in the same data set (P-lines) were reported by Hastings and Hill (1989).

3.3.1 0 - 7 GENERATIONS DATA SET.

A small data set from base plus 7 generations of selection was initially obtained to estimate unbiased estimates of the genetic parameters in the base population because lines had not diverged greatly by generation 7. These initial parameters can be used as "good" starting values for the complete data set analyses. Another advantage was the ease of computation and reduction of computation time.

Table 3.3 shows the estimates of variance components and genetic parameters for lean mass in the 0 to 7 generations data

Table 3.2: Summary Statistics for Lean Mass.

Line / Replicate	Over 20 generations Mean (g)	SD (g)	CV (%)	20th generation Mean (g)	SD (g)	CV (%)
Replicate 1	29.47	4.92	16.7	30.26	7.28	24.1
Replicate 2	28.95	4.71	16.3	28.58	6.05	21.2
Replicate 3	29.78	4.87	16.4	30.18	7.04	23.6
Overall	29.40	4.85	16.5	29.65	6.79	22.9
HIGH	33.07	4.42	13.4	38.05	3.04	8.0
CONTROL	28.64	2.99	10.4	27.48	2.14	7.8
LOW	26.02	2.83	10.9	23.04	1.74	7.6

Rep = Replicate, SD = Standard deviation, and
CV = Coefficient of variation.

Figure 3.1: Generation means for lean mass in different lines and replicates.

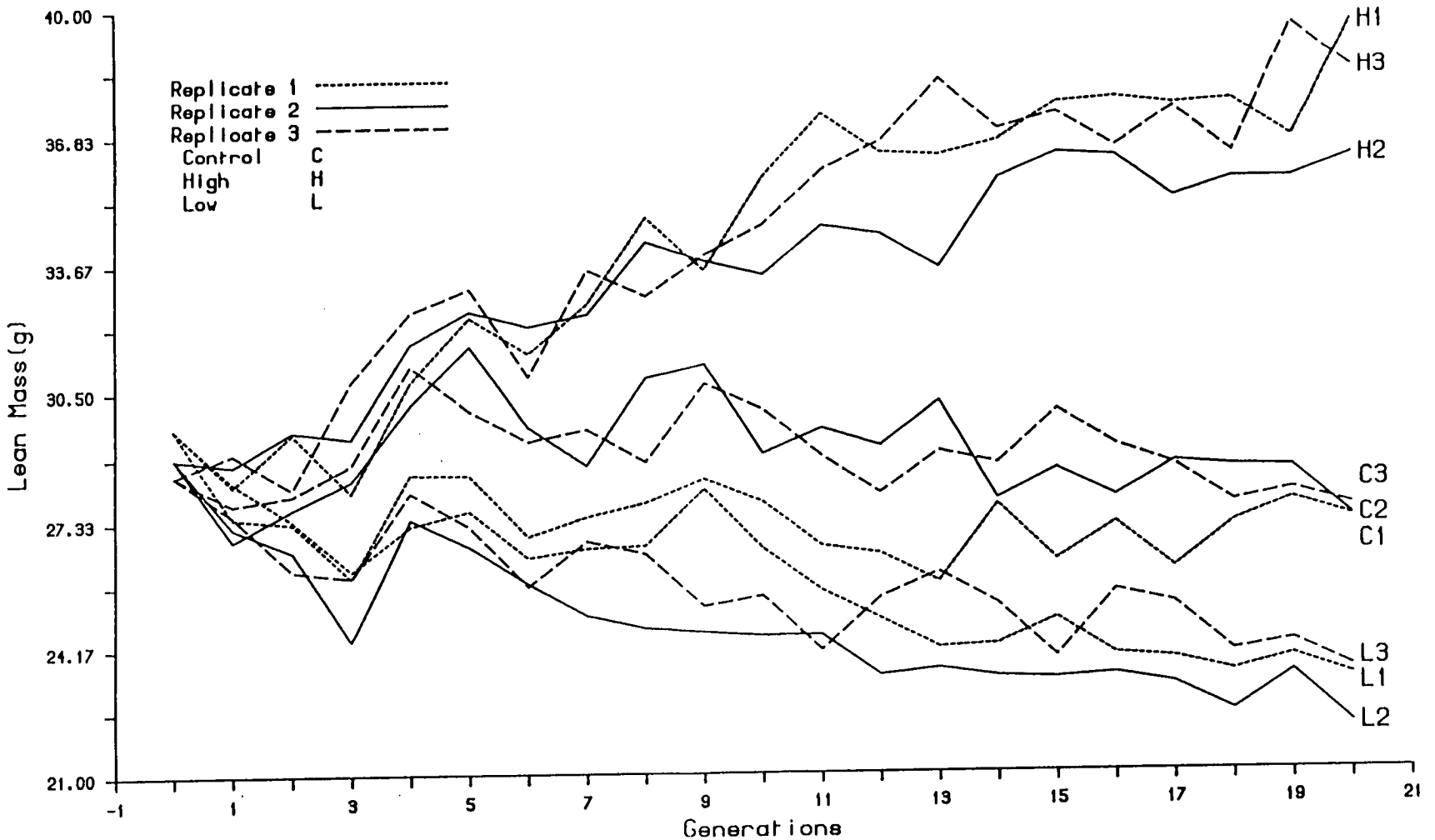


Figure 3.2: Generation means for lean mass in different lines averaged over replicates.

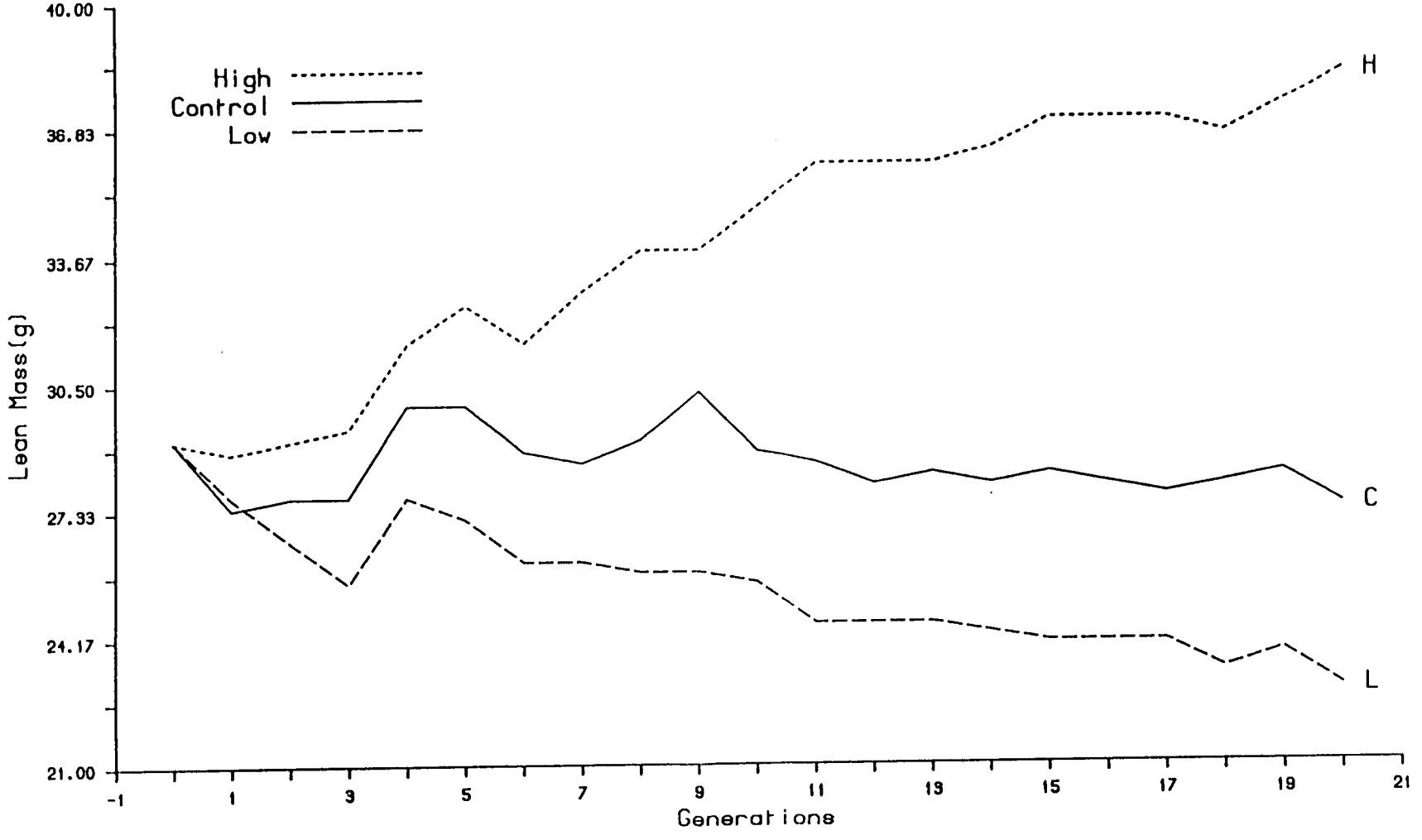


Table 3.3: Estimates of Variance Components and Genetic Parameters for Lean Mass.
Data Set : generations **0 to 7**.

Line/ Replicate	Variance Components				h^2	c^2
	V_a	V_c	V_e	V_p	± SE	± SE
Replicate 1 (C+H+L)	3.810	1.431	2.924	8.165	0.466 ±0.047	0.175 ±0.015
Replicate 2 (C+H+L)	5.046	1.961	2.231	9.238	0.546 ±0.054	0.212 ±0.028
Replicate 3 (C+H+L)	4.671	2.086	2.223	8.980	0.520 ±0.059	0.232 ±0.023
OVERALL	4.509	1.837	2.458	8.804	0.512 ±0.032	0.209 ±0.012
Control (Rep 1+2+3)	3.540	1.884	2.929	8.353	0.424 ±0.098	0.225 ±0.024
High (Rep 1+2+3)	2.639	2.510	4.613	9.762	0.270 ±0.091	0.257 ±0.020
Low (Rep 1+2+3)	2.413	1.045	2.757	6.215	0.388 ±0.096	0.168 ±0.022
Control+High (Rep 1+2+3)	4.642	2.370	3.068	10.080	0.461 ±0.053	0.235 ±0.016
Control+Low (Rep 1+2+3)	3.547	1.665	2.258	7.470	0.475 ±0.050	0.223 ±0.017

Table 3.4: Estimates of Variance Components and Genetic Parameters for Lean Mass.
Data Set: Log-transformed, generations **0 to 7**.

Line/ Replicate	Variance Components ($\times 10^{-2}$) V_a	V_c	V_e	V_p	h^2 ± SE	c^2 ± SE
Replicate 1 (C+H+L)	0.453	0.177	0.345	0.978	0.465 ±0.055	0.181 ±0.018
Replicate 2 (C+H+L)	0.594	0.226	0.267	1.088	0.546 ±0.049	0.208 ±0.026
Replicate 3 (C+H+L)	0.524	0.225	0.261	1.010	0.518 ±0.050	0.223 ±0.026
Control (Rep1+2+3)	0.415	0.212	0.353	0.979	0.424 ±0.097	0.216 ±0.020
High (Rep1+2+3)	0.303	0.249	0.480	1.032	0.294 ±0.097	0.241 ±0.020
Low (Rep1+2+3)	0.284	0.153	0.391	0.828	0.342 ±0.091	0.185 ±0.021
Control+High (Rep 1+2+3)	0.528	0.256	0.323	1.107	0.477 ±0.056	0.231 ±0.016
Control+Low (Rep 1+2+3)	0.431	0.207	0.308	0.946	0.455 ±0.050	0.219 ±0.017

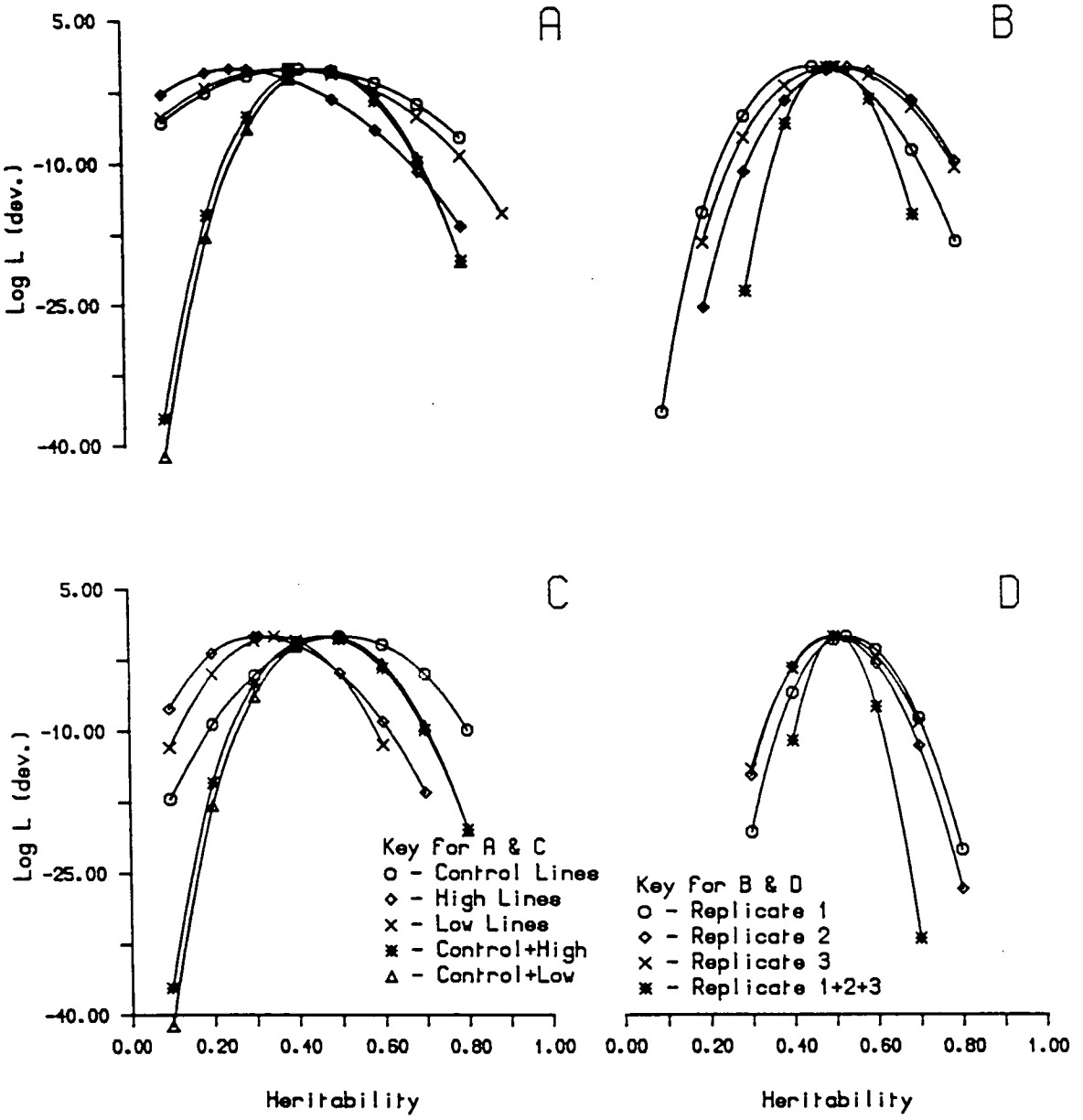


Figure 3.3: Likelihood curves for lean mass lines and replicates. Graphs A & B are for 0 - 7 generations and C & D are for 0 - 20 generations data sets. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

set. The likelihood curves for different lines and replicates are given in Figures 3.3A and 3.3B, respectively. The estimates of heritability in the base population from the three replicates ranged from 0.466 to 0.546, with an overall estimate of 0.512±0.032 using pooled data. The c^2 estimates, which are the ratios of variance due to common environment variance (Vc) to the phenotypic variance (Vp), ranged from 0.175 to 0.232 over replicates with an overall estimate of 0.209±0.012. Replicates 2 and 3 have similar estimates whereas replicate 1 showed a slightly lower estimate of heritability and c^2. The curvature of the log likelihoods (Figure 3.3B) for the overall data set was more sharp than that of the individual replicates, which corresponds with the lower estimate of standard error.

When selected lines were analysed individually, they revealed different estimates of genetic parameters as compared to those of the replicates each comprising high, low and control lines. The heritability and additive genetic variance estimates from high and low lines were lower than the control lines estimate. The c^2 estimates revealed an increase in the high lines and decline in the low lines, when compared to the control. The curvature of log likelihood curves for different lines (Figure 3.3A) were flat suggesting larger standard errors and that differences between the heritability estimates are primarily due to sampling.

The estimates from control+high and control+low lines and replicates individually were not different from that of control lines alone and pooled data of the three replicates. Therefore

the analyses of control plus selected lines seems to provide unbiased estimates of genetic parameters in the base population.

Preliminary analyses indicated some correlation between mean and variance of lean mass over the generations (within line correlation coefficients ranged from 0.15 to 0.79) while the coefficient of variation did not show much change, suggesting some scale effect. Therefore data were transformed to the natural logarithmic scale and reanalysed, the results of which are given in Table 3.4. The heritability and c^2 estimates from the transformed data were similar to the untransformed data.

3.3.2 0 - 20 GENERATIONS DATA SET.

The variance components and genetic parameter estimates for lean mass are presented in Tables 3.5 and 3.6 for untransformed and log transformed data, respectively, for the 0-20 generations data set. The log likelihood curves for this data set are given in Figures 3.3C and 3.3D for different lines and replicates, respectively.

The results in Table 3.5 revealed that the estimates in each of the three replicates were very similar, ranging from 0.499 to 0.533. The estimate of c^2 was lower (0.181±0.017) in replicate 1, than in replicates 2 and 3. When the control, high and low lines were analysed separately, substantially lower estimates of additive genetic variances were observed for the high and low lines than for the control lines. The estimates of heritabilty

Table 3.5: Estimates of Variance Components and Genetic Parameters for Lean Mass.
Data Set: generations **0 to 20**.

Line/ Replicate	Variance Components V_a	V_c	V_e	V_p	h^2 ± SE	c^2 ± SE
Replicate 1 (C+H+L)	4.447	1.511	2.384	8.342	0.533 ±0.033	0.181 ±0.017
Replicate 2 (C+H+L)	4.191	2.024	2.189	8.404	0.499 ±0.032	0.241 ±0.018
Replicate 3 (C+H+L)	4.358	1.976	2.216	8.550	0.510 ±0.045	0.231 ±0.018
Overall	4.309	1.914	2.276	8.500	0.507 ±0.022	0.225 ±0.015
Control (Rep1+2+3)	4.280	1.707	2.507	8.494	0.504 ±0.069	0.201 ±0.022
High (Rep1+2+3)	3.204	2.974	4.088	10.266	0.312 ±0.066	0.290 ±0.018
Low (Rep1+2+3)	2.999	0.876	1.778	5.653	0.530 ±0.073	0.155 ±0.013
Control+High (Rep1+2+3)	5.141	2.468	2.673	10.282	0.500 ±0.031	0.240 ±0.012
Control+Low (Rep1+2+3)	3.456	1.388	1.900	6.744	0.512 ±0.036	0.206 ±0.015

Figure 3.4: Fixed effect solutions for generations from different analyses of lean mass in Control(C), High(H), Low(L), Conrol+High(C+H ....) and Control+Low(C+L ----) lines.

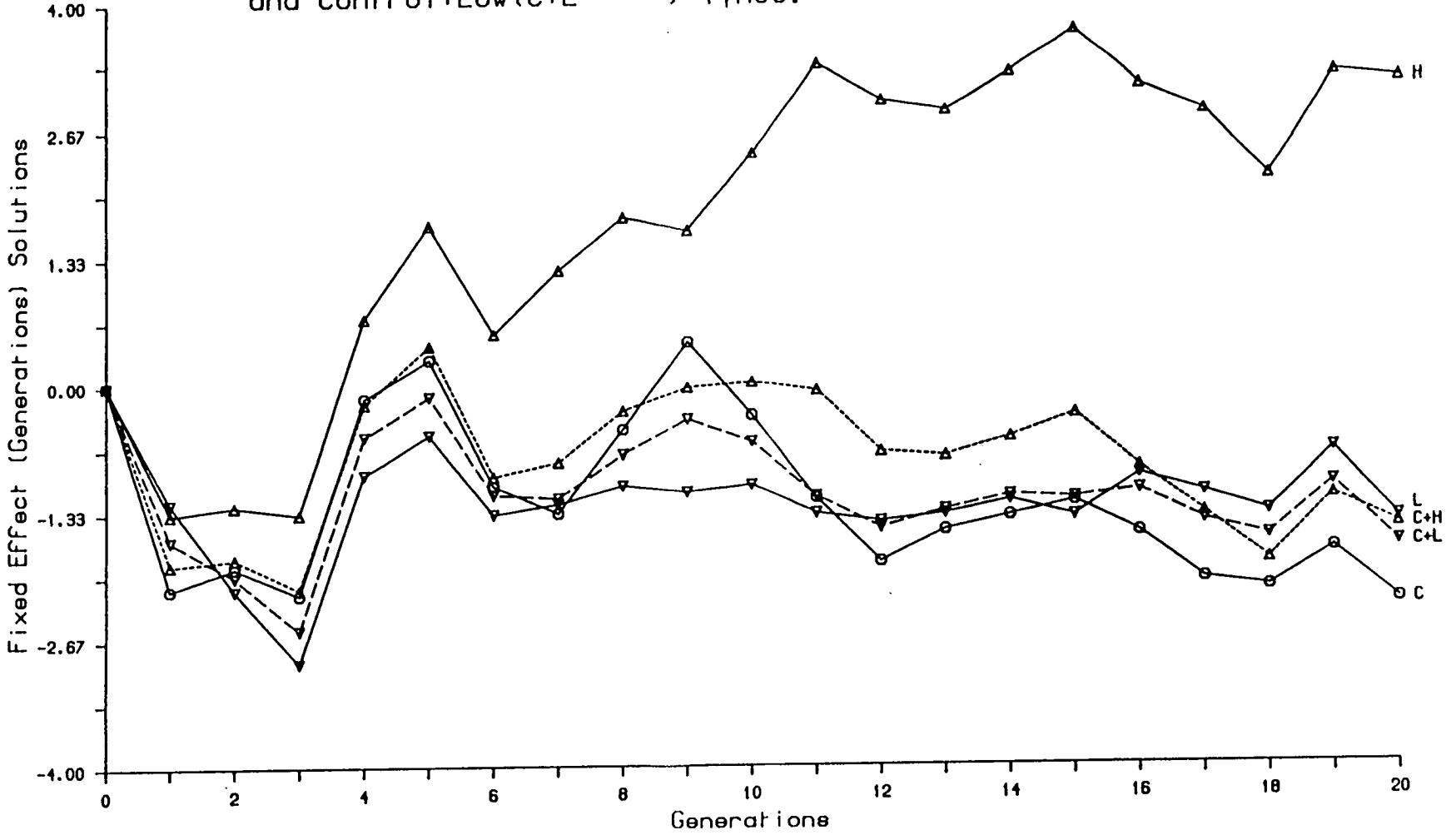


Table 3.6: Estimates of Variance Components and Genetic Parameters for Lean Mass.
Data Set: Log-transformed, generations **0 to 20**.

Line/ Replicate	Variance Components ($\times 10^{-2}$) V_a	V_c	V_e	V_p	h^2 ± SE	c^2 ± SE
Replicate 1 (C+H+L)	0.506	0.163	0.272	0.941	0.538 ±0.036	0.173 ±0.018
Replicate 2 (C+H+L)	0.500	0.220	0.246	0.966	0.518 ±0.038	0.228 ±0.018
Replicate 3 (C+H+L)	0.479	0.205	0.242	0.926	0.517 ±0.040	0.221 ±0.018
Overall (Rep1+2+3)	0.492	0.207	0.255	0.954	0.516 ±0.024	0.217 ±0.015
Control (Rep1+2+3)	0.512	0.202	0.295	1.009	0.508 ±0.071	0.200 ±0.021
High (Rep1+2+3)	0.367	0.250	0.359	0.976	0.376 ±0.072	0.256 ±0.022
Low (Rep1+2+3)	0.362	0.143	0.287	0.792	0.457 ±0.069	0.180 ±0.017
Control+High (Rep1+2+3)	0.547	0.233	0.260	1.040	0.526 ±0.035	0.224 ±0.013
Control+Low (Rep1+2+3)	0.431	0.181	0.270	0.882	0.489 ±0.038	0.205 ±0.012

in the control and low lines were similar, however, because there was also a reduction in the phenotypic variance of the low lines. The c^2 estimate of high lines was much higher (0.290±0.018) than the estimate from low lines (0.197±0.013), and that from the control lines was intermediate (0.201±0.022). The curvatures of the likelihood curves of each line (Figure 3.3C) were flat compared to the curves for the replicates (Figure 3.8D).

All these estimates refer back to the base population parameters. Since each line originated from the same base population, we would expect similar estimates for the additive genetic variance and heritabilty from the control, high and low lines, which is not the case in the present analysis. The estimates of the heritability had large standard error and differences could be due to sampling. Another possibility for the lower estimate in the selected lines could be that by fitting generations as fixed effect in the model, it might have removed the response due to selection, as the response and environmental fluctuations of generations are confounded.

To look into this possibility, the generation means (expressed as deviations from the overall mean, μ) obtained from the REML analyses as the fixed effect solutions were investigated. These are given in Figure 3.4 for different analyses. The generation means obtained from the high lines analysis showed an increasing trend, but there was no trend for the control and low lines. If response is included in the high lines generation means, the means of the low lines are also expected to show a decreasing trend.

But the fixed effect solutions have been obtained by using the heritability estimates obtained from the respective analysis. The heritability estimates for the low and control lines were more alike, resulting in a similar trend in the two lines; whereas the high lines had a lower heritability estimate, hence different generation means. Therefore, the fixed effect solutions are a funtion of the heritability estimates and the hypothesis of removal of response by fitting generations as a fixed effect could not be supported. However, the generation means from the control + high and control + low lines analyses showed a similar pattern (Figure 3.4) to that of the control lines, perhaps indicating an adequate correction for environmental effects.

These analyses of high+control and low+control resulted in estimates of hertabilities which were not different from the control lines and overall heritabilty estimates (Table 3.5). The sharp curvature of log likelihood curves indicate an increase in the precision of the estimates, as there was more information from two lines because they have common parents in the base population and more data. This approach suggests an alternative method to obtain unbiased estimates of genetic parameters from the selected lines in divergent selection experiment where the controls are maintained.

The estimates of heritability given in Table 3.6 for the log transformed data were not different from the estimates from untransformed data. However, the differences between the error variance of different lines were considerably reduced, hence the

remaining analyses were carried out only for the log transformed data.

3.3.3 ANALYSES WITH HETEROGENEITY OF ADDITIVE GENETIC VARIANCE.

The analyses of control and selected lines individually revealed that they differ considerably in their additive genetic variance components. Hence, the analyses of control + selected lines assuming that they have similar additive genetic variance, as has been done in previous analysis, may result in biased estimate. Therefore, control + selected lines were reanalysed by taking into account their heterogeneous variances. Not only this, it also gives an opportunity to estimate the additive genetic component at different time periods and to assess the changes in variance components, if any, as a consequence of selection.

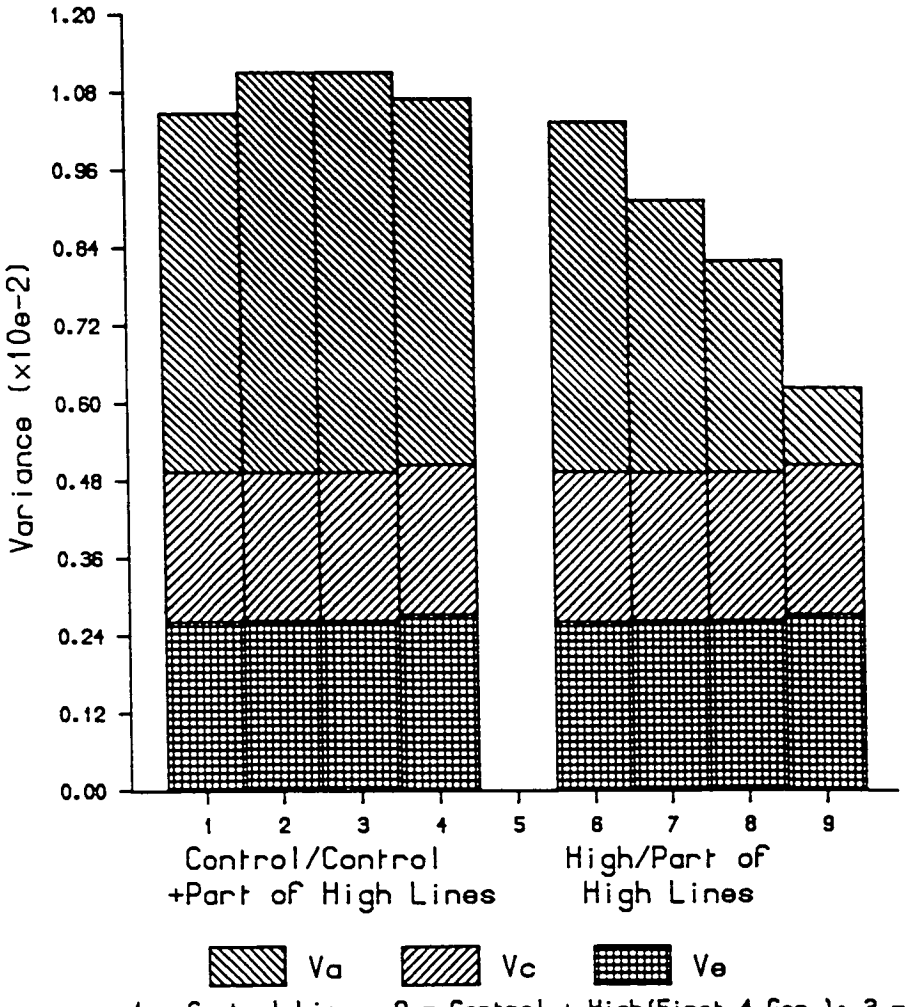
The results of these analyses are presented in Tables 3.7 and 3.8 for control + high and control + low lines, respectively, for log transformed data. The change in the additive genetic variance components over different generations have been shown in Figures 3.5 and 3.6 for high and low lines, respectively.

The analysis of control + high data (Table 3.7) provided separate estimates of additive genetic variance (Va) for high and control lines; these were similar to each other and also to the Va estimate from the analysis of control + high lines without taking into account any heterogeneity of variance (Table 3.6). This suggests that estimates of genetic parameters from control + high

Table 3.7: Estimates of Variance Components and Genetic Parameters for Lean Mass in Control + High lines. Analysed with heterogeneous additive genetic variance assumptions.
Data Set: Log-transformed, generations **0 to 20**.

	Group Description	h^2	c^2	Variance components (10^{-2}) V_p	V_a	V_c	V_e
-	Control+High (No Het.Option)	0.526	0.224	1.040	0.547	0.233	0.260
1	Control Lines (0-20 generations)	0.529	0.222	1.048	0.554	0.233	0.261
2	High Lines (0-20 generations)	0.523	0.225	1.034	0.541		
1	Control+First 4 gen.of High Lines	0.556	0.207	1.111	0.618	0.230	0.262
2	High Lines (5-20 generations)	0.461	0.252	0.913	0.421		
1	Control+First 7 gen.of High Lines	0.556	0.207	1.111	0.618	0.230	0.263
2	High Lines (8-20 generations)	0.398	0.281	0.819	0.326		
1	Control+First 14 gen.of High Lines	0.529	0.216	1.071	0.567	0.230	0.272
2	High Lines (15-20 generations)	0.190	0.372	0.623	0.118		

Figure 3.5: Change in the additive genetic variance of lean mass in the high lines.



1 = Control Lines; 2 = Control + High(First 4 Gen.); 3 = Control + High(First 7 Gen.); 4 = Control + High; (First 14 Gen.); 6 = High(0-20 Gen.); 7 = High(5-20 Gen.); 8 = High(8-20 Gen.); 9 = High(15-20 Gen.)

Table 3.8: Estimates of Variance Components and Genetic Parameters for Lean Mass in Control + Low lines. Analysed with heterogeneous additive genetic variance assumptions.
Data Set: Log-transformed, generations **0 to 20**.

	Group Description	h^2	c^2	Variance Components (10^{-2}) V_p	V_a	V_c	V_e
-	Control+Low (No Het.Option)	0.489	0.205	0.882	0.431	0.181	0.270
1	Control Lines (0-20 generations)	0.562	0.169	1.017	0.571	0.172	0.274
2	Low Lines (0-20 generations)	0.431	0.220	0.783	0.337		
1	Control+First 4 gen.of Low Lines	0.541	0.179	0.984	0.532	0.176	0.275
2	Low Lines (5-20 generations)	0.344	0.256	0.688	0.236		
1	Control+First 7 gen.of Low Lines	0.524	0.186	0.953	0.499	0.177	0.276
2	Low Lines (8-20 generations)	0.288	0.279	0.637	0.183		
1	Control+First 14 gen.of Low Lines	0.503	0.202	0.910	0.458	0.184	0.268
2	Low Lines (15-20 generations)	0.250	0.305	0.602	0.150		

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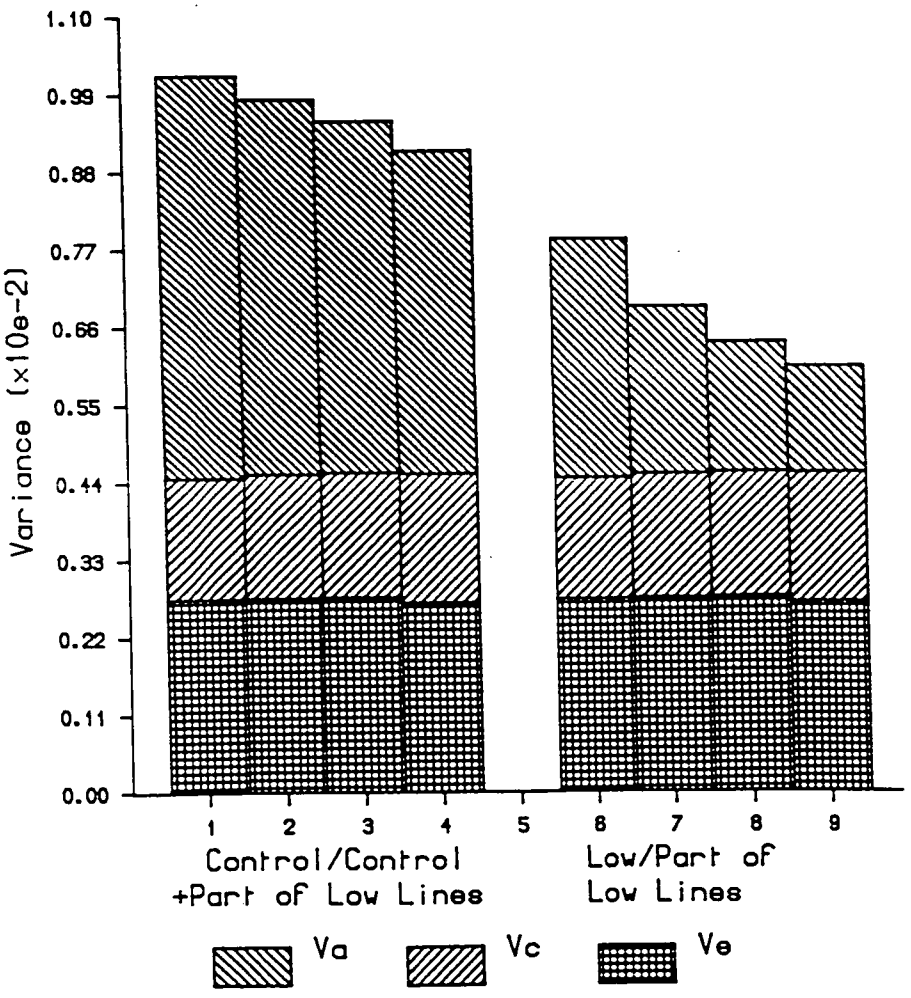
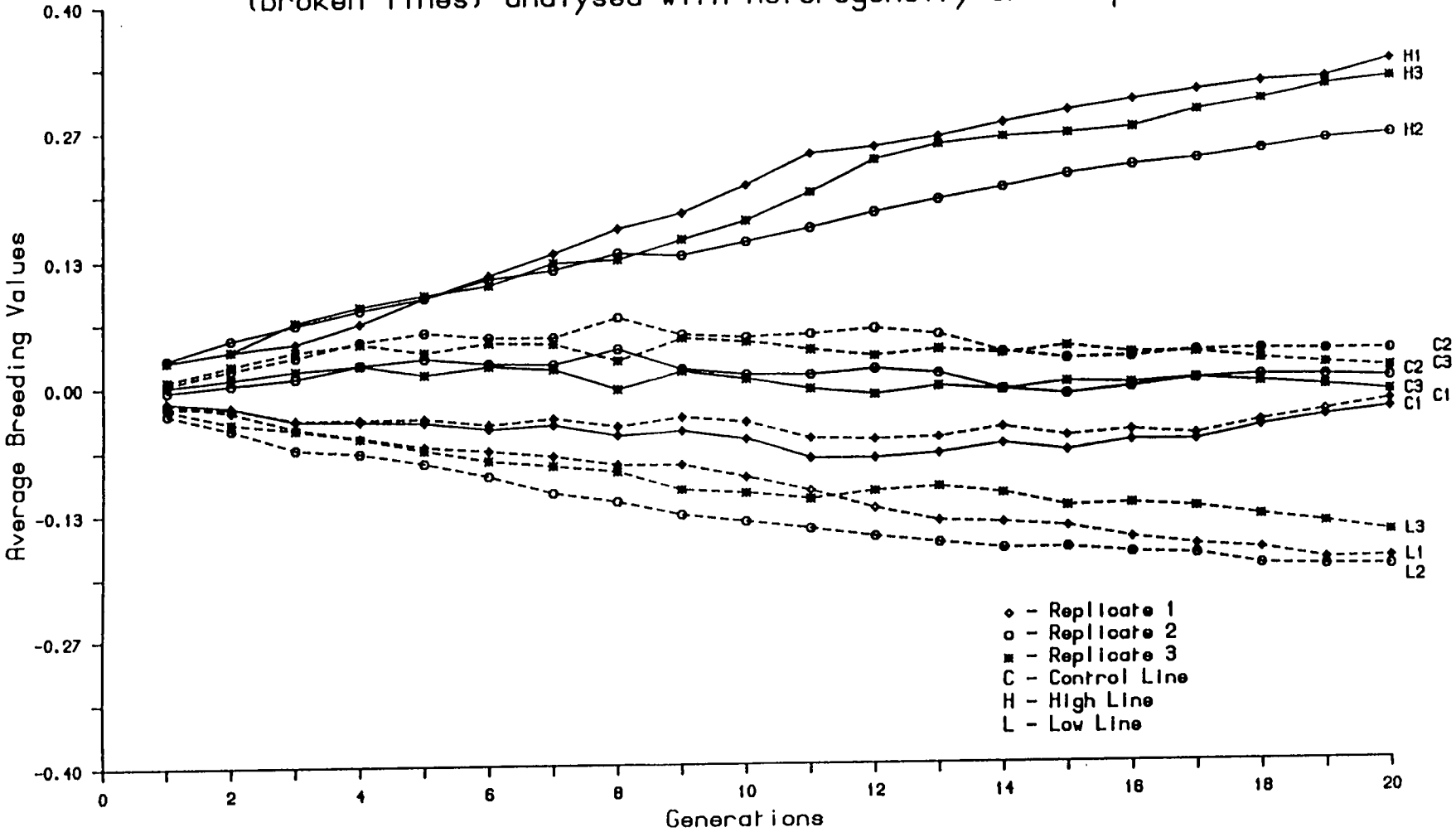
Figure 3.6: Change in the additive genetic variance of lean mass in the Low Lines.



1 = Control Lines; 2 = Control + Low(First 4 Gen.); 3 = Control + Low(First 7 Gen.); 4 = Control + Low; (First 14 Gen.); 6 = Low(0-20 Gen); 7 = Low(5-20 Gen.); 8 = Low(8-20 Gen.); 9 = Low(15-20 Gen.)

Figure 3.7: Predicted response to the Selection in lean mass.
Conrol + High (Continuous lines) and Control + Low lines
(Broken lines) analysed with heterogeneity of Va option.



without hetergeneity of variance assumptions in Table 3.6 were not biased, because the additive genetic variances were similar in the two lines. Therefore, it also indicates that the estimate of Va from the analysis of the high lines alone (Table 3.6) was an underestimate. The estimates for the c^2 term in the high and control lines were also similar, because their phenotypic variances (Vp) were equal.

Further analyses were carried out to see if the additive genetic variance changes as a consequence of selection in the high lines. The high lines data were partitioned at different generations to make two unequal groups (*i.e.* group 1 = control + part of high, and group 2 = remaining part of high lines) and the estimates of Va in the two groups were obtained separately by analysing the control + high lines data together. The complete pedigrees of the control and high lines were used as before in all the analyses. The estimate of Va in group 1 refers back to the base population, whereas in the group 2 refers to the generation from which the data of the high lines begins.

The data in group 2, for the high lines, were reduced to 5-20 generations and group 1 had the base population, all the control generations and the first four generations of the high lines. Analysis of these two groups with the heterogeneity of Va assumptions revealed that Va estimated from the 5-20 generations data set of the high lines was reduced to 0.421×10^{-2} in generation 5 from 0.541×10^{-2} in the base population, but Vc and Ve did not change. The heritability estimate also dropped from 0.523 to

0.461 and the c^2 term increased from 0.225 to 0.252 because of the decline in Vp. The data of the high lines (group 2) were further reduced to 8-20 and 15-20 generations and reanalysed. These analyses indicated a further drop in Va as data were restricted to the later generations. The estimates of Va for the 8-20 and 15-20 generations data sets of the high lines were 0.326×10^{-2} in generation 8 and 0.118×10^{-2} in generation 15 and estimates of the heritability were 0.398 and 0.190, respectively. The Vc and Ve components did not show any change in these analyses.

The high lines estimate of Va (0.541×10^{-2}) in the base population from the 0-20 generations data set may be considered as consisting of the Va components from the 0-4(VaA), 5-7(VaB), 8-14(VaC) and 15-20(VaD) generations data set. These components can be derived from the Va estimates for different generations given in Table 3.7 by weighting them with the appropriate number of observations assuming that each observation gives equal information.

There were in total 3645 observations in the control+high lines data, the proportions of observations in the high lines were 0.63(n_a), 0.43(n_b), 0.29(n_c) and 0.13(n_d) in the 0-20, 5-20, 8-20 and 15-20 generations data sets, respectively. The estimate of VaD = 0.118×10^{-2} from Table 3.7. The high lines estimate of Va (0.326×10^{-2}) from 8-20 generations can be considered as consisting of Va from 8-14(VaC) and 15-20(VaD) generations. Therefore, $0.326 \times 10^{-2} = (n_c \text{ VaC} + n_d \text{ VaD}) / (n_c + n_d)$ giving VaC = 0.419×10^{-2}. Similarly the estimate of Va (0.421×10^{-2}) from 5-20 generations

consists of Va from 5-7(VaB), 8-14(VaC), and 15-20(VaD) generations giving VaB = $0.514x10^{-2}$. The estimate of Va ($0.514x10^{-2}$) from 0-20 generations can also be decomposed as Va from 0-4(VaA), 5-7(VaB), 8-14(VaC) and 15-20(VaD) generations giving VaA = $0.708x10^{-2}$. The Va estimates ($x10^{-2}$) from 0-4, 5-7, 8-14 and 15-20 generations data sets were thus 0.708, 0.514, 0.419 and 0.118, respectively. These results indicate a steady decline in Va estimates of the high lines as a result of selection.

Similar analyses were carried out for the control + low lines. The results (Table 3.8) show almost the same trend as that of the control + high except that when the base to 20 generations data set were assigned to group 1 for the control and group 2 for the low lines, the estimate of Va ($0.337x10^{-2}$) was much lower in the low lines than the control ($0.517x10^{-2}$). The additive genetic variance showed a steady decline in the later generations of selection, consequently, the heritability estimates also showed a decreasing pattern in the low lines. As discussed for the control+high lines analysis, the estimate of Va ($0.337x10^{-2}$) in the low lines was partitioned in different components, which again indicated a steady decline. The estimates of Va($x10^{-2}$) for 0-4, 5-7, 8-14 and 15-20 generations were 0.477, 0.285, 0.197 and 0.150, respectively, in the low lines.

The Vc and Ve components in different grouping patterns remained unchanged.

The estimates of genetic parameters obtained for the control +

high and control + low lines from the analyses with heterogeneity of additive genetic variance assumptions were used to predict the breeding value of each individual for lean mass. The predicted response in the breeding values are given in Figure 3..7. The trend in the predicted response for lean mass was similar to that of the observed means in the experiment suggesing a close agreement between two.

3.3.4 ANALYSES WITH HETEROGENEITY OF BOTH ADDITIVE GENETIC AND COMMON ENVIRONMENT VARIANCES

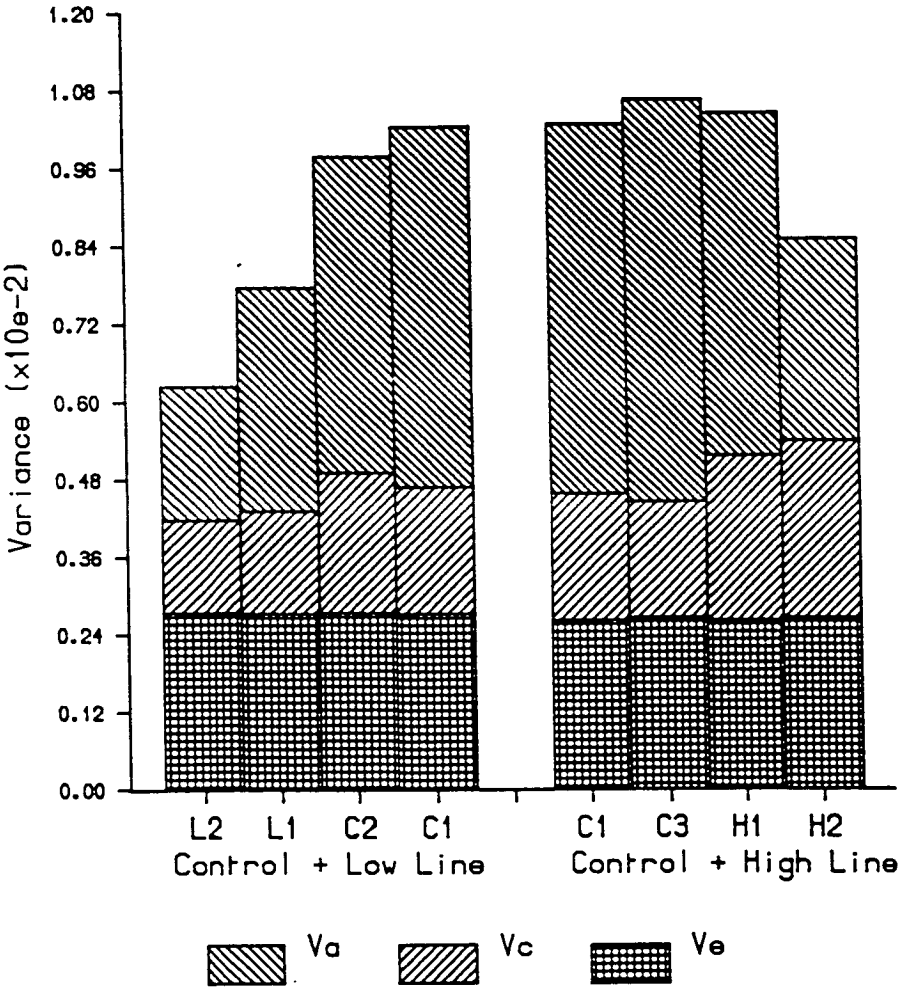
The results in Tables 3.7 and 3.8 revealed that the estimates of the common environment variance (Vc) are different between the high + control and low + control pairs of lines, the estimate of Vc there being higher in the high lines and lower in the low lines. This is further supported by the common environment variance components in Table 3.6 for analysis of each line separately. Hence, the data were reanalysed allowing for heterogeneity of Vc in addition to heterogeneity of Va in the selected lines.

The results of these analyses are presented in Table 3.9 and Figure 3.8. These indicate a higher Vc component in the high lines ($0.256x10^{-2}$) than in the controls ($0.196x10^{-2}$), using the 0-20 generations data set. When 8-20 generations (*i.e.* the later half) data set of the high lines was considered in group 2, the estimate of the Vc component was increased to $0.275x10^{-2}$.

Table 3.9: Estimates of Variance Components and Genetic Parameters for Lean Mass in Control + High and Control + Low Lines. Analysed with heterogeneous additive genetic and common environment variances. Data Set: Log-transformed, generations **0 to 20**.

Group Description	h^2	c^2	Variance Components (10^{-2}) Vp	V_a	V_c	V_e
1 Control Lines (0-20 generations)	0.555	0.191	1.031	0.573	0.196	0.262
2 High Lines (0-20 generations)	0.506	0.244	1.048	0.530	0.256	
1 Control+First 7 gen.of High Lines	0.583	0.168	1.069	0.623	0.180	0.266
2 High Lines (8-20 generations)	0.366	0.322	0.852	0.312	0.275	
1 Control Lines (0-20 generations)	0.544	0.191	1.027	0.559	0.196	0.272
2 Low Lines (0-20 generations)	0.445	0.205	0.777	0.346	0.159	
1 Control+First 7 gen.of Low Lines	0.499	0.222	0.981	0.490	0.218	0.273
2 Low Lines (8-20 generations)	0.331	0.231	0.624	0.207	0.144	

Figure 3.8: Change in the additive genetic and common litter environment variances of lean mass in low and High Lines.



CONTROL + LOW:
L2 = Low lines(8-20 Gen.); L1 = Low Lines(0-20 Gen.);
C2 = Control + Low(First 7 Gen.); C1 = Control Lines;
CONTROL + HIGH:
C1 = Conrol Lines; C3 = Control + High(First 7 Gen.);
H1 = High Lines(0-20 Gen.); H2 = High(8-20 Gen.).

The analyses of the low+control data showed that the low lines have a substantially lower Vc than the control lines, the estimates being 0.159×10^{-2} and 0.196×10^{-2} for low and control lines, respectively, for the 0-20 generations data set. When the 8-20 generations data set was considered, the estimate of Vc in the low lines decreased to 0.144×10^{-2}. However, the estimates of Va and Ve did not change much as compared to the previous analyses (Tables 3.7 and 3.8). These results suggests that common environmental variance is increasing in the high lines and decreasing in the low lines in the later generations of selection.

3.4. DISCUSSION AND CONCLUSIONS

The analyses were carried out by REML using an animal model with litters as additional random effects. The trait was considered to be governed by numerous unlinked loci, each having a small effect, *i.e.* the infinitesimal model, and the gene frequencies were expected to change negligibly in the course of selection. Under these assumptions, the additive genetic variance may change as a result of inbreeding and linkage disequilibrium but not due to change in gene frequencies. All the analyses had pedigree information back to the base population, hence estimates of variances and genetic parameters refer back to the base population. In this experiment, all the replicates and selected lines originated from the same base population, thus all the estimates are expected to be same.

Analyses of the three replicates with 0-7 and 0-20 generations data sets gave very similar estimates of heritability for lean mass in the base population, as expected. The overall estimate of heritability for the three replicates (0.516±0.024) is in close agreement with the realised heritability estimates of Sharp *et al.* (1984) for lean mass obtained from the 11 generations.

When selected and control lines were analysed separately, the estimates of additive genetic variance in the high and low lines were substantially lower than the estimates obtained from controls and from selected and controls together in each of the three replicates. As discussed in section 3.3.2, no reason could be assigned to the decline in the additive genetic variance in the high and low lines. This requires futher investigation.

However, the estimates of heritability obtained from the analyses of control + high and control + low lines were not different, which is as expected, because they refer to the same base population. However, Hastings (1989) has reported realized heritability estimates for the high lines slightly higher (0.53) and for the low lines substantially lower (0.31) than the estimates obtained in this study for lean mass from the same data set of 0-20 generations. The univariate REML analyses using an animal model, used in this study, include all the covariances among animals and a common litter environment effect, it is therefore expected that the estimates obtained would be unbiased. The estimates of the c^2 term, *i.e.* the ratio of common environmental variance (Vc) to the total phenotypic variance (Vp),

were around 20 to 25 percent. The high lines, however, had a higher whilst the low lines had a lower c^2 term than the controls suggesting different magnitude of maternal effects in the two divergently selected lines.

The analyses with the heterogeneity of Va assumptions provided different estimates of Va for the control and selected lines. These analyses including heterogeneous Va suggested that the control and high lines have similar heritabilities, whereas the estimates from the low lines in the base population was lower suggesting that variance is changing faster in the low lines during the selection, and this is not being accounted for by the analyses. The change in Va during the course of selection was assessed by partitioning the data at generation 5-, 8- and 15 - 20. The additive genetic variance and heritability estimates showed a consistent decline over generations in both the high and low lines. If the infinitesimal model assumptions held for lean mass, we would not expect the estimates of Va from the high and low lines to change.

Similarly the heterogeneity of common environment variance (Vc) was included because the estimates of Vc in the high and low lines were different from that in the control lines. In this analysis the decline in Va was also observed. The c^2 estimates showed an increase in the high lines and a decline in the low lines indicating that the common litter environment variance is also changing with selection. Body weight and litter size are correlated positively to the lean mass (Chapters 5 and 6).

Therefore the change in the lean mass resulted in the change in number and size of pups born. In the case of high lines, the litters are large and with heavy pups, if milk production may have not increased in proportion to the demand of pups, the differences in growth between litters would reflect maternal nourishment and perhaps thereby increasing the common litter variance estimate. However, in the low lines where the litters are small and light in body weight, the differences in the growth of different litters would be less due to maternal nourishment, giving a lower estimate of common litter variance.

The estimates of additive genetic variances showed a consistent decline over generations in the selected lines, even after accounting for the reductions due to linkage diseqilibrium among the unlinked loci and inbreeding suggesting that the infinitesimal model did not hold for lean mass data. There may be a possibility of presence of some gene(s) with large effect governing the trait, which may have been fixed or lost during the course of selection and thus reducing the additive genetic variance and heritability estimates. A similar decline in the heritability estimates has also been observed by Hastings (1989) in these P-lines when analysing 20 generations in two parts, *i.e.* 0-11 and 12-20 generations, to estimate the realised heritabilities in the base population and generation 11. The later generation data set had lower heritability estimates for high and low lines. In another selection experiment, Bunger *et al.*, (1990) have also reported a decline in realized heritability estimates for six week body weight and protein amount in a long

term selection experiment for growth traits spanning 50 generations. The presence of a major gene has been suggested by Calvert *et al.*(1984) and Hastings (1990) in growth traits of mice. Therefore further investigations are required by considering major gene(s). Perhaps this may give some more insight into the reasons for decline in additive genetic variance.

CHAPTER 4

GENETIC PARAMETERS FOR 10 WEEK BODY WEIGHT USING UNIVARIATE ANALYSES

4.1 INRODUCTION

In the previous chapter we investigated lean mass in divergently selected mice lines, where the selection criterion was an Index of lean mass (body weight - 8 x gonadal fat pad weight) at 10 weeks of age. Sharp *et al.* (1984) reported a correlation of 0.94 between this index and 10 week body weight in the P-line, suggesting that weight at 10 weeks of age would be a reasonable guide to the lean mass and dissection of mice to record gonadal fat pad weight can be avoided. The selection criterion in the lean growth lines (P-Lines) was changed from the lean mass index on male to 10 week body weight in both sexes from generation 21 onwards. This study uses data on 38 generations of selection in these mice. This should provide some information about the consequences of long term selection experiments as records on 10 week body weight were available throughout. Further the high line did not respond to selection in the later generations, rather it declined in body weight, whereas the low lines showed a consistent response. It would be useful to investigate the later generations to ascertain the possible reason for no response in the high line.

Theoretically, there are selection limits to any trait as gene fixation leads to the exhaustion of genetic variance and this may have been observed in practice. Falconer and King (1953) selected for high body weight in mice and attributed the lack of response in later generations to the loss of genetic variance due to fixation of loci by selection. Similar observations have also been made by Al-Murrani (1974) and Roberts (1974). However,

Falconer and Robertson (1956) suggested that the genes affecting the expression of a selected trait may do so either directly or by increasing the susceptibility of the individual to environmental variation. A study of Barria and Bradford (1981) indicated that large lines do not plateau only because of a lack of genetic variation. They selected a line for rapid weight gain until it reached a limit after 33 generations. At that point it was subjected to continued selection, reverse selection and relaxed selection. Although no further response occurred on upward selection, a response consistent with the realised heritability estimate was observed on reverse selection and the mean of the relaxed line regressed towards the base population. They also found a negative association between weight gain and fertility in these lines indicating that the limit was possibly due to natural selection against large body size. The reduction in fertility was due to increased embryonic mortality in large mothers and reduced fertility in large males.

There are many selection experiments with mice for increasing the body weight at a given age. A comprehensive review of the realised heritability estimates obtained in such experiments is given in Table 4.1. The realised heritability estimates for body weight reported in the literature ranged from 0.13 to 0.55 depending upon the age of measurement and origin of the base stock. These realised heritability estimates are primarily the description of response and they may not be an unbiased estimate of heritability in the base population (Falconer, 1989), as discussed in Chapter 1.

Table 4.1: Realised heritability estimates for body weights at different ages in mice.

Age (Week)	Gen	Method	Realised heritability	References
3	14	W	0.17	Frahm and Brown (1975)
5	15	W	0.39	McCarthy and Doolittle (1977)
6	11	W	0.22	Falconer (1953)
6	11	W	0.50(L)	Falconer (1955)
6	7	M	0.25	Rutledge *et al.* (1973)
6	10	W	0.40	Falconer (1973)
6	10	W	0.33(L)	Falconer (1973)
6	14	M	0.13	Cheung and Parker (1974)
6	12	M	0.55	Eisen (1978)
6	12	M	0.42	Nagai *et al.* (1978)
8	20	M	0.19	Wilson *et al.* (1971)
8	14	M	0.28	Bakker (1974)
10	15	W	0.32	McCarthy and Doolittle (1977)
10	14	W	0.23*	Hastings (1989)
10	14	W	0.50(L)*	Hastings (1989)

Gen = Generations of selection experiment. Realised heritability based on M = Mass or W = Within family selection method for increased body weight except where marked with L (L = Low body weight). * = Used same data set as in this study and the base population (P-lines) was highly selected.

The present study was undertaken to examine the body weight at 10 weeks of age from the base to 38 generations of selection in the P and P6-lines using REML analyses. As discussed in the previous chapters, animal model analyses with the complete relationship information back to the base population provide unbiased estimates of the genetic parameters, after accounting for the changes in additive genetic variance due to inbreeding and selection, assuming an inifinitesimal model. Out of the 38 generations, selection in the first 20 generations was practised on lean mass and thereafter on body weight at 10 weeks of age. Therefore, the univariate analysis of body weight from the first 20 generations may give biased estimates of genetic parameters due to selection on lean mass, as two traits are correlated. The univariate analysis assumes that the correlation between the traits under analysis and under selection is zero. An analysis including all the traits, i.e. a multivariate analysis, would be ideal as it accounts for the selection bias in the correlated traits. Such analyses are computationally very demanding, but a limited analyses are given in chapter 5.

The univariate analysis is computationally less demanding and gives an opportunity to examine body weight in greater detail. This study was undertaken with the following objectives: (a) to estimate variance components and genetic parameters by univariate REML analyses using the animal model for 10 week body weight in mice, from a divergent selection experiment, (b) to assess the change in genetic parameters as a consequence of selection, and (c) to investigate the high line, especially in the later

generations when it did not respond to selection.

4.2 MATERIAL AND METHODS

This analysis includes 10 week body weight data from P and P6-lines spread from generation 0 to 38 of selection. The P-lines (generations 0-20) had three replicates, namely replicate 1, 2 and 3, each of which contained a high, low and control line. Only one replicate was available the P6-lines (generations 21-38) which contained a high and low selected line and no control was maintained. Replicate 6 was produced by crosses among the three replicates of the P-lines within each selection treatment as detailed earlier. The details of number of records and litters in the data file and number of animals used in the pedigree files for various analyses of different lines and replicate are given in Table 2.1.

The REML univariate analyses fitting an Individual Animal Model were carried out with the infinitesimal model assumptions. The details of the model and assumptions are given in Chapter 2. The analyses of pairs of lines were carried out assuming heterogeneous variances (additive genetic and common litter environment) in addition to the usual analyses. All the analyses were carried out on log transformed data to reduce the scale effect. The random effects considered in the model were animal, common litter environment and residual environment. For the analyses of body weight in the P-lines, generations and replicates were fitted as fixed effects. The P6 and P+P6-lines were analysed

by fitting a sex x line interaction in addition to generations and replicates because body weight was recorded only on the males in P-lines and on both sexes in the P6-lines. Litter size at birth was included as a covariate in all the analyses. Each analysis was carried out including the complete pedigrees back to the base population relevent to the data set. The log likelihood curves for body weight are not presented here because the shape of curves for different lines and replicate in the P-lines were similar to that of the lean mass given in Figure 3.3. However, the standard errors for the estimates are given.

4.3 RESULTS

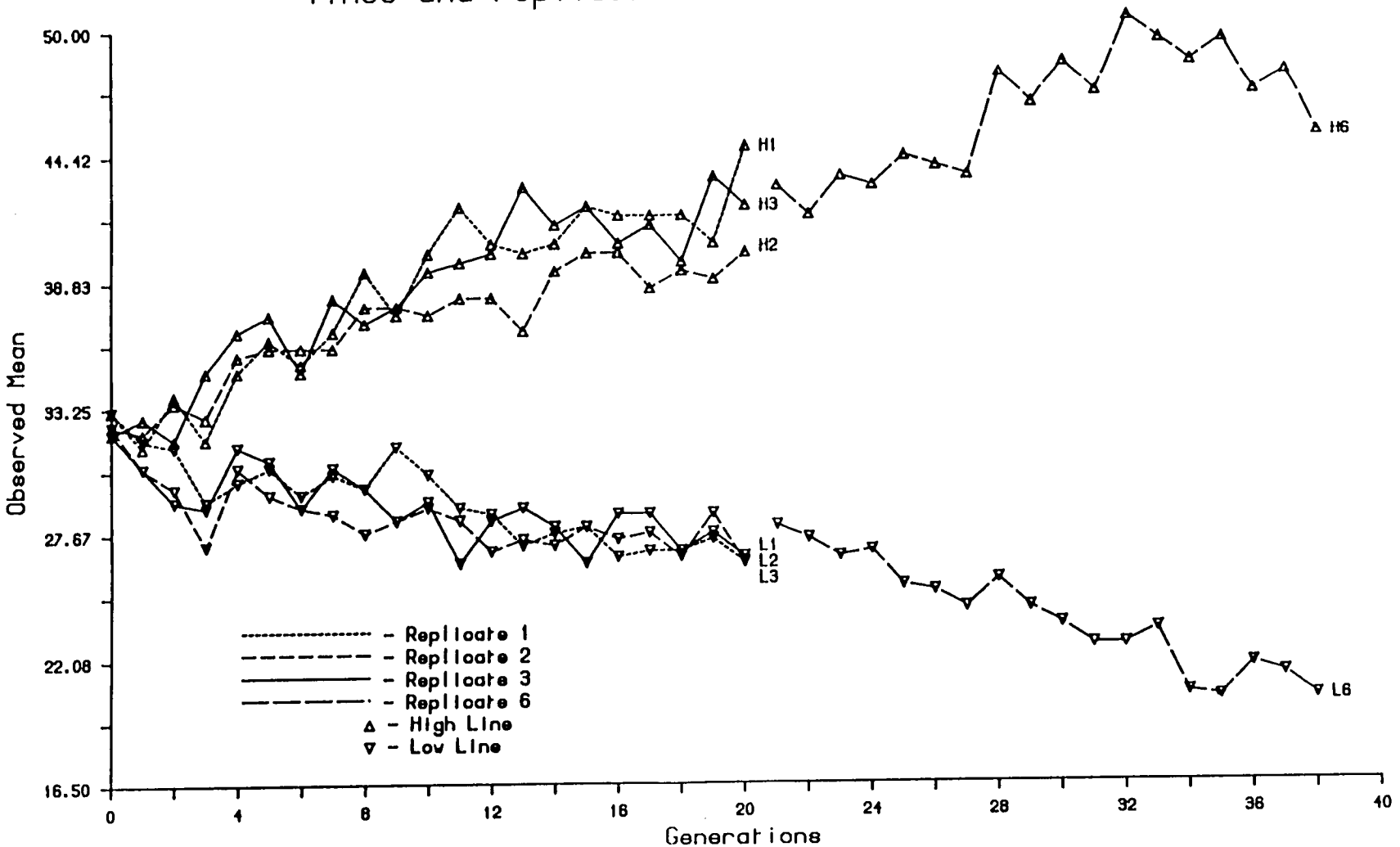
In all, 7949 records on 1768 litters and 9380 animals (Table 2.1, Chapter 2) from the high and low lines were used in the analysis of 10 week body weight from generations 0-38. The summary statistics are given in Table 4.2 and the generation means for high and low lines are plotted in Figure 4.1. The selected P-lines showed a substantial divergence in the 20^{th} generation. The high lines averaged over three replicates were heavier by 11.9g and low lines (averaged over three replicates) were lighter by 4.0g than the control lines mice in the 20^{th} generations; and in terms of phenotypic standard deviation units (σ_p), the differences were 4.4 and 1.5, respectively. Replicate 6 showed a divergence of 23.2g in the 38^{th} generation. There was no difference between the high lines means of generation 20 of P- and generation 38 of P6-Lines, which was the result of a decline in body weight after generation 32 (Figure 4.1). However, the low

Table 4.2: Mean, Standard Deviation (SD) and Coefficient of Variation (CV,%) for 10 Week Body Weight(g) in the P and P6 lines.

Line / Replicate	Over 20 generations* Mean	SD	CV	20th generation** Mean	SD	CV
High Lines:						
Replicate 1	37.27	5.20	14.0	44.85	4.18	9.3
Replicate 2	36.22	4.37	12.1	40.03	1.62	4.0
Replicate 3	37.47	5.12	13.7	42.37	4.98	11.7
Overall (Rep 1+2+3)	37.00	4.95	13.4	42.36	4.26	10.1
Replicate 6	42.51	5.99	14.1	42.10	6.60	15.7
Control Lines:						
Replicate 1	31.13	3.21	10.3	30.05	2.18	7.3
Replicate 2	32.29	3.36	10.4	30.18	2.06	6.8
Replicate 3	33.18	3.82	11.5	31.34	3.62	11.5
Overall (Rep1+2+3)	32.21	3.57	11.1	30.49	2.67	8.8
Low Lines :						
Replicate 1	29.80	3.39	11.4	26.43	1.83	6.9
Replicate 2	28.90	3.03	10.5	26.36	1.33	5.0
Replicate 3	29.43	3.19	10.8	26.80	2.77	10.3
Overall (Rep 1+2+3)	29.37	3.23	11.0	26.53	2.02	7.6
Replicate 6	21.81	3.57	16.4	18.90	2.33	12.3

* = Over 18 generations and ** = at the 38th generation for Replicate 6.

Figure 4.1: Generation means for 10 week body weight in different lines and replicates of mice.



lines (P6) showed a consistent decline up to 38 generations.

4.3.1 0 - 20 GENERATIONS DATA SET

The variance components and genetic parameter estimates are presented in Table 4.3 for log-transformed, 10 week body weight data on males. The estimates of heritability (h^2) and c^2 obtained from replicate 2 and 3 were similar. Replicate 1 indicated slightly different estimates of h^2 and c^2 as compared to the other two replicates. Although the differences among h^2 estimates were non-significant, the c^2 estimate from replicate 1 was significantly lower than that from replicates 2 and 3, judged by comparing the differences between the estimates with their standard errors.

The analyses of control, high and low lines individually revealed that the estimates of h^2 and c^2 from the control lines (0.51, 0.24) were in close agreement with those from each of the three replicates having control+high+low lines data, and estimates from control lines were close to the average of the three replicates. The analysis of high and low lines revealed substantially lower estimates of additive genetic variance (Va) and consequently lower heritabilities as compared to the control. The c^2 estimate in the high lines was higher (0.30) than the estimate from the control. The low lines had a lower estimate of common litter variance (Vc) than the control lines, although there was no difference in the c^2 estimates of the two lines because of a decline in both Vc and Vp (phenotypic variances) of the low

Table 4.3: Estimates of Variance Components and Genetic Parameters for 10 Week Body Weight in P and P6 lines

Line/ Replicate	Variance Components ($\times 10^{-2}$) V_a	V_c	V_e	V_p	h^2 ± SE	c^2 ± SE
P-Lines:						
Replicate 1 (C+H+L)	0.562	0.196	0.271	1.030	0.546 ±0.042	0.191 ±0.019
Replicate 2 (C+H+L)	0.490	0.280	0.251	1.021	0.480 ±0.043	0.274 ±0.028
Replicate 3 (C+H+L)	0.533	0.290	0.267	1.090	0.489 ±0.049	0.266 ±0.020
Control (Rep 1+2+3)	0.555	0.264	0.275	1.093	0.507 ±0.073	0.242 ±0.028
High (Rep 1+2+3)	0.379	0.320	0.369	1.068	0.355 ±0.074	0.300 ±0.024
Low (Rep 1+2+3)	0.374	0.209	0.315	0.898	0.416 ±0.068	0.233 ±0.032
C + H (Rep 1+2+3)	0.583	0.298	0.257	1.138	0.513 ±0.038	0.262 ±0.020
C + L (Rep 1+2+3)	0.455	0.247	0.286	0.988	0.461 ±0.040	0.250 ±0.022
H + L (Rep 1+2+3)	0.512	0.263	0.270	1.044	0.490 ±0.026	0.252 ±0.018
P6-Lines:						
High (Rep 6)	0.170	0.378	0.486	1.034	0.164 ±0.103	0.366 ±0.053
Low (Rep 6)	0.351	0.166	0.417	0.934	0.376 ±0.074	0.178 ±0.032
H + L (Rep 6)	0.367	0.281	0.412	1.060	0.346 ±0.029	0.265 ±0.023

C = Control, H = High, L = Low line, and Rep = Replicate.

lines. The possible reasons for the lower estimates of h^2 in high and low lines, discussed in section 3.3.2, Chapter 3, also apply in the case of body weight, hence that part is not repeated here. To overcome this problem, selected lines were analysed with controls. The estimates of h^2 and c^2 from control+low lines (0.46, 0.25) were slightly lower than the estimates from control+high data (0.51,0.26) but the differences were nonsignificant. The estimates from these analyses were also not significantly different from those obtained from control lines.

4.3.2 21-38 GENERATIONS DATA SET

Only one replicate (Replicate 6) was available from generation 21 to 38 for the high and low lines and there was no control line. The high, low and high+low lines were analysed with complete pedigrees from generation 0 to 38. The fixed effects fitted for the analyses of high and low lines were generations and sex; and for the high+low analysis were generations, sex and line. The h^2 and c^2 estimates (Table 4.3) in the high line were 0.16±0.10 and 0.37±0.05; and in the low line were 0.38±0.07, 0.18±0.03, respectively. When the high and low were analysed together, the estimates were 0.35±0.03 and 0.26±0.02, respectively.

Although the estimates of heritability were obtained using the data only from 21 to 38 generations, the estimates refer to the base population, as the complete pedigrees back to the base population are included in the analysis. The estimates of heritability in the base population using generations 21 to 38

were significantly lower than the estimates from the 0 - 20 generations data set of control, suggesting a decline in additive genetic variance of selected lines in the later generations.

4.3.3 ANALYSES WITH HETEROGENEITY OF BOTH ADDITIVE GENETIC AND COMMON LITTER ENVIRONMENT VARIANCES

The results given in section 4.3.1 and 4.3.2 were obtained assuming that the high, low and control lines have similar variances when analysing selected line plus control data. This may not be true when lines have undergone selection for many generations. Therefore data were reanalysed assuming that additive genetic and common environment variances differ between the high and low lines and results of these analyses are given in Table 4.4. The predicted responses in 10 week body weight for different lines and replicates are given in Figure 4.2

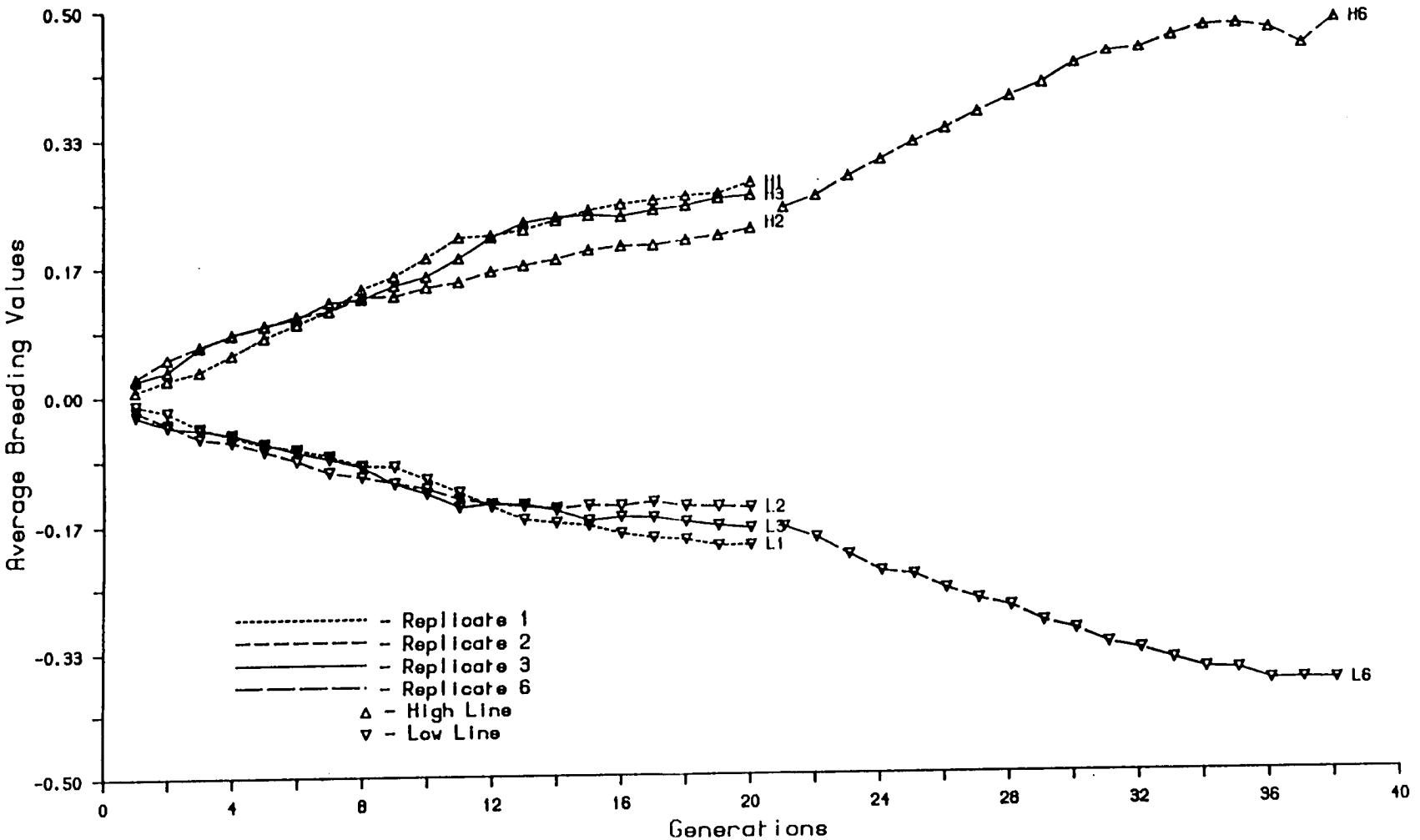
The analyses of the high+low lines data set using heterogeneity of variances (Va and Vc) were done by partitioning the total data into two groups, group 1 had data from the high lines and group 2 data from the low lines. They were analysed together to estimate the variance components of each group, and the details are given Chapter 2. The complete pedigrees back to the base population were included in each analysis, therefore the estimates of each group refer to the base population from which they originated.

The analysis of data from the high+low lines for the first 20

Table 4.4: Estimates of Variance Components and Genetic Parameters for 10 Week Body Weight. Analysis of High + Low Lines (P and P + P6 data), assuming heterogeneous additive genetic and common litter environment variances.

Group Description	h^2	c^2	Variance Components ($\times 10^{-2}$) V_p	V_a	V_c	V_e
P - Line:						
1 High Lines (0-20 generations)	0.502	0.264	1.149	0.577	0.303	0.269
2 Low Lines (0-20 generations)	0.474	0.235	0.926	0.439	0.218	
P + P6 - Lines:						
1 High Lines (0 - 29 generations)	0.433	0.276	1.062	0.460	0.293	0.308
2 Low Lines (0-29 generations)	0.448	0.221	0.934	0.419	0.207	
P + P6 - Lines:						
1 High Lines (0-38 generations)	0.385	0.305	1.135	0.437	0.346	0.352
2 Low Lines (0-38 generations)	0.409	0.214	0.935	0.383	0.200	

Figure 4.2: Predicted response to Selection in 10 week body weight of P & P6 Lines.
Parameters from the analysis with Heterogeneity of Additive Variance assumptions.



generations, where three replicates were available, revealed slightly lower estimates of h^2 and c^2 in the low lines (0.47, 0.24) as compared to the high lines (0.50, 0.26). The estimates from the high lines were similar to those obtained from controls as was expected because all lines originated from the same base population. When the data from generation 21 to 29 of the P6-lines were added to the previous data set, the estimates of heritability in the high and low lines decreased to 0.43 and 0.45, respectively, indicating a decline in the additive genetic variance in the 21 to 29 generation data set of replicate 6. The estimate of c^2 was increased in the high lines and decreased in the low line. A similar trend was observed when the complete data set of 0 to 38 generations was analysed. This indicates a further decrease in the additive genetic variance of later generations (30-38 generation) of replicate 6. The environmental variance (Ve) showed a consistant increase over later generations of replicate 6.

However, the predicted responses (Figure 4.2) in 10 week body weight using the parameters of the high and low lines estimated in this analysis agreed very well with the observed responses (Figure 4.1).

4.4 DISCUSSION AND CONCLUSIONS

As discussed earlier, the analyses were carried out by Restricted Maximum Likelihood using an Animal Model with litters as an additional random effect. In all the analyses the complete

pedigrees back to the base population were fitted, thus all the estimates refer to the base population. The use of the complete relationship matrix and complete data back to the base population with the animal model should account for the inbreeding and linkage between unlinked loci due to selection. The high, control and low lines originated from the same base population, hence the estimates of genetic parameters from the selected and control lines are expected to be the same if the infinitesimal model holds true for this data set. This appears to be the case from the results of three replicates in Table 4.3. The estimates from control, high and low separately did not agree with each other. However, when the selected plus control lines were analysed together, the estimates of heritability and c^2 from high and low were quite similar. But these analyses assume homogeneous variance in the control and selected lines, which is not realistic.

Replicate 6 (21 to 38 generations) showed very low estimates of h^2 from the analyses of the high, low and high+low lines, indicating a considerable decline in the additive genetic variance. Another possible explanation could be that in the analysis of high and low lines (Replicate 6), the observations were considered from generation 21 onwards, although the complete back pedigree was included. This omits the records from previous generations on which selection was based, and hence may give biased estimates. A similar observation has also been made in a simulation study by Van der Werf and De Boer (1990). They concluded that the estimate of σ_a^2 is unbiased when all data and

relationships are included in the analysis. Omitting data from selected parents causes biased estimate of σ_a^2 due to not accounting for all linkage disequilibrium.

To reduce such bias, the data from previous generations were included and reanalysed with the assumption that the selected lines and control lines have different variances (Table 4.4). The estimates of h^2 in high and low lines increased substantially over the previous analysis (Table 4.3) indicating that the analysis of a selected line alone underestimates the heritability. However, the parameters obtained with the heterogeneous variance assumptions for high and low lines were not different from the estimates obtained by control+high and control+low analyses in the Table 4.3. But analysis of selected lines with heterogeneous variance assumptions may help in the situations where no control line is maintained.

Further addition of data from the later generations of replicate 6 reduced the estimate of additive genetic variance, indicating a decline of additive genetic variance (Va) in the later generations. If the infinitesimal model held, Va is expected not to change when considering increasing number of generations. The possible reasons for a decline in additive genetic variance in a selection experiment are: (a) Linkage between unlinked loci caused by selection, (b) Inbreeding, and (c) Change in gene frequency. Reasons (a) and (b) are taken into account in the animal model analysis by using the complete relationship matrix. Hence the change in gene frequency remains

the most likely cause of a drop in genetic variance. In such circumstances the assumption of the infinitesimal model does not seem to be true. There could be some gene(s) with large effect governing 10 week body weight.

The c^2 estimates were quite different in the high and low lines. The c^2 estimates showed an increasing trend in the high lines and decreasing trend in the low lines with the progress of selection over the generations. As discussed in Chapter 3, body weight and litter size have a positive and moderate genetic correlation (Chapter 6) thereby large mothers produce large litters with heavy pups at birth and vice versa for small dams. The maternal environmental factors, e.g. milk production and intra-uterine environment, may lead to more differences in growth of different litters and thus an increase in the estimate of common litter environment variance in high lines. However, these differences are expected to be less in low lines due to smaller litters.

The observed response (Figure 4.1) revealed a decline in 10 week body weight of the high line (Replicate 6) from generation 32 onwards, although the low line was responding to the selection. The genetic parameters from the 0 to 38 generation data set were used to predict the breeding values. The predicted responses in breeding values are given in Figure 4.2. The trends in predicted responses were observed to be similar to that of observed responses and later generations of high (Rep 6) showed a decline in the predicted breeding values. The observed and predicted

Figure 4.3: Observed and predicted divergence between the high and low lines for 10 week body weight (P & P6-lines).

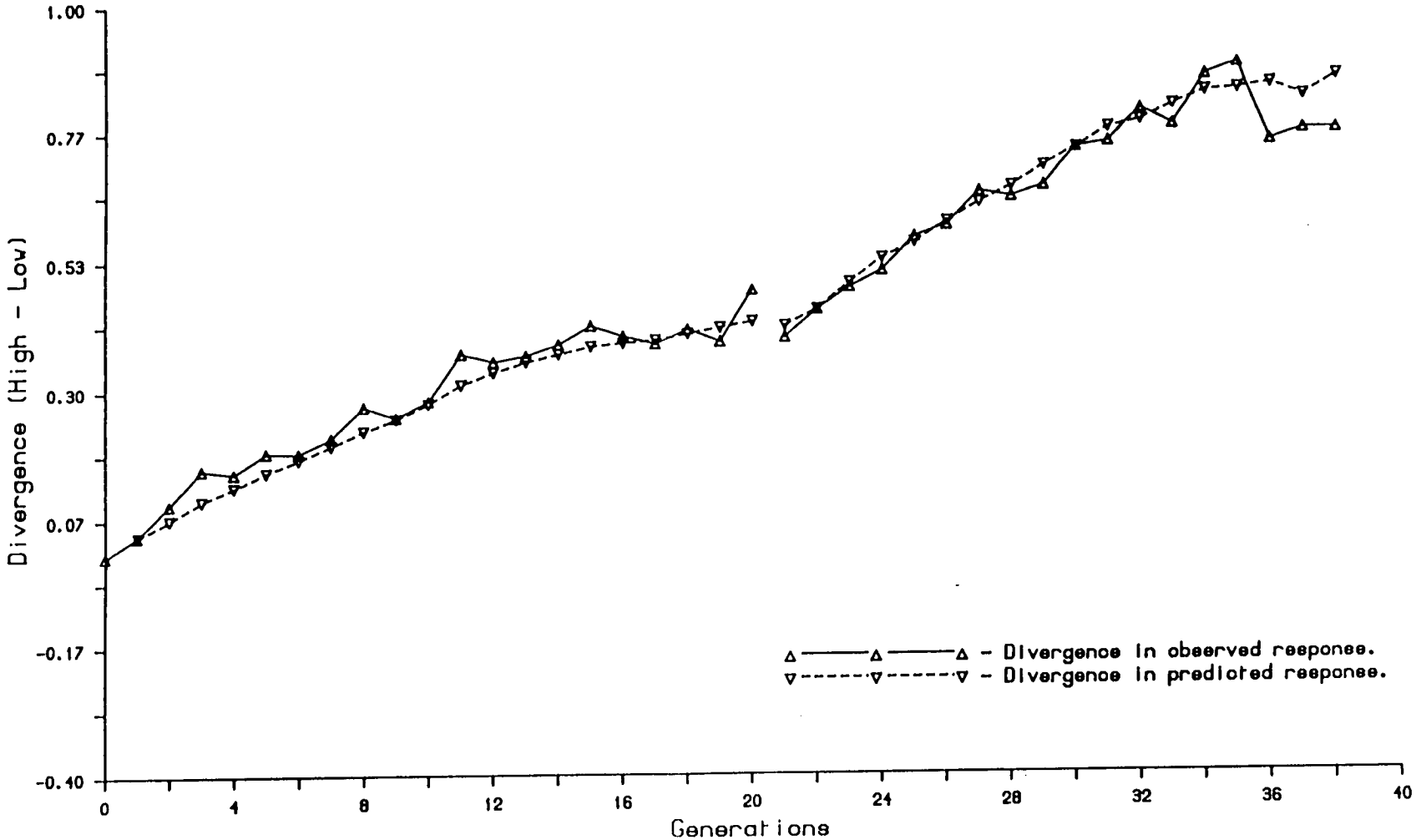


Figure 4.4: Selection Differentials for lean mass in P-lines and 10 week body weight in P6-lines.

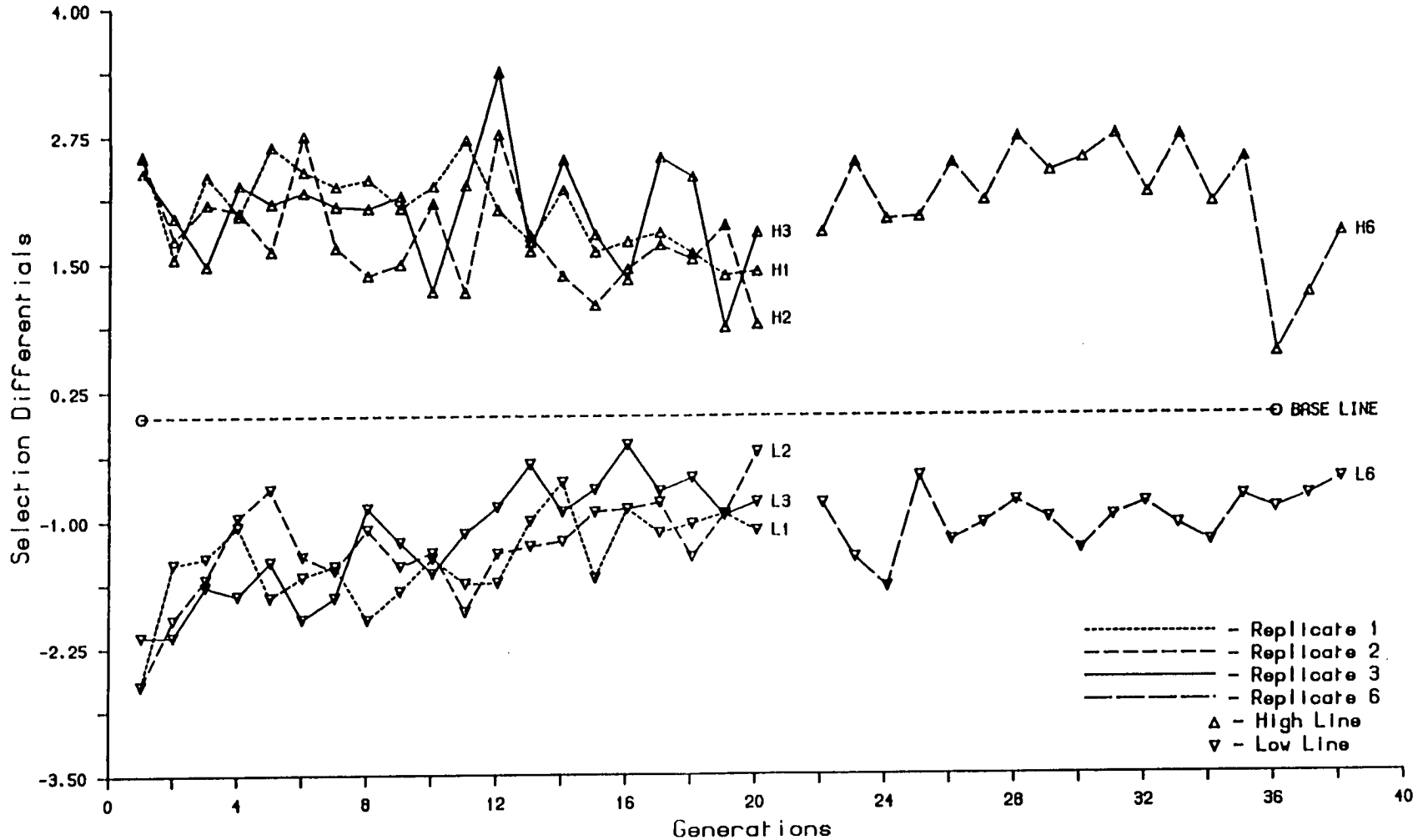
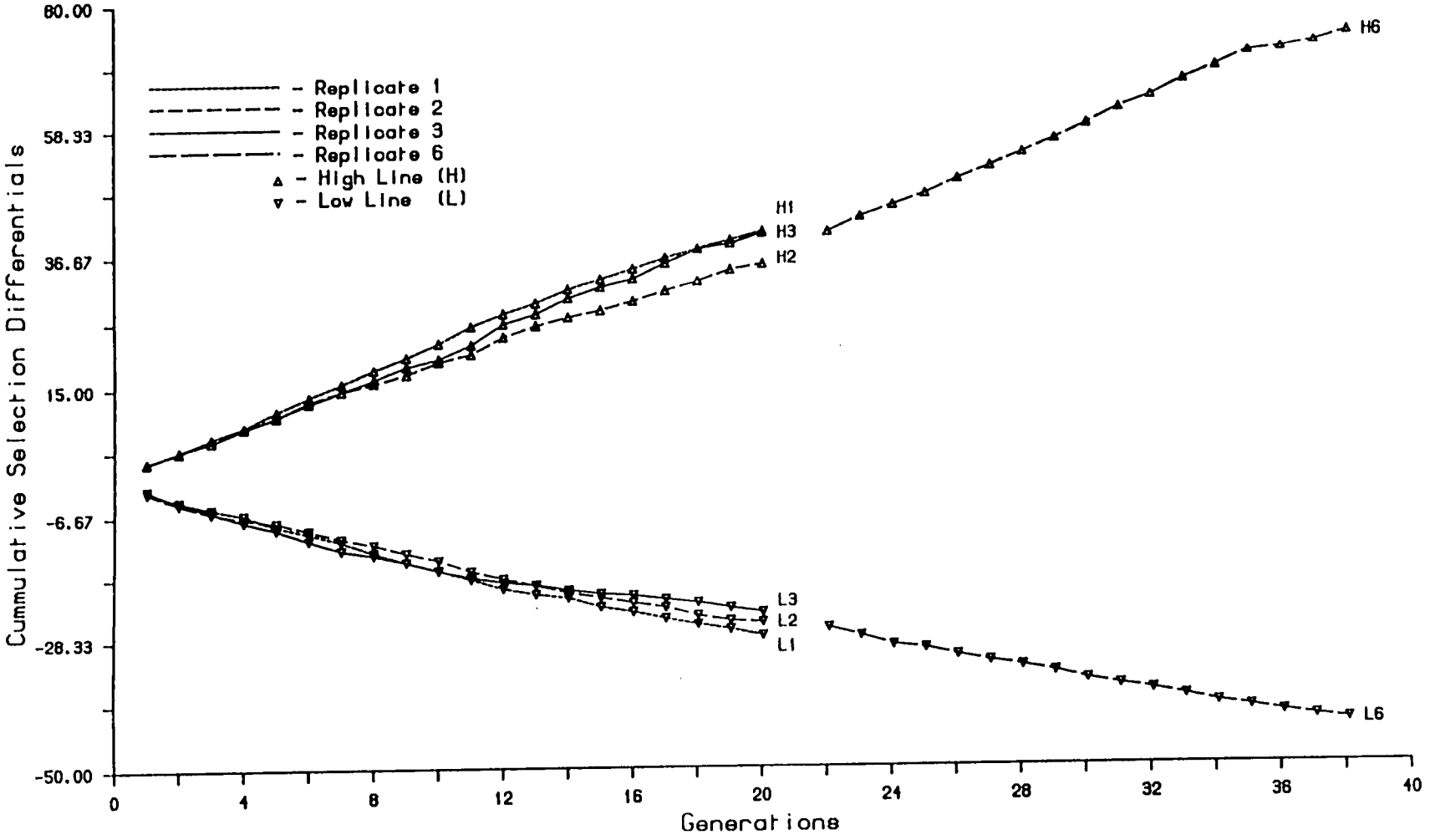


Figure 4.5: Cumulative selection differentials for lean mass (P-Lines) and 10 week body weight (P6-Lines).



divergences between high and low lines, given in Figure 4.3, showed a steep decline in the observed divergence in generation 36 and thereafter not much increase. The divergence in predicted response was higher in generations 36 to 38 as compared to the observed but did not show an increasing trend.

This suggests that the population is approaching a selection limit for increased body size. The fixation of genes due to a selection may cause exhaustion of genetic variation leading to selection limit. The results in the previous sections, however, showed the presence of a substantial amount of additive genetic variation in the high line (Replicate 6), so exhaustion of genetic variation seems unlikely. Another possibility could be that natural selection acted against the large body size because extreme animals were less fit. Roberts (1967) and Barria and Bradford (1981) have reported a negative association between extremely large size and fertility. In such a situation it is possible that the reduced fertility may have reduced the selection differential. The selection differentials and cumulative selection differentials for the high and low lines have been presented in Figure 4.4 and 4.5, respectively. The selection differential in each generation of the high line (Replicate 6) did not show much change except in generation 36, where it was reduced substantially and did not recover to the level of generation 35 in the later generations. However, cumulative selection differentials did not show reduction in the high (P6-line). Therefore, selection differential does not seems to be a limiting factor for response in the high P6-line.

From this analysis two conclusions can be drawn. (a) The decline in the additive genetic variance over 38 generations could not be explained by the infinitesimal model assumptions. One possibility for the reduction of variance could be the presence of gene(s) with large effect. (b) The decline in the response of the high line in later generations does not seem to be due to lack of additive genetic variation and selection differential.

CHAPTER 5

MULTIVARIATE ANALYSIS OF LEAN MASS, BODY WEIGHT AND COMPOSITION TRAITS

5.1 INTRODUCTION.

Selection experiments to change growth in mice invariably result in correlated response in body weight and/or body composition traits. Selection for lean mass leads to changes in body weight in the direction of selection (Sharp *et al.*, 1984, Bishop and Hill, 1985, Hastings and Hill, 1989, and see Chapter 4). McLellan and Frahm (1973) selected mice on the weight of the hind leg muscle (HLM) system at 12 weeks of age to increase lean mass, and observed a realised genetic correlation of HLM with 9- and 12-week body weight of 0.59±0.20 and 0.77±0.11, respectively. Contrary to this, the weight of hind carcass as a percentage of body weight at 12 weeks of age is negatively correlated with body weight (realised genetic correlation -0.61±0.09; Eisen,1987). This negative genetic correlation is expected as the percentage of hind carcass weight is derived as a ratio trait with body weight in the denominator.

Body composition, particularly fat percentage, changes during selection for growth. The results show significant differences among studies, perhaps indicating the presence of some genetic strain differences. Fowler (1958) reported that in two strains selected for six week body weight, one increased in fat percentage and the other showed relatively little change. Similarly, in three lines selected for postweaning weight gain by Biondini *et al.* (1968), two lines increased in fat percentage while the third line showed no change. Lang and Legates (1969) noted no change in fat deposition in the lines selected for large six week body

weight, while Timon *et al.* (1970) found a significant increase in the lines selected for post-weaning weight gain. Robinson and Bradford (1969) showed an increase in fat percentage due to selection for post-weaning weight gain. Selection for feed consumption also resulted in an increase in percent fat (Sutherland *et al.*,1974). However, Hastings and Hill (1989) observed a small effect on body composition in the lines selected for food intake corrected for body weight and P-lines selected for lean mass (same line as used in this study).

The realised genetic correlation (r_{gr}) of fat proportion (Gonadal fat pad weight/body weight) with the predicted lean mass at 10 weeks of age (Body weight - 8 x Gondal fat pad weight) has been observed to be low (r_{gr}=0.12, Sharp *et al.*,1984). Eisen (1987a,b) selected lines at 12 weeks of age for epididymal fat pad weight (EFPW) and hind carcass weight (HCW) as a percentage of body weight. The r_{gr} between EFPW and HCW was -0.67±0.05. The EFPW was negatively correlated with the lean index (BW - 8x2xright side epididymal fat pad weight at 12 weeks of age), the estimate of r_{gr} being -0.41±0.03. This estimate of r_g is considerably different from the findings of Sharp *et al.* (1984). The EFPW in Eisen's study had a realised genetic correlation of 0.57±0.05 with body weight at 12 weeks of age.

The univariate REML analysis of body weight (Chapter 4) gives more accurate estimates of the genetic parameters than the realised heritability estimation method; as discussed earlier, it takes into account the changes in the additive genetic variance

and covariance structure among all the individuals. However, it may give biased estimates of the genetic parameters for body weight due to selection on lean mass, as the two traits are correlated. The univariate analysis of body weight assumes a zero correlation with the trait under selection, and the same is the case for univariate analyses of the body composition traits. A Multivariate REML analysis would reduce the selection bias in the estimates by including all the traits in the model. Therefore, the objectives of this study were to use Multivariate Restricted Maximum Likelihood (REML) method: (a) to obtain more accurate estimates of genetic parameters for body weight and body composition traits, which are not under direct selection but are correlated to the directly selected trait, lean mass, and (b) to estimate correlations between different components of the lean mass index (LM = BW - 8 x Gonadal fat pad weight).

5.2 MATERIAL AND METHODS.

The data on lean mass, body weight and gonadal fat pad weight were available only up to generation 20, after which only body weight was recorded. Therefore a multivariate analysis was carried out for the 20 generation P-lines, using untransformed data sets containing only two traits for computational convenience. The body weight and gonadal fat pad weight data were used in the analysis as they were relatively less correlated than the body weight and lean mass. The (co)variances for lean mass were calculated from (co)variances of body weight and gonadal fat pad weight as the lean mass is a linear combination of these two

traits. To assess the change in the proportion of gonadal fat pad weight to body weight as a consequence of selection on lean mass, the correlations between the two traits were also calculated from the (co)variances obtained previously. The details are given in Chapter 2, section 2.4.2.

5.3 RESULTS

The genetic (r_g) and phenotypic (r_p) correlation and heritability estimates from different data sets are given in Table 5.1 and correlations due to common litter environment and residual environment along with c^2 estimates are given in Table 5.2. The direct and correlated responses of different traits are given in Figures 5.1, 5.2 and 5.3 for the high, low and control lines, respectively; see also Figures 3.1, 3.2 and 4.1 in the previous chapters. The univariate estimates for heritability and c^2 are also given in Tables 5.1 and 5.2, respectively for the comparison. The univariate estimates for the gonadal fat pad weight were estimated only for comparison purposes. However, genetic parameters for gonadal fat pad weight were almost similar to the estimates of lean mass and body weight, and hence are not included in the previous chapters.

The results given in Table 5.1 reveal that the heritability estimates were similar from univariate and multivariate analyses for all the traits in different data sets. The variance components were also the same from the two analyses, and hence are not given here. The proportion of gonadal fat pad weight (FP) was

Table 5.1: Estimates from Multivariate REML analysis for Lean Mass (LM), Body Weight (BW), Gonadal Fat Pad Weight (FW) and ratio of Gonadal fat to body weight (FP) at 10 weeks of age. Within each multivariate analyses, heritabilities are on diagonals, genetic correlations on upper off-diagonals and phenotypic correlations on lower off-diagonals. Heritability estimates from the univariate analyses are also given for comparison. Data set for 20 generations, untransformed.

Data Set	Traits	Multivariate LM	BW	FW	FP	Univariate (h^2)
Control	LM	0.516	0.943	0.220	-0.099	0.504
	BW	0.934	0.503	0.531	0.237	0.511
	FW	0.211	0.545	0.437	--	0.435
	FP	-0.083	0.278	--	0.436	--
Control+High	LM	0.485	0.932	0.183	-0.100	0.500
	BW	0.938	0.471	0.528	0.268	0.474
	FW	0.242	0.564	0.504	--	0.503
	FP	-0.047	0.303	--	0.522	--
Control+Low	LM	0.510	0.928	0.171	-0.121	0.512
	BW	0.921	0.484	0.526	0.257	0.483
	FW	0.199	0.565	0.437	--	0.436
	FP	-0.072	0.323	--	0.446	--

Table 5.2: Estimates from Multivariate REML analysis for Lean Mass (LM), Body Weight (BW), Gonadal Fat Pad Weight (FW) and ratio of Gonadal fat to body weight (FP) at 10 weeks of age. Within each multivariate analyses, c^2 are on diagonals, correlations due to common litter (r_c) on upper off-diagonals and environmental correlations (r_e) on lower off-diagonals. c^2 estimates from the univariate analyses are also given for comparison. Data set for 20 generations, untransformed.

Data Set	Traits	Multivariate LM	BW	FW	FP	Univariate (c^2)
Control	LM	0.195	0.939	0.462	0.239	0.201
	BW	0.921	0.248	0.739	0.558	0.245
	FW	-0.007	0.383	0.280	--	0.282
	FP	-0.289	0.106	--	0.243	--
Control+High	LM	0.234	0.955	0.495	0.242	0.240
	BW	0.937	0.275	0.730	0.519	0.274
	FW	0.120	0.459	0.250	--	0.251
	FP	-0.185	0.170	--	0.213	--
Control+Low	LM	0.207	0.931	0.423	0.196	0.206
	BW	0.903	0.253	0.723	0.539	0.254
	FW	0.069	0.490	0.257	--	0.259
	FP	-0.187	0.252	--	0.228	--

Figure 5.1: Direct response in Lean Mass (LM) and correlated responsee in Body Weight (BW), Gonadal Fat Pad Weight (GFPW) and Percent GFPW/BW for High P-Line.
(LM ———, BW ----------, GFPW o----o----o, Percent GFPW/BW o—o—o)

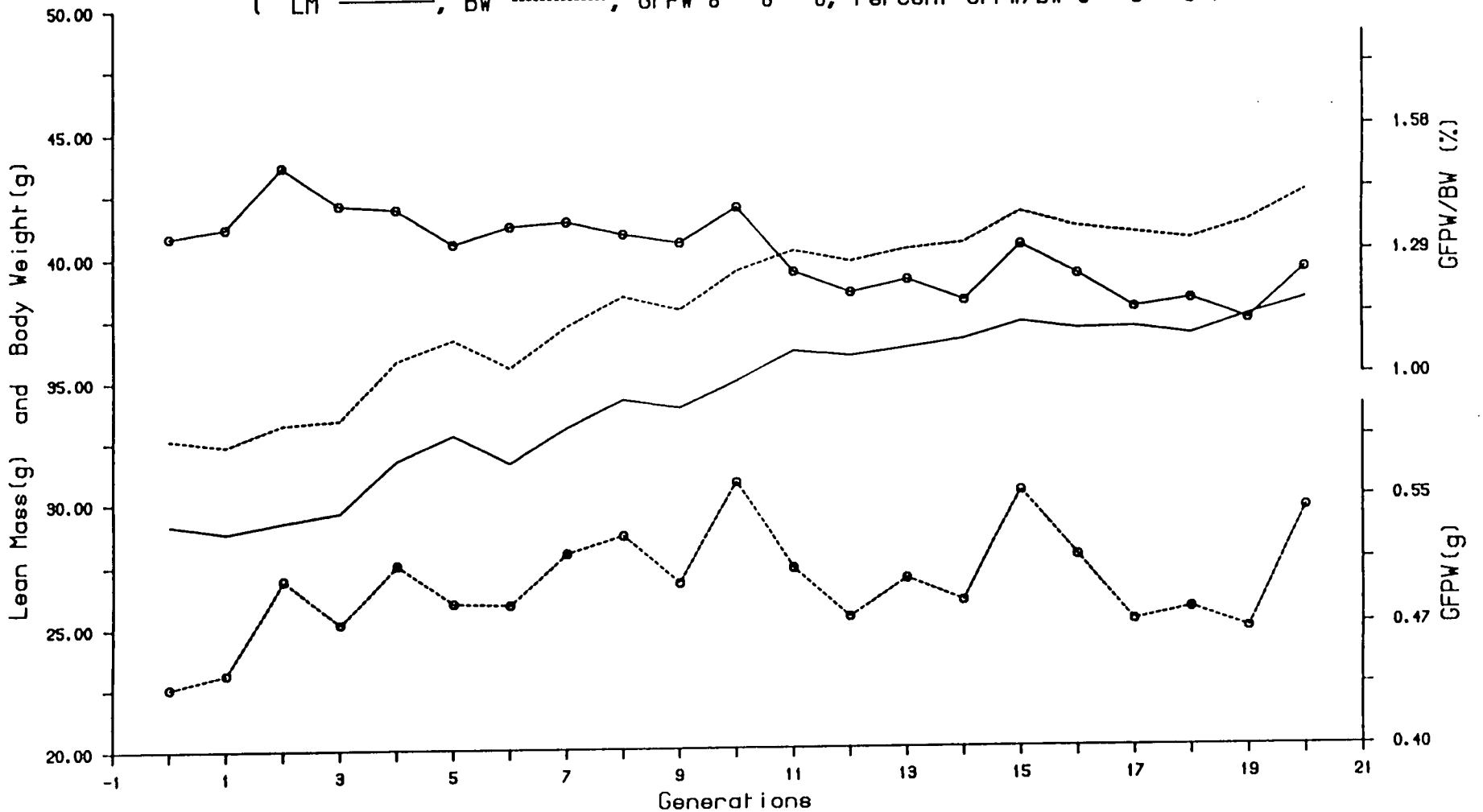


Figure 5.2: Direct response in Lean Mass (LM) and correlated responses in Body Weight (BW), Gonadal Fat Pad Weight (GFPW) and Percent GFPW/BW for Low P-Line.
(LM ———, BW ----------, GFPW o----o----o, Percent GFPW/BW o—o—o)

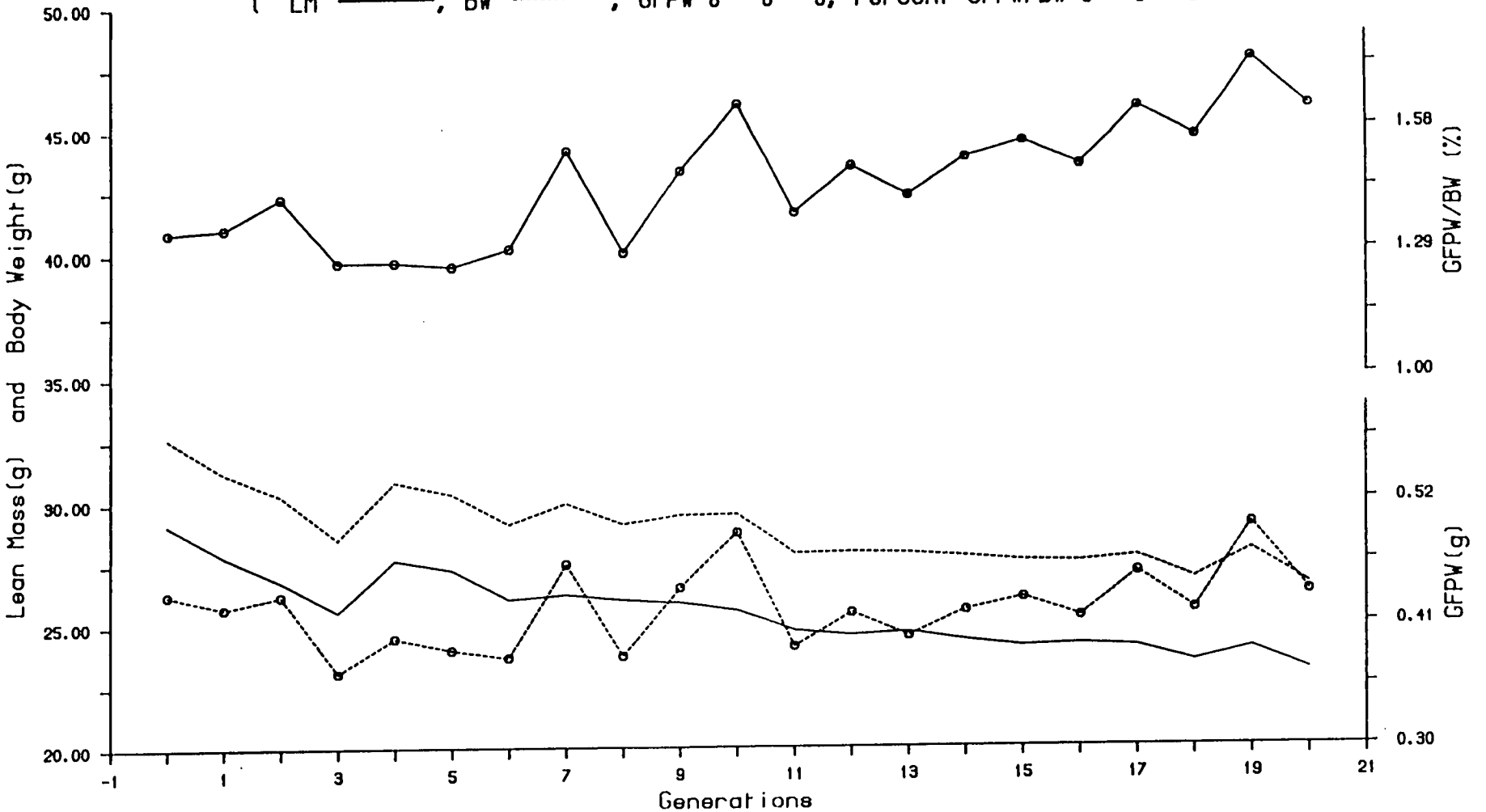
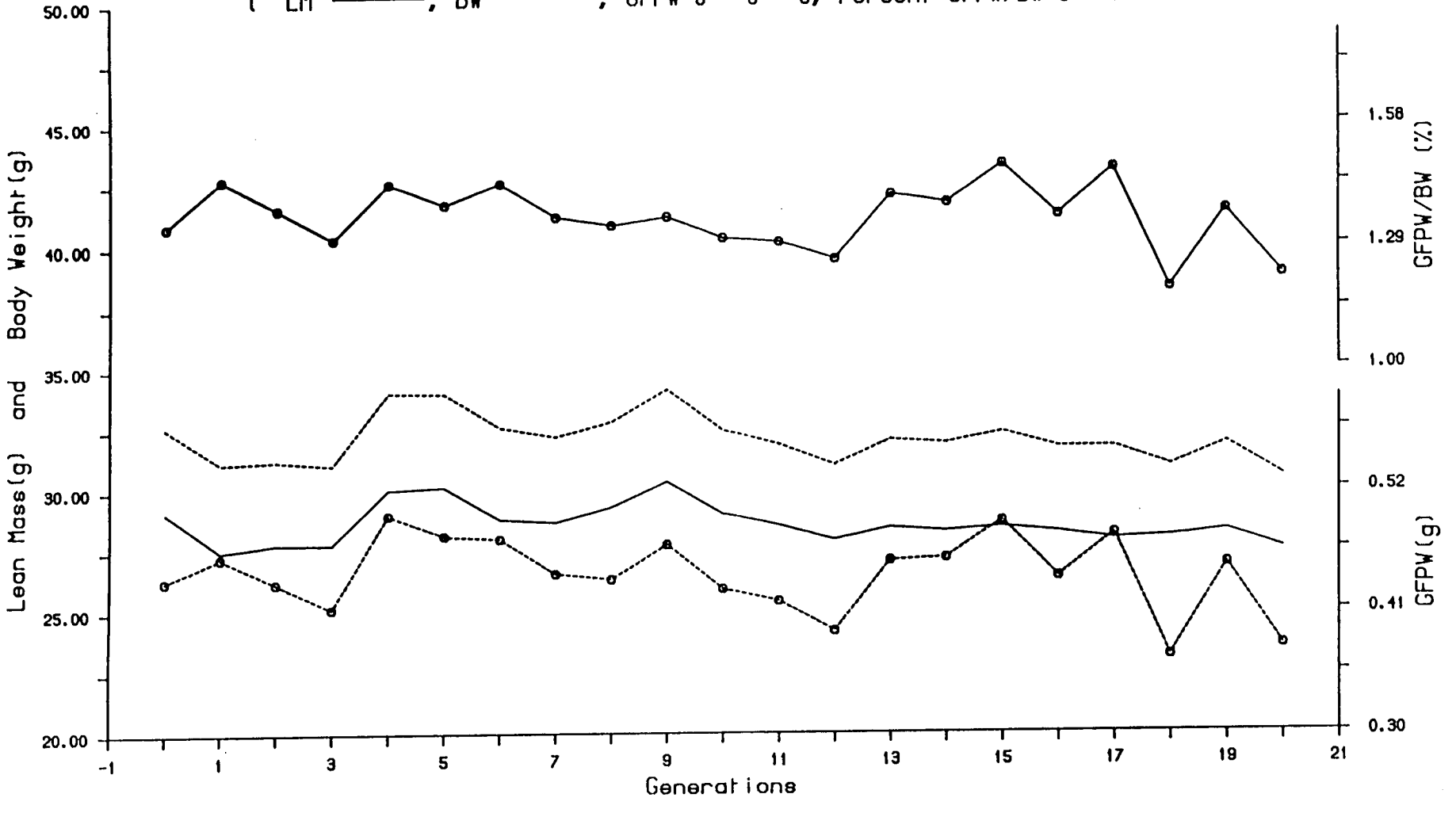


Figure 5.3: Direct response in Lean Mass (LM) and correlated responses in Body Weight (BW), Gonadal Fat Pad Weight (GFPW) and Percent GFPW/BW For Control P-Line.
(LM ———, BW ----------, GFPW o----o----o, Percent GFPW/BW o—o—o)



not analysed by the univariate method. The estimates of (co)variances for this trait were calculated from the lean mass (LM) and gonadal fat pad weight (FW) (co)variances obtained by the multivariate analysis. The estimates of heritability for FP ranged from 0.44 to 0.52 in different data sets, similar to the heritability estimates of LM, BW and FW.

The genetic (r_g) and phenotypic (r_p) correlations between LM and BW were positive and very high (more than 0.9) in all the data sets. A consistently similar trend was also observed for direct and correlated responses in LM and BW of high and low lines (Figures 5.1 and 5.2).

The estimates of r_g and r_p between LM and FW were also positive but low in magnitude in all the data sets. The estimates of r_p were higher than the estimates of r_g in the control + High and control + low lines because of positive residual environmental correlations (r_e). Figure 5.1 reveales a positive and small correlated response in FW of the high lines whereas it was negligible in the low lines (Figure 5.2), which was the objective for development of the lean growth lines. This was further evidenced by negative genetic and phenotypic correlations of LM with FP. The observed correlated responses in FP indicated a steady decline in high and increase in low lean growth lines which are in accordance with the genetic correlations between LM and FP. However, the correlation coefficients (r_g and r_p) of BW with FW and FP were higher than the estimates with LM and ranged from 0.53 to 0.57 and 0.24 to 0.32, respectively.

The c^2 estimates (Table 5.2) were similar from the multivariate and univariate analyses for all the traits in different data sets. The c^2 estimates for FP from multivariate (co)variances ranged from 0.21 to 0.24 in different data sets, similar to the c^2 estimates of LM, BW and FW. The residual (r_e) and common litter (r_c) environment correlations between LM and BW were very high (>0.9). The estimates of r_c between LM and FW ranged from 0.42 to 0.46 and between BW and FW ranged from 0.72 to 0.74 and the corresponding r_e ranged from -0.01 to 0.12 and 0.38 to 0.49, respectively. The estimates of r_e of FP with LM were negative.

5.4 DISCUSSION AND CONCLUSIONS

The heritability and c^2 estimates obtained by the univariate and multivariate analyses are almost the same for all the traits. Similar estimates from the two analyses were expected for lean mass (LM) as selection was practised on this trait, and thus the univariate analysis should give unbiased estimates for its genetic parameters.

Similar estimates of genetic parameters from the univariate and multivariate analyses were also obtained for body weight (BW) and gonadal fat pad weight (FW) at 10 weeks of age, although selection was on lean mass. The genetic and phenotypic correlations were similar in LM, BW and FW traits for all the data sets, as were the heritability estimates. Therefore, it is likely

that the univariate analysis gives unbiased estimates of genetic parameters. This can be shown as follows:

Consider regression of traits 1 and 2 (Y_1, Y_2) of an offspring on these traits of a parent (X_1, X_2). The prediction equations are:

$$Y_1 = b_{11} X_1 + b_{12} X_2 \qquad \ldots (1)$$

$$Y_2 = b_{21} X_1 + b_{22} X_2 \qquad \ldots (2)$$

The regression coefficient matrix can be written as $\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}$ from $\mathbf{Pb} = \mathbf{G}$. The elements of the matrices are:

$$\begin{bmatrix} \sigma^2_{p1} & r_p\sigma_{p1}\sigma_{p2} \\ r_p\sigma_{p1}\sigma_{p2} & \sigma^2_{p2} \end{bmatrix} \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} = \tfrac{1}{2} \begin{bmatrix} \sigma^2_{a1} & r_g\sigma_{a1}\sigma_{a2} \\ r_g\sigma_{a1}\sigma_{a2} & \sigma^2_{a2} \end{bmatrix}$$

or

$$\begin{bmatrix} \sigma^2_{p1} & r_p\sigma_{p1}\sigma_{p2} \\ r_p\sigma_{p1}\sigma_{p2} & \sigma^2_{p2} \end{bmatrix} \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} = \tfrac{1}{2} \begin{bmatrix} h^2_1\sigma^2_{p1} & r_g h_1\sigma_{p1} h_2\sigma_{p2} \\ r_g h_1\sigma_{p1} h_2\sigma_{p2} & h^2_2\sigma^2_{p2} \end{bmatrix}$$

and if $h^2_1 = h^2_2 = h^2$ and $r_g = r_p$, then

$$\begin{bmatrix} \sigma^2_{p1} & r_p\sigma_{p1}\sigma_{p2} \\ r_p\sigma_{p1}\sigma_{p2} & \sigma^2_{p2} \end{bmatrix} \begin{bmatrix} b_{11} & b_{12} \\ b_{21} & b_{22} \end{bmatrix} = \tfrac{1}{2} \begin{bmatrix} h^2\sigma^2_{p1} & r_g h^2\sigma_{p1}\sigma_{p2} \\ r_g h^2\sigma_{p1}\sigma_{p2} & h^2\sigma^2_{p2} \end{bmatrix}$$

$$\mathbf{G} = \tfrac{1}{2}h^2\,\mathbf{P} \qquad \ldots . (3)$$

$$\mathbf{b} = \mathbf{P}^{-1}\,\mathbf{G} = \tfrac{1}{2}\mathbf{P}^{-1}\mathbf{P}\,h^2 = \tfrac{1}{2}h^2\,\mathbf{I} \qquad \ldots (4)$$

As per the equation (4), $b_{11} = b_{22} = \frac{1}{2}h^2$ and $b_{12} = b_{21} = 0$, therefore the prediction equations 1 and 2 can be written as:

$$Y_1 = \tfrac{1}{2}h^2 X_1 \text{ , and}$$

$$Y_2 = \tfrac{1}{2}h^2 X_2$$

Therefore, when **G** is proportional to **P** of the form $\mathbf{G} = h^2 \mathbf{P}$, the prediction of one trait depends on that trait only. In this case regression of Y_i on X_i gives an unbiased estimate of the heritability. Perhaps a similar result holds for the analysis of this selection experiment where genetic prediction of one trait depends only on that trait. In such a situation, the multivariate analysis may give estimates of genetic parameters similar to those from the univariate analysis.

The genetic and phenotypic correlations between LM and BW were positive and very high, indicating that the faster growing and heavier mice have more lean tissue than the slow growing and lighter mice. The genetic and phenotypic correlations between LM and FW were positive and low, the estimates were around 0.18 in the selected lines. There was a very small correlated response in FW of the selected lines (Figures 5.1 and 5.2). The realised genetic correlation between LM and FW was also calculated from the divergence between the high and low lines. The genetic parameters used here in the calculations are taken from the multivariate analysis and converted to within family estimates (h^2_w, σ^2_w).

Lean Mass (LM)	Gonadal Fat Pad Weight (FW)
Direct Response = 15.0151	Correlated Response = 0.1025
Va = 4.3951, Ve = 2.4645	Va = 0.0106, Ve = 0.0068
$h^2_w = 0.4714$; $h_w = 0.6866$	$h^2_w = 0.4380$; $h_w = 0.6618$
$\sigma^2_w = 4.6621$; $\sigma_w = 2.1592$	$\sigma^2_w = 0.0121$; $\sigma_w = 0.1100$

For FW:

$$\text{Correlated response} = i.r_a.h_{w(FW)}.h_{w(LM)}.\sigma_{w(FW)}$$

$$0.1025 = i.r_a.(0.04998)$$

For LM:

$$\text{Direct Response} = i.h^2_{w(LM)}.\sigma_{w(LM)}$$

$$15.0151 = i.(1.0178)$$

$$r_a = 0.139$$

Where, i = selection intensity, r_a = genetic correlation, h^2_w = within family heritability (= ½ Va / (½ Va + Ve)), σ^2_w = within family variance (= ½ Va + Ve), and σ_w = within family standard deviation.

The realised genetic correlation estimate between lean mass and gonadal fat pad weight was 0.139, which is slightly lower than the estimate from the multivariate analysis. The low genetic correlation and correlated response in gonadal fat weight are due to the negative weighting given to the gonadal fat pad weight in the lean mass index used for selection. Negative phenotypic and genetic correlations, though low, between LM and FP lead to an increase in body weight as the gonadal fat pad weight remained more or less unchanged. This suggests that faster growing lean mice have little tendency to put on gonadal fat but its proportion in the body decreases. Contrary to this observation, Sharp *et al.* (1984) observed a positive and low genetic correlation between LM and FP from the 10 generations of this data set, perhaps due to a lesser number of generations of selection.

The genetic correlation of FW with BW was relatively high

(r_g=0.5) as compared to its correlation with LM. The index of lean mass (BW-8xFW) might indicate that FW has a large weight as compared to the body weight, but if this index is considered in terms of standard deviation units (within family) i.e. $(LM/\sigma_w LM)2.16 = (BW/\sigma_w BW)5.80 - 8x(FW/\sigma_w FW)0.11$, where σ_wLM=2.16, σ_wBW=5.80 and σ_wFW=0.11, body weight has about 2.3 times more weighting than FW. This perhaps explains a large change in BW without much change in FW and the considerably different genetic correlations of FW with LM and BW. The genetic and phenotypic correlations of BW with FP are positive and moderate, suggesting that selection on body weight alone (as in the P6-Line after 20 generations) is likely to increase the proportion of fat in the body. This does not agree with the literature reports. McCarthy (1982) in his review from several selection experiments concluded that there is relatively little change in the percentage of fat when selected lines of body weight are compared at the age of selection. In the P-lines, Hastings and Hill (1989) analysed the proportion of total fat in the gonadal fat pad weight at generation 20 and observed little difference between the high and low lines.

The common litter environmental correlations (r_c) were positive and medium to high in magnitude. This suggests that because of good maternal environment or maternal nourishment the mice have higher amount of lean tissue and are heavy at 10 weeks of body weight with a tendency to increase the gonadal fat pad weight and proportion of fat in the body. However, the negative residual environmental correlation (r_e) and genetic correlation between LM

and FP gives a negative phenotypic correlation between the two traits, checks the increase in FP and leads to a decline in the proportion of fat in the body.

CHAPTER 6

GENETIC PARAMETERS FOR LITTER SIZE USING UNIVARIATE AND BIVARIATE ANALYSES

6.1 INTRODUCTION

Reproductive performance is one of the crucial factors determining profitability in many animal production systems. Therefore, genetic control of reproductive traits and their inter-relationships with other economic traits are essential to the animal breeders. The mouse has been used extensively as a model to understand the basic genetic and physiological mechanisms involved in the traits of economic importance in domestic livestock species.

Reproductive performance has been studied in mice either by selecting for litter size at birth or its components, namely ovulation rate and early embryonic survival, or by studying it as a correlated trait to selection for other trait(s). There are many reports from selection experiments for increased litter size. The realised heritability values obtained in various experiments for direct selection of litter size are given in Table 6.1. The estimates of realised heritability ranged from 0.08 to 0.2, and are much less than the realised heritability estimates for body weights and weight gains. Nevertheless there is additive genetic variance which can provide response to direct selection for higher litter size.

In various published reports of reproductive performance as a correlated trait in mice, selection has been practised for growth rate or body weight; these have been extensively reviewed by Roberts (1965), McCarthy (1982) and Eisen (1989). Litter size at

birth is one of the traits used as a measure of reproductive performance and usually has changed in the direction of selection for growth or body weight (MacArthur, 1949; Falconer, 1953; Rahnefeld *et al.*, 1966 and Brien *et al.*, 1984, etc.). In rats, Baker and Chapman (1973) and Rios *et al.* (1986) observed a correlated response in litter size at birth in the same direction as that of selection for post-weaning weight gain. However, Bradford (1971) reported no correlated response in litter size for the lines selected for 3 to 6 week body weight gain in mice. Eisen (1987b) also did not observe any consistent correlated response in litter size, percentage of infertile matings and pre-weaning mortality in any of the mice lines selected for 10 generations for high and low epididymal fat pad percentage and hind carcass percentage of body weight at 12 weeks of age, but an index of reproductive fitness combining the three traits showed a decrease in all four selection treatments.

The positive genetic correlation between body weight / growth and litter size results in a correlated response in litter size; the estimates of genetic correlations have been summarised in Table 6.2. The realised genetic correlation (r_g) of litter size with 3 and 6 week body weight ranged from 0.3 to 0.6, whereas the realised genetic correlation of litter size with post-weaning gain ranged from 0.15 to 0.89. The estimate of 0.15 was not significantly different from zero (Rahnefeld, *et al.*, 1962) and the estimate of 0.89 was based on a genetic covariance of 0.20±0.22 (Rahnefeld *et al.*,1966). However, Bakker *et al.* (1978) observed non-significant correlated responses for 21 and 56 days

Table 6.1: Realised heritability estimates from selection experiments for increased litter size.

Realised heritabilty	Gener-ations	Method[1]	References
0.13	20	WF	Falconer (1960)
0.25±0.07	8	WF	Bradford (1968)
0.22±0.06	11	M	Bradford (1968)
0.17±0.09	8	M	Doolittle *et al.* (1972)
0.16±0.06	15	M	Eklund and Bradford (1977)
0.20±0.01	15	M	Joakimsen and Baker (1977)
0.11±0.01	30	M	Baker *et al.* (1978)
0.19±0.04	12	M	Eisen (1978)
0.08±0.01	13	M	Gion *et al.* (1990)

1. WF = Within family; M = Mass selection.

Table 6.2: Genetic correlations (r_g) of litter size with 3 week body weight (3-BW), 6 week body weight (6-BW) and 3 to 6 weeks body weight gain (WG).

Trait	Method	r_g	References
WG	Realised	0.15	Rahnefeld *et al.*(1962)
WG	ANOVA	0.89	Rahnefeld *et al.*(1966)
WG	ANOVA	0.58	Hanrahan and Eisen (1974)
3-BW	ANOVA	0.34	Hanrahan and Eisen (1974)
6-BW	ANOVA	0.36	Hanrahan and Eisen (1974)
3-BW	Realised	0.32	Joakimsen and Baker (1977)
6-BW	Realised	0.62	Joakimsen and Baker (1977)
6-BW	Realised	0.52	Eisen (1978)

Realised = Realised genetic correlation.
ANOVA = Analysis of (co)variance.

body weights in the lines selected for litter size. Nevertheless most reports suggest a positive and moderate genetic correlation between litter size and body weights / growth.

Ovulation rate has been observed to be the primary contribution to changes in litter size (MacArthur, 1944; Fowler and Edwards, 1960; Land, 1970, Durrant *et al.*, 1980, Brien *et al.*,1984 and Rios *et al.*, 1986). There is a positive genetic correlation between ovulation rate and body weight (r_g=0.4; Land, 1970). Bradford (1971) indicated that although ovulation rate increased in the lines selected for faster post-weaning gain in mice, litter size remained unchanged because of higher pre- and post-implantation losses. The increase in ovulation rate seems to be due to higher ovarian responsiveness to gonadotrophins and/or reduced sensitivity of the hypothalamus to feedback of ovarian steroids (Land, 1970 and Durrant *et al.*, 1980).

From the selection experiments at the Institute of Animal Genetics, Edinburgh, Brien *et al.* (1984) analysed 10 generations of the lean growth lines (P-Lines) data set, a part of the data set used in this study, and observed a difference of 1.0 pups in litter size between high and low lines. Hastings (1989) has analysed litter size of fat lines (gonadal fat pad weight / body weight at 10 weeks of age), appetite lines (food intake between 4 and 6 weeks of age, corrected for 4 week body weight) and lean growth lines. There was little difference between the litter size of high and low fat lines up to generation 28, after which they showed a slight difference, with an increase in litter size of the

high fat lines. The appetite and lean growth lines showed large differences in litter size. The differences between high and low lines in litter size in the later part of the experiment (average of 32 to 34 generations) were 1.6, 3.5 and 4.6 pups for fat, appetite and lean growth lines, respectively.

The objectives of this study were to estimate the genetic parameters of litter size by univariate and multivariate Restricted Maximum Likelihood method using an animal model. In contrast to realized heritability estimates, this method takes into account variances and covariances between all individuals. The incorporation of complete relationships in the pedigrees, back to the base population, takes into account the change in variance due to inbreeding and provides unbiased estimate of variances in the base population under infinitesimal model assumptions.

The univariate analyses of litter size in the selected lines may give biased estimates because it assumes zero correlation with traits under selection (Johansson and Sorensen, 1990 and Meyer 1991). For example, the univariate analysis of litter size assumes that its correlation with lean growth / body weight etc. is zero, which seems very unlikely and may provide biased estimates of the genetic parameters for litter size. A multivariate (bivariate in this case) analysis of litter size and lean growth is therefore required to account for such correlations between the traits. This would provide precise estimates of genetic parameters for litter size and more importantly its genetic correlation with lean growth.

6.2 MATERIAL AND METHODS

Litter size was analysed firstly by univariate Restricted Maximum Likelihood (REML) using an individual animal model, the details of which are given in Chapter 2. The additional feature in the analysis of litter size is to account for the heterogeneity of error variance. The model for litter size, given in chapter 2, assumes the same σ_e^2 in two lines while analysing high+low data. Since the high and low, P+P6-Lines differed in their error variance when analysed separately, they were analysed together with the assumptions of heterogeneous additive and error variances in high and low lines. Therefore assumptions for the model were modified as: $V(a) = \mathbf{A}_1 \sigma_{a1}^2 + \mathbf{A}_2 \sigma_{a2}^2$, and $V(e) = \mathbf{I}_1 \sigma_{e1}^2 + \mathbf{I}_2 \sigma_{e2}^2$. To accommodate additional Ve in the computing program, an additional random effect was fitted to provide estimates of Ve for each line.

To take into account the covariance of litter size with lean growth and to estimate the correlation between two traits, the litter size and lean growth were analysed simultaneously using a bivariate REML approach. The analysis was carried by fitting a different model for each of the two traits, *i.e.* an unequal design matrix. The model for lean growth had animals and litters as random effects, whereas the model for litter size had only animals as random effects apart from the error component in both traits. The common litter environment as an additional random effect could not be fitted for litter size because only one litter per female

was available and also only one female per family was usually used as a parent for the next generation. In all there were six parameters to be estimated in this analysis viz. σ^2_{a1}, σ^2_{a2}, σ_{a1a2}, σ^2_{c1}, σ^2_{e1}, and σ^2_{e2}, where 1 and 2 represent lean mass and litter size traits, respectively. The component of environmental covariance between litter size and lean growth could not be fitted as the records for lean growth were available only on males and litter size only on females. As this analysis required large computational time, the standard error of only the genetic correlation (r_g) was estimated by evaluating the log likelihoods for some fixed points of r_g around that at the maximum log likelihood.

Since the multivariate analyses were computationally very demanding, lean mass and litter size of only the complete data set (control+high+low) of the P-lines for generations 0-20 were analysed. However, univariate analyses were carried out on all the sub data sets; they also provide good starting values for multivariate analysis. The litter size of the P+P6 data set could not be analysed by the multivariate analysis because (a) lean mass was not available on the P6–lines, and (b) the selection criterion was changed from lean mass on males (P-lines) to body weight on males and females (P6-lines), thus it would require inclusion of all the three traits in the analysis which makes it computationally very demanding.

6.3 RESULTS

Table 6.3 gives the summary statistics of different data sets used for the analysis of litter size. The phenotypic means in different generations are plotted in Figure 6.1 to indicate the trend in litter size of different lines.

In the P-Lines, average litter size over 20 generations differed by 1.3 pups between high and low lines, compared to the average litter size in the control lines of 10.11. Figure 6.1 reveales some underlying increasing trend in the high and a decreasing trend in the low lines as compared to the control lines, although it was not consistent. The 20^{th} generation means of high and low lines differed by 3 pups (1.25 σ_p).

The average litter size in P6-Lines (over 17 generations) showed a difference of about 6 pups (= 2.4 σ_p). A large increase in litter size of both high and low lines was observed in generation 21 (Figure 6.1), presumably due to heterosis resulting from crossing the three replicates of P-Lines within each selection treatment. The high P6-Line did not show any consistent trend in litter size from generations 21 to 37. However, the low P6-Line revealed a consistent decline in the litter size. The average litter size of high and low lines in the complete data set (P+P6 Lines) differed by 2.4 pups. This difference is less than the difference in P-Lines at generation 20 because high P6-Line showed a decline in later generations.

Table 6.3: Summary Statistics for Litter Size.

Line/Replicate	N	Mean	SD	CV(%)
P - LINES:				
Replicate 1 (C+H+L)	619	10.38	2.56	24.6
Replicate 2 (C+H+L)	608	10.22	2.25	22.0
Replicate 3 (C+H+L)	583	10.03	2.49	24.8
Overall (Rep1+2+3)	1810	10.22	2.44	23.9
Control (Rep1+2+3)	574	10.11	2.42	23.9
High (Rep1+2+3)	616	10.92	2.51	23.0
Low (Rep1+2+3)	620	9.61	2.21	23.0
P6 - LINES:				
High	282	13.52	3.61	26.7
Low	290	7.65	2.05	26.8
P + P6 LINES:				
High (Rep1+2+3+6)	898	11.55	3.06	26.5
Low (Rep1+2+3+6)	910	9.15	2.35	25.6

N.B. N = Number of observations, SD = Standard deviation within line, CV = Coefficient of variation, Rep = Replicate, C,H,L = Control, High and Low Lines.

Figure 6.1: Correlated response in different lines for litter size averaged over replicates.

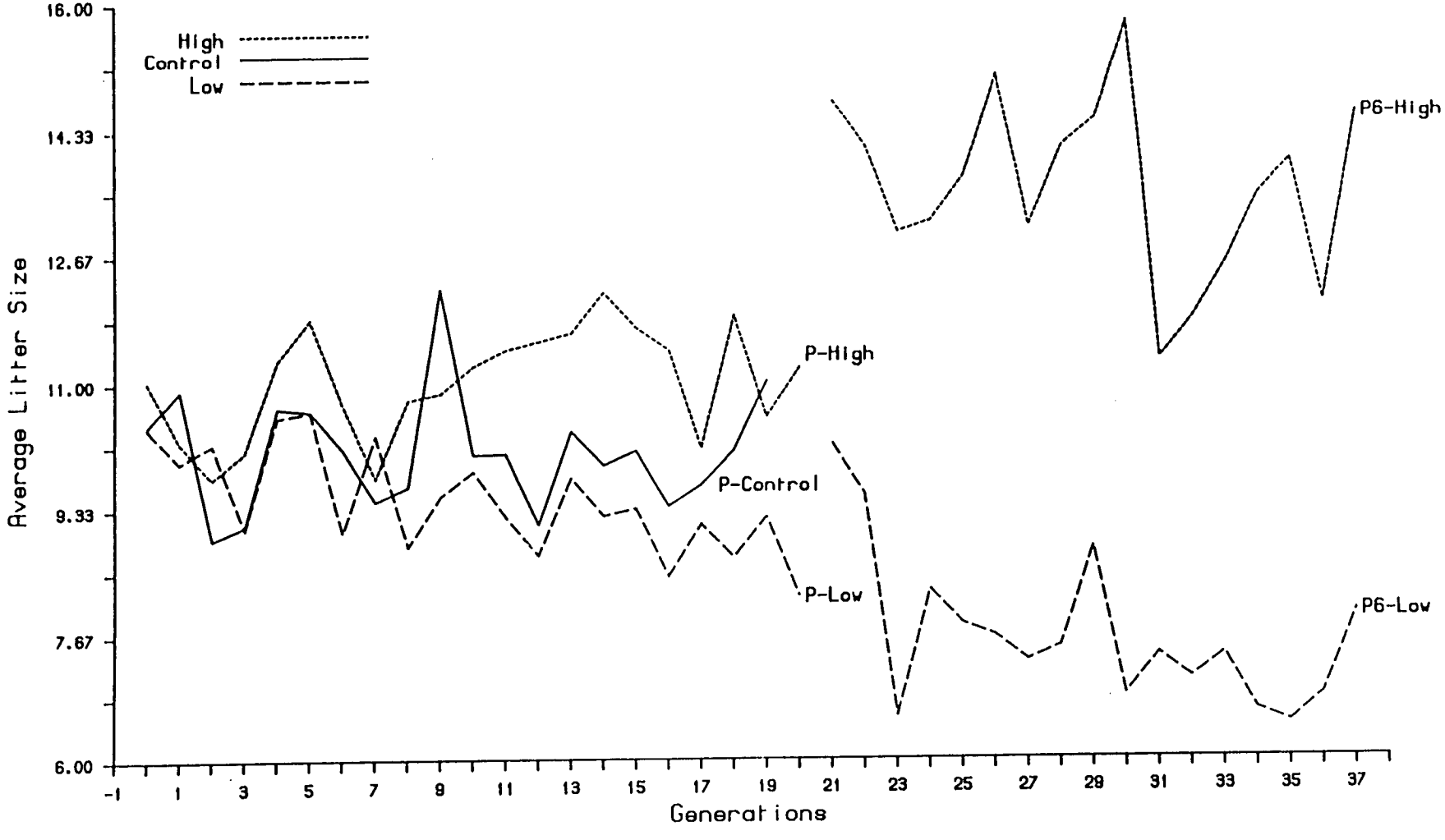


Table 6.4: Average Inbreeding Coefficients (F) in P and P6 Lines data sets.

F in P-Lines data set			F in P6-Lines data set		
Gen	High	Low	Gen	High	Low
0	0.00	0.00	21	0.00	0.00
1	0.00	0.00	22	0.08	0.09
2	0.00	0.00	23	0.10	0.09
3	0.00	0.00	24	0.09	0.12
4	0.01	0.00	25	0.10	0.12
5	0.02	0.02	26	0.11	0.14
6	0.03	0.04	27	0.12	0.13
7	0.05	0.04	28	0.13	0.15
8	0.05	0.06	29	0.14	0.18
9	0.06	0.06	30	0.14	0.17
10	0.07	0.07	31	0.17	0.18
11	0.08	0.08	32	0.17	0.20
12	0.11	0.09	33	0.19	0.21
13	0.12	0.11	34	0.19	0.21
14	0.14	0.13	35	0.20	0.23
15	0.15	0.15	36	0.23	0.23
16	0.16	0.16	37	0.23	0.24
17	0.18	0.18	38	0.22	0.25
18	0.19	0.20			
19	0.21	0.22			
20	0.22	0.23			

The average inbreeding coefficients of high and low lines in each generation are given in Table 6.4. In the last generations of the P and P6 lines, inbreeding coefficients ranged from 22 to 25 percent.

6.3.1 UNIVARIATE ESTIMATES OF GENETIC PARAMETERS

6.3.1.1 0 - 20 GENERATIONS DATA SET (P-LINES)

The estimates of variance components and genetic parameters for litter size in the P-Lines are given in Table 6.5. The likelihood curves for analyses are presented in Figure 6.2. These curves were obtained by plotting the log likelihood values at fixed points of heritability (h^2). The h^2 estimate of litter size in the three replicates ranged from 0.077 to 0.217. Replicate 2 had the lowest h^2 estimate. In Figure 6.2(B), the curvature of the likelihood curve for replicate 2 was flat as compared to the other replicates indicating a high standard error associated with the h^2 estimate. The overall h^2 estimate, obtained by combining the data from the three replicates, was 0.166±0.043 and the likelihood curve had more curvature than the individual replicates.

The analysis of different lines revealed that the h^2 and additive genetic variance (Va) estimates for the control lines were similar to those obtained from the analysis of the overall data set. The estimates of h^2 and Va obtained from the high lines were substantially lower than from the control lines, and negligible estimates for these parameters were obtained from the

Table 6.5: Univariate estimates of Genetic Parameters for Litter Size in P-Lines (0-20 generations) using untransformed data.

Line/Replicate	Fixed Effects	h^2 ± SE	Variance Components Vp	Va	Ve
Replicate 1 (C+H+L)	Gen	0.217 ± 0.026	5.684	1.233	4.451
Replicate 2 (C+H+L)	Gen	0.077 ± 0.089	4.884	0.376	4.508
Replicate 3 (C+H+L)	Gen	0.144 ± 0.081	6.014	0.863	5.151
Overall (Rep 1+2+3)	RepXGen	0.166 ± 0.043	5.545	0.922	4.623
Control (Rep 1+2+3)	Rep,Gen	0.181 ± 0.093	5.533	1.004	4.530
High (Rep 1+2+3)	Rep,Gen	0.051 ± 0.081	5.713	0.291	5.422
Low (Rep 1+2+3)	Rep,Gen	0.000 ± 0.091	4.531	<0.001	4.531
Control + High	Rep,Gen	0.156 ± 0.053	5.789	0.903	4.895
Control + Low	Rep,Gen	0.093 ± 0.061	5.099	0.472	4.627
High + Low	Rep,Gen	0.168 ± 0.048	5.428	0.913	4.515

Va,Vc,Vp = Additive genetic, Common Litter and Phenotypic Variances; SE = Standard Error; Gen = Generations; Rep = Replicates; C,H,L = Control, High and Low Lines.

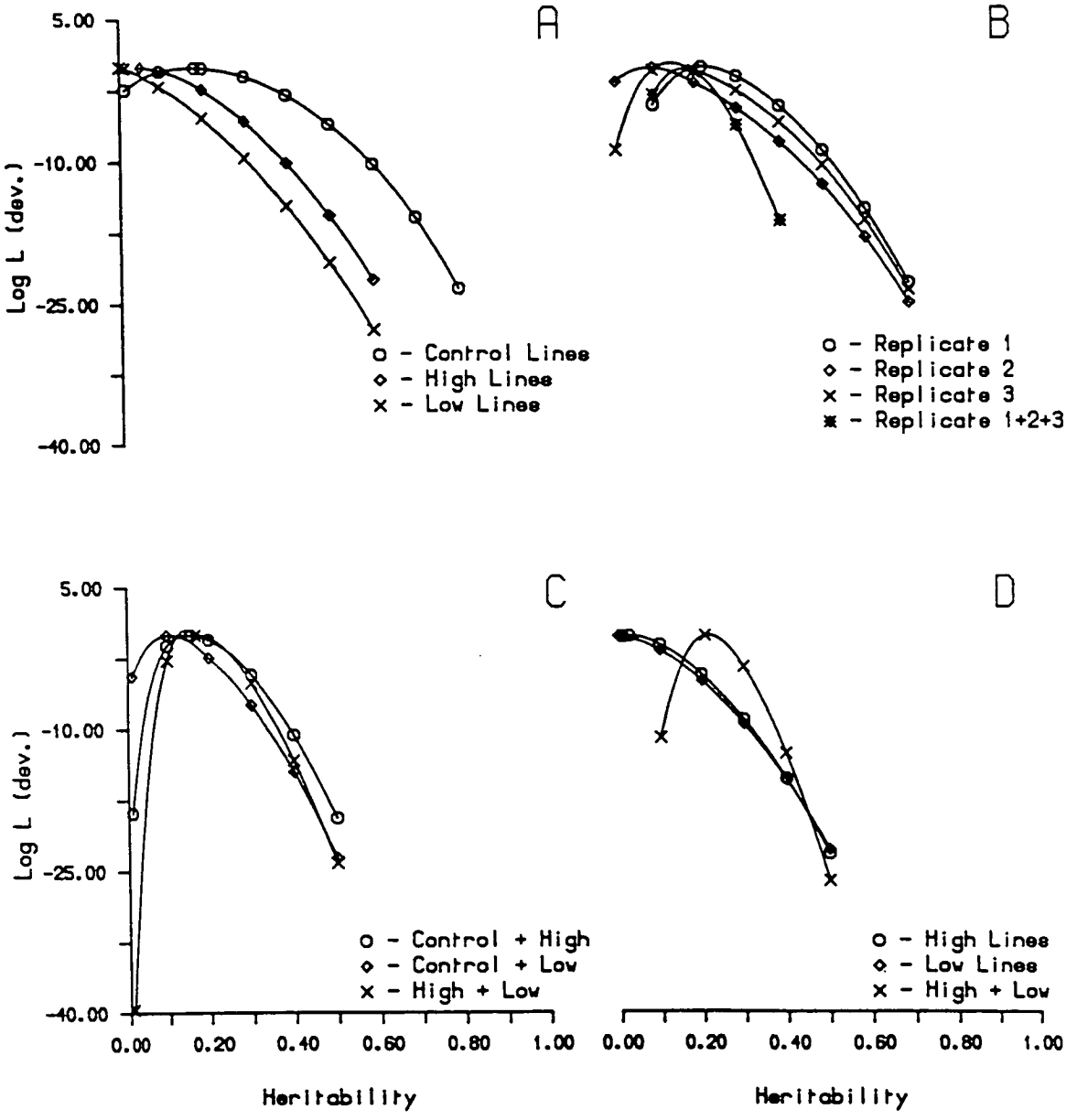


Figure 6.2: Likelihood curves for litter size. Graphs A, B and C are for P-Lines and D is for P+P6 data set. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

Table 6.6: Univariate estimates of Genetic Parameters for Litter Size in P-Lines (0-20 generations) using log-transformed data.

Line/Replicate	Fixed Effects	h^2 ± SE	Variance Components ($\times 10^{-2}$) Vp	Va	Ve
Replicate 1 (C+H+L)	Gen	0.181 ± 0.045	7.69	1.39	6.30
Replicate 2 (C+H+L)	Gen	0.068 ± 0.081	5.98	0.40	5.58
Replicate 3 (C+H+L)	Gen	0.126 ± 0.082	9.45	1.19	8.26
Overall (Rep 1+2+3)	RepXGen	0.144 ± 0.040	7.71	1.11	6.60
Control (Rep 1+2+3)	Rep,Gen	0.157 ± 0.092	8.63	1.35	7.27
High (Rep 1+2+3)	Rep,Gen	0.031 ± 0.065	6.90	0.21	6.69
Low (Rep 1+2+3)	Rep,Gen	0.000 ± 0.086	6.66	<0.001	6.66
Control + High	Rep,Gen	0.147 ± 0.059	7.94	1.16	6.78
Control + Low	Rep,Gen	0.062 ± 0.066	7.67	0.47	7.20
High + Low	Rep,Gen	0.140 ± 0.054	7.11	0.99	6.12

Va,Vc,Vp = Additive genetic, Common Litter and Phenotypic Variances; SE = Standard Error; Gen = Generations, Rep = Replicates; C,H,L = Control, High and Low Lines.

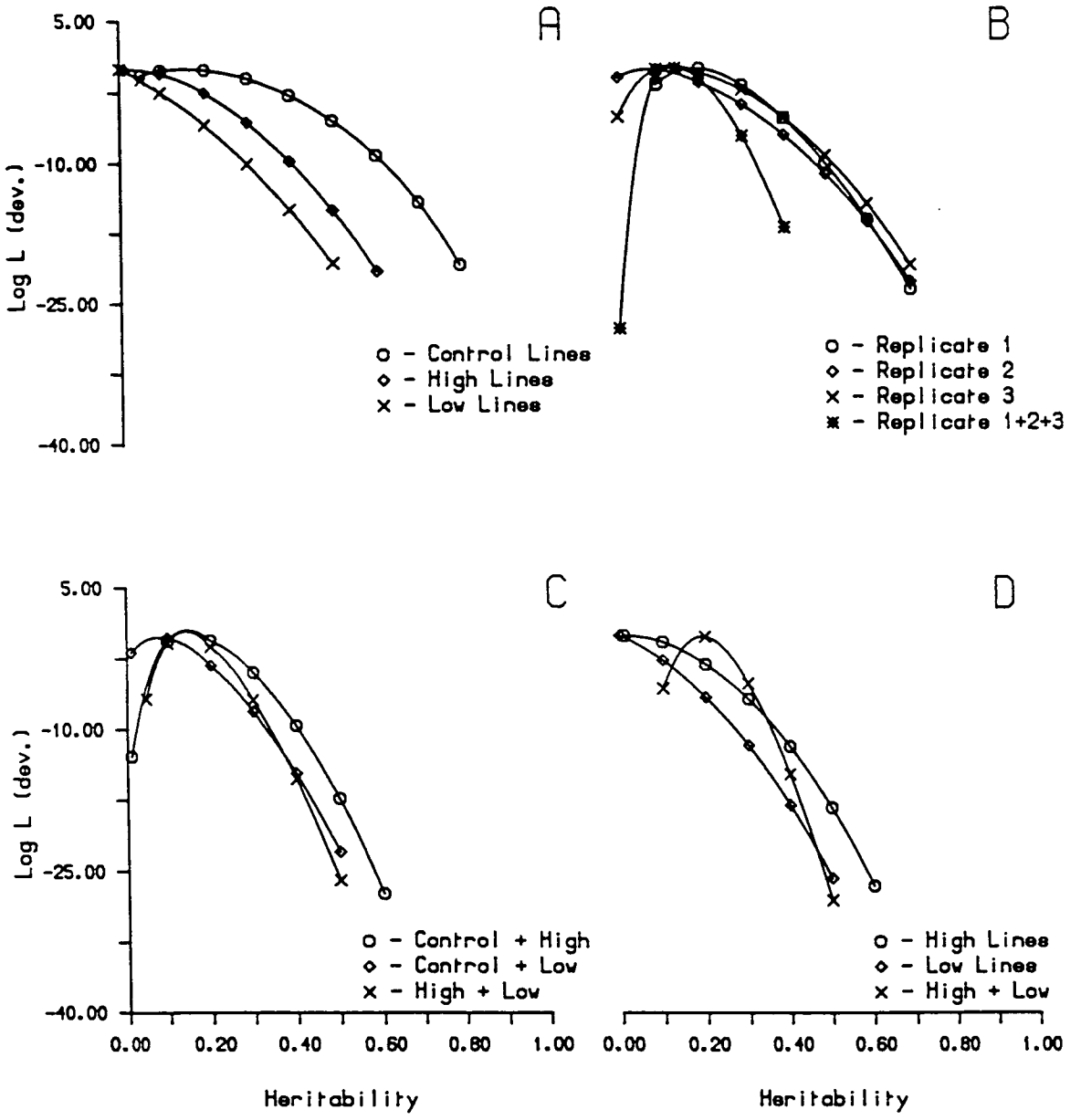


Figure 6.3: Likelihood curves for litter size using log-transformed data. Graphs A, B and C are for P-Line and D is for P+P6 data set. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

Table 6.7: Univariate stimates of Genetic Parameters for Litter Size in P-Lines data. Analysis of different lines assuming heterogeneous additive genetic variance. Fixed effects: Replicates and generations.

Analysis	Group	h^2	Variance components		
			V_p	V_a	V_e
Control+High	Control	0.143	5.694	0.816	4.878
	High	0.178	5.934	1.055	
Control+Low	Control	0.191	5.589	1.069	4.520
	Low	0.000	4.52	<0.001	
High+Low	High	0.266	6.140	1.632	4.508
	Low	0.008	4.546	0.038	

low lines analysis. When each selected line was analysed with the control in one data set, the estimates of h^2 and Va increased. The estimates of h^2 and Va obtained from the control+high lines analysis were 0.156 and 0.903, respectively, and these are very close to the estimates from the control lines. For the control+low lines much lower estimates were obtained as compared to the control lines, the estimates being 0.093 and 0.472 for h^2 ans Va, respectively. The analysis of high+low lines revealed estimates of h^2 and Va very close to estimates from the control alone and from the overall data as it takes into account the litter size differences between high and low lines. Figure 6.2(C) shows a sharp curvature for the likelihood curves for selected + control data sets as compared to the curves in fig 6.2(A) for each line individually.

There was some evidence of correlation between mean and variance of litter size, and the coefficient of variation did not change much. Therefore the P-Lines were reanalysed using log transformed data to reduce the scale effect. The estimates of variance components and heritability are given in Table 6.6 and likelihood curves in Figure 6.3. The estimates (Table 6.6) obtained were similar to those of estimates from the untransformed data (Table 6.5) except they were slightly lower in magnitude, suggesting that the scale effect did not affect the parameter estimation much. Therefore results from the analyses of untransformed data are given for remaining analyses.

Since the estimates of additive genetic variances (Va) in

control and selected lines were different, they were analysed together by taking into account the heterogeneous additive variance (Va) between them. The estimates of variance components and heritability obtained from the analyses with the assumption of heterogeneity of Va are given in Table 6.7. The analysis of control+high data gave estimates of h^2 and Va for the high lines of 0.178 and 1.055, respectively, very close to those obtained from the control lines (Table 6.5). However, the estimates for the low lines from the analysis of control+low data were still close to zero. When the high+low lines data set was analysed, the estimates for the low lines were still negligible, although the estimates for the high lines were slightly higher than that from the control+high analysis.

6.3.1.2 0 - 37 GENERATIONS DATA SET (P+P6 - LINES)

The data sets of high and low from P+P6-Lines were analysed including the complete pedigree back to the base population. Data on the control lines were not available after 20 generations, therefore analysis of only high+low lines has been given in this section. The estimates of variance components and h^2 are given in Table 6.8.

Table 6.8 indicates that the estimates of Va and h^2 are very low in the high lines and almost zero in the low lines. However, when high and low data were analysed together, the estimate of h^2 was 0.210±0.027, which is close to the estimate from the control lines (0.181±0.093) in the analysis of generations 0-20 (Table

Table 6.8: Univariate estimates of Genetic Parameters for Litter Size in P+P6-Lines data. Analysis of different lines with/without heterogeneous additive genetic variance.
Fixed effects: Replicates and generations.

Analysis	Group	h^2	Variance components V_p	V_a	V_e
Without Heterogeneity option:					
High	--	0.028 ± 0.065	7.532	0.207	7.325
Low	--	0.000 ± 0.084	4.201	0.001	4.200
High+Low	--	0.210 ± 0.027	6.470	1.359	5.111
With Heterogeneity option:					
High+Low (Het. Va option)	High	0.436	8.293	3.615	4.679
	Low	0.000	4.679	<0.001	
High+Low (Het. Va and Ve options)	High	0.267	8.547	2.278	6.269
	Low	0.055	4.201	0.232	3.969

6.5). The analysis of high+low with the heterogeneity of Va assumptions resulted in a very high estimate of h^2 (0.436) for the high lines. This analysis takes into account the different Va of high and low but assumes the same error variance in two lines. The error variance estimates from the analyses of high and low lines separately were 7.3 and 4.2, respectively. These different error variance components violate the assumption of the same σ_e^2 in two lines and may give biased estimates of genetic parameters. Therefore, the high+low lines data set was reanalysed taking into account different additive genetic and error variances. The estimates of h^2 and Va from this analysis were 0.267 and 2.278, respectively, in the high lines, and 0.055 and 0.232, respectively, in the low lines. The h^2 estimate for the high lines was higher than the estimate obtained from the P-lines (0-20 generations) analysis of control+high with the heterogeneity of Va assumption (Table 6.7). The low lines still showed a very low estimate of h^2.

6.3.2 SIMULATION OF LITTER SIZE

The P and P+P6 lines data revealed a nearly zero additive genetic variance estimate in the low lines but not in the high lines, although both lines originated from the same base population. To look into this aspect and to provide reassurance that the analytical procedures are adequate, a Monte Carlo simulation of litter size was done using the pedigree structure of the actual data. Simulation also provides an opportunity to compare the empirical standard error with the standard error

estimates obtained from analysis of each simulated data set.

The litter size was simulated as an independent trait with the infinitesimal model (Bulmer, 1980) assumptions of many genes each having small effect. The model used for simulation was

$$Y_i = \mu + a_i + e_i$$

Where Y_i is the phenotypic value of litter size for i^{th} individual, a_i is its breeding value and e_i is a residual value for non-additive and environmental effects. The μ is the phenotypic mean of litter size in the base population.

The data for 20 generations were simulated from a base population with phenotypic mean of 10.0 and variance of 5.5 and the heritability was assumed to be 0.2. The pedigree structures from the actual data of the control, high and low lines, each having three replicates, were used in the simulation to generate the phenotypic value of progenies without any selection. However, the terms control, high and low in the text refers to the simulated data with pedigree structures of control, high and low lines of the actual data. The inbreeding coefficient of each individual in the pedigree was calculated from the actual inbreeding coefficient of sire and dam in the real pedigree.

The random values of e_i were drawn from a normally and independently distributed population with mean zero and variance σ_e^2. The breeding values of individuals in the base population were taken from a NID (0, σ_a^2) population. Breeding values in subsequent generations were simulated as

$$a_i = \frac{1}{2} a_{si} + \frac{1}{2} a_{di} + \Phi_i$$

Where a_{si} and a_{di} are breeding values of sire and dam of individual i, and Φ_i is the residual genetic value resulting from Mendelian sampling. The residual genetic value for each individual (Φ_i) was drawn from a normal distribution with mean zero and variance equal to,

$$\text{Var}(\Phi_1) = \left[\frac{1}{4} (1 - F_{si}) + \frac{1}{4} (1 - F_{di}) \right] \sigma_a^2$$

Where F_{si} and F_{di} are the inbreeding coefficients of the sire and dam of individual i. Simulations were replicated 10 times for each line and referred to as "Simulates" in the text to avoid confusion between the replicates of real data and simulations.

The data for each simulate were analysed by REML using the animal model. Generations were fitted as a fixed effect. The estimates were averaged over 10 simulates in each line and are given in Table 6.9 with their empirical standard errors. The likelihood curves for different simulates in each line are presented in Figures 6.4.1 to 6.4.5.

The true parameters for h^2, Va, Ve and Vp are 0.2, 1.1, 4.4, and 5.5. Table 6.9 reveals that the estimates of h^2 and variance components averaged over 10 simulates in different analyses are very close to the true parameters. The estimates of h^2 and Va from control, high and low lines were not significantly different from the true parameters. Similar non-significant differences were also observed between true parameters and estimates from selected plus control line analyses, though the latter were slightly higher than true parameters. The likelihood curves given

Table 6.9: Univariate estimates of Genetic Parameters for Litter Size from Simulated data. Results are averaged over 10 Simulates.

Line	h^2	SE(P)	SE(E)	Va ±SD	Ve ±SD	Vp ±SD
Control	0.205	0.086	0.073	1.278 ±0.492	4.898 ±0.404	6.185 ±0.041
High	0.180	0.083	0.087	0.895 ±0.433	4.099 ±0.491	4.994 ±0.204
Low	0.186	0.085	0.082	1.025 ±0.464	4.468 ±0.508	5.493 ±0.367
Control+High	0.220	0.053	0.060	1.253 ±0.359	4.416 ±0.345	5.679 ±0.255
Control+Low	0.239	0.047	0.065	1.440 ±0.412	4.552 ±0.361	5.991 ±0.268

SE(P) = Predicted Standard Error from REML analyses,
SE(E) = Empirical Standard Error,
Va,Ve,Vp = Additive genetic, Error and Phenotypic Variances,
SD = Standard deviation in 10 simulates.

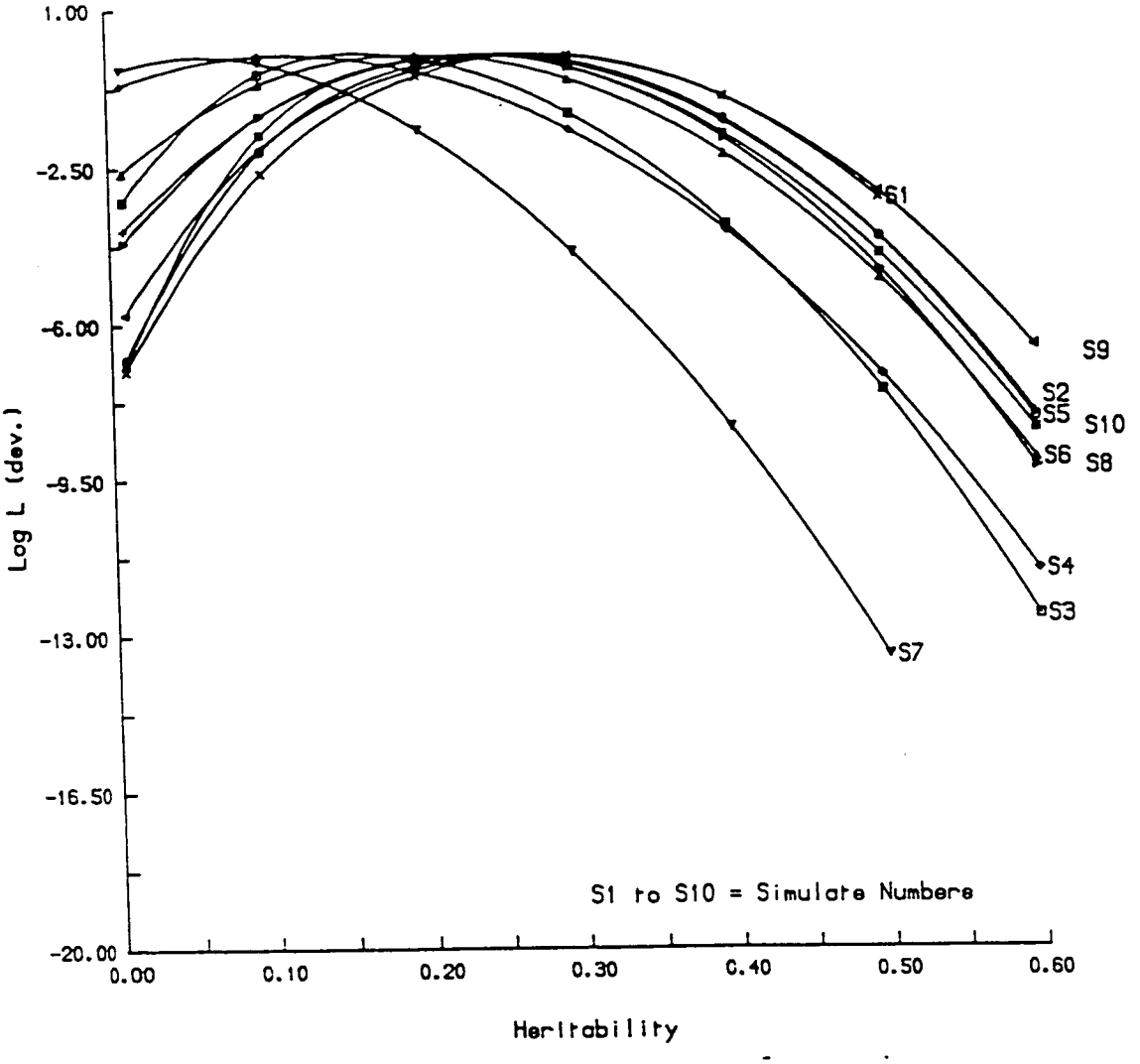


Figure 6.4.1: Likelihood curves for simulated litter size of control line. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L[dev.]].

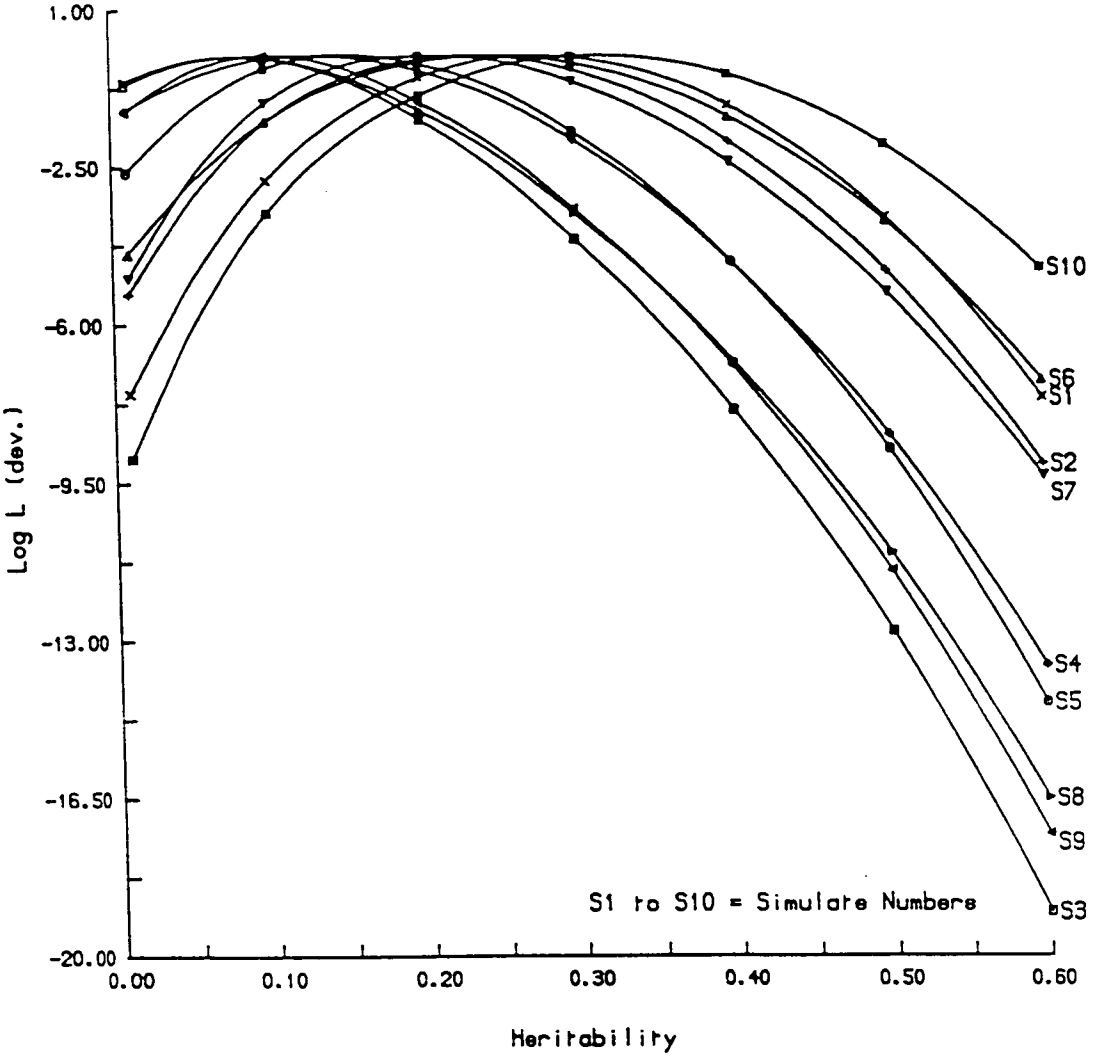


Figure 6.4.2: Likelihood curves for simulated litter size of high line. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

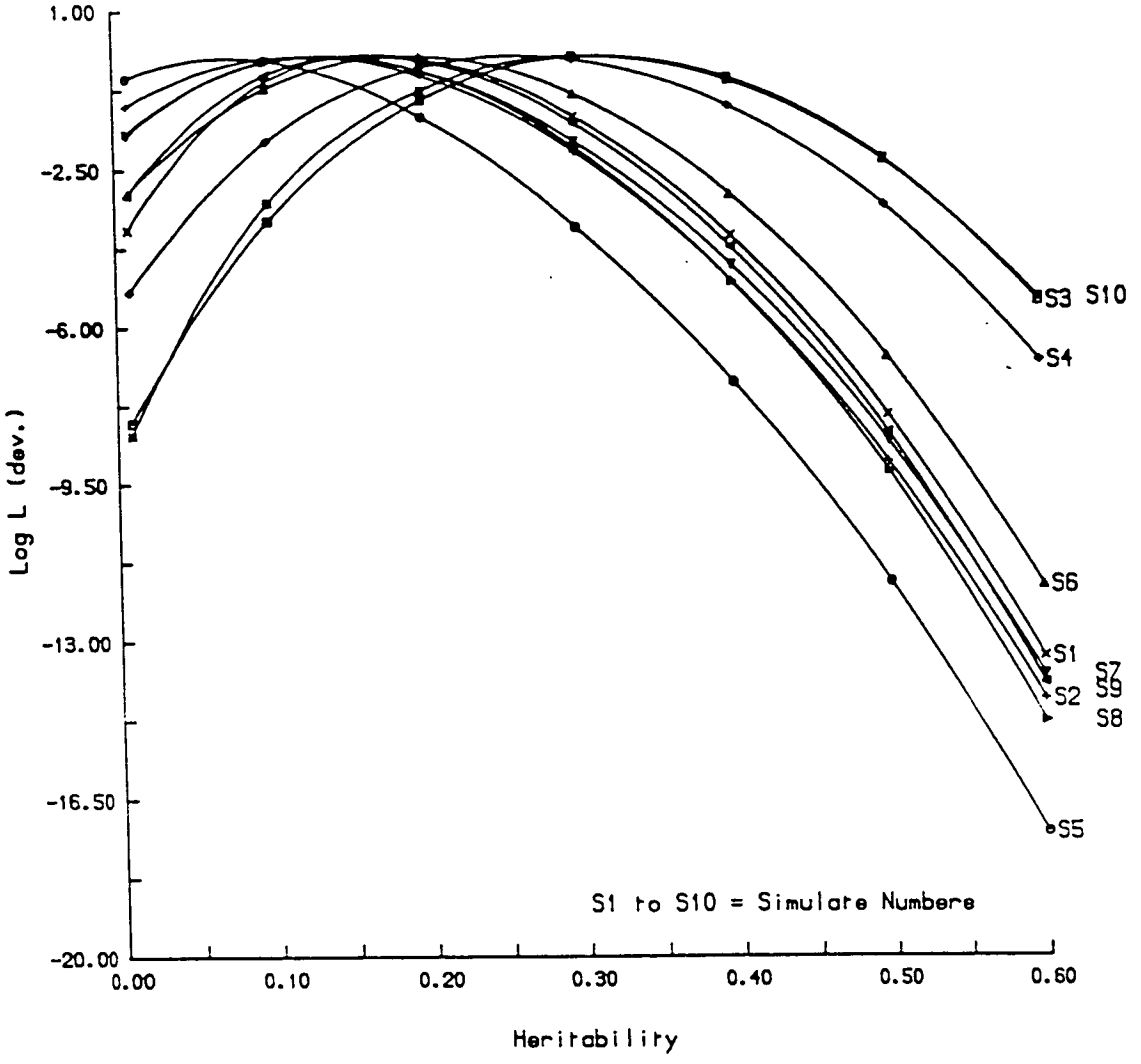


Figure 6.4.3: Likelihood curves for simulated litter size of low line. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

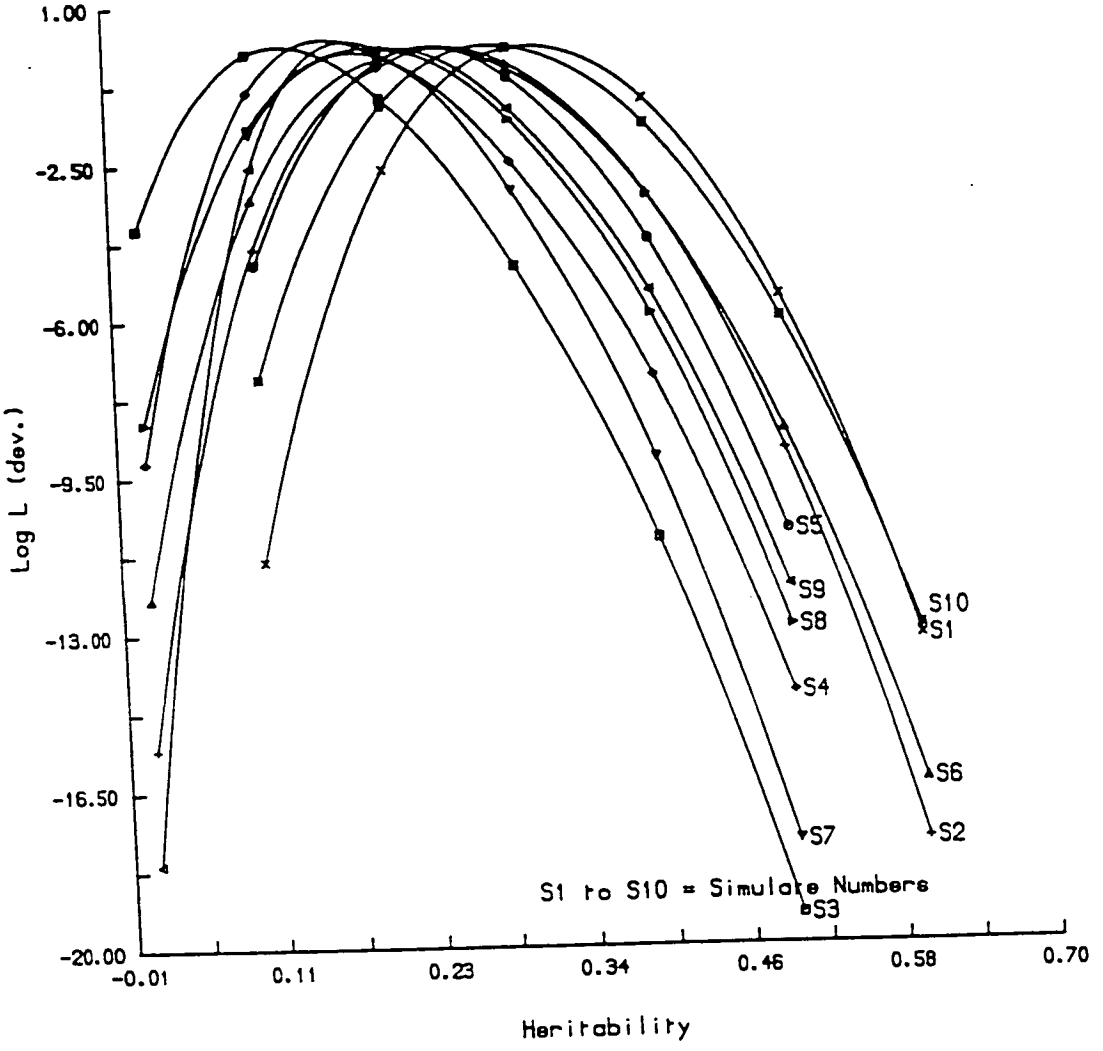


Figure 6.4.4: Likelihood curves for simulated litter size of control + high line. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

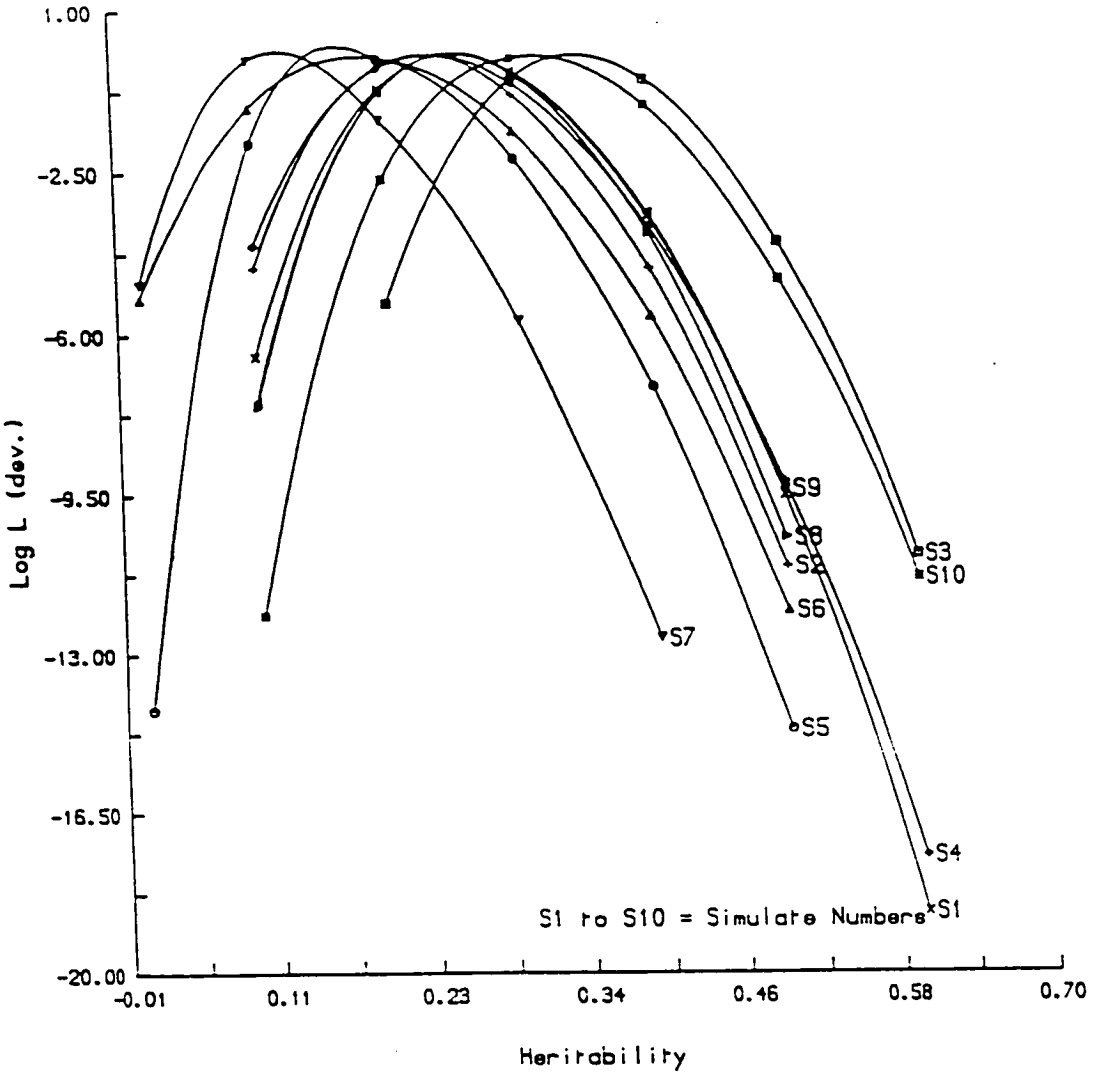


Figure 6.4.5: Likelihood curves for simulated litter size of control + low line. Maximum likelihood values for different heritabilities have been expressed as deviation from the global maximum likelihood value [Log L(dev.)].

in figures 6.4.1 to 6.4.5 revealed a pattern similar to that of the actual data (Figure 6.2). When the control, high and low lines were analysed alone, the curvatures of the likelihood curves were relatively flat indicating high standard errors associated with the h^2 estimates. However, the sharp curvature of the likelihood curves for selected plus control lines suggests lower standard error because more information is included in the analysis as the two lines have common parents in the base population. The average of predicted standard errors of heritabilites agreed very well with the empirical standard errors obtained from 10 simulates.

These results from simulation indicate that the analysis of the low line with the actual pedigree structure gives unbiased estimates of genetic parameters by taking account of the reduction in variance due to inbreeding. This excludes the possibility of any such error in the analysis of actual low line data which has shown nearly zero additive genetic variance. Here litter size has been simulated as an independent trait which is perhaps not the case with the real data. Possibly the correlation between litter size and body weight, which is not being taken into account in the univariate analysis, is leading to biased estimates of the genetic parameters in the actual data.

6.3.3 BIVARIATE ESTIMATES OF GENETIC PARAMETERS

The bivariate analysis of lean mass and litter size was carried out to take account of the covariance between litter size

Table 6.10: Genetic Parameters from Bivariate and Univariate analyses of Lean Mass and Litter size for Control+High+Low P-Lines data (0-20 generations).

Variance Components and Parameters	Lean Mass	Litter Size	Genetic Covariance/ Correlations
Bivariate analysis.			
Additive genetic Variance	4.052	0.561	0.412
Common Litter Variance	2.325	--	--
Error Variance	2.479	4.874	--
Phenotypic Variance	8.856	5.435	--
Heritability	0.458	0.103	0.273
± Standard Error	--	--	±0.100
c-square	0.263	--	--
Univariate analyses.			
Additive genetic Variance	4.309	0.922	
Common Litter Variance	1.914	--	
Error Variance	2.277	4.623	
Phenotypic Variance	8.500	5.545	
Heritability	0.507	0.166	
±Standard Error	±0.021	±0.043	
c-square	0.225	--	
±Standard Error	±0.015		

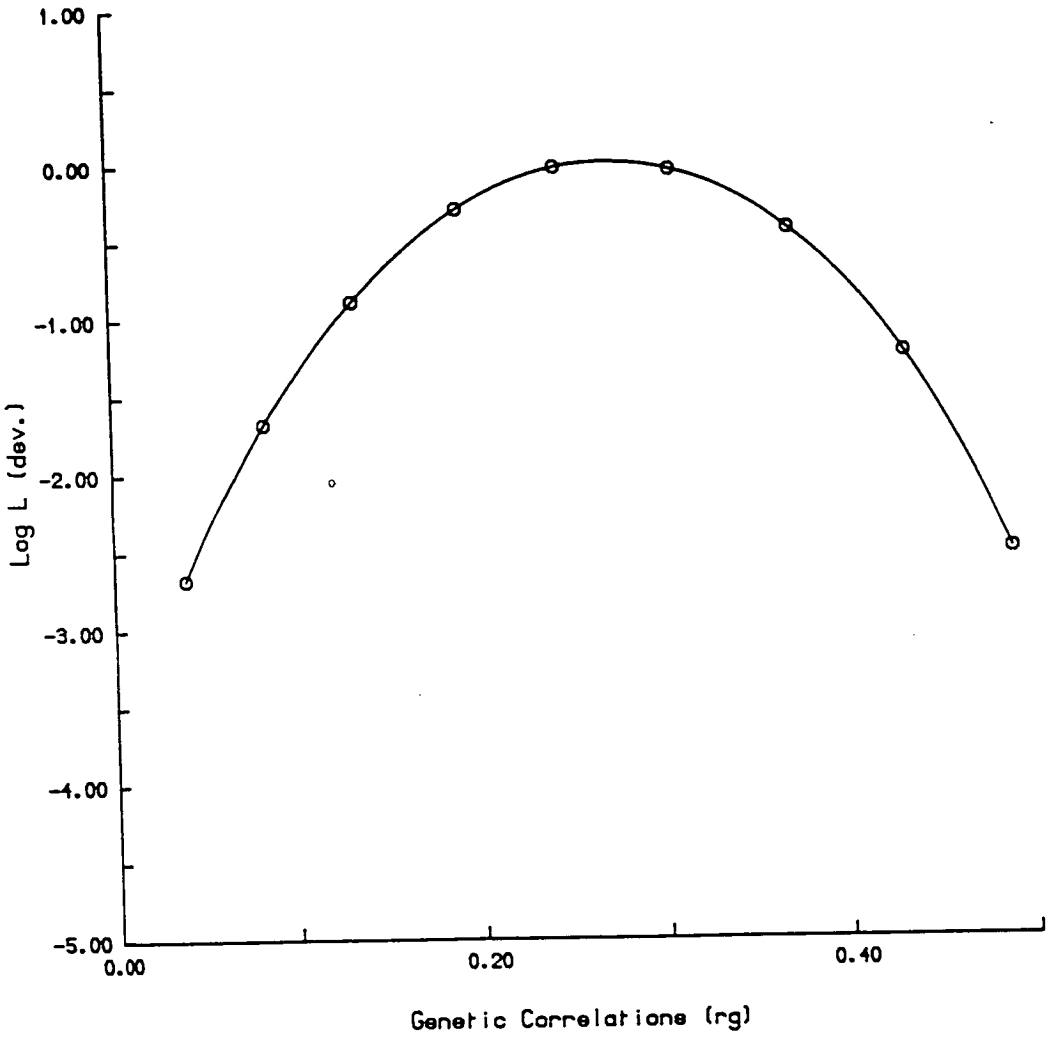


Figure 6.5: Likelihood curve for fixed points of genetic correlation (rg) between Lean Mass and Litter Size. Maximum likelihood values for different rg have been expressed as deviation from the global maximum likelihood value [Log L(dev.)]. Data : Control+High+Low for 20 generations.

and lean mass, because selection was practised on the latter trait, and to estimate the genetic correlation between the two traits. The results of bivariate analysis of lean mass and litter size for the control+high+low, 20 generations data set (P-lines) are given in Table 6.10 along with the results of the univariate analyses for comparison. The shape of the likelihood curve for the genetic correlation is given in Figure 6.5.

The results in Table 6.10 revealed that the estimate of Va for litter size is lower than from the univariate estimate, perhaps some of the variance has gone in the estimation of the covariance between the traits. Consequently the estimate of heritability is also lower, 0.103 compared to 0.166. The estimate of genetic correlation between litter size and lean mass was 0.273±0.10. The log likelihood curve (Figure 6.5) for different fixed points of the genetic correlation around the maximum log likelihood has relatively flat curvature, which is in line with a large standard error associated with the estimate. This could be due to less information on genetic covariance from the relatives or smaller sample size.

The estimates of heritability and additive genetic variance for lean mass from the bivariate analysis were also lower than the estimates obtained by univariate analysis, the heritability estimates being 0.458 and 0.507, respectively. The difference between the two estimates was tested by the likelihood ratio test. The log likelihoods obtained by the bivariate analysis for the heritabilities of 0.507 and 0.458 were -10656.64 and -10654.58,

respectively and differed by -2.06. Minus two times the difference of log likelihood has an approximate chi-square distribution with degrees of freedom equal to the number of parameters tested i.e. one in this case. The difference was found to be significant at 5 percent ($P \leq 0.05$) and non-significant at 1 percent ($P \geq 0.01$) levels of significance. The estimate of the variance within full-sib families ($\sigma_w^2 = (\sigma_a^2/2)+\sigma_e^2$) showed very little difference; the estimates were 4.43 and 4.50 from the bivariate and univariate analyses, respectively.

The standard errors for the bivariate heritability estimates of lean mass and litter size could not be calculated due to the high computational time requirement. For the same rasons, the control, high and low lines data sets could not be analysed individually by a bivariate analysis.

6.6 DISCUSSION AND CONCLUSIONS

The correlated changes in litter size of the P-Lines were observed to be in the direction of selection for lean mass and agree with other reports (MacArthur, 1949; Falconer, 1953; Rahnefeld *et al.*, 1966 and Brien *et al.*, 1984, etc.). Brien *et al.*, (1984) observed a difference of 1.0 pups between these high and low lines in generation 10, by generation 20 , the difference had increased to 3.0 as a consequence of selection on lean mass.

There was a sudden increase in the litter size (Figure 6.1) at the 21st generation when three replicates within each selection

treatment were crossed. The average inbreeding coefficient in the 20^{th} generation was more than 20 percent in the high and low lines (Table 6.4). The obvious reason for such a rise seems to be heterosis. The estimates of heterosis in this data set obtained by the difference between the average litter size of inbred dams carrying crossbred progenies in the 20^{th} generation and crossbred dams carrying crossbred progenies in the 21^{st} generation, were 31 and 24 per cent in the high and low lines, respectively, although the estimate is confounded with any environmental changes. These are very high estimates of heterosis as compared to the other reports. Bradford and Nott (1969) reported 6 to 11 percent heterosis in F_1 females of crosses of lines selected for litter size, primarily due to lower implantation losses. Bhuvanakumar *et al.*, (1985) observed a heterosis of only 4 percent for litter size by comparing pure bred and crossbred progenies of lines selected for 6 week body weight, but they had undergone many generations of relaxed selection before crossing. The observed estimate of heterosis in this study may be biased because the average of crossbred progenies includes only females selected to produce the next generation on the basis of body weight. The litter size for unselected females was not available, hence it was difficult to obtain unbiased estimates of heterosis in this data set. Further there was a change in the age of mating; from 9 weeks in the P-lines to 12 weeks in the P6-lines. The mating at the later age when animals have heavier body weight are likely to give large litters. This could be another source of bias in the heterosis esimates. This suggests that the increase in litter size at generation 21 is not only due to the heterosis, but also includes

environmental and other factors. However, crossing of three replicates did not show heterosis in body weight (Figure 4.1). Similar findings have also been reported by Roberts (1967). Perhaps body weight did not suffer much due to inbreeding depression.

The high P6–Line showed wide fluctuations in litter size with a decline in the later generations. The fluctuations were mainly due to weaning of very small families as there were no reserve matings in the P6-lines, whereas in the P–lines, such families were replaced by reserve matings. The decline in the litter size of later generations seems to be due to increase in the percentage of dead born in the high P6-line (figure 6.6). The increase in dead born could have two reasons. First, management or disease, but contemporary low P6-line did not show increase in born dead (figure 6.7). Second, uterine capacity could be a limiting factor for increase in the litter size thereby giving birth to dead pups who exceed the capacity. In an experiment with direct selection for litter size by Eklund and Bradford (1971), there was no response after 31 generations through to 45 generations but increase in number of dead born as a consequence of selection for litter size.

Falconer (1973) also observed a decline in litter size even after 5 generations of selection for six week body weight, which he attributed to inbreeding depression. Similar observations have also been made by Eisen *et al.* (1973) about the undesirable effects of inbreeding on fertility in selection for body weight.

Figure 6.6: Percentage of born dead mice in the High P6-Line.

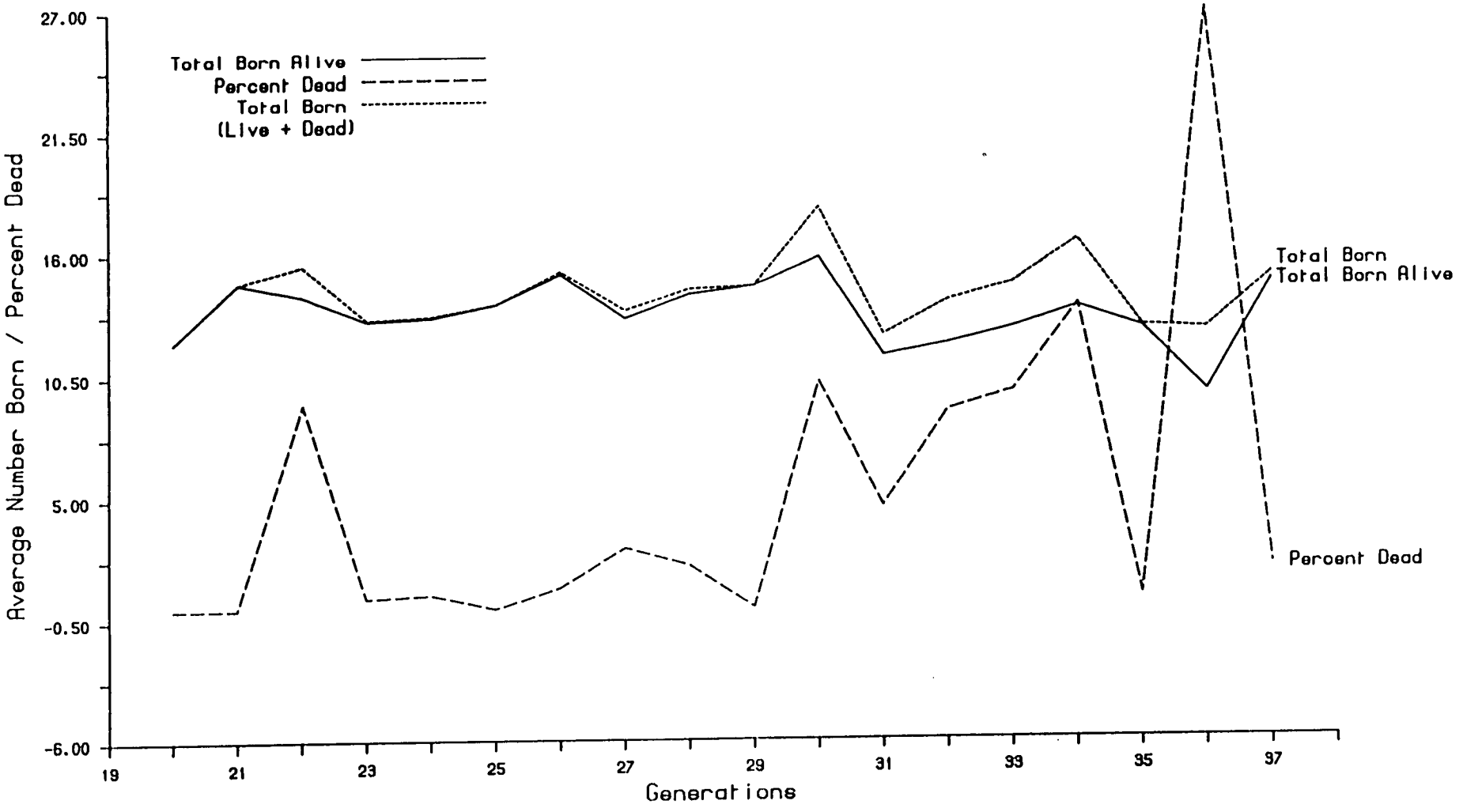
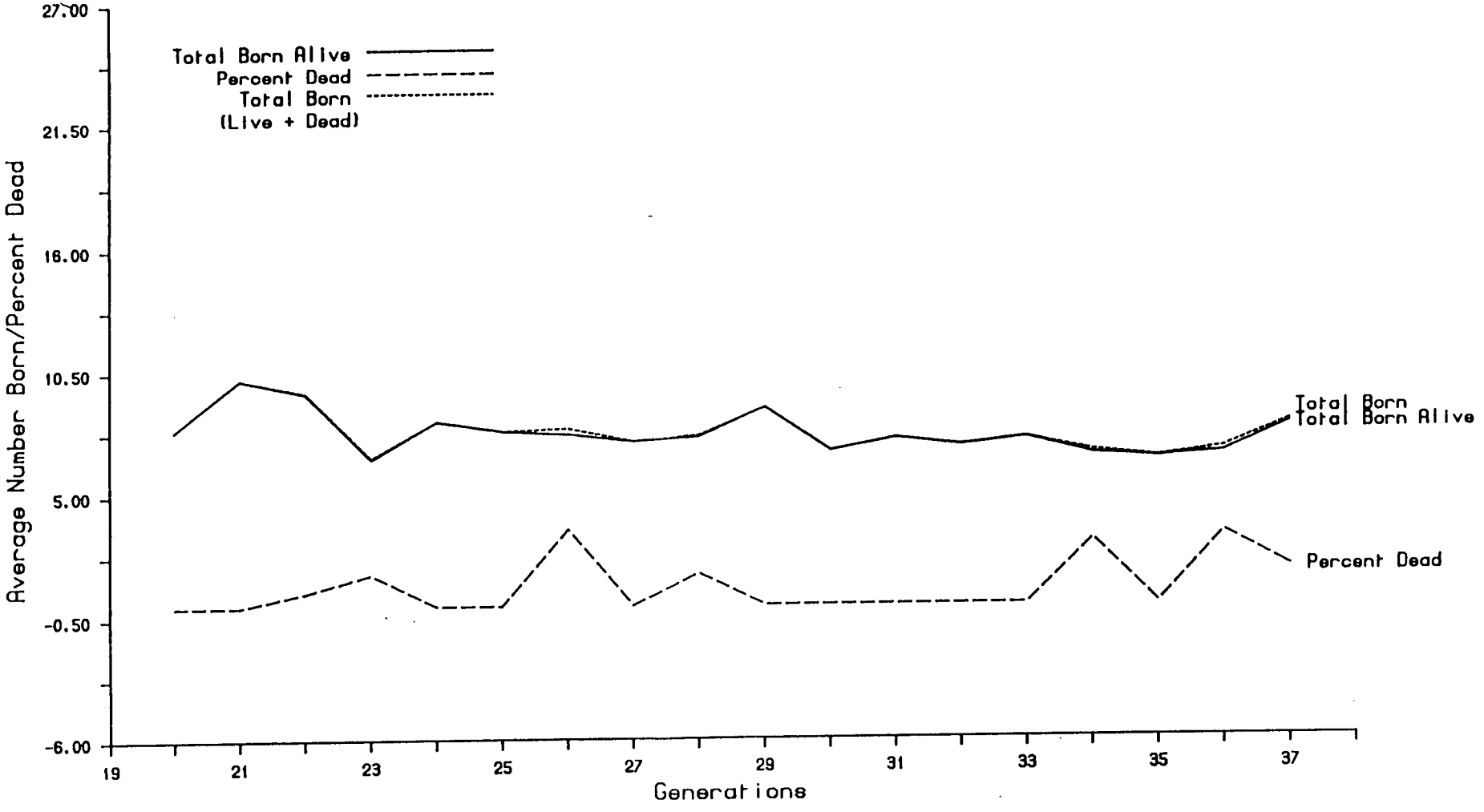


Figure 6.7: Percentage of born dead mice in the Low P6-Line.



The inbreeding coefficient (F) was 0.14 in generation 30 of the high P6-Line (Table 6.4), where the decline in litter size started. A similar value of F in the high P-Line replicates was observed in generation 14 and Figure 6.1 revealed a decline in litter size of the high P-Line after generation 14, though the decline was less marked as compared to the high P6-Line. The control P-lines also showed a decline in litter size (Figure 6.1) after generation 13. Hence, the inbreeding depression could be one of the possible explanations for the decline in litter size of the high P6-Line.

In a preliminary analyses inbreeding coefficient was fitted as a covariable to account for change in the mean due to inbreeding. The estimate of the regression coefficient (regression of litter size on inbreeding coefficient(F)) was not different from zero (16.6 ± 14.34 mice per unit of F for control lines) and estimates of variance components remained unchanged by inclusion of F as a covariate. The inbreeding coefficients of individuals within generations were similar due to the mating structure and the differences between generations were already accounted for by fitting the generations as a fixed effect, thus the estimates of variance components remained unchanged. Eisen *et al.* (1973) postulated that the sudden increase in infertility may result from a departure from an optimum body weight associated with an optimum degree of fatness. This is further supported by the findings of Hastings (1989) that high and low fat lines had a difference of only 1.6 pups in 32-34 generations. The body weight of high P6-Line in the 30^{th} generation was increased to 48g as compared to

32g in the beginning of the experiment but information on the fat contents of P6-lines were not available as the selection was on body weight. Hence it was not possible to assess the fatness. Barria and Bradford (1981) also observed a high incidence of infertility in a selected line. They have suggested that the pleiotropic genes with opposite effects on weight gain and infertility become important after a prolonged selection due to increase in their gene frequencies. It is difficult to provide any documentary evidence for such a hypothesis from the present analysis.

The estimates of additive genetic variance (Va) of litter size for control, high and low P-Lines were observed to be different. As detailed in Chapter 2, all the three lines and replicates originated from the same base population. As discussed earlier, REML with animal model, provides the variance components of base population under infinitesimal model assumptions of many genes, each with small effect. This method accounts for the change in variance due to inbreeding. Under such circumstances estimates of Va from control, high and low are expected to be the same from all the data sets, however, assuming that there is no genetic correlation between the litter size and selected trait.

In Table 6.5, the estimates of Va and h^2 for the high lines are lower than the estimates for the control lines and for the low lines they were close to zero, in the P-lines analyses. But a large estimate of standard error attached to them makes it inconclusive. However the analysis of control+high lines resulted

in estimates for high lines quite close to the estimates from the controls and three replicates together. This is as expected because the high and control lines originated from the same base population. The estimates from the low lines were very low even when analysed with control lines data (0.09 ± 0.06). and with large standard error.

The analyses incorporating heterogeneity of additive genetic variance (Table 6.7) yielded almost similar estimates in the control and high lines. There was not much change in the estimates of low lines even with analysis using heterogeneity of Va assumptions. The estimates for control lines in the above two analyses were slightly different, perhaps because the effects of generations may be different in control+high and control+low analyses.

The analysis of high+low of P+P6 data (Table 6.8), assuming heterogeneity of Va and Ve components, revealed that the residual environment component (Ve) was very high (factor of more than 1.5) in the high lines as compared to the low lines. The estimate of Ve in the high P-lines data set (Table 6.5) was 5.4; it increased to 7.3 in the high P+P6 data set. The Ve in low lines did not change much from P to P+P6 data set. This suggests that the high P-6 line in the later generations was becoming more susceptible to the environmental fluctuations as compared to the high P-lines. The Va also showed an increase in P+P6 data set, but the estimates of heritability were similar to the estimates obtained for high+low P-lines using the heterogeneity of Va computing option

(Table 6.7), because Va and Ve of P+P6 data set increased by similar amounts.

All these analyses suggest that Va in low lines is practically zero, which seems unlikely because the low lines are showing a correlated response in figure 6.1. The estimates for the low lines usually had a large standard error, indicating that these estimates could be low due to sampling. Another possibility could be the incorrect assumption of the infinitesimal model, that is the possibility of few genes with large effect which may have been fixed as a consequence of selection for lower body weight. In such circumstances, the assumption of the infinitesimal model does not seem to hold in the case of the low lines, giving biased estimates of genetic parameters.

To give a little more insight, litter size was simulated for 20 generations as an independent trait using pedigrees of the actual data and their inbreeding coefficients. The results (Table 6.9) showed no difference between the estimated and true parameters in all the analyses. The true h2 of 0.2 was estimated as 0.186±0.082 and 0.239±0.066 from low and control+low data sets, respectively for low line. This suggests that the negligible estimates of low lines are at least neither due to inbreeding (i.e. due to reduction in heterozygosity), which is being taken care of by this method nor due to calculation artifact or pedigree errors. The simulation also provided an opportunity to compare the actual and predicted standard errors. They agreed very well in the simulated data.

The heritability estimates for lean mass and litter size from the bivariate analysis were observed to be lower than the estimates from the univariate analysis. The estimate of h^2 for lean mass should be similar from bivariate and univariate analyses because the selection was practised on lean mass. The differences between the heritability estimates of lean mass from bivariate and univariate analyses were significant at the 5 but not at the 1 percent level. This suggests that the bivariate estimate is different from the univariate estimate because of inclusion of the litter size trait. The lean mass is available only on the males and there is no information on females, so litter size in the bivariate analysis might be providing some information on females as it is correlated to body weight and lean mass. The comparison of variances within full-sib families from bivariate and univariate analyses showed very little difference, indicating that these differences could also be due to sampling. The heritability estimate of litter size was lower from bivariate analysis than from the univariate analysis suggesting that the univariate estimates were slightly biased due to genetic correlation between litter size and lean mass. The genetic correlation between litter size and lean mass was positive and moderate.

The genetic parameters and variances obtained from the bivariate analysis of 0-20 generations were used to deduce the genetic correlation from correlated and direct responses of litter size and lean mass, respectively. Since the selection was within family, the parameters were converted to within family parameters

and are given below.

Litter Size (LS)	Lean Mass (LM)
Va = 0.561 Ve = 4.874	Va = 4.052 Ve = 2.479
h^2_w= 0.0544 h_w= 0.2333	h^2_w= 0.4497 h_w= 0.6706
σ^2_w= 5.1545 σ_w= 2.2704	σ^2_w= 4.5050 σ_w= 2.1225
Correlated response = 1.3140	Direct response = 15.015

$$\text{Correlated response in LS} = i.r_a.h_{w(LS)}.h_{w(LM)}.\sigma_{w(LS)}$$

$$1.3140 = i.r_a.(0.2333).(0.6706).(2.2704)$$

$$\text{Direct response in LM} = i.h^2_{w(LM)}.\sigma_{w(LM)}$$

$$15.015 = i.(0.4497).(2.1225)$$

$$r_a = 0.235$$

Where, i = Selection intensity, r_a = Genetic correlation, h^2_w = within family heritability { = ½ Va / (½ Va + Ve)}, σ^2_w and σ_w are within family phenotypic variance (= ½ Va + Ve) and standard deviation.

The genetic correlation was observed to be 0.23; which is very close to the estimated genetic correlation of 0.27 from the bivariate analysis. This indicates that the observed correlated response in litter size agrees with the estimates of genetic parameters for litter size in this data set. The estimate of genetic correlation obtained in this study is lower than the realised estimates of genetic correlation between body weight/weight gain and litter size reported in the published literature. Most reports have used 3- and 6-week body weight and post-weaning weight gain. The genetic correlation between litter size and body weight at 10 weeks in the P-lines was not estimated

because (a) selection was on lean mass, (b) the genetic correlation between body weight and lean mass was more than 0.9 (previous chapter).

CHAPTER 7

GENERAL DISCUSSION

7.1 SUMMARY

Mice provide an excellent alternative to commercial livestock species for undertaking long-term selection experiments because of their short generation interval, large litter size and early maturity. Such experiments can be replicated even with a large population size, as they are less expensive and easier to handle compared to the commercial livestock species.

In the analysis of animal breeding data for genetic parameter estimation and prediction, the infinitesimal model (a very large number of loci, each having a small effect, to determine the trait) is implied under multivariate normal distribution assumptions. The motivation of this study was to examine the infinitesimal model assumptions in long-term selection experiments. Data from long-term selection experiments with livestock species are rarely available, therefore information from an experiment with mice was used.

The mouse lines selected for lean mass and body weight for 38 generations were used for this study. The high, low and control lines each had three replicates and originated from one base population. The use of Restricted Maximum Likelihood with the complete pedigrees in an animal model analysis would account for the reductions in additive genetic variance due to selection and inbreeding. Hence, it is expected that the separate analysis of selected lines and controls should provide similar estimates of the genetic parameters, if the infinitesimal model held true. As

stated in Chapter 1, the objectives of this study were: (a) to analyse lean mass and body weight in mice using univariate REML analyses to estimate the genetic parameters, (b) to assess the change in genetic parameters over the selection period, (c) to estimate correlations of lean growth with other associated traits using multivariate REML analyses, and (d) to analyse litter size to examine the effects of selection on this trait.

The lean mass in the P-lines selected for 20 generations showed a divergence of 7 phenotypic standard deviation units (σ_p), indicating that the lean mass can be altered substantially in mice (Figure 3.1) using a phenotypic lean mass index, body weight - 8 x gonadal fat pad weight at 10 weeks of age, to predict the lean mass. The correlated responses in body weight and litter size were in the direction of selection for lean mass (Figures 4.1 and 6.1). After 20 generations, three replicates were crossed within selected lines and the selection criterion was changed from lean mass to body weight at 10 weeks of age, as the two traits were highly correlated; and lines selected for body weight were designated as the P6-lines. The divergence between the high and low P6-lines was 23.2g (8.7 σ_p) for body weight in generation 38 of selection. However, body weight and litter size declined after 31 generations in the high P6-line.

The univariate REML analyses of lean mass in the P-lines revealed the estimates of heritability (h^2) close to 0.5 from different data sets (Table 3.5). The estimates of additive genetic variance (Va) in the high and low lines were lower than that

from the control line. If the infinitesimal model held, the estimates of Va from the selected lines should not be different from that of the controls. The h^2 estimates from the high and low lines had high standard error estimates, indicating that these differences could be due to sampling. The estimates of h^2, however, from the analysis of each selected line plus control data resulted in estimates similar to that of the control line, which could be due to more information in the analysis as the two lines have common parents in the base population and more number of observations in the analysis. The estimates of c^2 ranged from 0.19 to 0.29; the high line estimates were higher than the low line, with the controls being intermediate. The analyses of control+high and control+low, by taking into account their heterogeneous additive and common litter variances and partitioning the data of high line at different generations, showed a consistent decline in Va of the selected lines over the generations (Tables 3.7, 3.8 and 3.9).

The h^2 and c^2 estimates for 10 week body weight from the univariate analyses of the first 20 generations were similar to those for lean mass (Table 4.3). Again the analyses of the high and low lines gave lower estimates of Va and h^2 than the control lines. However, the analysis of combined selected lines (high+low) and selected plus control data (i.e. high+control and control+low) resulted in estimates of h^2 similar to those from the control lines and pooled data of three replicates.

The analysis of P6-lines i.e. body weight data from 21 to 38

generations, by fitting the complete pedigrees (P+P6 lines) resulted in lower estimates of h^2 from each of the selected lines investigated individually and selected lines combined. This is, perhaps, due to ignoring the data from the first 20 generations (P-line) and thereby omitting information on selection prior to the 21st generation (Van der Werf and De Boer, 1990). Therefore to obtain unbiased estimates of genetic parameters, data of the P-lines were also included in the analysis of P6-lines.

The analysis with the assumptions of heterogeneity of additive genetic and common litter variances of P-lines plus 9 generations of P6-lines (i.e. a total of 29 generations) revealed a decline in the estimates of Va and h^2 in both high and low lines as compared to the P-lines (Table 4.4). These estimates were further reduced when data from 38 generations were analysed. This clearly indicates that the additive genetic variance of body weight is decreasing consistently over the generations, although Va is not completely exhausted in the high and low lines. As shown in Figure 4.1, the high lines did not respond to selection after generation 31, but this is clearly not due to the complete exhaustion of additive genetic variance. Amongst the other possible reasons explored in this study were decline in the litter size (Figure 6.1) and increased percentage of born dead (Figure 6.6), although there was a positive selection differential in each generation and the cummulative selection differential (Figures 4.4 and 4.5) did not show any substantial decline in the high lines. Further Hastings (personal communication) compared the body weights of sterile and fertile females and observed no significant

difference, suggesting that the body weight is not a limiting factor to the response.

The multivariate analyses (Tables 5.1 and 5.2) of lean mass, body weight, gonadal fat pad weight and the proportion of gonadal fat pad weight to body weight for 20 generations (P-lines) gave estimates of h^2 and c^2 similar to those from the univariate analyses. Similar estimates from the univariate and multivariate analyses are expected for the trait on which selection was practised i.e. lean mass in this case. However, the similar univariate and multivariate estimates for body weight and gonadal fat pad weight were due to the fact that the heritabilities of these traits were similar, as were the genetic and phenotypic correlations among these traits, which may be a coincidence in these data. In such a situation the **G** matrix is proportional to the **P** matrix and the prediction of one trait depends only on that trait and univariate estimates are unbiased.

The genetic and phenotypic correlations between lean mass and body weight at 10 weeks of age were positive and very high (>0.9), indicating that the fast growing and heavier mice have more lean tissue than the slow growing and lighter mice. Lean mass had a low and positive genetic correlation with gonadal fat pad weight, predicting little change in the latter trait, though in the positive direction of selection for lean mass, i.e. an increase in the high lines and a decline in the low lines. If the gonadal fat pad weight was considered as a proportion of body weight, it showed a decline in the high lines and an increase in the low

lines, due to its negative correlation with lean mass. This predicts that the high lean growth lines would have a lower proportion of fat tissue in the body as compared to the low lines at generation 20; which is in agreement to the body composition analyses of these lines by Hastings and Hill (1990). Body weight had a positive and moderate (≈ 0.5) genetic and phenotypic correlation with gonadal fat pad weight. This suggests a possible increase in the fat tissue of high P6-line (after 20 generations) where the selection criterion was changed to 10 week body weight; the body composition analyses of the P6-lines, however, are not available.

Litter size was analysed using univariate and bivariate analyses (Chapter 6) as it is correlated trait to the selection criteria, lean mass or body weight. The litter size of the first 20 generations showed an increase in the high lines and a decline in the low lines (Figure 6.1), the difference in the 20th generation being 3 pups. The replicates within high and low lines were inter-crossed to produce a new base for the P6-lines in the 21st generation. The litter size of generation 21 showed a sudden increase which may have been due to heterosis by crossing the replicates, but also included factors like environmental changes and change in the age of mating from 9 to 12 weeks. The litter size after generation 21, i.e. in the P6-lines, showed wide fluctuations with a decline after generation 31 in the high line. There was an increase in the percentage of dead born in the later generations of the high P6-line (Figure 6.6) that led to the decline in litter size.

The univariate analysis of litter size (Table 6.5) gave a heritability (h^2) estimate of 0.17 from the analysis of pooled data of three replicates each comprising of control, high and low lines and 0.18 from the control lines. The estimate of h^2 from the high lines alone was very small and in the low line it was zero. These estimates had a very high standard error associated with them. The analyses of control+high and high+low lines resulted in estimates of h^2 similar to that of overall pooled data of the three replicates, but the estimate from the control+low line was still very low as compared to the estimate from the pooled data.

The analysis of litter size with the assumptions of heterogeneous additive genetic and residual environment variances (Table 6.8) revealed an increase in h^2 from the P+P6-lines data (0.27) over that of controls in the P-lines (0.16); but in the case of the low lines it was still very low. The residual environmental (error) variance was also increased in the high lines suggesting an increase in the environmental fluctuations.

Litter size and lean mass of control + high + low data in the P-lines were analysed using a bivariate REML analysis because the univariate analysis of litter size may have given biased estimates of the parameters as selection was on lean mass and the two traits are correlated. The estimates of h^2 from the bivariate analysis for lean mass and litter size were lower than from the univariate analyses, the estimates being 0.46 and 0.10, respectively. The

genetic correlation between lean mass and litter size was observed to be 0.27, which was very close to the realised genetic correlation of 0.24 between the two traits. The estimates of h^2 for lean mass are expected to be the same from univariate and bivariate analyses as the selection was practised on the lean mass. The difference in the estimates from the two analyses could be due to the fact that lean mass was measured only on males whereas the litter size is a trait of females, and it is possible that the litter size is providing some information about the lean mass of females through its correlation with body weight.

7.2 GENERAL DISCUSSION

In this investigation it was expected that the estimates of genetic parameters for the base population should be similar from the analyses of high, low and control lines individually as they originated from the same base population. This expectation is based on the assumption that the animal model analysis with complete pedigrees takes account of the bias in the estimates of genetic parameters due to selection and inbreeding; and no change is expected in additive genetic variance due to change in gene frequency as they are very small if the trait is determined by a very large number of genes, each having a small effect.

The observations in this study are that the high and low lines, when analysed individually, give lower estimates of Va and h^2 for the base population than do the control lines or the pooled data of the three replicates, each having high, low and control

lines. These observations are in contradiction to the findings of Sorensen and Kennedy (1984), where they have shown with a simulation study that using an animal model and a complete relationship matrix, the estimates of Va in the base population are not biased due to inbreeding and linkage disequilibrium. However, they simulated only 5 generations of selection with the assumptions of a large number of unlinked loci affecting the trait. In this study, selection was practised for 38 generations and the possibility of some major gene(s) can not be ruled out. However, further research is required, perhaps by simulating long-term selection data and finite number of genes.

The analysis with the assumptions of heterogeneity of variance components can be useful for analysis of two selected lines together,but where no controls are maintained, a usual situation in the livestock species. This approach would provide estimates of variance components in each selected line separately.

The high P6-line failed to respond to selection after 31 generations, though the low line was showing consistent response up to generation 38. This study has shown that there is still additive genetic variance available to make further progress in the high line. The availability of additive genetic variance could further be investigated experimentally in the high line by relaxed and reverse selection. There is no evidence of reduced selection differential in the high P6-line indicating that the reduced litter size and increased dead born have not reduced the selection differential. The plateau could be due to the natural

selection by reducing the fitness e.g. animals of large body weight may not survive. If, for example, there are genes with large effect and overdominant gene action, heterozygotes would be selected in every generation and their segregation would result in variation being maintained in every generation with the selection pressure on other genes reduced. Therefore selection may not give a response as the same genotypes are being selected in each generation.

To assess the change in Va during the course of selection, the analyses of control plus selected lines were carried out using the assumptions of heterogeneity of variance components. In such analyses data were partitioned into two groups, i.e. control+part of the selected lines and the remaining part of the selected line e.g. 5-20, 8-20 and 15-20 generations data sets, and only two groups were fitted at a time due the limitations of the computing program. The estimates of additive genetic variance of different parts, i.e. 0-4, 5-7, 8-14 generations were calculated by weighting the variance components obtained in the analyses with the appropriate number of observations in each group. This showed a decline from the start of the experiment and thereafter. However, this procedure is a approximation with the assumption that each observation contribute equal information, which may not be the case as the observations in the later generations contribute more information. It would be more appropriate to fit control data and different parts of the high lines data simultaneously to estimate the variance components in each group.

This study has shown that the additive genetic variance is decreasing over the generations in the high and low lines due to selection, which is not expected under the infinitesimal model as discussed earlier. Apparently this seems to be due to the change in gene frequency, which is large enough to change the additive genetic variance. This indicates that the infinitesimal model did not hold and there may be some genes with large effects on body weight. Similar observations have also been made by Hastings (1990) by backcrossing the progeny of a cross between the high and low P6-lines to both parental lines. Further investigations are required by considering major gene(s) and polygenes simultaneously for the growth traits.

Mutations could be an important factor contributing to response and affecting the genetic variance in long-term selection experiments, depending upon the rate at which they are generated (Hill, 1982). Ignoring the mutational variance (Vm) in the model may inflate the estimates of Va. The model considered in this study assumes zero Vm. If there is some Vm, which may be likely in long-term selection experiments, the estimates of Va are confounded with Vm, thus the true Va still may be lower than the observed in this study. In this analysis we have considered an additive infinitessimal model ignoring the dominance and epistatic variances for computational ease. If dominant and epistatic gene actions are present, they are likely to give biased estimate of Va. However, common litter variance fitted in the model is likely to largely account for the variance due to dominance.

7.3 IMPLICATIONS

The implications of this investigation can be summarised as follows:

(a) There was a substantial amount of common litter litter variance in the selected lines. Ignoring the common litter variance in the analysis of data from the litter producing species would result in biased estimates of genetic parameters.

(b) Analysis of selected lines alone, from long-term selection, may provide underestimates of additive genetic variance.

(c) In situations where no controls are maintained, usual in livestock species, two divergently selected lines can be analysed together to give the estimates of variance components of each selected line separately.

(d) The analyses of the selected lines have shown that the infintesimal model assumptions did not held even in the first four generations. Therefore generality of the infinitesimal model assumptions in the animal breeding data analyses may not be realistic.

CHAPTER 8

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