# Single-gene Effects on Body Weight in Selected and Unselected Mouse Lines Detected by Bayesian Marker-free Segregation Analysis

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## Summary

Segregation analysis of body weight at 21, 42, and 63 days in eleven generations of unselected (2813 individuals of C line) and selected (1410 individuals of HGC line, derived from C line) mice was performed. The Gibbs sampling algorithm was applied to obtain posterior density distributions of model parameters. Moderate heritability via polygenic model was estimated. The results suggest a mixed inheritance model (major gene + polygenic) for the body weight. The proportion of single gene variance to phenotypic variance ranged from 9.7 to 41 percent. The estimated additive heritabilities for these traits varied between 0.25 and 0.47 for the C line and 0.37 and 0.41 for the HGC line. Considerable differences in estimated major gene frequencies were found between unselected and selected lines.

## Introduction

Over the last decades, a number of single genes with considerable effects on production and reproduction traits have been identified in livestock and laboratory animals. Mice were often proposed as model animals for human and livestock obesity as well as other characters. Specially created mouse lines (selected inbred, single mutant, congenic, chromosome substitution, transgenic etc.) give a unique opportunity to understand the physiological pathways and genetic background of weight. In some mouse lines mutations with a large effect on body weight were found: Leptin (*Zhang et al., 2004*), Leptin receptor (*Tartaglia et al., 1995*), Myostatin (*Szabó et al., 1998*) and also many QTL studies were performed (*Brockmann & Bevova, 2002*). However, it should be stressed that the effects are often population specific, and even large effects of single genes may not be seen in other populations. The costs of molecular analysis limit population size, which reduces the power of QTL detection. Some methods like selective DNA pooling were developed to reduce the number of genotypings (Darvasi & Soller, 1994). On the other hand marker-free segregation analysis introduced by Elston & Steward (*1971*) and developed by Morton & MacLean (*1974*) can indicate the presence or absence of major genes segregating within the population, based on performance records of a pedigree population. For large populations with complex pedigree structure, a Gibbs sampling algorithm was proposed by Guo & Thompson (*1994*) and Janss et al. *(1995)*. This approach allows an estimation of genotype effects and allele frequencies. The Gibbs sampling algorithm has many advantages (*Van Tassel et al., 1995*).

Segregation analysis was suggested to be the most powerful statistical method to identify a single gene when DNA marker information is unavailable (*Ilahi & Kadarmideen, 2004*). The method was successfully applied for studies of the genetic background

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of economically important traits in several species: dairy cattle (*Pan et al., 2001; Ilahi & Kadarmideen, 2004*), pigs (*de Moraes Gonçalves et al., 2005; Kadarmideen & Janss, 2005*), sheep (*Walling et al., 2001*), poultry (*Szydłowski & Szwaczkowski, 2001*) and dogs (*Mäki et al., 2004*).

The objective of our study was to perform segregation analyses of body weight in unselected and selected mice populations within the Bayesian framework. Heritabilities of three measurements of body weight were also estimated.

### *Materials and Methods*

The data were recorded in two mouse lines, C and HGC, where the C line was created as a genetic pool by crossing a random sample of mice acquired from petshops in Berlin for four generations (*Weniger et al., 1974*), then rotating 40 families in turn through 32 generations and starting random selection and mating in the 37th generation by the use of random numbers.

In the HGC line, derived in 1999 from generation 65 of the C line, mass selection was performed on body weight at 42 days of age. Eleven generations were included with 2813 and 1410 individuals from unselected C and selected HGC lines, respectively. Body weight at 21, 42 and 63 days was measured for all individuals. Mice were kept in Macrolon cages (type 2 by EBECO, E.Becker u. Co GmbH, Hermannstr. 2 – 8, D 44759 Castrop-Rauxel, Germany) on standard litter (Altromin type S 80150 by Altromin Spezialfutter GmbH u. Co. KG, Lange Str. 42, D 32791 Lage, Germany). They were weaned and separated for sex at 21 days of age. Animals were fed standard feed (Zuchtfutter fuer Ratten und

Maeuse Nr. 1314 by Altromin Spezialfutter GmbH u. Co. KG, Lange Str. 42, D 32791 Lage, Germany) *ad libitum*. Temperature varied between 20 and 24 °C and relative humidity between 50 and 65 percent. Descriptive statistics are given in Table 1. The Kruskal-Wallis test was employed to evaluate the differences in population means (*SAS, 2002-2003*). Two single-trait models were applied.

Model I (full model):

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{ZWm} + \mathbf{Zu} + \mathbf{e},$$

where:

**y** is the nx1 vector of observations,

**ß** is the rx1 vector of fixed sex effects,

**u** is the sx1 vector of random additive polygenic effects,

**e** is the nx1 vector of random errors.

**X** and **Z** are the nxr and nxs design matrices for fixed and random effects, respectively.

**W** is the sx3 matrix of unknown genotype configurations

**m** is the 3x1 vector of genotypic effects (AA, Aa, aa).

Model II (reduced model):

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e},$$

where: **y, ß, u, X, Z, e-** as above.

The Gibbs sampling algorithm was applied to obtain posterior density distributions of model parameters. The mixing properties of the Gibbs sampling process were monitored by a visual inspection of suitable plots. In every analysis 200 000 rounds of algorithm were generated and the first 50 000 of them were discarded as a burn-period. The autocorrelation among the generated chains was small for every parameter so it was decided to keep the results from each of the 10th round as the important

**Table 1.** Descriptive statistics of the analyzed traits

|  | **C line** (n = 2813) | | | | **HGC line** (n = 1410) | | |
|---|---|---|---|---|---|---|---|
|  | **BW21** | **BW42** | **BW63** |  | **BW21** | **BW42** | **BW63** |
| **Mean** | 12.59 | 25.05 | 28.05 |  | 13.64 | 28.16 | 32.11 |
| **STD** | 1.65 | 3.01 | 3.69 |  | 1.90 | 3.98 | 5.07 |
| **Skewness** | 0.54 | 0.28 | 0.30 |  | 0.54 | 0.17 | 0.13 |
| **Kurtosis** | 1.86 | -0.27 | -0.43 |  | 1.43 | -0.41 | -0.44 |

sample. The point marginal posterior estimators of unknown parameters have been calculated as the mean values from the generated chains. Numerical properties of the applied algorithm were discussed by Skotarczak et al. (*2007*).

An autosomal gene with two alleles in Hardy-Weinberg equilibrium was assumed to influence body weight apart from polygenes. For numerical reasons, the dominance effect was not included (*Kadarmideen & Janss, 2005*). Therefore, $\sigma^2_{QTL}$ was equal to $2pq(m_{AA})^2$, where $p$ and $q$ are allele frequencies and $m_{AA}$ is the QTL dominance homozygote effect. Significance of QTL effects was verified by 95% Highest Posterior Density Regions (HPDR) derived by the shifted histogram method of Scott (*1992*). In addition, the classic genetic additive model was used for the comparison of results. The following parameters were estimated:

Based on model I:
additive heritability
$$h^2_{add} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{QTL} + \sigma^2_e}$$

proportion of variance explained by QTL
$$h^2_{QTL} = \frac{\sigma^2_{QTL}}{\sigma^2_a + \sigma^2_{QTL} + \sigma^2_e}$$

total heritability
$$h^2_T = \frac{\sigma^2_a + \sigma^2_{QTL}}{\sigma^2_a + \sigma^2_{QTL} + \sigma^2_e}$$

Based on model II:
polygenic heritability
$$h^2_{pol} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e}$$

where: $\sigma^2_a$ is the additive genetic variance, $\sigma^2_{QTL}$ is the QTL variance and $\sigma^2_e$ is the error variance.

### Results and Discussion

Averages over generations for these traits studied in both lines are presented in Figures 1 and 2. Highly significant differences between ages and lines were observed. In the C line (Fig. 1.) generation means fluctuated randomly about the mean and - as expected in an unselected population - specific trends in phenotypic means were not observed. However, for the HGC line (Fig. 2.) significant positive trends appeared in the phenotypic means for all weights registered.

Heritability estimates via full and reduced models are given in Figures 3 and 4. In general, heritability of body weight is known to be at a moderate to high level, which results from a relatively high propor-
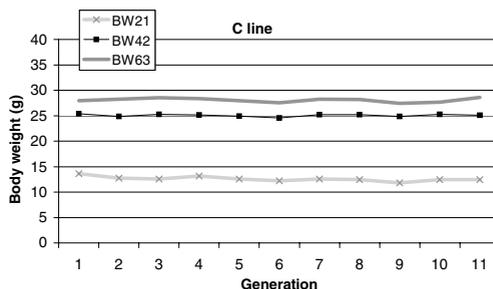


**Figure 1.** Phenotypic trends of body weight in the C line.
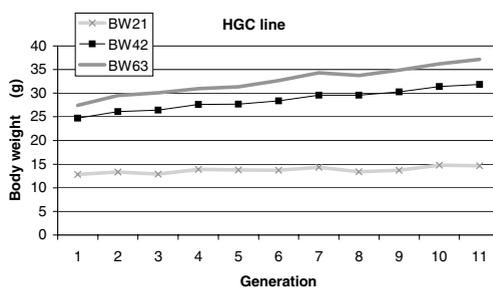


**Figure 2.** Phenotypic trends of body weight in the HGC line.

tion of genetic component in determination of this trait. Estimates based on a polygenic model were similar in both lines and close to 0.5 for all ages. Basically, the estimated polygenic heritabilities correspond with results obtained by Leamy et al. (*2005*). A moderate level of both realized heritability and REML estimates were reported by Siewerdt et al. (*2000*) and Eisen (*1978*). Much higher heritability estimates found by Bachmanov et al. (2002) by ANOVA methodology were not confirmed by selection experiments. A genetic and environmental (residual) variability summarised by heritability estimate is influenced by a number of factors specified for a given population. These differences observed for heritabilities estimated via both models indicate various genetic backgrounds to body weight. Some authors concluded that other polygenic effects (for instance, maternal effects) are implicated in controlling mouse body weight (*Leamy et al., 2005*). However, apart from the polygenic background
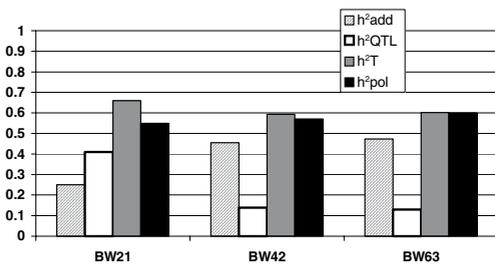
**Figure 3.** Heritability estimates in the C line
Note on symbols: h²add – additive polygenic variance to total variance (included QTL); h²QTL – QTL variance to total variance; h²T – sum of additive polygenic and QTL variances to total variance; h²pol – additive polygenic variance to total variance (without QTL).
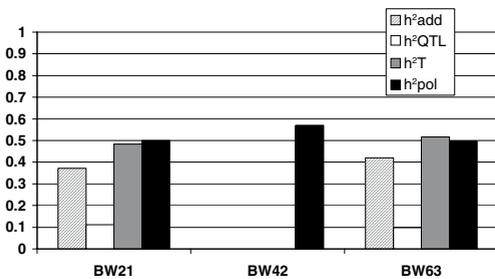


**Figure 4.** Heritability estimates in the HGC line
Note on symbols – as above.



**Figure 5.** Marginal posterior density of major gene effect for body weight at 21 day in C line.
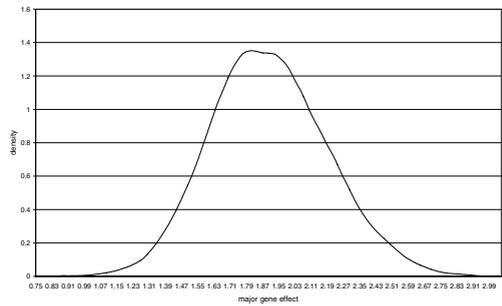


**Figure 6.** Marginal posterior density of major gene effect for body weight at 42 day in C line.
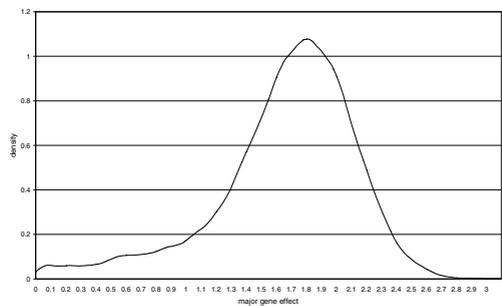


**Figure 7.** Marginal posterior density of major gene effect for body weight at 63 day in C line.

major genes could also contribute to body weight. Marginal posterior density of single gene effect for respective traits are presented in Figures 5 and 9. In the case of body weight at the 42nd day in the HCG line, no effect of single locus could be estimated since the frequency of dominant allele was evaluated as one, whereas for others traits studied, the results indicate a mixed inheritance model.

Keightley (*1998*) reported that mutations with large effects could be responsible for a significant proportion of the selection response in inbred mouse lines. The existence of single mutations with large effect and results from multiple QTL studies support the idea that genes with large effects segregate in dif-

ferent mouse populations (*Brockmann & Bevova 2002*). In our study, a mixed inheritance model for all body weight measurements was suggested prior to molecular analysis. In the C line, estimated addi-
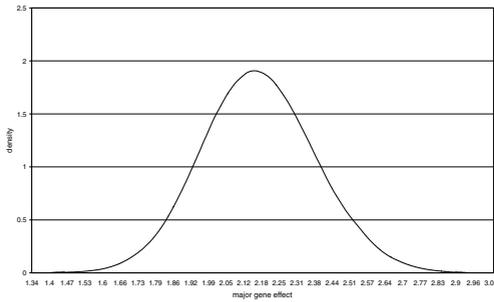
**Figure 8.** Marginal posterior density of major gene effect for body weight at 21 day in HGC line.
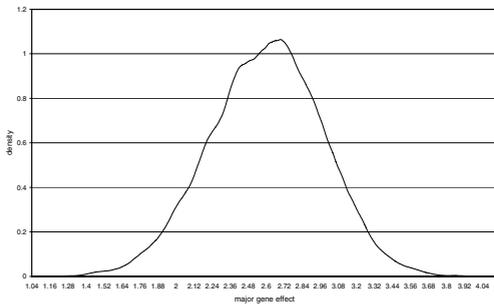


**Figure 9.** Marginal posterior density of major gene effect for body weight at 63 day in HGC line.

tive heritability ranged from 0.25 to 0.53. The highest proportion of variance was attributed to QTL for early body weight. An increase of heritability and additive QTL variance with age was reported by Kramer et al. (*1998*), who used intercrosses between inbred mice lines. Heritability according to the polygenic model gave an accurate approxima-

tion of total heritability based on the mixed inheritance model.

In the selected HGC line the proportion of QTL variance and additive heritability remained on a constant level over time. No reduction of polygenic heritability was observed compared to an unselected line, which could result from a relatively small number of selected generations.

However, the frequency of the dominant allele was higher in the selected line and reached the value of one for the trait, which was the direct selection objective. There seems to be agreement between the result obtained and molecular data. From the DNA analysis it was shown that in some of the selected mouse lines derived from the C line, a mutation in the myostatine gene was present (*Schlote W, personal communication*). This gene is known to cause muscular hypertrophy that leads to the so-called compact phenotype and is one of suggested candidate genes for body weight (*Szabo et al., 1998; Brockmann et al., 2000*).

The differences between allele frequencies were on a constant level of about 10 percent. The estimated QTL effect ranged from 1.7 to 2.3g (Table 2) with slightly higher effects in the selected line. Differences of the effects between lines tended to increase with age. None of the 95% Highest Posterior Density Regions included 0; therefore QTL effects were significant.

As a conclusion of the study, a mixed inheritance model of body weight in mice was suggested with noticeable differences between selected and unselected lines in gene frequencies and effects.

**Table 2.** Allele frequencies and effects in the studied populations

|  | Allele frequency | | QTL effect and its HPDR | |
|---|---|---|---|---|
|  | **C line** | **HGC line** | **C line** | **HGC line** |
| **BW21** | 0.829 | 0.965 | 1.984 (1.777-2.193) | 2.174 (1.767-2.596) |
| **BW42** | 0.902 | 1.000 | 1.906 (1.356-2.494) | *** |
| **BW63** | 0.829 | 0.912 | 1.718 (0.556-2.476) | 2.256 (1.852-3.326) |

*** - the frequency of dominant allele was estimated as one; therefore no effect could be estimated.

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