



Commission on Animal Genetics
Session G4.4
(Abstract No. 414)

# Marker assisted introgression of a sex linked major gene into a mouseline with extreme growth

**Lutz Bünger[1], Fiona Oliver[2], Ulla Renne[3], Peter Keightley[2], William G. Hill[2]**

email: L.Bunger@ed.sac.ac.uk

[1]Scottish Agricultural College, Sustainable Livestock Systems Group, Bush Estate, Penicuik, EH26 0PH, UK

[2] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK

[3] Research Institute for Biology of Farm Animals, Department of Genetics, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

## Summary

Earlier studies found evidence of a substantial sex-linked effect accounting for over 20% of the divergence between two mouse lines (EDH, EDL) divergently selected for body weight and a follow-up marker-based study indicated a single QTL for body weight at 70d (BW 70) at about 23 cM from the proximal end of the X-chromosome. The estimates of the additive genotypic effects were 2.6 g in both males (half the difference between hemizygotes) and females (half the difference between homozygotes) corresponding to 20% and 17% of mean BW70 in females and males in the F2-population. The average male BW 70 in the developed inbred EDH (EDHi) is ca 47g. Reciprocal F1 crosses showed that another long term growth selected and inbred line (DUHi), the heaviest known inbred mouse line with a male BW70 of ca 80g, had a low allele and males hemizygous for the high X-QTL were 9.4g (17%) heavier then the low QTL carriers at 70d. To test whether the growth of this extreme line can be further improved by introgressing the X-linked high QTL, and whether the increase in performance is by a similar proportion, marker assisted introgression (MAI) was initiated in which a region of the X chromosome of EDHi was backcrossed into DUHi. In a replicated MAI experiment (> 2 and 7 backcross generations) no significant effects on body weight were found in females and a small effect in males between individuals having the DUH or EDH X-chromosomal region. This suggests epistatic effects acted on the high body weight background.

## 1. Introduction

Despite all progress in QTL mapping (for reviews on identified QTL for body weight see: Pomp, 1997; Brockmann & Bevova, 2002; Corva & Medrano, 2001), we are far from having a list of the number, location, and effects of all individual genes contributing to variation in growth in the mouse, or indeed in other species, but we know of a few individual genes that contribute to variation. Further, there is substantial evidence of epistatic interactions between genes affecting growth traits and other genetic background genes, posing special challenges for future genetic analysis and for the identification of modifier genes (e.g.Glazier *et al.*, 2002; Nadeau, 2001).

In earlier studies in our lab (Hastings, 1990; Hastings & Veerkamp, 1993; Veerkamp *et al.*, 1993) line crossing was used to investigate gene action underlying selection responses, and it was found that 20-25% of the difference in body weight between divergently selected lines from the same base population (EDH, EDL) was accounted for by one major effect locus on the X chromosome. This was followed by a marker-based study in an F-2 population generated from a reciprocal F1 between an inbred low line derived from the EDL and the outbred EDH line. The analysis of data on body weight at 70d indicated a single QTL of large effect situated towards the proximal end of the chromosome, with a 95% confidence interval for the QTL location of 8 cM. The estimates for the additive genotypic effects were 2.6g in both males and females (half the differences between hemizygous males and between homozygous females), or 17% and 20% of the body weight at 70d in males and females respectively (Rance *et al.*, 1997b). Subsequently segments of the X chromosome from the EDH were backcrossed onto an inbred line derived from the EDL, thereby removing contributions from the autosomes and linked segments of the X chromosome. Sublines containing a region at the proximal end of the X chromosome were found to account for almost all the difference between the lines. This QTL was mapped to a region of about 6 cM, and no evidence for QTLs elsewhere on the chromosome was found (Rance *et al.*, 1997a). An interval-specific congenic strain with progeny testing of recombinants for markers flanking a QTL with 39 recombinants in a 12 cM region of the X chromosome was then used to map the QTL to a region of approximately 2 cM (Liu *et al.*, 2001b).

Here we report two experiments. The first was undertaken to detect whether the Dummerstorf High line (DUH), the heaviest known mouse line (Bünger *et al.*, 2001b; Bünger *et al.*, 2001a) carries a high or a low X-QTL allele. Reciprocal crosses were made between DUH and EDH and EDL, and it was found that DUH had a low X-QTL allele. Second, we then practised marker assisted introgression (MAI) to introduce the high X-QTL into DUH to test whether the weight of DUH could be increased to the extent predicted from the effect of the QTL on lower weight backgrounds, as a model MAI breeding programme.

## 2. Material and Methods

*(i) Experiment 1: Reciprocal crosses between DUH(i) and EDHi*
A reciprocal cross is a powerful method for detecting the effects of X-linked genes and to test if the X-chromosome (X-Chr) of different lines differs in its effect on body weight: male progeny inherit their X-Chr from their mother, whereas females inherit one X-Chr from each parent, and so a difference between reciprocal crosses in the sex effect on the trait is indicative of X chromosome effects. The aim of these reciprocal crosses was to test if the DUH/DUHi mice have a "low X-Chr". Mice from four different mouse lines, DUH, DUHi, EDHi and EDLi, were used for such reciprocal crosses; their average male body weights at 70d were approximately 83, 77, 47 and 16g, respectively (Bunger *et al.*, 2001).

**DUH** and **DUHi**: Dummerstorf high outbred and Dummerstorf high inbred (Bünger *et al.*, 1983; Bünger *et al.*, 1990; Bünger *et al.*, 2001a). The DUH mice were long-term selected in Dummerstorf for high body weight at 42d from a base population produced by a cross of 4 inbred and 4 outbred strains (Schüler, 1985). An inbred line (DUHi) was derived by full sib matings in the Edinburgh lab from a sub-population of the line (Bünger *et al.*, 2001a).
**EDHi** and **EDLi**: Edinburgh high inbred and low inbred (Sharp *et al.*, 1984; Bünger & Hill, 1999; Bünger *et al.*, 2001a). The base population was an F1 of two inbreds populations crossed to an outbred. There were initially three replicates with divergent selection for the first 20 generations on an index of lean mass.

Replicates lines were then crossed and the subsequent divergent selection continued on body weight at 70d, giving lines EDH and EDL. Inbred lines were later obtained by sib mating. Generation numbers given here refer to generations of inbreeding.

Five replicate sets of reciprocal crosses were made (R1 to R5) and involved the following lines and generations (G), undertaken either in the Edinburgh or Dummerstorf labs:

| | | | |
|---|---|---|---|
| R1 | DUH (G4) | EDHi (most G11) | Edinburgh |
| R2 | DUH (G5) | EDHi (G12); EDLi (G16) | Edinburgh |
| R3 | DUH (G8), DUHi (G1) | EDHi (G16); EDLi(G19) | Edinburgh |
| R4 | DUH (G13) | EDH (G71) | Dummerstorf |
| R5 | DUH (G14) | EDH (G72) | Dummerstorf |

Animals were weighed at 42, 70 and 84d. For the X-chromosome genotype the following nomenclature was used. Females: X/X homozygous for two (high) EDH–X-chromosomes, x/x homozygous for two (low) DUH-X-chromosomes, and X/x heterozygotes. Males: X/y having the EDH-X, or x/y having the DUH-X-chromosome.

*(ii) Experiment 2: Marker assisted introgression*
The introgression was repeated resulting in two lines: DUHi(1)$^{X}$ and DUHi(2) $^{X}$. The F1 for DUHi(2) $^{X}$ was set up contemporaneous to G6 of DUHi(1)$^{X}$ as DUHi(1)$^{X}$ seemed to suffer from some fertility problems that were jeopardising the experimental aim.

**DUHi(1)$^{X}$**: Initiated in parallel to the beginning of the inbreeding in line DUHi, so generations numbers correspond (Figure 1) and in the later generations most contribution came from DUHi with higher degree of inbreeding. Heterozygous females and hemizygous males from R3 were used to initiate this line and afterwards animals with this genotype were used for recurrent backcrossing to DUHi animals. The F1 was followed by 7 backcross generations, re-establishing the expected DUHi background to 99.6%. In G9 only a few backcross matings were set up. Mostly heterozygous females and hemizygous males were used in G9 for *inter se* matings to produce all three female and both male genotypes, but produced insufficient animals for final analysis. Generation 10 was set up for further backcrosses increasing the expected DUHi background to at least 99.8%. Generation 11 comprised again mostly *inter se* matings. For the final analysis all offspring produced in G9 to 11 were analysed, with the generation number as a fixed effect.

**DUHi(2)$^{X}$**: As this line was initiated 1.5 years later, the inbreeding in the recurrent line DUHi was much further progressed. The F1 was followed by 2 backcross generations (DUHi background re-established on average to 87.5%) before a first set of *inter se* matings produced all three female and both male genotypes. Again numbers achieved were insufficient for a final analysis. Two further backcross generations then reduced the EDHi background to ca 3%, which was followed by a final round of *inter se* matings to produce offspring of both sexes and all genotypes.

**Microsatellite genotyping**: Genomic DNA was extracted from either tail clip or ear clip tissue using the HotSHOT method (Truett *et al.*, 2000). Microsatellite genotyping was carried out based on a protocol described by Routman & Cheverud, 1994). PCR products were separated using 20cm long 6% acrylamide gels, which were run vertically at 200V for approximately 2 hours. Gels were then stained using ethidium bromide and photographed under ultraviolet light for scoring.

**Marker search:** 164 markers were checked using tissues from 2 DUHi and 2 EDHi mice to find polymorphisms distinguishing the lines. Markers found to be polymorphic were further checked with a panel of 90 DUHi samples to confirm that there were no genetic differences at the marker within the DUHi line. Any markers found to segregate within the DUI line were excluded.

**Introgression**: Initially the marker assisted introgression was based on markers DXMit55 and DXMit143, which were known to span the region including the QTL proximal to DXMit68 (Liu et al., 2001). By generation 08 of DUHi(1)$^{X}$ markers DXMit164 and DXMit68 were also used. After the introgression all

animals used in this analysis were typed for all five markers (see below) and animals showing recombinant genotype at any marker position were excluded.

**Marker Positions on X Chromosome:** (Source: Mouse Genome Database. (Mouse Genome Database (MGD), The Jackson Laboratory, Bar Harbor, Maine. http://www.informatics.jax.org/).

| | |
|---|---|
| DXMit55 | 1.4cM |
| DXMit164 | 6.7cM |
| DXIcp7 | 14.2 cM (close to DXMit48) |
| DXMit68 | 17.25 cM |
| DXMit 143 | 26.0 cM |

iii) *Data analysis*

Data on body weights in experiment 1 (reciprocal crosses) were analysed using the following model:

$Y = M + T + R + S + F(R) + TS + e$

where $M$ is an overall mean, $T$ is the effect of type of mating (line of mother and of father) (1-4), $R$ is a replicate effect, $S$ (1-2) is the sex effect, $F(R)$ is a family effect nested in replicate, $TS$ is the interaction and $e$ is the residual error. The model to analyse body weight data in experiment 2 (marker assisted introgression) was similar:

$Y = M + C + G + S + F(G) + CS + e$

where $C$ is the effect of genotype (X/X, X/x and x/x in females; X/y and x/x in males), $G$ the generation effect and $CS$ the interaction. All effects were fitted as fixed except $F(R)$, $F(G)$ and $e$, fitted as random. ANOVA was undertaken using the GLM-procedure of the SAS System for Windows Release 6.08 (SAS Institute Inc., Cary, NC 27513, USA).

## 3. Results

*(i) Experiment 1: Reciprocal crosses between DUHi and EDHi*

The results of the common analysis of the first three replicated crosses involving both the EDHi and EDLi lines in reciprocal crosses with the DUH animals indicate that the male-female difference in reciprocal crosses of EDLi and DUH, ca. 8g at 42d and about 8-9g at 70d (Table 1), does not differ significantly between the two reciprocal crosses, regardless of which is the mother. In contrast, the reciprocal cross has a substantial influence on the difference in weight between male and female littermates: 19.8g at 70d from EDHi mothers and 10.8g from DUH mothers. This implies a difference in weight of males due to the different X-chromosomes of 9.0g at 70d, and similarly 5.1g at 42d. This is reflected also in higher male/female ratios in the EDHi x DUH (1.40 at 70d) than in the reciprocal cross (1.23).

Results of the same approach applied to all five replicated crosses but restricted to lines DUH and EDH (at Dummerstorf) or EDHi (at Edinburgh) are shown in Table 2. Females born by the larger DUH mothers are heavier at all three ages than females born by EDHi mothers, reflecting probably a better maternal environment provided by the DUH mothers. However males born by DUH mothers are similar to EDHi mothered males at 42d, but much lighter at 70 and 84d. The male-female difference, which accounts for different maternal environments between the two lines of mother, is significantly higher in EDH x DUH crosses. The average differences are 5.5, 9.4 and 11.9g at 42, 70 and 84d, corresponding to 12, 17 and 22%, respectively, of body weight.

*(ii) Experiment 2: Marker assisted introgression*

Mean male body weights at 70d of relevant lines illustrate the difference between the "QTL donor" line EDHi and the "QTL recipient" line DUH (Figure 1). Whereas the EDHi males reach average weights of 47g, those of DUH and DUHi have average weights of ca 80g and nearly 90g. The introgression lines DUHi(1)$^X$ and DUHi(2)$^X$ (representing here a mix of all X chromosome genotypes) reach DUHi weights in only a few generations after the F1 cross. But they do not achieve higher levels than DUHi as would be expected from the introgression of the X-QTL; the effect estimated from the reciprocal crosses at 70d was over 9g and a substantial proportion of the males should have a X/y genotype.

Table 1: Body weights (g) at 42d (BW42) and 70d (BW70) of male (M) and female (F) offspring of reciprocal crosses between lines DUH, EDHi and EDLi (line of mother shown first) (replicates 1-3)

| | DUH x EDLi | | | EDLi x DUH | | | DUH x EDHi | | | EDHi x DUH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | LSM | se | n | LSM | se | n | LSM | se | n | LSM | se |
| BW42 F | 42 | 26.1 | 0.39 | 50 | 24.5 | 0.36 | 60 | 38.2 | 0.33 | 44 | 38.4 | 0.40 |
| BW42 M | 50 | 34.2 | 0.40 | 53 | 32.3 | 0.35 | 81 | 48.5 | 0.27 | 47 | 53.7 | 0.43 |
| M-F diff | | 8.1 | | | 7.9 | | | 10.3 | | | 15.4 | |
| M/F ratio | | 1.31 | | | 1.32 | | | 1.27 | | | 1.40 | |
| BW70 F | 37 | 30.5 | 0.54 | 55 | 28.3 | 0.45 | 63 | 46.3 | 0.42 | 43 | 46.4 | 0.51 |
| BW70 M | 50 | 40.0 | 0.51 | 63 | 36.3 | 0.41 | 86 | 57.1 | 0.34 | 48 | 66.2 | 0.53 |
| M-F diff | | 9.4 | | | 7.9 | | | 10.8 | | | 19.8 | |
| M/F ratio | | 1.31 | | | 1.28 | | | 1.23 | | | 1.43 | |

Table 2: As Table 1, but crosses between lines DUH and either EDHi or EDH (EDH(i)), and including body weights (g) at 84d (BW84) (replicates 1-5)

| | DUH x EDH(i) | | (n=114-144) | EDH(i) x DUH | | (n=62-78) |
|---|---|---|---|---|---|---|
| | LSM | M-F | M/F | LSM | M-F | M/F |
| BW42 F | 42.8 | 8.3 | 1.19 | 38.4 | 13.8 | 1.36 |
| BW42 M | 51.1 | | | 52.1 | | |
| BW70 F | 49.7 | 8.6 | 1.17 | 47.0 | 18.0 | 1.38 |
| BW70 M | 58.4 | | | 65.0 | | |
| BW84 F | 53.4 | 7.2 | 1.13 | 47.6 | 18.9 | 1.40 |
| BW84 M | 60.5 | | | 66.5 | | |

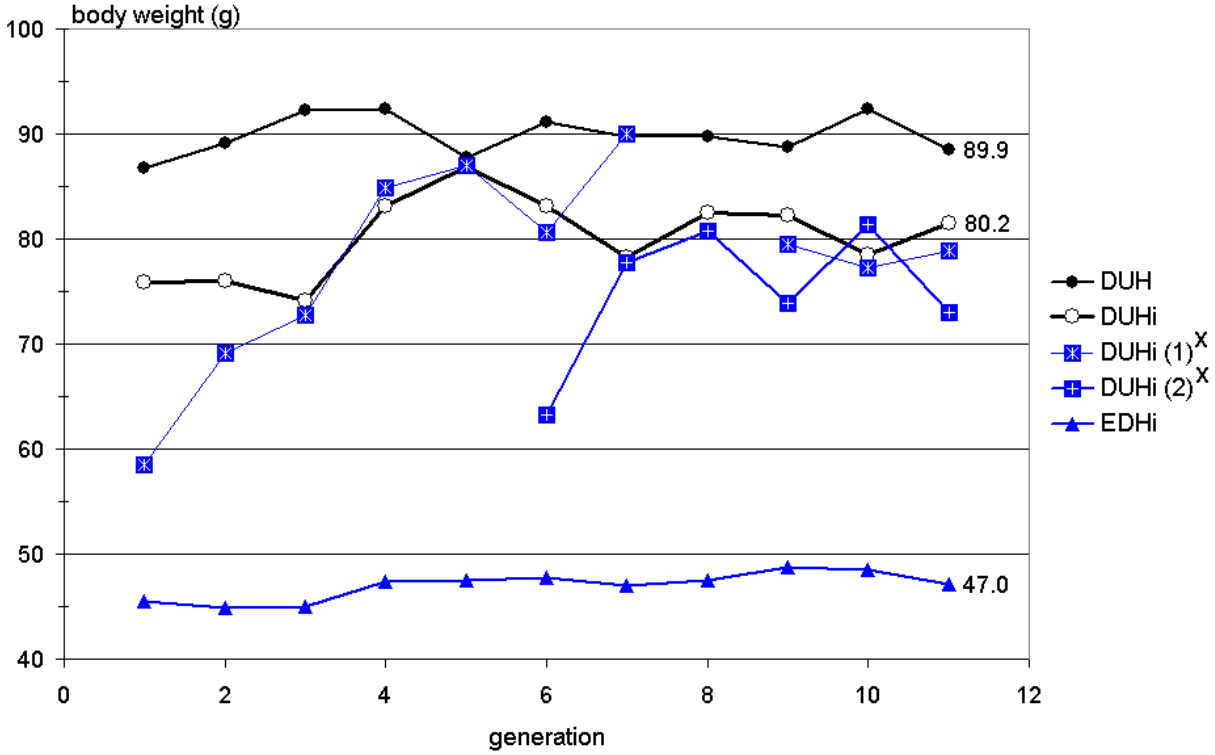
The detailed analysis of DUHi(1)$^{X}$ related data also does not suggest the large effects expected from the reciprocal cross (Table 3 vs. Table 2). There are no significant differences between the three X-Chr genotypes for females at either age. A similar result was observed for males at 42d, but at 70d X/y hemizygous males were 4.6g (6%) heavier (P = 0.005) than x/y males. Similar minor effects were observed in the other introgression line, DUHi(2)$^{X}$ (Table 3). None were significant at 42d in both sexes, although X/X females were almost 4g (9%) heavier than x/x females (P = 0.062). However at 70d X/X females were 6.3g (11%) significantly heavier (P = 0.012). Males with the high X-QTL were not heavier than x/y at 42d, but heavier by 2.4g (3%, NS, P = 0.072) at 94d.

Table 3: Body weights (g) at 42d (BW 42) and 70d (BW70) in introgression line DUHi(1)$^{X}$ in generations 9 to 11 and in introgression line DUHi(2)$^{X}$ in generations 4 to 7

| | Females | | | | | | | Males | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X/X | | X/x | | x/x | | | X/y | | x/y | | |
| | n | LSM | n | LSM | n | LSM | se pooled | n | LSM | n | LSM | se pooled |
| | Introgression line 1: DUHi(1)$^{X}$ | | | | | | | | | | | |
| BW 42 | 6 | 49.6$^{b}$ | 69 | 50.8$^{b}$ | 25 | 51.2$^{b}$ | 0.82 | 24 | 63.2$^{a}$ | 73 | 61.8$^{a}$ | 0.74 |
| BW 70 | 10 | 60.7$^{c}$ | 62 | 63.5$^{c}$ | 14 | 60.9$^{c}$ | 1.25 | 24 | 82.3$^{a}$ | 64 | 77.7$^{b}$ | 1.08 |
| | Introgression line 2: DUHi(2)$^{X}$ | | | | | | | | | | | |
| BW 42 | 29 | 49.8$^{b}$ | 49 | 48.2$^{b}$ | 9 | 45.8$^{b}$ | 0.94 | 45 | 58.1$^{a}$ | 69 | 58.2$^{a}$ | 0.71 |
| BW 70 | 27 | 65.4$^{b}$ | 46 | 63.5$^{b,c}$ | 13 | 59.1$^{c}$ | 1.22 | 43 | 78.3$^{a}$ | 74 | 75.9$^{a}$ | 0.86 |

Means sharing a common character in their superscript are not significantly different (P > 0.05)

Figure 1: Male body weight at 70d in the selected lines and in the introgression lines




DUH and DUHi: Dummerstorf high outbred and inbred. EDHi: Edinburgh high inbred; DUHi(1)$^{X}$ and DUHi(2)$^{X}$: introgression lines with DUHi as recurrent parent, following a cross between EDHi and DUHi/DUH. Generation 1 is the first of inbreeding in DUHi and the F1 cross to form DUHi (1)$^{X}$, and corresponds to inbred generation 17 in EDHi.

## 4. Discussion

*Reciprocal crosses* Reciprocal crosses between the DUH and EDHi lines showed clearly that the daughter weights were very similar for DUH and EDHi dams, although somewhat higher in females born by DUH dams, presumably due to a better maternal environment provided by the substantial heavier DUH-dams. The males however inherit their X-Chr from their mother and there are substantial differences in body weight between males with a high X-Chr from the EDHi mother and males with a low X-Chr from the DUH mother. Using the male-female difference, which accounts for the difference in the maternal environment, the average effects of the EDHi-X-Chr were 5.5, 9.4 and 11.9g at 42, 70 and 84d, respectively, corresponding to 12, 17 and 22% of the body weight, but showed a considerable variation between the replicates. This indicates that (i) the DUH-line has a "low X-chromosome", (ii) the effect seems to be proportional to body weight, which means a much higher effect in DUH than in EDH or EDL background in absolute terms, and (iii) a substantial further increase in body weight of the already extreme line DUH/DUHi would be expected if the EDHi-X-QTL were introgressed.

As the subsequent introgression resulted in a much smaller effect than expected from the reciprocal crosses, it seems worthwhile to review the effects previously estimated from previous reciprocal crosses between EDH and EDL. These are quite consistent. Hastings & Veerkamp, 1993) found the X QTL effect in all three original replicates indicating that the polymorphism on the X-Chr causing the increased growth was probably already present in one of the founder lines. A similar reciprocal cross between EDH and EDL in G45 showed no difference in BW70 for the females in the reciprocal halves but a 4.6g (16%) effect in males (Rance *et al.*, 1994). The effect of the X-QTL on the EDLi background was recorded by Liu *et al.*, 2001a) from birth to 70d of age, using a congenic line and an EDL inbred line without the X-QTL segment. They also showed an increase in the absolute effect over age in both sexes reaching 3.0 and 3.4g in females and males at 70d, respectively, but a similar proportional effect at each age (17, 17, 8 and 20% in males and 28, 24, 16 and 19% in females at birth, 21d , 42d and 70d, respectively).

*Marker assisted introgression* The replicated marker assisted selection of the X-QTL originating from the EDH line into an extreme growth line, DUH, did not increase the body weight to the extent expected from the reciprocal crosses. In the first introgression line, DUHi(1)$^{X}$, no effect on BW42 was shown in either sex, and no effect in females at 70d, but a moderate and significant effect of 4.6g (6%) in males, which is of similar magnitude in absolute terms to that found in reciprocal EDH x EDL crosses, but is smaller as a proportion of the mean. This overall picture is supported by the second introgression line DUHi(2)$^{X}$, where no significant effects were found in either sex at 42d (although X/X females were 4g (9%) heavier than x/x females). At 70d X/X-females were 6.3g (11%) significantly heavier than x/x females, and X/y males were slightly but not significantly heavier (2.4g or 3%) than x/y males. Obviously the effect of the X-QTL is smaller after marker assisted introgression than in the DUHi and EDHi crosses, especially at 42d. The coefficients of variation were close to 10-12%, so the effect at 70d was roughly 0.5 sd.

The reasons for the lower effect found after MAI than that estimated from reciprocal crosses are unknown and remain speculative at this stage. The loss of the X chromosome QTL can be ruled out as it was mapped to within a 2 cM region in the range covered by the markers we applied, and the data shown in Table 3 are only on individuals that showed no recombinant genotype (i.e. markers all from EDHi or all from DUHi). The small reciprocal differences in EDL and DUH crosses and large reciprocal differences in EDH and DUH crosses can not be explained by DUH carrying the high X-QTL but decreasing alleles elsewhere on the X chromosome or by an effect of the DUH Y-chr. Epistatic effects (e.g. modifiers) must surely have to be invoked to explain why the reciprocal crosses (EDH vs. DUH) gave indication for substantial effects and the MAI showed much smaller effects.

*Epistasis?* As modifier loci exert their effects on body weight only in the presence of the high X-QTL, the higher body weight of males that have the whole X-chr from the EDH might be explained by a combined action of high X-QTL and one or more modifiers. A similar situation was found for example by Varga *et al.*, 2003) in relation to a murine myostatin mutation (*Compact*, *Mstn*$^{Cmpt\text{-}dl1Abc}$), where modifiers increased muscularity considerably in the presence of the myostatin mutation but not otherwise. A recent study on the introgression of *Compact* into DUHi also showed evidence for substantial interaction of the mutation with the genetic background (Bünger *et al.*, 2004). A similar situation has been seen in a study of UK beef herds where the allele of the myostatin gene that is associated with the double muscling in continental breeds was also found to be present in several breeds that do not have extreme musculature (Smith *et al.*, 2000; J.L. Williams, pers. com.). The phenotype associated with this allele is highly variable: from extreme double muscling in the Belgian Blue breed (where there is also anecdotal evidence that its effect has become more extreme through selection for increased muscling (Charlier *et al.*, 1995), through a variable phenotype in the South Devon (Wiener *et al.*, 2002), to no distinct phenotype in the Highland breed (J.L. Williams, pers. com.), showing that the myostatin mutation must interact with modifier loci.

Overall, our experiment shows that marker assisted introgression may not be successful in increasing the performance of an extreme line, even though a QTL having large effect in a different background is introgressed. There is also indication that the increased improvements expected by MAI have not been realized in plant breeding. Bouchez *et al.* ( 2002) introgressed QTL among elite lines of maize. They concluded that for simple traits such as silk date and grain moisture, QTL effects in the MAI lines generally accorded with those expected from those initially detected, while for yield, a more complex trait, results were generally poorer than expected and one putative high yielding allele finally exhibited a negative effect. In addition to epistatsis, however, some genotype x environment interaction effects may have contributed to these observations. In conclusion, it seems that an assumption of no epistatic effects on an extreme background is not tenable, that caution should be taken in adopting MAI in breeding programmes without assessment of interactions, and that the mode of action of major genes in highly selected backgrounds merits further attention.

We are grateful to the BBSRC and SEERAD for financial support.

References


Bouchez,A., Hospital,F., Causse,M., Gallais,A. & Charcosset,A. (2002). Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. *Genetics* **162**, 1945-1959.

Brockmann,G.A. & Bevova,M.R. (2002). Using mouse models to dissect the genetics of obesity. *Trends Genet.* **18**, 367-376.

Bünger,L., Herrendörfer,G. & Renne,U. (1990). Results of long term selection for growth traits in laboratory mice. *Proc. 4th World Congr. on Genetics appl. to Livestock Prod., Edinburgh* **XIII**, 321-324.

Bünger,L. & Hill,W.G. (1999). Inbred lines derived from long-term divergent selection on fat content and body weight. *Mammalian Genome* **10**, 645-648.

Bunger,L., Laidlaw,A., Bulfield,G., Eisen,E.J., Medrano,J.F., Bradford,G.E., Pirchner,F., Renne,U., Schlote,W. & Hill,W.G. (2001). Inbred lines of mice derived from long-term growth selected lines: unique resources for mapping growth genes. *Mamm. Genome* **12**, 678-686.

Bünger,L., Laidlaw,A.H., Bulfield,G., Eisen,E.J., Medrano,J.F., Bradford,G.E., Pirchner,F., Renne,U., Schlote,W. & Hill,W.G. (2001a). Inbred lines of mice derived from long-term on growth selected lines: unique resources for mapping growth genes. *Mammalian Genome* **12**, 678-686.

Bünger,L., Ott,G., Varga,L., Schlote,W., Rehfeldt,C., Renne,U., Williams,J.L. & Hill,W.G. (2004). Marker assisted introgression of the *Compact* mutant *myostatin* allele: *Mstn*$^{Cmpt\text{-}dl1Abc}$ into a mouse line with extreme growth - effects on body composition and muscularity. *Genetical Research*, in press

Bünger,L., Renne,U., and Buis,R.C. (2001b). Body weight limits in mice - Long-term selection and single genes. *Encyclopedia of Genetics* (Reeve,E.C.R., ed) pp. 337-360, Fitzroy Dearborn Publishers, London, Chicago

Bünger,L., Schüler,L., Kupatz,B. & Renne,U. (1983). Selection for growth in model animals (laboratory mice) 2. Direct selection response (G). *Archiv fur Tierzucht-Archives of Animal Breeding* **26**, 281-293.

Charlier,C., Coppieters,W., Farnir,F., Grobet,L., Leroy,P.L., Michaux,C., Mni,M., Schwers,A., Vanmanshoven,P., Hanset,R. & Georges,M. (1995). The mh gene causing double-muscling in cattle maps to bovine chromosome-2. *Mammalian Genome* **6**, 788-792.

Corva,P.M. & Medrano,J.F. (2001). Quantitative Trait Loci (QTLs) mapping for growth traits in the mouse: A review. *Genet. Sel Evol.* **33**, 105-132.

Glazier,A., Nadeau,J.H. & Aitman,T.J. (2002). Finding Genes that underlie complex traits. *Sci.***298**, 2345-2349.

Hastings,I.M. (1990). An investigation of mouse lines selected on body weight for the presence of major genes. *Proc. 4th WCGALP,Edinburgh* **XIII**, 317-320.

Hastings,I.M. & Veerkamp,R.F. (1993). The genetic basis of response in mouse lines divergently selected for body weight or fat content. I. The relative contributions of autosomal and sex-linked genes. *Gen. Res.* **62**, 169-175.

Liu,X., Bünger,L. & Keightley,P.D. (2001a). Characterization of a major X-linked quantitative trait locus influencing body weight of mice. *Journal of Heredity* **92**, 355-357.

Liu,X., Oliver,F., Brown,S.D., Denny,P. & Keightley,P.D. (2001b). High-resolution quantitative trait locus mapping for body weight in mice by recombinant progeny testing. *Genetical Research* **77**, 191-197.

Nadeau,J.H. (2001). Modifier genes in mice and humans. *Nature Reviews. Genetics* **2**, 165-174.

Pomp,D. (1997). Genetic dissection of obesity in polygenic animal models. *Behavior Genetics* **27**, 285-306.

Rance,K.A., Hastings,I.M., Hill,W.G. & Keightley,P.D. (1994). Mapping of putative QTL influencing body weight on the X-chromosomes in mice. *Proc. 5th WCGALP, Guelph* **21**, 268-271.

Rance,K.A., Heath,S.C. & Keightley,P.D. (1997a). Mapping quantitative trait loci for body weight on the X chromosome in mice. II. Analysis of congenic backcrosses. *Gen. Res.* **70**, 125-133.

Rance,K.A., Hill,W.G. & Keightley,P.D. (1997b). Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F-2 population. *Gen. Res.* **70**, 117-124.

Routman,E. & Cheverud,J. (1994). A rapid method of scoring simple sequence repeat polymorphisms with agarose gel electrophoresis. *Mammalian Genome* **5**, 187-188.

Schüler,L. (1985). The mice outbred strain Fzt: DU and its application in animal breeding research (G). *Archiv für Tierzucht-Archives of Animal Breeding* **28**, 357-363.

Sharp,G.L., Hill,W.G. & Robertson,A. (1984). Effects of selection on growth, body composition and food intake in mice. I. Responses in selected traits. *Genetical Research* **43**, 75-92.

Smith,J.A., Lewis,A.M., Wiener,P. & Williams,J.L. (2000). Genetic variation in the bovine myostatin gene in UK beef cattle: allele frequencies and haplotype analysis in the South Devon. *Animal Genetics* **31**, 306-309.

Truett,G.E., Heeger,P., Mynatt,R.L., Truett,A.A., Walker,J.A. & Warman,M.L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* **29**, 52, 54.

Varga,L., Müller,G., Szabo,G., Pinke,O., Korom,E., Kovacs,B., Patthy,L. & Soller,M. (2003). Mapping modifiers affecting muscularity of the myostatin mutant (*Mstn*$^{Cmpt\text{-}dl1Abc}$) Compact mouse. *Genetics* **165**, 257-267.

Veerkamp,R.F., Haley,C.S., Knott,S.A. & Hastings,I.M. (1993). The genetic-basis of response in mouse lines divergently selected for body-weight or fat-content .2. The contribution of genes with a large effect. *Genetical Research* **62**, 177-182.

Wiener,P., Smith,J.A., Lewis,A.M., Woolliams,J.A. & Williams,J.L. (2002). Muscle-related traits in cattle: The role of the myostatin gene in the South Devon breed. *Genetics Selection Evolution* **34**, 221-232.