RFLPs for Somatotropic Genes Identify Quantitative Trait Loci for Growth in Mice

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ABSTRACT

Restriction fragment length polymorphisms for somatotropic genes were tested for associations with body weight and postweaning growth rate in mice. Polymorphisms for growth hormone (GH) and insulin-like growth factor 2 (IGF-2) genes were identified in stock population lines which had been subjected to long-term selection for high 42-day body weight (H lines) or randomly mated (FP and C lines). Two F₂ populations of mice (5F₂ and MF₂) were generated from crosses between a single H line of mice and two unselected control lines and subsequently, two divergently weight selected sublines were generated from each F₂ population. The *GH^h* allele which had originally been fixed in three of four H lines and absent from all FP and C lines was found to have a significant ($P < 0.01$) effect on 42-day weight and postweaning growth rate in the F₂ populations. However, *GH^h* was associated with lower 42-day weight in the F₂ populations, suggesting that the positive association between *GH^h* and weight in the stock population was unique to the high weight selected genetic background of those lines. In agreement with this, the frequency of *GH^h* increased in sublines selected for high 42-day weight and decreased in sublines selected for low 42-day weight. The *IGF-2^h* allele was associated with higher weights in a sex-dependent manner in 5F₂. In the high selected subline derived from 5F₂, a significant increase in the frequency of *IGF-2^h* was observed. Therefore this allele, in contrast to *GH^h*, appears to be a positive indicator of growth irrespective of genetic background.

WE are interested in developing genetic models which would predict phenotypic values based on how the genes and environmental effects influence the metabolic status of animals. Successful models would identify genotypes which would constitute components of breeding goals tailored to defined production systems. The identification of quantitative trait loci (QTLs) for economically important traits in livestock is essential for genetic modeling of those traits. In the approach we have taken, candidate genes selected on the basis of known relationships between physiology and a production trait, are tested as putative QTLs for the development of a genetic model. QTLs so identified can also be used directly as aids to selection in livestock breeding programs. The importance of new sources of data in animal breeding programs is increasing as current statistical methodologies approach maximum accuracy (HILL and KNOTT 1990). Confirmed QTLs provide a new source of information and their incorporation into selection indices would increase the accuracy of breeding value estimation (SMITH 1967). If DNA markers are used to identify QTLs they have the further advantages of being available at, or even before, birth; they are present in all animals and they are unaffected by environmental conditions. Therefore, these markers

provide ideal tools for marker assisted selection either as parameters incorporated into selection indices (SALES and HILL 1976), or for selection of juveniles in two-stage selection programs (WOOLLIAMS and SMITH 1988).

In our research, we have focused on growth traits in mice as a pilot system for genetic modeling. The reasons for examining growth traits were twofold. First, extensive research has identified many of the gene products that influence mammalian growth and, as discussed below, many of the specific effects elicited by growth factors and the interactions between them are known (SPENCER 1985). Second, the likelihood of being able to detect QTLs is maximized because most growth-related traits in mice are of moderate to high heritability (*i.e.*, $h^2 > 0.3$) (EISEN and PRASETYO 1988). And finally, major gene effects on growth, producing phenotypes such as *little* (EICHER and BEAMER 1976), *Ames dwarf* (SCHAIBLE and GOWAN 1961), *Snell dwarf* (BARTKE 1965) and *obese* (GREEN 1981) have been observed in mice. This pilot system was also chosen because of the economic importance of growth and growth-related traits such as feed efficiency to the livestock industry.

The central hormone of the somatotropic axis is pituitary growth hormone (GH) which, in conjunction

with insulin-like growth factors (IGFs), is essential for postnatal growth in animals (BOYD and BAUMAN 1989; FROESCH *et al.* 1985). GH has growth-promoting, galactopoeitic, lipolytic and diabetogenic activity (EV-OCK *et al.* 1988). Numerous studies have shown that treatment with exogenous GH affects many growth-related traits such as rate of gain, feed efficiency and lean meat deposition (reviewed in KOPCHICK and CIOFFI 1991). Some studies have also indicated that metabolic parameters of GH secretion which may be genetically controlled are correlated with breeding values for some production traits in livestock (*e.g.*, KLINDT 1988; ARBONA *et al.* 1988). Also pertinent to the development of a genetic model for growth-related traits, is the fact that regulation of many of the growth factors and receptor-hormone interactions is influenced by environmental effects such as nutrition and stress (reviewed by BREIER and GLUCKMAN 1991).

In this study, we have attempted to assess the effects of restriction fragment length polymorphisms (RFLPs) of somatotrophic genes on growth traits in mice. RFLPs for a given gene can be used to identify allelic variants at that locus. Two haplotypes at the GH locus in our stock mouse populations were previously identified in this laboratory (SALMON *et al.* 1988). One of the haplotypes, designated GH^h , was found exclusively in the four stock population lines of mice which had been subjected to long term selection for high 42-day weight. The GH^h haplotype was fixed in three of these lines while the remaining selected line was polymorphic. The alternate haplotype, designated GH^c , was fixed in all five of the unselected control and foundation population lines. The fixation of the GH^c haplotype in unselected lines suggests that, in the absence of selection for increased body weight, natural selection favors the GH^c haplotype. Alternately, selection for high 42-day body weight appears to favor the GH^h haplotype. It was, therefore, postulated that the GH RFLPs might identify a QTL for 42-day body weight with the allele designated GH^h having a positive effect on body weight (WINKELMAN, QUERENGESSER and HODGETTS 1990). To test this, segregation of the two GH alleles with body weight was examined in an F_2 population generated from crosses between a line selected for high 42-day weight and a control line (WINKELMAN, QUERENGESSER and HODGETTS 1990). Interestingly, while a significant correlation between body weight and GH genotype was observed, the GH^h allele was associated with lower weights.

The present study was undertaken to examine the generality of our previous results by creating two different F_2 populations from crosses between selected and unselected lines in which GH^h and GH^c , respectively, were fixed. Following the construction of the new F_2 populations, we discovered an IGF-2 polymor-

FIGURE 1.—Response to selection for high 42-day weight in strain 5. Line 5C was maintained without artificial selection and 42-day weights were not recorded between generations 37 and 67. Strain 5H was an amalgam at generation 72 of two replicates (512a and 512b) upon which selection was initiated at generations 6 and 9, respectively, and continued until generation 72. Generation mean 42-day weights are actually three-generation running averages and the points plotted were chosen to illustrate real trends.

phism in the high weight selected stock population line used to generate these F_2 populations. The IGF-2 polymorphism was also tested for correlations with growth traits in the F_2 populations. Both the IGF-2 and the GH polymorphisms were then monitored in divergently selected lines derived from the F_2 populations in an attempt to assess their effects in selected *vs.* nonselected genetic backgrounds.

MATERIALS AND METHODS

Stock population lines: The mice used in this experiment were descendants of the Lacombe Mouse Colony obtained from the Canada Department of Agriculture Research Station in Lacombe, Alberta. The lines we obtained from Lacombe were developed to compare the effects of inbreeding and selection in two sizes of populations (SUMNER 1974). The origins of these lines were two strains developed from various source lines imported from the Institute of Animal Genetics, Edinburgh, Scotland, in 1961. Strain 2 was an amalgam of four outbred strains: Q, NB, LX and JH, while strain 5 was bred directly from the Q strain.

H lines for strains 2 and 5 (2H and 5H) were developed by long-term selection for high 42-day weight. The H lines were maintained with 15 full-sib families per generation using a hierarchical mating system with each of the five males used mated to three females for each generation. Randomly mated control (C) lines were also developed for strains 2 and 5 using the same mating system as was employed for the H lines. Foundation population (FP) lines for each strain were maintained without selection with 25 single pair matings per generation. Mice from three of the stock population lines were used in this experiment: line 5H which had been subjected to selection for 72 generations and two unselected lines, 5C and 2FP. The response to selection that was obtained in 5H is shown in Figure 1.

Experimental populations: For this study, two F_2 populations were generated and, from each of these, two divergently selected sublines were developed. Litters in all generations were culled to five pups per full-sib family seven days after parturition. Matings between full sibs were avoided throughout the experiment and only first parity litters were used. The two F_2 populations were: 5 F_2 from crossing 5H with 5C and MF $_2$ from crossing 5H with 2FP.

FIGURE 2.—Breeding scheme used to generate F_2 populations of mice from crosses between high 42-day weight selected (H) and randomly mated (C) lines of mice.

In both cases, F_1 populations were generated from 20 single pair matings using equal numbers of males and females from each stock line. The F_2 populations consisted of 150 individuals from 30 single pair matings between F_1 mice. The details of the breeding scheme are summarized in Figure 2.

Divergently selected lines were generated from each F_2 population. Fifteen males and 15 females from 5 F_2 were randomly selected and single pair mated to create 5 F_3 , from which 5Hi and 5Lo, selected for high and low 42-day weight, respectively, were generated. Sublines MHi and MLo were created from F_2 in an analogous fashion. Sublines 5Hi and 5Lo were selected for nine generations; six generations of selection were carried out on MHi and MLo. Selected sublines consisted of from 7 to 13 families in each generation. Variations in the number of families between generations resulted from relatively high infertility encountered in some of the sublines, particularly 5Lo. The first five generations of selection in 5Hi and 5Lo and the first three generations of selection in MHi and MLo were carried out using single pair matings and selecting breeding replacements for each male and female from their progeny using 42-day weight as the criterion of selection. Selected mice within each subline were mated at random with no matings made between full sibs. The remaining generations incorporated a hierarchical mating scheme with each male mated to two females. For these latter generations, replacement breeders for each female were selected by 42-day weight from their progeny and the required number of males were selected from all of the families by individual 42-day weight, using no more than one male from each full sib family. By this method, some sires would yield two sons for the next generation while others would yield none, but all would be represented by at least two females.

Southern analysis: Genomic DNA was extracted from individual livers as previously described (WINKELMAN, QUERENGESSER and HODGETTS 1990). Aliquots of approximately 10 μ g DNA were completely digested with the restriction enzyme *Hind*III, usually in the presence of 5 mM spermidine. Digested DNA samples were fractionated by electrophoresis on 0.7% agarose gels and transferred to Gene Screen Plus (NEN) membranes. A 0.8-kb cDNA of the rat GH gene (SEEBURG *et al.* 1977) and a 0.66-kb cDNA of the human IGF-2 gene (BELL *et al.* 1984) were used to make 32 P-oligolabeled probes (as described by FEINBERG and VOGELSTEIN 1983) which were individually hybridized with the membranes at an approximate concentration of 10 ng/ml (10^6 cpm/ml) in 50% formamide, 1% sodium dodecyl sulfate (SDS), 1 M NaCl, and 10% dextran sulfate overnight at 42°. Membranes were then washed twice in $2 \times$ SSC at room temperature, twice in $2 \times$ SSC, 1% SDS at 65° and twice in $0.1 \times$ SSC at room temperature, then exposed to

Kodak XAR film overnight. From these films, GH and IGF-2 RFLP patterns for each mouse of the F_2 populations and the final generation of the selected sublines were determined.

Each gene was characterized by two distinguishable restriction patterns following digestion with *Hind*III. The GH^h restriction pattern revealed a single band at 4.5-kb while the alternate allele, GH^c , showed a single band at 8.0-kb (SALMON *et al.* 1988). 5H was monomorphic for GH^h , 5C and 2FP were both monomorphic for GH^c . The $IGF-2^{H^S}$ allele was characterized by a 9.4-kb band in place of a 7.5-kb band observed for the alternate allele, $IGF-2^{H^A}$. The two IGF-2 restriction patterns displayed a common band at 7.2 kb. 5H was polymorphic at the IGF-2 locus while 5C and 2FP were monomorphic for $IGF-2^{H^A}$.

Methods of analysis: The growth traits measured were weaning (21-day) weight, mature (42-day) weight and post-weaning (21 days to 42 days) growth rate. An analysis of variance was carried out on each F_2 population, using the model shown below, to determine if the GH and/or IGF-2 genotypes contributed to the variance of the traits measured. The model included the family effect which is expected to account for differences due to parent weight and litter size (MALLARD, KENNEDY and WILKIE 1990). These data were analyzed using the general linear models, GLM, procedure of SAS (1982).

Model:

$$Y_{ijklm} = \mu + FAM_i + SEX_j + GENOGH_k + GENOIGF_l + SEXGENOGH_{jk} + SEXGENOIGF_{jl} + e_{ijklm}$$

where:

Y_{ijklm} is the 21-day weight (g), 42-day weight (g) or post-weaning growth rate (g/day) of the individual,

μ is the overall mean for the trait analyzed,

FAM_i is the effect of the i th family ($i = 1, 2, \dots, 30$),

SEX_j is the effect of the j th sex ($j = 1, 2$)

$GENOGH_k$ is the effect of the k th genotype at the GH locus ($k = 1, 2, 3$),

$GENOIGF_l$ is the effect of the l th genotype at the IGF-2 locus ($l = 1, 2, 3$),

$SEXGENOGH_{jk}$ is the effect of the interaction between the j th sex and the k th genotype at the GH locus

$SEXGENOIGF_{jl}$ is the effect of the interaction between the j th sex and the l th genotype at the IGF-2 locus

e_{ijklm} is the residual error.

Average additive gene substitution effects of the growth traits were calculated for each allele of each gene which was found to have a significant effect on the trait using the

TABLE 1

Least squares means of 42-day weight (g) for the three GH genotypic classes in the F₂ populations developed for this study

Population	Genotype		
	<i>GH^hGH^h</i>	<i>GH^hGH^c</i>	<i>GH^cGH^c</i>
5F ₂	29.2	30.2	31.4
MF ₂	34.6	34.9	38.8

The two F₂ populations, 5F₂ and MF₂, were derived from crosses between a single selected line, 5H, and two unselected lines, 5C and 2FP. The *GH^h* allele was fixed in the selected 5H line while the alternate allele, *GH^c*, was fixed in both unselected lines. The overall means and standard deviations were as follows: 5F₂ males 32.7 ± 2.7 g (*N* = 79), 5F₂ females 27.5 ± 1.9 g (*N* = 69); MF₂ males 38.7 ± 4.0 g (*N* = 79) and MF₂ females 32.7 ± 2.7 g (*N* = 63).

method outlined by FALCONER (1981). Least squares means, calculated from type III sums of squares, of the genotypic classes for the traits analyzed were used to determine average additive gene substitution effects. The probability of genetic drift being responsible for changes in allele frequencies observed in the divergently selected sublines were calculated according to the methodology reviewed by HEDRICK (1983).

RESULTS AND DISCUSSION

Analysis of GH alleles in F₂ populations: The two GH alleles, *GH^h* and *GH^c*, were both fixed in the stock population lines used in this study: the selected line, 5H, was monomorphic for *GH^h* while the unselected lines, 5C and 2FP, were monomorphic for *GH^c*. We hypothesized that selection for increased 42-day weight resulted in the fixation of *GH^h*, therefore, we predicted that *GH^h* would be associated with higher 42-day weights in the F₂ populations generated for this study. The exclusion of *GH^h* from all of the unselected lines in our stock population suggests that, in the absence of selection for higher weight, *GH^h* is a detrimental allele.

The analysis of variance of the F₂ populations indicated that the GH genotype had significant (*P* < 0.01) effects on 42-day body weights and postweaning growth rate in both 5F₂ and MF₂. Least squares means of the three GH genotypic classes are given in Table 1 and the distributions of 42-day weight for the three GH genotypic classes are shown in Figures 3 and 4 for 5F₂ and MF₂, respectively. No association (*P* > 0.05) was found between GH genotype and 21-day weight. These results indicate that the effect of the GH variants on 42-day weight is mediated through an alteration in the rate of postweaning growth rather than prenatal or preweaning growth. This effect was also seen in the stock population lines in which either *GH^h* or *GH^c* was fixed since the selected and unselected lines did not differ in 21-day weight although differences in 42-day weight were significant (*P* < 0.01) (data not shown). The postweaning effect on growth rate of the GH variants corresponds with the effects

of endogenous GH on growth rate which typically commence at puberty (BOYD and BAUMAN 1989); this postpubertal effect on growth rate has also been seen in transgenic mice carrying heterologous GH genes (MATTHEWS *et al.* 1988).

Additive gene substitution effects calculated for the GH haplotypes are given in Table 2. Contrary to expectations, the *GH^h* haplotype, fixed in the selected stock population lines, had an apparent negative effect on 42-day weight and postweaning gain in the F₂ population. This observation parallels the results of our previous study (WINKELMAN, QUERENGESSER and HODGETTS 1990) performed with the 3F₂ population which was developed from lines genetically distinct from those used in this study. The present study also incorporated a larger number of animals from the stock lines than was used for the 3F₂ population. The size of the base population was increased to determine if the negative correlation between *GH^h* and 42-day body weight previously observed may have been due to recombination between the variant GH haplotype and a QTL affecting 42-day weight. Confirmation of our previous results indicates that the negative association between *GH^h* and 42-day weight seen in the F₂ populations is more likely due to the effects of the *GH^h* and *GH^c* haplotypes in a genetic background not selected for 42-day weight. It should be noted here that the magnitude of the effect of *GH^h* on 42-day weight in MF₂ was twice that observed in 5F₂ (Table 2). Furthermore, *GH^h* appeared to act as a dominant allele in MF₂ and a codominant allele in 5F₂ (Figures 3 and 4) further supporting the hypothesis that the effect of GH alleles on body weight is dependent on genetic background. Genetic background-dependent effects of QTLs have also been reported for QTLs mapped using RFLPs in tomatoes (TANKSLEY and HEWITT 1988). It is, therefore, essential that the effects of QTLs be determined in the populations to which this information is to be applied.

Analysis of IGF-2 alleles in F₂ populations: The variant IGF-2 restriction pattern, *IGF-2^{H5}*, was found only in the selected line 5H. It was not known if this allele was present in any of the source strains used to create the stock population lines or if it arose in 5H after the selected lines were created. Therefore, it was not possible to make any *a priori* speculations concerning associations between the IGF-2 RFLPs and weight parameters.

IGF-2 genotypes were found to have no effect on 21-day weight, 42-day weight or postweaning gain in the MF₂ population where the frequency of *IGF-2^{H5}* was 0.36. However, in the 5F₂ population, IGF-2 genotype had a significant (*P* < 0.01) effect on both 42-day weight and 21-day weight with no effect on postweaning growth rate. Least squares means for the IGF-2 genotypic classes are given in Table 3. In the

FIGURE 3.—Distribution of observations from the 5F₂ population for the three GH genotypic classes, *GH^hGH^h* (striped bars), *GH^hGH^c* (solid bars), and *GH^cGH^c* (stippled bars).

FIGURE 4.—Distribution of observations from the MF₂ population for the three GH genotypic classes, *GH^hGH^h* (striped bars), *GH^hGH^c* (solid bars), and *GH^cGH^c* (stippled bars).

TABLE 2

Average additive gene substitution effects of GH alleles on 42-day weight (g) of F₂ mice derived from crosses between high 42-day weight selected and unselected lines

Population	<i>GH^h</i>	<i>GH^c</i>	σ^2
5F ₂	-0.52*	+0.49*	0.21
MF ₂	-1.03**	+1.09**	0.38

* Indicates that the value was significant, $P < 0.05$; ** indicates that the value was significant, $P < 0.01$.

5F₂ population the frequency of *IGF-2^{H5}* was 0.16. The IGF-2 genotype by sex interaction effect was significant ($P < 0.01$) for both 21-day weight and 42-day weight. Average additive gene substitution effects for IGF-2 alleles are given in Table 4. These effects were found to be sex-dependent: 42-day weights of females and 21-day weights of males were significantly

($P < 0.05$) associated with IGF-2 genotypes. This suggests a possible interaction between RFLP identified allelic variants of IGF-2 and sex-determining genes. In both cases, the *IGF-2^{H5}* allele which was contributed by the selected line, 5H, was associated with higher weight. Since it is known that interactions between sex steroid hormones and somatotropins affect growth patterns (BREIER, GLUCKMAN and BASS 1988), a sex-dependent effect observed for a somatotropic RFLP indicates another venue which could be pursued in the development of a genetic model for growth.

The 5F₂ and MF₂ populations were analyzed separately to test for possible effects of interactions between GH and IGF-2 genotypes on weight traits. No interaction effect was found in the MF₂ population for any of the traits analyzed ($P > 0.1$). The analyses revealed that interactions between GH and IGF-2

TABLE 3

Least squares means of 21-day and 42-day weight (g) for IGF-2 genotypic classes in F₂ populations of mice derived from crosses between high 42-day weight selected and unselected lines

Population	Genotype					
	IGF-2 ^{Hs} IGF-2 ^{Hs}		IGF-2 ^{Hs} IGF-2 ^{HA}		IGF-2 ^{HA} IGF-2 ^{HA}	
	21-day	42-day	21-day	42-day	21-day	42-day
5F ₂ (males)	17.2	33.3	16.1	33.8	15.6	32.6
5F ₂ (females)	15.4	29.9	15.3	28.3	14.8	27.1
MF ₂	18.3	35.2	18.7	36.2	18.1	35.4

Overall means and standard deviations for 42-day weights are given in the legend to Table 1. The 21-day means and standard deviations of the 42-day animals were: 5F₂ males 15.9 ± 1.7 g, 5F₂ females 14.8 ± 1.9 g; MF₂ males 18.2 ± 2.4 g and MF₂ females 18.4 ± 1.8 g.

TABLE 4

Average additive gene substitution effects of IGF-2 haplotypes on 21-day weight (g) and 42-day weight (g) of F₂ mice

Trait	IGF-2 ^{Hs}	IGF-2 ^{HA}	σ^2
21-Day weight			
Male	+0.47*	+0.10	0.21
Female	+0.39	+0.07	0.25
42-Day weight			
Male	-0.13	+0.03	0.79
Female	+1.11**	-0.19	0.62

* Indicates that the value was significant, $P < 0.05$; ** indicates that the value was significant, $P < 0.01$.

genotypes might have had an effect on 42-day weight in the 5F₂ population ($P = 0.08$). However the low frequency of IGF-2^{Hs} (i.e., 0.16) in this population rendered the number of observations in the interaction subclasses in this population too small to evaluate the interaction effects.

Analysis of selected sublines: To assess the effects of the different alleles of GH and IGF-2 in weight selected genetic backgrounds, divergent selection for 42-day weight was applied to the 5F₃ and MF₂ populations. Responses to divergent selection of the high and low 42-day weight selected sublines derived from 5F₃ and MF₂ are shown in Figures 5 and 6, respectively. The average selection intensity in 5Hi was 0.42 for males in the first five generations and 0.21 for the remaining generations after the hierarchical mating system was instituted; the corresponding selection intensities for males in MHi were 0.27 and 0.18. The average selection intensities for females were 0.44 and 0.40 for 5Hi and MHi, respectively. Average increases in 42-day weight of 0.39 and 1.12 g/generation in 5Hi and MHi, respectively, were observed. The greater response observed in MHi may have been due to greater genetic heterogeneity in the MF₂ population than in the 5F₂ population since the MF₂ population was developed from lines of two different strains. The average selection intensity in 5Lo was 0.36 in the first five generations and 0.18 after the institution of hierarchical matings; corresponding se-

lection intensities in MLo were 0.29 and 0.17. The average selection intensities for females were 0.44 and 0.43 for 5Lo and MLo, respectively. Sublines 5Lo and MLo showed decreases in average 42-day weight of 0.35 and 0.69 g/generation, respectively. Again, the magnitude of the response was greater in the subline derived from the more heterogeneous MF₂. These results suggest the presence of heterotic gene effects influencing 42-day weight in these populations in accordance with results reported for other populations of mice (NAGAI 1971; EISEN and PRASETYO 1988).

Analysis of GH in selected sublines: The changes in allele frequency which occurred concomitantly with selection are given in Table 5. Concurrent with the original identification of the GH^h haplotype exclusively in high 42-day weight selected stock lines, we observed an increase in the frequency of GH^h in sublines selected for high 42-day weight and a decrease in sublines selected for low 42-day weight. The average change in allele frequency for all of the lines selected for high 42-day body weight was 0.04/generation. This increase in GH^h in the two upward selected lines is unlikely ($P < 0.01$) due to drift alone. Therefore, it can be stated that selection for high body weight in mice produces an increase in the frequency of GH^h. The decrease in the frequency of GH^h averaged 0.02/generation in lines 5Lo and MLo. Selection for decreased body weight did not have as great an effect on the frequency of the GH alleles as did selection for increased body weight. As discussed previously, the absolute change in weight in the selected sublines was highly variable, suggesting the effects of a number of other segregating loci affecting body weight.

Our results show that in the appropriate genetic background, namely one which has been selected for 42-day weight, the GH^h allele has a positive effect on 42-day weight. Since there was no significant difference in weaning weights of high and low selected sublines, the effect of the GH^h allele is manifested through an increased rate of postweaning gain.

FIGURE 5.—Response to nine generations of divergent selection for 42-day weight in lines 5Hi (dotted line) and 5Lo (solid line). These lines were selected from the 5F₃ population developed from crossing 5H (a selected line) with 5C (an unselected line).

FIGURE 6.—Response to six generations of divergent selection for 42-day weight in lines MHi (dotted line) and MLo (solid line). These lines were selected from the MF₂ population developed from crossing 5H (a selected line) with 2FP (an unselected line).

The contrasting results between the F₂ populations and the sublines selected from them indicate that it will be necessary to evaluate some QTLs in a selected line which better represents the genetic background the breeder is aiming for than in F₂ or backcross populations. It would be possible to estimate the effects of isolated QTLs in selected populations since they are a function of change in allele frequency, the selection intensity and the number of generations of selection (HILL and KNOTT 1990). Genetic background-dependent effects of QTLs might also be assessed in large populations of livestock if we consider the elite animals to represent a selected population and compare allele frequencies between elite animals and the remainder of the population. The latter method may be particularly advantageous if we can identify the composite genotypes which represent the goals of selection for a given production program.

Analysis of IGF-2 in selected sublines: The frequency of the *IGF-2^{H5}* allele remained unaffected by selection on MF₂ but increased more than twofold in 5Hi (Table 5). These results reflect the effects of the variants of this gene in the two F₂ populations: in MF₂ no effect of the *IGF-2* genotype on body weight was observed whereas in 5F₂ the *IGF-2^{H5}* allele was found to have a positive additive effect on body weight. The absence of any change in allele frequency in 5Lo can be attributed to the initial low frequency of *IGF-2^{H5}* in the 5F₂ population. From these results we conclude that it is possible to evaluate the effects of some QTLs in F₂ populations of animals as has been previously carried out in plants (PATERSON *et al.* 1988).

CONCLUSIONS

This study was designed as the first step in the development of a genetic model for growth which

TABLE 5

Frequencies of GH and IGF-2 alleles, as identified by RFLP haplotypes, in F₂ populations and selected sublines of mice

Population	Allele frequency	
	GH ^a	IGF-2 ¹⁵
5F ₂	0.50	0.16
5Hi	0.84	0.39
5Lo	0.34	0.18
MF ₂	0.50	0.36
MHi	0.76	0.39
MLo	0.39	0.37

Allele frequencies for 5Hi and 5Lo were determined after nine generations of selection for high and low body weight, respectively; allele frequencies for MHi and MLo were determined after six generations of selection for high and low body weight, respectively.

would eventually provide the connections between allelic variants of growth-determining genes, metabolic parameters, environmental influences and phenotype. Our results have shown that allelic variants of somatotrophic genes, identified by RFLPs, can act as QTLs for growth traits. The absolute effect of these QTLs was found to vary with the genetic background in which they were studied. This genetic background effect stresses the importance of finding those genotypes which optimize the effects of identified QTLs. It also indicates that naturally occurring variants in elite animals would most likely provide us with realistic selection goals; the effects of genetic background are likely to have even more profound effects if we attempt to manipulate the genotype through the use of transgenics.

Previous research has shown that there is individual variation in circulating and pituitary levels of GH in mice (SINHA, SELBY and VANDERLAAN 1974; YANAI and NAGASAWA 1968). Variant forms of GH (LEWIS 1984) and variation in GH transcript processing (DeNOTO, MOORE and GOODMAN 1981) are also known to exist within species. Uncovering the genetic basis for these variations would greatly aid the development of our genetic model. Further research should allow us to determine the relationship between RFLPs of somatotrophic genes and variations in protein structure or metabolic parameters and the determination of capacity for growth traits. Ultimately, this information could be exploited in animal breeding programs by identifying optimum genotypes for different production systems; these genotypes would then provide the breeder with defined, environment-specific components of selection indices. In the interim, research in this area should provide us with information concerning the identification of advantageous genotypes for some loci which could be incorporated into selection indices. Moreover, if the development of a genetic model focuses on candidate genes rather than random markers, relationships between identified

QTLs and phenotypic values should be consistent between populations.

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