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Identification of a variant growth hormone haplotype in mice selected for high body weight

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Summary

Restriction site analysis revealed a variant growth hormone gene haplotype fixed within growth-selected mice (High line-3; HL-3) exhibiting growth rates 1·5 times greater than those of unselected Foundation population (FP-3) mice. Relative to the FP-3 haplotype, the HL-3 haplotype exhibited restriction fragment length polymorphisms for each of seven different restriction enzymes. Three of the polymorphic sites lie within 1·1 kb of the 5′ end of the structural gene; a fourth polymorphism exists within the structural gene. The HL-3 haplotype was also fixed within an additional three growth-selected lines (including a replicate of HL-3). This identification of an association, between the natural variant of a growth regulating gene and a growth-related phenotype, is the prototype of experiments that could lead to the isolation of variant genes which enhance livestock production characters.

1. Introduction

Animal geneticists have long sought accurate biochemical indicators of an animal's production potential. Previously, researchers attempted to correlate various blood serum protein polymorphisms with economically important traits including growth rate and body size (Neimann-Sorensen & Robertson, 1961; Stansfield *et al.* 1964; Smith, 1967). The unreliability of such correlations (Stansfield *et al.* 1964; Smith, 1967) illustrates the need to seek biochemical indicators with an established physiological involvement in the manifestation of the production character. Since several of the major genes regulating body growth are known (Schuler & First, 1985), we are employing molecular techniques in an attempt to identify naturally occurring variant genes which make a positive contribution to growth. The frequency of such variants should be increased by artificial selection for growth-related parameters. Molecular analysis of growth-selected populations should, thereby, aid in the identification of major variants. The identification of such variants will aid in the selection of replacement animals for use in traditional breeding programmes. More importantly, the introduction of such variant genes via gene transfer, into populations lacking these variants, could significantly accelerate the rate of animal improvement. To investigate the feasibility of this novel approach to animal improvement, our prototype experiments have involved phenotypic and molecular comparisons between growth-selected lines of mice and their unselected Foundation and control populations. The study reported here employed Southern analysis of genomic DNA to investigate the hypothesis that the selection pressure could have identified, and acted upon, natural variation at the growth hormone (GH) locus.

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2. Materials and Methods

(i) *Mouse lines*

The two primary lines of mice employed in this study (FP-3 and HL-3) were obtained from the Agriculture Canada Research Station at Lacombe, Alberta. The Foundation mouse population (FP-3) was established by crossing representatives of four highly inbred strains of mice (A/J, C57-BR/cd, DBA/1, RF/J) obtained from Jackson Memorial Laboratory. The mating scheme allowed each of the four inbred lines to contribute equally to this population (Farid-Naeini, 1986). The FP-3 was maintained through 25 single-pair matings per generation. Each mating produced only one litter; from each of these litters, one male and one female replacement was chosen at random.

To minimize inbreeding and genetic drift, replacements were mated according to a cyclical mating plan (Farid-Naeini, 1986). The FP-3 mice used in this study were from generation 80. At generation 80, the inbreeding coefficient of FP-3 was 0·66.

At the eighth generation of the FP-3, a subline was established in which replacements were selected, on a within-litter basis, for high 42-day body weight. This subline (High line-3; HL-3) was maintained at a population size of 15 litters per generation (5 males each mated to 3 females). Selection for high 42-day body weight was continued for 69 generations. The HL-3 mice used in this study were from generation 72. At this generation, the inbreeding coefficient of HL-3 was 0·93.

After consideration of the results of the phenotypic and molecular characterization of HL-3 and FP-3, variation at the GH locus was investigated in twelve additional mouse lines (2 representatives per line). The background of these lines was:

(*a*) DBA/1, A/J, C57-BR/cd, RF/J: The four inbred lines used to establish the original FP-3 were obtained from Jackson Laboratory. DBA/1 was a subline of a mouse line established by the mating of a single pair of mice in 1909. During the first 20 generations, descendants of this pair of mice were mated brother to sister or parent to offspring. Twice during that period, the line of descent narrowed to a single mating pair (Farid-Naeini, 1986). The A strain of mice was established in 1913 through one single-pair mating of representatives of two albino stocks. A/J was a subline of the A strain which exhibited a low incidence of mammary tumours (Jackson Memorial Laboratory, 1955). C57-BR was established in 1921 through the mating of two black littermates. Brother-to-sister mating of the progeny of this pair, which segregated as black and brown, resulted in two strains: C57-BR and C57-BL. The history of the inbred RF strain dates back to 1928 (Farid-Naeini, 1986).

(*b*) High line-3a: HL-3a was a subline of FP-3. This subline was established by the Lacombe Research Station at the same time as HL-3 and was maintained at a population size of 15 litters per generation (5 males each mated to 3 females). Replacements were selected, on a within litter basis, for high 42-day body weight. HL-3a was, thus, a replicate of HL-3. The present study sampled HL-3a at generation 78 (inbreeding coefficient = 0·95).

(*c*) Control line-3: CL-3 was a subline of FP-3. This subline was established by the Lacombe Research Station at the same time as HL-3 and was maintained at a population size of 15 litters per generation (5 males each mated to 3 females). Replacements were chosen at random. The present study sampled CL-3 at generation 78 (inbreeding coefficient = 0·95).

(*d*) FP-2, HL-2, CL-2: FP-2 was established at the Lacombe Research Station by crossing four outbred lines of mice (JH, NB, XL, Q) obtained from the Institute of Animal Genetics in Edinburgh. The JH strain was a high litter-size selection line derived from a heterogeneous base population of mixed origin (J-stock). The base population had been generated from crosses between Bateman's high lactation line, Goodale's and MacArthur's high body-weight selected lines and four mutant stocks with the C57-BL inbred lines as part of their ancestry (Bowman & Falconer, 1960). The Q strain also had a highly heterogeneous background. One half of the ancestry of the Q strain was from a cross between the JU inbred line and the JC control line (both derived from J-stock). Another half was from crosses between MacArthur's and Goodale's high body-weight selected lines, MacArthur's low body-weight line, the JH strain and a sample of the J-stock (Falconer, 1960; 1973). The NB strain was a non-inbred strain which had been formed from crosses between non-selected strains. The XL was made by crossing four strains plateaued for large body size (Farid-Naeini, 1986).

The mating scheme employed by the Lacombe Station allowed each of the JH, Q, NB and XL strains to contribute equally to the establishment of FP-2 (Farid-Naeini, 1986). The population size and mating scheme of FP-2 was exactly the same as that described for FP-3. FP-2 was maintained through 25 single-pair matings per generation. Each mating produced only one litter; from each of these litters, one male and one female was chosen at random. Replacements were mated according to a cyclical mating plan.

At the eighth generation of FP-2, the HL-2 and CL-2 sublines were established. Both of these sublines were maintained at population sizes of 15 litters per generation (5 males each mated to 3 females). Within HL-2, replacements were selected on a within-litter basis for high 42-day body weight. Selection was continued for 69 generations. Within CL-2, replacements were chosen at random. FP-2, HL-2 and CL-2 were, therefore, exactly analogous to FP-3, HL-3 (HL-3a) and CL-3, respectively. The present study sampled: FP-2 at generation 81 (inbreeding coefficient = 0·67); HL-2 at generation 73 (inbreeding coefficient = 0·94); CL-2 at generation 73 (inbreeding coefficient = 0·94).

(*e*) FP-5, HL-5, CL-5: Mice, used to establish FP-5, were members of the Q strain and were obtained by the Lacombe Research Station from the Institute of Animal Genetics in Edinburgh. The population size and mating pattern of FP-5 was exactly the same as that described for FP-3 and FP-2. The FP-5 was maintained through 25 single-pair matings per generation. Each mating produced only one litter; from each of these litters, one male and one female replacement was chosen at random. To minimize inbreeding and genetic drift, replacements were mated according to a cyclical mating plan.

At the eighth generation of FP-5, the HL-5 and CL-5 sublines were established. Both of these sublines were maintained at population sizes of 15 litters per

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generation (5 males each mated to 3 females). Within HL-5, replacements were selected on a within litter basis for high 42-day body weight. Selection was continued for 72 generations. Within CL-5, replacements were chosen at random. FP-5, HL-5 and CL-5 were, therefore, exactly analogous to FP-3, HL-3 (HL-3a) and CL-3, respectively. The present study sampled: FP-5 at generation 83 (inbreeding coefficient = 0·67); HL-5 at generation 75 (inbreeding coefficient = 0·94); CL-5 at generation 75 (inbreeding coefficient = 0·94).

(ii) *Calculation of inbreeding coefficients*

Inbreeding coefficients were calculated using the following equation

$$F_t = 1-(1-1/2N)^t(1-F_0),$$

where F_t = inbreeding coefficient at generation t, N = effective population size and F_0 = inbreeding coefficient at generation zero.

(iii) *Average 42-day body weights*

During the selection experiment, Agriculture Canada personnel calculated the average 42-day body weights of FP-3 and HL-3 mice at each generation. Weights of FP-3 mice were not recorded between generations 33 and 63.

(iv) *Logistic growth patterns of HL-3 and FP-3 mice*

Mice were weaned at 21 days of age. Male and female mice were caged separately (4/cage). Fifteen mice of each sex were individually weighed every 3rd day from 21 to 84 days of age. The logistic function was fitted to the weight-age data of each individual mouse by a generalized least squares non-linear estimation procedure. The logistic function for the nth mouse is represented by

$$Y_n(t) = A_n(1-b_n\,e^{-k\,n^t})^{-1}$$

where $Y_n(t)$ = body weight (g) at time t(d), b_n = integration constant, k_n = rate at which logarithmic function of weight changes linearly per unit of time (maturing rate) and A_n = asymptotic (mature) weight. Other traits derived from this function were age (t_n^*) and weight (y_n) at the point of inflection, age at maturity ($t_n 0{\cdot}99$) and mean absolute growth rate (ν_n). Each of the estimated parameters of the growth function was analyzed separately using a generalized least squares procedure. The following statistical model was assumed for this analysis

$$Y_{ijk} = \mu + L_i + S_j + (LS)_{ij} + e_{ijk}$$

where Y_{ijk} = observation on the kth mouse of the ijth subclass, μ = population mean, L_i = effect of the ith genetic line ($i = 1, 2$), S_j = effect of the jth sex ($j = 1, 2$), $(LS)_{ij}$ = interaction effect of the ith line and the jth sex and e_{ijk} = random effect term.

(v) *Restriction site analysis of GH gene*

Genomic DNA was prepared from liver tissue, digested with the appropriate restriction endonuclease and fractionated by electrophoresis on 0·7–1·5% agarose gels. Fractionated DNA was transferred to GeneScreenPlus membranes (NEN Research). Membranes were probed with a 0·8 kb *Hin* dIII fragment containing the complete rat GH cDNA (Seeburg *et al.* 1977). The probe was ^{32}P-labelled by synthesis using random oligonucleotide primers. Hybridization was carried out for 24–48 h at 42 °C in 50% formamide, 500 μg ml^{-1} sheared, denatured salmon sperm DNA, 1·0 M-NaCl, 1·0% Sarcosyl, 50 mM-Pipes pH 7·0, 5X Denhardts, 20 μg ml^{-1} yeast tRNA and 10% dextran sulphate. Filters were washed twice in 100 ml of 2X SSC (5 min each at room temperature); twice in 200 ml of 1X SSC, 1·0% SDS (30 min each at 65 °C); twice in 100 ml of 0·1X SSC (30 min each at room temperature). Filters were then exposed to Kodak XAR-5 film with intensifying screens (Dupont Cronex Lightening Plus) at −70 °C.

The orientation of the GH gene was determined with enzymes known to cut within the mouse GH coding sequence (Linzer & Talamantes, 1985). The resulting GH fragments were identified as 5′ or 3′ with 5′- and 3′-specific rat GH cDNA probes. The positions of the two identified internal *Pvu* II sites are conserved in the human, rat and mouse GH coding sequences (Seeburg *et al.* 1977; DeNoto *et al.* 1981; Linzer & Talamantes, 1985). Furthermore, in the rat and human GH genes, the 5′ end of the structural gene lies approximately 400 bp upstream from the 5′ *Pvu* II site; the 3′ end lies approximately 100 bp downstream from the 3′ *Pvu* II site (Barta *et al.* 1981; DeNoto *et al.* 1981). The 5′ and 3′ ends, of the mouse GH structural gene, have been predicted on the basis of this information.

3. Results

(i) *Body growth patterns*

As reflected in Fig. 1, the average 42-day weight of FP-3 mice remained relatively constant between generations 0 and 69 of the experiment. However, in response to the selection pressure, the average 42-day weight of HL-3 mice increased approximately 1·5-fold during this period. Figure 1 is representative of the general relationship between the additional selected lines and their respective foundation and control lines. At 42 days of age: HL-3 and HL-3a were 1·6-fold heavier than CL-3; HL-2 was 1·3-fold heavier than FP-2 and 1·4-fold heavier than CL-2; HL-5 was 1·3-fold heavier than FP-5 and 1·5-fold heavier than CL-5.

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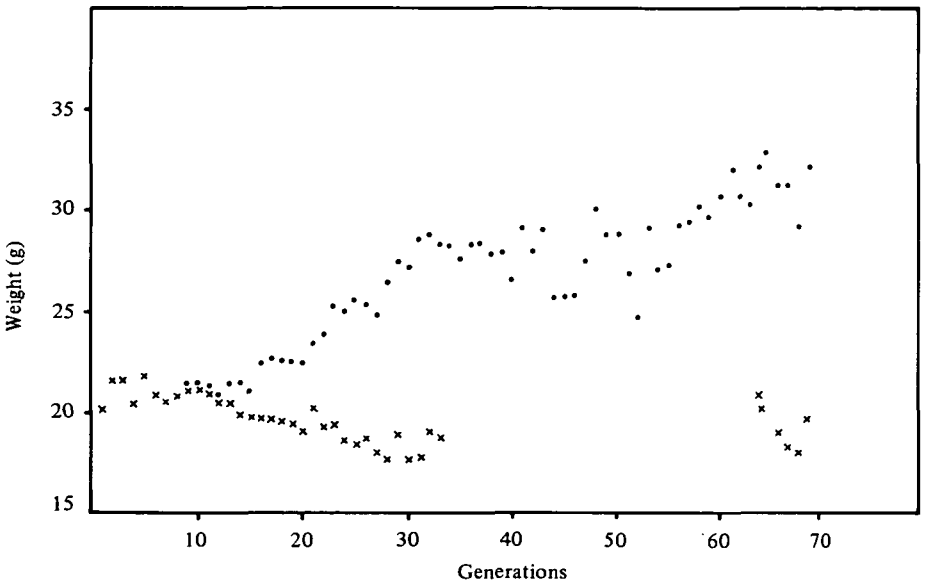

Fig. 1. Response to selection for high 42-day body weight. Average 42-day weight of HL-3 mice is denoted by a dot; average FP-3 42-day weight is denoted by an ×.

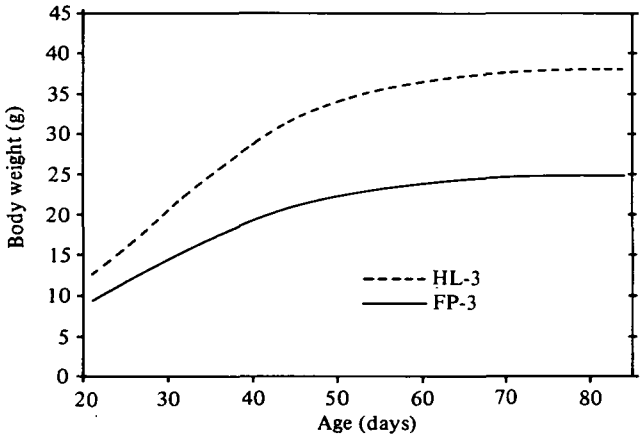

Fig. 2. Logistic growth curves of HL-3 and FP-3 mice.

The logistic growth curve analysis of HL-3 and FP-3 mice indicated that selection had directed a significant increase in the mean absolute growth rate (v) of HL-3 mice (Table 1). As a consequence of this accelerated rate of growth, HL-3 mice were 1·5 times heavier than FP-3 mice by approximately 25 days of age; this weight difference was maintained to maturity (see growth curves in Fig. 2). However, selection had little effect upon the rate of maturation (k), age at point of inflection (t^*) or shape of the growth curve (weight at inflection, y^*, relative to asymptotic weight, A) (Table 1).

(ii) *Restriction site analysis of FP-3 and HL-3*

The molecular analysis revealed between-line restriction fragment length polymorphisms (RFLP's) for each of the seven restriction enzymes employed to digest FP-3 and HL-3 DNA (Fig. 3). In contrast to this between-line variation, a survey of 50 nonsibs from each line revealed no within-line RFLP's. Each of these two lines, therefore, possessed a unique, fixed GH gene haplotype. The two GH haplotypes were transmitted as alleles of the GH gene; all progeny from HL-3 × FP-3 matings were heterozygous for the two haplotypes (Fig. 4). Densitometric scanning of the F_1 autoradiographic signals revealed that the HL-3 and FP-3 associated signals do not differ in intensity. This result suggested that there had been no amplification of the GH gene copy number during the selection process.

As indicated in Fig. 5, the identified polymorphic restriction sites occurred primarily in both the 5′ and 3′ flanking regions. However, the analysis indicated that sequence variation also existed within the structural gene itself. Resolution on high percentage agarose gels revealed that one of the HL-3 *Pvu* II/*Xmn* I fragments of the structural gene (indicated by an asterisk in Fig. 5) is approximately 15 bp shorter than the equivalent FP-3 *Pvu* II/*Xmn* I fragment. In both the human and the rat GH gene, this *Pvu* II/*Xmn* I fragment would include the 3′ end of the second exon, all of the second intron and the 5′ end of the third exon (Barta *et al.* 1981; DeNoto *et al.* 1981).

(iii) *Restriction site analysis of additional lines*

The GH haplotypes, in one representative of each of the four FP-3 parental lines, are shown in Fig. 6: the representatives of C57-BR/cd, DBA/1 and A/J exhibited the 'HL' *Hin* dIII haplotype while the representative of RF/J exhibited the 'FP' *Hin* dIII haplotype. This distribution of haplotypes was confirmed in DNA samples from a second representative of each line.

The GH *Hin* dIII haplotypes of one representative of each of the other foundation, growth-selected and

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Table 1. *Means and standard errors of logistic growth curve parameters*[a]

Parameter	FP-3	HL-3
Asymptotic weight (A)	25·05 ± 1·00	38·20 ± 0·95**
Age at maturity ($t_{0.99}$)	81·92 ± 3·88	79·52 ± 3·67
Weight at inflection point (y^*)	12·53 ± 0·50	19·10 ± 0·47**
Age at inflection point (t^*)	25·38 ± 0·64	27·14 ± 0·61
Maturing rate (k)	0·090 ± 0·006	0·098 ± 0·006
Mean absolute growth rate (v)	0·369 ± 0·27	0·602 ± 0·026**

** FP-3 and HL-3 parameters on the same line are significantly different ($P < 0·01$).

[a] The logistic function is detailed in the Materials and Methods.

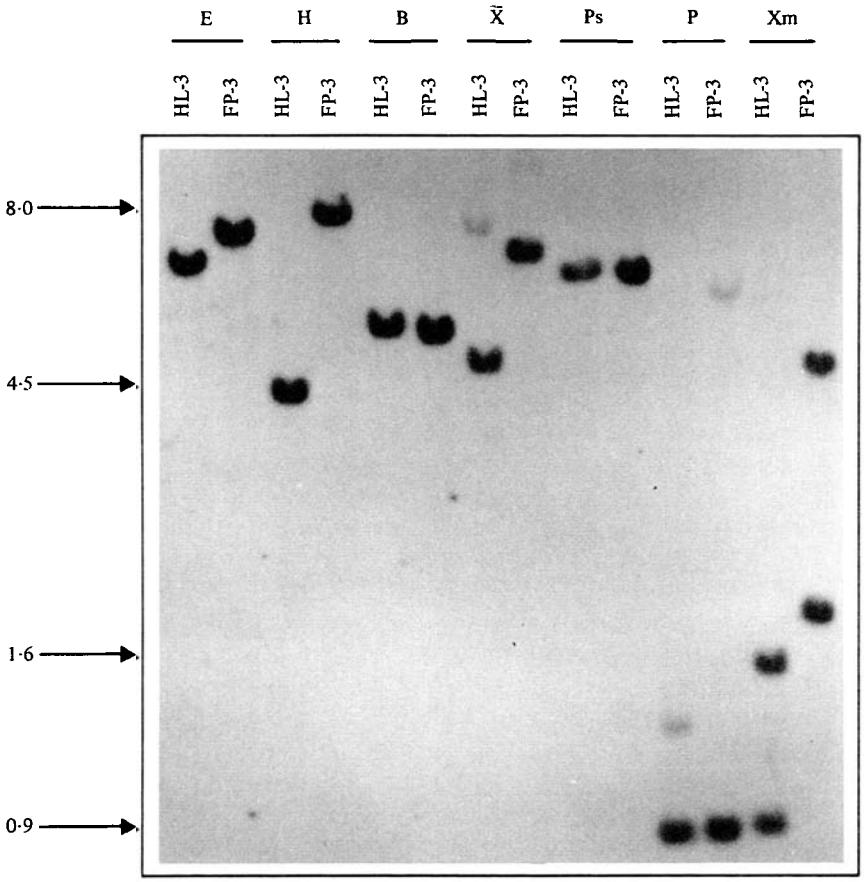

Fig. 3. Comparison of HL-3 and FP-3 restriction fragments. Symbols: E, *Eco* RI; H, *Hin* dIII; B, *Bam* HI; X, *Xba* I; Ps, *Pst* I; Xm, *Xmn* I; P, *Pvu* II. The lower intensity bands in the *Pvu* II and *Xba* I lanes represent 5′ fragments with only short regions of homology to the probe (see Fig. 5).

control lines were compared to FP-3 and HL-3 in Fig. 7. As shown, the 'HL' *Hin* dIII haplotype was found within every growth-selected line (including HL-3a, the replicate of HL-3). This observation has been extended to one additional animal from HL-3a and four additional animals from both HL-2 and HL-5. Within the unselected lines (FP-2, CL-2, FP-5, CL-5, CL-3), both the HL and the FP haplotypes have been identified. Five animals from CL-2 exhibited the HL haplotype, whereas five representatives of CL-5 and two representatives of CL-3 exhibited the FP pattern. Interestingly, and in contrast to FP-3, further sampling of FP-2 and FP-5 (five individuals/line) has indicated that the HL haplotype, in addition to the FP haplotype shown in Fig. 7, is segregating.

4. Discussion

A previous genetic analysis of weight selected mice (Pidduck & Falconer, 1978) indicated that an increased amount, or activity, of GH was involved in the selection response. This weight-selected line of mice was a subline of the Q-strain which contributed to both the FP-2 and FP-5 populations studied here.

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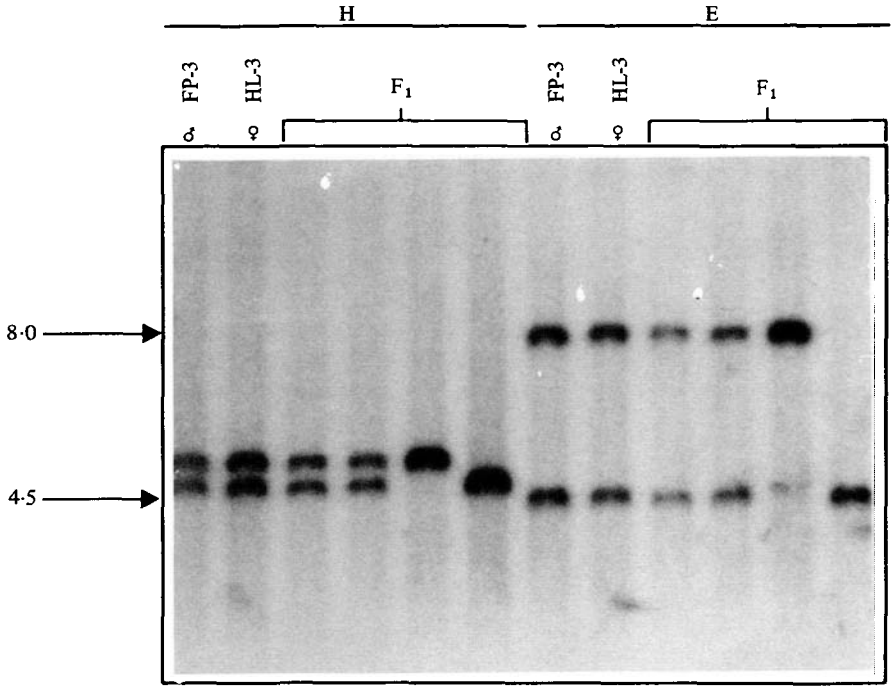

Fig. 4. Segregation of restriction fragment length polymorphisms in F_1 generation. DNA from an FP-3 male, an HL-3 female and four of their offspring was restricted with *Eco* RI (E) and *Hin* dIII (H) to examine the restriction fragment lengths of F_1 animals.

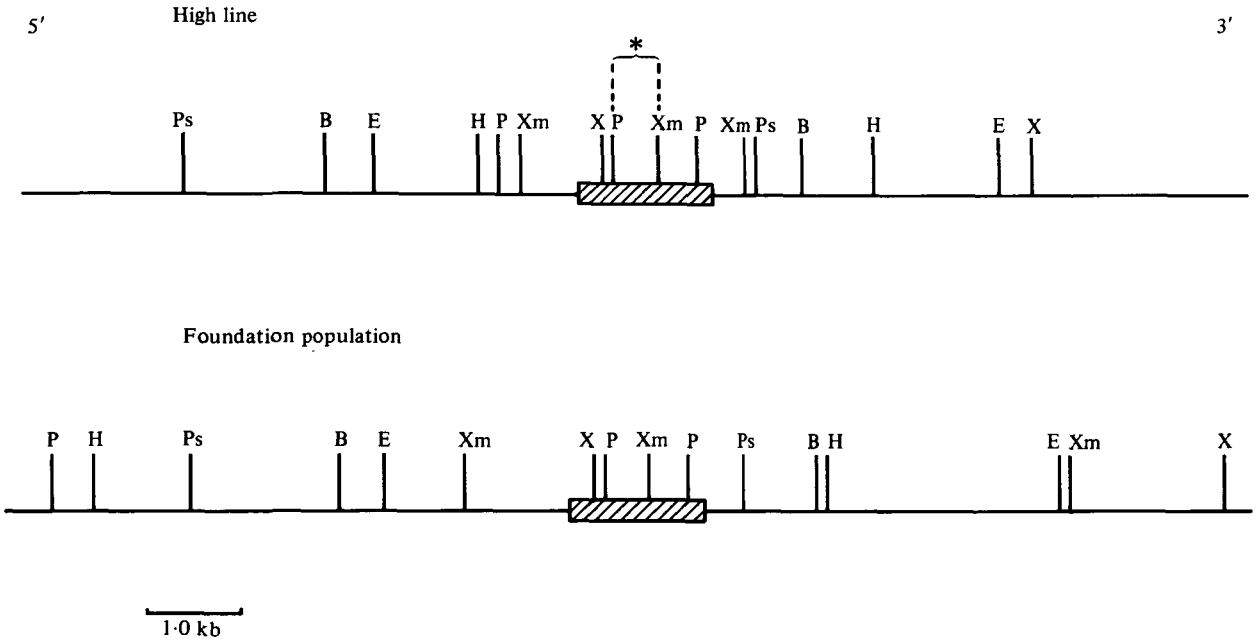

Fig. 5. Map of restriction sites identified by Southern analysis. The GH structural gene is designated by the cross-hatched box. The HL-3 *Pvu* II/*Xmn* I fragment marked with an asterisk is approximately 15 bp shorter than the equivalent FP-3 *Pvu* II/*Xmn* I fragment. Symbols: E, *Eco* RI; H, *Hin* dIII; B, *Bam* HI; X, *Xba* I; Xm, *Xmn* I; P, *Pvu* II; Ps, *Pst* I.

While the genetic analysis of Piddick & Falconer (1978) did not allow elucidation of the specific mechanism through which the GH endocrine unit was acting, natural variation in both circulating and pituitary GH levels has been documented in mice (Yanai & Nagasawa, 1968; Sinha *et al.* 1974). Furthermore, variant forms of the GH molecule (Lewis, 1984), as well as variation in the processing of the GH primary transcript (DeNoto *et al.* 1981), have been identified in rats and humans.

Despite this evidence of variation in GH gene expression, two earlier reports were unable to identify any GH gene sequence variation between mice exhibiting diverse patterns of growth (Parks *et al.*

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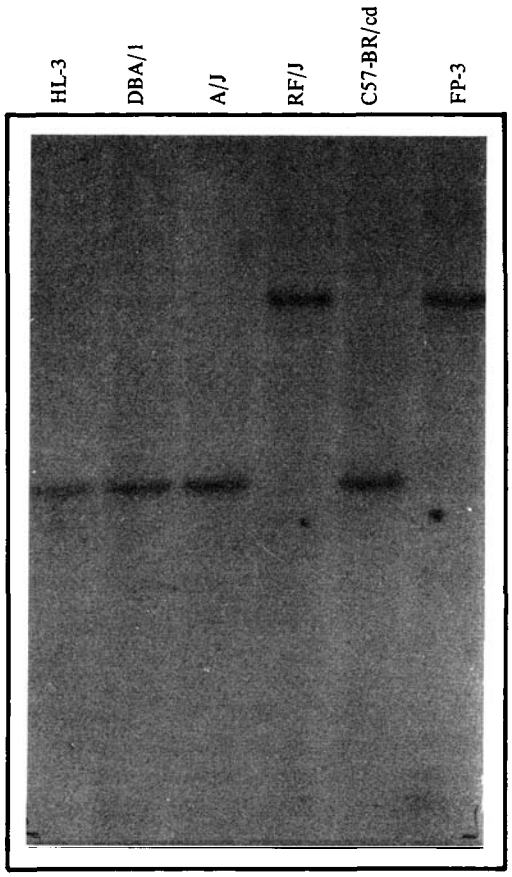

Fig. 6. Comparison of GH *Hin* dIII haplotypes in the four FP-3 parental lines.

1982; Phillips *et al.* 1982). The study described herein, however, identified two GH haplotypes which may be differentiated on the basis of seven polymorphic restriction sites which exist in both the 5′ and 3′ flanking regions of the GH gene as well as within the structural gene itself (Fig. 3 and Fig. 5). Considering the different histories of these lines (see Materials and Methods), the existence of only two haplotypes is very interesting. Another interesting aspect of our results concerns the large number of polymorphic restriction sites by which we can distinguish the two haplotypes. We have preliminary data on three other loci in these lines and have yet to uncover a single polymorphism (unpublished observation).

As the inbreeding coefficient approaches unity, there is a high probability that populations will eventually become fixed for a specific allele. Thus, while only 2 representatives of each of the highly inbred C57-BR/cd, DBA/1, A/J and RF/J were examined, it is likely that each line was fixed for the specific GH allele identified within its representatives. Since each of the 4 inbred lines (C57-BR/cd, DBA/1, A/J, RF/J) contributed equally to the establishment of FP-3, the frequency of the HL and FP alleles in the original FP-3 population was probably 0·75 and 0·25, respectively. The probability of fixation of the HL and FP alleles in any ideal subpopulation (as the inbreeding coefficient approaches unity) is expected to equal the frequency of the alleles in the initial population (i.e. 0·75 and 0·25, respectively). Therefore, with a proba-

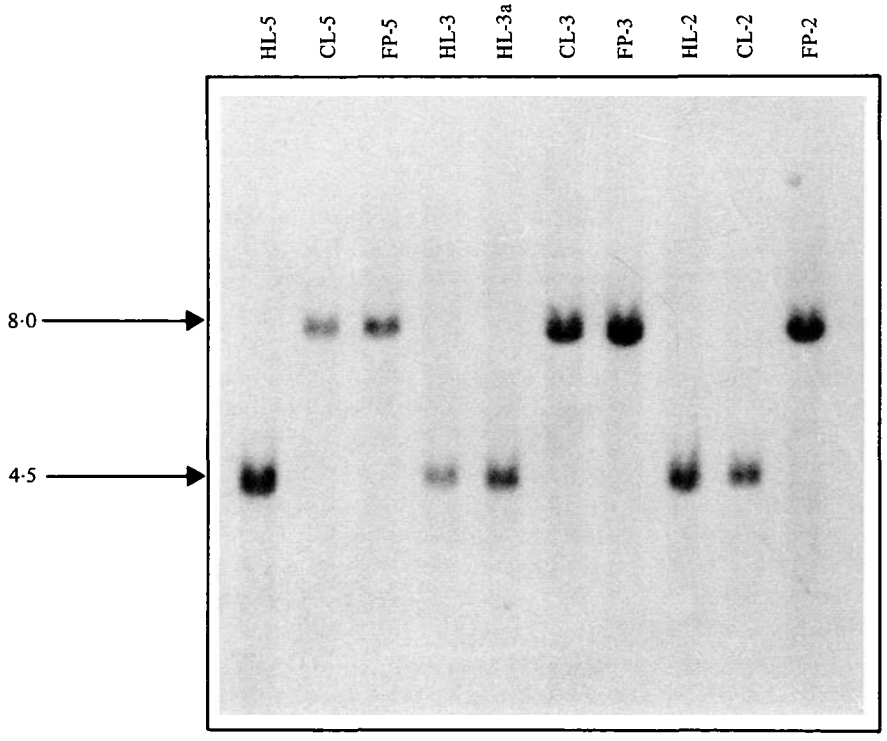

Fig. 7. Comparison of GH *Hin* dIII haplotypes in the growth-selected and unselected lines of mice.

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bility of 0·25, chance fixation of the FP allele within the unselected FP-3 and CL-3 lines was considerably less probable than fixation of the HL allele. The likely fixation of the FP allele was, thus, rather unexpected. A possible explanation for this phenomenon is that, in the absence of high body weight selection, the FP allele was most compatible with the general fitness of the mice. The breeding system, employed for these lines, left little potential for such a natural selective pressure to act upon either fertility or litter size. However, natural selection could have influenced the frequency of the GH alleles by acting upon viability.

The initial HL allele frequency (0·75) clearly favoured its chance fixation within HL-3 and HL-3a. However, any natural selective force acting upon the unselected lines (favouring the fixation of the FP allele), must also have acted upon these growth-selected lines. Therefore, the probable fixation of the HL allele within HL-3 and HL-3a may reflect not random fixation, but fixation as a result of the imposed high body weight selection pressure. The presence of only the HL allele within the five representatives of both HL-2 and HL-5 further supports this suggestion. The segregation of both GH haplotypes in FP-2 and FP-5, from which HL-2 and HL-5 were derived, indicates that the possibility of fixing either allele did exist. Unfortunately, we cannot comment on the likelihood of chance fixation because the initial allele frequencies in the founding animals of these two sets of lines could not be determined. Nevertheless, at present we are pursuing the concept that the association, between a specific GH haplotype and accelerated growth, has functional significance.

According to selection theory, high body-weight selection should identify, and act upon, functional variation at the loci of genes affecting growth; alleles, or combinations of alleles, which make a positive contribution to growth will be favoured within a line selected for the enhancement of a growth-related parameter. The study described herein identified sequence variation in both the 5′ and 3′ flanking regions of the GH gene as well as within the structural gene itself (Fig. 5). Of particular interest is the sequence variation associated with the 3 polymorphic sites (*Hin* dIII, *Pvu* II, *Xmn* I) which occur within 0·6–1·1 kb of the 5′ end of the structural gene. In the rat GH gene, this 5′ region includes elements which mediate thyroid regulation (Casanova *et al.* 1985). Moreover, natural sequence variation in the distal 5′ flanking sequences of several other genes has been associated with variation in both gene expression (Muskavitch & Hogness, 1982; Estelle & Hodgetts, 1984) and phenotypic expression (Rotwein *et al.* 1983). The identified sequence variation in the proximal 3′ flanking region is also notable as a result of the demonstration that sequences in this region are important in determining the precise site of poly-adenylation in bovine GH mRNA (Rottman *et al.* 1986). Moreover, the variation within the structural gene has the potential to generate both qualitative and/or quantitative variation in the gene product (Darnell, 1982). Thus, the HL GH haplotype may represent a GH allele which is functionally different from the FP GH allele and has, thereby, reached fixation as a result of the imposed selection pressure. To further investigate the relationship, between the HL haplotype and accelerated growth, HL-3 and FP-3 mice have been mated to generate an F_2 population; once this population has been established, the growth patterns of the three F_2 GH genotypes will be compared. The ratio of the F_2 genotypes may also reveal information regarding an association between the two alleles and viability. To substantiate that the divergent growth patterns are a direct result of the GH alleles, and not due to variation at a linked 'growth-regulating' gene locus, molecular studies have been initiated to evaluate the expression of the two GH alleles.

Agriculturally important variant alleles undoubtedly exist in many livestock populations. Knowledge of the existence of such alleles will greatly aid the selection of replacement breeding animals. In addition to phenotypic considerations, the selection of replacements could include an evaluation of the specific alleles carried by each animal. Further, gene transfer techniques will eventually allow combinations of important variants to be introduced into a population much more rapidly than traditional breeding procedures. The potential of gene transfer to influence growth rate and body size has been illustrated by the accelerated growth of mice bearing metallothionein-I/growth hormone (MT-I/GH) fusion genes (Palmiter *et al.* 1982, 1983). This fusion construct effectively disrupts all feedback mechanisms which regulate GH gene expression via the natural GH 5′ regulatory elements. Unfortunately, in addition to the correlated acceleration of growth in mice carrying this construct, the disruption of the natural GH regulatory region is also associated with physiological changes which reduce the overall fitness of the transgenic animal: the sexual differentiation of certain liver functions is abnormal (Norstedt & Palmiter, 1984) and the fertility of females is impaired (Hammer *et al.* 1984). Consequently, while the MT-I/GH construct has already been introduced into two livestock species (Hammer *et al.* 1985), this disruption of all natural 5′ regulation may create a deleterious regulatory variant with limited application in livestock populations. However, since natural growth-enhancing variants evolve as a functional component of the genome, their expression in recipient genomes should not cause the undesirable effects associated with fusion genes. While the growth rates of HL-3 and FP-3 mice differ markedly, the two populations do not differ in their fertility, mortality or litter size (R. K. Salmon, unpublished data). Therefore, naturally occurring variants may offer a superior

alternative, to fusion constructs, for the enhancement of animal production characters through gene transfer technology.

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References

Barta, A., Richards, R. I., Baxter, J. D. & Shine, J. (1981). Primary structure and evolution of rat growth hormone gene. *Proceedings of the National Academy of Sciences U.S.A.* **78**, 4867–4871.

Bowman, J. C. & Falconer, D. S. (1960). Inbreeding depression and heterosis of litter size in mice. *Genetical Research* **1**, 262–274.

Casanova, J., Copp, R. P., Janocko, L. & Samuels, H. H. (1985). 5′-flanking DNA of the rat growth hormone gene mediates regulated expression by thyroid hormone. *Journal of Biological Chemistry* **260**, 11744–11748.

Darnell, J. E. (1982). Variety in the level of gene control in eucaryotic cells. *Nature* **297**, 365–371.

DeNoto, F. M., Moore, D. D. & Goodman, H. M. (1981). Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. *Nucleic Acids Research* **9**, 3719–3730.

Estelle, M. A. & Hodgetts, R. B. (1984). Insertion polymorphisms may cause stage specific variation in mRNA levels for dopa decarboxylase in drosophila. *Molecular and General Genetics* **195**, 442–451.

Falconer, D. S. (1960). Selection of mice for growth on high and low planes of nutrition. *Genetical Research* **1**, 91–113.

Falconer, D. S. (1973). Replicated selection for body weight in mice. *Genetical Research* **22**, 291–321.

Farid-Naeini, A. (1986). Inbreeding and litter size in mice. Ph.D. thesis, University of Alberta.

Hammer, R. E., Palmiter, R. D. & Brinster, R. L. (1984). Partial correction of murine hereditary growth disorder by germ-line incorporation of a new gene. *Nature* **311**, 65–67.

Hammer, R. E., Pursel, V. G., Rexroad, C. E., Wall, R. J., Bolt, D. J., Ebert, K. M., Palmiter, R. D. & Brinster, R. L. (1985). Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* **315**, 680–683.

Jackson Memorial Laboratory (1955). Descriptions of strains maintained in the 'inbred nucleus' and 'market colonies'. Jackson Memorial Laboratory, Bar Harbor, Maine. Mimeograph, p. 5.

Lewis, U. J. (1984). Variants of growth hormone and prolactin and their posttranslational modifications. *Annual Review of Physiology* **46**, 33–42.

Linzer, D. I. H. & Talamantes, F. (1985). Nucleotide sequence of mouse prolactin and growth hormone mRNAs and expression of these mRNAs during pregnancy. *Journal of Biological Chemistry* **260**, 9574–9579.

Muskavitch, M. A. T. & Hogness, D. S. (1982). An expandable gene that encodes a *Drosophila* glue protein is not expressed in variants lacking remote upstream sequences. *Cell* **29**, 1041–1051.

Neimann-Sorensen, A. & Robertson, A. (1961). The association between blood groups and several production characteristics in three Danish cattle breeds. *Acta Agricultural Scandinavica* **11**, 163–196.

Norstedt, G. & Palmiter, R. D. (1984). Secretory rhythm of growth hormone regulates sexual differentiation of mouse liver. *Cell* **36**, 805–812.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans, R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300**, 611–615.

Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. (1983). Metallothionein-human GH fusion genes stimulate growth of mice. *Science* **222**, 809–814.

Parks, J. S., Herd, J. E., Wurzel, J. M. & Martial, J. A. (1982). Structural analysis of rodent growth hormone genes; application to genetic forms of hypopituitarism. *Endocrinology* **110**, 1672–1675.

Phillips, J. A., Beamer, W. G. & Bartke, A. (1982). Analysis of growth hormone genes in mice with genetic defects of growth hormone gene expression. *Journal of Endocrinology* **92**, 405–407.

Pidduck, H. G. & Falconer, D. S. (1978). Growth hormone function in strains of mice selected for large and small size. *Genetical Research* **32**, 195–206.

Rottman, F., Camper, S., Goodwin, E., Hampson, R., Lyons, R., Sakai, D., Woychik, R. & Yao, Y. (1986). Structure and regulated expression of bovine prolactin and bovine growth hormone genes. In *Molecular and Cellular Aspects of Reproduction* (ed. D. S. Dhindsa and O. P. Bahl), pp. 281–299. New York: Plenum Press.

Rotwein, P. S., Chirgwin, J., Province, M., Knowler, W. C., Pettitt, D. J., Cordell, B., Goodman, H. M. & Permutt, M. A. (1983). Polymorphism in the 5′ flanking region of the human insulin gene: a genetic marker of non-insulin dependent diabetes. *New England Journal of Medicine* **308**, 65–71.

Schuler, L. A. & First, N. L. (1985). *Regulation of Growth and Lactation in Animals*, 1st edn. University of Wisconsin, Madison.

Seeburg, P. H., Shine, J., Martial, J. A., Baxter, J. D. & Goodman, H. M. (1977). Nucleotide sequence and amplification in bacteria of structural gene for rat growth hormone. *Nature* **270**, 486–494.

Sinha, Y. N., Selby, F. W. & Vanderlaan, W. P. (1974). The natural history of prolactin and GH secretion in mice with high and low incidence of mammary tumors. *Endocrinology* **94**, 757–764.

Smith, C. (1967). Improvement of metric traits through specific genetic loci. *Animal Production* **9**, 349–358.

Stansfield, W. D., Bradford, G. E., Stormant, C. & Blackwell, R. L. (1964). Blood groups and their associations with production and reproduction in sheep. *Genetics* **50**, 1357–1367.

Yanai, R. & Nagasawa, H. (1968). Age, strain and sex differences in the anterior growth hormone content of mice. *Endocrinology Japonica* **15**, 395–402.