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ASSOCIATION BETWEEN RESTRICTION FRAGMENT LENGTH POLYMORPHISMS FOR SOMATOTROPIC GENES AND BODY WEIGHT IN MICE

BY

DIANNE CHRISTINA WINKELMAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF PhD

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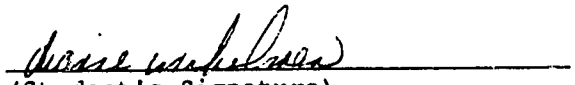

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SUBMITTED BY DIANNE CHRISTINA WINKELMAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

PhD

Ross Hodgetts

Francis Yeh

Michael Russell

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Date: July 2, 1991

for fun

This work is dedicated to Sevaar and Vern who played polo.

ABSTRACT

In this study, restriction fragment length polymorphisms (RFLPs) in somatotropic genes were tested for correlations with body weight and postweaning growth rate in mice.

Four F_2 populations of mice ($3F_2$, $5F_2$, $2F_2$, and MF_2) were generated from crosses between lines of mice subjected to long term selection for high 42-day weight and unselected controls. RFLPs segregating in these crosses were for the growth hormone (GH), insulin-like growth factor 2 (IGF-2), and growth hormone releasing factor (GRF) genes.

The GH^h haplotype which had originally been fixed in three of four selected lines and absent from all unselected lines was found to have a significant ($P<0.01$) negative gene substitution effect on 42-day weight and postweaning growth rate in three of the F_2 populations. We suggest that this is the result of interactions between the growth hormone haplotype and other genes in the genetically mixed population. The positive association originally seen between GH^h and weight in the stock lines may have been particular to the high weight selected genetic background.

The IGF-2^{H5} haplotype was associated with increased 21-day and 42-day weight in a sex-dependent manner in one of the two F_2 populations ($5F_2$) in which it was analyzed. No associations were found between RFLPs for GRF and any of the growth parameters studied in the three F_2 populations polymorphic at this locus. There was, however, some indication that the GRF haplotypes acted in conjunction with RFLPs of GH and IGF-2 to affect 42-day weight. The analysis of interactions also indicated that certain combinations of GH and IGF-2 haplotypes yielded heavier mice than other combinations.

Three of the F_2 populations ($5F_2$, $2F_2$, and MF_2) were subjected to divergent selection for 42-day weight and RFLP frequencies were determined at the termination of selection. We believed that by recreating a selected genetic background, the GH^h haplotype from the

original high lines would segregate with high weight. The selected lines also allowed us to monitor the effects of selection on associations between polymorphisms of IGF-2 and GRF and weight parameters. In all of the selected sublines, changes in the frequency of the GH RFLPs occurred in accordance with our predictions: the frequency of GH^h increased in all three lines selected for high 42-day weight. We also observed a decrease in the frequency of GH^h in two of the three lines selected for low 42-day weight. This suggests that GH^h has a positive effect on 42-day weight in a weight-selected genetic background, regardless of whether the genetic background is selected for high or low weight.

Changes in frequency of the RFLPs for IGF-2 coincided with expectations: IGF-2^{H5} increased in the high selected subline derived from $5F_2$, the population in which it was found to be positively associated with 42-day weight. No changes in the frequency of GRF haplotypes were correlated with selection.

We have shown that RFLPs for somatotropic genes may act as QTLs for weight parameters but the actual effect of the RFLP is dependen. on the genetic environment and its effects must, therefore, be evaluated in the appropriately bred background. Our results also indicate that the simultaneous evaluation of polymorphisms at numerous somatotropic loci may lead to the identification of desirable genotypic combinations which could constitute breeding goals.

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INTRODUCTION

The modern domestic animal is the product of centuries of artificial selection by mankind. Selection has been performed, with varying degrees of intensity, throughout the history of domestication; choosing as breeding stock those animals expected to produce better progeny in the next generation. Originally, selection was probably approached in a negative manner, through the slaughter of less fit or unmanageable animals thereby selecting for improved fitness and disposition. Castration of males in many livestock species has also long been practised to render them more tractable, leaving only the more docile of the males for breeding and thus selecting for increased manageability. Current methods take a more positive approach, with the goal of selection being the identification of breeding animals with the highest genetic potential for traits of economic importance. In some production systems, the goal is even more defined, being the identification of the precise matings which will yield the highest genetic potential in their offspring.

I. SELECTION OF QUANTITATIVE TRAITS

Accurate assessment of the genotypic values of livestock for the maximization of rates of genetic gain is the ultimate goal of the animal breeder. This area is, however, fraught with difficulties which arise from the quantitative nature of most economically important production traits. The manifestation of these characters is physiologically complex and is, therefore, under the control of numerous segregating genes

(Wright, 1968) which determine genetic potential. The actual expression of these quantitative traits is also influenced by continuously variable environmental effects (Falconer, 1981). Furthermore, genotype by environment interactions may have profound influences on the expression of quantitative traits (Bray et al., 1962) and breeding values may also be biased as a result of correlations between genotype and environment. This is of particular concern for traits evaluated in multiparous animals when the traits are significantly influenced by maternal effects. Environmental effects and genotype by environment interactions constitute background "noise" which must be eliminated in the calculations of genotypic values. An additional impediment to the determination of genotypic values for economically important traits arises when they cannot be measured on potential breeding animals. This situation is encountered with sex-limited traits such as milk and egg yield as well as with traits that are properties of the slaughtered carcass. Sex-limited traits are of concern when they are exclusive to the female since most of the selection pressure in any breeding programme is applied on the males.

Typically, genotypic values of animals for a given trait have been determined using phenotypic values for that trait. Individual or mass selection simply involves the selection of superior animals on the basis of their own phenotype. Mass selection is expected to yield relatively high rates of genetic progress for traits of high heritability (*i.e.* $h^2>0.5$) that can be measured on the individual (Lush, 1935). Family selection involves the selection of families of sibs as parents for the

next generation utilizing full or half sib family means as the criterion of selection. This should be more effective than mass selection for traits of low heritability (*i.e.* $h^2<0.25$) when the correlation between breeding values of family members is high relative to the correlation between phenotypes of family members (Lush, 1947; Osborne, 1957). Family selection can be particularly useful when traits are more economically measured on groups than on individuals as with feed consumption by groups of penned animals.

The phenotypic values of animals related to the animal being assessed can also be used to estimate genotypic values. Progeny testing can yield very accurate estimates of breeding values for traits of low heritability when a large number of progeny records are available (Lush, 1935). This method has been extensively and successfully utilized in the evaluation of dairy sires. In most other situations, however, the increase in the accuracy of evaluation over other methods is not sufficient to compensate for the concomitant increase in the generation interval and the resulting retardation in the absolute rate of genetic progress (Lerner and Hazel, 1947). Significant increases in the accuracy of genotypic value estimates for traits of low heritability can be attained with indices incorporating information on the individual and its relatives (Sales and Hill, 1976a). The application of index selection in the evaluation of animals allows for the incorporation of all available information on relatives. The effect of additional information on the accuracy of the selection index is a function of the relationship between the relative contributing the information and the

animal being evaluated as well as the heritability of the trait. However, increases in accuracy are always realized with the incorporation of additional information (Sales and Hill, 1976a).

II. INDIRECT SELECTION

With indirect selection, the focus is placed on the response of main traits when the criterion of selection is a correlated trait. Indirect selection has been suggested as an alternative to progeny testing for traits of low heritability (*i.e.* $h^2<0.25$). This method could alleviate the extended generation interval invoked by the necessity of obtaining a large number of progeny records. The selection response attained with indirect selection will be greater than that for individual selection only if $r_A h_Y > h_X$; where r_A is the genetic correlation between the two traits, h^2_Y is the heritability of the correlated trait and h^2_X is the heritability of the trait of interest (Falconer, 1981). Obviously, a breeding programmed based solely on indirect selection requires that the indicator trait be of high heritability and that there exists a good correlation between the indicator trait and the trait of interest. Such a situation has been observed in Merino sheep where there is a high genetic correlation between the highly heritable polled trait and infertility, a trait of low heritability which requires extensive testing to measure (Wiesner, 1960). Indirect selection can also be of great value when individual selection is not possible or requires a prolonged waiting period to obtain the requisite information.

The main interest in indirect selection methods has been in their potential utilization in the preliminary step of two-stage selection programmes. Two-stage selection programmes entail an initial selection of juveniles to be maintained as potential breeders with a second stage of selection to choose actual breeders or those breeders which will be more extensively used. These programmes are applied in systems where production records are not available until later stages of life or where progeny testing is the ultimate method of evaluation.

Evaluation of animals by selection indices can also benefit from the incorporation of indicator or correlated traits into the index used. The aim of the development of a selection index is to derive an index which is maximal with respect to the correlation between the index value (I) and the true aggregate genotypic value (T) (*i.e.* maximize r_{TI}) (Hazel, 1943). Animals at the extremes of the phenotypic distribution for a trait are easily identified, however, selection indices are necessary to distinguish genotypic values of animals of intermediate phenotypic values. The importance of selection indices for evaluating animals of intermediate phenotypic value arises from the higher variability of progeny about parental values and greater within-family variance than is observed for animals at the limits of the distribution (Hill, 1978). Improvements in the accuracy of breeding value estimates are realized by incorporating additional information into the selection index. Therefore, the addition of indicator traits which are genetically correlated with the trait of interest will result in increased accuracy of the index, even if the genetic correlation between the indicator

trait and the trait of interest is relatively low (*i.e.* $r_{XY}<0.3$) (Sales and Hill, 1976b; Woolliams and Smith, 1988).

Indirect selection can also be important when only the correlated trait can be measured on potential breeding animals and has, therefore, received much attention from dairy bull breeders. In the dairy industry, indicator traits, measured on bulls can be incorporated into selection indices consisting of records on relatives to increase the accuracy of estimated breeding values (Woolliams and Smith, 1988). For example, Klindt (1988) examined growth hormone and prolactin secretory profiles in Holstein bulls and found a negative correlation between growth hormone peak frequency and Predicted Differences (PD) for milk, fat and protein yield. Consequently, addition of growth hormone peak frequency to ancestor merit in the model for PD milk increased the accuracy from 0.31 to 0.59. Correlations between prolactin baseline concentration and PD for fat and protein yield and frequency of prolactin peaks and PD for milk, fat and protein yield were also found in this study.

Numerous studies have been undertaken to examine relationships between metabolic parameters and production characters. The purpose of research in this area is two-fold: it furthers our understanding of the physiological basis of production traits and allows the identification of metabolic parameters which might serve as indicator traits for indirect selection. The results of an analysis of heritabilities for serum and production traits in Holstein cows indicated that selection for decreased levels of alkaline phosphatase would theoretically yield

38%, 88% and 87% of the response acquired with direct selection for milk, fat and protein yield, respectively (Peterson et al., 1982).

Investigations aimed at determining relationships between somatotropin metabolism and production traits have been of particular interest for our research which is focused on determining how polymorphisms for somatotropic genes might influence production traits. Lean tissue growth has been found to be positively correlated with average daily growth hormone secretion in cattle (Trenkle and Topel, 1978), pigs (Machlin, 1972) and lambs (Wagner and Veenhuizen, 1978). These observations are correspondent with the effects of exogenous growth hormone treatment which typically results in increased lean tissue deposition (reviewed in Webster, 1989). Conversely, Landrace pigs selected for maximum weight at 200 days were found to have lower baseline concentrations of growth hormone than unselected controls although other parameters of growth hormone profiles did not differ between the two lines (Arbona et al., 1988). It was also found that growth hormone levels of phenotypically normal Hereford cattle heterozygous for the dwarf gene were found to be higher than those of either homozygous class (Dev and Lasley, 1969). These apparently contradictory results may be due to the effects of interactions between circulating growth hormone and other polypeptides or receptors of the somatotropic axis. For instance, reduction in hepatic or circulating growth hormone receptors would result in both decreasing the metabolic effects of growth hormone and increasing the levels of circulating growth hormone (Smith and Talamantes, 1987). It is also possible that

the larger animals with lower growth hormone levels have greater fat deposition than those animals with higher levels of circulating growth hormone which reduces fat accumulation.

It has been suggested that metabolic parameters such as these might provide heritable markers for indirect selection of production traits. Thus, metabolic parameters could provide the criterion for juvenile selection in two-stage selection programmes or could be used in conjunction with other records in index selection. However, since levels of circulating somatotropic hormones are cyclic (Haynes, 1986) and influenced by numerous environmental factors (Houseknecht et al., 1988), it is often difficult to obtain accurate and meaningful measures of these parameters. Nevertheless, elucidation of the relationship between metabolic parameters and production traits would improve our understanding of the physiological processes controlling the expression of such traits. And, from this, we will be in a better position to develop the genetic model for the determination of production traits.

III.MARKER ASSISTED SELECTION

The possibility of indirect selection at an even more basic level than metabolic parameters is currently being approached with marker assisted selection utilizing protein variants and/or restriction fragment length polymorphisms (RFLPs) of genomic or cytoplasmic DNA. The major advantages realized with molecular markers result from the fact that they are free of influences from environmental effects and they can

be identified at an early age. Therefore, the use of molecular markers as selection criteria can considerably reduce the generation interval thereby increasing the potential rate of genetic improvement. Furthermore, since it is possible to identify allelic polymorphisms for many of the markers used, their transmission patterns can be predicted giving us traits of high heritability on which to carry out selection. It has also been suggested that, as methods for estimating breeding values are becoming optimal, improvements in the accuracy of index values will, of necessity, be derived from novel sources such as molecular markers (Hill and Knott, 1990).

Marker assisted selection as the basis of mapping quantitative trait loci (QTLs) to chromosomal segments was proposed by Geldermann (1975). This approach recognizes the possibility of following the transmission of chromosomes from parents to progeny by means of gene markers (Thoday, 1961). Geldermann (1975) posited that the effect of chromosome segment substitution on the phenotype for a metric trait of a progeny group could be quantitatively estimated because (1) monogenic variants at the molecular level are common in the population, (2) every gene locus marks a chromosome section which encompasses all loci linked to the marker gene, and (3) the variance of metric characters is influenced by genes within chromosome sections with statistically identifiable additive gene effects. The finding of an association between a marker gene and the variation of a quantitative character is indicative of either a pleiotropic effect of the marker gene itself or linkage between the marker gene and one or more segregating loci which

affect the trait (Mather and Jinks, 1971). The observation of such associations constitutes the preliminary step in the mapping of loci controlling a specific quantitative character. The strength of the association between a marker gene and the quantitative trait is a function of the degree of linkage between the marker and the QTL and the magnitude of the effects of the QTL (Jensen, 1989). QTLs, so identified, could influence underlying physiological or molecular parameters that directly control quantitative traits (Soller, 1978). This might be expected of variants in regulatory genes which influence hormone levels or with polymorphisms in control regions of structural genes which mediate the response to regulatory genes. They may also exhibit distinctive, highly heritable effects on specific components of production traits (Soller, 1978) as would be the case for polymorphisms in structural genes that result in protein variants.

Soller and Beckman (1983) indicated that the utilization of genetic markers for direct selection on genotypes could be advantageous in predictions of selection methods by reducing the amount of "noise" inherent in current statistical methodologies. Marker assisted selection appears to have great potential for the identification of desirable genes from resource germplasm which might be advantageously introgressed into domestic populations inferior for the affected trait(s) (Soller and Plotkin-Hazan, 1977; Soller and Beckman, 1983; Beckman and Soller, 1983). The potential usefulness of known loci in selection is dependent upon the proportion of the total additive genetic variance contributed by known loci relative to the heritability of the trait (Smith, 1967;

Lande and Thompson, 1990). Therefore, marker assisted selection is expected to have the greatest potential for traits of low heritability. Marker assisted selection has also been considered for breeding programmes where sex-limited traits or carcass traits, which cannot be measured on the animal being evaluated, are the focus of selection (Smith, 1967). Marker assisted selection might also be used for juvenile selection in two-stage selection programmes (Jung et al., 1989). The relative efficiency of marker assisted selection as compared to traditional methods should also be increased in situations where correlations between genotypic and environmental effects exist (Lande and Thompson, 1990). This would be possible because segregating alleles are identified directly and are unaffected by environment.

III.A.MARKER ASSISTED SELECTION WITH COMPLETE RFLP MAPS

Recently, the systematic mapping of QTLs has been approached utilizing numerous random RFLPs comprising densely populated genetic linkage maps spanning most of the genome (Paterson et al., 1988; Lander and Botstein, 1989). The methodology for the systematic testing of RFLPs as potential QTL markers involves scoring the progeny, from a cross between two inbred strains which differ in their expression of a quantitative trait, for the trait and a number of genetic markers (Lander and Botstein, 1989). The analysis of complete RFLP maps allows for interval mapping of QTLs by a modification of maximum likelihood and lod scoring methods from reverse genetics techniques used in the study of human disease (Orkin, 1986). Reverse genetics involves the mapping of

genes of interest by pedigree analysis, tracing cosegregation of disease and genetic markers from affected individuals in a population back through ancestors. The existence of numerous, multiallelic random RFLPs throughout the genome, characterized by codominance and expected to be without pleiotropic effects on economic traits, renders them particularly amenable to genetic studies (Beckman and Soller, 1983). With a complete RFLP map, inferences can be made about points throughout the genome and the confounding of phenotypic effects with recombination is avoided through the use of flanking genetic markers (Paterson et al., 1988). A further refinement of this technique has been proposed by Paterson et al (1990) for the fine mapping of QTLs by substitution mapping using overlapping chromosomal segments in the region of previously identified QTLs. In this manner, QTLs are mapped either to common regions of overlapping segments or to regions unique to one of a number of overlapping segments.

Genetic mapping of QTLs using extensive RFLP maps has, to date, been applied only to a few plant species; the application being limited to organisms for which such maps are available and where the necessary crosses and numbers of progeny can be screened. Paterson et al (1988) analyzed 237 backcross progeny from a cross between the domestic tomato, *Lycopersicon esculentum*, and the South American green fruited tomato, *Lycopersicon chmielewskii*, using 63 RFLPs and five isozyme markers to flank intervals of approximately 20 centimorgans covering 95% of the tomato genome. The analyses revealed six QTLs for fruit mass, four QTLs for soluble solids and five QTLs for fruit pH accounting for 58%, 44%

and 48% of the genetic variances of these traits. Further analysis of three of the markers associated with soluble solids revealed that the effect of one of them was dependent upon genetic background (Tanksley and Hewitt, 1988). This result emphasizes the necessity of a cautious approach to marker assisted selection in applied breeding programmes and indicates the importance of evaluating markers for QTLs in the proposed genetic milieu of application. A similar study was performed on sixty progeny-tested F_2 plants from a cross between two soybean species, *Glycine gracilis* and *Glycine soja*, utilizing 150 RFLP differences to cover 1200 centimorgans of the genome (Keim et al., 1990). From these analyses, one to five markers with significant effects, *i.e.* accounting for 16% to 24% of the phenotypic variance, were identified for each of eight reproductive and morphological traits. Only one of the traits scored was found to have no associated significant markers in this test. Extensive RFLP maps for the potato, *Solanum tuberosum L.* (Bonierbale et al., 1988), lettuce, *Lactuca sativa L.* (Landry et al., 1987), maize, *Zea mays L.* (Helentjaris, 1987), and rice, *Oryza sativa* (McCouch et al., 1988) make these species available for QTL mapping by this method. However, no livestock genomes have, as yet, been so extensively mapped and practical considerations may limit the feasibility of generating the requisite test populations for QTL mapping with complete RFLP maps.

A preliminary study using a limited set of random genetic markers, namely DNA fingerprint banding patterns observed after restriction of genomic DNA with HinfI and hybridization with Jeffreys' minisatellite probe 33.6 was carried out on chickens by Dunnington et al (1990).

Patterns were compared for two lines of White Plymouth Rock chickens which had been divergently selected for eight-week body weight for 31 generations. Significant banding pattern differences characterized the two lines: 48% of the bands were line specific, within-line bandsharing was 0.50 while between-line bandsharing was only 0.22. Further research in this area is currently under way to better define the associations between the DNA fingerprints and QTLs for eight-week body weight in these lines.

III.B.MARKER ASSISTED SELECTION WITH INDIVIDUAL MARKERS

In attempting to identify QTLs for quantitative traits in livestock it has been necessary to focus on a few known genetic systems. Lander and Botstein (1989) cited some of the problems associated with evaluating QTLs using one or a few genetic markers rather than a complete collection of RFLPs spanning the entire genome. These problems include the systematic underestimation of the phenotypic effects of QTLs and lack of resolution in locating QTLs because distant linkages are indistinguishable from small phenotypic effects. They also noted that a larger number of progeny is required for detection of QTLs by linked markers if only one or a few isolated markers are employed. Prior to the development of the complete RFLP maps utilized in the plant research, Geldermann (1975) suggested the simultaneous testing of a number of genetic markers to: (1) determine the specific effects of marked chromosome sections; (2) define the relative importance of two homologous chromosome sections; (3) assess the effects of combinations

of homologues or different chromosome sections; and (4) evaluate a number of chromosomes bearing QTLs of different efficiency, their distribution in the genome and frequencies in the population. The objectives cited by Geldermann (1975) can, in fact, be fulfilled by examining a relatively small number of genetic markers . Moreover, by focusing on polymorphisms for macromolecular substances known to be involved in the expression of the quantitative character or those already shown to exercise other influences on the quantitative character, it is possible that these selected genes will affect a polygenic character to a greater extent than the average of the remaining loci (Geldermann, 1976; Peterson et al., 1982).

I have chosen to focus on candidate genes rather than utilizing random molecular marker loci which are rarely expected to have a major influence on characters of economic importance themselves (Soller and Genizi, 1978; Lande and Thompson, 1990). This approach should minimize the reliance of marker assisted selection on linkage disequilibrium between marker loci and QTLs which is continually eroded by recombination. The reliance on linkage disequilibrium is minimized because the candidate genes define the markers employed and, thus, linkage between the marker and the QTL is maximized. Moreover, the identification of candidate genes which affect quantitative traits could provide a crucial link in the development of genetic models for quantitative traits which would ultimately define relationships between genes, physiology, environment and phenotype. It is expected that, for most quantitative characters, a large proportion of the variance can be

attributed to the effects of a relatively small number of QTLs (Kluge and Geldermann, 1982; Hanset, 1982) or clusters of loci acting as supergenes (Thoday, 1961; Lewontin, 1973). Lande and Thompson (1990) also indicated that the distribution of additive genetic variances contributed by QTLs may be approximated by a geometric series, so that a hierarchy of the magnitude of effects on the expression of quantitative characters exists.

III.B.1. EXPERIMENTAL RESULTS: MHC

Much of the research on markers for QTLs in livestock has involved testing for associations between the major histocompatibility complex (MHC) locus and various traits. This system has been widely used because of the availability of a variety of well-characterized, easily identified markers. The MHC locus in the cow is extremely polymorphic and the identification of many bovine lymphocyte antigens (BoLA) encoded by MHC genes has been established and standardized through international efforts (Newman et al., 1982). Predictably, much of this research has examined associations between MHC and disease resistance traits. Significant associations between MHC alleles and disease in man have previously been well documented (van Rood et al., 1981). Disease resistance in livestock has come to be of increased importance to the animal breeder. The reasons for this include the high costs of disease treatment and lost production, intensive housing systems which promote the spread of infectious disease, decreased heterozygosity in many livestock breeds and the association of disease resistance with general

vigour or health status of the animal. Since most forms of disease resistance are of low heritability (*i.e.* $h^2<0.20$), these traits are well suited to an approach utilizing marker assisted selection.

Mastitis is one of the most costly afflictions in the dairy industry and has, therefore, received much attention from animal scientists. The major focus for research on controlling the incidence of mastitis has been directed towards identifying the environmental factors that contribute to it, since it is known that the incidence of mastitis is a reflection of the hygienic conditions of dairy operations. There is, however, also a heritable component in susceptibility to mastitis. Although the relatively low heritability of resistance to mastitis makes it a poor candidate for direct selection, it also renders the trait amenable to indirect or marker assisted selection. The requirement for exposure to infectious organisms, which act as causative agents in mastitis, in order to directly evaluate the trait gives an additional advantage to indirect or marker assisted selection for mastitis resistance. A number of studies have been carried out to investigate possible associations between BoLA class I alleles and mastitis or mastitis-related traits such as somatic cell counts. Somatic cell counts are typically elevated in milk samples from infected animals and might be indicative of latent infection (Schultz, 1977). These studies revealed significant associations between some BoLA class I alleles and incidence of mastitis (Spooner et al., 1988) or somatic cell counts (Ostergard et al., 1989; Oddgeirsson et al., 1989). It should be noted that none of the alleles with significant effects on somatic cell counts

or mastitis was common between these studies and, furthermore, Ostergard et al (1989) reported significant breed effects in their results. Differences in MHC allele frequencies between various populations studied may be a major factor in determining which alleles are associated with significant effects on these traits. Another study examined the relationship between MHC and mastitis susceptibility using RFLPs of the BoLA class II locus (Lunden et al., 1990). The results of this study showed that one of the haplotypes examined in 196 Swedish Red and White bulls was associated with breeding value for susceptibility to clinical mastitis, accounting for 2.2% of the variance in the bull breeding value.

Relationships between MHC and other disease conditions which affect general vigour have also been examined. Associations between BoLA class I alleles and susceptibility to bovine leukemia virus, development of virus-dependent lymphocytosis and lymphosarcoma incidence were shown in analyses by Stear et al (1988a) and Lewin (1988). Resistance to parasites in 247 Zebu cross cattle (Hetzel et al., 1989) and in 132 Africander-Hereford cross cattle (Stear et al., 1988b) was also found to be associated with the presence of some BoLA class I alleles. As reported for the studies on mastitis resistance, the identity of effective alleles again depended upon the test population analyzed. Relationships between MHC loci and disease resistance have also been examined in some other species. Ruff and Lazary (1988) found MHC class I alleles associated with predisposition to sarcoid development in horses and susceptibility to virus-induced arthritis in goats.

It has been suggested that allelic MHC molecules differ in their influence on the immune response and that this, in turn, is reflected in differences in resistance to pathogens (Klein, 1986). Since natural selection appears to act to maintain polymorphism of MHC molecules (Andersson et al., 1987), interactions between the many MHC alleles may be of importance. Thus, the influence of any particular allele on disease resistance will be a function of the allele frequencies in the population

MHC alleles have also been studied in conjunction with production traits in cattle. Batna et al (1989) found significant effects of BoLA haplotypes on body weight at 350 days and at first calving, age at first heat and milk and protein yield in 179 cows. Birth weight, preweaning gain and postweaning gain were found to be affected by the substitution of some BoLA class I antigens in 739 cattle of nine breeds analyzed by Stear et al (1989). In a study of carcass characteristics, Beever et al (1990) found that only rib-eye area was significantly associated with MHC haplotype. The results of these and other studies have consistently shown associations between BoLA alleles and various traits in cattle in conjunction with inconsistency in the specific alleles involved. This suggests that either the BoLA alleles are linked to QTLs affecting a number of traits or that pleiotropic effects of BoLA alleles on phenotype are heavily influenced by genetic background.

Similar results have been reported for associations between RFLPs of swine lymphocyte antigens (SLA) class I genes and production traits in Hampshire and Duroc boars. In one study (Jung et al., 1989),

significant effects of SLA-I haplotypes on average daily gain, backfat thickness, age at 104 kg, selection index and loin muscle area were identified. MHC haplotypes were not found to have any significant effects on production traits in a study of 942 Large White and Swiss Landrace sows, although some alleles were observed to have significant effects on reproduction traits (Gautschi and Gaillard, 1990). Interestingly, this study revealed that identity of MHC haplotypes between boar and sow significantly depressed litter sizes at birth and at weaning for both breeds. This observation indicates the importance of heterozygosity at MHC loci to viability which might be utilized for selection of breeding pairs in swine production systems.

In general, the results of these studies on the relationships between MHC and various traits suggest that much information on metabolic function affecting overall fitness and productivity might be gleaned from further research on MHC loci. The significant associations between MHC alleles and such a wide variety of traits is consistent with the proposition that natural selection acts to maintain polymorphism of MHC molecules which appear to affect the general health status of the animal. However, the importance of polymorphism of MHC loci may hinder its potential use as a parameter for selection, since the effect of any given allele may depend on which other alleles are present. Thus, the identification of desirable alleles will vary between populations with different allele frequencies and will also change as selection alters the corresponding allele frequencies. MHC markers might best be used for selecting breeding pairs to promote heterozygosity or to obtain

particularly favourable combinations of alleles in an applied breeding programme.

III.B.2. EXPERIMENTAL RESULTS: OTHER POLYMORPHISMS AND PRODUCTION TRAITS

Other molecular markers which have been tested for relationships with production traits consist mostly of erythrocyte and serum protein polymorphisms. The possibility of marker assisted selection has been most widely explored by dairy cattle breeders whose interest stems from the sex-limited nature of the major production traits and the extensive time taken to obtain production records. Geldermann (1975) examined marked chromosome sections of 1457 German Fresian cattle comprising three sire groups. In two of the sire groups, markers significantly correlated with milk production traits were found. Effective chromosome sections were identified by three blood group systems, RBC-Z, RBC-C and RBC-S, as well as postalbumin, amylase, ß-lactoglobulin and α_{s1}-casein. It should be noted that markers showing significant effects differed between sire groups and no markers were found to have significant effects in the third sire group. The highest correlations in the study were between milk production traits and chromosome sections marked by milk protein constituents. Possible effects of milk protein variants on milk production traits are of particular interest since some of these variants have been found to affect the technological properties of milk (Schaar et al., 1985). Thus, milk protein variants could be utilized both as a selection goal for improving the processability of milk and as

indicator traits for milk yield. Correlations between ß-casein and ß-lactoglobulin genotypes and milk production traits of dairy cattle were found by Bech and Kristiansen (1990) and Ng-Kwai-Hang et al (1984). Further studies with blood group systems in dairy cattle showed RBC-F alleles to be associated with milk and fat yield and RBC-B alleles with fat percentage in an extensive 18 year trial covering 3107 lactations (Brum et al., 1967). Blood group markers have long been utilized because they can be easily typed; the associations which have been found, however, seem to be more likey due to the effects of linked genes than to pleiotropic effects of the marker genes. The study by Brum et al (1967) also revealed an association between transferrin alleles and milk and fat yield. Brown et al (1989), in a study of the effects of cytoplasmic DNA on milk production traits, found that decreased ($P<0.001$) milk fat percentage was associated with the presence of a HpaII restriction site at nucleotide 360 of mitochondrial DNA.

Many of the markers examined in conjunction with milk production traits in dairy cattle have also been examined in other species. Milk protein variants were used in a study examining the effects of genotype at the ß-lactoglobulin and α_{s1}-casein loci in 207 Sardinian ewes showed that the former had a significant effect on total milk, fat and protein yield while the latter influenced fat and protein percentages (Bolla et al., 1989). Genotypes at the transferrin locus in sheep also influenced milk fat percentage in 222 Sardinian ewes but there were no effects on other milk traits (Rizzi et al., 1989). It has been suggested that the associations between milk protein variants and milk production traits

may be causative with the milk protein genes acting as QTLs for milk production (Geldermann, 1975). Associations of erythrocyte and serum protein polymorphisms with production traits have also been examined. A study of 146 Aberdeen-Angus half sibs scored for various serum proteins showed no association between beef production traits and RBC-C, RBC-FV, transferrin or group specific component whereas RBC-B genotypes were associated with 205 day weight, 365 day weight, preweaning average daily gain and fat thickness (Beever et al., 1989).

III.B.3. EXPERIMENTAL RESULTS: LABORATORY MODELS

Extensive studies on associations between markers for QTLs and various quantitative traits can be quite easily carried out on laboratory animals such as mice with minimal expense and time. Conservation of linkage groups between species and common effects of basic metabolism on the expression of quantitative traits suggest that results found with studies on experimental animals might be applicable to livestock species. Such studies may be particularly useful if the markers examined are QTLs rather than linked markers. Kluge and Geldermann (1982) examined the effects of four marked chromosome sections on body length, body weight, dry weight, dry matter, fat weight and fat content of 2321 backcross generation mice. The markers used in this study were not expected to directly influence any of the traits examined, therefore any significant associations found would be indicative of linkage between these markers and QTLs. Three of the marked chromosome sections had significant effects on traits examined.

Notable differences between traits influenced in males and females suggest that hormonal interactions with QTLs influence the phenotypic expression of these traits. Differential effects on males and females indicate that RFLPs may be of more value than metabolic parameters in assessing males for breeding female-expressed production traits. The results of the study by Kluge and Geldermann (1982) indicated that a large proportion of the genetic variation of some quantitative traits might be under the influence of a few chromosomal sections.

IV. THE SOMATOTROPIC AXIS

In this study, I have focused on the relationship between RFLPs for genes known to be involved in regulating growth and body weight of mice. This system was chosen because many of the proteins involved in growth regulation have been identified and their genes cloned. I wished to examine markers for genes which might potentially act as QTLs themselves. The identification of such QTLs could then be extended to similar studies in livestock. Extensive research in this area has also revealed much information concerning the mechanisms by which many of these growth factors act (reviewed in Spencer, 1985). Furthermore, the studies previously cited which have shown associations between metabolic parameters of somatotropins and production traits suggest that polymorphisms of these genes may influence genetic potential for production traits.

The best characterized of the polypeptide growth factors is pituitary growth hormone (GH) or somatotropin which is involved in the regulation of postnatal somatic growth (Albertsson-Wikland and Isaksson, 1976). GH levels are regulated by two hypothalamic hormones: growth hormone releasing factor (GRF) and somatostatin (SRIF) which, respectively, induce and depress secretion of GH. The growth promoting effects of GH are thought to be mediated, for the most part, by peptide growth factors or somatomedins such as insulin-like growth factors 1 (IGF-1) and 2 (IGF-2). The GH-dependent somatomedins are powerful mitogenic agents and might be considered the ultimate hormones in the somatotropic axis (Spencer, 1985). Insulin is involved in mediating the interaction between GH and the somatomedins through regulation of the GH receptors which act to stimulate production of the somatomedins (Rosen, 1987). Thyroid hormone (TH), in turn, mediates the mitogenic effects of somatomedins through its role as a regulator of somatomedin receptors on target cells (Froesch et al., 1976). TH secretion, like GH secretion, is regulated in part by somatostatin and levels of TH also influence GH synthesis and secretion (Samuels et al., 1979). The interactions between the known growth factors, other hormones and environmental influences are complex and much remains to be clarified concerning their roles in the regulation of growth. However, for my purposes, the identification of numerous growth factors and their receptors and the availability of the cloned genes is of the most relevance.

The mouse GH gene is a single copy gene located on chromosome 11 (Jackson-Grusby et al., 1988) within a 3.2 kb region of DNA (Parks et

al., 1982) which contains both the mRNA coding regions and four introns. Mouse GH is translated as a 216 amino acid precursor from which the leader sequence is cleaved to yield a mature protein of 190 amino acids (Linzer and Talamantes, 1985). GRF is a 44 amino acid peptide (Speiss et al., 1983) secreted episodically by the hypothalamus causing induction of GH secretion via an increase in cAMP in the pituitary (Bilezikjian and Vale, 1983). Similarly, the inhibitory effect of SRIF on GH secretion is mediated through a depression of pituitary cAMP (Bilezikjian and Vale, 1983). Hypothalamic SRIF is found in two biologically active forms of 14 and 28 amino acids produced by differential processing of the 116 amino acid translation product, preprosomatostatin (Funckes et al., 1983). The single copy SRIF gene is located on chromosome 16. The somatomedins, IGF-1 and IGF-2, are also single copy genes which encode mature peptides of 70 and 67 amino acids, respectively (Tricoli et al., 1984). The chromosomal location of IGF-1 in the mouse is not known although IGF-1 is known to be non-syntenic with IGF-2 in humans (Brissenden et al., 1984). Mouse IGF-2 has been mapped to chromosome 17. The genomic sequence of IGF-2 has been well-characterized in the rat, where it was found to span 12kb of DNA (Frunzio et al., 1986); this large genomic area suggests that many polymorphic sites might be expected within this gene.

V. EXPERIMENTAL DESIGN

The purpose of this study was to determine if polymorphisms at somatotropic gene loci might function as QTLs for growth traits.

The experiment was carried out in three stages using nine lines of mice from three strains. Four of these lines had previously been subjected to long term selection for high 42 day weight and five were unselected control lines. Firstly, I examined high 42-day weight selected and unselected control lines of mice for RFLPs at somatotropic gene loci. RFLPs which differed in frequency between selected and unselected lines or which were present in only one of the lines were then proposed as potential markers associated with the differences between the lines. Secondly, the segregation of these RFLPs and growth traits in F_2 populations derived from crosses between selected and unselected lines was analyzed to determine if there were any significant associations between the RFLPs and growth parameters in the mixed populations. Lastly, divergently selected lines of mice were generated from the F_2 populations. RFLP frequencies in the selected lines were then assessed to determine if selection for high or low 42-day weight from the mixed populations had any concomitant effect on the frequency of these RFLPs.

This approach was taken to determine if RFLPs which differed in frequency between high 42-day weight selected lines and unselected lines would be associated with high 42-day weight in a mixed genetic background. It also provided the opportunity to assess the effects of RFLPs for somatotropic genes which may have spontaneously arisen in any one of the lines or which may have been lost in certain lines either as the result of selection or due to genetic drift. Furthermore, having established the frequencies of these RFLPs in the F_2 populations and

their effects on the F_2 growth traits we examined how these parameters were affected by divergent selection for 42-day body weight.

The hypothesis proposed was that haplotypes present in selected lines at higher frequencies than in control lines might have a positive additive effect on 42-day weight. Such haplotypes are expected to segregate with higher 42-day weight in F_2 populations. Furthermore, I postulated that the frequency of haplotypes with a positive additive effect on 42-day weight would increase in lines selected for high 42-day weight and decrease in lines selected for low 42-day weight.

MATERIALS AND METHODS

I. EXPERIMENTAL POPULATIONS

All mice were housed in a room maintained at 22°C with 50% relative humidity. Mice were kept in 8-inch by 10-inch shoebox cages with wire tops and deep-drawn feeders. A maintenance feed of 4% fat (Teklad Premier, Wayne Laboratory Animal Diets) was supplied *ad libitum* and diets of breeding females were supplemented with feed of 10% fat (Rodent Blox, Wayne Laboratory Animal Diets) until litters were weaned.

I.A. STOCK 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 obtained from Lacombe were originally designed to compare the effects of inbreeding and selection in two sizes of populations (Sumner, 1974). The origins of these lines were three strains developed from various sources as follows: Strain 2 was an amalgam of four outbred strains, Q, NB, LX and JH, imported from the Institute of Animal Genetics, Edinburgh, Scotland in 1961; Strain 3 was developed from intercrossing four inbred strains, C57BR/cd, A, DBA and RF, acquired from Jackson Laboratories, Bar Harbor, Maine in 1962; and Strain 5 was bred directly from the Q strain which also contributed to Strain 2.

Foundation Population (FP) lines were established for each strain. The FP lines were maintained with 25 single pair matings for each generation with all breeders replaced by first parity offspring. A

rotational mating plan (Farid-Naeini, 1986) was utilized to minimize inbreeding. FP lines for strains 2, 3, and 5 were acquired by the University of Alberta at generations 96, 96, and 99, respectively. These lines have been designated 2FP, 3FP, and 5FP.

Selected and control lines were derived from generation 8 of each FP line. These were established and maintained with fifteen matings per generation utilizing a hierarchical mating scheme, mating each of five males with three females. Successive generations were propagated by replacing each breeding male and female with one of their first parity offspring. Inbreeding was kept to a minimum by utilizing a rotational mating plan (Farid-Naeini, 1986). For the selected lines, replacement breeders were chosen on the basis of highest 42-day weight. These three lines, designated 2H, 3H, and 5H underwent selection for 69, 64, and 72 generations, respectively. An additional line, 3Ha, is a replicate of 3H. Control lines, 2C, 3C, and 5C, were established and maintained in the same manner as the selected lines, the only difference being that replacement breeders were chosen at random from sire and dam families. Following relaxation of selection, experimental lines were maintained with twelve single pair matings per generation. Replacements for each generation were taken at random from first parity progeny. All experimental lines were acquired in the 26th or 27th generation of random mating.

All lines except 2C were maintained for fifteen generations with twelve single pair matings per generation. The 2C line was lost due to high infertility and postnatal losses. Litters were culled at weaning.

Three males and three females were randomly selected from each family where possible. Obviously sick or unfit animals were culled. Breeding replacements were taken at random from these progeny groups and a rotational mating scheme was employed. Data on time from mating to parturition, infertility and litter sizes at weaning were recorded for all fifteen generations. Weights at 21 and 42 days of age were recorded for generations 10 through 15.

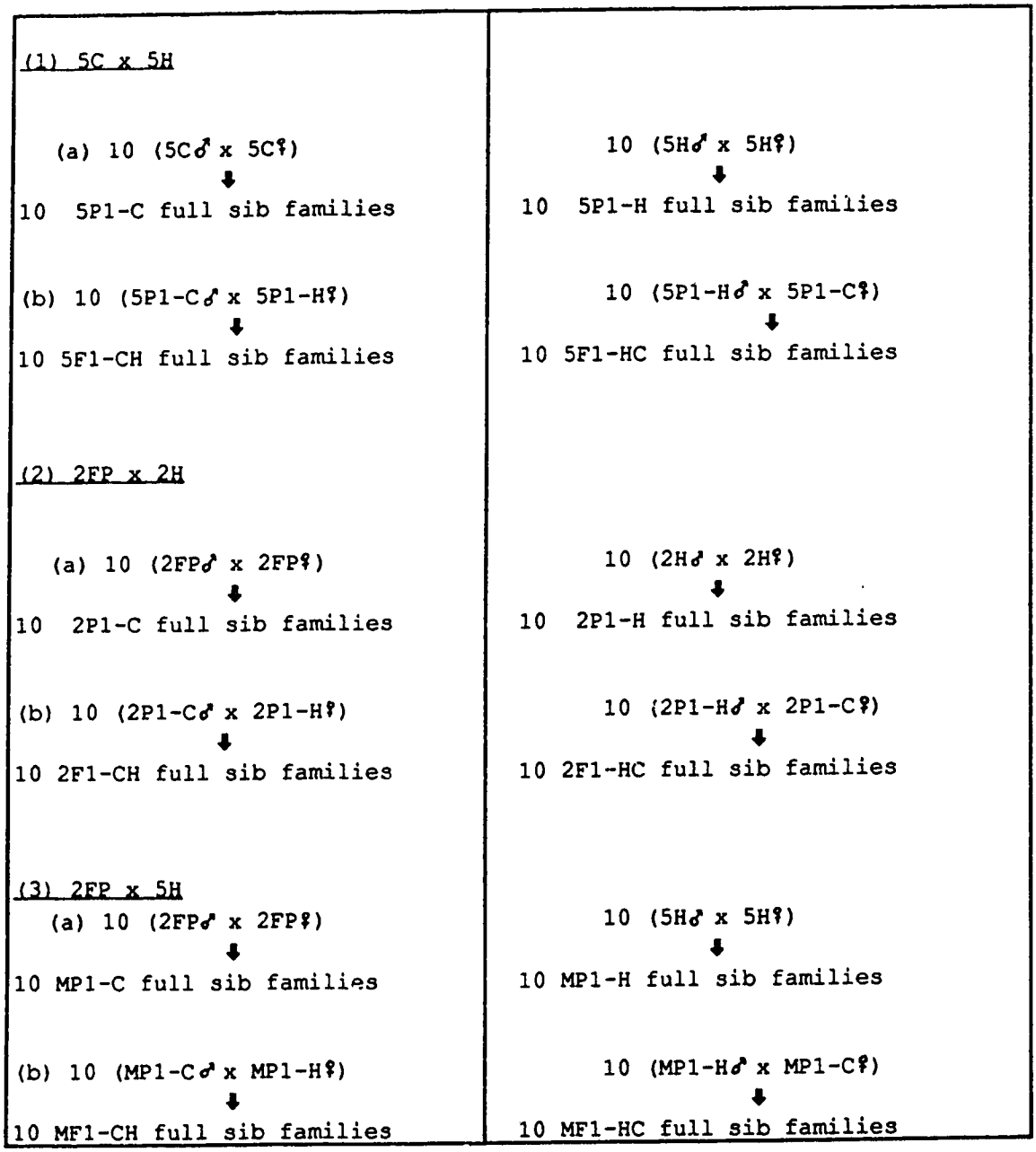
I.B. F_2 POPULATIONS

The first F_2 population developed for this study, $3F_2$, was derived from crosses between 3FP and 3H (L. Querengesser, 1986. personal communication). Eight $3F_1$ families were obtained by mating one 3FP male with four 3H females and, reciprocally, two 3H males were each mated with two 3FP females. Twenty-eight single pair matings between the $3F_1$ generation progeny yielded 263 $3F_2$ individuals. Matings between full sibs were avoided throughout the breeding programme. Weights were recorded for all $3F_1$ and $3F_2$ mice at weaning, between 19 and 24 days of age, and every three days thereafter for a minimum of four weeks. Litter sizes at weaning were recorded for all matings.

Three additional F_2 populations generated in this study incorporated a broader genetic base to remove the confounding effects of genetic drift which may have been present in $3F_2$. Also, litters were culled to five progeny seven days after parturition in an attempt to remove the effects of litter size on 42-day weight. Litter sizes at seven days were recorded for all matings. Individual mice were weighed

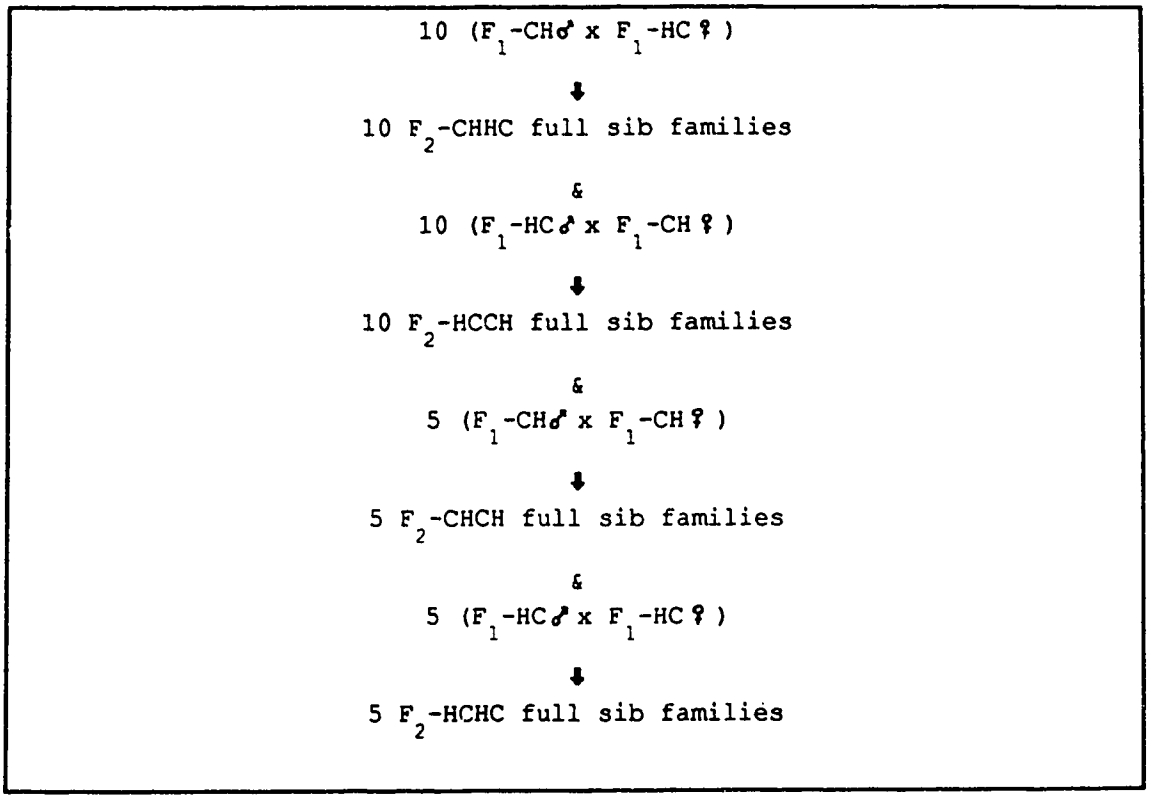
at 21, 28, 35, and 42 days of age. These populations were obtained from crosses between (1) 5C and 5H, (2) 2FP and 2H, and (3) 2FP and 5H. The resulting F_2 populations were designated $5F_2$, $2F_2$, and MF_2, respectively. The crosses were made using lines which were identified as polymorphic for IGF-2 and GRF so that polymorphisms for both of these markers in addition to GH were present in all of the F_2 generations. A single mating design was used for all populations. Reciprocal crosses were incorporated into the design to test for maternal effects. Ten single pair matings were made from the original lines to generate ten full-sib P_1 families for each set. The F_1 populations were each composed of twenty full sib families obtained from reciprocal crosses between P_1 lines. Thus, ten of the F_1 families, designated CH, were the produced by the mating of ten C or FP males, each with a single H female; the remaining ten F_1 families, designated HC, were produced by the mating of ten H males, each with a single C or FP female (Figure 1). Crosses within each F_1 population were made to generate 30 full sib F_2 families from single pair matings. All possible combinations between CH and HC mice from the F_1 populations were made. Ten CH males mated with ten HC females yielded CHHC families, ten HC males mated with ten CH females yielded HCCH families, five HC males mated with five HC females produced HCHC families, and five CH males mated with five CH females gave CHCH families (Figure 2). No matings were made between full sibs. This mating plan yielded a potential 150 F_2 progeny for each set with recorded weights for parents (F_1) and grandparents (P_1).

FIGURE 1. Mating scheme used to generate experimental F_1 populations consisting of 20 full sib families from crosses between selected and unselected base population lines.



Each full sib family was comprised of five progeny after culling at seven days of age.

FIGURE 2. General mating scheme used to generate experimental F_2 populations consisting of 30 full sib families from F_1 populations.



This mating scheme was used to generate the three F_2 populations: $5F_2$, $2F_2$, and MF_2 from their corresponding F_1 populations.

Each full sib family was comprised of five offspring after culling at seven days of age.

I.C. LINES DEVELOPED BY DIVERGENT SELECTION FROM F_2 POPULATIONS

Fifteen randomly selected $5F_2$ males were mated with fifteen $5F_2$ females to produce a $5F_3$ population. Divergent selection for high and low 42-day weight was commenced on $5F_3$, $2F_2$, and MF_2 populations, creating six new selected sublines. Sublines 5Hi and 5Lo, from $5F_3$ were selected for nine generations; six generations of selection were carried out on 2Hi and 2Lo, from $2F_2$, as well as on MHi and MLo from MF_2. Selected sublines consisted of from seven to thirteen families in each generation. Variations in the number of families between generations resulted from relatively high infertility encountered in some of the sublines.

The first five generations of selection in 5Hi and 5Lo and the first three generations of selection in the other sublines were carried out using single pair matings and selecting breeding replacements for each male and female from their progeny. The remaining generations incorporated a hierarchical mating scheme with each male mated to two females. Replacement breeders for each female were selected from their progeny. The required number of males were selected from all of the families, 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 dams for the next generation.

II. IDENTIFICATION OF RFLPs FOR SOMATOTROPIC GENES

II.A. EXTRACTION OF GENOMIC DNA

Genomic DNA samples were prepared from mice randomly sampled from each of the selected, control and foundation population lines. The mice were sacrificed by cervical dislocation and livers were dissected out. Each liver was placed in a labeled tube and immediately frozen in liquid nitrogen. Frozen livers were kept at -40°C until used.

Tissue samples weighing 200 to 300 mg (approximately 1/4 of the whole liver) were ground in liquid nitrogen to the consistency of a fine powder with a chilled mortar and pestle. The ground livers were placed in 5 to 6 ml lysis buffer (0.5 M EDTA, pH8.0; 100 µg/ml proteinase K; 0.5% Sarcosyl) and incubated in a 50°C water bath for a minimum of three hours. Following lysis, DNA was phenol extracted by adding an equal volume of phenol to the lysis mixture. Tubes were then gently rotated at 4°C for at least 2 hours, centrifuged for 15 min at 4000 rpm and the aqueous upper phase was removed to a clean tube. Two phenol-chloroform extractions and one chloroform extraction of the DNA was then done to remove all protein. The total volume of the final aqueous phase was brought to 15 ml with the addition of 1 mM Tris-Cl, pH 7.6. The DNA was ethanol precipitated with two volumes of cold 95% ethanol after the addition of 1 ml 3 M sodium acetate, pH 7.0. The precipitate was rinsed twice with cold 70% ethanol and vacuum-dried. DNA was suspended in 1.5 ml deionized-distilled water and dialyzed against TE buffer (10 mM Tris-Cl, pH 8.0; 1 mM EDTA) overnight. After dialysis, DNA solutions were

concentrated by precipitation with 95% ethanol, washed in 70% ethanol and suspended in 1.5 to 2.0 ml deionized-distilled water.

Ten µl aliquots of each DNA solution were run in 0.7% agarose gels at 30 to 40 V for two hours to assess relative concentrations and to assure an absence of degradation. Typically, the DNA formed a single broad band with a molecular weight slightly greater than 50 kb.

II.B SOUTHERN BLOTS

Genomic DNA from the stock populations was tested for restriction fragment length polymorphisms of growth hormone releasing factor (GRF), insulin-like growth factor 1 (IGF-1), insulin-like growth factor 2 (IGF-2) and somatostatin (SRIF). Aliquots of genomic DNA preparations containing approximately 10 µg DNA were digested with various restriction enzymes according to the suppliers' directions (BRL or Boehringer-Mannheim). Samples which proved refractory to digestion were replaced and digestions were carried out with the addition of 5 mM spermidine. Digested DNA samples were fractionated by electrophoresis on 0.7% to 0.8% agarose gels and transferred to Gene Screen Plus (NEN) membranes using the manufacturer's protocol.

^{32}P-oligolabeled probes were prepared from cDNA clones of rat GH (rGH), rat GRF (rGRF), human IGF-1 (hIGF-1), human IGF-2 (hIGF-2) and human SRIF (hSRIF) as described by Feinberg and Vogelstein (1983). The clones used in this study are described in Table 1. Labeled probes were used individually for hybridization with the membranes prepared as

described above at a concentration of approximately 10 ng/ml (approximately 10^6 cpm/ml) in 50% formamide, 1% SDS, 1M NaCl and 10% dextran sulfate overnight. All hybridizations except that with rGRF were carried out at 42°C; hybridizations with rGRF were carried out at 30°C. Following hybridization, membranes were washed twice in 2XSSC at room temperature for 5 minutes, twice in 2XSSC, 1% SDS at 65°C for 30 minutes and twice in 0.1XSSC at room temperature. Membranes were then exposed to Kodak XAR film overnight at -40°C or -70°C and restriction fragment patterns were assessed from these films. Between hybridizations with different probes, membranes were stripped of labeled probe by boiling in 0.1XSSC, 1% SDS for 30 to 60 minutes.

Those probes which were found to yield polymorphic restriction patterns were subsequently used for hybridization with Southern blots prepared from genomic DNA extracted from the F_2 populations and selected sublines. Southern blots prepared from the F_2 populations and selected sublines were also probed with rGH.

TABLE 1. Description and source of the cDNA probes used to test for RFLP's and determine restriction fragment patterns in experimental mouse populations.

PLASMID	VECTOR	HOST	SIZE	SOURCE	REFERENCE
prGH-1	pBR322	HB101	800	Seeburg[1]	Seeburg et al 1977
prGH-1B*	Blue-scribe	DH5A	800	subcloned from prGH-1	
prGRF-2	pUC-8	JM83	350	Kelly Mayo[2]	Mayo et al 1983
phIGF-1B	pGEM-1	JM83	1130	Peter Rotwein[3]	Rotwein 1986
phIGF-2	pKT·218	HB101	660	Graeme I. Bell[4]	Bell et al 1984
phSRIF	pBR322	JM83	239	W.J. Rutter[5]	Shen et al 1982

sizes given are in bp.

*plasmid used in this study

[1] Metabolic Research Unit, University of California, San Francisco

[2] Salk Institute for Biological Studies, San Diego

[3] Washington University School of Medicine

[4] Howard Hughes Medical Institute Research Laboratories, Chicago

[5] Hormone Research Institute, University of California, San Francisco

III. METHODS OF ANALYSIS

III.A. ANALYSIS OF STOCK POPULATION LINES

Data obtained from stock population lines were analyzed to ascertain if differences in fertility, fecundity, 21-day body weight and 42-day body weight were present between these lines. All data were tested for skewness and kurtosis as described by Snedecor and Cochran (1982). Two measures of fertility were included in the analysis, namely, the percent of matings producing progeny and the number of days which elapsed from time of first mating until parturition. Fecundity was measured as the number of offspring weaned at 21 days per fertile mating. Analysis of variance was carried out on fertility and fecundity with Model 1 using the general linear models, GLM, procedure of SAS (1982). All components of the model were considered fixed. Variance components were estimated using Type III sums of squares which allows the estimation of each component with remaining effects taken into account and allows the estimation of interaction effects when empty cells are present in the data. Least squares means were calculated for each line within strain.

MODEL 1:

$$Y_{ijkl} = \mu + STR_i + LN(STR)_{j(i)} + GEN_k + STRGEN_{ik} + e_{ijkl}$$

where;

Y_{ijkl} is fertility (days) or fecundity (number of offspring) of each productive mating,

μ is the overall mean fertility or fecundity,

STR_i is the effect of the ith strain (i=1,2,3)

$LN(STR)_{j(i)}$ is the effect of the jth line within the ith strain (j=1,2,3,4),

GEN_k is the effect of the kth generation (k=1,2,...15), and

$STRGEN_{ik}$ is the effect of the interaction between the ith strain and the kth generation

e_{ijkl} is the residual error.

21-day and 42-day body weights were also analyzed for the stock population lines. These data were analyzed by Model 2 using the general linear models, GLM, procedure of SAS (1982) considering all components of the model fixed. Least squares means were calculated for each line within strain using Type III sums of squares. Postweaning growth rates for each line within strain were calculated as the difference between least squares means for 42 day weight and 21 day weight.

Model 2:

$$Y_{ijklmn} = \mu + STR_i + LN(STR)_{j(i)} + GEN_k + FEC_l + SX_m + STRGEN_{ik} + STRFEC_{il} + GENFEC_{kl} + e_{ijklmn}$$

where;

Y_{ijklm} is the 21-day or 42-day body weight (gm) of the individual,

μ is the overall mean 21-day or 42-day body weight,

STR_i is the effect of the ith strain (i=1,2,3),

$LN(STR)_{j(i)}$ is the effect of the jth line within the ith strain (j=1,2,3,4),

GEN_k is the effect of the kth generation (k=1,2,..5),

FEC_l is the effect of the lth litter size (l=1,2,..13),

SX_m is the effect of the jth sex (j=1,2)

$STRGEN_{ik}$ is the effect of the interaction between the ith strain and the kth generation

$STRFEC_{il}$ is the effect of the interaction between the ith strain and the lth litter size

$GENFEC_{kl}$ is the effect of the interaction between the kth generation and the lth litter size

e_{ijklmn} is the residual error.

Regression analysis of 21-day weight and 42-day weight on litter size at weaning was also carried out.

III.B. ANALYSIS OF EXPERIMENTAL POPULATIONS

The experimental populations generated from the crossing of 3C with 3H were analyzed separately from the other experimental populations because differences in the mating schemes used would have affected the extent of the genetic base. In all models, the analysis of 21 day and 42 day weights included the effects of sex and litter size. Analysis of F_1 populations also included the effect of cross to determine if there were differences between the reciprocal crosses due to maternal effects. Fecundity or litter size was also assessed in each generation of the experimental populations. However, for stock population lines and for populations $3F_1$ and $3F_2$, litter size at weaning was the trait recorded while for the other experimental populations the trait was litter size at seven days after parturition; therefore, the two traits are not directly comparable.

Responses to divergent selection for 42-day weight were assessed for each of the strains generated by Model 3 using the general linear models, GLM, procedure of SAS (1982) considering all components of the model to be fixed. Least squares means were calculated for 21-day and 42-day weight for each generation within a direction of selection using Type III sums of squares. Weights of selected sublines were individually compared with the source population to determine the response attained for each direction of selection. Additionally, pairwise comparisons between Hi and Lo sublines from each population for the last generation of selection were made to determine the divergence in weights attained with selection.

Model 3:

$$Y_{ijkl} = \mu + DIR_i + GEN(DIR)_{j(i)} + SX_k + DIRSX_{ik} + e_{ijkl}$$

where:

Y_{ijkl} is the 21-day or 42-day body weight (gm) of the individual,

μ is the overall mean 21-day or 42-day body weight,

DIR_i is the effect of the ith direction of selection (i=1,2),

$GEN(DIR)_{j(i)}$ is the effect of the jth generation within the ith direction (j=1,2..9) for 5Hi and 5Lo, (j=1,2..6) for 2Hi, 2Lo, MHi and MLo,

SX_k is the effect of the kth sex (k=1,2),

$DIRSX_{ik}$ is the effect of the interaction between the ith direction of selection and the kth sex, and

e_{ijkl} is the residual error.

The relationships between RFLPs and growth traits were assessed using two models. Firstly, raw 21-day weights, 42-day weights and postweaning growth rates from 21 to 42 days of age were analyzed. This analysis was carried out on each F_2 population and the model included family and sex effects. The family effect is expected to account for differences in parent weight and litter size. These data were analyzed by model 4 using the general linear models, GLM, procedure of SAS (1982) considering all effects in the model to be fixed. Least squares means were calculated for each genotypic class of each locus examined using Type III sums of squares.

Model 4:

$$Y_{ijkl} = \mu + FAM_i + SX_j + GENO_k + SXGENO_{jk} + e_{ijkl}$$

where:

Y_{ijkl} is the 21-day weight, 42-day weight, or postweaning growth rate, in gm, of the individual

μ is the overall mean for the trait analyzed

FAM_i is the effect of the ith family (i=1,2...30),

SX_j is the effect of the jth sex (j=1,2),

$GENO_k$ is the effect of the kth genotype (k=1,2,3),

$SXGENO_{jk}$ is the effect of the interaction between the jth sex and the kth genotype, and

e_{ijkl} is the residual error.

Twenty-one-day weights and 42-day weights measured as deviations from the midparent were the traits analyzed in the second model used for assessing the association between growth traits and RFLPs. Measures of

deviations from the midparent were tested because the original selection scheme was based on within family selection. Deviations from the midparent used rather than raw weights also gives an indication of how much of the variance accorded to family effects in the previous model is due to differences between parent weights of the families. These data were analyzed by Model 5 using the general linear models, GLM, procedure of SAS (1982) with all effects in the model considered as fixed effects. Least squares means were calculated for each genotypic class of each locus studied using Type III sums of squares.

Model 5:

$$Y_{ijkl} = \mu + SX_i + FEC_j + GENO_k + SXGENO_{ik} + e_{ijkl}$$

where:

Y_{ijkl} is the 21-day or 42-day weight, in gm, of the individual measured as the deviation from the midparent

μ is the overall mean 21-day or 42-day weight

SX_i is the effect of the ith sex (i=1,2),

FEC_j is the effect of the covariate, litter size at weaning or seven days,

$GENO_k$ is the kth genotypic class (k=1,2,3),

$SXGENO_{ik}$ is the effect of the interaction between the ith sex and the kth genotype, and

e_{ijkl} is the residual error.

The final analysis carried out on 21-day and 42-day weights of the F_2 populations was used to determine if interactions between the genotypes of the various loci examined could be detected. This analysis

was considered as preliminary since many of the cells in the interaction effects were empty, distribution between cells was markedly uneven and few data were available for each class of interaction. These data were analyzed using Model 6 with all effects in the model considered as fixed by the general linear models, GLM, procedure of SAS (1982).

Model 6:

$$Y_{ijklmn} = \mu + SX_i + FAM_j + GNGH_k + GNIGF_l + GNGRF_m + GNGHIGF_{kl} + GNGHGRF_{km} + GNIGFGRF_{lm} + e_{ijklmn}$$

where:

Y_{ijklmn} is the 21-day or 42-day weight, in gm, of the individual,

μ is the overall mean 21-day or 42-day weight,

SX_i is the effect of the ith sex (i=1,2)

FAM_j is the effect of the jth family (j=1,2,...30)

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

$GNIGF_l$ is the effect of the lth genotype at the IGF-2 locus (k=1,2,3)

$GNGRF_m$ is the effect of the mth genotype at the GRF locus (m=1,2),

$GNGHIGF_{kl}$ is the effect of the interaction between the kth genotype at the GH locus and the lth genotype at the IGF-2 locus,

$GNGHGRF_{km}$ is the effect of the interaction between the kth genotype at the GH locus and the mth genotype at the GRF locus,

$GNIGFGRF_{lm}$ is the effect of the interaction between the lth genotype at the IGF-2 locus and the mth genotype at the GRF locus, and

e_{ijklmn} is the residual error.

Average additive gene substitution effects on the growth traits were calculated for each RFLP of each gene which was found to have a significant effect on the trait. Least squares means of the genotypic classes for the traits analyzed were used. Calculations were made using Equation 1 following the method outlined by Falconer (1981).

Equation 1:

$$\alpha_1 = q [a + d (q-p)]$$

$$\alpha_2 = -p [a + d (q-p)]$$

where:

α_1 and α_2 are the additive effects of alleles 1 and 2,

p and q are the corresponding allele frequencies of alleles 1 and 2

a is the deviation of the homozygous classes from the midpoint of the two homozygous classes, and

d is the deviation of the heterozygous class from the midpoint of the two homozygous classes.

RESULTS AND DISCUSSION

I. STOCK POPULATION

Stock population lines from strains 2, 3, and 5 were first analyzed with respect to 42-day weight. Lines 2H, 3H, 3Ha, and 5H were developed by selection using 42-day weight as the criterion for selection. It was necessary to determine if the differences in 42-day weight between selected and unselected lines realized by selection were maintained after relaxation of selection. This was of relevance to our study since these differences constituted the basis of our experimental design and were assumed to be present at the commencement of the breeding programme. The data on weights were collected from generations 11 through 15 at the University of Alberta. Thus, the data was obtained from generations 37 through 41 of random mating following the termination of selection.

The distributions of 42-day weight for selected lines (2H, 3H, 3Ha, and 5H) and unselected lines (2FP, 3FP, 3C, 5FP, and 5C) are shown in Figures 3A and 3B, respectively. As can be seen in Figure 3, the distributions of 42-day weight were positively skewed; this effect was significant only in the unselected lines. The deviation from normalcy in the distribution of weights for the unselected lines was due mainly to the relatively high weights of line 2FP; removal of 2FP data from the analysis showed weights to be normally distributed.

FIGURE 3A. Distribution of 42-day weights of selected stock lines.

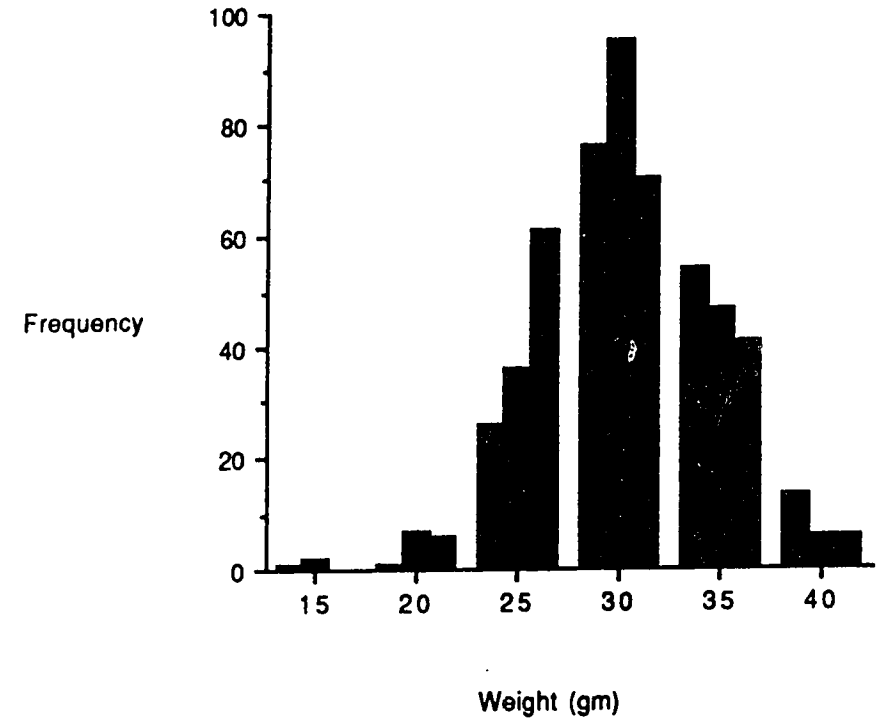
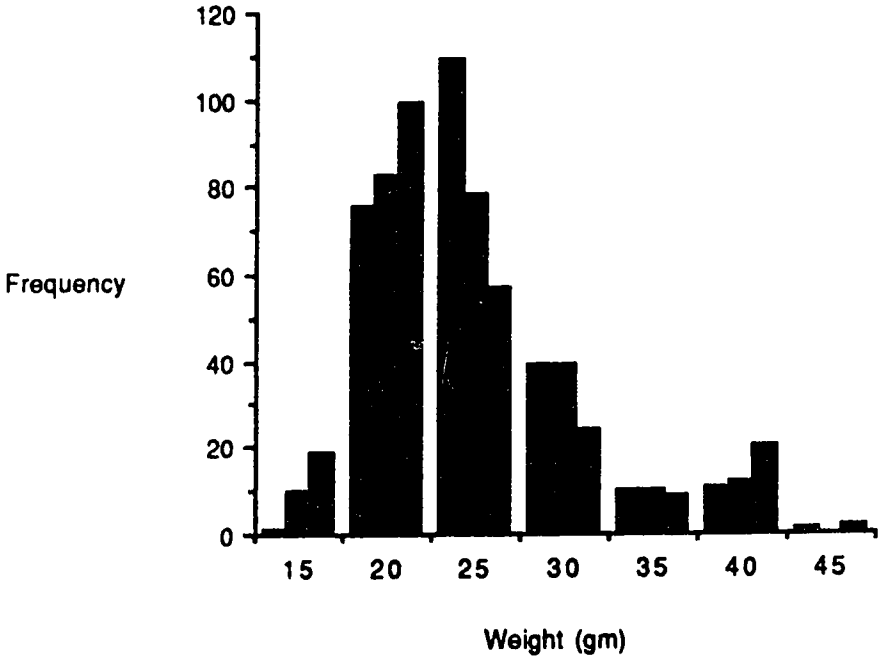


FIGURE 3B. Distribution of 42-day weights in unselected stock population lines.



The results of the analysis of variance of 42-day weight are shown in Table 2. The effects of interactions between components in the model were not significant ($P>0.05$) and were, therefore, omitted. These results revealed that sex, strain, line within strain, generation and litter size all contributed significantly ($P<0.01$) to the variance of 42-day weight. The model used accounted for 80% of the variance of 42-day weight. In accordance with the results of previous studies (*e.g.* Salmon et al., 1990; Eisen and Coffey, 1990), males were heavier than females at 42 days of age. Approximately 30% of the variance of 42-day weight was accounted for by the line within strain effect.

Mean 42-day weights of the stock population lines are given in Table 3. For strains 3 and 5, differences in 42-day weight between selected and unselected lines were still extant after 41 generations of random mating. The difference in 42-day weight between selected and unselected lines of both strains 3 and 5 was about 60% of that observed at the termination of selection. In strain 2, however, the mean 42-day weights of the selected and unselected lines were essentially the same and the mean 42-day weight of the unselected line 2FP was, in fact, greater than that of any of the other lines. This observation accounts for the effect of line 2FP on the distribution of 42-day weights in unselected lines previously observed. The differences in mean 42-day weight between 2FP and the selected lines were not significant ($P>0.05$), but this line was significantly ($P<0.01$) heavier than any of the other unselected lines. The comparatively high 42-day weight of 2FP may have been due, in part, to inadvertent selection for larger individuals which

would appear more vigorous. This was done in an attempt to salvage 2FP from the fate experienced by 2C which was lost due to high infertility and postnatal mortality.

The extensive use of 2FP in the propagation of the experimental populations was commenced prior to generation 11 when we began recording weights of mice in the stock lines. It was, therefore, unfortunately done without prior knowledge of the current status of the lines involved. The variances of 42-day [illegible] males tended to be somewhat higher than those of females [illegible] given line although these differences were not significant ([illegible]). Forty-two day weights of the selected lines for strains 3 and 5 were associated with higher ($P<0.05$) variances than those of the unselected lines. This observation suggests that the lines which had previously undergone selection retained a greater potential response to selection than the control lines which had been developed by random mating.

Regression analysis of 42-day weight on litter size at weaning yielded regression coefficients of -0.66±0.08 and -0.47±0.07 for males and females, respectively. Thus, it can be seen that the effects of litter size on body weight for litters which have not been culled are still present at 42 days of age, three weeks after weaning.

TABLE 2. Analysis of variance of 42-day weights (gm) of mice in stock population lines calculated from data collected on generations 11 through 15.

SOURCE OF VARIANCE	DF	SS	P
Strain	2	20015.33	0.0001
Line within strain	6	21874.42	0.0001
Generation	4	97.19	0.0001
Litter size	12	1014.56	0.0001
Sex	1	12452.86	0.0001
Residual error	2083	15440.30	

TABLE 3. Mean 42-day weights (gm) of stock population lines of mice calculated from data collected on generations 11 through 15.

		MALES		FEMALES	
STRAIN	LINE	N	WEIGHT	N	WEIGHT
2	FP	117	39.33 (3.10)	104	29.95 (2.97)
	H	120	36.17 (3.19)	110	29.71 (2.91)
3	FP	131	23.43 (2.09)	131	18.59 (1.54)
	C	132	24.31 (2.02)	141	19.53 (1.66)
	H	93	30.69 (4.25)	78	26.62 (3.16)
	Ha	109	32.77 (3.45)	102	28.56 (2.78)
5	FP	98	28.57 (2.57)	108	23.47 (2.04)
	C	142	27.79 (2.41)	127	23.12 (1.84)
	H	132	35.30 (3.10)	135	28.55 (2.73)

FP and C lines were unselected controls.

H and Ha lines were selected for high 42-day weight for 69 (Strain 2), 64 (Strain 3), and 72 (Strain 5) generations; these weights were collected from generations 37 through 41 of random mating following the termination of selection.

Numbers given in brackets are standard deviations

Weaning weights, measured at 21 days of age, were analyzed to determine if the higher 42-day weight of selected lines was the result of increased preweaning or postweaning growth rate. The distributions for 21-day weights of selected lines (2H, 3H, 3Ha, and 5H) and unselected lines (2FP, 3FP, 3C, 5FP, and 5H) are shown in Figures 4A and 4B, respectively. Twenty-one day weights of selected lines were normally distributed although there was some indication of kurtosis. The distribution of 21-day weights of unselected lines was positively skewed; as was found for the distribution of 42-day weights in these lines, this effect was due mainly to the 2FP line.

The analyses of variance of weaning weight revealed significant ($P<0.01$) effects of sex, strain, line within strain, generation, and litter size at weaning as shown in Table 4. The interactions tested in the model were not significant ($P>0.05$) and were, therefore, excluded. The model used accounted for 50% of the variance of weaning weight. Most of the variance of weaning weight in the stock population lines that was accounted for in the model was due to the strain effect. The only difference in 21-day weights between selected and unselected lines was found in strain 2, where both males and females of the selected line, 2H, were lighter ($P<0.01$) than those of 2FP. In the remaining strains, 3 and 5, there were essentially no differences in weaning weights of selected and unselected lines.

FIGURE 4A. Distribution of 21-day weights of selected stock lines.

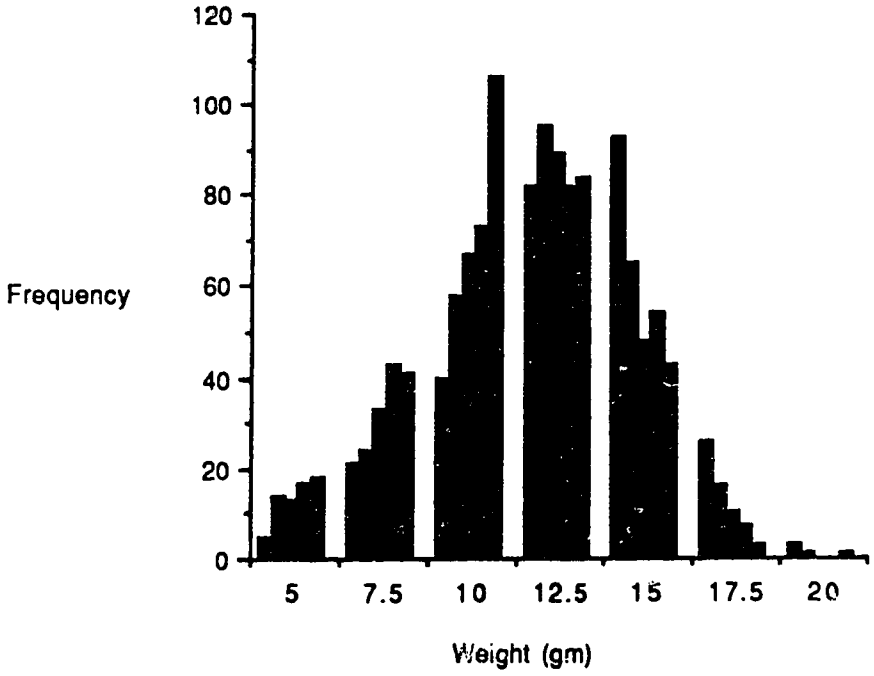
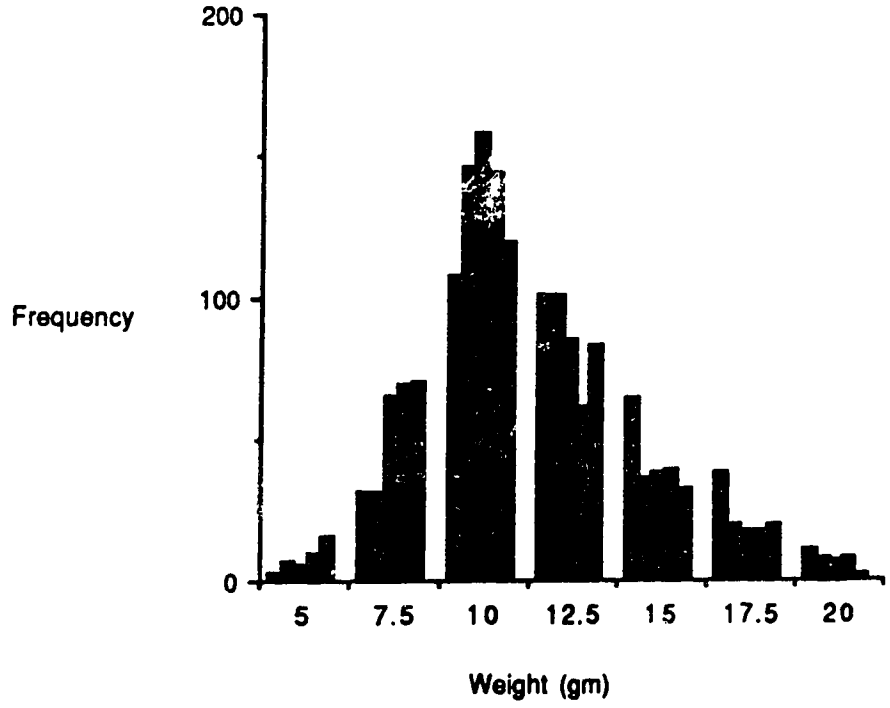


FIGURE 4B. Distribution of 21-day weights of unselected stock population lines.



These results indicate that any higher 42-day weights in the selected lines as compared to the unselected lines must be due to increases in postweaning growth rate rather than increases in either prenatal or postnatal, preweaning growth rates. This also suggests that the increased mature body weight is unlikely to be strictly due to the maternal effect of increased dam size because if this were the situation we would expect to see weight differences between lines manifested at an earlier age. Predictably, litter size accounted for a larger proportion of the variance of 21-day weight than of 42-day weight. Regression analysis of 21-day weight on litter size yielded a regression coefficient of -0.49±0.03 for both males and females.

TABLE 4. Analysis of variance of 21-day weights (gm) of mice in stock population lines calculated on data collected on generations 11 through 15.

SOURCE OF VARIANCE	DF	SS	P
Strain	2	5674.79	0.0001
Line within Strain	6	2149.11	0.0001
Generation	4	509.92	0.0001
Litter size	12	3571.53	0.0001
Sex	1	300.36	0.0001
Residual error	3006	15348.05	

Figures 5A and 5B show the differences between mean 42-day weights and mean 21-day weights of the stock population lines for males and females, respectively. These data represent the mean growth rate of litters from weaning to 42 days of age. The sexes are shown separately to account for the differences in growth rate patterns between them. From this figure, it is clearly seen that the higher 42-day weights in the selected lines relative to the unselected lines of strains 3 and 5 are due, at least in part, to increased postweaning growth rates in these lines. This observation is relevant to our experimental approach in that the effects of many of the somatotrophs, particularly those of growth hormone, are exerted only after the onset of puberty.

Stock population lines were analyzed for differences in fertility and fecundity to determine if selection for increased 42-day weight had had any concomitant effect on reproductive performance. Two measures of fertility were assessed. The first of these, the percentage of matings yielding progeny, showed no differences between strains or between selected and unselected lines. The overall percentage of fertile matings amongst the stock population lines ranged from 83% to 95%.

The second measure of fertility, the number of days between first mating and parturition, showed significant ($P<0.01$) variance attributable to strain and to line within strain as determined by the analysis of variance given in Table 5. The generation effect also contributed to the variance of this fertility trait although no distinguishable pattern in the fluctuations of fertility levels could be discerned. However, there were no consistent differences between

selected and unselected lines within strains (Figure 6), indicating that variations in this trait are not due to selection for increased mature body weight. The dsitribution of this trait was markedly skewed with an extremely long tail at the upper end of the distribution. However, it should be noted that this measure of fertility includes the effects of numerous fertility traits such as time to conception and length of gestation. Moreover, since males were left with females until the latter were obviously pregnant, first pregnancies which aborted before term would appear as lengthened days to parturition.

Fecundity was measured as the number of offspring weaned, *i.e.* the number of offspring surviving to 21 days of age, per litter. There was no correlation ($P>0.05$) between fecundity and either measure of fertility. Mean litter sizes for the stock population lines are shown in Figure 7. There were significant ($P<0.01$) differences between lines, but strain did not contribute significantly to the variance of fecundity. As with fertility, differences in fecundity between lines could not be attributed to selection for body weight since no consistent patterns were observed in comparisons of selected and unselected lines. These results indicate that selection for high 42-day body weight in these strains has had no concomitant effect on reproductive performance.

FIGURE 5A. Differences between mean 42-day weight and mean 21-day weight (gm) of males in the stock population lines of mice.

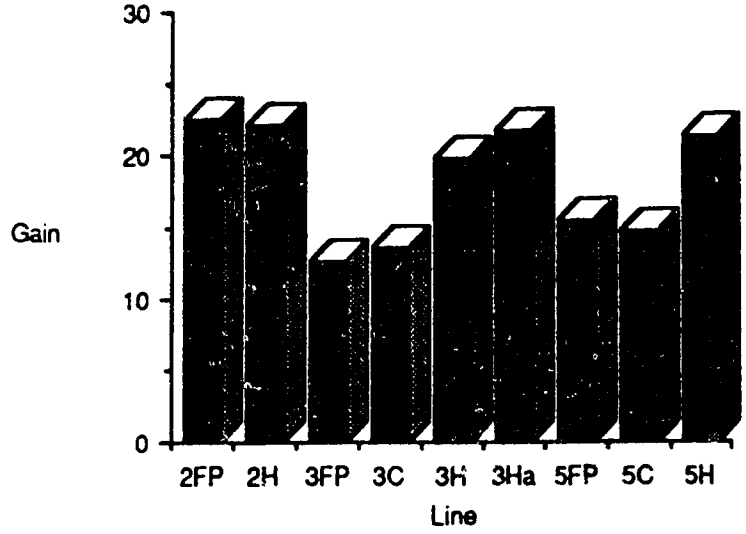
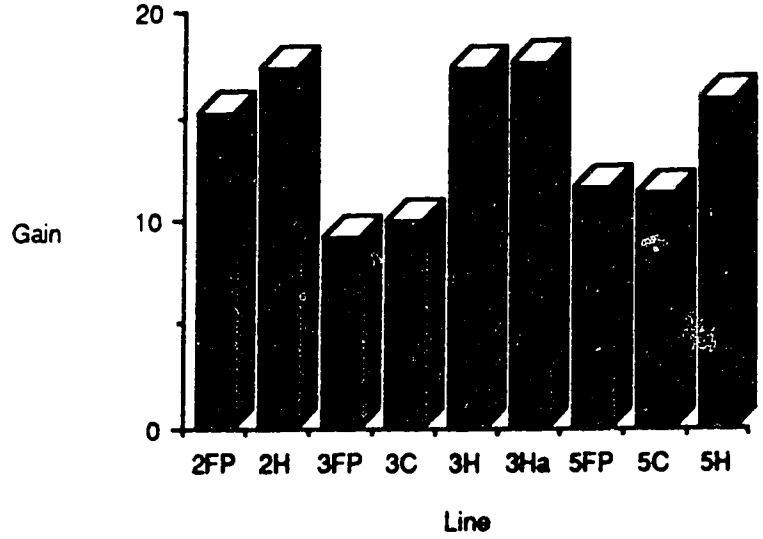


FIGURE 5B. Differences between mean 42-day weight and mean 21-day weight (gm) of females in the stock population lines of mice.



Weights were measured from generations 11 through 15 at the University of Alberta.

TABLE 5. Analysis of variance of fertility, measured as the number of days from first mating until parturition, of stock population lines of mice calculated on data obtained on generations 1 through 15.

SOURCE OF VARIANCE	DF	SS	P
Strain	2	374.92	0.0002
Line within Strain	6	2623.25	0.0001
Generation	14	745.00	0.0001
Residual error	1537	32729.02	

FIGURE 6. Mean fertility, measured as number of days from first mating until parturition, of stock population lines of mice.

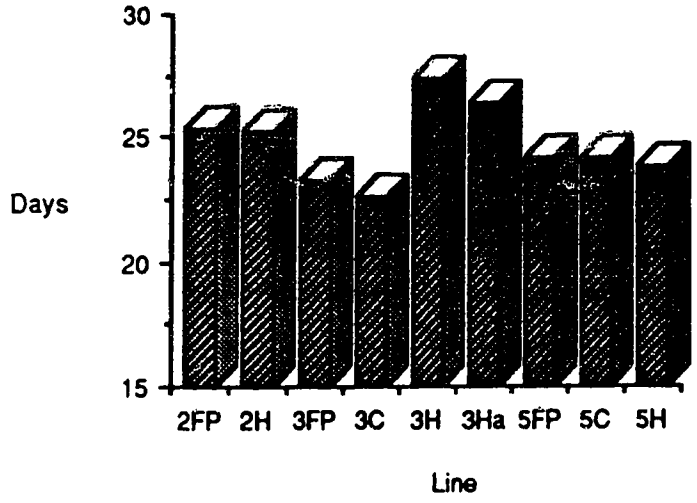
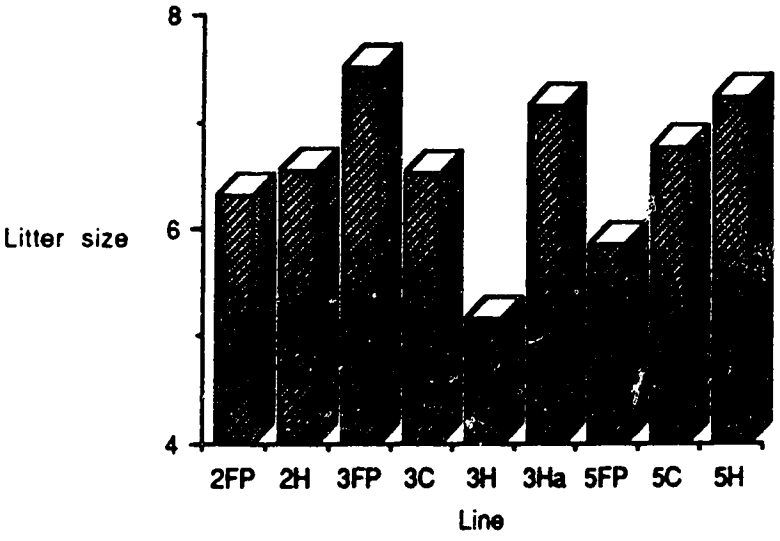


FIGURE 7. Mean litter size, measured as number of offspring weaned per mating, of stock population lines of mice.



Fertility and litter sizes were recorded for generations 1 through 15 at the University of Alberta.

II. RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF SOMATOTROPIC GENES

A RFLP for growth hormone (GH) was identified in our laboratory in the stock mouse lines (Salmon et al., 1988) when genomic DNA was restricted with HindIII as shown in Figure 8. This polymorphism, one of many identified (Figure 9), was used because the large difference in fragment size, *i.e.* 3.5 kb, and the simple banding pattern facilitated genotype scoring. The polymorphic restriction sites associated with these GH haplotypes were detailed by Salmon et al (1988). The 8.0 kb GH fragment obtained with digestion of genomic DNA with HindIII, designated GH^C was fixed in lines 2FP, 3FP, 3C, 5FP and 5C. The alternate haplotype, designated GH^h, was fixed in all of the high 42-day weight selected lines except 2H, in which it segregated with GH^C. The GH^h haplotype is defined by a 4.5 kb fragment obtained after digestion with HindIII. GH^CGH^C homozygotes show a single band at 8.0 kb; GH^hGH^h homozygotes are identified by a single band at 4.5 kb; and the GH^hGH^C heterozygotes are characterized by two bands at 8.0 kb and 4.5 kb.

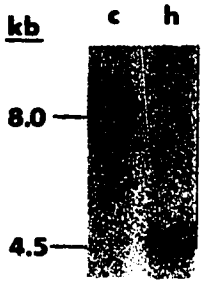
Line 2H was found to be polymorphic for the GH haplotypes. In contrast to Strains 3 and 5, where the GH^h haplotype was fixed in the selected lines and the selected lines were significantly ($P<0.01$) heavier than the unselected control lines (Table 3), the polymorphic 2H line did not have a higher 42-day weight than the corresponding control

line. This suggests that the fixation of GH^h in a given strain is required for increased 42-day weight as compared to the foundation population. Thus, GH^h appears to act as a recessive allele with homozygotes being heavier at 42 days than their counterpart heterozygotes or GH^c homozygotes. As discussed in the introduction, previous reports have indicated that the effect of a QTL may be limited by the genetic background in which it is found. Thus, it is possible that the GH^h haplotype has no effect on 42-day weight in the genetic environment that characterizes Strain 2. This would differ from the effect of GH^h in strains 3 and 5 where fixation of this allele in all three selected lines indicates a definite association between the GH haplotypes and a QTL for weight. The exclusion of GH^h from all of the randomly mated FP and C lines suggests that natural selection may act against this allele or that selection for higher weight is necessary for the continued retention of this allele. It is also possible that selection for high weight acts effectively as selection against the GH^c allele which might then be expected to have a lowering effect on 42-day weight. Exploration of the effects of the GH haplotypes on weight parameters will be the focus of section IV.

FIGURE 8. Haplotypes of the GH gene as identified following digestion of murine genomic DNA with the restriction enzyme HindIII.

The GH^h haplotype was found in all of the selected lines of mice in the stock population; in three of the four selected lines it was fixed while the fourth selected line was polymorphic.

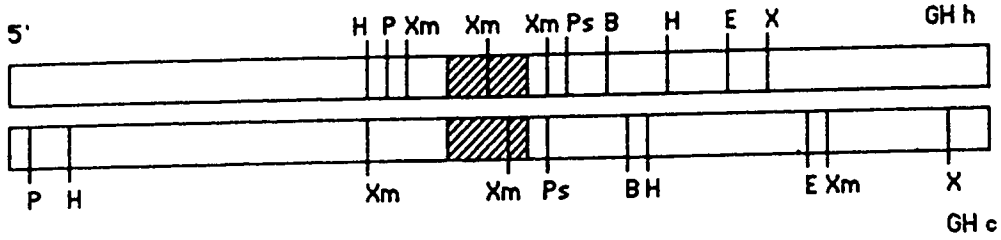
The GH^c haplotype was fixed in all of the unselected control lines in the stock population.



PROBE: rGH

ENZYME: Hind III

FIGURE 9. Polymorphic restriction sites that distinguish the GH^h haplotype from the GH^c haplotype (from Salmon et al., 1988).



The symbols used in the figure are as follows: E=Eco RI, H=HindIII, B=BamHI, X=XbaI, Xm=XmnI, P=PvuI, and Ps=PstI.
The hatched area represents the structural GH gene in the mouse.

Restriction patterns for IGF-2 were examined for polymorphisms following digestion with each of eight different restriction enzymes. The restriction enzymes used on the genomic DNA samples were: BamHI, EcoRI, EcoRV, HindIII, PstI, SstI, XbaI, and XmnI. Polymorphisms were found with two of these enzymes, BamHI and HindIII. Digestion with BamHI revealed a common band at 12 kb and three variable bands resulting from a polymorphic site ($IGF2^B$) present in line 2FP. The variant pattern in line 2FP, $IGF\text{-}2^{B2}$, resulted from the absence of a BamHI restriction site. Thus, the $IGF\text{-}2^{B2}$ haplotype was identified by a single band at

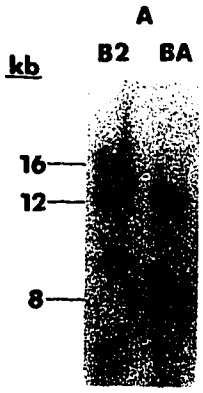
16.5 kb in place of the two bands at 8.2kb and 8.3 kb which characterized the common haplotype, IGF-2^{BA}. The two restriction fragment patterns for IGF-2 generated with BamHI are shown in Figure 10A. These restriction patterns indicate that the mouse IGF-2 gene must cover more than 8.2 kb of genomic DNA; in light of the 12 kb of genomic DNA spanned by the rat IGF-2 gene (Frunzio et al., 1986), this is not surprising.

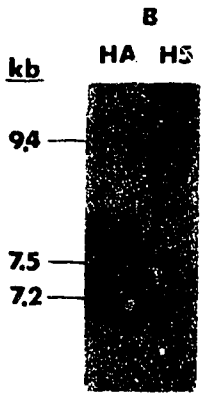
A second polymorphic site for IGF-2 was found following digestion of genomic DNA with HindIII (IGF-2^{H}). This polymorphism (Figure 10B) was present only in line 5H which exhibited both haplotypes. The variant haplotype found in line 5H was designated IGF-2^{H5} while the common haplotype of the other lines was designated IGF-2^{HA}. The variant pattern was the result of the loss of a HindIII restriction site which produced a 9.4 kb band in place of the 7.5kb band observed in the common restriction pattern. As the polymorphic BamHI and HindIII sites in IGF-2 were each present in only a single line, it is not possible to determine their origin. Without knowing whether or not these sites were present in the base populations prior to the divergence of the selected and control lines, we cannot give any *a priori* speculation concerning the possible effects of these haplotypes on 42-day weight.

FIGURE 10. Haplotypes of the IGF-2 gene as identified following digestion of murine genomic DNA with the restriction enzymes (A) BamHI and (B) HindIII.

(A) The IGF-2^{B2} haplotype was found in line 2FP which was polymorphic. All other lines showed only the IGF-2^{BA} haplotype following digestion with BamHI.

(B) The IGF-2^{H5} haplotype was present in line 5H which was polymorphic. All other lines showed only the IGF-2^{HA} haplotype following digestion with HindIII.





PROBE: hIGF-2

ENZYME: BamHI

PROBE: hIGF-2

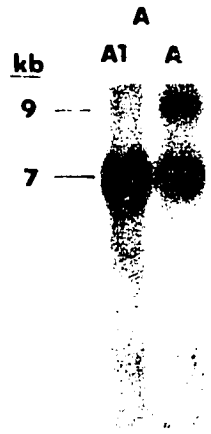
ENZYME: Hind III

Polymorphisms were also sought in GRF restriction fragment patterns of the stock lines following digestion of genomic DNA with each of thirteen restriction enzymes: ApaI, BamHI, DraI, EcoRI, EcoRV, HindIII, HpaI, KpnI, PstI, PvuI, SstI, TaqI, and XbaI. Polymorphisms were found with only two of these enzymes: ApaI and HpaI. The polymorphism obtained after digestion with ApaI, shown in Figure 11A, was found in strain 5; this polymorphism was not pursued further in this study. The second polymorphism, shown in Figure 11B, was observed following digestion of genomic DNA with Hpa I. The variant pattern obtained for GRF with HpaI was present in lines 2FP and 5H and was designated GRF^{HpV}. The common pattern for the remaining lines was designated GRF^{HpA}. The GRF^{HpA} haplotype showed two bands at 8.7 kb and 0.4 kb; whereas the GRF^{HpV} haplotype was identified by the presence of a 6.2 kb band along with the 8.7 kb band, while the 0.4 kb band was missing. The variant pattern resulted from the loss of the HpaI restriction site which defined the small 0.4 kb band. Because of difficulties involved in identifying the presence of the 0.4 kb band while resolving the 8.7 kb and 6.2 kb bands, genomic DNA samples were only screened for the presence or absence of the 6.2 kb band. Thus, GRF^{HpV} homozygotes and heterozygotes were classed together since both showed the 6.2 kb band which was absent from GRF^{HpA} homozygotes. The presence of GRF^{HpV} in both 2FP and 5H which shared a common ancestral strain, Falconer's Q Strain, indicates that this haplotype was likely present in the foundation populations of both Strains 2 and 5. Since the variant haplotype is seen in both a selected line, 5H, and an unselected

control line, 2FP, it would not be expected to act as a QTL for higher weight unless this effect were confined to the genetic background of Strain 5. However, since the unselected 2FP line was, at the time of this study, actually the heaviest of the stock population lines (Table 3) it is possible that this variant GRF haplotype has an effect on increasing 42-day weight such that its continued presence in the unselected line was due to there being no direct selection against higher weight in the control line.

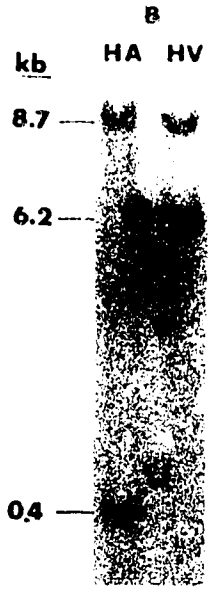
No polymorphisms were found for either SRIF or IGF-1 with the enzymes we tried which consisted of BamHI, EcoRI, EcoRV, HindIII, PstI, SstI, and XmnI.

FIGURE 11. Haplotypes of the GRF gene as identified following digestion of murine genomic DNA with the restriction enzymes (A) ApaI and (B) HpaI.
(A) All lines of Strain 5 were polymorphic for the haplotypes obtained following digestion with ApaI, no other strains were tested with this enzyme and the observed restriction patterns for Strain 5 were not characterized.
(B) The GRF^{HpV} haplotype was present in lines 2FP and 5H which were polymorphic. All other lines whowed only the GRF^{HpA} haplotype following digestion with HpaI.



PROBE: rGRF

ENZYME: Apa I



PROBE: rGRF

ENZYME: Hpa I

III. GROWTH CHARACTERISTICS OF EXPERIMENTAL POPULATIONS

III.A. THE $3F_2$ POPULATION

The following information of the $3F_2$ population was previously published by Winkelman et al (1990). The $3F_1$ population of mice consisted of 74 individuals from eight litters; each litter produced between six and eleven offspring. Twenty-six $3F_1$ males were mated to 28 $3F_1$ females to generate 28 $3F_2$ families. The $3F_2$ litter sizes ranged from two to fourteen pups yielding 263 $3F_2$ individuals. Weaning weights and 42-day weights of the $3F_1$ and $3F_2$ populations were analyzed separately for males and females to account for the differences in variances between the sexes. Means and variances of weaning weight and 42-day weight of the two generations are given in Table 6. The means and variances of weaning weights within each generation were similar for males and females. There was a slight increase in weaning weights from generation $3F_1$ to $3F_2$. This may have been due to a heterotic maternal effect on birth weight or preweaning gain, with crossbred dams having a positive effect on the productive traits of their progeny. Weaning weights for the lines from which the $3F_1$ population was derived were not measured for the generation used in this breeding plan. Therefore, we could not compare the weaning weights of $3F_1$ or $3F_2$ mice with those of the parental lines.

Males were heavier ($P<0.01$) than females at 42-days. The variance of 42-day weight was also greater for males than females in both the $3F_1$ and $3F_2$ populations. Forty-two day weights were essentially the same for

the two populations, making the postweaning growth rate of $3F_2$ mice lower than that of $3F_1$ mice. Predictably, variances of both weaning weight and 42-day weight were greater in $3F_2$ than in $3F_1$. Litter size was found to account for 15% of the variance of weaning weight and 5% of the variance of 42-day weight in the $3F_2$ mice. Regression analysis of 42-day weight on litter size yielded regression coefficients of -0.46±0.11 gm and -0.29±0.07 gm for males and females, respectively.

TABLE 6. Means and variances of weaning weight (gm) and 42-day weight (gm) of generations $3F_1$ and $3F_2$.

	$3F_1$			$3F_2$		
WEIGHT AT:	N	MEAN	σ^2	N	MEAN	σ^2
21 DAYS						
MALE	35	10.16	1.93	125	13.32	5.62
FEMALE	39	10.63	1.06	137	12.67	5.29
42 DAYS						
MALE	35	25.00	4.93	125	26.44	7.78
FEMALE	39	22.68	2.16	137	22.68	3.72

III.B. THE $5F_2$, $2F_2$ AND MF_2 POPULATIONS

The P_1 generations created for the three other populations used in this experiment (Figure 1) were analyzed with respect to fecundity, 21-day weight, and 42-day weight. These data are summarized in Table 7. Litter sizes at seven days post-parturition did not differ between $5P_1$-H and $5P_1$-C or between MP_1-H and MP_1-C; $2P_1$-H contained more offspring per litter than did $2P_1$-C. Twenty-one day weights of $5P_1$-H and MP_1-H were lower than those of their respective P_1-C populations. This relationship was still seen for MP_1-H and MP_1-C at 42 days. Therefore, in this cross, MP_1-C mice were actually heavier than those of MP_1-H at 42 days of age; this a[illegible] result of using the stock line 2FP to create MP_1-C. Postwea[illegible]wth rates of MP_1-H and MP_1-C were similar. Forty-two day weights of $5P_1$-H were greater than those of $5P_1$-C as a result of a higher postweaning growth rate in $5P_1$-H. There were no significant ($P<0.01$) differences in the 21-day weights or the 42-day weights of $2P_1$-H and $2P_1$-C.

Analyses of variance were carried out on 21-day and 42-day weights of the F_1 generations obtained from reciprocal crosses between corresponding P_1-H and P_1-C mice. The analyses showed that line, cross within line, sex and the covariate, litter size, all contributed significantly to the variance of body weight at these two ages. The model used accounted for 50% of the variance of 21-day weight and 78% of the variance of 42-day weight. The variance accounted for by the cross

within line effect was due, mainly, to differences in the 21-day and 42-day weights of offspring from the reciprocal crosses between MP_1-H and MP_1-C. Twenty-one day and 42-day weights of MF_1-HC were 2.56±0.89 gm and 3.00±0.76 gm greater than MF_1-CH. The difference in these reciprocal crosses may be partially due to maternal effects since the MP_1-C population had higher 42-day weights than MP_1-H. There were no differences in either 21-day or 42-day weight between the other reciprocal crosses indicating an absence of maternal effects on productive traits for these populations. Culling the litters at seven days of age did not completely remove the effect of litter size on 21-day or 42-day weights; therefore, corrections for litter size were retained in further analyses. Computations for further populations were simplified by including litter size as a covariate; this was necessary because of smaller population sizes and the existence of many empty cells. Heritabilities for 21-day and 42-day weight, calculated by regression of offspring on midparent, were 0.27±0.07 and 0.37±0.12, respectively, for the F_1 population. These estimates are comparable to previously reported estimates for heritability of body weight in mice. For example, Falconer (1953) obtained a heritability estimate of 0.35 for six-week body weight of mice and Nishida (1972) reported heritability estimates of 0.13 and 0.41 for 21-day and 42-day weight, respectively.

TABLE 7. Summary of fecundity and weight data of the P_1 populations.

				WEIGHT (gm) AT:	
	LITTER SIZE	SEX	N	21 DAYS	42 DAYS
$5P_1$-H	7.56 (1.16)	M	25	14.40 (2.58)	33.83 (2.93)
		F	16	13.26 (2.47)	27.89 (2.49)
$5P_1$-C	7.90 (2.47)	M	22	15.61 (1.61)	28.15 (1.79)
		F	25	15.26 (1.20)	24.11 (1.61)
$2P_1$-H	7.60 (3.50)	M	26	17.54 (3.07)	37.66 (2.84)
		F	17	16.81 (2.50)	30.03 (1.57)
$2P_1$-C	6.10 (3.36)	M	24	17.08 (4.12)	35.87 (4.92)
		F	12	16.18 (1.49)	29.37 (1.48)
MP_1-H	6.78 (1.93)	M	21	16.02 (2.42)	34.37 (2.96)
		F	28	15.51 (2.04)	27.85 (3.08)
MP_1-C	6.44 (2.63)	M	21	17.46 (2.94)	37.37 (3.33)
		F	24	16.65 (3.05)	30.35 (3.11)

Numbers shown in brackets are the standard deviations.

Analyses of variance were also carried out on 21-day and 42-day weights of the F_2 populations. The results of the analyses of variance are given in Tables 8a and 8b. Line, sex and the covariate, litter size, were all found to contribute significantly to the variances of 21-day and 42-day weight. The model used accounted for 37% of the variance of 21-day weight and 69% of the variance of 42-day weight in the F_2 populations. The overall variance of 21-day weight in the F_2 generation was not different from that in the F_1 whereas there was a large increase in the the variance of 42-day weight. Heritabilities for 21-day weight and 42-day weight were also calculated from the F_2 data by regression of offspring on midparent. The values obtained, 0.21 ± 0.05 gm for 21-day weight and 0.36 ± 0.07 gm for 42-day weight, were similar to those calculated from the F_1 data. There were no differences in mean 21-day or 42-day weights between the F_1 and F_2 generations. Reproductive traits appeared to be influenced by heterotic effects as shown by a significant ($P<0.01$) increase in litter size in the F_2 generation when compared with the F_1 generation. Litter size in these populations could not be directly compared with litter size in the stock lines since the stock line data were obtained at 21 days after parturition and these data were obtained seven days after parturition.

TABLE 8a. Analysis of variance of 21-day weights (gm) of F_1 mice generated from reciprocal crosses betweem P_1-H and P_1-C.

SOURCE OF VARIANCE	DF	SS	P
Line	2	729.24	0.0001
Cross within Line	3	168.26	0.0001
Sex	1	75.36	0.0001
Litter size	1	112.65	0.0001
Residual error	253	1009.83	

TABLE 8b. Analysis of variance of 42-day weights (gm) of F_1 mice generated from reciprocal crosses between P_1-H and P_1-C.

SOURCE OF VARIANCE	DF	SS	P
Line	2	2000.29	0.0001
Cross within Line	3	231.28	0.0001
Sex	1	4139.39	0.0001
Litter size	1	173.94	0.0001
Residual error	253	1778.50	

III.C. LINES CREATED BY DIVERGENT SELECTION FOR 42-DAY WEIGHT

Lines 5Hi and 5Lo, generated from $5F_3$, were subjected to divergent selection for 42-day weight for nine generations in the course of this study. The response to selection by generation is shown in Figure 12. Selection of individuals for the first five generations was carried out on a within family replacement basis; all families were the result of single pair matings and one male and one female were selected from each family to be used as parents for the next generation. Seven matings were made for each line in each generation. Selected mice were paired randomly with no matings between full sibs. During this time, response to selection was moderate but successful in yielding divergence of mean 42-day weights. Commencing in generation five, selection intensity on males was increased and a hierarchical mating scheme was instituted with each male mated to two females. A single female replacement for the next generation was selected from the offspring of each female on the basis of highest or lowest 42-day weight. The required number of males were those with the highest or lowest 42-day weights selected from all families, using no more than one male from each full sib family. Selected mice were again paired randomly with no matings between full sibs. Six to ten full sib families were produced in each generation for each line. This selection method constitutes a combination of within-family and individual selection and is expected to yield a faster rate of response to selection than pure within-family selection (Von Butler et al., 1984). Response to selection increased following implementation of the hierarchical mating scheme and at the end of nine generations the

two lines differed significantly ($P<0.01$) in mean 42-day body weight. The difference in 42-day weight attained with nine generations of selection was 6.72±1.06 gm, somewhat greater than the difference of 5.17±1.62 gm between $5P_1$-H and $5P_1$-C in the foundation generation for these lines. This represents an average increase of 0.39 gm/generation in the high 42-day weight selected line and a decrease of 0.35 gm/generation in the low 42-day weight selected line. Differences in 21-day weights between 5Hi and 5Lo followed essentially the same pattern as those of the 42-day weights although the difference of 1.40±0.86 gm was not significant ($P>0.05$) after nine generations. Thus, much of the difference in 42-day weights observed between 5Hi and 5Lo after nine generations of selection was due to differences in postweaning growth rates. Litter size was highly variable between generations but no changes were correlated with either high or low selection.

FIGURE 12. Response to divergent selection for 42-day weight in lines 5Hi and 5Lo.

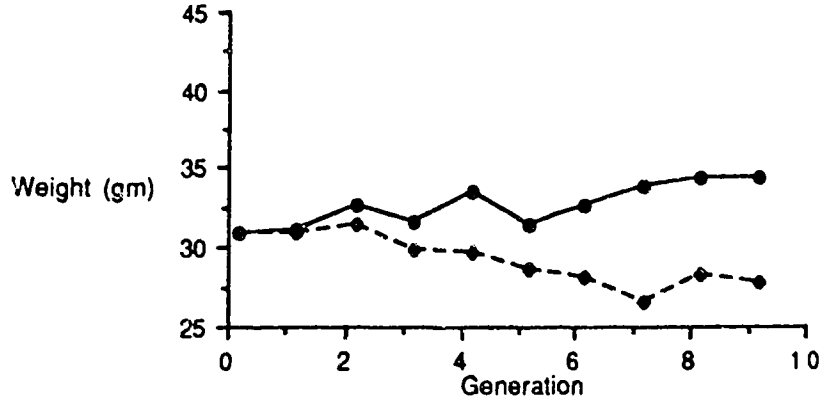


FIGURE 13. Response to divergent selection for 42-day weight in lines 2Hi and 2Lo.

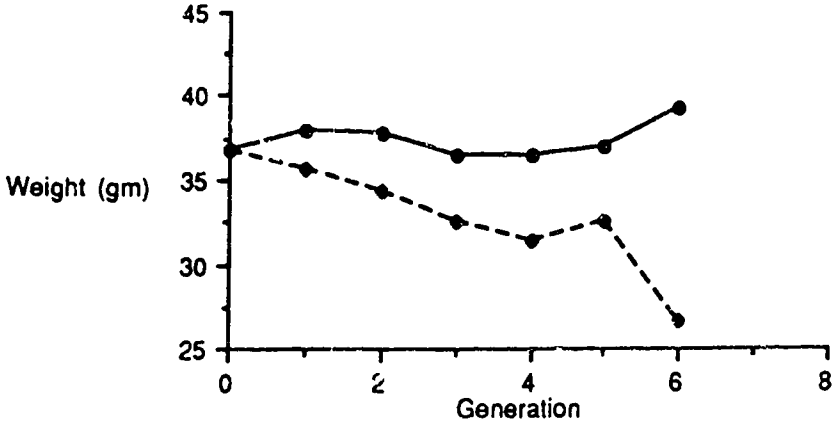
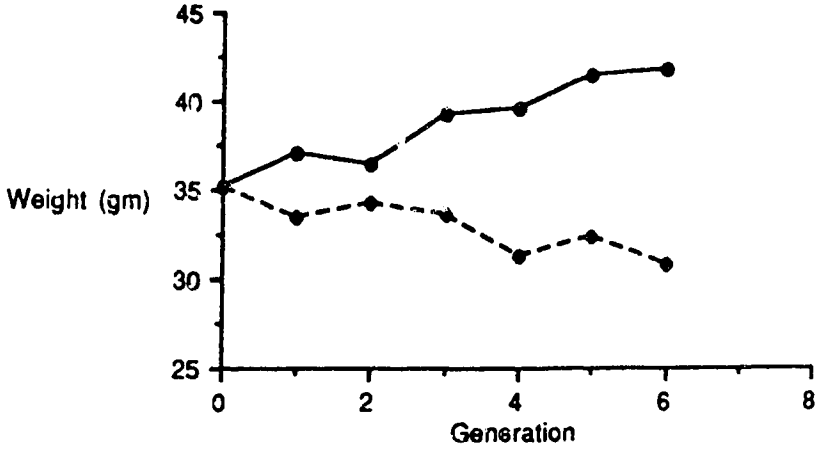


FIGURE 14. Response to divergent selection for 42-day weight in lines MHi and MLo.



Lines selected for high weight are shown with solid lines.
Lines selected for low weight are shown with dashed lines.

Divergent selection was also carried out on lines 2Hi and 2Lo from $2F_2$, and MHi and MLo from MF_2 for six generations in the course of this experiment. The responses to divergent selection for these two populations are shown in Figures 13 and 14, respectively. The selection programme and mating system used was the same as that described for 5Hi and 5Lo. Eight to ten full sib families were produced in each generation for lines 2Hi and 2Lo; ten to fourteen full sib families were produced in each generation of lines MHi and MLo. For these lines, selection intensity on males was increased by the implementation of a hierarchical mating scheme in generation 3. Response to selection for high 42-day weight in 2Hi was minimal and after six generations the mean 42-day weight of 2Hi was not significantly ($P>0.05$) different from that of the base population, $2F_2$. The average increase in size in the 2Hi line was 0.44 gm/generation; however, weights of both the $2F_2$ and selected lines were characterized by very high variances. Line 2Lo did, however, respond favourably to selection for decreased 42-day weight and, after six generations, had a mean 42-day weight significantly ($P<0.01$) less than that of both the base population and 2Hi. The final weight difference in 2Lo represented an overall decrease of 1.68 gm/generation. Such asymmetric response to divergent selection for body weight in mice has frequently been observed in selection experiments (e.g. MacArthur, 1949; Falconer, 1955; and Von Butler et al., 1984). Interestingly, one of the four strains originally used to develop Strain 2 was the Q Strain from Edinburgh for which the same asymmetry in response to divergent selection for 42-day weight was observed by Falconer (1953) who attained

an increase of 0.27±0.05 gm/generation for upward selection and -0.62±0.046 gm/generation for downward selection. It is notable that the mean 42-day weights reported by Falconer (1953) were much lower than those of the $2F_2$ population used as the source for 2Hi and 2Lo. It is possible that the $2F_2$ 42-day weight may have been approaching the upper limit for this strain thus limiting the potential response to selection for increased 42-day weight. However, the upswing in the trend observed in the final generation suggests that the absence of response prior to this may have been overcome with further selection. The response to divergent selection observed in MHi and MLo was essentially symmetric and a productive response was observed for both directions selected. A difference of 12.223±2.41 gm was realized between MHi and MLo with six generations of selection. This result is interesting in light of the fact that the variances of 42-day weights were not significantly ($P>0.05$) different in the MF_2 and $2F_2$ populations and we would, therefore, predict similar responses to selection for these two populations. The genetic origins of these populations are also similar in that they were both derived from the same ancestral populations.

IV. THE ASSOCIATION OF RFLPs FOR GROWTH HORMONE WITH BODY WEIGHT

The GH^h haplotype was originally found exclusively in lines which had been selected for high 42-day body weight. This haplotype was, in fact, fixed in three of four selected lines of diverse genetic backgrounds. The GH^h haplotype was not found in any of the unselected control lines derived from the same genetic stocks as the selected lines. The exclusion of GH^h from all of the control lines suggests that the alternate allele, GH^c, may be associated with increased fitness in the absence of selection for high weight (Salmon et al., 1988). We hypothesized that the presence of GH^h in all of the high lines is due to a positive effect of GH^h on body weight and that this haplotype acts as a QTL for increased 42-day weight. From this, we postulated that the GH^h haplotype would segregate with 42-day weight in F_2 populations derived from crosses between selected and control lines.

IV.A. ANALYSIS OF $3F_2$

Our first analysis was carried out on the $3F_2$ population to determine if the GH^h haplotype which was fixed in the original selected line, 3H, would segregate with high body weight in the $3F_2$. These reports were previously published by Winkelman et al (1990). GH genotypes were identified for 258 of the 263 individuals obtained in the $3F_2$ population. The mice were then grouped into one of the following genotypic classes: GH^hGH^h, homozygous for the GH^h haplotype; GH^cGH^c, homozygous for the GH^c haplotype; and GH^hGH^c for the heterozygous mice

carrying both haplotypes. The total number of mice in each of the genotypic classes was 72 GH^hGH^h, 75 GH^cGH^c, and 111 GH^hGH^c. The allele frequencies in the $3F_2$ population were calculated to be 0.49 and 0.51 for GH^h and GH^c, respectively. These allele frequencies are in accordance with the allele frequencies of 0.5 expected in an F_2 population generated from crosses between two P_1 populations in which the alternate alleles were fixed. Although the two homozygous classes were represented by equal numbers of individuals, the proportion of heterozygotes in the $3F_2$ population was somewhat lower than the 50% expected in this class. This observation, in conjunction with the observation that in eight of nine stock lines only one of the two haplotypes could be found, suggests that heterozygotes for the GH RFLPs may be at a selective disadvantage.

The results of the analysis of variance carried out on 42-day weights of the $3F_2$ population are given in Table 9. The addition of GH genotypic class to the model accounted for an additional 3% of the phenotypic variance of 42-day weight; this effect was significant ($P<0.01$). Similar results were obtained with the analysis of postweaning growth rate from 21 to 42 days of age whereas in the analysis of 21-day weights, the effect of genotypic class was not significant ($P>0.05$). These results are evidence that there is an association between GH RFLP haplotypes and 42-day weight in the $3F_2$ population and that this association is effected through the rate of postweaning growth.

TABLE 9. Results of the analysis of variance of 42-day weights (gm) in $3F_2$ mice.

SOURCE OF VARIANCE	DF	SS	P
Line	2	729.24	0.0001
Cross within Line	3	168.26	0.0001
Sex	1	75.36	0.0001
Litter size	1	112.65	0.0001
Residual error	253	1009.83	

Tne distribution of 42-day weights, measured as deviations from the midparent, for each genotypic class were also calculated. From these distributions, the average additive substitution effects for GH^h and GH^c were determined to be -0.6186 gm and +0.5888 gm (σ^2=0.3645), respectively. Although these results show that the GH haplotypes are associated with 42-day weight in the $3F_2$ population, the association was the converse of that predicted. In this population, the GH^h haplotype which was fixed in the selected 3H line was associated with lower 42-day weight and postweaning growth rate in the F_2 population.

Our observations do not exclude the possibility that the GH haplotypes act directly as QTLs for postweaning growth rate and, subsequently, 42-day weight. It may be that both the direction and the magnitude of the effect of the QTL is dependent on genetic background. A genetic background effect has been previously reported for a QTL affecting soluble solids in tomatoes (Tanksley and Hewitt, 1988). Moreover, it may be that the fixation of GH^h in the selected lines was

not due to a positive effect of GH^h on 42-day weight but rather to a negative effect of GH^c in a selected genetic background. However, if the GH^c haplotype was associated with lower 42-day weight in the high weight selected genetic background, this effect must be strain-dependent since the stock line, 2H, was polymorphic for GH haplotypes. It is also possible that the GH RFLP is linked to a QTL for postweaning growth rate. Since the genetic base of the $3F_2$ population was quite small, consisting of only six 3H and five 3C mice, recombination between the GH RFLPs and a linked QTL would have had major confounding effects on the results.

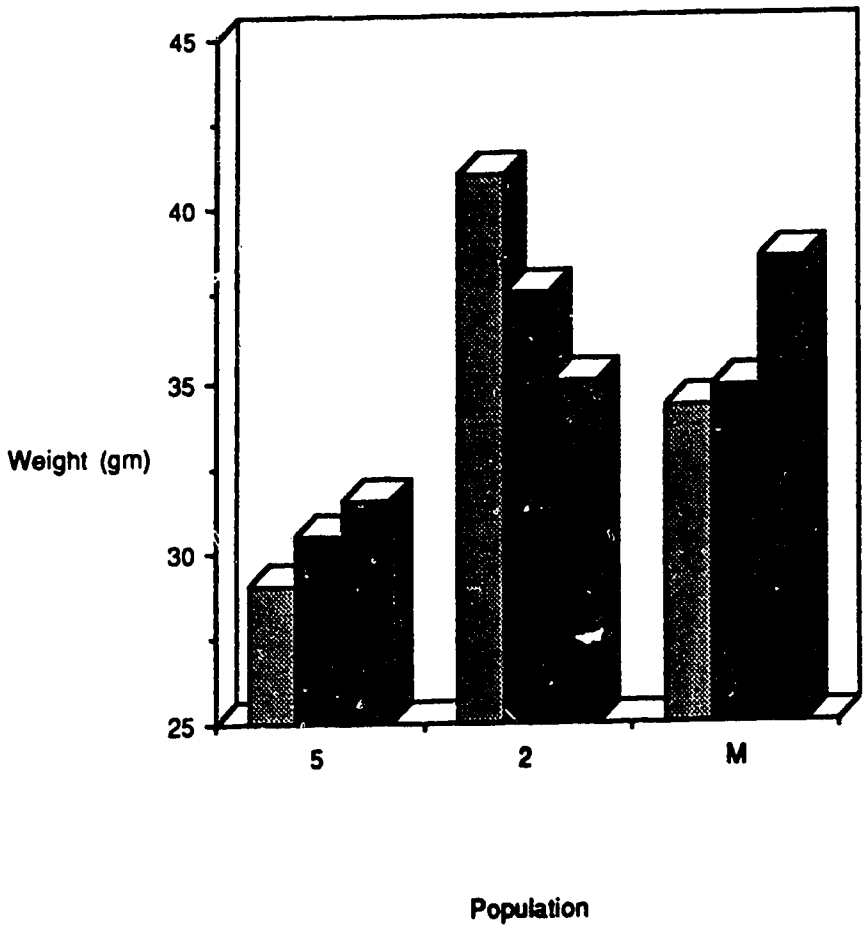
In order to distinguish between the possibilities of linkage between a QTL for weight and the GH haplotype and a genetic environment effect on the GH QTL in the $3F_2$ population, we analyzed the association of GH haplotypes and growth parameters in the remaining F_2 populations generated for this study. More extensive P_1 populations were employed to create these other F_2 populations so that we could determine if the negative association between GH^h and 42-day weight observed in the $3F_2$ population was due to recombination between the GH haplotype and a linked QTL for 42-day weight. Furthermore, the remaining F_2 populations were developed utilizing strains of diverse genetic backgrounds to allow for further testing of interactions between GH haplotypes and genetic background.

IV.B. ANALYSIS OF $5F_2$, $2F_2$ AND MF_2

Data from the remaining F_2 populations were analyzed to determine the extent of the associations between GH haplotypes and the production traits, 42-day weight, 21-day weight, and postweaning growth rate from 21 to 42-days of age. In the first analysis, actual 42-day weights with the effects of family and sex included in the model, were examined. In the second analysis, 42-day weights measured as deviations from the midparent value with the effects of sex and litter size included in the model, were evaluated. The two models yielded similar results: the GH genotypic classes contributed significantly ($P<0.01$) to the phenotypic variance of 42-day weight in all three F_2 populations. Mean 42-day weights for each genotypic class of the three F_2 populations are shown in Figure 15. The average additive gene substitution effects of GH^h and GH^c on 42-day weight, measured as the deviation from the midparent, are given in Table 10. In two of the F_2 populations, $5F_2$ and MF_2, GH^h was found to have a negative effect on 42-day weight as it did in $3F_2$. This suggests that the effect observed in the $3F_2$ population was not an artefact resulting from the small number of individuals in the founder P_1 generation. These results also indicate that the effect observed in the $3F_2$ population did not result from recombination between the GH RFLP and a linked QTL for 42-day weight. Therefore, it seems likely that the GH haplotype is a QTL for 42-day weight. However, its effect is dependent on the genetic environment in which it occurs: in the selected parental lines GH^h increased in frequency with selection for high 42-day

weight whereas in the F_2 populations, GH^h was correlated with lower 42-day weight.

FIGURE 15. Forty-two day weights of genotypic classes in F_2 populations of mice derived from crosses between selected and control lines.



GH^C homozygotes are represented by solid bars, GH^h homozygotes by stippled bars and heterozygotes by dashed bars.

TABLE 10. Average additive gene substitution effects (in gm) of GH^h and GH^c on 42-day body weight (measured as the deviation from the midparent) in F_2 populations of mice derived from crosses between selected and unselected lines.

POPULATION	EFFECT OF GH^h	EFFECT OF GH^c	σ^2
$5F_2$	-0.5177	+0.4902	0.2146
$2F_2$	+1.7583	-0.7389	0.5934
MF_2	-1.0338	+1.0938	0.3842

For $5F_2$ and MF_2, the allele frequencies of GH^h were 0.4864 and 0.5141, respectively. Unlike the $3F_2$ population, these two populations exhibited no deficiency in the GH^hGH^c heterozygous class. There were 71 $5F_2$ and 70 MF_2 heterozygotes in populations of 147 and 142 individuals, respectively. This indicates that either the GH^hGH^c heterozygote is only at a selective disadvantage in some populations or that the reduced proportion of individuals in this class in the $3F_2$ population was simply a sampling error. A replication of the study which generated the $3F_2$ population would be necessary to distinguish between these possibilities.

In contrast to the results obtained in the $3F_2$, $5F_2$ and MF_2 populations, GH^h was associated with increased 42-day weight in the $2F_2$ population. This observation further supports the hypothesis that the effect of the GH^h RFLP is dependent on the genetic environment. However, interpreting the results obtained with the $2F_2$ population requires

caution. They may have been caused by the low allele frequency of GH^h in this population and the consequent dearth of GH^hGH^h homozygotes. The frequency of GH^h in the $2F_2$ population was 0.30, and there were only 8 GH^hGH^h homozygotes of 147 individuals. The lower frequency of GH^h in $2F_2$ as compared with the other F_2 populations was a consequence of polymorphism at the GH locus in 2H. Since the additive gene substitution effect of an allele is dependent on allele frequency (Equation 1; Page 48), this frequency must be considered as one of the features of the genetic environment which influences the effect of a QTL. This interaction will be explored further in the analysis of GH haplotypes in the selected lines.

In the analysis of 21-day weights, the GH genotypic class was not found to have a significant effect on the phenotypic variance ($P>0.05$) in either $5F_2$ or $2F_2$ populations. Consequently, the GH haplotypes were associated with postweaning growth rates in $5F_2$ and $2F_2$, although the direction of the effect of each haplotype was reversed between the two populations. Thus, GH^h was associated with lower postweaning growth rate in $5F_2$ and higher postweaning growth rate in $2F_2$. GH^h and GH^c act as codominant alleles in their effects on postweaning growth rates in these two populations, with the GH^hGH^c heterozygotes having values between the two homozygous classes.

GH genotypic class was also associated with postweaning growth rate in the MF_2 population; the postweaning growth rate of GH^cGH^c mice was significantly ($P<0.01$) greater than that of GH^hGH^c or GH^hGH^h mice. There was no difference in the postweaning growth rates of GH^hGH^c and

GH^hGH^h mice. Thus, GH^h appears to act as a dominant allele associated with lower postweaning growth rate in the MF_2 population unlike its apparent codominant mode of action in $5F_2$ and $2F_2$. This is interesting in light of the fact that all of these F_2 populations were derived from the same ancestral strains and, therefore, we might expect a common mode of action for a given allele in these populations. In the MF_2 population, a significant association between GH genotype and 21-day weight was found, with GH^cGH^c having a higher 21-day weight than GH^hGH^c and GH^hGH^h which were essentially the same. This was the only F_2 population where GH haplotypes were found to be associated with 21-day weight.

In summary, analysis of the effects of GH haplotypes in the $5F_2$, $2F_2$, and MF_2 populations yielded the following results. (1) GH genotypes had a significant ($P<0.05$) effect on 42-day weight in all of the populations; the effect of GH^h was negative in two and positive in one of the F_2 populations. (2) GH genotypes had a significant ($P<0.05$) effect on 21-day weight in only one of the three F_2 populations; GH^h was associated with lower weight in this population. (3) GH genotypes had a significant ($P<0.05$) effect on postweaning growth rate in all of the populations; GH^h acted as (i) a codominant allele associated with lower postweaning growth rate in $5F_2$, (ii) a codominant allele associated with higher postweaning growth rate in $2F_2$, and (iii) a dominant allele associated with lower postweaning growth rate in MF_2. These results stress the importance of interactions between putative QTLs and genetic background.

Further investigation of genetic background effects was undertaken in the examination of how GH haplotype frequencies changed with selection. Hypothesizing that the diverse effects of the GH haplotypes observed in the F_2 populations were the result of interactions between the haplotypes and the genetic background, we postulated that reconstituting a weight-selected genetic background should restore the positive association of GH^h with body weight.

IV.C. ANALYSIS OF SELECTED LINES

The change in GH haplotype frequency for the six divergently selected lines created in this study is given in Table 11. In the 5Hi and MHi lines, which were selected for high 42-day body weight, GH^h was found to increase in frequency while it decreased in frequency in the corresponding lines selected for low 42-day weight. These results parallel our initial observations that GH^h was fixed in the selected stock population lines while the alternate allele, GH^c, was fixed in the unselected control lines. This result suggests that, for these populations, GH^h increases 42-day weight when the genetic environment has been selected for body weight. This effect is seen with selection for both high and low weight which, respectively, resulted in increased and decreased frequencies of GH^h. The average change in allele frequency for all of the lines selected for high 42-day body weight was 0.04/generation. This constant increase in GH^h in all upward selected lines is unlikely ($P<0.01$) due to chance or drift alone. Therefore, it can be stated that selection for high body weight in mice produces an

increase in the frequency of GH^h. This change in the frequency of GH^h was accompanied by average increases in body weight of 0.39, 0.44, and 1.12 gm/generation in 5Hi, 2Hi, and MHi, respectively. The absolute change in weight in these lines was highly variable, suggesting the effects of a number of other segregating loci affecting this trait. The decrease in the frequency of GH^h averaged 0.02/generation in lines 5Lo and MLo which showed weight decreases of 0.35 and 0.69 gm/generation, respectively. Therefore, selection for decreased body weight does not have as great an effect on the frequency of the GH haplotypes as does selection for increased body weight. This further supports our hypothesis that the positive effect of GH^h is most dramatic in a high weight selected genetic background.

TABLE 11. GH^h allele frequencies in lines of mice selected for high and low 42-day weight from the corresponding F_2 populations.

LINE	$q(GH^h)$	LINE	GENERATION	N	$q(GH^h)$
$5F_2$	0.50	5Hi	9	28	0.84
		5Lo	9	26	0.34
$2F_2$	0.30	2Hi	6	35	0.52
		2Lo	6	38	0.45
MF_2	0.50	MHi	6	38	0.76
		MLo	6	32	0.39

Selection in both 2Hi and 2Lo resulted in an increase in the frequency of GH^h from the original frequency of 0.30 in $2F_2$ (Table 11). The allele frequency in 2Hi was somewhat higher than that in 2Lo after six generations of selection, but this difference was not significant ($P>0.05$). Since the selection response in 2Hi was minimal (Figure 13), the effect of GH^h on 42-day weight in 2Hi and 2Lo appears to be negative, although GH^h was associated with a positive effect on 42-day weight in $2F_2$. Thus, in all of the selected lines generated, the change in the frequency of GH^h in response to selection was the opposite of that expected from the analysis of the F_2 populations. This not only stresses the importance of testing QTLs in the appropriate strains, it indicates that interactions between QTLs should also be considered. It seems imperative, therefore, that we attempt to simultaneously analyze loci which are known to be interactive. These observations are not entirely surprising in view of what is known about the complexity of the interactions between the many growth factors, their receptors and environmental influences as they affect growth.

V. THE ASSOCIATION OF RFLPs FOR INSULIN LIKE GROWTH FACTOR 2 WITH BODY WEIGHT

The IGF-2^{H} variant, IGF-2^{H5}, was tested for associations with the growth traits 42-day weight, 21-day weight and postweaning growth rate in the $5F_2$ and MF_2 populations. The source line of this variant haplotype for both populations was 5H.

V.A. ANALYSIS OF $5F_2$

The frequency of IGF-2^{H5} in $5F_2$ was 0.16. The number of individuals in each of the genotypic classes was: five in the homozygous variant class, IGF-2^{H5}IGF-2^{H5}; 104 in the homozygous common class, IGF-2^{HA}IGF-2^{HA}; and 38 in the heterozygous class IGF-2^{HA}IGF-2^{H5}. This distribution is in agreement with predictions for Hardy-Weinberg equilibrium ($P<0.01$) with the given allele frequencies. In the first analysis, the IGF-2^{H} genotypic class was found to contribute significantly to the phenotypic variances of 42-day weight ($P<0.01$) and 21-day weight ($P<0.05$) with family and sex included in the model although it only accounted for 1.6% and 1.2% of the total variance in the two analyses. The IGF-2^{H} genotypic class was not found to be significant ($P>0.05$) in the analysis of postweaning growth rate. Further analysis of the association between IGF-2^{H} haplotypes and weights at 42 and 21 days revealed that the effect of IGF-2^{H5} was sex-dependent. Forty-two day weight of females and 21-day weight of males were associated with IGF-2^{H} haplotypes. However, there was no association between 42-day weight of males or 21-day weight of females with IGF-2^{H}

RFLP haplotypes. The IGF-2^{H5} haplotype was associated with higher weight at 42 and 21 days for females and males, respectively. The additive gene substitution effects of IGF-2^{H5} and IGF-2^{HA} are given in Table 12. Although IGF-2^{H5} was found to have a positive effect on weight in the two situations discussed, the alternate haplotype, IGF-2^{HA} did not significantly affect weight. These results suggest that IGF-2^{H5} may act as a QTL for increased body weight in the $5F_2$ population but that the timing of its action is related to the presence of other hormones. This situation clearly demonstrates one way by which the effects of a QTL may be dependent on the genetic environment. Since there is much information available concerning the genetic and hormonal determinants of sex, this system may be exploited in the development of a genetic model for growth parameters by combining information from somatotropic genes and sex-determining genes.

TABLE 12. Average additive gene substitution effects of IGF-2^{H5} and IGF-2^{HA} on 21-day and 42-day weights in the $5F_2$ population.

WEIGHT AT	EFFECT OF IGF-2^{H5}	EFFECT OF IGF-2^{HA}	σ^2
21 DAYS			
MALES	0.4689*	0.1010	0.2113
FEMALES	0.3928	0.0677	0.2512
42-DAYS			
MALES	-0.1264	0.0272	0.7852
FEMALES	1.1110*	-0.1916	0.6213

* indicates that the value is significant ($P<0.05$).

V.B. ANALYSIS OF 5Hi AND 5Lo

The allele frequencies for IGF-2^{H5} were determined for lines 5Hi and 5Lo after nine generations of selection for 42-day body weight. These allele frequencies are given in Table 13. The frequency of IGF-2^{H5} was greater in 5Hi, which had been selected for increased 42-day weight, than it was in the $5F_2$ population. There was no concomitant change in the frequency of IGF-2^{H5} with selection for decreased 42-day weight in 5Lo as compared with $5F_2$. These observations, in conjunction with the results reported for the $5F_2$ population, show that the IGF-2^{H5} haplotype acts as a QTL for increased body weight in Strain 5 mice. The absence of any change in 5Lo does not contradict this, when we take into consideration the initial low frequency of IGF-2^{H5} upon which negative selection is unlikely to have much effect.

TABLE 13. Frequency of the IGF-2^{H5} haplotype in $5F_2$ and MF_2 populations and their respective selected sublines.

		SELECTED SUBLINE	
POPULATION	F_2	HIGH	LOW
$5F_2$	0.16	0.39	0.18
MF_2	0.36	0.39	0.37

V.C. ANALYSIS OF MF_2, MHi AND MLo

The frequency of IGF-2^{H5} in the MF_2 population was 0.36; this frequency is much higher than that observed in the $5F_2$ population. The discrepancy in these values was unexpected since 5H, which was the source of IGF-2^{H5}, contributed equally to the two populations. This difference may have been due to sampling from the stock line or there may have been a change in the allele frequency in the stock line between the time of sampling for $5F_2$ and MF_2 four generations later. The analysis of 42-day weight, 21-day weight and postweaning growth rate revealed no effect ($P>0.05$) of IGF-2^{H} genotypic class on any of these traits in MF_2. Nor was there any change in gene frequency concomitant with divergent selection for 42-day weight in MHi or MLo following six generations of selection. It should be noted that the frequency of IGF-2^{H5} in the MF_2 population was of an intermediate value and, therefore, would likely have been affected by selection for a trait which it affected.

These results indicate that the IGF-2^{H} haplotypes do not act as QTLs for the studied growth traits in this population. This is particularly evident in the absence of any change in gene frequencies in the selected lines where selection would be expected to have a greater effect on alleles at the intermediate frequencies observed.

In contrast to the results obtained in the analysis of GH haplotypes, the response of the frequency of the IGF-2 haplotypes to divergent selection paralleled the effects of this haplotype in the F_2

populations. Comparing the results from $5F_2$ and MF_2, indicates that any further examination of the effects of these RFLPs on growth traits should be carried out in lines developed from the $5F_2$ population which appears to provide a more conducive genetic background for the expression of their effects.

V.D. ANALYSIS OF $2F_2$, 2Hi AND 2Lo

In the $2F_2$ population, the IGF-2^B variant, IGF-2^{B2}, from the stock line 2C, was tested for association with the growth traits measured in this study. The frequency of IGF-2^{B2} in the $2F_2$ population was 0.2755. Only one of the analyses carried out showed any significant ($P<0.05$) effect of the IGF-2^B genotypic class on a growth trait. The single instance where IGF-2^B was associated with the phenotypic variance of a trait was for 21-day weight measured as the deviation from the midparent. The results of this analysis are given in Table 14. The IGF-2^B genotypic class accounted for 6.4% of the total variance of this trait; the effect of sex was not significant. An examination of least squares means for the genotypic classes showed that the heterozygous class, IGF-2^{B2}IGF-2^{BA} had a somewhat lower value than either of the homozygous classes. However, the difference between the means of the heterozygous class and the class homozygous for the common haplotype, IGF-2^{BA}, was not significant and the class homozygous for the alternate allele, IGF-2^{B2}, was represented by only ten records. The results obtained with this analysis are inconclusive and there is no apparent

additive effect of IGF-2^{B2} on the growth traits examined in this population.

TABLE 14. Results of the analysis of variance on 21-day weights, measured as deviations from the midparent, in the $2F_2$ population.

SOURCE OF VARIANCE	DF	SS	P
SEX	1	18.80	0.2057
LITTER SIZE	1	193.99	0.0001
GENOTYPE	2	133.72	0.0040
RESIDUAL ERROR	142	1652.31	

The genotype refers to IGF-2^{B} genotypic classes.

There was a slight increase in the frequency of IGF-2^{B2} in the high 42-day weight line, 2Hi, and a slight decrease in the low 42-day weight line, 2Lo, after six generations of selection. However, the gene frequencies at the termination of the selection programme were not significantly different ($P>0.05$) from those at the commencement. Therefore, there seems to be no indication that further study of the IGF-2^{B} RFLP's in this population would be fruitful. It might be productive to examine the effects of IGF-2^{B} RFLPs in the MF_2 population and its derived selected lines where they should be present in conjunction with the IGF-2^{H} RFLPs.

VI. THE ASSOCIATION OF RFLPs FOR GROWTH HORMONE RELEASING FACTOR WITH BODY WEIGHT

The GRF variant haplotype, GRF^{HpV}, was expected to be present in all of the F_2 populations since it had originally been found in both the 2FP and 5H stock lines. The presence of this haplotype in the unselected line, 2FP, would not preclude a possible association with high growth parameters since this line was found to be the heaviest in the stock population. Because of the difficulty encountered in characterizing the complete RFLP patterns for GRF, homozygotes for the variant pattern and heterozygotes were grouped together. These two classes were distinguishable from the remaining homozygous class, $GRF^{HpA}GRF^{HpA}$, by the absence of the 6.7 kb band in the latter class. The frequency of GRF^{HpV} was, therefore, calculated indirectly assuming Hardy-Weinberg equilibrium in the F_2 populations. Estimated gene frequencies for GRF^{HpV} are given in Table 15. The estimated frequency of GRF^{HpV} was lowest in $5F_2$ (from 5H x 5C), intermediate in MF_2 (from 5H x 2FP), and highest in $2F_2$ (from 2H x 2FP). These data indicate that GRF^{HpV} was more common in the stock line 2FP than in 5H.

In none of the experimental F_2 populations was the presence of the GRF^{HpV} associated with the phenotypic variance of either 42-day weight or 21-day weight. In the $2F_2$ population, the presence of the GRF^{HpV} band was associated with a higher ($P<0.05$) rate of postweaning gain; the association was unique to this population. In the absence of any other association between the presence of the GRF^{HpV} haplotype and growth

traits in the experimental populations, it is suggested that GRF^{HpV} is not a QTL for 42-day body weight.

TABLE 15. Estimated frequency of the GRF^{HpV} haplotype in experimental F_2 populations and their respective selected sublines.

		SELECTED SUBLINE	
POPULATION	F_2	HIGH	LOW
$5F_2$	0.13	0.73	0.81
MF_2	0.21	0.26	0.29
$2F_2$	0.35	0.34	0.29

The results observed in the characterization of the GRF banding patterns in the divergently selected lines are also given in Table 15. No change in the frequency of occurence of the GRF^{HpV} haplotype was observed between the lines selected for high and low 42-day weight. The only lines which showed a change in the frequency of this haplotype in the selected lines as compared with the original F_2 populations were those derived from $5F_2$. Both the high and low 42-day weight selected lines derived from $5F_2$ showed a dramatic increase in the frequency of GRF^{HpV}. It is possible that this dramatic increase in the frequency of GRF^{HpV} haplotypes is due to interactive effects of the GRF haplotypes and other loci in the genome. These results support the conclusion that the GRF RFLPs studied have no effect on 42-day weight in these populations.

VII. THE EFFECTS OF INTERACTIONS BETWEEN RFLPs FOR SOMATOTROPIC GENES ON BODY WEIGHT

Data from the experimental F_2 populations, $5F_2$, $2F_2$, and MF_2, were analyzed to test for interactions between haplotypes for different somatotropins and their effects on the measured growth parameters. These analyses were carried out only as a preliminary study, since the small numbers of observations in the classes tested for the effects of interactions limit the possibility of accurate assessment. However, it was hoped that the results might give some indication for potentially fruitful venues to pursue in this area. If interactions between RFLPs for different somatotropic genes can be identified and defined, then these would provide a basis for building a model to determine which RFLPs act as QTLs and which RFLPs would make up part of the genetic environment conducive to the action of the QTLs. The model used to analyze the F_2 populations included genotypes for GH, IGF-2, and GRF as well as all possible interactions between genotypic classes.

In the analysis of the $5F_2$ population, only one interaction was noted and its effect was on 42-day weight ($P=0.08$). This interaction was between RFLPs for GH and IGF-2^{H}; both of which were found to be significantly ($P<0.01$) associated with 42-day weight in previous analyses. The interaction between these two loci was effected through an increase in the 42-day weight of the $GH^{C}GH^{C}$ genotypic class when the IGF-2^{H5} haplotype was present.

No interactions were found in the analysis of the MF_2 population using a level of significance of 0.1 as the criterion for accepting an effect as being associated with the trait analyzed.

The analysis of 42-day weight in the $2F_2$ population using the model including all loci and all possible interactions between loci accounted for 93% of the total variance. Interactions between GH and GRF genotypes (P=0.06) and between GRF and IGF-2^B genotypes (P=0.03) were noted. Previous analyses had revealed no effect (P>0.9) of the GRF genotypic class alone on 42-day weight. The 42-day weight of the GH^CGH^C genotypic class was greater in the presence of the GRF^{HpV} variant band than in its absence. The interaction between GRF and IGF-2^B haplotypes was such that higher 42-day weights were observed for the classes carrying either both or neither of the variants than in classes carrying one variant and one common haplotype.

Due to the small population sizes involved in assessing the effects of interactions between the loci studied, the results cannot be considered definitive. However, there is some indication that further study on these interactions may be beneficial. It should, moreover, be possible to find RFLPs for most known somatotropic genes and their receptors. The simultaneous analysis of a number of somatotropic loci would reveal whether any of them act as QTLs for growth traits in a concerted manner. If this is the case, then we could develop breeding schemes with the view of developing the best combinations of QTLs.

CONCLUSIONS

For this study, we utilized nine lines of mice from three genetically diverse strains. The mice were acquired from the Lacombe Agricultural Research Station (Lacombe, Alberta) where at least one line from each strain had been subjected to long-term selection for high 42-day body weight. The remaining lines were maintained as unselected controls. When we began our experiment, the lines had been maintained with a random mating scheme without selection for 20 to 30 generations following the termination of selection.

Prior to the commencement of this study, Salmon et al (1988) found between-line RFLPs for the GH gene. One of the GH haplotypes, GH^h, was found exclusively in the selected lines. The GH^h haplotype was present in all four selected lines; in three of these it was fixed. It was postulated that the GH^h haplotype might act as a QTL for high 42-day body weight in mice.

In the first stage of this study, we tested four more somatotropic genes for RFLPs in the stock mouse lines. Polymorphisms were found for two of these, IGF-2 and GRF. No polymorphisms were found for either IGF-1 or somatostatin, although these were subjected to limited testing only and the possibility of polymorphisms at these loci should be further explored. In addition to the polymorphisms found here for IGF-2 and GRF, the RFLPs previously found for GH were included in the second and third stages of this study. Genes for numerous other somatotropins and hormones known to interact with somatotropins as well as receptors for

many of these are available; the possibility of RFLPs at these loci warrents investigation.

In the second stage of this study, associations between RFLPs for polymorphic somatotropins and growth parameters were tested in F_2 populations derived from crosses between selected and unselected lines of mice. We proposed that the GH^h haplotype which was fixed in three of the four selected lines in the stock population would segregate with high 42-day weight in the F_2 populations. Furthermore, testing of RFLPs for IGF-2 and GRF in the F_2 populations would allow us to determine if haplotypes for either of these genes might act as QTLs for any of the weight parameters studied. Significant ($P<0.05$) associations were found for both GH and IGF-2 in these analyses. For each of these genes, a significant additive gene substitution effect on 42-day weight was observed for at least one haplotype. However, both the magnitude and the direction of the gene substitution effect of a given haplotype was found to depend on the population in which it was tested. GH^h was found to be associated with lower 42-day weight in three of the F_2 populations and with higher 42-day weight in the remaining F_2 population. This effect of the GH^h haplotype which we had supposed would segregate with high 42-day weight we now believe to be due to interactions between the haplotype and the unselected or mixed genetic background of the F_2 populations. The IGF-2^{H5} haplotype which was originally found exclusively in a single selected line was associated with high weight in one of the F_2 populations derived from this line. The association between IGF-2^{H5} and body weight was sex-dependent with females carrying IGF-2^{H5} being

heavier at 21 days of age and males carrying IGF-2^{H5} being heavier at 42-days of age. There was some indication that haplotypes of GRF^{HpV}, which by themselves were not correlated with weight, did influence the effects of the haplotypes for the other genes studied. All of these observations indicate that the potential for determining the effects of isolated QTLs may be limited because of variable effects due to different genetic backgrounds. Such effects may be of particular importance in evaluating markers such as those used here which may, in fact, be involved in hormonal interactions. In such cases, it may be more promising to attempt to identify interactive QTLs which could, in essence, define the necessary genetic background. Moreover, such studies might provide more information on how somatotropins regulate growth traits.

Lines selected for 42-day weight developed from the the F_2 populations were analyzed for changes in gene frequency associated with selection. We believed that, by reconstructing a high weight selected genetic background, we would reestablish the positive association between GH^h and 42-day weight. Therefore, we predicted that the frequency of the GH^h haplotype would increase in lines selected for high body weight. We further postulated that the frequency of the IGF-2^{H5} haplotype would increase in the high weight selected line derived from the F_2 population in which it was found to be associated with high weight. Changes in the frequency of the GH haplotypes were correlated with selection for high and low 42-day weight and completely in accordance with our predictions. The GH^h and IGF-2^{H5} both increased in

the high selected lines. No changes in the frequency of GRF RFLPs were correlated with selection for high or low 42-day weight; both selected lines derived from $5F_2$ showed an increase in the frequency of the variant GRF haplotype while none of the other selected lines showed any change.

These results stress the importance of evaluating QTLs in the appropriate genetic background. It also seems that if we could employ a battery of RFLPs for genes known to be involved in the somatropic axis, we might be able to define a number of highly productive genotypes on the basis of interactions between loci. Identification of optimum genotypes which might serve as selection goals gives more potential to the application of identified QTLs as selection criteria than would single identified QTLs and would also maintain polymorphisms in the population which might provide genetic diversity for later selection. Defined interactions between somatotropic loci which affect growth parameters might be directly applicable to livestock species. The potential for defining effective genotypes for other economically important growth related traits such as feed efficiency or fat deposition also exists within our model system.

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