CHARACTERIZATION OF DNA POLYMORPHISMS IN THREE POPULATIONS OF HEREFORD CATTLE AND THEIR ASSOCIATIONS WITH GROWTH AND MATERNAL TRAITS IN USDA LINE 1 HEREFORDS

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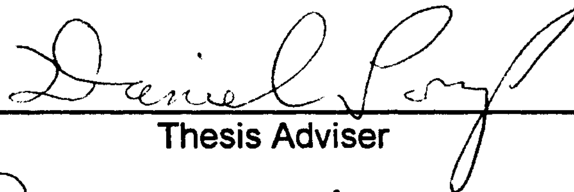

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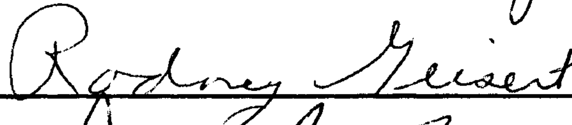

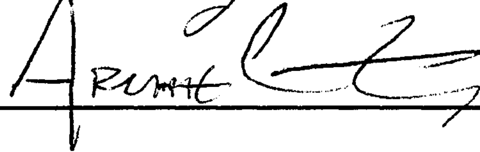

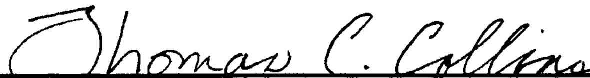

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LIST OF NOMENCLATURE

B-Lac beta lactoglobulin

BWT birth weight

D_A disequilibrium coefficient

EPD expected progeny difference

GH growth hormone

GHR growth hormone receptor

H_I heterozygosity coefficient

IGF-I insulin-like growth factor I

K-Cas kappa casein

LSM least squares means

MAS marker assisted selection

MILK maternal ability

MTDFREML multiple trait derivative free reduced maximum likelihood

PCR polymerase chain reaction

PCR-RFLP polymerase chain reaction based restriction fragment length polymorphism

PIT1 pituitary transcriptional activator 1

PRL prolactin

QTL quantitative trait loci

RFLP restriction fragment length polymorphism

SSCP single stranded conformational polymorphism

WWT weaning weight

YWT yearling weight

CHAPTER 1

REVIEW OF THE LITERATURE

Hormonal Regulation of Growth

Introduction

Growth is a complex, highly integrated process involving interactions among nutrients, environment, genotype, enzymes, and many different metabolic hormones. Interrelationships among hormones, as well as the interaction between circulating hormones and their receptors, are critical factors involved in normal growth and development. Hormonal stimulation and regulation of growth are primarily carried out by growth hormone and the somatomedins, which will be the focus of this chapter. However, production of these hormones and their receptors is regulated by many other hormones. Table 1 summarizes many of the effects of hormones which have regulatory roles in the growth axis.

Table 1. Summary of hormonal regulators of growth[a].

Hormone	Biological action regarding growth
Thyroid Hormones	• Influence GH synthesis and secretion • Regulate somatomedin receptors • Hypothyroidism and hyperthyroidism cause growth retardation
Insulin	• Permissive role in growth • Regulate hepatic GH receptors • Important effects on levels of other hormones and their receptors
Glucocorticoids	• Inhibit growth in immature animals • Inhibit DNA synthesis in liver, muscle, kidney and heart • Increase fractional degradation rate in muscle
Androgens	• Accelerate linear growth and weight gain • Increase muscle growth • Stimulate epiphyseal plate closure
Estrogens	• Increase basal and stimulated GH levels • Decrease somatomedin levels • Stimulate epiphyseal plate closure

[a]References are presented in review by Spencer (1985).

Growth Hormone

Identification. Evans and Long (1921) first suggested a growth-promoting action of a pituitary derived factor. This factor, now known as Growth Hormone (GH) or somatotropin, was isolated from bovine pituitary by Li and Evans (1944), and from human pituitary by Li and Papkoff (1956) and Raben (1957). GH is a 22,000 dalton single-chain polypeptide protein (Woychik et al., 1982).

Human GH has been described as representing a family of proteins with heterogeneity resulting from differences in alleles, posttranscriptional events, posttranslational events, postsecretory events, and metabolic conversions, as reviewed by Baumann (1991). Seavey et al. (1971) reported two forms of bovine

GH. The A and B forms were characterized by a leucine and valine amino acid, respectively, at amino acid position 127. A variant bovine mRNA, resulting from missed splicing of the last intron and a frameshift in the last exon, was reported by Hampson and Rottman (1987). However, the predicted higher molecular weight protein has not been isolated.

The three dimensional structure of GH has been described in porcine (Abdel-Meguid et al., 1987) and in humans (de Vos et al., 1992). GH is characterized as a four-helical bundle. The helices are arranged in an up-up-down-down configuration, which differs from the more usual up-down-up-down configuration. Porcine and human GH configurations differ at the connections between helices 1 and 2 (although this observed difference may be a result of receptor binding in human GH), and the connection between helices 2 and 3 (de Vos et al., 1992). The primary structures of GH show a high degree of homology among higher vertebrates as previously compared by Palanivelu and Dharmalingam (1993).

Growth Hormone Gene. The bovine GH (bGH) gene has been sequenced by Woychik et al. (1982) and Gordon et al. (1983). The bGH gene is approximately 1.8-kb and is composed of five exons (13, 161, 117, 162 and 201 bp each) and four introns (248, 227, 229 and 274 bp each). The gene has been assigned to bovine chromosome region 19q26-qter by Hediger et al. (1990).

Several restriction fragment length polymorphisms (RFLPs) in the bGH gene have been identified in different breeds of cattle. These RFLPs represent two different polymorphisms in the bGH gene: an insertion/deletion of approximately 0.9-kb in the 3' region of the gene, and a polymorphic MspI restriction site in the third intron (Hoj et al., 1993). These bGH RFLPs are summarized in Table 2.

Table 2. Summary of bGH RFLPs identified in cattle.

Restriction Enzyme	Breed	Reference
BglII, BamHI, EcoRI, HindIII	Holstein-Baladi x Hereford-Simmental-Charolais	Hallerman et al. (1987)
PstI, PvuII, TaqI, MspI, BglII	Holstein	Cowan et al. (1989)
BglII, BamHI, EcoRI, EcoRV, MspI, PstI, PvuII, TaqI	Belgian Blue	Hilbert et al. (1989)
TaqI	Angus, Brahma, Hereford, Holstein, Jersey	Rocha et al. (1992a)
BglII, DraI, EcoRV, MspI, PstI, PvuII, TaqI, XhoI	Red Danish	Hoj et al. (1993)

Polymerase chain reaction (PCR) has also been used to detect polymorphisms in the bGH gene. Kirkpatrick (1992a) reported a single strand conformation polymorphism (SSCP) in the fourth intron. A polymorphic MspI restriction site in PCR products was described by Hoj et al. (1993) and Zhang et al. (1993). Lucy et al. (1991) and Zhang et al. (1992) described another PCR-based method using the restriction enzyme AluI to distinguish the A and B forms of bGH originally described by Seavey et al. (1971).

Secretion of Growth Hormone. GH is secreted by somatotropic cells of the the anterior pituitary. The regulation of GH is primarily controlled by two hormones secreted by the hypothalamus: Growth Hormone Releasing Factor (GRF), which stimulates GH secretion, and Growth Hormone Release Inhibitory Factor (Somatostatin; SRIF), which inhibits GH secretion (Buonomo and Baile, 1990).

SRIF was first identified by Krulich et al. (1968) and was further purified and sequenced from ovine pituitary by Brazeau et al. (1973). SRIF is found in

high concentrations in the central nervous system (CNS), pancreas and gut. Administration of SRIF blocks secretion of endogenous as well as GRF-stimulated GH secretion, while immunoneutralization against SRIF increases plasma GH concetration (see Buonomo and Baile, 1990).

The presence of GRF was first described by Deuben and Meites (1964), and the GRF protein was sequenced from pancreatic tumor tissues by Guillemin et al. (1982) and Rivier et al. (1982). Ling et al. (1984) showed that human hypothalamic GRF was identical to pancreatic GRF. Administration of exogenous GRF stimulated growth in rats, GH-deficient children and swine, while immunoneutralization against GRF resulted in decreased GH and circulating IGF-1 concentrations in cattle, as well as decreased growth in rats and cattle (see Buonomo and Baile, 1990).

GH is secreted in a pulsatile manner which is regulated by episodic secretions of GRF and SRIF (Frohman et al., 1990). GRF peaks measured from the hypophyseal portal system were significantly correlated with GH peaks in sheep, but no correlation was observed between SRIF troughs and GH peaks (Frohman et al., 1990). These results indicate that GRF is a stronger regulator of pulsatile GH release than SRIF in sheep (Buonomo and Baile, 1990). The secretion of GRF and SRIF is regulated by a number of factors (see Buonomo and Baile, 1990, for review) including: neurotransmitters (histamine, acetylcholine, gamma amino butyric acid (GABA), and serotonin), neuropeptides (vasoactive intestinal peptide (VIP), motilin, galanin, substance P, and neurotensin), opiod peptides, α–adrenergic agonists, cytokines (tumor necrosis factor alpha, interleukin-1β, and interleukin-6), and glucocorticoids. GH is also capable of regulating its own secretion through negative feedback on the hypothalamus (Tannenbaum, 1980), although the exact mechanism of this feedback is not known. Long term negative feedback may also be carried out by

IGF-I on the hypothalamus and pituitary (Buonomo and Baile, 1990). Finally, GH secretion is influenced by changes in nutritional or metabolic status (Driver and Foster, 1981). Effects of these regulators on GH regulation are summarized in Table 3.

Table 3. Regulators of GH secretion[a].

Regulator	Effect on GH Secretion	Mechanism of Regulation
Serotonin	increase	increase GRF
Dopamine	decrease	increase SRIF
Opiod peptides	increase	increase GRF
α-adrenergic agonists	permissive	increase GRF
Cytokines	various	hypothalamus and pituitary
Glucocorticoids	decrease	increase SRIF
Growth Hormone	decrease	negative feedback at hypothalamus
Hypoglycemia	decrease	increase SRIF
Free Fatty Acids	decrease	hypothalamus and pituitary
Restricted feeding*	increase	decrease SRIF, effects pituitary

[a]References are presented in review by Buonomo and Baile, 1990.
*opposite effect observed in rats

Biological Actions of GH

Metabolic. GH has regulatory actions on metabolism and growth. Metabolic effects of GH may be described as insulin-like and insulin-antagonistic (diabetogenic) for acute and chronic effects, respectively (see Press, 1988). Acute, insulin-like effects of GH are observed 1 to 2 hours after GH administration and are more apparent in GH deficient animals. It has been suggested that normal levels of endogenous GH prevent the expression of insulin-like effects of GH (Isaksson et al., 1985). Some transient effects

observed as a result of GH administration in GH deficient animals include an increase in amino acid incorporation into proteins, a decrease in lipolysis and free fatty acids, and a decrease in plasma glucose (Press, 1988).

Chronic elevation of GH levels results in increases in insulin-like growth factors (IGFs) and insulin. IGF is known to be a mediator of growth stimulating effects of GH (see below), and it has been suggested that insulin may be a mediator of the anabolic effects of GH. Many of the metabolic changes observed after chronic elevation of GH levels are summarized in Table 4.

Table 4. Metabolic effects of GH[a].

Tissue	Physiological process affected
Adipose	↓ Glucose uptake and glucose oxidation ↓ Lipid synthesis if in positive energy balance ↓ Lipogenic enzyme activity ↑ Basal lipolysis if in negative energy balance ↓ Insulin stimulation of glucose metabolism ↑ Catecholamine-stimulated lipolysis ↑ Ability of insulin to inhibit lipolysis
Liver	↑ Glucose output ↓ Ability of insulin to inhibit gluconeogenesis
Intestine	↑ Absorption of Ca and P required for lactation or growth ↑ Ability of 1,25 vitamin D3 to stimulate calcium-binding protein
Systemic effects	↓ Blood urea nitrogen ↓ Glucose clearance ↓ Glucose oxidation ↓ Insulin sensitivity ↑ FFA oxidation if in negative energy balance

[a]Adapted from Etherton and Smith (1991).

Growth. Salmon and Daughaday (1957) showed in hypophysectomized rats that increased sulphation of costal cartilage, which was known to be closely correlated with growth rate, was stimulated by serum from GH treated animals, but not from GH itself. The unknown "sulphation factor" was later termed

"somatomedin" (Daughaday et al., 1972), and has since been identified as insulin-like growth factor I (IGF-I; Klapper et al., 1983). Since this work, the somatomedin hypothesis, which suggests the growth promoting actions of GH are carried out by circulating somatomedins rather than GH itself, has been widely accepted.

Although many of the growth promoting effects of GH are known to be carried out by increased levels of plasma somatomedins, direct stimulatory effects of GH have been demonstrated. Isaksson et al. (1982) and Russell and Spencer (1985) demonstrated that hGH administered directly into the cartilage growth plate of the proximal tibia of hypophysectomized rats resulted in an increase in unilateral longitudinal bone growth. Overall growth in the treated animals did not increase, indicating the observed response was not caused by indirect effects of GH on serum somatomedins. It was suggested that the increased bone growth was a result of increased cell proliferation in the growth plate caused by GH. Lindahl et al. (1986) showed that GH can potentiate the formation of large size chondrocyte colonies from chondrocyte cells isolated from epiphyseal plates of tibia of normal rats. The authors suggested that GH stimulates differentiation of epiphyseal chondrocyte stem cells or early proliferative chondrocytes, supporting previous evidence for a direct stimulatory effect of GH on longitudinal bone growth. GH was also shown to stimulate DNA synthesis in cultured chondrocytes from rabbit ear and rat rib growth cartilage (Madsen et al., 1983). Finally, the visual demonstration of GH receptors on human growth plate chondrocytes by Werther et al. (1990) provides additional evidence for direct actions of GH on chondrocytes.

<u>Circulating Growth Hormone Concentrations and Growth</u>. Serum GH concentrations have been shown to be correlated with growth in cattle. Verde and Trenkle (1987) found significantly higher serum GH concentrations in steers

with a high genetic potential for growth as compared to steers with a low genetic potential for growth. Similar results were observed by Ohlson et al. (1981) where higher GH concentrations were found in Simmental than Hereford bulls, and Grigsby and Trenkle (1986) where Simmental were reported to have higher GH concentrations than Angus or Limousin steers.

Arbona et al. (1988) compared GH secretory patterns and metabolic clearance rate between Landrace pigs selected 3 generations for increased 200-day weight, and randomly selected Landrace controls. Significantly greater baseline GH concentrations were observed in the selected pigs compared to the controls, but no differences in overall mean concentrations of GH, frequency of secretory episodes, amplitude of GH peaks or metabolic clearance rate were observed. Norton et al. (1989) studied GH plasma concentrations in two lines of pigs divergently selected for slow growth rate and fast growth rate for 5 generations. In contrast to the results reported by Arbona et al. (1988), Norton et al. (1989) observed significantly greater mean GH concentrations and plasma GH profiles in the line selected for slow growth rate compared to the line selected for fast growth rate.

Goddard et al. (1988) measured circulating GH concentrations in broilers selected for an increase in growth, broilers in which selection pressure was relaxed, and in White Leghorns. Goddard et al. (1988) found the highest GH levels in lines of birds with the slowest growth rate. Results from this study also indicated that selection within a genotype for a higher growth rate resulted in lower mean plasma GH levels. In reviewing related studies, Goddard et al. (1988) concluded that heavy lines have lower plasma GH levels regardless of the mode of increased growth (i.e. selection, introduction of a dwarfing gene, or natural genotype differences).

Pidduck and Falconer (1978) studied the effects of the dwarf gene *dw*, which causes hypopituitary dwarfism and a lack of GH, on GH status in three lines of mice previously selected for divergent growth rates. Repeated backcrossing was used to introgress the *dw* gene into lines of mice selected 21 generations for high or low 6-week body weight, and non-selected controls. Results indicated that increased growth in the large strain was partially due to an increase in amount or activity of circulating GH, while the decrease in growth observed in the small strain was due to a reduced sensitivity of target organs to GH.

Medrano et al. (1991) investigated the effects of the high growth gene (*hg*) on circulating and pituitary GH levels in three different genetic backgrounds of mice. The study also considered the effects of the *hg* gene on plasma levels of insulin-like growth factor I (IGF-I). The *hg* gene dramatically increases postweaning growth rate and mature size (Bradford and Famula, 1984) without significantly altering overall body composition (Calvert et al., 1985) in mice. Results from this study showed that mice carrying the *hg* gene had decreased levels of pituitary and plasma GH, and GH was not secreted in the normal pulsatile pattern. It was also observed that the interaction between different genetic backgrounds (selected and non-selected for high growth) and the *hg* gene was significant. This interaction may have resulted from negative feedback of increased IGF-I levels on GH production, or from other genetic mechanisms which developed due to selection pressures and interacted with the *hg* gene to regulate GH production.

Elevated GH Levels. Exogenously administered GH has been shown to stimulate nitrogen retention and body weight gain in swine, sheep and cattle (Davis et al., 1970; Machlin, 1972; Moseley et al., 1982; Muir et al., 1983; Chung et al., 1985; Etherton et al., 1987; Johnsson et al., 1987; Beermann et al., 1990;

Hancock and Preston, 1990; Smith and Kasson, 1990; Moseley et al., 1992). Average daily gain and feed efficiency improve in cattle treated with bGH, but studies have reported variability in the magnitude of response due in part to different management conditions among studies (Moseley et al., 1992). Average daily gain and feed efficiency in cattle are improved by bGH administration in either the growing or finishing phase (Moseley et al., 1992).

The development of transgenic animals has provided another method by which to study the effects of increased levels of GH. Palmiter et al. (1982) reported the production of transgenic mice which expressed high levels of rat GH from a mouse metallothionein I promoter-rat GH construct. These mice grew at a faster rate to a final size 1.9-fold larger than non-transgenic controls. Since then, size increases of 1.9-fold to 4.0-fold have been reported in mice expressing transgenes for rat, human and ovine GH (see Brem et al., 1989). Pomp et al. (1992) reported increased growth as well as increased efficiency of growth and lean tissue production in mice expressing a sheep metallothionein 1a-sheep GH transgene.

The introduction of a fusion gene containing human GH linked to a murine metallothionein promoter into transgenic rabbits, sheep and pigs was reported by Hammer et al. (1985). None of these animals grew at increased rates despite expression of the hGH transgene. Out of six transgenic pigs, Vize et al. (1988) reported one pig with increased growth due to the expression of a human metallothionein-IIA-porcine GH gene construct.

Pursel et al. (1989) studied the long term effects of elevated plasma GH levels in two lines of pigs expressing a bGH transgene. Two successive generations displayed significant improvements in daily weight gain and feed efficiency, and exhibited changes in carcass composition including a marked reduction in subcutaneous fat. However, the long term elevation of bGH was

detrimental to the health of the pigs as they demonstrated increased incidences of gastric ulcers, arthritis, cardiomegaly, dermatitis, and renal disease. The authors concluded that better control of the transgene, a different genetic background, or a modified husbandry regimen would be necessary in order to gain the beneficial, growth-promoting effects of elevated GH without a damaging decline in health and fitness of the animals (Pursel et al., 1989).

Pituitary Transcriptional Activator Pit-1

Required for GH and Prolactin Synthesis. Bodner and Karin (1987) first identified the pituitary cell-type specific factor GHF-1 as a transcription factor necessary for the expression of GH. GHF-1 was shown to bind to the promoter of the hGH gene at two sites upstream of the hGH TATA box. Binding of GHF-1 to both sites was necessary for maximal expression of hGH, suggesting that an interaction between GHF-1 molecules was required for optimal stimulation of transcription.

Nelson et al. (1988) used competition experiments to show that a single transcription factor was capable of activating both GH and Prolactin (PRL) genes. A comparison of the sequences of GH and PRL promoter elements and their binding affinities revealed a consensus sequence with a core of TATNCAT in both genes. The authors suggested that a common pituitary transcriptional activator, termed pituitary transcriptional activator-1 (Pit-1), recognized the consensus sequence and allowed the expression of GH and PRL by anterior pituitary somatotroph and lactotroph cells, respectively.

Conflicting results were observed by Castrillo et al. (1989). GHF-1 was purified from extracts of GH and PRL-expressing pituitary tumor cells and

identified as a 33 kilodalton polypeptide. DNase I footprinting experiments showed significant binding of GHF-1 to the GH promoter, but failed to show binding of GHF-1 to the PRL promoter. In contrast to the conclusions made by Nelson et al. (1988), Castrillo et al. (1989) suggested that two separate factors control the expression of GH and PRL genes.

The cDNA sequences of GHF-1 and Pit-1 were independently determined by Bodner et al. (1988) and Ingraham et al. (1988), respectively. Bodner et al. (1988) purified the GHF-1 protein to near homogeneity and determined a partial amino acid sequence. Complete cDNA sequences for the coding regions of bovine and rat GHF-1 were derived from cDNA libraries screened with synthetic oligonucleotide probes corresponding to the partial GHF-1 amino acid sequence. Ingraham et al. (1988) sequenced DNA complementary to Pit-1 mRNA, cloned on the basis of specific binding to AT-rich cell-specific elements in the rat PRL and GH genes. The cDNA sequences for GHF-1 and Pit-1 were identical. In accordance with recent literature, this pituitary specific transcription factor will hereby be referred to as Pit-1.

Homeobox-containing Protein. The Pit-1 cDNA sequence included an 873 nucleotide open reading frame encoding a 291 amino acid protein with a predicted molecular weight of 32.9 kilodalton (Ingraham et al., 1988). Identity between amino acid sequences predicted by rat and bovine cDNA's was 93% (Bodner et al., 1988). Comparison of the Pit-1 amino acid sequence with other known DNA binding proteins revealed a 60 amino acid sequence at the carboxyl terminus of Pit-1 that exhibited statistically significant similarity with several homeobox-containing proteins (Bodner et al., 1988; Ingraham et al., 1988). Homeobox-containing proteins have been identified as important gene regulators in Drosophila, and sequences homologous to the homeobox have been isolated from higher organisms (Gehring, 1987). The homeobox domain

encoded by homeotic genes mediates binding to specific DNA sequences. This allows the homeotic proteins to have a gene regulatory function which has been shown to specify the identity and spatial arrangement of body segments in Drosophila (Gehring, 1987).

The greatest similarity between Pit-1 and other homeobox-containing genes was found at the C-terminal one-third of the homeobox, which is the most highly conserved region among all known homeoboxes (Gehring, 1987). The entire Pit-1 homeobox displayed 73% identity with a homeobox consensus sequence derived from a large number of homeoboxes from different species (Bodner et al., 1988). This identity increased to 78% if conservative amino acid changes were taken into consideration. No significant similarities between Pit-1 and other homeobox-containing proteins were observed outside the homeobox domain (Bodner et al., 1988; Ingraham et al., 1988).

Member of the POU-domain Family. Pit-1 was further characterized as a member of the POU-domain family of genes, which was originally named for sequence similarity among the three mammalian proteins Pit-1, Oct-1 and Oct-2 and in the product of the unc-86 gene of the nematode *Caenorhabditis elegans* by Herr et al. (1988). The POU-domain is a 150- to 160-amino-acid long region comprised of two conserved subdomains (Herr et al., 1988; Rosenfeld, 1991); the POU-specific (POU_S) subdomain and the homeobox-related (POU_{HD}) subdomain previously described (Bodner et al., 1988; Ingraham et al., 1988). The POU_S subdomain is the region that most strongly characterizes the POU proteins as a distinct class and may have been conserved by functional constraints on the protein products (Herr et al., 1988). Unlike the homeobox-containing proteins which interact with DNA at only one location, POU-domain proteins contact DNA recognition sites at two locations, the POU_{HD} and the POU_S domains. Rosenfeld (1991) suggested that the POU_S domain interacts

with the sequence motif TATNCA while the POU_{HD} domain interacts with A/T rich sequences 5' of the TATNCA core of target DNA sequences. Both subdomains are required to permit high-affinity, site specific binding of POU proteins to their target DNA sequences (Rosenfeld, 1991). POU-domain proteins have also been implicated in early developmental regulation of gene transcription, the proliferation of specific cell types, progression and commitment events in organogenesis, and the stimulation of DNA replication (Rosenfeld, 1991).

Involvement in Expression of Other Genes. Expression of Pit-1 was originally thought to be limited to GH-secreting (somatotroph) and PRL-secreting (lactotroph) cells of the anterior pituitary (Bodner et al., 1988; Ingraham et al., 1988). Simmons et al. (1990) demonstrated that Pit-1 is also expressed in thyrotropin (TSH)-secreting cells (thyrotrophs). In contrast to the requirement of Pit-1 before GH and PRL expression by somatotrophs and lactotrophs, expression of TSH precedes expression of Pit-1 in thyrotrophs (Simmons et al., 1990). Steinfelder et al. (1992) provided evidence that Pit-1 is involved in the regulation of TSH expression by mediating thyroliberin (TRH) and cAMP stimulation of the TSHβ gene. The mechanism that allows Pit-1 regulation involves phosphorylation of Pit-1 for increased binding of Pit-1 to at least two Pit-1 binding sites in the TSHβ gene. Binding of phosphorylated Pit-1 allows for hormonal responsiveness to TRH and cAMP and increased expression of the TSHβ gene (Steinfelder et al., 1992).

Pit-1 has also been implicated in the expression of the receptor for GRF (Lin et al., 1992). GRF receptor transcripts were first detected two days after the first detection of Pit-1 transcripts, and were found only in tissues known to express Pit-1. GRF receptor transcripts were not detected in a line of *dw/dw* dwarf mice known to lack Pit-1. Lin et al. (1992) suggested that pituitary

hypoplasia observed in *dw/dw* mice was due in part to the absence of GRF receptor which was caused by a lack of functional Pit-1.

Mutations and Polymorphisms in the Pit-1 Gene. Several reports of mutations in the Pit-1 gene have resulted in combined pituitary hormone deficiencies. Homozygosity for a nonsense mutation in the Pit-1 gene caused cretinism along with hormone deficiencies of thyrotropin, GH, and PRL in humans (Tatsumi et al., 1992b). Three different Pit-1 point mutations were identified in the transactivation region, POU_S and POU_{HD} domains of three children with combined pituitary hormone deficiencies (Ohta et al., 1992). Pfaffle et al. (1992) reported a mutation in the POU_S domain of the Pit-1 gene which resulted in a protein capable of binding DNA response elements but unable to activate target genes, resulting in deficiencies of GH, PRL, and TSH. The mutant Pit-1 was capable of initiating other programs of gene activation required for the normal proliferation of somatotroph, lactotroph and thyrotroph cells. A different mutation in the POU_{HD} domain of Pit-1 identified by Radovick et al. (1992) produced a mutant protein that would bind DNA normally, but inhibited Pit-1 action in the pituitary of two strains of dwarf mice, resulting in hypoplasia of GH, PRL, and TSH secreting cells.

The Pit-1 gene has also been investigated for use as a genetic marker in livestock species. Tuggle et al. (1993) used a partial Pit-1 POU-domain cDNA probe to screen for RFLPs in different breeds of swine. Polymorphic BamHI fragments were found in the Chinese breed Meishan, but no polymorphisms were identified in five American breeds of swine. Polymorphic MspI bands were reported by Yu et al. (1993) in the Chinese breeds Fengjing, Meishan and Minzhu as well as the American Yorkshire breed using the same Pit-1 probe as described by Tuggle et al. (1993).

Growth Hormone Receptor

Identification. In order for GH to produce a biological response, it must bind with a receptor at a target tissue. There are at least three distinct but related families of receptors which bind GH and/or the structurally related hormones PRL and placental lactogen (PL) to carry out a physiological response (Roupas and Herington, 1989). These receptors may be identified as the somatotrophic receptor, the lactogenic receptor and the PL receptor. This review will focus on the somatotrophic receptor (GHR) because of its physiological effects on growth and metabolism.

GHR has been identified in a variety of tissues including adipose, heart, kidney, liver, fibroblasts, lymphocytes, muscle and ovary (see Roupas and Herrington, 1989). GHR has also been identified on human growth plate chondrocytes (Werther et al., 1990), and Glimm et al. (1990) provided evidence for the expression of GHR by lactating mammary tissue. GHR appears to be highly conserved across species with 84% amino acid identity observed between rabbit and human GHR amino acid sequences (Leung et al., 1987), and 70-80% amino acid similarty in bovine GHR compared to human, rabbit, mouse and rat GHR (Hauser et al., 1990). GHR contains distinct extracellular, transmembrane and intracellular components and is not similar in amino acid sequence to previously sequenced proteins, suggesting that GHR belongs to a unique family of transmembrane receptors (Leung et al., 1987).

GHR gene. Amino acid and nucleotide sequences have been determined for GHR mRNA in bovine (Hauser et al., 1990), ovine (Adams et al., 1990) and rat (Mathews et al., 1989). The complete human GHR gene has been described by Godowski et al. (1989). The human gene for GHR spans at least 87-kb

(Barton et al., 1989). Nine exons (numbered 2-10) encode the coding and 3' untranslated regions of the gene. Exons 2-9 range in size from 66 to 179 bp, while exon 10 is approximately 3400 bp. The putative secretion signal sequence for human GHR is encoded by exon 2; exons 3 to 7 are translated to the extracellular GH binding region; exon 8 encodes the transmembrane domain; and exons 9 and 10 encode the cytoplasmic domain and 3' untranslated region (Godowski et al., 1989). Abnormal GHR genes have been identified in human patients with Laron-type dwarfism (Godowski et al., 1989; Berg et al., 1993) and in chickens with sex-linked dwarfism (Burnside et al., 1992). The GHR gene has been mapped to human chromosome 5p14-p12 (Davisson et al., 1991) and chromosome 16 in pigs (Chowdhary et al., 1994).

Regulation of GHR Expression. Regulation of hormone receptors is an important regulator of hormone action. The regulation of GHR expression has been studied in a variety of species and tissues with many conflicting results. Stage of development, level of nutrition, insulin and GH itself all appear to have an effect on the regulation of GHR.

Mathews et al. (1989) reported developmental regulation of GHR in rat liver, kidney, heart and muscle tissue. Expression of GHR was significantly lower at birth than in adults. Expression increased through neo-natal development to reach a maximum at 5 to 8 weeks after birth for liver, kidney and heart tissues, while increased expression continued through post-pubertal development for muscle tissue. No significant differences in liver GHR were observed between male and female rats, although higher levels of GHR were observed in the livers of pregnant females. Earlier studies reviewed by Roupas and Herington (1989) cited similar evidence for developmental regulation of hepatic GHR, as well as a lack of difference between male and female rat adipocyte GHR. However, other studies cited in the same review reported

increased hepatic GHR in females compared to males for both mice and rats. Developmental regulation of GHR in rabbits indicates an increase in hepatic GHR during puberty with additional increase of GHR during pregnancy, but no sex differences observed for hepatic GHR binding. In contrast, ovine livers did not show an increase in GHR during the latter part of gestation (see Roupas and Herrington, 1989).

Nutritional status has been implicated in the regulation of GHR. Hepatic GHR capacity decreases during fasting in rats, and is restored upon refeeding (Baxter et al., 1981; Maes et al, 1983; Postel-Vinay et al., 1982). Breier et al. (1988a) examined the effects of low and high planes of nutrition on the presence of GHR in steers. It was observed that high-affinity GHR were present in steers on the high plane of nutrition, but not in steers on the low plane of nutrition. In steers fed for a high plane of nutrition, weight-gain was proportional to the high-affinity GHR, and a single injection of bGH produced an increase in circulating IGF-1 concentrations (Breier et al., 1988b), confirming the biological function of the high-affinity GHR.

Insulin has been implicated in the control of GHR, although its role has not been well defined. A decrease in insulin has been shown to result in decreased liver GHR in rats with chronic renal insufficiency (normal circulating GH and PRL, but decreased insulin; Finidori et al., 1980); and in rats with hypoinsulinemia due to fasting (Baxter et al., 1981). Baxter et al. (1980) showed that the loss of rat hepatic GHR that occurs during fasting may be reversed by insulin.

GH is involved in the regulation of its own receptor, but studies have presented conflicting observations concerning this regulation. Many of these studies were reviewed by Roupas and Herington (1989) and are summarized in Table 5. Roupas and Herington (1989) suggested that GH has a long-term role

in the maintenance of GHR, but recommended using caution to interpret observations of acute down-regulation by GH because of the irreversible binding of GH to GHR proposed by Donner et al. (1978) and Gorin et al. (1984), and the complex pathways involved in GHR turnover. More recently, Mullis et al. (1991) and Nilsson et al. (1990) both provided evidence for the regulation of GHR mRNA expression by direct actions of GH on human hepatoma cells and rat epiphyseal chondrocytes, respectively. Both conclude that the physiological significance of a rapid increase in GHR gene expression is difficult to determine due to the complexity of the regulation. This complexity is increased by the physiological pulsatile release of GH, and the elusive role of GH binding protein (Nilsson et al., 1990).

Table 5. Summary of observations of GH regulation of GHR[a].

Tissue	GH treatment	Effect on GHR
Rabbit, lamb liver	GH administration, post-hypohysectomy	GHR partially restored
Rat liver	Hypersomatotropic by GH-secreting tumor	GHR induced 2-3 times normal
Rat liver	rGH infusion in vivo	GHR induced
Rat adipocyte	GH infusion in vivo	GHR maintained
Rat adipocyte	Hypohysectomy	Decreased GH binding
Mouse liver	Snell dwarf (panhypopituitary)	Low GHR levels
Mouse	Little dwarf (GH deficient)	Normal GHR
Cultured human IM-9 lymphocytes	GH treatment	GHR decreased
Mouse fibroblast	GH treatment	GHR decreased
Rat adipocyte	Chronic hGH exposure	GHR decreased

[a]References presented in review by Roupas and Herington (1989).

Fate of GHR. Little is known about the turnover and degradation of GHR. Several studies have observed rapid turnover rates for GHR with $t_{1/2}$ values ranging from 45 minutes in adipocytes to 8 hours in human IM-9 lymphocytes (see Roupas and Herington, 1989). This turnover rate is much more rapid than the turnover rates observed for insulin and epidermal growth factor (EGF) receptors, but it is similar to turnover rates observed for PRL (see Roupas and Herington, 1989). GH and PRL differ from insulin and EGF in that GH and PRL are both secreted in a pulsatile manner. Baxter (1985) suggested that a rapid turnover rate of receptors would be necessary in order for receptors to recognize individual pulses of hormones and to allow pulse-related responses to occur, assuming hormone action is mediated or terminated by internalization and degradation of occupied receptors. Roupas and Herington (1988) showed that the turnover rate of GHR in cultured adipocytes was more rapid in the presence of GH, suggesting that receptor occupancy leads to a shorter half life.

The fate of GHR following GH binding remains unclear. Roupas and Herington (1989) suggest a model for GHR degradation whereby the turnover of GHR is rapid and constitutive with the turnover rate increasing in the presence of GH. Although evidence has not been reported, it is assumed that degradation occurs via classical mechanisms of cell surface redistribution and microaggregation in coated pits. Because GH does not disassociate from GHR at the pH of pre-lysosomal endosomes (Mellman et al., 1986), Roupas and Herington (1989) suggest the fate of GH and GHR are the same on a degradative pathway.

GH Binding Proteins. Serum proteins which specifically bind human GH have been identified by Ymer and Herrington (1985) and Baumann et al. (1986). Leung et al. (1987) and Spencer et al. (1988) demonstrated in rabbits that the amino terminal amino-acid sequences of GH binding protein (GHBP) and GHR

are identical, establishing the serum binding protein as the extracellular hormone-binding domain of the membrane bound receptor. Leung et al. (1987) originally suggested that GHBP may be formed by proteolytic cleavage of the binding domain of GHR. Baumbach et al. (1989) has since shown in rats that GHBP is formed by a mechanism of alternative splicing of the GHR gene. Laron dwarfs, which have normal circulating GH concentrations but low IGF-I concentrations, are thought to lack functional GHR. It has been shown that they have little or no GHBP activity (Baumann et al., 1987; Daughaday and Trivedi, 1987), which is consistent with the structural origin of GHBP.

GHBP slows the clearance rate of bound GH (Baumann et al., 1987) and may serve as a circulating reservoir and buffer for GH. GHBP inhibits GH binding to receptors and bioactivity in vitro, but can enhance GH bioactivity in vivo. This discrepency may be due to the extended half-life of bound GH in vivo (see Baumann et al., 1991).

Davis et al. (1992) reported the presence of a protein which specifically bound GH in ovine, bovine, chicken, human, goose, porcine and equine serum. Chicken, ovine and porcine GHBP were purified and showed higher binding affinity for human GH as compared to GH from the same species. Binding affinities for ovine placental lactogen and ovine GH were intermediate between the affinities of human GH and GH from the same species in chicken and swine Variation in binding affinities was observed within and between species with the highest affinity observed in porcine and lowest in ovine. Davis et al. (1992) concluded that even though GHBP does not bind GH as strongly in many domestic species as compared to humans, the functional significance of GHBP in domestic species cannot be disregarded without further investigation.

Insulin-like Growth Factors

Identification. Two insulin-like growth factors, IGF-I and IGF-II, have been purified from plasma and share roughly 40% amino acid identity with insulin (Rinderknecht and Humbel, 1978a,b). IGF-II is present in high concentrations in fetal plasma and tissues, but concentrations decline rapidly postnatally (DeChiara et al., 1991). Postnatally, IGF-I mediates many of the growth-promoting effects of GH. Other biological effects of IGF-I include: acting as an insulin mimic; stimulating cell differentiation; and stimulating renal Na transport (Etherton and Smith, 1991). IGF-I is a 70 amino acid, single-chain protein with a calculated molecular weight of 7500 Da. IGF-I is highly conserved among pigs, cattle, and sheep (Etherton and Smith, 1991), and is identical at 67 of 70 amino acid residues between rat and human (Shimatsu and Rotwein, 1987). IGF-I displays obvious homology with proinsulin, and its amino acid sequence is compatible for a conserved 3-dimensional structure with insulin (Rinderknecht and Humbel, 1978a).

IGF-I Gene. The IGF-I gene has been isolated and characterized in the human (Rotwein et al., 1986), rat (Shimatsu and Rotwein, 1987) and ovine (Dickson et al., 1991). The gene for IGF-I is large, spanning at least 45 kb in human (Rotwein et al., 1986) and 73 kb in rat (Shimatsu and Rotwein, 1987). It is organized in 5 exons and 4 introns, and is expressed as multiple mRNA species in human and rat (Rotwein et al., 1986; Shimatsu and Rotwein, 1987).

Rotwein et al. (1986) identified an RFLP near exon 5 of the human IGF-I gene using the restriction enzymes HindIII and PvuII. Kirkpatrick (1992b) described a microsatellite polymorphism in the IGF-I gene in bovine and porcine. Six alleles of IGF-I were observed in porcine, and 3 alleles were observed in

bovine (Kirkpatrick, 1992b). Bishop et al. (1991) identified an RFLP in Polled Hereford and Angus cattle from genomic DNA digested with PvuII and PstI restriction endonucleases.

Expression of IGF-I. Although IGF-I is produced primarily in the liver, D'Ercole et al. (1984) provided evidence that IGF-I is also produced locally by several tissues. By extracting IGF-I from tissues of hypophysectomized rats, it was shown that IGF-I concentrations from kidney, lung, heart, testis and liver increased following GH treatment. This provides support for an autocrine/paracrine mechanism of action for IGF-I produced locally in multiple tissues. This is in contrast to previous thought that IGF-I acted by an endocrine mechanism. D'Ercole et al. (1984) observed that at maximal response to GH, mean tissue IGF-I levels were higher than IGF-I concentration in whole blood, suggesting that IGF-I produced from multiple tissues determines serum IGF-I concentrations. Further evidence for local control of IGF-I production by GH was provided by Isgaard et al. (1988). These researchers found that GH treatment caused an increase in IGF-I mRNA in rib growth plate and liver of hypophysectomized rats. The increase in IGF-I mRNA observed in the rib growth plate was caused, in part, by an increase in the rate of transcription of the IGF-I gene in chondrocytes, and the IGF-I gene was specifically activated.

Mathews et al. (1988b) describe the overespression of human IGF-I (hIGF-I) in mice carrying a hIGF-I-mouse metallothionein promoter. Increased levels of hIGF-I were found in liver, pancreas, lung, kidney and brain of transgenic mice compared to non-transgenic controls. Mice expressing the hIGF-I transgene displayed plasma IGF-I levels 1.5 x normal levels, and grew to weights of 1.3 x the weights of non-transgenic controls. However, the increase in weight was not apparent until 6 weeks after birth and resulted from selective

organomegaly without an increase of skeletal size. In addition, expression of endogenous IGF-I and GH was inhibited by expression of the hIGF-I transgene.

Plasma levels of IGF-I were studied in mice with the high growth recessive mutation gene (*hg)* in lines which had been selected for growth (G) and in non-selected lines (C; Medrano et al., 1991). C and G lines without the *hg* mutation were studied as controls for each genetic background. Mice with the *hg* gene had significatly higher plasma IGF-I levels compared to controls at 3, 4.5 and 9 weeks of age in the C lines, and at 6 weeks of age in the G line. This study also considered plasma and pituitary GH levels and reported a significant decrease in GH levels in mice with the *hg* gene compared to controls. These results are consistent with results reported by Mathews et al. (1988b) from transgenic mice engineered to overproduce human IGF-I. These similarities suggest that elevated IGF-I levels in *hg* mice may be a direct result of the *hg* mutation due to a molecular alteration of the IGF-I gene. Observations of decreased plasma and pituitary GH from both studies also implicate IGF-I as a regulator of GH expression through a negative feedback mechanism (see Medrano et al., 1991).

Chow et al. (1994) studied the regulation of IGF-I and binding protein-3 expression in transgenic mice carrying the metallothionein Ia-ovine growth hormone (oMtIa-oGH) transgene. Expression of the transgene was activated or inactivated by the addition or removal of 25 mM zinc sulfate in the drinking water. Expression of the oMtIa-oGH transgene significantly increased plasma IGF-I levels, as well as hepatic IGF-I mRNA. However, the increase in hepatic IGF-I mRNA accounted for only a portion of the observed increase of plasma IGF-I. After a review of other studies considering the regulation of plasma IGF-I, the authors concluded that oGH-induced post-transcriptional regulatory mechanisms, production of IGF-I by other GH-responsive tissues, and elevated

plasma IGF binding protein levels may account for the additional rise in plasma IGF-I levels they observed in oMtla-oGH transgenic mice (see Chow et al., 1994).

IGF-I Action. The GH mediating actions of IGF-I were originally thought to be carried out by an endocrine mechanism, as suggested by the somatomedin hypothesis. Recently, this has received considerable debate. Schlechter et al. (1986) demonstrated that the direct growth promoting effects of GH on cartilage are due, at least in part, to local production of somatomedins. They found that direct infusion of both rGH and hIGF-I at the epiphyseal cartilage plate in hypophysectomized rats increased unilateral growth. However, the stimulatory effect of hGH was abolished by simultaneous infusion of antiserum to IGF-I. These results suggest that local somatomedin production may be important in stimulating growth.

An autocrine/paracrine mechanism of IGF-I activity has been supported by many studies. Kerr et al. (1990) failed to observe a decrease in growth of guinea pigs treated with a monoclonal antibody to IGF-I. Because the antibody bound and inactivated circulating IGF-I, they suggested that local production of IGF-I was sufficient to maintain normal growth. Skottner et al. (1987) observed little growth promoting action from administration of recombinant methionyl IGF-I (met-IGF-I) in hypophysectomized rats. Significant effects on body weight gain were observed only when high doses of met-IGF-I were used. Because met-IGF-I was shown to be biologically active in the hypophysectomized rat, the authors concluded that it is a relatively poor growth promoting agent when given systemically, and that somatomedins are more likely to act via an autocrine/paracrine rather than endocrine mechanism. Similar results were obtained by Skottner et al. (1989) in a study involving a mutant GH deficient dwarf rat. High, but not low doses of IGF-I resulted in body weight gain similar

to that observed after hGH treatment. However, IGF-I treatment resulted in specific tissue growth of kidney, adrenal and spleen while hGH resulted in greater bone growth. hGH treatment did not produce an increase in circulating IGF, providing further evidence that GH action is not carried out by circulating IGF alone.

Finally, in vitro studies have shown that IGF-I is capable of being produced and acting locally to stimulate bone growth. Nilsson et al. (1989) demonstrated that the IGF-I gene is expressed in the rat epiphyseal growth plate chondrocytes under the control of GH, and Scheven and Hamilton (1991) reported IGF-I stimulated growth in vitro of rat intact long bones.

Although these studies do indicate an important role of locally produced IGF-I, reports of significant effects of circulating IGF-I cannot be overlooked. Schoenle et al. (1982) reported that pure IGF-I stimulated growth in a dose dependent manner in in hypophysectomized rats. An increase in circulating IGF-I was observed prior to increased growth in mice carrying GH or GRF fusion genes (Mathews et al., 1988a). Guler et al. (1988) found that infused recombinant IGF-I and hGH caused similar increases in body weight, tibial epiphyseal width, longitudinal bone growth and trabecular bone formation in hypophysectomized rats, although differences in organ weights were observed between the two treatments. Thus, there is evidence supporting the somatomedin hypothesis with an endocrine function of IGF-I. However, this evidence does not exclude the possibility that many effects of IGF-I are carried out by locally produced IGF-I acting via an autocrine/paracrine mechanism.

IGF-I Binding Proteins. In serum, almost all IGF-I is complexed to one of several IGF binding proteins (IGFBP). There are at least 3 distinct classes of IGFBP designated as IGFBP-1, IGFBP-2 and IGFBP-3 (see Etherton and Smith, 1991). As reviewed by Pell and Bates (1990), several theories regarding the

function of IGFBP have been proposed. It has been suggested that the pool of protein-bound serum IGFBP may act as a storage depot or a reservoir for IGF-I, continuously releasing small amounts of IGF-I to bind to receptors. IGFBP have been shown to both stimulate and inhibit IGF-I action. It is not clear if they have both effects in vivo, and studies using IGFBP-I and IGFBP-3 have produced conflicting results (see Etherton and Smith, 1991). Finally, it has been suggested that the effect of GH on locally produced IGFBP, rather than the effect of GH on IGF-I, is the important step in regulating IGF-I action in response to GH (Etherton and Smith, 1991).

IGF-I Receptors. IGF-I will bind to three types of receptors: the insulin receptor, and type 1 and type 2 growth factor receptors (Rosenfeld, 1989). Type 1 and type 2 IGF receptors are similar in amino acid sequence to the insulin receptor, and the type 1 receptor has been shown to behave in a manner similar to the insulin receptor (Furlanetto, 1988). However, the insulin receptor modulates metabolic processes whereas the IGF-I receptor stimulates cell proliferation and differentiation (Pell and Bates, 1990).

Genetic Control of Growth

Heritability of Growth Traits

Growth is a quantitative trait controlled by several genes that each contribute a partial effect to the overall trait. Attempts have been made at estimating the number of loci involved in controlling body weight in mice, but the validity of these estimates is questionable because of their large range. As reviewed by Eisen (1980), estimates have varied from 10 to 180 loci. Despite

the difficulty in determining the actual number of loci influencing quantitative traits such as growth and body weight, these traits are almost certainly controlled by many genes with small effects, as well as some genes with large effects (Eisen, 1980).

Growth traits in beef cattle are generally considered to be moderately heritable. Recently reported heritabilites for growth traits ranged from .10 to .58 for a variety of populations, with average heritabilities of .37, .28 and .32 for birth weight, weaning weight and yearling weight, respectively. (See Table 6.)

Table 6. Summary of recent reports of heritabilites for growth traits.

Heritability Estimate				
BWT	WWT	YWT	Reference	Population
0.50	0.25		Nelsen, 1986	Hereford
0.39	0.34		Nelsen, 1986	Hereford
	0.12		Lamb. 1990	Hereford
0.45	0.33	0.50	Kriese, 1991	Hereford
0.21	0.24		Reynolds, 1991	Hereford
0.53	0.58		Johnson, 1992	Hereford
0.18	0.17		Vaseth, 1993	Hereford
0.28	0.10	0.33	Smith, 1989	Hereford, Angus, Red Angus males
0.27	0.14	0.29	Smith, 1989	Hereford, Angus, Red Angus females
0.51	0.30	0.36	Alenda, 1987	Angus males
0.41	0.21	0.18	Alenda, 1987	Angus females
0.37	0.49		Johnson, 1992	Angus
0.46	0.39	.040	Winder, 1990	Red Angus
0.28	0.19	0.17	Kriese, 1991	Brangus
0.37	0.28	0.32	Average	

Heritability estimates indicate the portion of phenotypic variance controlled by additive genetic variance. These estimates for growth traits indicate that a substantial portion of observed phenotypic variation in growth is controlled by additive genetic effects which will be passed from parent to

offspring. Therefore, growth traits should respond well to selection pressure for increased growth (Falconer, 1989).

Correlations Among Growth Traits

Correlations among growth traits are high, indicating that selection for increased growth at one age will result in increased growth at other ages. Recently reported correlations are summarized in Table 7.

Table 7. Genetic (above diagonal) and phenotypic (below diagonal) correlations among growth traits.

Trait	Birth Weight	Weaning Weight	Yearling Weight	Average Daily Gain
Birth Weight		.52(a) .25(b) .56(c) .57(d)	.58(a) .41(b) .57(c) .75(d)	.45(a) .57(b) .31(c) .61(d)
Weaning Weight	.26(a) .31(b) .38(c) .34(d)		.80(a) .84(b) .78(c) .89(d)	.56(a) .49(b) .18(c) .37(d)
Yearling Weight	.37(a) .35(b) .39(c) .37(d)	.68(a) .79(b) .63(c) .77(d)		.92(a) .84(b) .75(c) .90(d)
Average Daily Gain	.21(a) .33(b) .19(c) .24(d)	.05(a) .00(b) .10(c) .22(d)	.68(a) .58(b) .74(c) .79(d)	

References:
(a) Smith et al., 1989a
(b) Smith et al., 1989b
(c) Brinks et al., 1990
(d) Alenda and Martin, 1987

Variation in Genetic Merit for Growth

Considerable variation in growth rate and weights at particular ages is known to exist between and within breeds of cattle, as summarized by Cundiff et al. (1986) in a review of the cattle Germ Plasm Evaluation Program at the Roman L. Hruska U.S. Meat Animal Research Center. Performance data from progeny of topcrosses by 20 sire breeds were used to estimate breed averages for economically important traits, including growth. The range (R) between the highest and lowest breed averages for growth traits along with phenotypic standard deviations calculated within breed groups (σp) are presented in Table 8. This large amount of variation for growth traits increases the amount of response that would be expected when selection pressures are placed on growth traits (Falconer, 1989).

Table 8. Range (R) and phenotypic standard deviation (σp) within breed groups from estimates of breed averages from the cattle Germ Plasm Evaluation program.[a]

Trait	Breed Groups	R	σp	$2R/\sigma p$
Birth weight, kg	Maine Anjou, Charolais - Jersey	10	4.3	4.7
200-day weight, kg	Gelbvieh - Jersey	25	20.0	2.5
Postwean gain, g (steers)	Simmental - Red Poll, Sahiwal	222	117.0	3.8
424-day weight, kg (steers)	Charolais, Simmental - Red Poll	73	36.0	4.1
400-day weight, kg (heifers)	Maine Anjou - Jersey	55	27.0	4.1

[a]Cundiff et al., 1986

Selection for Growth Traits

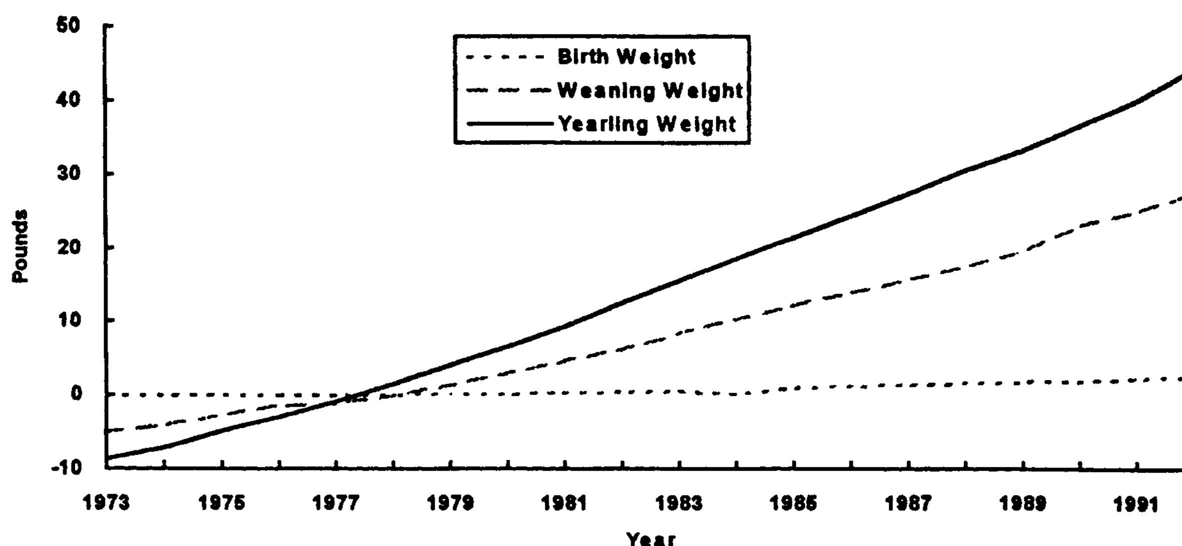
Beef Cattle. Selection for postweaning growth in Line 1 Hereford cattle was described by MacNeil et al. (1992). The expected selection differential for yearling weight, averaged over sexes, was 31.2 kg per generation. Correlated responses of increased birth weight, preweaning ADG and postweaning ADG were also observed. Breeding values of birth weight, weaning weight and yearling weight increased by a total of 3.2 kg, 14.5 kg and 62.4 kg, respectively, from 1935 to 1989. No selection plateaus have been observed after 13 generations of selection.

In comparison, data from calves produced over 21 years in a herd of Angus cattle selected for increased yearling weight was studied by Alenda and Martin (1987). A response of 15.18 kg per generation was calculated when yearling weight was the selection criterion. Correlated increases in birth weight, weaning weight, postweaning gain and post-birth gain were observed as a result of selection for increased yearling weight.

Additional indications of response to selection for increased growth in beef cattle may be found by examining field data reported to breed organizations. Because most cattle are marketed on the basis of weight, selection for increased weights at particular ages has been practiced in the beef industry for many years. Efficiency of this selection has been greatly increased by the publication of sire evaluations, presented as expected progeny differences (EPD), by breed organizations. Currently, 16 breeds sponsor sire and breed evaluation programs (Ludwig, 1993). The 1993 Hereford Sire Evaluation Report indicates that the Hereford breed has increased average birth

weight, weaning weight, and yearling weight by 1.14, 14.93, and 24.24 kg, respectively, from 1973 to 1992. These trends are shown in Figure 1.

Figure 1. Change, in pounds, of average birth weight, weaning weight, and yearling weight from 1973 to 1992 in Hereford cattle.



Pigs. Increases in growth and growth rate resulting from selection have also been well documented in pigs. Baird et al. (1952) reported two lines of Hampshire pigs divergently selected for high or low 150-day weight diverged by 28.1 kg in 180-day weight after nine generations of selection. Rahnefeld (1973) reported a realized heritability of .126 ± .029 in an experiment in which mass selection for increased postweaning average daily gain had been practiced for seven generations in Lacombe swine. A realized heritability of .198 ± .016 was reported after 11 generations of selection in the same population (Rahnefeld and Garnett, 1976). Mass selection for increased 70-day weight for six generations in Landrace pigs was reported by Kuhlers and Jungst (1990). The total weighted cummulative selection differential was 30.3 kg, representing 6.11 phenotypic standard deviations. Response per generation for 70-day weight was reported as .65 ± .29 kg with a realized heritability of .13 ± .06. Woltmann

et al. (1992) reported a difference in average daily gain of .16 kg from two lines of pigs divergently selected for fast and slow growth for four generations. Cumulative selection differentials of .335 and -.086 were observed in the fast and slow lines, respectively.

Mice. Long term selection experiments for increased growth in laboratory animals also demonstrate that growth and body weights can be significantly altered as a result of selection (see Eisen, 1980). LaSalle et al. (1974) reported an average increase in 21 to 42 day gain of .65 gram per generation in four replicate lines of mice which had undergone mass selection for increased 21 to 42 day gain for 12 generations. Wilson et al. (1971) reported an increase in average 60-day body weight of 18 g, representing 7 phenotypic standard deviation units, after 35 generations of selection in mice. Eisen (1972) observed an increase in 12-day litter weight for 17 generations of selection in mice. Each of these studies also reported selection limits after which no further increases in body weights were observed.

Alternate Selection Criteria. Despite the economic advantages of marketing animals with increased body weights, selection for increased weaning or yearling weights alone can result in undesirable correlated responses in other traits. Positive genetic and phenotypic correlations have been reported between calf birth weight and weaning and yearling weights (Smith et al., 1989a,b; Brinks et al., 1990; Alenda and Martin, 1987), and calf birth weight is a major factor contributing to dystocia and perinatal death loss in beef cattle (Laster et al., 1973). In addition, increased growth is often accompanied by increased mature body size, fatness, and reduced fertility (Scholtz and Roux, 1984) which increase the costs of beef cattle production. Therefore, there is considerable interest in selecting cattle for increased growth and weights at particular ages while limiting undesirable correlated responses in other traits.

Arnold et al. (1990) selected Angus sires for either high birth weight and high yearling weight EPD, or low birth weight and high yearling weight EPD in an attempt to increase yearling weight without a correlated increase in birth weight. Significant differences were observed in progeny for birth weight, but not for weaning weight, post-weaning gain, or yearling weight. These results indicate that the potential exists to increase genetic merit for growth to weaning and yearling without significantly increasing birth weight.

Selection of Hereford bulls for rate and efficiency of gain was studied by Mrode et al. (1990a,b). Bulls of two lines were selected for lean growth rate and lean feed conversion ratio. Results after 8 years of selection indicated that selection had been successful in both lines, with a greater response in the line selected for lean growth rate. Correlated responses of increased body weights were observed in males and females from the line selected for lean growth rate, but only in females from the line selected for lean feed conversion ratio. Correlated responses in lean proportion and food conversion ratio were found in both selection lines, while no adverse correlations, such as calving difficulty, calf mortality, or female reproductive performance, were observed in either line.

Environmental Effects. Another factor, besides genetics, that influences growth rates in animals is the environment. Godfrey et al. (1990) studied the effects of moving Hereford and Brahman bulls to different environments. Hereford bulls from Montana and Nebraska that were relocated to Montana, Nebraska, and Texas did not differ significantly in body wieght from controls maintained in their original environment, with the exception of lower body weights observed in bulls moved from Montana to Texas. In contrast, Brahman bulls from Texas and Louisiana that were moved to Montana and Nebraska had dramatically reduced body weights compared to control Brahman kept in Texas. Relocated Brahman bulls also had smaller scrotal circumference and paired

testis volume than controls. These results indicate that body and testis growth of Brahman bulls were hindered by relocation to northern environments, but comparable affects of relocation were not observed in Hereford bulls.

Butts et al. (1971) studied the possibility of genotype-environment interaction in cattle within the Hereford breed. Two herds of Hereford cattle originating from Montana and Florida were subdivided, and half of each transferred to the opposite location. Significant location-origin interactions were found for birth, weaning and yearling weights for both sexes. Performance to yearling ages favored cattle raised in their native location. Significant differences between location and between origin groups were found for several traits measured. Results from this study gave support for the presence of significant genotype-environment interactions in cattle within the same breed.

DeNise et al. (1988) and DeNise and Torabi (1989) studied the effects of stressful environments on the estimates of genetic parameters for preweaning and postweaning traits in Hereford cattle raised in the southwestern U.S. Results indicated that genetic parameters change in response to the level of environmental stress, and that the sexes respond differently to stressful conditions. It was suggested that the genetic parameters differed because of different genes affecting the traits of interest, or because of different relative contributions of single genes that depended on the environment.

Summary

Experimental evidence shows that growth and body weights can be influenced by selection. Growth traits are moderately heritable, and growth traits at different stages of develpment are highly correlated. Because of the

economic advantage of marketing animals at heavier weights, beef cattle producers have practiced selection for increased growth rates. However, there are limits due to increased cost of production and increased calving difficulty which often accompany intense selection for increased growth. Recent studies looking at alternative selection criteria have shown that genetic variation exists to allow for continued imporovement in growth without detrimental correlated responses in other traits. Finally, environment is important to the expression of genetic potential for growth traits and should not be overlooked.

Genetic Markers and Marker Assisted Selection

Genetic Markers

The idea of Marker Assisted Selection was first presented by Sax (1923). He suggested that if certain size factors, or genes for quantitative traits, could be identified that were linked to genes for qualitative traits, then it should be possible to study independently the genes for quantitative traits within each linkage group marked by a qualitative trait. Thoday (1961) expanded on this idea by describing the study of quantitative genetic differences using chromosomal markers. However, experimentation at that time was limited by the lack of availability of useful genetic markers.

Beckmann and Soller (1983) described a new type of marker known as a restriction fragment length polymorphism (RFLP). Unlike morphological or pigmentation markers previously used in plants, or isozymes used in animals, RFLPs are numerous and display important genetic traits such as a lack of dominance and multiple allelic forms (Beckmann and Soller, 1983). In addition,

RFLPs are located throughout the genome and provide a mechanism by which complete genome linkage maps may be constructed (Botstein et al., 1980). RFLPs are identified by extracting genomic DNA, digesting the DNA with a restriction enzyme and separating the resulting fragments using gel electrophoresis. The separated fragments are then transferred to a membrane using southern hybridization (Southern, 1975), and specific fragments are identified by a labelled probe which is complementary in DNA sequence to the genomic fragment of interest. Polymorphisms result when probes hybridize to fragments differing in size because of changes in restriction enzyme recognition sites, or insertions or deletions of DNA between restriction enzyme recognition sites (Beckmann and Soller, 1983).

The development of the polymerase chain reaction (PCR; Saiki et al., 1985) has allowed for an alternative method to detect RFLPs known as PCR-based RFLP or PCR-RFLP. This method involves the amplification of a specific portion of genomic DNA using PCR. The resulting amplification product may contain a polymorphic restriction enzyme recognition site. The polymorphism is detected by digesting PCR amplification products with appropriate restriction enzymes and then observing differences in the size of resulting digestion fragments using gel electrophoresis. Some of the earliest bovine PCR-RFLPs were reported by Medrano and Aguillar-Cordova (1990a, b) in genes for milk proteins.

PCR has also been useful in developing another type of genetic marker known as a microsatellite marker. Microsatellites are regions of tandem repeats of di- or trinucleotide sequences in the genome (Tautz, 1989). Polymorphisms result from different numbers of tandemly repeating units among different alleles. Polymorphisms may be detected using PCR to amplify across a microsatellite region and then determining which allele is present based on the size of the

amplification product. Microsatellite polymorphisms have been found to be highly polymorphic in all mammals, and are thought to be dispersed frequently throughout the genome (Weber and May, 1989; Georges et al., 1990; Love et al., 1990; Cornall et al., 1991; Fries et al., 1990).

Another type of genetic marker is a single-strand conformation polymorphism (SSCP). Orita et al. (1989) first described this type of polymorphism using genomic DNA which had been digested with restriction endonucleases. Single-stranded DNA fragments were electrophoresed on neutral polyacrylamide gels. Mobility shifts representing single base substitutions were observed in the single-stranded DNA fragments, possibly resulting from conformational changes in the DNA. This technique has also been successful in identifying polymorphisms in PCR amplification products (Hayashi, 1992).

Genetic Maps

Techniques from molecular biology have allowed large numbers of useful genetic markers to be identified in many species. In order to discern how these markers can be helpful to livestock production, markers which are closely linked to genes controlling quantitative traits of economic importance, or quantitative trait loci (QTL), must be identified. Until recently, one of the limitations to identifying markers linked to QTL in livestock species has been the lack of a genetic linkage map (Dentine, 1992). A sufficient number of highly polymorphic marker loci which are evenly distributed along the chromosomes is a prerequisite for mapping QTL (Fries, 1993). The minimum number of markers to

cover the bovine genome at a spacing of 40 cM is estimated at 100, assuming even distribution of crossovers along the chromosomes (Fries, 1993).

Recently, genetic linkage maps have been published for bovine (Barendse et al., 1994; Bishop et al., 1994), porcine (Rohrer et al., 1994) and ovine (Crawford et al., 1994). The bovine map described by Barendse et al. (1994) includes 201 loci that were genotyped across cattle reference families. These markers cover approximately 90% of the expected length of the cattle genome and include 35 linkage groups representing 29 of the 30 pairs of chromosomes. In comparison, the map described by Bishop et al. (1994) includes 30 linkage groups represented by 313 genetic markers distributed on 24 autosomal chromosomes, the X and Y chromosomes, 4 unanchored syntenic groups and 2 unassigned linkage groups. These maps provide the skeletal framework upon which more detailed genome maps may be built, and may be used in studies designed to investigate the genetic basis for quantitative traits (Barendse et al., 1994; Bishop et al., 1994).

The development of gene maps in numerous species provide a valuable resource for genetic analysis, as well as insight into the evolution of genome organization by comparing linkage relationships of homologous genes (O'Brien et al., 1993a). Comparative gene mapping involves the study of linkage associations between homologous genes of different species and considers persistent conservation of linkage order as evidence of functional or adaptive significance (O'Brien et al., 1993a). Comparative gene mapping has provided evidence for the evolutionary origin of human chromosome 21 (Threadgill et al., 1991), and has revealed several conserved gene clusters including the Hox, MHC, immunoglobulin and globin clusters (Farr and Goodfellow, 1992). Reviews comparing genomes among human, mouse, cattle, sheep, pig, rabbit,

rat, hamster, horse, mink, silver fox and dog have been compiled (O'Brien et al., 1993 a,b).

Genetic Markers and Major Genes

Most traits of economic importance in livestock production are quantitative or polygenic traits, implying that variation in the trait is determined by the action of alleles at several loci, together with environmental factors. However, examples of single genes which have major effects on the physiology and economics of livestock production can be found. In these situations, genetic markers resulting from mutations in the major gene, or genetic markers closely linked to the major gene are useful in selection programs designed to control the effects of the major gene.

In swine, pigs with the disease malignant hypothermia (MH) are more susceptible to stress and are more likely to produce pale, soft and exudative pork than normal pigs (O'Brien,1987). It has been thought that heterozygous carriers of MH produce a net economic advantage over MH free pigs due to an increase in lean yield (Simpson and Webb, 1989). However, more recent studies using more accurate tests for MH heterozygosity have failed to show an advantage of the heterozygous genotype (Webb et al., 1994; Goodwin et al., 1994). Malignant hypothermia can be induced in living pigs using halothane anesthesia to detect homozygous carriers of MH (Webb and Jordan, 1978), but susceptibility to halothane anesthesia is controlled by a recessive gene (Hal) and progeny testing has been required to identify heterozygous carriers. Fuji et al. (1991) identified a C to T transition in the ryanodine receptor (also known as the calcium release channel) gene that was detectable as a PCR-RFLP. This

mutation was subsequently identified as the mutation responsible for MH in swine (Houde et al., 1993; Otsu et al., 1991). This PCR-RFLP is currently being used by commercial swine companies to identify MH carriers and control the disease in swine populations.

In horses, hyperkalaemic periodic paralysis (HYPP) results in potassium-induced episodes of paralysis (Spier et al., 1990). A C to G transversion was identified in the sodium channel α subunit gene, resulting in a predicted phenylalanine to leucine amino acid substitution (Rudolph et al., 1992). In an HYPP quarter horse extended pedigree, all affected animals had the leucine substitution while all unaffected animals demonstrated the phenylalanine residue, indicating the sodium channel α subunit is a likely candidate gene for HYPP. The C to G transversion may be detected by TaqI restriction enzyme analysis of PCR-amplified genomic DNA (Rudolph et al., 1992).

Progressive degenerative myeloencephalopathy (weaver disease) is a genetic disease common in Brown Swiss cattle. The microsatellite locus TGLA116 was found to be closely linked to the weaver locus and may be useful to select against the genetic disease (Georges et al., 1993). Hoeschele and Meinert (1990) reported that weaver-carrier cows produced significantly more milk and more milk fat compared to weaver-free contemporaries. Therefore, the TGLA116 marker may also be useful to study its chromosomal region as a potential QTL for milk production (Georges et al., 1993).

Bovine leukocyte adhesion deficiency (BLAD) is an autosomal recessive genetic disease that has been characterized in Holstein cattle (Kehrli et al., 1990). Cattle homozygous for the disease have low resistance to infection and usually die within a few months of birth. A PCR-RFLP reported by Shuster et al. (1992) identifies a point mutation within the bovine gene encoding the adhesion protein CD18. The point mutation results in an amino acid substitution in a

region of the protein which has been shown to cause leukocyte adhesion deficiency in humans.

In sheep, Booroola ewes possess a major gene, Fec^B, that influences their ovulation rate (see Bindon, 1984). Homozygotes (BB), heterozygotes (B+) and non-carriers (++) of the Fec^B gene are identified on the basis of ovulation rate recordings of ≥5, 3 or 4 and 1 or 2, respectively (Davis et al., 1982). Montgomery et al. (1993) reported linkage between the Fec^B mutation and two microsatellite markers (OarAE101 and OarHH55) and epidermal growth factor from human chromosome 4q25. Lanneluc et al. (1994) also reported a cluster of seven minisatellite fragments as being linked to the Fec^B gene. Linkage of a genetic marker to the Fec^B gene could be useful in selecting sheep for increased prolificacy.

A final example of important effects from a major gene can be found in sheep where a mutation causing extreme muscle hypertrophy has recently been identified. Muggli-Cockett et al. (1993) reported a variable number of tandem repeat marker with five codominant alleles that was linked to the gene causing muscle hypertrophy, which has been named callipyge. The callipyge locus has since been mapped to ovine chromosome 18 (Cockett et al., 1994).

Genetic Markers and QTL: Candidate Gene Approach

In order for genetic markers to be useful in livestock production to assist in selection of quantitative traits, genetic markers which are closely linked to genes controlling quantitative traits, or quantitative trait loci (QTL), must be identified. One approach to identifying such markers is known as the candidate gene approach. Genes encoding proteins known to have important

physiological effects on the trait of interest are identified as candidate genes. Polymorphisms within these genes are then evaluated to determine if the candidate genes are closely linked to a QTL, or if the candidate genes themselves are involved in regulation of the trait of interest. Several examples of possible linkages between genetic markers and QTL have been reported as a result of this approach.

Rothschild et al. (1994) identified a polymorphism in the estrogen receptor gene having a major effect on litter size in pigs. Rocha et al. (1992) identified a significant effect of a GH-TaqI allele on birth weight as a maternal trait, and on shoulder width at birth in a five-breed diallel cross of beef cattle. A significant effect was also found between a parathyroid-MspI marker and measures of body size in the same study. Hoj et al. (1992) observed different allele frequencies for GH-MspI alleles in Red Danish cattle expressing high and low milk fat production. Andersson-Eklund and Rendel (1993) reported evidence for linkage between the amylase-1 locus and a QTL influencing milk fat content in offspring from heterozgous sires of the Swedish Red and White dairy breed. Several studies have also shown significant associations between polymorphisms in milk protein genes and milk production and composition traits (see Bovenhuis et al., 1992).

Genetic Markers and QTL: Interval Mapping

Another approach to identifying QTL has been made possible by the development of genetic linkage maps. This approach, known as interval mapping, utilizes linkage maps to select genetic markers evenly distributed throughout a genome. Intervals between pairs of flanking markers are examined

for evidence of the presence of a QTL. Lander and Botstein (1989) described methods using maximum likelihood and lod scores to determine intervals where QTL are most likely to exist. More sophisticated statistical analyses are being developed which will allow for more precise interval mapping as well as the fine resolution of multiple QTL (Haley et al., 1994; Zeng, 1994; Jansen and Stam, 1994; Zeng, 1993; Jansen, 1993).

Paterson et al. (1988) first reported the use of interval mapping for the identification of 15 QTL in the tomato. Approximately 95% of the total tomato genome was estimated to be detectably linked to a total of 68 markers, spaced at approximately 20 cM intervals, that were used in the study.

Jacob et al. (1991) developed 112 simple sequence repeat markers in rats which were estimated to cover 90% of the rat genome at a spacing of 30 cM. These markers were used to screen F2 crosses between hypertensive and normal rats. Using linkage analyses and LOD scores (Lander and Botstein, 1989), two markers showed significant linkage to blood pressure phenotypes. These markers were then mapped to mouse chromosomes 10 and 18. Although a genetic map was not used to select markers to be analyzed in this study, linkage was found by using interval mapping with a set of markers randomly distributed throughout a large portion of the genome.

In mice, interval mapping has been successful in identifying QTL for plasma cholesterol and obesity, as well as morphine preference in inbred strains (Warden et al., 1993; Berrettini et al., 1994). Both studies used the statistical analyses described by Lander and Botstein (1989) to localize QTL to specific chromosomal regions. The study by Warden et al. (1993) involved backcross progeny of two inbred lines of mice differing in plasma total cholesterol and total lipid concentrations. Chromosomal region 7 was found to have significant linkage to plasma total cholesterol and carcass lipid, and a locus on

chromosome 6 showed significant linkage to plasma total cholesterol. Berrettini et al. (1994) identified loci on chromosomes 1, 6 and 10 which were responsible for 85% of variation in oral morphine preference. F2 mice from an intercross of parental strains differing in their oral preference to addictive substances were used in the study.

Pomp et al. (1994) identified four QTL having significant effects on body weight, and four QTL having significant effects on body fat in mice. The average effect of these markers was in the range of 1-2% of the phenotypic variation, except for one marker which may be linked to a pleiotropic marker with larger effects. The population involved in the study was a backcross population created from two different inbred mice strains that differed in body size. Selective genotyping (Lander and Botstein, 1989) was used to identify markers with an increased likelihood of being linked to QTL. Least-squares procedures were used to quantitate the effect of inidividual markers on body weight and body fat.

Andersson et al. (1994) reported the presence of a QTL for growth rate, fatness, and length of the small intestine on chromosome 4 in pigs using an interval mapping approach. The study involved a cross between the European wild boar with the domesticated Large White pig and utilized 105 genetic markers assigned to 15 of the 18 autosomes. The study demonstrated that genetic maps can be useful in dissecting genetically quantitative traits in outbred livestock species and was an application of the analytical method based on least squares for the identification of QTL segregating in crosses between divergent outbred lines that was described by Haley et al. (1994).

Marker Assisted Selection

When Sax (1923) and Thoday (1961) first presented the idea of using genetic markers to study genes regulating quantitative traits, the primary obstacle was the lack of availability of useful genetic markers. The development of RFLPs, PCR-RFLPs, microsatellite and SSCP markers have allowed complete genomes to be represented by informative genetic markers. The obstacle currently facing animal geneticists is to use genetic markers and linkage maps to identify QTL important to livestock production. Recent publications of genetic linkage maps in livestock species (Barendse et al., 1994; Bishop et al., 1994; Rohrer et al., 1994; Crawford et al., 1994) along with ongoing development of new genetic markers will facilitate the systematic dissection of genomes to allow for detailed searches of QTL (Paterson, 1988; Lander and Botstein, 1989; Andersson et al., 1994; Haley et al., 1994).

Lander and Botstein (1989) discuss methods to increase the efficiency of identifying genetic markers linked to QTL including identifying promising crosses for QTL mapping, utilizing the full power of complete linkage maps, and decreasing the number of progeny to be genotyped. Experimental analysis and choice of populations in which to study genetic markers are also crucial to the successful identification of QTL. The statistical method of maximum likelihood (Lander and Botstein, 1989) has been successful in identifying QTL in inbred populations (Paterson et al., 1988; Jacob et al., 1991; Warden et al., 1993; Berrettini et al., 1994), but is computationally complex and difficult to extend to non-inbred populations (Haley et al., 1994). Analyses using ordinary least squares to test for QTL in crosses between divergent outbred lines are presented by Haley et al., (1994). Soller and Genizi (1978) compare the

efficiency of experimental designs using half-sib or full-sib families. The granddaughter design, which utilizes marker genotypes of sons from heterozygous sires and phenotypic data from daughters (or sons) of the genotyped sons, was proposed as a way to decrease the number of animals genotyped (Weller and Soller, 1990).

The next challenge will be to incorporate information gained from genetic marker analyses into existing selection programs. Selection based on molecular genetic information as well as individual phenotypes and breeding value estimations is known as Marker Assisted Selection (MAS). Although many details regarding the use of MAS in long term selection programs have yet to be determined, there is evidence that molecular genetic information has the potential to substantially increase the efficiency of artificial selection (Lande and Thompson, 1990). The efficiency of MAS will depend on several factors including the heritability of the trait being selected, the proportion of additive genetic variance associated with the marker, and the selection scheme being used (Lande and Thompson, 1990). The benefits of MAS will be gained through a decrease in generation interval, increase in accuracy of selection, and the ability to select animals not expressing a trait. However, it will be important to maintain realistic expectations of these benefits. MAS is a complex system with difficulties arising from differences due to environment, genetic backgrounds, and selection goals (Pomp, 1994). Researchers and the livestock industries must be willing to work together in order to identify specific goals and applications for MAS.

Marker assisted selection is still in its infancy, but many obstacles have already been overcome. Marker assisted selection presents an exciting new field of research for animal scientists, and has the potential to benefit livestock

CHAPTER 2

INTRODUCTION

Growth is an economically important trait in beef cattle production that is regulated by many genes. Traditional selection strategies to improve growth traits have been based on selecting cattle expressing superior phenotypes for growth traits. This approach assumes that animals with superior phenotypes also have superior genotypes, or combinations of genes, influencing growth traits. However, the combination of genes that creates a superior phenotype in one animal may not be inherited by the animal's offspring due to the sampling and recombination of genetic material that occurs during meiosis.

Estimates of the genetic merit for a specific trait that an animal will pass on to its offspring may be calculated by animal evaluation programs developed through breed organizations. These estimates are known as expected progeny differences (EPD) and are equal to one-half of the animal's estimated breeding value relative to other animals evaluated in the same program. EPD estimates are useful tools in the beef industry because they are calculated from an animal's own phenotypic data as well as data collected from ancestors, relatives and progeny of the animal. EPDs are valuable to selection programs because they allow selection decisions to be based on more information than simply the phenotype of an individual.

Techniques from molecular biology have now become available which may allow even more information to be considered when making selection decisions. These techniques allow for the evaluation of specific genes of an animal rather than the evaluation of complete phenotypes. By comparing

differences, or polymorphisms, in genes among different animals, it may be possible to identify regions of an animal's genome that are involved in the regulation of quantitative traits such as growth. Polymorphisms used to identify these regions, or quantitative trait loci (QTL), would be known as DNA or genetic markers. Marker assisted selection (MAS) would then incorporate information gained from examining an animal's DNA using genetic markers with EPD estimates to assist in making accurate selection decisions.

One obstacle that must be overcome before MAS can be a practical selection strategy is the identification of DNA markers located in close proximity to QTL. Many DNA markers have been identified in the bovine genome, but associations of markers with quantitative traits have not been clearly established. Another important consideration will be to understand the effects of a marker in the genetic background in which it will be used. The main objectives of this study are to characterize specific DNA polymorphisms in three populations of Hereford cattle, and to identify associations between DNA markers and growth or maternal traits in a large population of Hereford cattle.

The polymorphisms that were investigated in this study were chosen based on the candidate gene approach. In order to select candidate genes, biological factors known to be involved in the regulation of growth were identified. Polymorphisms in genes encoding these biological regulators were investigated in hope that the polymorphisms could be used as markers of closely linked genes involved in the regulation of growth, or that the polymorphisms themselves result in a biological effect that impacts growth.

Growth Hormone (GH) and insulin-like growth factor I (IGF-I) are hormones that are important stimulators of growth, and are required for normal growth to continue in animals. The pituitary transcription factor PIT1 is required for the expression of the GH gene, and growth hormone receptors (GHR) are

necessary in order for GH to carry out its biological action at target tissues. Genes encoding GH, IGF-I, PIT1 and GHR were chosen as candidate genes for the study of growth traits. The gene for the hormone prolactin (PRL) was identified as a candidate gene for the study of milk production because of the stimulatory action of PRL on lactation. Finally, polymorphisms in genes for the milk proteins kappa-casein (K-Cas) and beta-lactoglobulin (B-Lac) were investigated for their effects on milk production and as potential markers of QTL for growth.

The three populations of Hereford cattle used in the present study differed in terms of selection pressures, and in the amount of average inbreeding that has accumulated in the populations. Inbred populations were represented by the USDA Line 1 Herefords and Lents Anxiety 4th Herefords which have average inbreeding coefficients of ~.30 and ~.50, respectively. The USDA Line 1 Herefords have been selected for increased growth, but the Lents Axiety 4th Herefords have not. These populations were compared to a population of 27 Hereford sires chosen from the US Hereford Sire Summary for either high or low yearling weight EPD. Allele frequencies in these populations were characterized by evaluating differences in allele frequencies among the populations, estimating the average amount of genetic variation present in each population, and determining if alleles were segregating as expected under an assumption of Hardy-Weinberg equilibrium.

The USDA Line 1 Hereford population was used to test for associations between DNA polymorphisms and growth or maternal traits. Genotypes were determined for 420 cattle born in 1991, 1992 and 1993. Phenotypic data and EPD from these cattle were used to determine if the substitution of one allele for another allele at a particular locus had a significant effect on growth traits or maternal ability in the population. A significant effect due to allele substitution

could indicate the presence of a QTL in close proximity to the polymorphism, or it could indicate a significant biological effect caused by differences in gene products produced by different alleles.

CHAPTER 3

MATERIALS AND METHODS

Populations

USDA Line Herefords

The Line 1 population of Hereford cattle was established at the Fort Keogh Livestock and Range Research Laboratory (Miles City, MT) in 1931 as part of a project to develop a line of cattle superior in genetic merit for growth (MacNeil et al., 1992). Two sons of Advance Domino 13th, Advance Domino 20th and Advance Domino 54th, were the primary foundation sires of Line 1, and the population was closed to outside introduction of germplasm in 1935. Line 1 cattle are recognized for genetic superiority of growth traits and have been used extensively throughout the Hereford breed. The average expected progeny differences (EPD) of birth weight, weaning weight, and yearling weight in this herd for calves born between 1991 and 1993 are -.84, 23.0 and 40.3 lbs., respectively. These values compare to average EPDs for Hereford calves born after January 1, 1991 of +2.3, +26.0 and +42.0 lbs. for birth weight, weaning weight and yearling weight, respectively (Bennyshek et al., 1993).

Husbandry and management practices of Line 1 are described in MacNeil et al. (1992). Briefly, cows are placed in calving pastures in mid-March. Calving occurs from early April through mid-May. Cow-calf pairs are moved to native range spring pasture a few days after birth, and then moved to smaller mating pastures about June 1. After breeding season, cow-calf pairs are moved to

rangeland summer pastures. Since the mid-1970's calves have been weaned in mid-October at an average age of 180 days. Cows are moved to autumn range after weaning. Heifer and bull calves are brought into the feedlot for a two week adjustment period before being fed growing rations to about one year of age.

Almost 13 generations of selection for increased postweaning gain have been applied since the Line 1 population was closed. Progeny testing of potential herd sires was initiated in 1942 and continued until 1960. Bulls that were used in the Line 1 population during that time were selected from the results of the progeny test. Since 1960, selection has been based on individual performance. Adjusted yearling weight has been the primary selection criterion for male replacements, and 18-month weight has been emphasized in selection of female replacements. The Line 1 population has been divided into two sublines since 1976. One subline has been selected for increased yearling weight while the other subline has been selected by independent culling levels for increased yearling weight and decreased birth weight.

Trends in predicted breeding values for the Line 1 population were reported by MacNeil et al. (1992). Postweaning gain predicted breeding values have increased .29 kg/day, or 2.6 SD, in 13 generations of selection. A 3.2-kg genetic increase in birth weight has resulted from positive trends in direct and maternal breeding values for birth weight. Breeding values for 200-day weight have increased 14.5-kg between 1935 and 1989. However, accumulated effects of inbreeding have reduced expression of genetic potential for growth and maternal ability to the extent that the results of selection on 200-day weight have been slightly more than offset phenotypically.

Inbreeding in the Line 1 population increased rapidly in the late 1930's and early 1940's due to the mating of daughters of Advance Domino 20th to Advance Domino 54th and the mating of daughters of Advance Domino 54th to

Advance Domino 20th. Inbreeding has increased at a lesser rate since that time because of some avoidance of matings between close relatives. Since 1960, inbreeding has accumulated at a nearly constant annual rate of .22%/year to reach the current average inbreeding coefficient of .30. Trends in inbreeding coefficients in the Line 1 population from 1935 to 1989 are presented in MacNeil et al. (1992).

A total of 430 animals born in the Line 1 herd in 1991, 1992 and 1993 were involved in the present study. Blood samples were taken at weaning by jugular bleeding for use in DNA extraction and analysis. All calves were weighed within 24 hours after birth. Those surviving were weighed at weaning, at 28-day intervals after weaning, and at approximately 1 year of age. Twelve sires of 1991 and 1992 calves were used to represent the population for initial genotyping.

Lents Anxiety 4th Herefords

The Lents Anxiety 4th Herefords located in Indiahoma, Oklahoma, are owned by Joe and Jim Lents. This is a closed population of cattle with linebreeding tracing back to the bull Anxiety 4th, one of the founding bulls of the Hereford breed from the late 1800's. This herd is unique to most Hereford cattle because it has not been influenced by the Line 1 Herefords and because selection emphasis has not been placed on increased size.

Cattle from the Lents Anxiety 4th herd involved in this study include 8 herd sires used prior to and during 1992 and 58 calves born in 1992 and 1993. Blood samples were obtained by jugular bleeding for DNA extraction and analysis. All calves were weighed at birth and the 1992-born calves were

weighed at weaning. EPDs were not available for any animals from this herd. The average birth weight and weaning weight of calves included in this study were 73 lb and 404 lb, respectively. Inbreeding coefficients calculated for the 8 herd sires involved in this study were all greater than .50.

High Yearling Weight EPD Hereford Sires

Thirteen sires with high yearling weight EPDs that also had yearling weight EPD accuracies greater than .70 were selected from the 1992 U.S. Hereford Sire Summary (Table 9). This population was included in the study to represent Hereford cattle from different herds that have a high genetic potential for growth traits, as indicated by yearling weight EPD. Yearling weight EPDs of these sires ranged from +80 to +119 with an average of +96.5. This compares to the breed average of +48.0 lb. for yearling weight EPD in 1992. Semen samples were obtained from these sires for DNA extraction and analysis.

Low Yearling Weight EPD Hereford Sires

Fourteen sires with low yearling weight EPDs that had yearling weight EPD accuracies greater than .60 were also selected from the 1992 U.S. Hereford Sire Summary (Table 9). This population was studied as a comparison to the High yearling weight EPD sires. Yearling weight EPDs of this group ranged from -11 to +38 with an average of +17.2. Semen samples were obtained from these sires for DNA extraction and analysis.

Table 9: Sires selected from 1992 Hereford sire summary and their EPD and accuracies for birth weight, weaning weight and yearling weight.

Sire	Birth Weight		Weaning Weight		Yearling Weight	
Name	EPD	Acc	EPD	Acc	EPD	Acc
Tex Prime Time 7056	9.9	0.92	67	0.92	119	0.89
JBF Encore GEXPL	4.9	0.80	73	0.81	110	0.76
Power House	5.8	0.88	57	0.88	108	0.83
Knight Ryder	7.4	0.84	68	0.85	107	0.78
HH Magnum 9696	9.2	0.80	65	0.79	106	0.71
JR P183 Rival T025	7.4	0.80	59	0.82	97	0.75
SR Verdict 455	11.0	0.92	63	0.93	96	0.88
IHRY GK Race 4 ET	6.1	0.88	51	0.87	94	0.83
BB Domino 1087	5.7	0.87	50	0.91	86	0.90
MFR Stockman 764	3.0	0.84	44	0.82	84	0.77
WS DHR Duke 8430	6.2	0.93	51	0.94	84	0.91
Klondike Banner 469L	8.4	0.85	59	0.85	84	0.83
GK Explosion 412T	4.0	0.90	42	0.89	80	0.87
4M Hummer	1.2	0.78	21	0.82	38	0.77
OXH Mark 5155	2.1	0.79	17	0.78	36	0.79
CL1 Domino 0005	-1.5	0.91	14	0.91	25	0.89
Selkirk Lad H3	-0.3	0.77	16	0.85	24	0.83
HN Mark Domino 919	0.1	0.82	13	0.85	24	0.79
JCS Mont Donald 191	1.0	0.54	24	0.76	23	0.66
WCF L1 Domino 6164	-1.4	0.53	16	0.68	21	0.63
Selkirk Extra 367	1.7	0.77	18	0.75	20	0.71
L1 Advance 9129	-4.0	0.83	7	0.80	13	0.68
CL1 Domino 113	0.7	0.74	15	0.81	13	0.76
C1 Domino 334	-1.2	0.83	3	0.88	-1	0.87
CL1 Dom 1177	0.5	0.75	9	0.85	-1	0.86
S Britisher Lad 569	0.9	0.74	6	0.83	-2	0.81
TT Donald S406	0.1	0.80	-8	0.89	-11	0.87

DNA Extraction

Genomic DNA was extracted from blood samples taken from animals in the Line 1 and Lents Anxiety 4th populations. Genomic DNA for the Line 1 population was extracted at the Fort Keogh Livestock and Range Research Laboratory in Miles City, MT, and then sent to Stillwater, OK for further processing. All other DNA was extracted in Stillwater, OK. Genomic DNA was extracted from blood samples using the Salt Extraction Protocol (Appendix 1).

Genomic DNA was extracted from semen samples from bulls in the High and Low Yearling Weight EPD populations. Genomic DNA was extracted from semen samples using the Organic Solvent Extraction Protocol (Appendix 2).

The concentrations of all stock genomic DNA samples were determined using a Lambda 3B UV/VIS spectrophotometer (Perkin Elmer, Norwalk, CT). The absorbance of 10 μl of stock DNA solution diluted in 990 μl water was determined at wavelengths of 260 nm and 280 nm. The concentration of the stock solution was calculated using the formula (Boyer, 1993):

[DNA] in μg/ml = (A260)(100)(50μg/ml)

where A260 is the absorbance measured at a wavelength of 260 nm, 100 is the dilution factor used in determining the absorbance, and 50μg/ml is the extinction coefficient for double stranded DNA. Appropriate volumes of stock solutions were diluted with TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to make 200 μl of DNA working solutions of a final concentration of 50 ng/μl. DNA stock solutions were stored at -80°C and DNA working solutions were stored at 4°C. Sample calculations for determining stock DNA concentration and making 50 ng/μl DNA working solutions are shown in Appendix 3.

The ratio of absorbance at 260 nm to absorbance at 280 nm was used as an indicator of DNA quality. Ratios between 1.6 and 1.9 were desirable. Lower ratios indicated protein impurities while higher ratios indicated either RNA or solvent impurities. All samples were tested in a polymerase chain reaction, regardless of absorbance ratios. Samples with undesirable ratios that failed to produce a PCR amplification product were re-extracted using the Organic Solvent Protocol (Appendix 2) normally used for semen samples for additional purification. The DNA concentrations of the new samples were determined as previously described and new working solutions were made.

Genotyping

Polymerase Chain Reaction

All genotyping for this study was based on polymerase chain reaction (PCR) amplification of a portion of a single gene from genomic DNA. A diagram of a standard PCR including denaturation, primer annealing and primer extension steps is shown in Appendix 3. Typically, thermal cycling began with a single cycle of 2 minutes at 95°C, 1 minute at the annealing temperature specific for the primer being used in the reaction (55-60°C) and 2 minutes at 72°C. This was followed by 24-29 cycles of 1 minute at 94°C, 1 minute at the annealing temperature and 1 minute at 72°C. All reactions concluded with a final 9 minute extension at 72°C and were then held at 4°C.

All PCRs were carried out in a DNA Thermal Cycler 480 (Perkin Elmer, Norwalk, CT) or a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA). Taq polymerase enzyme and PCR buffer were obtained

from Boerhinger-Mannheim (Indianapolis, IN). Nucleotides (dATP, dGTP, dCTP and dTTP) were obtained from New England Biolabs (Beverly, MA) and combined in a dNTP working solution of 10 mM each nucleotide.

Oligonucleotides for PCR primers were obtained from the OSU Biochemistry Core Facility (Stillwater, OK). Lyophilized primers were resuspended in water. Working solutions of 5 μM each primer (forward primer and reverse primer) were made for each primer pair. Processing of lyophilized oligonucleotides and sample calculations are shown in Appendix 5.

Optimization of PCR conditions was carried out for each primer pair. All optimization reactions were completed in the DNA Thermal Cycler 480. Three concentrations of $MgCl_2$ (.75 mM, 1.5 mM and 2.25 mM), 2 concentrations of dNTPs (100 μM and 200 μM each dNTP) and 3 concentrations of primers (0.1 μM, 0.3 μM and 0.5 μM each primer) were used in a 3 x 2 x 3 factorial to determine the optimal concentrations of the reaction ingredients. All optimizations were carried out in a total volume of 25 μl using .875 units of Taq polymerase, PCR buffer resulting in final reaction conditions of 10 mM Tris-HCl and 50 mM KCl at pH 8.3 and 50 ng of genomic DNA. Annealing temperature for optimization PCR was first tested at 55°C. This temperature was increased if a more stringent reaction was required to produce a single PCR amplification product. Reaction conditions resulting in the strongest single amplification product were used for future PCRs with the optimized primer.

Polymorphisms

Two types of polymorphisms, PCR-based restriction fragment length polymorphisms (PCR-RFLP) and microsatellite polymorphisms, were used in this

study. PCR-RFLPs were represented by polymorphisms in genes for K-casein (K-Cas), β-lactoglobulin (B-Lac), growth hormone (GH) and pituitary transcription factor 1 (PIT1). This type of DNA polymorphism results from a change in the nucleotide sequence of one allele that either creates or eliminates a recognition site for a Type II restriction endonuclease. PCR-RFLPs were detected by digesting PCR amplification products with the appropriate restriction enzyme. Genotypes were determined using gel electrophoresis to separate the restriction enzyme digestion products in 3% agarose made with BioRad and NuSieve agarose in a 1:1 ratio (BioRad, Hercules, CA; Midwest Scientific, St. Louis, MO) gels. Digestion products were mixed with loading dye and 10 μl were loaded in the gel. Typically, two combs with 30 wells each were used to form two rows of wells in gels, and 60 samples were run per gel. DNA was visualized in the gel by staining for 30 minutes in 1.3 mM ethidium bromide, destaining 30 minutes in water, and photographing using a Foto Prep I ultraviolet light source and MP4 Instant Camera System (Fotodyne, Inc., Hartland, WI). Unique banding patterns were observed for each genotype because of differences in restriction enzyme recognition sites between alleles.

Microsatellite polymorphisms used in this study included polymorphisms in insulin-like growth factor I (IGF-I) and prolactin (PRL) genes as well as the random bovine microsatellite marker BM2113. Microsatellites are regions of tandem repeats of di- or trinucleotide sequences in the genome (Tautz, 1989). Polymorphisms result from different numbers of tandemly repeating units among alleles. Polymorphisms were detected using PCR to amplify across the microsatellite region. The size of the PCR product depended on the number of tandemly repeating units in the microsatellite region and was used to characterize different alleles. Genotypes were determined by the size of the PCR product from polyacrylamide gel electrophoresis. PCR products were run

as double stranded products on non-denaturing acrylamide gels (Fischer Scientific, Pittsburgh, PA) and were visulaized by staining in 1.3 mM ethidium bromide for 20 minutes, destaining in water for 20 minutes and photographing using a Foto Prep I ultraviolet light source and MP4 Instant Camera System (Fotodyne, Inc., Hartland, WI).

The actual base pair length of the PCR products was determined by end-labelling PCR amplification of each different allele. ^{33}P (DuPont and NEN®, Boston, MA) labelled PCR products were run as single stranded DNA on sequencing gels along with a sequencing standard of known size (see Appendix 6). The length of the PCR product was found by comparison with the known sequencing standard. After the actual base pair length of PCR products from DNA samples representing each allele was determined, those samples were used as standards to genotype additional samples using non-denaturing gels and ethidium bromide staining.

K-Cas and B-Lac genotyping

K-Cas and B-Lac genotypes were determined as described by Medrano and Aguilar-Cordova (1989a) and Medrano and Aguilar-Cordova (1989b), respectively, from a single PCR. The reaction ingredients included: 200 μM each dNTP, 0.1 μM each K-Cas primer (see Appendix 7), 0.1 μM each B-Lac primer (see Appendix 7), standard PCR buffer (1.5 mM $MgCl_2$, 10 mM Tris-HCl and 50 mM KCl at pH 8.3), .75 units of Taq polymerase and 50 ng genomic DNA in a total volume of 30 μl. Thermal cycling was carried out as previously described with an annealing temperature of 60°C for a total of 30 cycles.

Digestion of the PCR products with the restriction enzyme HinfI determined the K-Cas genotype (Medrano and Aguilar-Cordova, 1990a) from the 350 bp K-Cas PCR product. PCR products (11.125 µl) were digested with .125 µl (1.25 units) of HinfI and 1.25 of buffer 2 (New England Biolabs, Beverly, MA) in 12.5 µl reactions. Digestion reactions were carried out at 37°C for a minimum of 3 hours. Gel electrophoresis was carried out as previously described for 50 minutes at 72 volts. K-Cas genotypes were characterized by the following banding patterns: 134/132 and 84 bp (AA genotype); 266, 134/132 and 84 bp (AB genotype); and 266 and 84 bp (BB genotype). Three additional monomorphic bands of approximately 180, 50 and 32 bp resulted from digestion of products from the B-Lac PCR primers and did not influence the K-Cas genotype determination.

Digestion of the PCR products with the restriction enzyme HaeIII determined the B-Lac genotype (Medrano and Aguilar-Cordova, 1990b) from the 262 bp B-Lac PCR product. PCR products (11.125 µl) were digested with .125 µl (1.25 units) of HaeIII and 1.25 of buffer 2 (New England Biolabs, Beverly, MA) in 12.5 µl reactions. Digestion reactions were incubated at 37°C for a minimum of 3 hours. Gel electrophoresis was carried out as previously described for 50 minutes at 72 volts. B-Lac genotypes were characterized by the following banding patterns: 350, 153 and 109 bp (AA genotype); 350, 153, 109 and 74/79 bp (AB genotype); and 350, 109 and 74/79 bp (BB genotype). The 350 bp band was the K-Cas PCR product and did not influence the B-Lac genotypes.

GH genotyping

GH genotypes were determined as described by Zhang et al. (1992). PCR was carried out in 15 µl reactions with 200 µM each dNTP, .3 µM each GH primer (see Appendix 7), standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 58°C for a total of 30 cycles.

The restriction enzyme AluI was used to digest the 427 bp GH PCR products. PCR products (11.125 µl) were digested with .125 µl of AluI (1.25 units) and 1.25 µl of buffer 1 (New England Biolabs, Beverly, MA). Digestion reactions were incubated at 37°C for a minimum of 3 hours. Gel electrophoresis was carried out as previously described for 45 minutes at 72 volts. GH genotypes were characterized by the following banding patterns: 264, 96 and 51 bp (AA genotype); 264, 147, 96 and 51 bp (AB genotype); and 264 and 147 bp (BB genotype).

PIT1 genotyping

PIT1 genotypes were determined as described by Moody et al. (1994; Appendix 8). PCR was carried out in 15 µl reactions with 200 µM each dNTP, .1 µM each PIT1 primer (see Appendix 7), standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 55°C for a total of 30 cycles.

The restriction enzyme HinfI was used to digest the 1355 bp PIT1 PCR products. PCR products (11.125 µl) were digested with .125 µl of HinfI (1.25

units) and 1.25 µl of buffer 2 (New England Biolabs, Beverly, MA). Digestion reactions were incubated at 37ºC for a minimum of 3 hours. Gel electrophoresis was carried out as previously described for 45 minutes at 72 volts. PIT1 genotypes were characterized by the following banding patterns: 660, 425 and 270 bp (AA genotype); 660, 425, 385 and 270 bp (AB genotype); and 660, 385 and 270 bp (BB genotype).

GHR genotyping

GHR genotypes were determined as described by Moody and Pomp (1994; Appendix 9). PCR was carried out in 15 µl reactions with 200 µM each dNTP, .3 µM each GHR primer (see Appendix 7), standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 55ºC for a total of 30 cycles.

The restriction enzyme AluI was used to digest the 2.3-kb GHR PCR products. PCR products (11.125 µl) were digested with .125 µl of AluI (1.25 units) and 1.25 µl of buffer 1 (New England Biolabs, Beverly, MA). Digestion reactions were incubated at 37ºC for a minimum of 3 hours. Gel electrophoresis was carried out as previously described for 75 minutes at 72 volts. GHR genotypes were characterized by the following banding patterns: 785, 670, 375, 195 and 115 bp (AA genotype); 785, 670, 475, 375, 195 and 115 bp (AB genotype); and 785, 475, 375, 195 and 115 bp (BB genotype). Only the A allele of the GHR polymorphism was observed in animals involved in this study, so GHR genotype was not included in statistical analyses.

IGF-I genotyping

IGF-I genotypes were determined using PCR primers (see Appendix 7) designed to amplify the microsatellite described by Kirkpatrick (1992). PCR was carried out in 15 μl reactions using 200 μM each dNTP, 0.1 μM each IGF-I primer, standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 55°C for a total of 25 cycles. Fewer cycles were required in order to clearly visualize genotypes from polyacrylamide gels.

PCR products were run on non-denaturing polyacrylamide gels. A 12% 24:1 (wt:wt) acrylamide:bisacrylamide gel was used to separate the two IGF-I alleles. PCR products were mixed with 3 μl of 6X loading dye and 8 μl were loaded in the gel. Typically, 2 gels with 20 wells each were poured. Fifteen IGF-I PCR products were loaded in each gel along with standards of known AB genotype. The gels were run for 30 minutes at 385 volts (192.5 v-hr) and 15 more samples were loaded in each gel. Gels were then run 5 hours at 385 volts (1925 v-hr) to genotype a total of 60 samples. DNA was visualized as previously described. A 173 bp product was designated as the A allele and corresponded to the 130 bp allele described by Kirkpatrick (1992), and a 171 bp PCR product was designated as the B allele and corresponded to the 128 bp allele described by Kirkpatrick (1992).

PRL genotyping

PRL genotypes were determined using PCR primers designed to flank a microsatellite region in the 5' region of the bovine prolatin gene (Wolf et al.,

1990). PCR was carried out in 15 μl reactions using 200 μM each dNTP, .2 μM each PRL primer (see Appendix 7), standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 55°C for a total of 25 cycles.

PRL PCR products were run on 12% 24:1 (wt:wt) acrylamide:bisacrylamide non-denaturing polyacrylamde gels as described for IGF-I, except PRL PCR products were run 30 minutes longer for a total of 2118 v-hr. A 204 bp PCR product was designated as the A allele and a 200 bp PCR product was designated as the B allele.

BM2113 genotyping

BM2113 microsatellite marker genotypes were determined as described by Sunden et al. (1993). PCR was carried out in 15 μl reactions using 200 μM each dNTP, .2 μM each BM2113 primer (see Appendix 7), standard PCR buffer, .5 units of Taq polymerase and 50 ng genomic DNA. Thermal cycling was carried out as previously described with an annealing temperature of 55°C for a total of 25 cycles.

BM2113 PCR products were run on 12% 24:1 (wt:wt) acrylamide:bisacrylamide non-denaturing polyacrylamde gels as described for IGF-I, except gels were loaded only once and were run 4 hours at 385 for a total of 1540 v-hr. BM2113 PCR products of 133,135,139 and 141 bp were observed in these populations.

Statistical Analyses

The genotypic data obtained in this study was analyzed in two parts. The objectives of Part I were: to determine allele frequencies and variances at each polymorphism genotyped; to compare allele frequencies among the 4 unique populations; to determine the amount of genetic variance or heterozygosity present in the populations; and to describe the effects of inbreeding on gene frequency in the Lents Anxiety 4th and Line 1 populations. The objective of Part II was to use EPD, phenotyic and genotypic data from the Line 1 population to test for the presence of a quantitative trait locus (QTL) linked to a DNA polymorphism. This was accomplished by using regression, least squares, and animal model analyses.

Part I: Description of Allele and Genotype Frequencies

Genotype Frequencies, Allele Frequencies and Variances. Genotype and allele frequencies were determined for each polymorphism in each population using all available genotypes. Genotype frequencies (P_{uu}) were determined for each possible genotype as the ratio of the number of animals of uu genotype to the total number of animals genotyped in a population for a given locus. Allele frequencies (p_u) were determined for each allele as the ratio of one particular allele (u) to the total number of alleles in the population at a given locus. The variances of allele frequencies [Var (p_u)] were calculated using the formula (Weir, 1990):

$$\text{Var}(p_u) = 1/2n(p_u + P_{uu} - 2p_u^2)$$

where: n = total number of animals in population

p_u = frequency of u allele

P_{uu} = frequency of uu genotype

<u>Comparison of Allele Frequencies.</u> Allele frequencies were compared using Chi-Square goodness of fit analyses. Preliminary analyses compared allele frequencies between High Yearling Weight EPD and Low Yearling Weight EPD populations. No significant differences in allele frequencies were observed between the two groups, so they were pooled as one population (pooled EPD) representing Hereford sires with a wide range in genetic potential for growth traits. A χ^2 test statistic was then determined to test for significant differences in allele frequencies among the 3 populations (Line 1, Lents and pooled EPD) at each locus (l). A continuity correction factor was used to account for the fact that a continuous χ^2 distribution was used to test a hypothesis with discrete genotypic counts. The χ^2 test statistic was determined as follows (Weir, 1990):

$$\chi^2_l = \Sigma_p \Sigma_u \chi^2_{lpu}$$

where: χ^2_l = χ^2 test statistic for locus l

χ^2_{lpu} = χ^2 test statistic for allele u of locus l in population p

$$\chi^2_{lpu} = (|obs_{lpu} - exp_{lpu}| - 0.5)^2 / exp_{lpu}$$

where: obs_{lpu} = observed number of u alleles in population p at locus l

exp_{lpu} = expected number of u alleles in population p at locus l

0.5 = continuity correction

$$exp_{lpu} = (n_u / n)(n_p)$$

where: n_u = total number of u alleles in all populations being compared

n = total number of all alleles in all populations being compared

n_p = total number of all alleles in population p

χ^2_l test statistics comparing the three populations were compared to a table value of 5.99 for a .05 significance level at two degrees of freedom. If allele frequencies were significantly different among the three populations, then comparisons were made between two populations using the Chi -square analysis to determine which populations differed. When two populations were compared, a table value of 3.84 for one degree of freedom was used to determine significance at the .05 level.

Similar analyses were performed to test for significant differences in genotype frequencies among populations. Calculation of the χ^2 test statistic was the same, except the number of animals in each genotypic class was used in place of numbers of alleles. Test statistics comparing three populations were compared to a table value of 9.49 for a .05 significance level with four degrees of freedom, and comparisons of two populations were compared to a table value of 5.99 for two degrees of freedom at a .05 significance level.

Genetic Variation. The amount of genetic variation present in each population was described by calculating a heterozygosity coefficient (H_l). H_l was determined for each polymorphism in each population as the ratio of the number of heterozygotes to the total number of animals genotyped in the population for the polymorphism. The H_l calculated for all polymorphisms were averaged within populations to estimate the amount of genetic variation in the 4 populations. The variance of H_l was calculated using the following formula (Weir, 1990):

$$Var(H_l) = (1/n)(H_l)(1-H_l)$$

where: n = number of a animals in the population

H_l = heterozygosity coefficient.

Comparison with Expectations from Hardy-Weinberg Equilibrium. The number of animals in each genotypic class was compared to the number of animals expected under an assumption of Hardy-Weinberg equilibrium using Chi-square goodness of fit analyses. These analyses were carried out in the Line 1 population for K-Cas, B-Lac, GH, IGF-I and PIT1 polymorphisms and in the Lents Anxiety 4th population for K-Cas and GH polymorphisms. The BM2113 polymorphism was only genotyped in sires representing the two populations. The remaining polymorphisms were not genotyped in the total populations because of high frequencies of one allele in sires representative of the total populations.

The expected number of animals in each genotypic class was calculated from allele frequencies in each population and Hardy-Weinberg equilibrium assumptions using the following formulas (Falconer, 1989):

Expected number of AA = p^2n

Expected number of AB = $2pqn$

Expected number of BB = q^2n

where: p = frequency of allele A

q = frequency of allele B

n = number of animals in the population

The χ^2 test statistic, including a continuity correction factor, used to test for significant differences between observed and expected genotype frequencies was calculated as follows (Weir, 1990):

$$\chi^2 = \sum_{uu} \chi^2_{uu}$$

$$\text{where } \chi^2_{uu} = \frac{(|\text{Observed number of UU - Expected number of UU}| - 0.5)^2}{\text{Expected UU}}$$

for each possible genotypic class, UU.

χ^2 test statistics were compared to a table value of 5.99 for a .05 significance level at 2 degrees of freedom.

The same analyses were done after adjusting the expected number of animals in each genotypic class for known inbreeding coefficients (f) of .30 and .50 in the Line 1 and Lents Anxiety 4th populations, respectively. Adjusted expected numbers of animals in each genotypic class were calculated from the following formulas (Falconer, 1989):

Adj. expected number of AA = $(p^2 + pqf)n$

Adj. expected number of AB = $(2pq - 2pqf)n$

Adj. expected number of BB = $(q^2 + pqf)n$

where: f = inbreeding coefficient

p = frequency of allele A

q = frequency of allele B

n = number of animals in the population.

Finally, assumptions of Hardy-Weinberg equilibrium were tested using the disequilibrium coefficient (D_A). The D_A and its variance for each polymorphism was calculated using the formulas (Weir, 1990):

$$D_A = P_{AA} - p^2$$

$$\mathrm{Var}(D_A) = \frac{1}{n}[p^2(1-p)^2 + (1-2p)^2 D_A - D_A^2]$$

where: P_{AA} = frequency of genotype AA

p = frequency of allele A

n = number of animals in the population

The standard normal variate, z, where (Weir, 1990):

$$z = (D_A - 0)/\sqrt{\mathrm{Var}(D_A)}$$

was used to test the hypothesis Ho:D_A=0, which is equivalent to testing for Hardy-Weinberg equilibrium expectations because D_A is expected to be 0 in a

population at Hardy-Weinberg equilibrium. A z test statistic greater than 1.96 or less than -1.96 indicated a significant ($P<.05$) deviation from Hardy-Weinberg equilibrium expectations caused by either an excess or deficiency of heterozygotes (H_A:D_A not 0). Specifically, a z test statistic greater than 1.64 indicated a significant ($P<.05$) decrease in the number of heterozygotes (H_A:$D_A>0$), and a z test statistic less than -1.64 indicated a significant ($P<.05$) increase in the number of heterozygotes (H_A:$D_A<0$) expected from assumptions of Hardy-Weinberg equilibrium.

Part II: Analyses for QTL

Regression Analyses. Regression analyses were performed to determine the average effect of allele substitution as described by Falconer (1989). Birth weight (BWT), weaning weight (WWT), yearling weight (YWT) and maternal (MILK) expected progeny differences (EPD) were obtained from the American Hereford Association National Cattle Evaluation for cattle from the USDA Line 1 population. Regression analyses were performed in which EPDs were the dependent variables and genotype was the independent variable (EPD Regression). Genotypes were coded as 0 (AA), 1 (AB) or 2 (BB) to represent the number of B alleles present for the K-Cas, B-Lac, GH, IGF-I and PIT1 polymorphisms. Regression analyses were also performed in which phenotypic data for birth weight, weaning weight and yearling weight were the dependent variables (Regression model 1). Analyses with phenotypes included a combined fixed effect of year-sex-age of dam, and calving date was included as a covariate in birth weight analyses. Regression analyses using phenotypic data

were also performed including sire as an additional fixed effect (Regression model 2).

Each point in the regression analyses was weighted by the frequency of the genotype it represented (Table 10). The genotypic frequencies were calculated for each dependent variable and then averaged across EPD or phenotype measurement within each marker. Only the genotypes of animals that were included in the analyses were used to calculate these genotype frequencies. Genotypes of animals that did not have an EPD or phenotypic measurement were not considered.

Table 10. Genotype frequencies used to weight regression analyses.

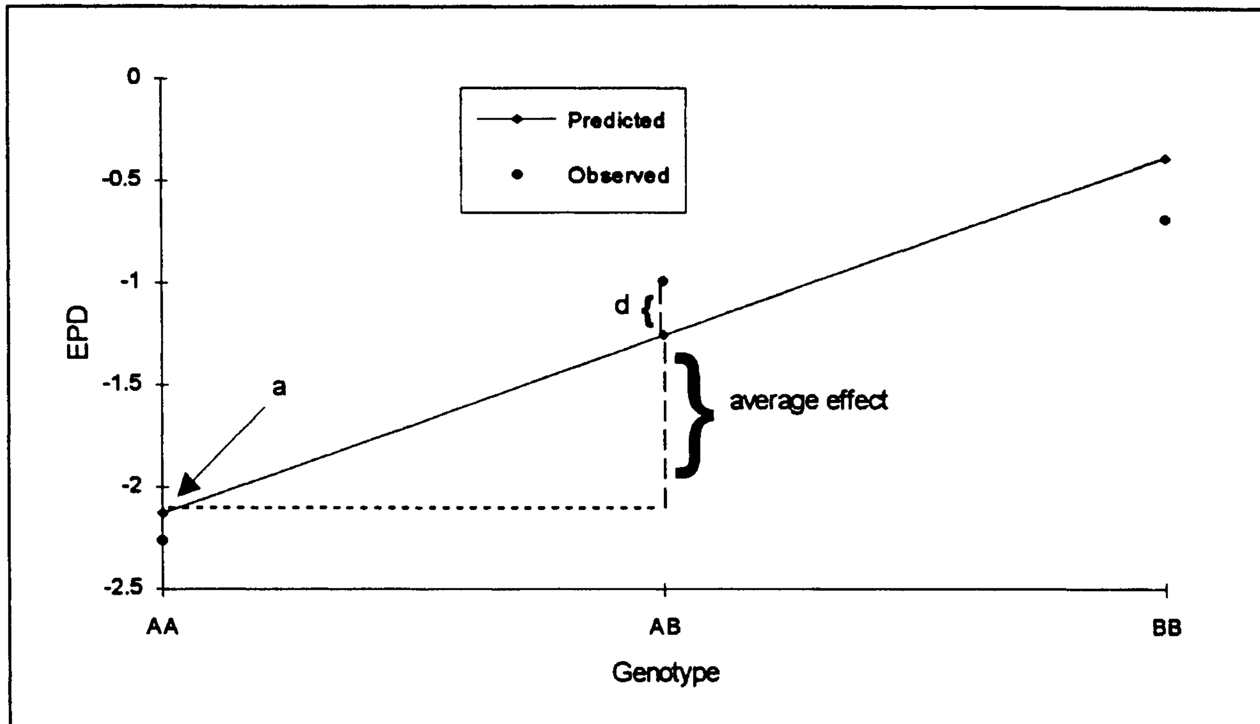
	AA		AB		BB	
	EPD[a]	Phenotype[b]	EPD	Phenotype	EPD	Phenotype
K-Cas	.452	.453	.376	.376	.172	.171
B-Lac	.523	.520	.395	.400	.082	.080
GH	.108	.105	.442	.452	.450	.442
IGF-I	.427	.427	.399	.394	.174	.179
PIT1	.018	.020	.186	.188	.796	.793

[a]Frequencies were calculated from animals with EPD estimates included in the regression analyses.
[b]Frequencies were calculated from animals with phenotyic records included in the regression analyses.

As described by Falconer (1989), the regression coefficient (α) estimates the average effect of allele substitution, or the the average effect of replacing an A allele with a B allele (Figure 2). The y intercept of the regression line (a) estimates the average value of the AA genotype. Dominance deviations are estimated by the difference between observed and predicted values at the AB genotype (d), and the coefficient of determination, R^2, estimates the percentage of variability of the dependent variable (EPD or phenotype) that is explained by genotype. The percentage of total variability in phenotype that was explained

by genotype, and the percentage of residual variability explained when genotype was added to the model which already contained fixed effects were also calculated.

Figure 2: Regression analysis to determine the average effect of allele substitution.



Legend: 'a' represents the average EPD for animals of AA genotype; d represents the amount of dominance deviation; and the average effect represents the effect on EPD of replacing an A allele with a B allele.

Least Squares Analyses. Least squares analyses, weighted for genotype frequencies (Table 10), were performed on BWT EPD, WWT EPD, YWT EPD and MILK EPD with genotype (AA, AB or BB) included in the model as a fixed effect (EPD LSM). Linear comparisons tested for differences between genotypes with additive effects defined as AA - BB and dominance effects defined as AB - .5(AA + BB).

Least squares analyses, weighted for genotype frequencies (Table 10) were performed on birth weight, 200-day weight and 365-day weight phenotypes using three different models. LSM model 1 included fixed effects of genotype and year-sex-age of dam subclasses. Calving date was included as a covariate in birth weight analyses. LSM model 2 included sire as an additional fixed effect along with genotype and year-sex-age of dam, while LSM model 3 included a random effect of sire nested within genotype. The random effect of sire nested within genotype was used as the denominator of the F-test for genotype in LSM model 3.

LSM models 1, 2 and 3 were performed with all possible 2-way interactions among genotype, year, sex and age of dam. All non-significant interactions were eliminated from the models and final analyses were performed using fixed effects and all interactions that approached significance ($P<.20$). Linear comparisons tested for differences between genotypes with additive effects defined as AA - BB and dominance effects defined as AB - .5(AA + BB).

Animal Model Analysis. An animal model using multiple trait derivative free reduced maximum likelihood (MTDFREML; Boldman et al., 1993) was used to analyze BWT, 200-day weight and 365-day weight phenotypes. A total of 395 animals had complete genotype records. All 395 were included in the BWT analysis, 390 were included in the 200-day weight analysis and 387 were included in the 365-day weight analysis. For all three traits, an animal effect was fitted to the data. For BWT and 200-day weight, a maternal genetic effect and an effect due to permanent environment (repeated records on dams) was added to the model.

Six fixed effects were fitted to the data. They included a combined year-age of dam-sex effect plus the five genotype effects (K-Cas, B-Lac, GH, IGF-I and PIT1). Individual AA, AB and BB genotypes were coded 1, 2 or 3 for the

analysis. In addition, a covariate for calving day was fit to birth weight. The numerator relationship matrix contained 6769 individuals and included all animals described by MacNeil et al. (1992), plus animals born up to and including 1993. The total number of equations solved were 13801 for BWT, 13799 for 200-day weight and 6806 for 365-day weight. Linear contrasts were calculated for each trait and each genotype for additive (AA - BB) and dominance (AB - .5[AA + BB]) effects.

CHAPTER 4

RESULTS

Part I. Description of Allele and Genotype Frequencies

Allele and Genotype Frequencies

GH, B-Lac and K-Cas allele and genotype frequencies are shown in Figures 3 and 4, respectively; PIT1, IGF-I and PRL allele and genotype frequencies are shown in Figures 5 and 6, respectively; and BM2113 allele frequencies are shown in Figure 7. Both A and B alleles were segregating in the High EPD and Low EPD sire populations at the GH, B-Lac, K-Cas, PIT1, IGF-I and PRL polymorphisms. Animals in the High EPD and Low EPD populations displayed 5 and 4 alleles, respectively, of the BM2113 microsatellite marker. A and B alleles of the GH, B-Lac, K-Cas, PIT1 and IGF-I polymorphisms were both present in the Line 1 population, but the PRL polymorphism was fixed for the A allele. Only 2 alleles (139 and 135 bp) of the BM2113 marker were present in the Line 1 population. A and B alleles of the GH and K-Cas polymorphisms were segregating in the Lents population, but the PIT1 and PRL polymorphisms were fixed for the B and A alleles, respectively. One animal from the Lents population (n=58) was heterozygous at the B-Lac polymorphism while all others were homozygous for the B allele. One heterozygous sire was identified in the Lents population for the IGF-I polymorphism while the remaining seven sires were homozygous for the A allele. At the BM2113 polymorphism, seven Lents sires were homozygous for the 139 bp allele and one was heterozygous with 139

and 141 bp alleles. Therefore, the Lents population appears to be close to fixation for the B, A and 139 bp alleles of the B-Lac, IGF-I and BM2113 polymorphisms, respectively. The A allele of the GHR polymorphism was fixed in all populations, which is consistent with the observation of fixation of the A allele in all bos taurus cattle (see Appendix 9).

Comparison of Allele and Genotype Frequencies

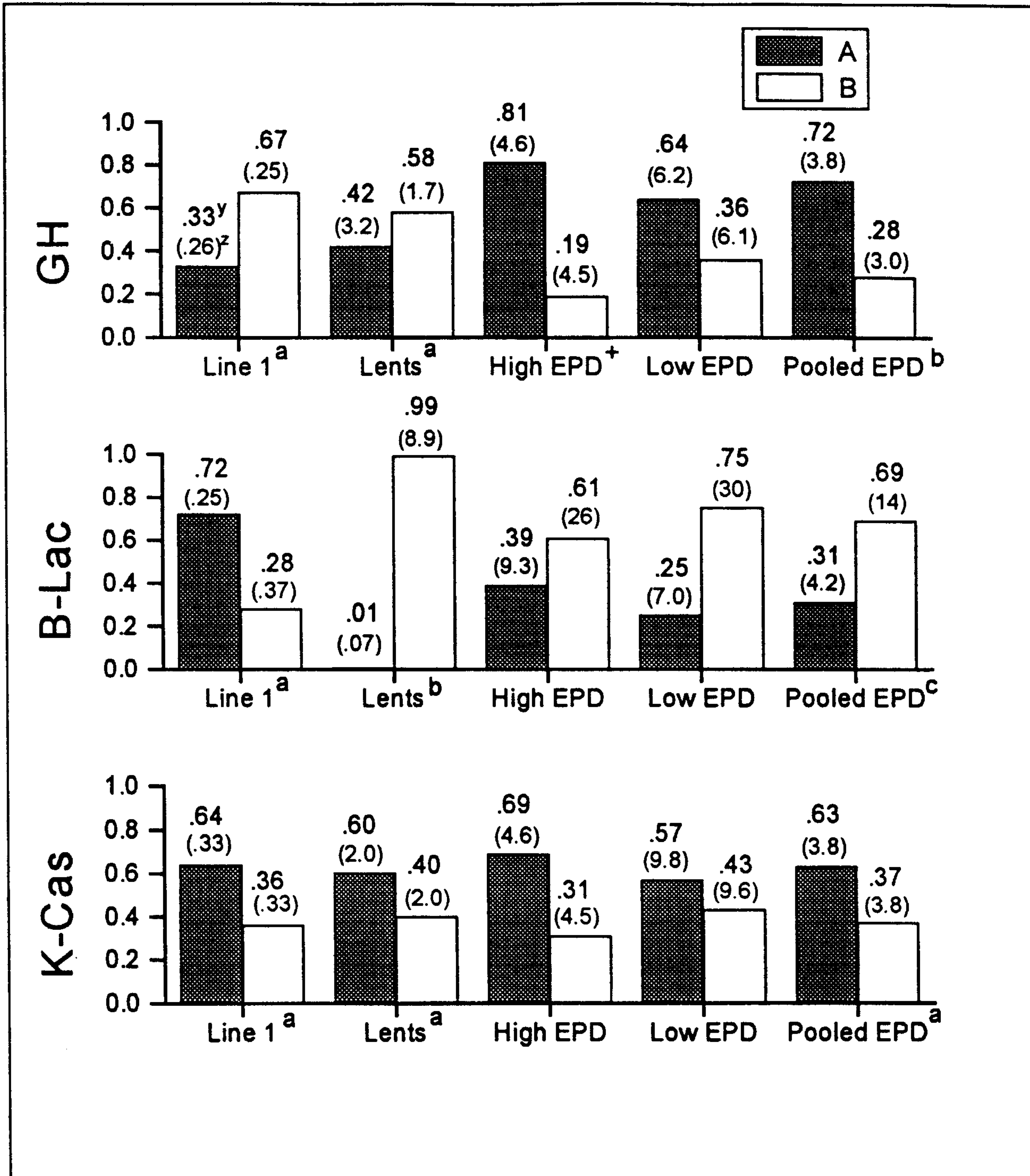
Table 11 shows the Chi-square test statistics that were calculated when testing for differences in allele frequencies among Line 1, Lents, and the pooled High EPD and Low EPD populations. (Differences in allele frequencies between the High EPD and Low EPD populations were non-significant for all genotypes). Values shown in parentheses indicate that at least one expected value in the analysis was less than five. Because expected values occur in the denominator of the Chi-square test statistic, small expected values may greatly inflate the test statistic. When comparing these values to a table value of 5.99 for a .05 significance level at 3 degrees of freedom, these results indicate that significant differences (P<.05) in allele frequencies among the three populations exist at the B-Lac, GH, IGF-I, PRL, PIT1 and BM2113 polymorphisms.

Results from two-way comparisons (Table 11) indicate that all three populations had different (P<.05) allele frequencies for the B-Lac polymorphism. GH and BM2113 allele frequencies in the pooled EPD population differed (P<.05) from frequencies in both the Line 1 and Lents populations, but frequencies in the Line 1 and Lents populations did not differ from each other. IGF-I allele frequencies in the Lents population differed (P<.05) from allele frequencies in both the Line 1 and pooled EPD populations, but allele

frequencies in the Line 1 and pooled EPD populations did not differ from each other. Allele frequencies for the PIT1 polymorphism were different (P<.05) between the pooled EPD and Lents populations, but allele frequencies in the Line 1 population did not differ from either the pooled EPD or Lents population frequencies. Allele frequencies for the PRL polymorphism were different (P<.05) between the pooled EPD and Line 1 populations, but allele frequencies in the Lents population did not differ from either the pooled EPD or Line 1 population frequencies.

Table 12 shows the Chi-square test statistics that were calculated when testing for differences in genotype frequencies among Line 1, Lents and the pooled EPD populations. These results indicate that differences (P<.05) in genotype frequencies among the three populations exist at the GH, B-Lac, PIT1 and IGF-I polymorphisms. Results from two-way comparisons indicate that differences in genotype frequencies at the GH, B-Lac, PIT1 and IGF-I polymorphisms are the same as the differences in allele frequencies previously described.

Figure 3: Allele frequencies for K-Cas, B-Lac and GH polymorphisms in each population.



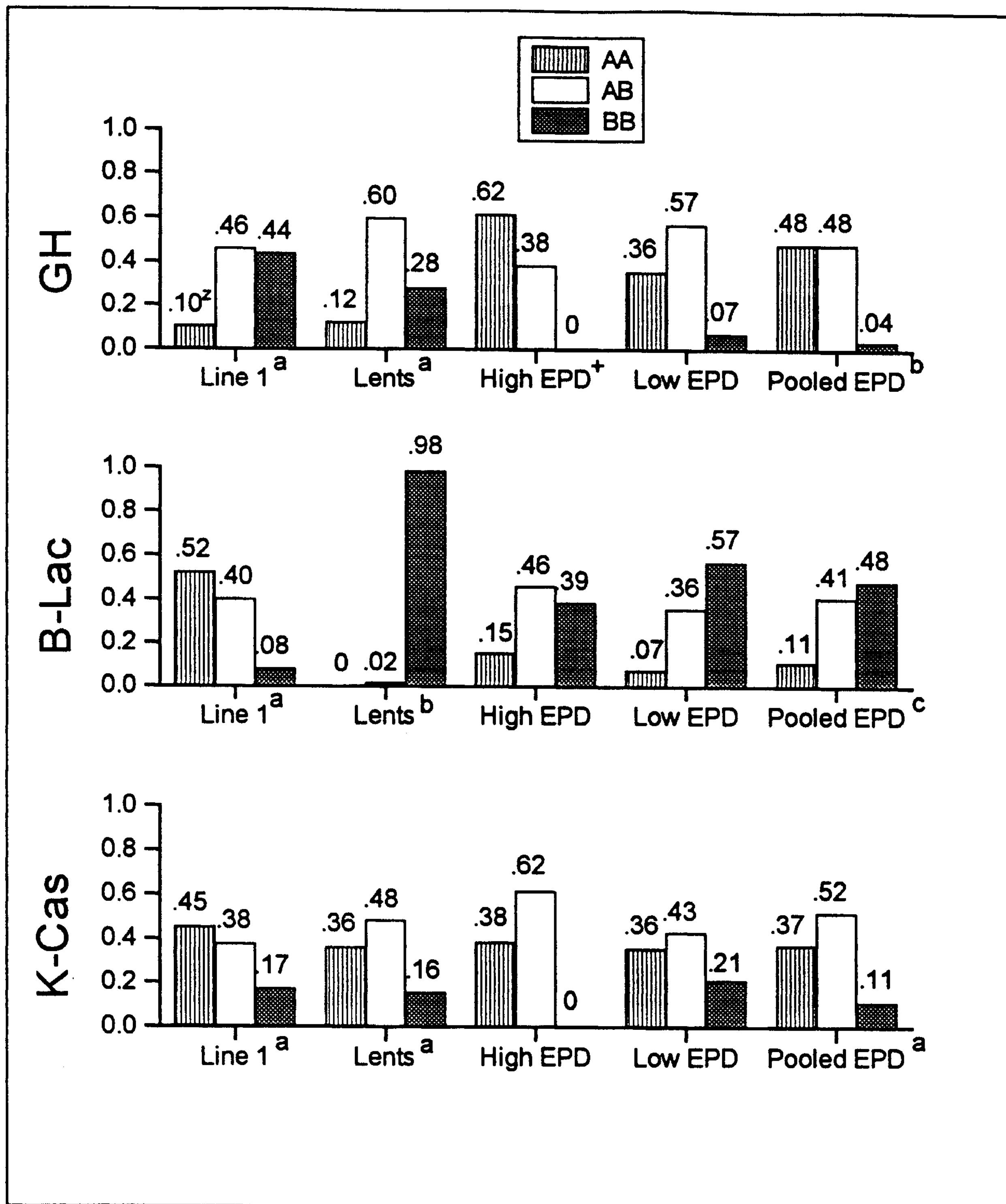
a,b,c populations with different superscripts have significantly different allele frequencies

y actual allele frequencies

z allele frequency variance (x 10^{-3})

+ no differences in allele frequency between High and Low EPD sires were significant

Figure 4: Genotype frequencies for GH, B-Lac and K-Cas polymorphisms in each population.

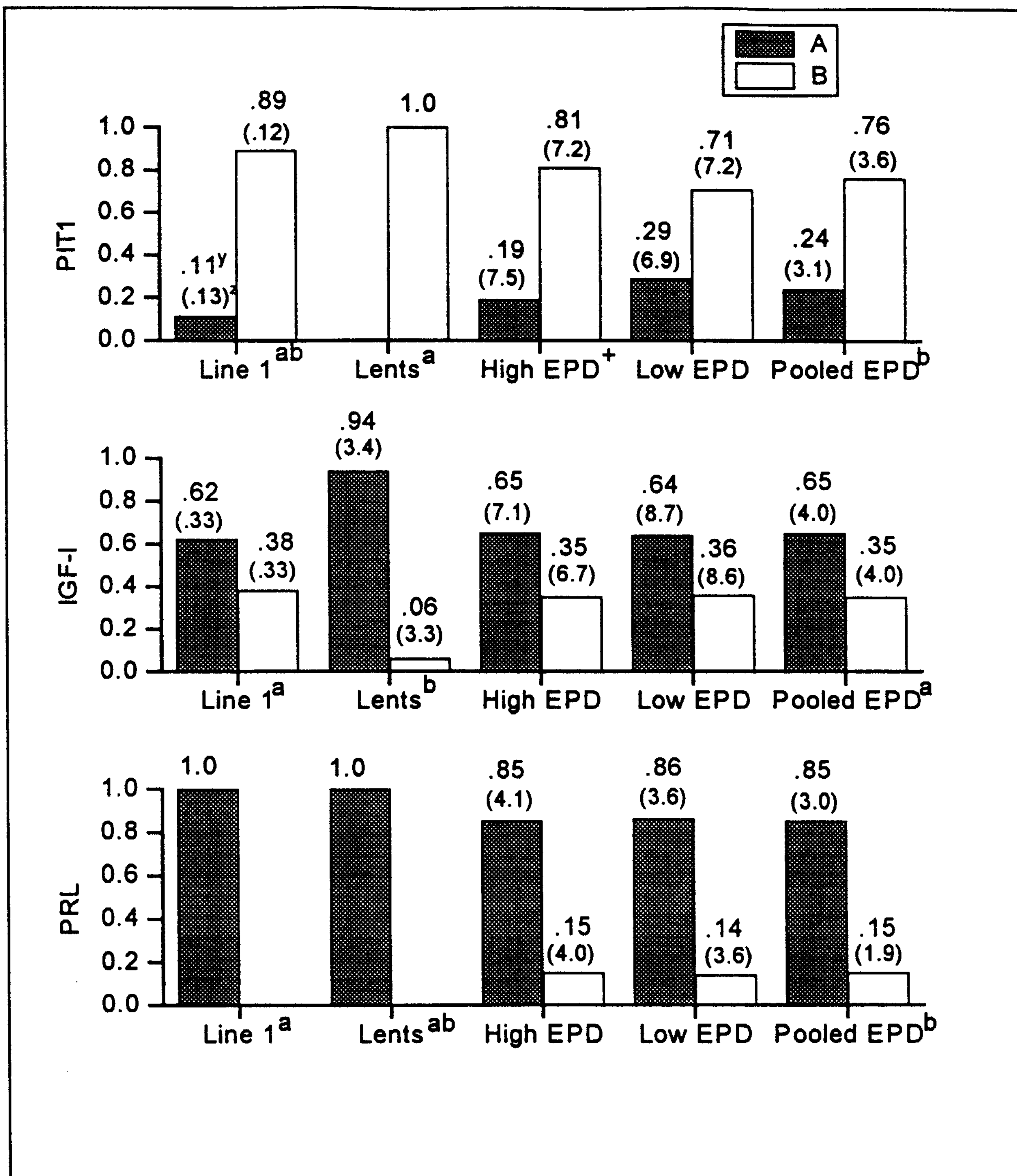


[a,b,c]populations with different superscripts have significantly different genotype frequencies

[z]actual genotype frequencies

[+]no differences in genotype frequencies between High and Low EPD sires were significant

Figure 5: Allele frequencies for PIT1, IGF-I and PRL polymorphisms in each population.



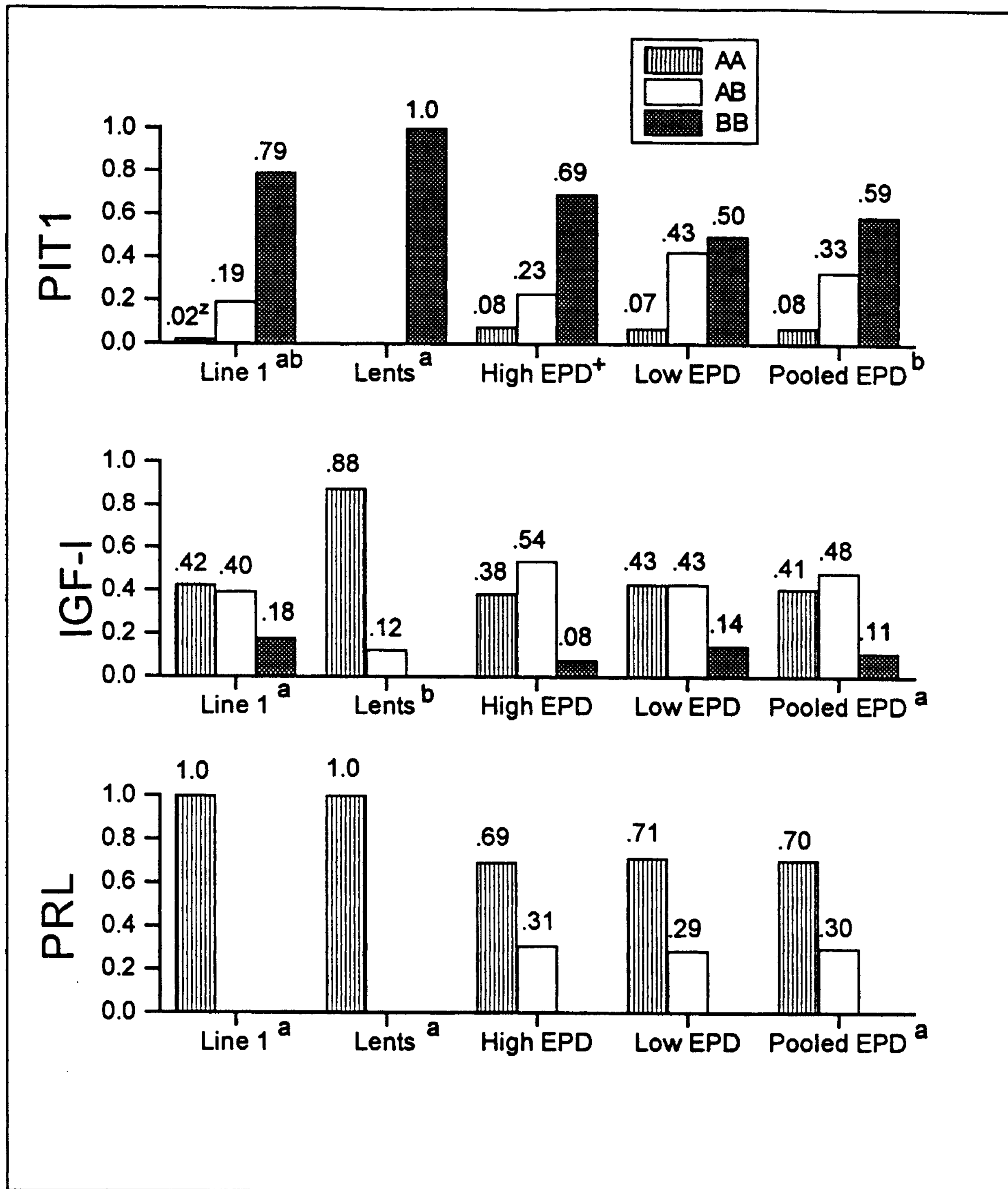
[a,b]populations with different superscripts have significantly different allele frequencies

[y]actual allele frequencies

[z]allele frequency variance (x 10^{-3})

[+]no differences in allele frequency between High and Low EPD sires were significant

Figure 6: Genotype frequencies for PIT1, IGF-I and PRL polymorphisms in each population.

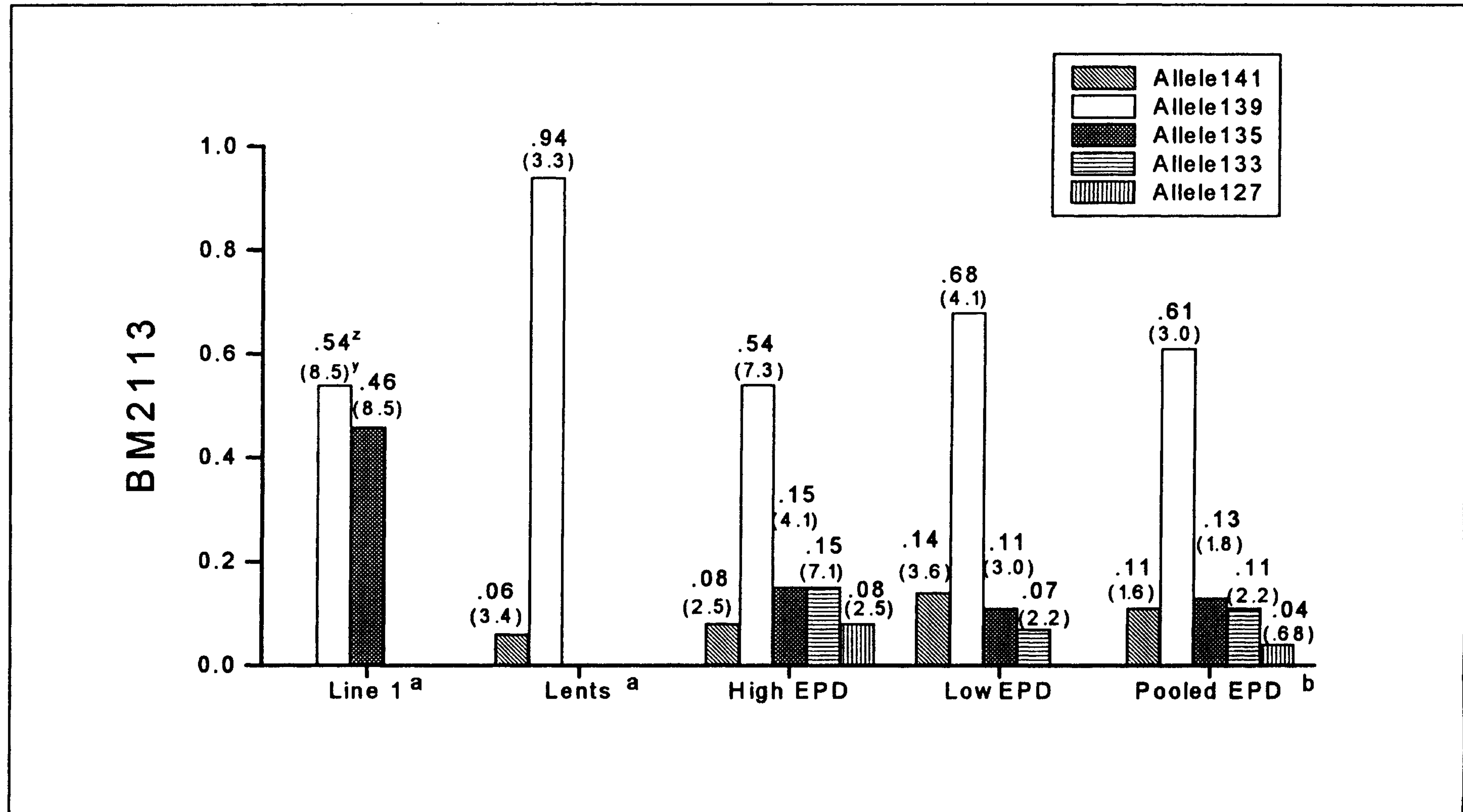


[a,b]populations with different superscripts have significantly different genotype frequencies

[z]actual genotype frequencies

[+]no differences in genotype frequencies between High and Low EPD sires were significant

Figure 7: Allele frequencies for the BM2113 polymorphism in each population.



a,b populations with different superscripts have significantly different allele frequencies

y actual allele frequencies

z allele frequency variances (x 10^{-3})

Table 11. Chi-square test statistics[a] calculated for comparisons of allele frequencies among populations.

Polymorphism	EPD/Line1/Lents	Line1/Lents	Line1/EPD	Lents/EPD
K-Cas	0.60	-	-	-
B-Lac	238.63*	221.09*	38.87*	35.63*
GH	34.55*	3.37	33.07*	13.35*
IGF	7.48*	7.00*	0.56	(4.84*)
PIT1	10.07*	(3.08)	6.76*	(5.41*)
PRL	(8.29*)	(0.26)	(4.90*)	(3.49)
BM2113	(47.47*)	(4.40)	(24.02*)	(14.61*)

*Indicates a significant difference (P<.05) in allele frequency
[a]Values shown in parentheses indicate at least one expected value in the analysis was less than five.

Table 12. Chi-square test statistics[a] calculated for comparisons of genotype frequencies among populations.

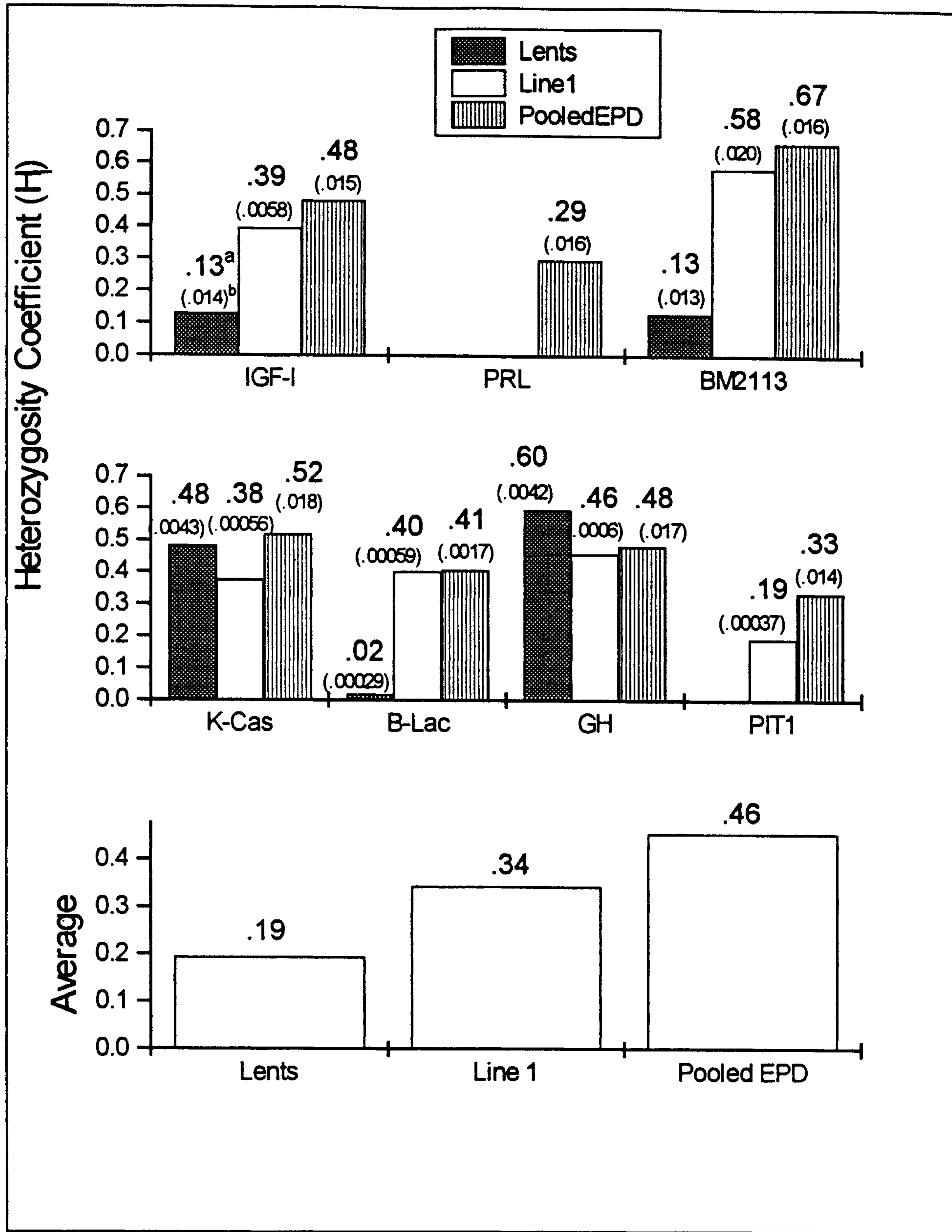
Polymorphism	EPD/Line1/Lents	Line1/Lents	Line1/EPD	Lents/EPD
K-Cas	4.78	-	-	-
B-Lac	268.10*	266.35*	45.66*	30.49*
GH	(41.45*)	5.17	(36.58*)	15.82*
IGF	(10.08*)	(7.51*)	(2.53)	(6.66*)
PIT1	(8.87)	-	-	-
PRL	(10.77*)	(5.71)	(5.17)	(6.76*)

*Indicates a significant difference (P<.05) in allele frequency
[a]Values shown in parentheses indicate that at least one expected value in the analysis was less than five.

Genetic Variation

Heterozygosity coefficients (H_l) and variances for each polymorphism in each population are shown in Figure 8, along with the average H_l. The average H_l indicates that the pooled EPD population had the highest, and the Lents population the lowest average genetic variation. This trend was observed specifically at the IGF-I, PIT1 and BM2113 polymorphisms. Genetic variation at the PRL polymorphism existed in the pooled EPD population (H_l=0.29) but not in the Line 1 or Lents populations (H_l=0). Almost no variation at the B-Lac polymorphism was present in the Lents population (H_l=.02), but genetic variability was similar in the Line 1 (H_l=.40) and pooled EPD (H_l=.41) populations for the B-Lac polymorphism. For the K-Cas and GH polymorphisms, the least amount of genetic variation was observed in the Line 1 population (K-Cas H_l = .38; GH H_l = .46). The pooled EPD population had the greatest genetic variability at the K-Cas polymorphism (H_l=.52), while the Lents population had the greatest genetic variability at the GH polymorphism (H_l=.60).

Figure 8: Heterozygosity coefficients observed in Lents, Line 1 and Pooled EPD populations.



[a]Actual H_I
[b]Var(H_I)

Comparisons with Expectations of Hardy-Weinberg Equilibrium

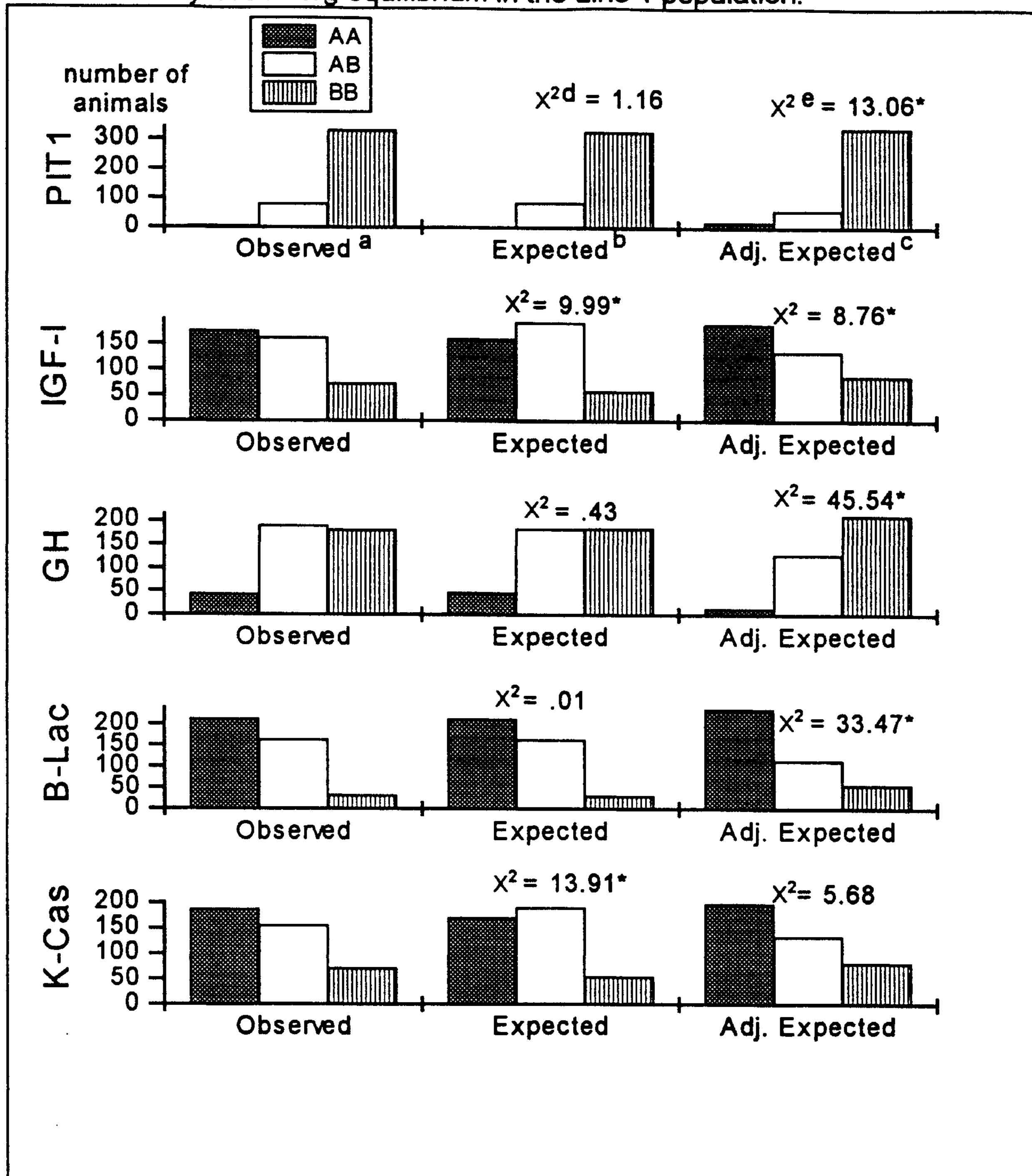
Results from the Chi-square goodness of fit analyses to test assumptions of Hardy-Weinberg equilibrium in the Line 1 and Lents populations are shown in Figures 9 and 10, respectively. Numbers of animals in each genotypic class in the Line 1 population did not differ significantly (P>.05) from expectations of Hardy-Weinberg equilibrium at the PIT1, GH or B-Lac polymorphism, but differed (P<.05) after adjusting expected numbers for known inbreeding (f=.30). The difference from adjusted expected numbers resulted from a lack of increase in the number of homozygotes that is expected in inbred populations (Falconer, 1989). The observed numbers of animals in each genotypic class at the IGF-I polymorphism were intermediate between the expected numbers and the adjusted expected numbers. An increase in the number of homozygotes expected based on an assumption of Hardy-Weinberg equilibrium was observed, resulting in a difference (P<.05) between observed and expected numbers. However, this increase was not as large as the increase predicted by adjusting expected values for known inbreeding, resulting in a significant difference (P<.05) between observed and adjusted expected numbers at the IGF-I polymorphism. Observed numbers of animals in each genotypic class of the K-Cas polymorphism did display the increase in homozygosity that was predicted when adjustments for inbreeding were made (P>.05), but differed (P<.05) from the expected numbers without adjustments for inbreeding.

In the Lents population, the observed numbers of animals in each genotypic class at the K-Cas and GH polymorphisms did not differ (P>.05) from expected numbers, but did differ (P<.05) from expected numbers that were adjusted for a known inbreeding coefficient of .50.

Table 13 shows results obtained when testing assumptions of Hardy-Weinberg equilibrium using disequilibrium coefficients (D_A; Weir, 1990). When considering departures from Hardy-Weinberg equilibrium due to either an excess or deficiency of homozygotes (H_0: D_A=0 vs. H_A: D_A not 0), the Line 1 population differs ($P<.05$) from Hardy-Weinberg equilibrium expectations at the IGF-I and K-Cas polymorphisms but not at the B-Lac, GH or PIT1 polymorphisms ($P>.05$). The same analysis in the Lents population indicates no difference ($P>.05$) from Hardy-Weinberg equilibrium expectations at either the K-Cas or GH polymorphism.

When considering only an excess (H_0: D_A=0 vs. H_A: D_A<0; 5% significance level at $z < -1.64$) or only a deficiency (H_0: D_A=0 vs. H_A: D_A>0; 5% significance level at $z > 1.64$) of heterozygotes, results indicate a deficiency of heterozygotes ($P<.05$) at the K-Cas and IGF-I polymorphisms in the Line 1 population. Results also indicate a significant ($P<.05$) excess of homozygotes in the Lents population for the GH polymorphism.

Figure 9: Results from Chi-square goodness of fit analyses to test assumptions of Hardy-Weinberg equilibrium in the Line 1 population.



[a]Number of Line 1 animals in each genotypic class

[b]Expected number of animals in each genotypic class based on an assumption of Hardy-Weinberg equilibrium

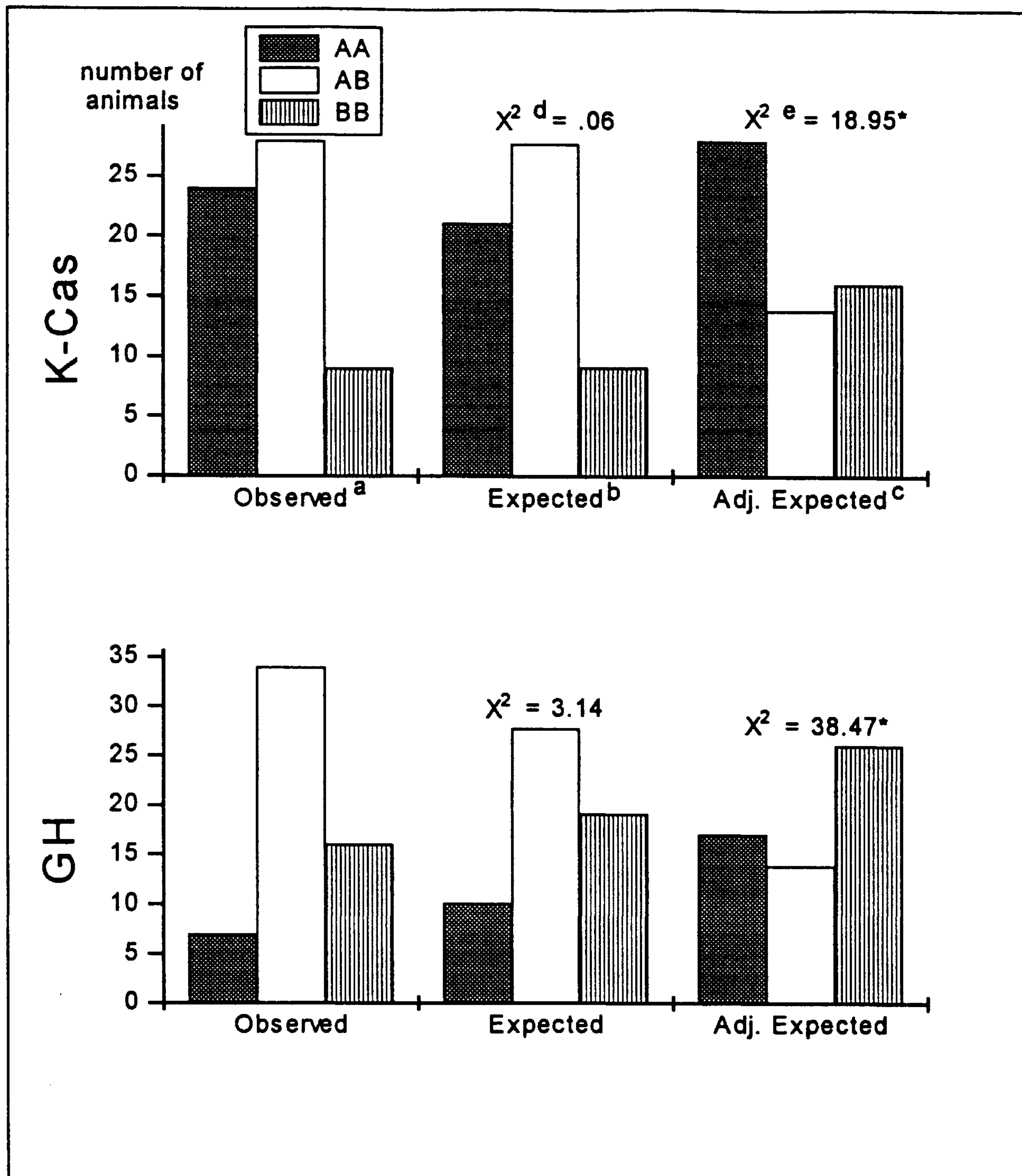
[c]Expected number of animals in each genotypic class based on an assumption of Hardy-Weinberg equilibrium after adjusting for known inbreeding level

[d]Chi-square test statistic comparing expected numbers to observed numbers

[e]Chi-square test statistic comparing adjusted expected numbers to observed numbers

* indicates a significant difference ($P<.05$) between observed numbers expected or adjusted expected numbers in each each genotypic class

Figure 10: Results from Chi-square goodness of fit analyses to test assumptions of Hardy-Weinberg equilibrium in the Lents population.



[a]Number of Line 1 animals in each genotypic class

[b]Expected number of animals in each genotypic class based on an assumption of Hardy-Weinberg equilibrium

[c]Expected number of animals in each genotypic class based on an assumption of Hardy-Weinberg equilibrium after adjusting for known inbreeding level

[d]Chi-square test statistic comparing expected numbers to observed numbers

[e]Chi-square test statistic comparing adjusted expected numbers to observed numbers

* indicates a significant difference ($P<.05$) between observed numbers expected or adjusted expected numbers in each each genotypic class

Table 13. Results from tests of assumptions of Hardy-Weinberg equilibrium in Line 1 and Lents populations using disequilibrium coefficients.

	D_A[a]	$var(D_A)$[b]	z[c]
Line 1			
PIT1	0.0061	0.0002	3.60
IGF-I	0.0369	0.0003	0.09
GH	0.0067	0.0001	0.27
B-Lac	0.0009	0.0001	2.20
K-Cas	0.0427	0.0001	0.41
Lents			
K-Cas	-0.0015	0.0010	-0.05
GH	-0.0545	0.0010	-1.75

[a] $D_A = P_{AA} - P_A^2$

[b] $var(D_A) = 1/n[p_A^2(1-p_A)^2 + (1-2p_A)^2 D_A - D_A^2]$

[c] $z = (D_A - 0)/[var(D_A)]^{1/2}$

Part II: QTL Analysis

Regression Analyses

The number of observations, means and standard deviations for EPDs and phenotypic data used in statistical analyses for markers of QTL in the Line 1 population are presented in Table 14. The number of observations varies because of missing EPDs or phenotypic data.

Table 14: Summary of Line 1 EPD and phenotypic data included in QTL analyses.

Trait	n	Mean[a]	Standard Error[a]
BWT EPD	401	-0.8441	0.1196
WWT EPD	402	23.0075	0.3115
YWT EPD	402	40.3159	0.5219
MILK EPD	388	-2.6675	0.3058
Birth Weight	418	80.8589	0.6000
Weaning Weight	413	457.4201	3.6597
Yearling Weight	410	847.0567	6.5561

[a]pounds

Results from the regression analyses of BWT, WWT, YWT and MILK EPD on genotype (EPD regression) are shown in Table 15. Substitution of an A allele with a B allele had significant effects on BWT EPD for the K-Cas, B-Lac and IGF-I polymorphisms (P<.01), and for the PIT1 polymorphism (P<.05). The effect of substituting a B allele for an A allele on WWT EPD was significant for K-Cas (P<.01) and IGF-I (P<.05) polymorphisms, while the effect of allele substituion on YWT EPD was significant only for the K-Cas polymorphism

(P<.01). The effect of allele substitution on MILK EPD was significant (P<.01) for the K-Cas, GH and IGF-I polymorphisms.

The percentage of variability in EPD explained by genotype is also presented in Table 15 and ranges from 0.01% to 11.33%. The largest percentage of variability in BWT EPD was explained by K-Cas (9.04%) and IGF-I (6.44%) polymorphisms. B-Lac and PIT1 polymorphisms also had significant effects on BWT EPD, but explained a smaller percentage of BWT EPD variability (2.06% and 1.07%, respectively). K-Cas and IGF-I genotype accounted for 2.05% and 1.07% of the variability in WWT EPD while K-Cas genotype explained 2.13% of YWT EPD variability. K-Cas genotype explained 11.33% of the variabilty in MILK EPD while the significant effects of GH and IGF-I accounted for 1.66% and 2.88%, respectively, of MILK EPD variation. Estimates of dominance deviations were not significant for any traits. EPD means observed for each genotype and the predicted means estimated from regression analyses are shown in Appendix 10.

Results from the regression analyses of birth weight, 200-day weight and 365-day weight on genotype without sire in the model (Regression Model 1) are shown in Table 16. Substituting a B allele for an A allele had a significant effect on BWT for the K-Cas polymorphism (P<.01) and for the B-Lac and IGF-I polymorphisms (P<.05). The effect of allele substitution on 200-day weight was significant for the K-Cas polymorphism (P<.05) and approached significance for the IGF-I polymorphism (P<.10). The effect of allele substitution on 365-day weight was not significant for any of the polymorphisms analyzed.

The percentage of total variability as well as the percentage of residual variability explained by genotype for each trait are also presented in Table 16. The largest amount of variabilty explained by genotype was 1.53% of total and 2.23% of residual variability in BWT explained by K-Cas genotype. B-Lac

genotype explained 1.05% of total and 1.57% of residual variability in BWT while IGF-I genotype accounted for 0.81% of total and 1.20% of residual variability in BWT. K-Cas genotype explained 0.63% of total and 1.23% of residual variability in 200-d WT and IGF-I genotype accounted for 0.40% of total and 1.20% of residual variability in 200-d WT. Estimates of dominance deviations were not significant for any traits analyzed. Estimates of a, or the average weight of animals with the AA genotype, are not presented because the predicted value from the regression analysis is biased.

Results from the regression analyses of birth weight, 200-day weight and 365-day weight on genotype with sire included in the model as a fixed effect (Regression model 2) are shown in Table 17. The effect of allele substitution at the B-Lac polymorphism on birth weight ($P<.01$) was the only significant effect. B-Lac genotype accounted for 0.97% of total and 1.81% of residual variability of BWT in this analysis.

Results of the three different regression models (EPD Regression, Regression Model 1 and Regression Model 2) are summarized in Table 18. Significant effects from EPD Regression and Regression Model 1 analyses were similar, with the exception of significant effects of K-Cas and PIT1 genotypes on BWT EPD that were not observed for BWT from Regression Model 1. Inclusion of sire in the model (Regression Model 2) removed all significant effects of allele substitution on phenotype, with the exception of the the effect of B-Lac genotype on birth weight.

Table 15: Results from regression analyses (EPD regression) of birth weight (BWT EPD), weaning weight (WWT EPD), yearling weight (YWT EPD) and maternal (MILK EPD) EPD.

	a[a]	α[b]	d[c]	% var[d]
K-Cas				
BWT EPD	-0.051 ± 0.148	-1.113 ± 0.178**	-0.076 ± 0.210	9.04
WWT EPD	24.000 ± 0.403	-1.388 ± 0.483**	-0.303 ± 0.543	2.05
YWT EPD	42.070 ± 0.666	-2.342 ± 0.798**	-0.426 ± 0.946	2.13
MILK EPD	-0.422 ± 0.376	-3.158 ± 0.453**	-0.072 ± 0.512	11.33
B-Lac				
BWT EPD	-0.509 ± 0.150	-0.662 ± 0.232**	-0.029 ± 0.175	2.06
WWT EPD	22.690 ± 0.391	0.461 ± 0.604	-0.073 ± 0.522	0.15
YWT EPD	39.769 ± 0.650	0.970 ± 1.001	-0.036 ± 0.848	0.24
MILK EPD	-2.126 ± 0.385	-0.788 ± 0.597	0.197 ± 0.511	0.46
GH				
BWT EPD	-0.958 ± 0.339	0.130 ± 0.217	0.054 ± 0.175	0.09
WWT EPD	22.882 ± 0.885	0.133 ± 0.814	0.053 ± 0.485	0.01
YWT EPD	38.681 ± 1.487	1.322 ± 0.949	0.174 ± 0.846	0.49
MILK EPD	-4.460 ± 0.861	1.392 ± 0.549**	0.086 ± 0.440	1.66
IGF-I				
BWT EPD	-1.516 ± 0.162	0.971 ± 0.187**	0.123 ± 0.188	6.44
WWT EPD	22.256 ± 0.427	1.016 ± 0.493*	-0.119 ± 0.502	1.07
YWT EPD	39.739 ± 0.724	0.740 ± 0.836	-0.479 ± 0.841	0.20
MILK EPD	-3.853 ± 0.429	1.642 ± 0.491**	0.244 ± 0.468	2.88
PIT1				
BWT EPD	1.068 ± 0.292	-1.069 ± 0.516*	0.002 ± 0.262	1.07
WWT EPD	24.936 ± 2.702	-1.098 ± 1.379	-0.011 ± 0.681	0.16
YWT EPD	44.788 ± 4.538	-2.510 ± 2.315	0.001 ± 1.167	0.30
MILK EPD	0.711 ± 2.790	-1.889 ± 1.422	0.048 ± 0.597	0.46

**P<.01, *P<.05, +P<.10

[a]average EPD of the AA genotype

[b]average effect of allele substitution (pounds)

[c]dominance deviation (pounds)

[d]percentage of variability of EPD explained by genotype.

Table 16: Results from regression analyses (Regression model 1) of birth weight, 200-day weight and 365-day weight on genotype.

	α^a	d^b	% Tot[c]	% Res[d]
K-Cas				
BWT	-2.427 ± 0.815**	-0.296 ± 0.824	1.53	2.23
200-d WT	-93468 ± 4.328*	-1.360 ± 3.963	0.63	1.23
365-d WT	-9.776 ± 6.649	-0.497 ± 6.370	0.21	0.56
B-Lac				
BWT	-2.467 ± 0.998*	-0.133 ± 0.766	1.05	1.57
200-d WT	-2.143 ± 5.408	-0.532 ± 4.233	0.04	0.04
365-d WT	-5.347 ± 8.237	-1.237 ± 6.813	0.04	0.11
GH				
BWT	0.037 ± 0.965	0.054 ± 0.708	0.00	0.00
200-d WT	0.522 ± 5.102	0.477 ± 3.882	0.00	0.00
365-d WT	-0.742 ± 7.810	0.761 ± 5.756	0.00	0.00
IGF-I				
BWT	1.772 ± 0.819*	0.359 ± 0.817	0.81	1.20
200-d WT	7.318 ± 4.236+	-1.891 ± 4.348	0.40	0.78
365-d WT	4.665 ± 6.579	-1.425 ± 6.922	0.05	0.14
PIT1				
BWT	-1.437 ± 2.214	-0.100 ± 1.149	0.07	0.11
200-d WT	-9.655 ± 11.663	0.109 ± 6.370	0.09	0.18
365-d WT	-20.038 ± 18.132	0.715 ± 9.394	0.12	0.32

**P<.01, *P<.05, +P<.10

[a]average effect of allele substitution (pounds)

[b]dominance deviation (pounds)

[c]percentage of total variance explained by genotype

[d]percentage of residual variance explained by genotype

Table 17. Results from regression analyses (Regression model 2) of birth weight, 200-day weight and 365-day weight on genotype with sire included in the model as a fixed effect .

	α[a]	d[b]	% Tot[c]	% Res[d]
K-Cas				
BWT	-0.589 ± 0.989	-0.429 ± 0.750	0.06	0.10
200-d WT	-3.802 ± 5.451	-2.021 ± 3.733	0.06	0.13
365-d WT	-2.061 ± 8.315	-2.673 ± 5.749	0.01	0.02
B-Lac				
BWT	-2.777 ± 1.078**	-0.116 ± 0.739	0.97	1.81
200-d WT	-4.264 ± 6.122	-0.434 ± 3.979	0.06	0.14
365-d WT	-9.869 ± 9.308	-1.456 ± 6.279	0.10	0.32
GH				
BWT	-0.201 ± 0.984	0.043 ± 0.638	0.01	0.01
200-d WT	0.813 ± 5.471	0.336 ± 3.573	0.00	0.01
365-d WT	-4.065 ± 8.207	0.868 ± 5.163	0.02	0.07
IGF				
BWT	-1.506 ± 1.005	-0.028 ± 0.717	0.33	0.61
200-d WT	-1.658 ± 5.479	-2.543 ± 4.044	0.01	0.03
365-d WT	-4.493 ± 8.271	-1.650 ± 6.353	0.03	0.08
PIT1				
BWT	-0.500 ± 2.229	-0.148 ± 1.048	0.01	0.01
200-d WT	-6.016 ± 12.296	0.055 ± 6.509	0.03	0.07
365-d WT	-14.400 ± 18.745	0.609 ± 9.835	0.05	0.16

**P<.01, *P<.05, +P<.10

[a]average effect of allele substitution (pounds)

[b]dominance deviation (pounds)

[c]percentage of total variance explained by genotype

[d]percentage of residual variance explained by genotype

Table 18: Comparison of regression analyses to determine the average effect of allele substitution for each polymorphism.

Model	Birth Weight	Weaning Weight	Yearling Weight	Milk
K-Cas				
EPD	**	**	**	**
Model 1	**	*	ns	
Model 2	ns	ns	ns	
B-Lac				
EPD	**	ns	ns	ns
Model 1	*	ns	ns	
Model 2	**	ns	ns	
GH				
EPD	ns	ns	ns	**
Model 1	ns	ns	ns	
Model 2	ns	ns	ns	
IGF-I				
EPD	**	*	ns	**
Model 1	*	+	ns	
Model 2	ns	ns	ns	
PIT1				
EPD	*	ns	ns	ns
Model 1	ns	ns	ns	
Model 2	ns	ns	ns	

** P<.01, * P<.05, + P<.10, ns P>.10

Least Squares Analyses

Results from least squares analyses of EPDs (EPD LSM) are shown in Table 19. Main effects of K-Cas (P<.01), IGF-I (P<.01) and B-Lac (P<.05) genotypes on BWT EPD were significant. Results from contrasts indicated significant (P<.01) additive gene action on BWT EPD due to K-Cas and IGF-I genotype, but neither additive nor dominant gene action was significant for the B-Lac polymorphism (P>.10). The main effect of K-Cas genotype on WWT EPD was significant (P<.01), and the main effect of IGF-I genotype on WWT EPD approached significance (P<.10). In both cases, the effect was caused by additive gene action. The main effect of K-Cas genotype on YWT EPD was significant (P<.01), and was due to additive gene action (P<.05). Main effects of K-Cas (P<.01), IGF-I (P<.01) and GH (P<.05) genotype on MILK EPD were significant. Additive gene effects of K-Cas (P<.01) and IGF-I (P<.05) genotype were significant, and additive gene effects of GH genotype (P<.10) approached significance.

The largest percentage of variability in BWT EPD was explained by K-Cas genotype (9.13%) followed by IGF-I (6.70%) and B-Lac (2.10%) genotype. K-Cas genotype accounted for 2.26% of the variability in WWT EPD and 2.29% of the variability in YWT EPD, while IGF-I explained 1.11% of WWT EPD variability. The largest percent of variability in MILK EPD was explained by K-Cas genotype (11.34%) followed by IGF-I (3.04%) and GH (1.72%) genotype.

Results from least squares analyses of phenotypic data including significant two-way interactions (LSM Model 1) are shown in Table 20. The main effect of K-Cas genotype on BWT was significant (P<.01), and the significant effect was due to additive gene action (P<.05). The main effect of IGF-I

genotype on BWT approached significance (P<.10), but neither contrast testing for additive nor dominant gene action was significant (P>.10). The main effect of B-Lac genotype on BWT was significant (P<.01) when no interactions were included in the model, but was non-significant when interactions of B-Lac x age of dam, B-Lac x year, age of dam x year, age of dam x sex and sex x year were included in the analysis. The main effect and additive gene effect of K-Cas genotype on 200-day weight approached significance (P<.10), and the additive gene effect of IGF-I genotype on 200-day weight was significant (P<.05).

K-Cas genotype accounted for 1.67% of total and 2.34% of residual variability of BWT while IGF-I genotype explained 0.88% of total and 1.27% of residual variability. K-Cas genotype explained 0.67% of total and 1.29% of residual variability in 200-day weight while IGF-I genotype accounted for 0.54% of total and 1.03% of residual variability in 200-day weight.

No main effects of genotype were significant when either sire or sire nested within genotype were included in the least-squares model (LSM Model 2 and LSM Model 3). Results from least squares analyses (EPD LSM, LSM Model 1, LSM Model 2 and 3) are summarized in Table 21. Significant effects of K-Cas genotype on 365-day weight and B-Lac genotype on BWT were observed from the EPD LSM analysis but not from the LSM Model 1 analysis. Otherwise, least squares analyses of EPDs and phenotypic data were similar.

Table 19. Results from least squares analyses (EPD LSM) of birth weight (BWT EPD), weaning weight (WWT EPD), yearling weight (YWT EPD) and maternal (MILK EPD) EPD.

	ME[a]	Additive[b]	Dominance[c]	% Var[d]
K-Cas				
BWT EPD	**	2.074 ± 0.431**	-0.179 ± 0.285	9.13
WWT EPD	**	2.164 ± 1.170+	-0.715 ± 0.771	2.26
YWT EPD	**	3.827 ± 1.934*	-1.002 ± 1.275	2.29
MILK EPD	**	6.159 ± 1.115**	-0.175 ± 0.725	11.34
B-Lac				
BWT EPD	*	0.993 ± 0.979	-0.204 ± 0.529	2.10
WWT EPD	ns	-1.758 ± 2.556	-0.512 ± 1.381	0.19
YWT EPD	ns	-2.346 ± 4.242	-0.249 ± 2.292	0.24
MILK EPD	ns	3.787 ± 2.518	1.358 ± 1.362	0.72
GH				
BWT EPD	ns	-0.681 ± 0.829	0.290 ± 0.404	0.22
WWT EPD	ns	-0.682 ± 1.902	0.286 ± 1.054	0.03
YWT EPD	ns	-4.009 ± 3.194	0.940 ± 1.769	0.56
MILK EPD	*	-3.483 ± 1.876+	0.476 ± 1.036	1.72
IGF-I				
BWT EPD	**	-1.674 ± 0.451**	0.308 0.290	6.70
WWT EPD	+	-2.295 ± 1.190*	-0.302 ± 0.764	1.11
YWT EPD	ns	-2.539 ± 2.016	-1.216 ± 1.295	0.42
MILK EPD	**	-2.754 ± 1.177*	0.618 ± 0.756	3.04
PIT1				
BWT EPD	ns	2.027 ± 5.387	0.058 ± 2.742	1.07
WWT EPD	ns	2.831 ± 14.398	-0.329 ± 7.328	0.16
YWT EPD	ns	4.945 ± 24.176	0.038 ± 12.304	0.30
MILK EPD	ns	1.354 ± 14.285	1.261 ± 7.281	0.47

**P<.01, *P<.05, +P<.10, ns:P>.10

[a]main effect of genotype

[b]contrasts testing for additive gene action

[c]contrasts testing for dominant gene action

[d]percentage of variability in EPD explained by genotype

Table 20. Results from least squares analyses (LSM Model 1) of birth weight, 200-day weight, and 365-day weight.

	ME[a]	Additive[b]	Dominance[c]	% Tot[d]	% Res[e]
K-Cas					
BWT	**	4.375 ± 1.970*	-0.672 ± 1.298	1.67	2.34
200-d wt.	+	17.221 ± 10.481+	-2.439 ± 0.937	0.67	1.29
365-d wt.	ns	18.177 ± 16.121	-1.905 ± 10.562	0.22	0.58
B-Lac					
BWT	ns	1.370 ± 5.513	-1.335 ± 2.890	0.55	0.89
200-d wt.	ns	-1.066 ± 23.096	-4.694 ± 12.406	0.07	0.14
365-d wt.	ns	-6.228 ± 35.010	-9.623 ± 18.819	0.06	0.16
GH					
BWT	ns	-1.157 ± 3.290	0.374 ± 1.811	0.14	0.20
200-d wt.	ns	-6.739 ± 17.194	3.184 ± 9.424	0.02	0.04
365-d wt.	ns	-7.062 ± 26.175	4.076 ± 14.360	0.01	0.02
IGF-I					
BWT	+	-2.657 ± 1.917	0.943 ± 1.248	0.88	1.27
200-d wt.	ns	-19.958 ± 9.985*	-5.866 ± 6.493	0.54	1.03
365-d wt.	ns	-8.966 ± 15.714	3.644 ± 10.138	0.03	0.09
PIT1					
BWT	ns	6.637 ± 20.995	-1.933 ± 10.723	0.08	0.12
200-d wt.	ns	12.029 ± 110.814	1.382 ± 56.628	0.05	0.10
365-d wt.	ns	-0.356 ± 170.097	18.423 ± 86.977	0.09	0.24

**P<.01, *P<.05, +P<.10, ns:P>.10

[a]main effect of genotype

[b]contrasts testing for additive gene action

[c]contrasts testing for dominant gene action

[d]percentage of total variability explained by genotype

[e]percentage of residual variability explained by genotype

Table 21: Comparison of results from least squares analyses of EPDs, Model 1 and Models 2 and 3. Significance levels are presented for the main effect of genotype.

	Birth Weight	Weaning Wt	Yearling Wt	MILK EPD
K-Cas				
EPD	**	**	**	**
Model 1	**	+	ns	
Model 2,3	ns	ns	ns	
B-Lac				
EPD	*	ns	ns	ns
Model 1	ns	ns	ns	
Model 2,3	ns	ns	ns	
GH				
EPD	ns	ns	ns	*
Model 1	ns	ns	ns	
Model 2,3	ns	ns	ns	
IGF-I				
EPD	**	+	ns	**
Model 1	+	ns	ns	
Model 2,3	ns	ns	ns	
PIT1				
EPD	ns	ns	ns	ns
Model 1	ns	ns	ns	
Model 2,3	ns	ns	ns	

**P<.01, *P<.05, +P<.10, ns P>.10

Animal Model Analysis

Results from the Animal Model analysis are shown in Table 22. Contrasts testing for additive and dominant gene action indicated no significant effects of genotype on birth weight, weaning weight or yearling weight. These results are consistent with results from regression and least squares analyses which included sire as a fixed effect in the model.

Table 22. Results from animal model analysis of BWT, 200-day weight and 365-day weight for contrasts (pounds) for additive and dominance effects[a].

	BWT	200-day weight	365-day weight
K-Cas			
additive	1.2 ± 1.8	11.8 ± 8.8	7.5 ± 15.5
dominance	-1.3 ± 1.1	-5.1 ± 5.5	-8.4 ± 9.2
B-Lac			
additive	2.3 ± 2.1	-9.3 ± 9.6	-9.1 ± 17.1
dominance	-1.0 ± 1.2	-0.1 ± 5.8	-8.3 ± 10.1
GH			
additive	0.7 ± 2.0	-1.3 ± 9.3	1.4 ± 16.3
dominance	0.3 ± 1.2	2.6 ± 5.6	6.4 ± 9.7
IGF-I			
additive	2.1 ± 1.9	-5.0 ± 8.8	-0.7 ± 15.7
dominance	0.2 ± 1.1	-2.7 ± 5.3	-5.9 ± 8.9
PIT1			
additive	3.0 ± 4.1	-6.6 ± 21.7	-22.7 ± 33.6
dominance	-1.8 ± 2.2	4.6 ± 11.9	25.5 ± 18.5

[a]No contrasts were statistically significant ($P<.05$).

CHAPTER 5

DISCUSSION

Part I: Characterization of DNA Polymorphisms in 4 Populations of Hereford Cattle

Allele Frequencies

Frequencies of the K-Cas A allele were .64, .60 and .63 in the Line 1, Lents and pooled EPD populations, respectively. These frequencies compare to earlier reports of .85 for Holstein (Medrano, 1990) and .10 for Jersey (Medrano and Aguilar-Cordova, 1990a). Medrano (1990) reported a B-Lac A allele frequency of .40 in Holstein, which is similar to .31 observed for the pooled EPD population, but much lower than .72 observed in the Line 1 population, and higher than .01 observed in the Lents population.

Zhang et al. (1993) reported GH A allele frequencies of .91, .73, .74 and .65 for Holstein, Simmental, Angus and Herefords, respectively. GH A allele frequency observed in the pooled EPD population (.72) is similar to the previous report for Herefords, but GH A allele frequencies observed in Line 1 (.33) and Lents (.42) populations are considerably lower. Kirkpatrick (1992) reported an A allele frequency of .26 for IGF-I in a mixed-breed population, which is much lower than observations of .62, .94 and .65 for the Line 1, Lents and pooled EPD populations, respectively. These results indicate significant between-breed and within-breed variation in allele frequency at many loci.

Differences in Allele Frequencies in Hereford Populations

Changes in allele frequencies in a population may be caused by several factors including selection, random genetic drift, migration and mutation. Inbreeding enables gene frequencies of a population to drift more rapidly by limiting the sample of gametes available for the next generation. Differences in allele frequencies between populations may also result from differences in allele frequncies among the founder animals of the different populations, and from sampling error in determining actual allele frequencies.

When considering the differences in allele frequencies that were observed at six of the seven polymorphisms studied in three different Hereford populations, it is not clear which factor(s) are responsible for the observed differences. Differences in selection emphasis has likely influenced allele frequencies of the populations. The Line 1 population has undergone continuous selection pressure for increased yearling weight, while selection emphasis in the Lents population has been less specific. Sires in the High EPD population were chosen to represent Hereford cattle in which selection for increased growth had been successful, and sires in the Low EPD were chosen to represent Hereford cattle in which superior growth traits are not observed. If selection was the primary force influencing the allele frequencies of these populations, it could be expected that allele frequencies in the Line 1 population would be similar to allele frequencies of the High EPD sires, and allele frequencies of the Lents population would be similar to the Low EPD sires. This expectation was not observed for any of the seven polymorphisms genotyped. No significant differences in allele frequencies between the High and Low EPD sires were observed. One reason for this may have been the limited number of

sires representing each group (n = 13 and 14). Pooling the two EPD groups was done so that a larger sample of sires representing the Hereford breed as a whole could be used in comparisons with the Lents and Line 1 populations.

An important unknown factor when considering the effects of selection on specific allele frequencies is the genetic background in which the frequencies occur. Even though all three populations involved in this study represented the Hereford breed, results clearly show that differences in allele frequencies exist among the populations. Therefore, a favorable allele for growth in one population may be unfavorable in other populations due to interactions of the allele with background genes.

Genetic drift is another factor which may influence allele frequencies. Genetic drift is a random process, and may eventually lead to the fixation of any allele. The fixation of a more common allele at a particular locus is more likely, but the fixation of an allele originally present at a low frequency is also possible. Because this study determined genotypes for only one generation, changes in allele frequencies within populations over time cannot be known, and effects of genetic drift cannot be determined. However, differences in allele frequencies observed at the B-Lac polymorphism may represent changes due to genetic drift. At this polymorphism, the A allele was more common in the Line 1 population, the B allele more common in the Lents population, and frequencies in the pooled EPD population were intermediate between the Line 1 and Lents populations. One possible explanation is that original frequencies of Line 1 and Lents populations were similar to the pooled EPD population, but genetic drift has led toward fixation of the A allele in the Line 1 population, and fixation of the B allele in the Lents population.

Changes in allele frequencies due to genetic drift may have been amplified in the Line 1 and Lents populations because of inbreeding which

accumulated in those herds. Both the Lents and Line 1 are closed, relatively small populations. Because of limited numbers of animals in the populations, the mating of related animals could not be avoided completely. As a result, the effective population size of the herds decreased and inbreeding accumulated, causing a reduction in the pool from which gametes of the next generation were obtained. As the gametic pool decreased, the chance of obtaining gametes for the next generation with different allele frequencies from the preceeding generation increased, increasing the possibility of random genetic drift.

Migration may be another cause of changes in allele frequencies. The Line 1 and Lents populations have both been maintained as closed populations, meaning no new germplasm has been introduced to the herds since they were first established. In contrast, the pooled EPD population represents several diverse genetic lines that have developed within the Hereford breed since the Line 1 and Lents populations have been closed. Four of the sires included in the pooled EPD population are polled, indicating a wide range of genetic lines within the Hereford breed are represented. As these lines have developed, factors previously discussed may have caused allele frequencies to differ among the lines. The pooled EPD population represents many of these different lines which have not influenced the Line 1 or Lents populations. Observed differences in GH allele frequencies may represent changes due to migration of different germplasm into the pooled EPD population that is not present in the Line 1 or Lents populations. The B allele of the GH gene was more common in the Line 1 and Lents populations, but the A allele was more common in the pooled EPD population. This could indicate that the A allele has recently become more common in the Hereford breed because of its prevalence in lines that are currently popular, whereas the B allele is more common in older lines.

This result was unexpected because the Line 1 population has had a significant impact on the Hereford breed.

A similar situation would exist if the animals which were used as the foundation of the different populations, specifically Line 1 and and Lents, had different allele frequencies. If these allele frequencies have not been significantly changed over time, then different allele frequencies would be observed in the present populations. Allele frequencies at the B-Lac and IGF-I polymorphisms were significantly different between the Line 1 and Lents populations, and may represent differences in allele frequencies of the founder animals of these populations.

Mutation is a potential cause of differences in allele frequencies, but has likely not been a factor in this situation. The polymorphisms genotyped in this study are polymorphisms that are known to exist in other populations. In order for mutation to have influenced observed allele frequencies, a point mutation or deletion changing one allele to the exact sequence of the alternative allele must have occured. The probability of this happening is negligible.

Finally, the numbers of animals representing each population of this study need to be considered. The Line 1 population was represented by ~420 animals, the Lents population by 58, and the pooled EPD population by 24. One problem with Chi-square test statistics is that they can be sensitive to small expected values, and small expected values (less than five) were predicted in several analyses. Bootstrapping (Weir, 1990) may be a more appropriate method of analyzing these data. Bootstrapping operates by drawing random samples of the same size as the original sample from that sample, and then providing confidence intervals for allele frequencies. Significantly different allele frequencies could then be determined by non-overlapping confidence intervals.

Differences in Heterozygosity

The primary effect of inbreeding is to increase the probability that offspring will inherit the same allele from its sire and dam, thus lowering the percentage of heterozygotes in the population (Lush, 1945). Therefore, it was expected that the Lents population, which has the highest level of inbreeding, would have the lowest percentage of heterozygotes (H_l), and the pooled EPD population, which has the lowest level of inbreeding, would have the highest H_l. These expectations were observed at the IGF-I, PRL, BM2113 and PIT1 polymorphisms. Expectations were not observed at the K-Cas (lowest H_l observed in Line 1), B-Lac (equal H_l observed in Line 1 and pooled EPD) or GH (highest H_l observed in Lents) polymorphisms. However, inbreeding refers to all of the genes of an animal, not specific loci.

The inbreeding coefficient (f) measures the percentage of genes which were heterozygous in the base population but have become homozygous because of inbreeding. Using the H_l of the pooled EPD population as the basic population, a decrease of 50% in the Lents population (f=.50) and a decrease of 30% in the Line 1 population (f=.30) would be expected. The average H_l of the Lents and Line 1 populations actually decreased by 59% and 26%, respectively, relative to the H_l of the pooled EPD population. Thus, the average H_l from all seven polymorphisms closely reflects the decrease in heterozygosity that is expected due to inbreeding, even though some individual polymorphisms fail to meet expectations.

Expectations of Hardy-Weinberg Equilibrium

In the Line 1 population, B-Lac, GH and PIT1 alleles appear to be segregating as predicted for a population in Hardy-Weinberg equilibrium, despite violation of many of the associated assumptions. IGF-I genotype frequencies display some increase in homozygosity, while K-Cas genotype frequencies illustrate the expected effects of inbreeding for a population in Hardy-Weinberg equilibrium. In the Lents population, K-Cas alleles appear to be segregating as predicted for a population in Hardy-Weinberg equilibrium, but there is a significant increase in heterozygosity at the GH polymorphism. This latter result is exactly opposite of what is expected when considering the high level of inbreeding in the Lents population. However, if the less common A allele is undergoing a process (selective or random) toward fixation, then selection of AA and AB genotypes would be advantageous.

The conformity to Hardy-Weinberg equilibrium expectations in these populations was surprising because the Line 1 and Lents populations are relatively small (n= 420 and 58, respectively), mating was not random, i.e, a limited number of sires were selected for the next generation, and selection for increased growth was practiced in the Line 1 population. These are all violations of assumptions associated with the Hardy-Weinberg equilibrium theory (Falconer, 1989). The differences that were observed when comparisons were made using expected values that had been adjusted for inbreeding were also surprising because results from the previous section indicated that the amount of heterozygosity had decreased as expected for the amount of inbreeding in each population. However, results from the previous section considered average H_I, which included all seven polymorphisms studied, while

comparisons to expectations of Hardy-Weinberg equilibrium could only be done for individual loci. Perhaps the most unusual result was the significant increase of heterozygotes observed for the GH polymorphism in the Lents population. The explanation for this observation is unclear, but the A and B alleles of the GH polymorphism do represent different forms of the GH protein that differ in their amino acid sequence. Therefore, a physiological selective advantage to the production of both forms of GH may exist, and this may cause a natural selective advantage for the AB genotype of GH in the Lents population.

Part II: Associations Between DNA Polymorphisms and Growth and Maternal Traits

Potential Genetic Markers

Before DNA polymorphisms can be considered as potential markers of QTL, some basic differences in types of polymorphisms must be understood. In this study, the K-Cas, B-Lac and GH polymorphisms result from point mutations in one allele of their respective genes. When translated, the two different alleles of each of these loci code for different forms of each protein, which differ in their amino acid sequence. The different amino acid sequences of the protein products may cause different physiological effects for responses to the protein. This difference in physiological response could then have a significant effect on a quantitative trait. Therefore, genotypes determined at these loci identify different protein products which have the potential of directly influencing a quantitative trait through different physiological responses to differences in

amino acid sequences. In addition, these genotypes may be used as markers of the chromosomal region in which they are located.

IGF-I and PIT1 polymorphisms also serve as markers of chromosomal regions, but are not translated to different protein products. DNA polymorphisms that serve as markers of chromosomal regions enable the inheritance of that region to be monitored from parent to offspring. This allows for possible associations between the chromosomal region and effects on quantitative traits to be identified by comparing offspring which inherited one allele to offspring that inherited an alternative allele. In this situation, the cause of the effect may not be the marker itself, but polymorphisms in other genes (QTL) closely linked to the marker. However, the possibility that the effect is caused by the gene containing the marker is not eliminated. Before markers may be used in a marker assisted selection program, it is necessary to understand the linkage phase, or the linkage relationship between the marker and the QTL causing the effect. For example, the A allele of the marker may be linked to the favorable allele of the QTL in one family, but linked to the unfavorable allele of the QTL in another family. This linkage phase may also change within families due to recombination events that take place between the marker and QTL. The chance of a recombination event changing the linkage phase within a family decreases with closer linkage between the marker and QTL. In order to identify effects of marker polymorphisms on quantitative traits, and to determine their linkage phase, marker polymorphisms must be analyzed within large families whose chromosomes are segregating at the marker locus. Otherwise, significant effects associated with a chromosomal region may not be detected because of differences in linkage phase among families.

Milk Production. Based on the regression analysis of EPD data, the effect of allele substitution on MILK EPD for the K-Cas, GH and IGF-I polymorphisms

was significant. These results indicate that these polymorphisms may be markers of QTL involved in milk production. In addition, it is possible that the polymorphisms in the K-Cas and GH genes may have direct effects on milk production.

Despite significant effects of allele substitution, the magnitude of effect was small for GH and IGF-I polymorphisms. The percentage of variability in MILK EPD explained by genotype was 1.66% for GH and 2.88% for IGF-I. In contrast, K-Cas genotype accounted for 11.33% of the variability in MILK EPD. The mean MILK EPD for cattle of K-Cas genotype AA was 6.2 pounds greater compared to cattle of K-Cas genotype BB. Because of this relatively large effect, the K-Cas polymorphism deserves further attention to investigate its usefulness as a genetic marker for milk production in a marker assisted selection program.

Several studies have investigated the effects of the K-Cas and B-Lac polymorphisms on milk yield in dairy cattle, and many conflicting results have been reported for the effect of K-Cas genotype. Significant advantages to milk production from the K-Cas A allele have been reported (Bovenhuis et al., 1992; Gonyon et al., 1987; Ng-Kwai-Hang et al., 1986), which are consistent with the results from the present study. However, others have reported significant advantages to milk production from the K-Cas B allele (Cowan et al., 1992; Van Eenennaam and Medrano, 1990; Lin et al., 1989), and still others found no signifcant effect of K-Cas genotype on milk yield (Aleandri et al., 1990; Ng-Kwai-Hang et al., 1990). Possible explanations for these discrepancies include differences in statistical models, differences in the type of milk yield data analyzed, and differences in populations. Another likely explanation is that the observed effect is not a direct result of the K-Cas polymorphism, but the polymorphism is acting as a marker for a linked QTL influencing milk production.

Bovenhuis et al. (1992) showed that the K-Cas gene was linked to the β-casein gene in the Dutch dairy cattle population, and that a significant effect of K-Cas genotype on milk production was actually due to the β-casein gene. This result was confirmed by a later study (Bovenhuis and Weller, 1994). Cowan et al. (1992) concluded that the inconsistencies reported for the effect of K-Cas genotype on milk yield suggest that the linkage phase between K-Cas and QTL influencing milk yield differs among populations. This is the first report of the effects of K-Cas genotype on milk production in beef cattle.

Studies of the effects of B-Lac genotype on milk yield report a significant advantage for the B-Lac A allele (Bovenhuis et al., 1992; Cowan et al., 1992, Aleandri et al., 1990; Ng-Kwai-Hang et al., 1986; Geldermann et al., 1985), which was also observed in the present study. Consistencies among these studies indicate that B-Lac may have a direct effect on milk yield. Nevertheless, other studies have failed to determine a significant association between B-Lac genotype and milk yield (Van Eenennaam and Medrano, 1991; Ng-Kwai-Han et al., 1990; Lin et al., 1989; Hanlein et al., 1987; Gonyon et al., 1987).

Ziehe et al. (1993) considered the effects of K-Cas and B-Lac polymorphisms on milk production and calf performance in Angus and Polled Hereford sired beef heifers. A significant advantage of the K-Cas A allele on milk production during early lactation, and on calf weaning weight was reported for Angus-sired heifers, which is consistent with results of the present study. However, no heifers with the K-Cas BB genotype were included in the study and a similar effect was not observed in Polled Hereford-sired heifers. No significant genotype effect was observed for the B-Lac polymorphism, but only the AB and BB genotypes were represented. The actual role of K-Cas and B-Lac genotype in milk production remains unclear. As results of studies which evaluate these effects in different populations are published, perhaps the role of these genes in

milk production will become better understood. However, it does appear clear that K-Cas is a QTL or is closely linked to a QTL for milk production.

Birth Weight. Based on the regression of BWT EPD on genotype, the effect of allele substitution was significant for the K-Cas, B-Lac, IGF-I and PIT1 polymorphisms. Results from the regression of BWT phenotypic data on genotype confirmed significant effects of allele substitution for the K-Cas, B-Lac and IGF-I polymorphisms. These results suggest that K-Cas, B-Lac and IGF-I polymorphisms may be markers of QTL influencing birth weight in this population. K-Cas and IGF-I genotypes are likely to be more useful markers of BWT EPD because they explained 9% and 6%, respectively, of the variability in BWT EPD compared to 2% of variability explained by B-Lac genotype. K-Cas, B-Lac and IGF-I explained 1.5%, 1.0% and 0.8%, respectively, of the total variation in birth weight phenotype, suggesting effects of similar magnitude from the three polymorphisms.

Weaning Weight. Based on the regression of WWT EPD on genotype, the effect of allele substitution was significant for the K-Cas and IGF-I polymorphisms. Regression of 200-day weight on genotype confirmed the significant effect of K-Cas. However, the magnitude of the effect of K-Cas was small, explaining 2% of the variability in WWT EPD and .6% of the total varaibility in 200-day weight. Because of this small magnitude of effect, the use of K-Cas genotype as a marker for weaning weight in a marker assisted selection program may not be practical.

Yearling Weight. Regression of YWT EPD on genotype indicated a significant effect of allele substitution for the K-Cas polymorphism. The magnitude of the effect was small, explaining only 2% of the variability in YWT EPD. Regression of 365-day weight on genotype failed to confirm a significant association between K-Cas genotype and yearling weight. Because of the small

magnitude of effect on YWT EPD, and the lack of significant effect on 365-day weight, the K-Cas polymorphisms should not be considered a marker of QTL influencing yearling weight without confirmation from additional studies. However, the effects of K-Cas genotype on WWT and YWT EPD may indicate that K-Cas is linked to a gene influencing growth in general in this population.

Statistical Analyses

Regression Analysis. Results from the regression analyses of EPD and phenotypes on genotype were similar; all significant gene effects were additive in nature. In general, lower significance levels were observed from analyses using phenotypes compared to analyses using EPD. These minor differences between analyses were not unexpected. Analyses using EPD as the dependent variable considered the effects of genotype on additive genetic effects, as estimated by EPD. Analyses using phenotypes as the dependent variable considered the effects of genotype on phenotype, which is influenced by additive, dominant and epistatic gene effects, as well as environment. Error in the analyses using phenotypes may have been inflated by fixed effects that were unaccounted for, and by non-additive genetic variation at loci other than those genotyped. Therefore, regression analysis of EPD is likely more informative in estimating effects of DNA polymorphisms on additive gene action.

The percentage of variability of EPD, the percent of total variability in phenotype, and the percent of residual variability in phenotype remaining after variability due to sire, year, sex and age of dam were removed were low (2% and less) for several effects that were detected as significant (K-Cas genotype on weaning and yearling weight, B-Lac genotype on birth weight, GH genotype on

MILK EPD, and PIT1 genotype on birth weight). Because of the relatively small population size used in this study, the statistical power of the analyses was probably not great enough to detect such small effects. The fact that these small effects were detected as significant indicates the results may be spurious. Two significant results would be expected by chance from the 35 analyses included in the EPD regression and Regression model 1 analyses at a 5% significance level. Therefore, significant results having small magnitudes of effects should be interpreted with caution until they can be confirmed or rejected by additional studies.

When sire was included as a fixed effect in the analyses of phenotypes, the only significant effect observed was the effect of B-Lac genotype on BWT. The effect of sire was significant in all analyses, accounting for a large portion of the phenotypic variability. Some of this phenotypic variability that was accounted for by sire may have resulted from the genotype of the sire that was passed on to his offspring. A more appropriate analysis would be to analyze the effects of genotype within sire families. Unfortunately, the small numbers of animals within half sib families of heterozygous sires prevented this analysis from being informative for this population.

Least Squares Analysis. Results from the least squares analysis were similar to results from regression analysis. This was expected due to the similar nature of the two analyses. The effect of B-Lac allele substitution on BWT phenotype from regression analysis was significant with and without sire included in the model as a fixed effect. However, least squares analysis failed to reveal significant effects for these analyses. Significant interactions for B-Lac x age of dam and B-Lac x year were observed through least squares analysis. These interactions were not included in regression analyses and may have

influenced those results. Other minor differences resulted from a decrease of significance level in the least squares compared to regression analyses.

Animal Model. Kennedy et al. (1992) suggest that ordinary least squares analysis may result in finding an excess of spurious significant effects of single genes when no effect exists. The bias of the analysis is directly proportional to the heritability of the polygenes influencing the trait, and increases with increasing selection intensity. Kennedy et al. (1992) show that the use of mixed model equations under an animal model with single-locus genotype treated as a fixed effect results in unbiased estimates of the genotype effect.

The phenotypic data in the present study were analyzed using an animal model, and linear contrasts were calculated to determine additive and dominance gene effects. Unlike the other models which considered the effects of each locus individually, the animal model tested the effect of each locus with all genotypes included in the model. Even though the five polymorphisms segregated independently due to their locations on different chromosomes, the possibility of interactions resulting from epistatic effects remains. Results from the animal model analysis indicated no significant effects of genotype on growth traits in the Line 1 population, which is in agreement with regression and least squares results when sire was included in the model as a fixed effect.

The implications of these results are not clear. The animal model analysis is designed to adjust for relationships among animals and provide estimates of the additive genetic merit of individuals. Therefore, results from the animal model analysis were expected to be similar to regression and least squares analyses using EPD data. The most likely cause for the discrepancies of results is the size of the population involved in the study. A total of 395 animals had complete genotype and phenotype records to be analyzed using the animal model, which is designed to be used with much larger data sets. The

number of animals involved in this study limited the power of the animal model analysis and may have prevented the detection of significant effects. Another possibility is that there simply were no significant effects of genotype on the traits analyzed. Significant effects of genotype on WWT EPD and YWT EPD were observed from regression analysis, but the small magnitude of these effects (<2% of variability explained) cause the validity of these results to be questionable. In addition, significant associations between genotype and 365-day weight phenotype were not observed.

In contrast to the effects of genotype on weaning and yearling weights, the magnitude of effects of K-Cas, B-Lac, IGF-I and PIT1 genotype on birth weight were larger (1-9% of BWT EPD variability explained). Significant effects of K-Cas, B-Lac and IGF-I genotype on BWT phenotype were also observed, which further supports the validity of the results. However, the lack of significant effects of genotype on BWT EPD and phenotype resulting from the animal model analysis cannot be overlooked completely. Further investigation involving larger populations will be needed in order to better understand the actual effects of these genotypes on birth weight.

Limitations

Population Size and Structure. The fields of marker assisted selection and molecular genetics applied to the improvement of livestock are still being explored and established. Several different approaches have been taken to investigate associations between DNA markers and quantitative traits in cattle (Georges et al., 1993; Andersson-Eklund and Rendel, 1993; Ziehe et al., 1993; Rocha et al., 1992; Hoj et al., 1992; Bovenhuis et al., 1992). A common obstacle

faced by each study was the identification of informative populations in which to study the effects of DNA markers. It has been suggested that several thousands of animals would be required to detect a QTL accounting for additive genetic variance equal to 1% of total phenotypic variance at 90% power (Soller and Genizi, 1978). However, the accuracy of these estimates remain to be proven experimentally. Pomp et al. (1994) detected markers linked to four QTL for body weight and fat in mice which accounted for 1 to 2% of the total phenotypic variation, and one marker which may be linked to a pleiotropic QTL with larger effects. A total of 424 animals were genotyped in the study, which is much less than the predicted number of animals (Soller and Genizi, 1978) required to achieve those results. In pigs, Andersson et al. (1994) identified a region of chromosome 4 that accounted for a large portion of variation in growth rate and fatness. A total of 200 F2 offspring of a cross between the European wild boar and the domesticated Large White pig were involved in the study. Rothschild et al. (1994) successfully identified a polymorphism having a major effect on litter size in pigs based on 85 first parity records. In all of these studies, the populations utilized were carefully designed in order to maximize the probability of identifying a QTL. Examples such as these indicate that the proper design of populations may enable QTL to be identified from many fewer animals than originally suggested by Soller and Genizi (1978).

The present study considered a very unique population, which presented both advantages and disadvantages to the project. The Line 1 population has undergone 60 years of selection for increased growth (MacNeil et al., 1992). Selection is successful because it increases the frequency of favorable alleles and decreases the frequency of unfavorable alleles that influence the trait being selected. Therefore, some QTL which have large effects on growth traits may have become fixed for favorable alleles, while unfavorable alleles may have

been eliminated from the Line 1 population. The variation in growth traits that does remain in the Line 1 population is probably due to several loci that each contribute a small effect to growth traits. Because of this, the identification of a marker for growth traits may be more difficult in the Line 1 population than in a non-selected population. However, the Line 1 population offered the advantage of having data available for growth traits of all ancestors in the population. These data increased the accuracy of the predictions of EPD and were useful in the animal model analysis.

The Line 1 population is also unique because it has been closed to the introduction of outside germplasm, accumulating an average inbreeding coefficient of approximately .30 (MacNeil et al., 1992). This indicates that the genetic background among animals in the Line 1 population is very similar. A homogeneous genetic background is desirable, as long as variation exists at polymorphisms being studied, because interactions among markers and the genetic background would be similar among animals. Effects from background genes are less likely to be a source of variation in an inbred population compared to a non-inbred population.

The structure of a population used for this type of study is also an important consideration. An F2 backcross or intercross population from two genetically distinct lines (Barinaga, 1994) is perhaps the "ideal" population in which to identify DNA markers of QTL. This type of population can be developed in laboratory animals and livestock species with short generation intervals, but it is a time-consuming and expensive process in cattle. The granddaughter design (Weller et al., 1990), which involves genotyping grandsires and sons, and analyzing phenotypic data from granddaughters, has been employed in studies of milk composition and production traits in dairy cattle (Cowan et al., 1992). This design reduces the amount of genotyping required

(Weller et al., 1990), but requires phenotypic data from large numbers of granddaughters from grandsires that must be heterozygous for DNA markers evaluated.

The Line 1 population was made up of several small half-sib families representing 24 sires. Because of the limited number of offspring from each sire, the data could not be analyzed within sire families. Therefore, the effect of alternative alleles from a heterozygous sire could not be determined. One of the limitations of this study is that it is not known if effects associated with K-Cas, B-Lac and GH polymorphisms are due to a direct biological effect caused by differences in proteins produced by alternative alleles, or if the polymorphisms act as markers of a chromosomal region containing QTL influencing the traits of interest. The structure of the Line 1 population prevents investigation to determine if observed effects are due to a direct or marker effect of the polymorphisms. Large segregating families in which the effects of alternative alleles could be followed from parent to offspring would be required in order to better understand the nature of the observed effect.

A final characteristic of the Line 1 population that deserves consideration is the genotypic frequency of each polymorphism. Both A and B alleles were segregating for five of seven polymorphisms genotyped in the population, but the PIT1 AA genotype was represented by only eight animals. If these eight animals had extreme values for the phenotypic traits analyzed, results of the analysis would be biased. Weighting of analyses for genotypic frequencies was done to avoid such bias, but resulted in large standard errors from genotypic classes with low frequencies. The use of markers with equal allele frequencies would help to avoid this problem because genotypic classes would less likely be represented by few animals with extreme values that may not be truly representative of the actual mean of the genotypic class.

Applications to Marker Assisted Selection Programs. The most important result from this study was the identification of the effect of K-Cas genotype on MILK and BWT EPD. These effects were statistically significant and accounted for a large portion of the variability in EPD. However, due to the limitations of this study, these results do not mean that selection for decreased birth weight or increased milk production should be based on K-Cas genotype. Instead, they do indicate that K-Cas deserves further attention as a potential marker of these traits.

Several considerations must be made before any DNA marker is incorporated into a selection program. First, the true effect of a polymorphism must be clearly understood. Markers which have a direct biological effect on the traits they influence should be expected to have a consistent effect in different families of similar genetic background, whereas the effects of markers which are linked to genes or QTL influencing a trait will depend on the linkage phase between the marker and QTL. The linkage phase between a marker and a QTL, the closeness of linkage between a marker and a QTL, and the magnitude of effect attributable to a marker should be understood for a marker in the genotype and physical environment in which it is to be used. The present study identified the K-Cas A allele as favorable for MILK EPD and the B allele as favorable for BWT EPD, and estimated the magnitude of each effect. However, further study is required to understand the cause of these effects and how K-Cas might be used in a marker assisted selection program.

It must also be considered that results of one study may be limited to the genetic environment in which they were obtained. In this example, the Line 1 Herefords are a very unique population because of selection pressures that have been placed on the herd and the amount of inbreeding that has accumulated. Associations between DNA polymorphisms and growth and

maternal traits that were identified in this study may be very different in a different genetic background. The effect of K-Cas genotype on milk production and protein percentage is an example of a marker that appears to have different effects in different populations (Bovenhuis et al., 1992). Even within the Holstein breed, Cowan et al. (1992) reported opposite effects of K-Cas alleles on protein percentage in two different families. The K-Cas example provides an important warning that selection based on a genetic marker should not be promoted until the effects of the marker are fully defined.

Finally, correlated responses due to marker selection for a specific trait cannot be overlooked. In this study, K-Cas and IGF-I polymorphisms had significant effects on both MILK and BWT EPD. The addition of a B allele affected both traits in the same direction indicating that selection for increased maternal ability would also result in an increase in birth weight. This is an undesirable correlation in many situations and should be considered before using these markers in a marker assisted selection program. In contrast, the GH polymorphism had a significant effect on MILK EPD but no effect on BWT EPD, while B-Lac genotype had a significant effect on BWT EPD but no effect on MILK EPD. Thus, GH may be a useful marker when the selection goals are to increase maternal ability without increasing birth weight and B-Lac may be a useful marker to achieve a decrease in birth weight without sacrificing maternal ability. The response in BWT EPD and MILK EPD to K-Cas and IGF-I is only one example of a possible correlated response to marker assisted selection. Many other correlated responses, both desirable and undesirable, may exist and should be considered before genetic markers are included in selection programs.

Potential for Future Research

The most definitive conclusion that can be drawn from this study is that more research is needed if the effects of DNA polymorphisms on quantitative phenotypic traits are to be understood and used as selection criteria in marker assisted selection programs. At the time this project was initiated, bovine gene maps had not yet been developed. For that reason, the candidate gene approach was used to identify genes whose protein products were known to have a biological effect on growth and maternal traits as potential markers of these traits. Success of this approach has recently been documented in pigs (Rothschild et al., 1994)

The development of the bovine genetic map (Barendse et al., 1994; Bishop et al., 1994), more efficient experimental designs (Weller et al., 1990) and the refinement of statistical techniques to accommadate molecular data (Lander and Botstein, 1989; Zeng, 1993; Jansen, 1993; Haley et al., 1994; Zeng, 1994; Jansen and Stam, 1994) now allow for more sophisticated strategies to be employed in order to identify regions of the genome that are associated with quantitative traits. Several studies in mice have utilized murine genetic linkage maps to select markers distributed throughout the genome in order to identify QTL influencing quantitative traits (Berretini et al., 1994; Pomp et al., 1994; Warden et al., 1993). Although the bovine map is not as saturated as the murine map, the same type of approach may now be used in cattle. Results from previous projects using the candidate gene approach will be useful to future research in order to target specific regions of the genome to study. For example, the polymorphism in the K-Cas gene, which has been mapped to bovine chromosome 6, was found to have a significant effect on BWT EPD in the

Line 1 population. Future studies may begin by choosing other polymorphisms linked to K-Cas on chromosome 6 in order to identify a marker more closely associated with a QTL influencing birth weight. Similarly, chromosomes 6 and 11 may be logical starting points for the study of QTL influencing growth because these chromosomes harbor K-Cas and IGF-I polymorphisms, respectively, which were identified as having significant effects on weaning weight. Ideally, a more efficient approach will be to utilize the genetic map first to identify regions of the genome having an effect on a QTL, and then use the candidate gene approach to investigate genes located within that region in order to identify the specific gene responsible for the effect.

Another source of information to consider is the identification of chromosomal regions which affect quantitative traits in other species, for example mice or pigs. As more Type I markers are added to genetic maps, it will become possible to identify homologous chromosomal regions between cattle and other species. If QTL influencing quantitative traits are located in homologous chromosomal regions in different species, then the identification of QTL in mice or pigs could be helpful in identifying QTL for the same traits in cattle. For example, Pomp et al. (1994) identified a QTL influencing body weight and fat percentage on chromosome 2 in mice. Other genes located in the same region of mouse chromosome 2 have been mapped to bovine chromosome 13. Therefore, markers on bovine chromosome 13 would be logical choices for investigation of markers of QTL influencing growth or fatness in cattle. In pigs, Andersson et al. (1994) identified a QTL influencing growth and fatness located on chromosome 4. Unfortunately, no Type 1 markers are present on chromosome 4 of the current porcine genetic map (Rohrer et al., 1994), so a homologous region in the bovine genome cannot yet be identified. This

emphasizes the need for more Type 1 markers to allow for the comparison of genetic maps across species.

Although the identification of DNA markers linked to QTL is an essential step in the process of making MAS a reality, it is not the final step. Markers of QTL define pieces of a genetic puzzle. The next step will be to put those pieces together. In doing this, it will be important to keep in mind that complete animals with complex genomes will ultimately be selected. Just as the phenotypic performance of an animal is influenced by the environment, the performance of a specific genotype will also depend on the genetic and physical environment in which it is expressed. It should be emphasized that the goal is marker *assisted* selection, not marker *based* selection. DNA markers will not replace information gained from performance testing and phenotypic appraisal, but DNA markers will add to the information available from which to make intelligent selection decisions.

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APPENDIX 1

DNA Extraction from Blood Samples:

Salt Extraction Protocol

Solutions

Red Blood Cell (RBC) Lysis Buffer

.017 M Tris
.14 M NH_4Cl

For 1 liter of 1X RBC lysis buffer:

17.0 ml 1M Tris (pH 7.65)
7.49 g NH_4Cl
Dilute in water for a total volume of 1 liter.
Warm to 37°C in water bath before use.

Nuclei Lysis Buffer

.01 M Tris-HCl pH 8.0
.4 M NaCl
.002 M EDTA pH 8.0

For 1 liter of 1X Nuclei Lysis Buffer:

5.0 ml 2M Tris-HCl pH 8.0
80.0 ml 5M NaCl
4.0 ml .5 M EDTA pH 8.0
Dilute in water for a total volume of 1 liter.

TE (pH 8.0)

10 mM Tris-HCl (pH 8.0)
1 mM EDTA (pH 8.0)

For 1 liter of 1X TE:

1.576 g Tris-HCl
.372 g EDTA
Dilute in water for a total volume of 1 liter.

Other Reagents Needed

- .15 M NaCl
- 6M NaCl (saturated salt solution)
- 10% SDS
- Iso-propanol
- Proteinase-K (20 mg/ml)
- 70% ethanol

Procedure

1. Centrifuge blood tubes at 2000 rpm for 10 minutes at 4°C, accelerate 8, decelerate 8.
2. Aspirate off plasma using a transfer pipet; discard (optional).
3. Carefully pull off buffy coat plus any red blood cells that tag along using a transfer or glass pipet. Place in a 15 ml centrifuge tube and add 37°C RBC Lysis Buffer to bring volume to 14.5 ml.
4. Incubate at 37°C for 10 minutes.
5. Centrifuge at 2000 rpm for 10 minutes at 4°C, accelerate 8, decelerate 8.
6. Pour off supernatant and blot tube upside down on kimwipe.
7. Resuspend pellet in 5.0 ml of .15 M NaCl.
8. Centrifuge at 2000 rpm for 10 minutes at 4°C, accelerate 8, decelerate 8.

9. Repeat steps 6 and 7 if needed to remove red blood cells.

10. Pour off supernatant and blot tube upside down on kimwipe. Resuspend pellet in 4.0 ml of Nuclei Lysis Buffer (be sure to resuspend pellet before adding SDS). Add 300 μl of 10% SDS and 50.0 μl of Proteinase-K (20 mg/ml) and incubate 1 to 3 hours with shaking at 60°C.

11. Samples should be a thin, runny liquid at this point. If not, add 50.0 μl more Proteinase-K and incubate with shaking for an additional hour at 60°C. Let samples cool at room temperature for 10 minutes.

12. Add 1.3 ml of saturated salt solution (6M NaCl) and shake vigorously for 15 seconds. Let stand at room temperature for 5 minutes.

13. Centrifuge at 2700 rpm for 10 minutes at 4°C, accelerate 8, decelerate 8.

14. Draw off supernatent into another 15 ml centrifuge tube and add an equal volume of Iso-propanol. Gently rock the tube until DNA precipitates (it should look like white or clear angel hair). Remove DNA with a curved glass pipet hook. Rinse with 70% ethanol.

15. Place pipet in a 1.5 ml tube and dry in vacuum oven (no heat) for 2-12 hours. Resuspend in 500 μl TE buffer.

APPENDIX 2

DNA Extraction from Bull Semen:

Organic Solvent Protocol

Solutions

STES Digestion Buffer

10 mM Tris-HCl (pH 8.0)
10 mM EDTA
50 mM NaCl2
2% SDS

TE (pH 8.0)

10 mM Tris-HCl (pH 8.0)
1 mM EDTA (pH 8.0)

For 1 liter of 1X TE:

1.576 g Tris-HCl
.37224 g EDTA
Dilute in water for a total volume of 1 liter.

Other Reagents Needed

- Proteinase-K (20 mg/ml)
- DTT (1 M in 10 mM NaAc)
- Tris buffered phenol
- 3 M sodium acetate (NaAc)
- 100% ethanol at -20°C
- 70% ethanol

- Tris buffered phenol/chloroform:iso-amyl alcohol
- Chloroform:iso-amyl alcohol

Procedure

1. Empty entire straw or transfer .5 ml from ampule of bull semen into a 2.0 ml microcentrifuge tube. Add 1 ml ddH2O, mix briefly, then centrifuge at 8000 rpm for 5 minutes to pellet the sperm.
2. Carefully pour or aspirate off the supernatant, being careful not to disturb the pellet. If floating milky substance sticks to the inside of the tube, remove it with a sterile cotton swab being careful not to touch the pellet with the swab.
3. Resuspend the pellet in 750 µl of STES digestion buffer and mix well.
4. Add 15 µl of Proteinase-K and 40 µl of DTT and mix by rocking back and forth. Incubate overnight at 37°C or 1 to 3 hours at 60°C.
5. Add 750 µl of Tris buffered phenol. Put samples on rotator or mix back and forth by hand 5 minutes (be sure caps are securely closed).
6. Centrifuge for 5 minutes at 3000 rpm.
7. Transfer the top aqueous phase into a new 2 ml microcentrifuge tube. Add TE to restore volume to 750 µl . Add 750 µl of Tris buffered phenol/chloroform:iso-amyl alcohol and mix by rocking for 5 minutes.
8. Centrifuge for 5 minutes at 3000 rpm.
9. Transfer the top aqueous phase into a new 2 ml microcentrifuge tube. Add TE to restore volume to 750 µl. Add 750 µl of chloroform:iso-amyl alcohol and rock for 5 minutes to mix.
10. Centrifuge for 5 minutes at 3000 rpm.
11. Transfer the top aqueous phase into a 2 ml tube, note volume, then pour into a 5 ml Falcon tube.

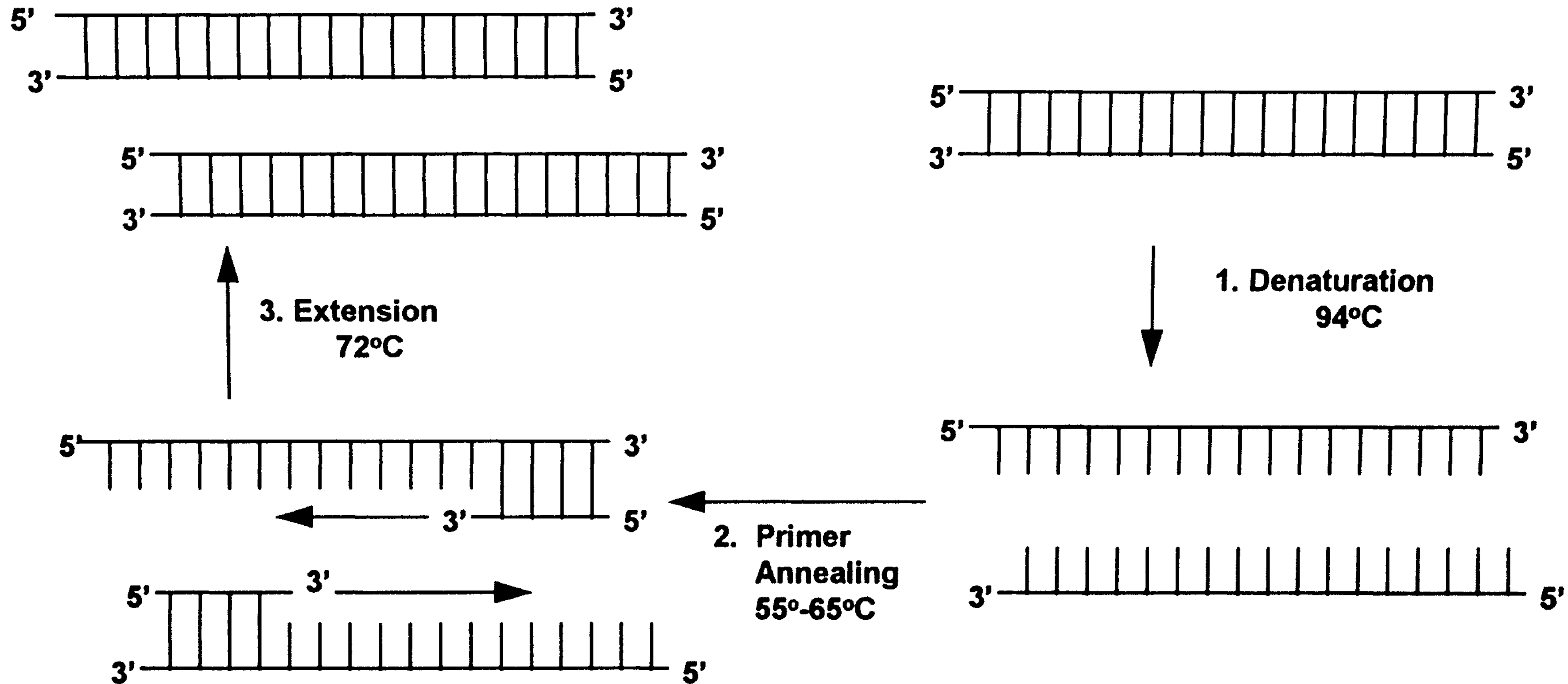
12. Add 1/10 volume of 3 M NaAc, cap tubes and mix well by rocking back and forth.

13. Add 2 volumes of -20°C 100% ethanol and rock back and forth until DNA precipitates (it should look like white or clear angel hair). Remove DNA with a curved glass pipet hook. Rinse with 70% ethanol. Place pipet in a 1.5 ml tube and dry in vacuum oven (no heat) 2-12 hours. If no DNA is visible, place tube in -80°C for one hour. Centrifuge at 10,000 rpm for 15 minutes at 4°C. Pour or aspirate off the supernatant and dry pellet in vacuum oven.

14. If DNA was present in the previous step without centrifugation, then resuspend in 200 µl of TE. If centrifugation was necessary, then resuspend in 50 µl of TE.

Appendix 3:

Polymerase Chain Reaction



Legend:
1. Denaturation of double stranded DNA template to two single strands.
2. Annealing of primers to complementary sequence of template DNA.
3. Extension of primers using Taq polymerase to make two double stranded copies of the template DNA.

APPENDIX 4

Sample Calculations for Determining DNA Concentrations and Making Working Solutions

DNA Concentration:

[DNA] μg/ml = (A_{260})(DF)(50 μg/ml)

where: A_{260}= Absorbance of stock solution at 260 nm

DF = dilution factor

50 μg/ml = constant

Example:

A_{260} = 0.100

DF = 100 (10 μl stock DNA diluted in 990 μl water)

[DNA] μg/ml = (0.100)(100)(50)

= 500 μg/ml

Working Solution: 200 μl of 50 ng/μl final concentration

(50 ng/μl)(200 μl) = 10000 ng of DNA needed

μl of stock solution to add = (10000 ng) / [DNA] μg/ml

(Note: ng/μl = μg/ml)

μl of TE to add = 200 μl - μl of stock solution to add

Example:

μl of stock solution to add = (10000 ng) / (500 μg/ml)

= 20 μl of stock solution

μl of TE to add = 200 μl - 20 μl

= 180 μl of TE

APPENDIX 5

Working Solutions for PCR Primers from Lyophilized Oligonucleotides

Dissolve Oligonucleotides

1. Add 100 µl of PCR water to each tube of lyophilized primer. Leave tubes undisturbed at room temperature overnight.

2. Vortex all tubes containing primer. Pulse-spin. Combine all tubes (4) for each primer into one 1.5 ml microcentrifuge tube. This will be the stock primer solution. Vortex and pulse-spin

Measure Absorbance

1. Place 998 µl of water in a 1.0 ml cuvette and zero at a wavelength of 260 nm.

2. Add 2 µl of primer from the stock solution, mix and record absorbance at a wavelength of 260 nm.

3. This is a 1:500 dilution, and 400 - 2 = 398 µl of primer stock solution remain.

Dilution

1. Determine the volume necessary to dilute the primer stock solution to a final concentration of 50 µM:

   A) From the primer information sheet, obtain the following values:
   µg/OD
   Molecular Weight

   B) Calculate the µM concentration of the primer stock solution:

μg/ml = (OD260) (Dilution factor = 500) (μg/OD)
ng/ml = (μg/ml) (1000)
μM = (ng/ml) / Molecular Weight

C) Calculate the total volume for a 50 μM primer stock solution:
total volume (μl) = [(μM stock) (μl stock remaining)] / 50 μM

D) Calculate the volume needed to dilute the stock solution to 50 μM:
μl to add = total volume (μl) - μl stock remaining

E) Be sure to label stock solutions as either forward or reverse primer

2. Add the appropriate amount of PCR water to dilute the primer stock solution to 50 μM. Remember, this solution contains a single primer, either forward or reverse. This stock solution may be stored at -20°C.

3. Make 300 μl of 5 μM working solution, forward and reverse primer, for PCR:
   30 μl of 50 μM stock, forward primer +
   30 μl of 50 μM stock, reverse primer +
   240 μl of PCR water

4. Working solutions may be stored at -20°C and are ready for use in PCR.

APPENDIX 6a

M13 DNA Sequencing Standard Protocol

Sequenase Version 2.0

(United States Biochemical Corporation, Cleveland, OH)

Equipment Needed: ♦ Water bath at 65°C ♦ Heat block at 37°C
♦ Water bath or PCR machine at 80°C before loading

Thaw ^{33}P in hood in designated area.

Thaw ingredients at room temperature, then place on ice:
- ddNTPs ♦ DTT ♦ Sequenase buffer
- -40 primer ♦ dGTP labelling mix ♦ Stop solution
- ssM13 DNA ♦ Enzyme dilution buffer

Place sequenase on ice.

Label microcentrifuge tubes:

- Four .6 ml tubes labelled G, A, T, C ♦ Labelling mix tube
- Reaction tube ♦ Enzyme dilution tube

In Reaction tube, mix well on ice (for 1X reaction):

1 µl -40 primer
2 µl Sequenase buffer
7 µl ssM13 DNA
10 µl PCR water

- Heat reaction to 65°C for 4 minutes
- Remove and incubate at room temp for 15-30 minutes

Labelling mix, mix well on ice:

4 µl dGTP labelling mix
16 µl PCR water

Enzyme dilution, mix well on ice:

APPENDIX 6a

M13 DNA Sequencing Standard Protocol

Sequenase Version 2.0

(United States Biochemical Corporation, Cleveland, OH)

Equipment Needed: ♦ Water bath at 65°C ♦ Heat block at 37°C
♦ Water bath or PCR machine at 80°C before loading

Thaw ^{33}P in hood in designated area.

Thaw ingredients at room temperature, then place on ice:
- ddNTPs ♦ DTT ♦ Sequenase buffer
- -40 primer ♦ dGTP labelling mix ♦ Stop solution
- ssM13 DNA ♦ Enzyme dilution buffer

Place sequenase on ice.

Label microcentrifuge tubes:

- Four .6 ml tubes labelled G, A, T, C ♦ Labelling mix tube
- Reaction tube ♦ Enzyme dilution tube

In Reaction tube, mix well on ice (for 1X reaction):

1 µl -40 primer
2 µl Sequenase buffer
7 µl ssM13 DNA
10 µl PCR water

- Heat reaction to 65°C for 4 minutes
- Remove and incubate at room temp for 15-30 minutes

Labelling mix, mix well on ice:

4 µl dGTP labelling mix
16 µl PCR water

Enzyme dilution, mix well on ice:

2 μl Sequenase
14 μl Enzyme dilution buffer

Termination tubes, place in 37°C block to warm:

2.5 μl ddNTP (i.e., ddATP in tube A, ddGTP in tube G, etc.)

When Reaction has incubated at least 15 minutes at room temp., add in order:

1 μl DTT
2 μl Labelling mix
1 μl ^{33}P dATP
2 μl Diluted enzyme

- Incubate 5 minutes at room temperature

Then add 3.5 μl from Reaction tube to each GATC termination tube.

- Incubate at 37°C for 5 minutes.

Then add 4 μl of stop solution to each termination tube.

Store at -20°C.

Before loading (2.5 μl), heat to 80°C for 3-5 minutes, then place on ice.

APPENDIX 6b

End Labelling PCR

End Labelling Reaction

Dilute forward and reverse primers separately to 5 μM from 50 μM primer stock:

2 μl of 50 μM primer stock (one primer only) + 18 μl PCR water

Labelling Reaction:

1.2 μl 10X kinase buffer
0.8 μl T4 Kinase
2.0 μl of 5 μM primer to be labelled
8.0 μl γ-^{32}P-ATP

Incubate at 37°C for 30 minutes, then 65°C for 10 minutes.

Add:

94 μl of PCR water
10 μl of each 5 μM unlabelled primer (forward and reverse)

PCR

Ingredients (25 μl total volume):

16.1 μl ddH_2O
2.5 μl 10X PCR buffer
.25 μl dNTPs
.15 μl Taq polymerase
.5 μl primer mix (includes end-labelled, forward and reverse primers)

Mix reaction ingredients together, adding primer mix last. Aliquot 24 μl to PCR tubes. Then add 1 μl of 50 ng/μl genomic DNA and start the reaction. Denature PCR products for 5 minutes at 80°C and place on ice before loading 5 μl on a sequencing gel with a known DNA sequencing standard.

APPENDIX 7

Primer sequences used in PCR.

Locus	Forward primer, Reverse primer	Reference
K-Cas	5-ATCATTTATGGCCATTCCACCAAAG-3, 5-AGACAATGTCTCTTCCGCTTTACCCG-3	Medrano and Aguilar-Cordova, 1990a
B-Lac	5-TGTGCTGGACACCGACTACAAAAAG-3, 5-GCTCCCGGTATATGACCACCCTCT-3	Medrano and Aguilar-Cordova, 1990b
GH	5-CCGTGTCTATGAGAAGC-3, 5-GTTCTTGAGCAGCGCGT-3	Lucy et al., 1991
Pit-1	5-CAATGAGAAAGTTGGTGC-3, 5-TCTGCATTCGAGATGCTC-3	Moody et al., 1994
GHR	5-CAGATGAACCCATCTGCATGT-3, 5-AATGTCACTGCTAGCCCAAGT-3	Moody and Pomp, 1994
IGF-I	5-AGCTGAGATTTGAATGACATCAT-3 5-CCACTGTTCATATTTTTCTGCATAA-3	Pomp, unpublished
Prolac	5-CAGTTTGTGTAACCTTACCC-3, 5-TGAATCCTCCAACATACGTTGC-3	Pomp, unpublished
BM2113	5-GCTGCCTTCTACCAAATACCC-3, 5-CTTCCTGAGAGAAGCAACACC-3	Sunden et al., 1993

APPENDIX 8:

SHORT COMMUNICATION:

Restriction Fragment Length Polymorphism in Amplification Products of the Bovine PIT1 Gene and Assignment of PIT1 to Bovine Chromosome 1

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Abstract

A polymorphism was identified in the bovine PIT1 gene by digesting polymerase chain reaction (PCR) products with the restriction enzyme HinfI. This polymorphism was segregating in five diverse breeds of cattle. PIT1 was sublocalized to the centromeric region of bovine chromosome 1 by linkage analysis.

Keywords: PIT1, mapping, genetic marker, bovine, PCR, RFLP

PIT1 has been identified as a pituitary specific transcription factor that regulates the expression of growth hormone (GH) and prolactin (PRL) genes in the anterior pituitary (Bodner *et al.*, 1988; Ingraham *et al.*, 1988; Nelson *et al.*, 1988). The objectives of this study were to identify a polymorphism in the bovine PIT1 gene, determine its chromosomal location, and estimate allelic frequencies in diverse breeds of cattle.

PIT1 cDNA has been sequenced for cattle (Bodner *et al.*,1988) and human (Tatsumi *et al.*, 1992). Ohta *et al.* (1992b) characterized the human PIT1 gene and provided exon/intron organization of the human PIT1 gene (personal communication). Bovine cDNA sequence (Bodner *et al.*, 1988; EMBL accession number X12657) corresponding to portions of human exons 5 and 6 was used to design primers to amplify a region of bovine PIT1 flanking an expected intron of ~1.1-kb. Primer sequences were: 5'-*primer:* 5'-CAATGAGAAAGTTGGTGC-3'; and 3'-*primer:* 5'-TCTGCATTCGAGATGCTC-3'.

PCR (15 µl final volume) was performed using 50 ng of genomic DNA, 200 µM each dNTP, 0.1 µM each primer, 0.5 units of Taq polymerase and PCR reaction buffer (Tris-HCl, 10 mM; $MgCl_2$, 1.5 mM; KCl, 50 mM; pH 8.3). Thermal cycling began with an initial cycle of 95°C for 2 min, 55°C for 1 min and 72°C

for 2 min followed by 29 cycles of 1 min at 94, 55 and 72°C, and concluded with a final extension at 72°C for 9 min. The reaction resulted in a single product of ~1.35-kb (Figure 1). These primers were also successful in amplifying genomic DNA from ovine, but not porcine or murine DNA.

A panel of 18 purebred cattle representing Angus, Brahman, Brangus, Gelbvieh, Hereford, Holstein, Limousin, and Simmental breeds was tested for polymorphisms in the PIT1 PCR product. Products were digested with 19 different restriction enzymes and separated in 3% ethidium bromide stained agarose gels. One restriction enzyme, Hinfl, revealed two different alleles (A and B) from the PIT1 PCR fragment (Figure 1). The remaining 18 restriction enzymes failed to reveal polymorphisms within the PIT1 PCR product.

No departure from Mendelian inheritance of the PIT1 alleles was observed in 14 full sib families including 155 offspring. A total of 103 purebred animals were genotyped to determine allele frequencies in different breeds. Allele A frequencies of .45, .26, .21, .18 and .10 were observed in Angus (n=19), Holstein (n=17), Hereford (n=45), Gelbvieh (n=17) and Brahman (n=5) breeds, respectively, with an overall frequency of .25.

A total of 185 animals representing 14 informative families from the International Bovine Reference Family Panel (IBRP) was utilized to determine linkage relationships between PIT1 and previously mapped genes. The PIT1 genotypes obtained on the IBRP were merged with the Cattle Genotypic Database. All possible pairwise comparisons were performed using CRI-MAP (v2.4 SunOS). PIT1 was localized on chromosome 1 using the BUILD option of CRI-MAP and the odds for alternative orders were calculated using the ALL option (Barendse et al., 1994).

PIT1 was sublocalized to the centromeric region of bovine chromosome 1, located midway between TGLA57 and RM95 (Figure 2). This makes a linkage

group of TGLA49-RM95-PIT1-TGLA57 (log odds of 5.36 over the next best order TGLA49-PIT1-RM95-TGLA57). The superoxide dismutase 1 gene (SOD1) is located proximal to this linkage group (Barendse et al., 1994), but showed no recombinants to TGLA49.

PIT1 is conserved on human chromosome 3, and more distal to it are other loci (CRYG8 and UMPS; Barendse et al., 1993) that are also conserved on human chromosome 3. More proximal to the centromere is SOD1 and COL6A1 (Schmutz et al., 1994), conserved on human chromosome 21, while other loci conserved on human chromosome 21 are located much further towards the telomere of bovine chromosome 1 (O'Brien et al., 1993). PIT1 thus contributes to defining the boundary of an evolutionary breakpoint on bovine chromosome 1, and extends previous mapping efforts (Threadgill et al., 1991; Barendse et al., 1993) to understanding the evolutionary conservation of this chromosome.

Acknowledgements

We thank J. E. Womack and D. J. S. Hetzel for distributing DNA from the International Bovine Reference Family Panel, and K. Ohta and I. Matsuda for providing exon/intron organization of the human PIT1 gene. We also gratefully acknowledge S.M. Armitage, M. A. Cushman and P. A. Tank for technical assistance. This work was supported in part by the Oklahoma Beef Industry Council (D.P) and the Meat Research Corporation of Australia (W.B.). Approved for publication by the Director, Oklahoma Agricultural Experiment Station.

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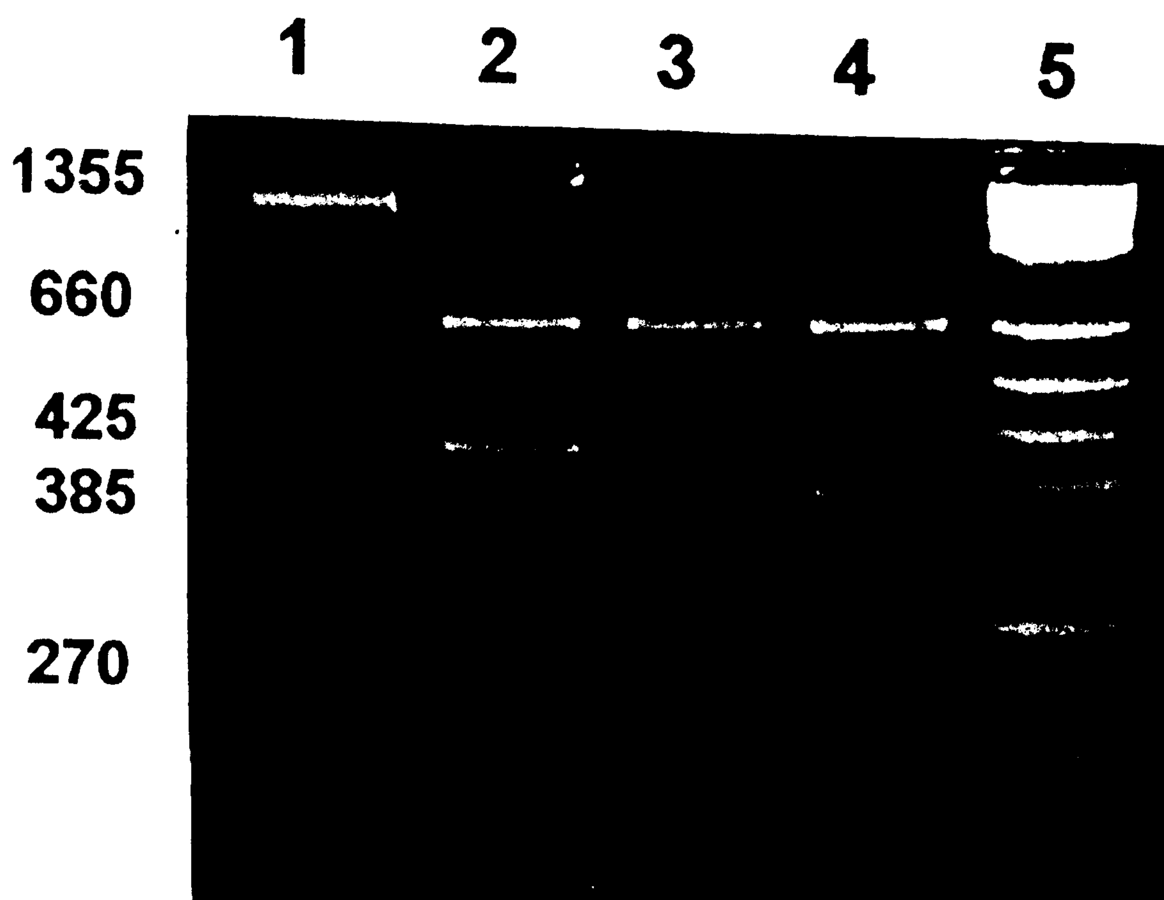
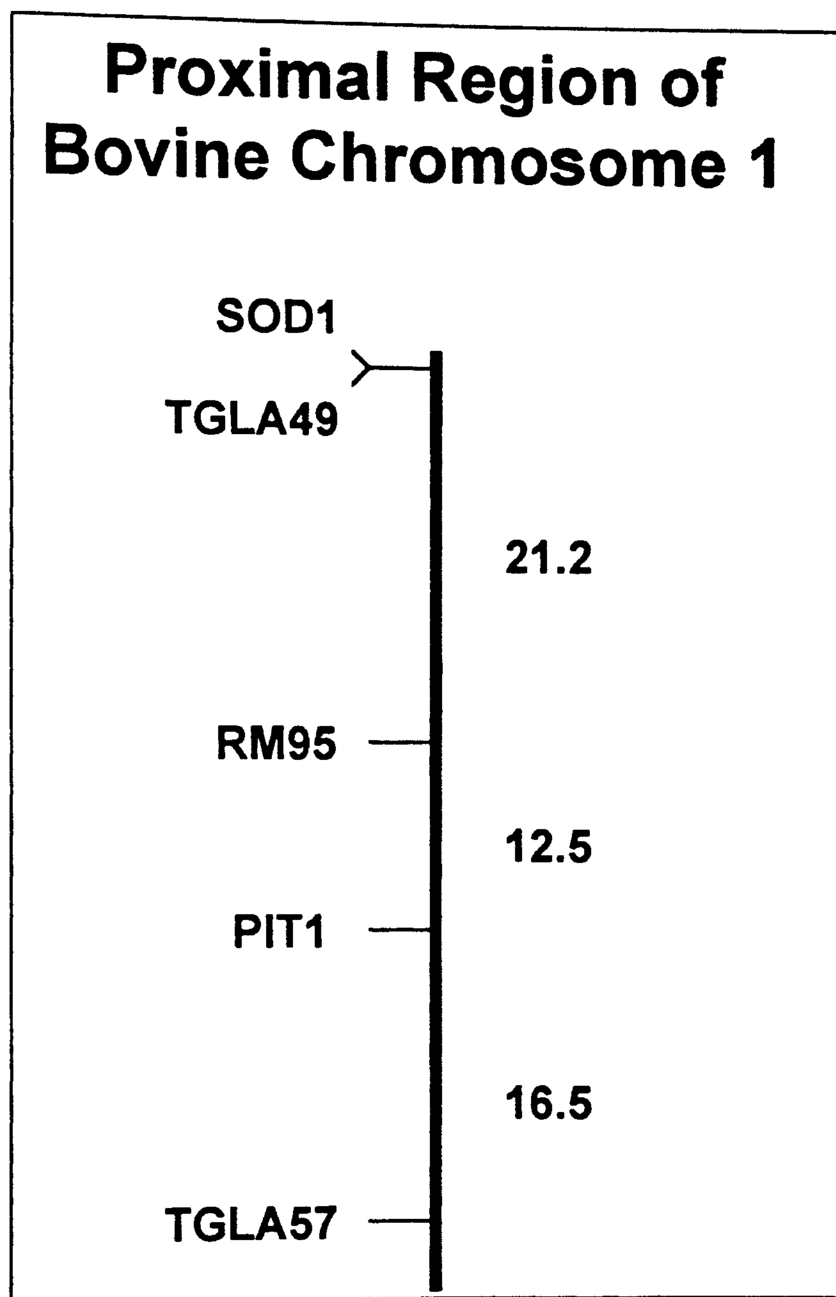
Fig. 1. HinfI restriction fragment length polymorphism in bovine PIT1 PCR products.



Lane 1 is the undigested PIT1 PCR product of ~1355 bp. Lanes 2, 3 and 4 are HinfI digested PIT1 PCR products from cattle of AA (660, 425 and 270 bp), AB (660, 425, 385 and 270 bp) and BB (660, 385 and 270 bp) genotypes, respectively. A 40 bp fragment not visible in the gel was likely the result of an additional HinfI recognition site in the B allele. Lane 5 is Boehringer Mannheim (Indianapolis, IN, USA) DNA Molecular Weight Marker VI.

Fig. 2. Sublocalization of the PIT1 gene to the centromeric region of bovine chromosome 1. Kosambi cM map distances are shown for a sex averaged genetic linkage map. SOD1 showed no recombinants to TGLA49.



APPENDIX 9

Rapid Communication: A PCR-Based Restriction Fragment Length Polymorphism in the Bovine Growth Hormone Receptor Gene[1]

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Polymorphism

AluI polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of the bovine Growth Hormone Receptor (GHR) gene.

Source and Description of Primers

Primers were designed from bovine GHR cDNA sequence (Hauser et al., 1990) with consideration of consensus with human exon/intron boundaries (Godowski et al., 1989). The amplified products spanned bovine cDNA nucleotides 946 through 1037 described by Hauser et al. (1990). This region of the bovine GHR gene corresponds to portions of exons 7 and 8 of the human GHR gene, which are separated by a ~3-kb intron (Godowski et al., 1989). PCR amplification of bovine genomic DNA resulted in products of ~1945 bp, indicating the presence of an intron in a similar location but of smaller size than described for the human GHR gene. The PCR product was sequenced to verify identity to published bovine cDNA GHR sequence.

Primer Sequence

5' Primer: 5'-CAGATGAACCCATCTGCATGT-3'. 3' Primer: 5'-AATGTCACTGCTAGCCCAAGT-3'.

Method of Detection

PCR (15 μl final volume) was performed using 50 ng of bovine genomic DNA, 200 μM each dNTP, 0.5 μM each primer, 0.5 units of Taq polymerase and PCR reaction buffer (Tris-HCl, 10 mM; $MgCl_2$, 1.5 mM; KCl, 50 mM; pH 8.3). Thermal cycling began with an initial cycle of 95°C for 2 min, 55°C for 1 min and 72°C for 3 min, followed by 29 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 3 min, and concluded with a final extension at 72°C for 9 min. PCR products were digested with 1.25 units of the restriction enzyme AluI in 12 μl

reactions. Digested products were separated in 3% agarose gels stained with ethidium bromide.

Description of Polymorphism

AluI digestion of the approximately 2.0-kb PCR product of the bovine GHR gene revealed 2 different alleles (Figure 1). The A allele was characterized by bands of 785, 670, 375 and 115 bp. The B allele had an additional AluI restriction site within the 670 bp fragment resulting in bands of 785, 475, 375, 195 and 115 bp.

Inheritance Pattern

Departure from Mendelian inheritance of the two GHR alleles was not observed in 11 full sib families including 119 offspring.

Frequency

While the A allele of the GHR gene was fixed in a total of 94 animals representing Hereford, Polled Hereford, Angus, Gelbvieh and Holstein breeds, the A and B GHR alleles were segregating in cattle of *bos indicus* descent. Five unrelated Brahman sires were homozygous for the B allele. Twenty unrelated animals of at least partial *bos indicus* descent included as parents in the International Bovine Reference Family Panel (IBRP) had frequencies of .42 for the B allele and .58 for the A allele.

Chromosomal location

The bovine GHR gene was mapped to chromosome 20, distal to TGLA126 and proximal to GMBT41 (Barendse et al., 1994), by linkage analysis utilizing the IBRP and the cattle genotypic database. Hexosaminidase B and 5-hydroxytryptamine receptor 1A genes have also been mapped to bovine chromosome 20 (Barendse et al., 1994). These genes, along with GHR, have been mapped in humans to chromosome 5 (see O'Brien et al., 1993). The addition of GHR to the bovine map provides further evidence that homology

exists between bovine chromosome 20 and human chromosome 5. Collaborative efforts are underway to determine the chromosomal location of GHR using physical mapping techniques.

Comments

Growth Hormone Receptor is the cell surface receptor for Growth Hormone (GH) and is required for GH to carry out its effects on target tissues. It has been suggested that serum growth hormone-binding protein is also a product of the GHR gene by a mechanism of alternative splicing (Baumbach et al., 1989). Mutations in the GHR gene have been associated with sex-linked dwarfism in chickens (Burnside et al., 1992) and Laron-type dwarfism in humans (Godowski et al., 1989).

This polymorphism should be useful in studies to identify Quantitative Trait Loci (QTL) in resource populations developed from crosses between *bos indicus* and *bos taurus* cattle. Primers designed to amplify bovine genomic DNA were also successful in amplifying genomic DNA from sheep, but not from porcine or murine DNA. Polymorphisms in sheep were not investigated.

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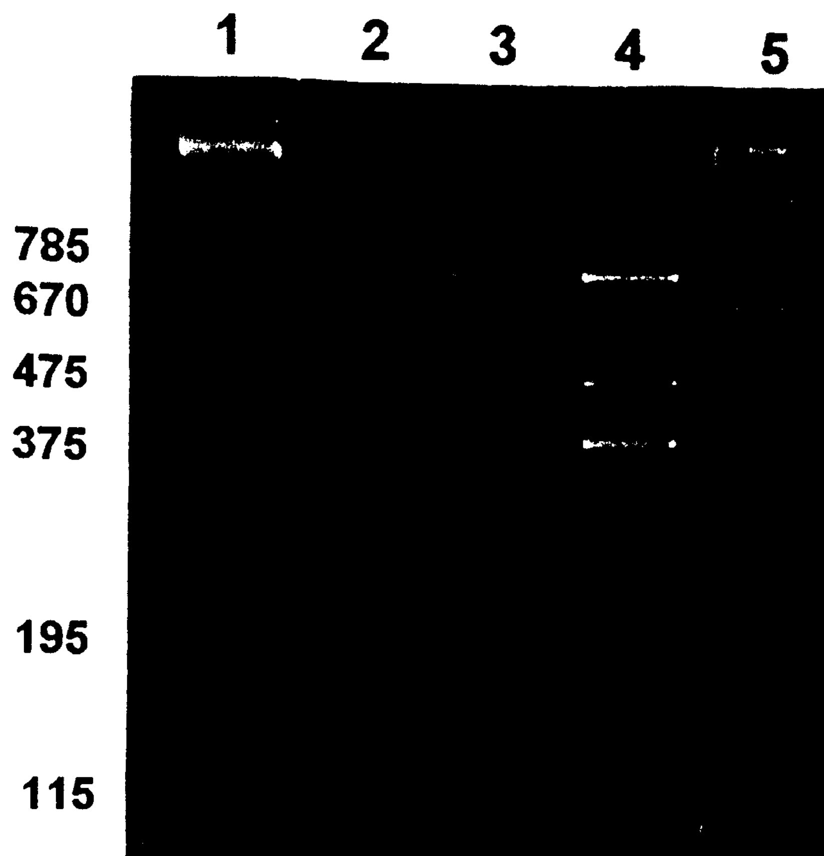
Key Words: Bovine, Growth Hormone Receptor, Polymorphism, Genetic Marker, PCR, RFLP

Figure 1. AluI restriction fragment length polymorphism of bovine growth hormone receptor PCR products.

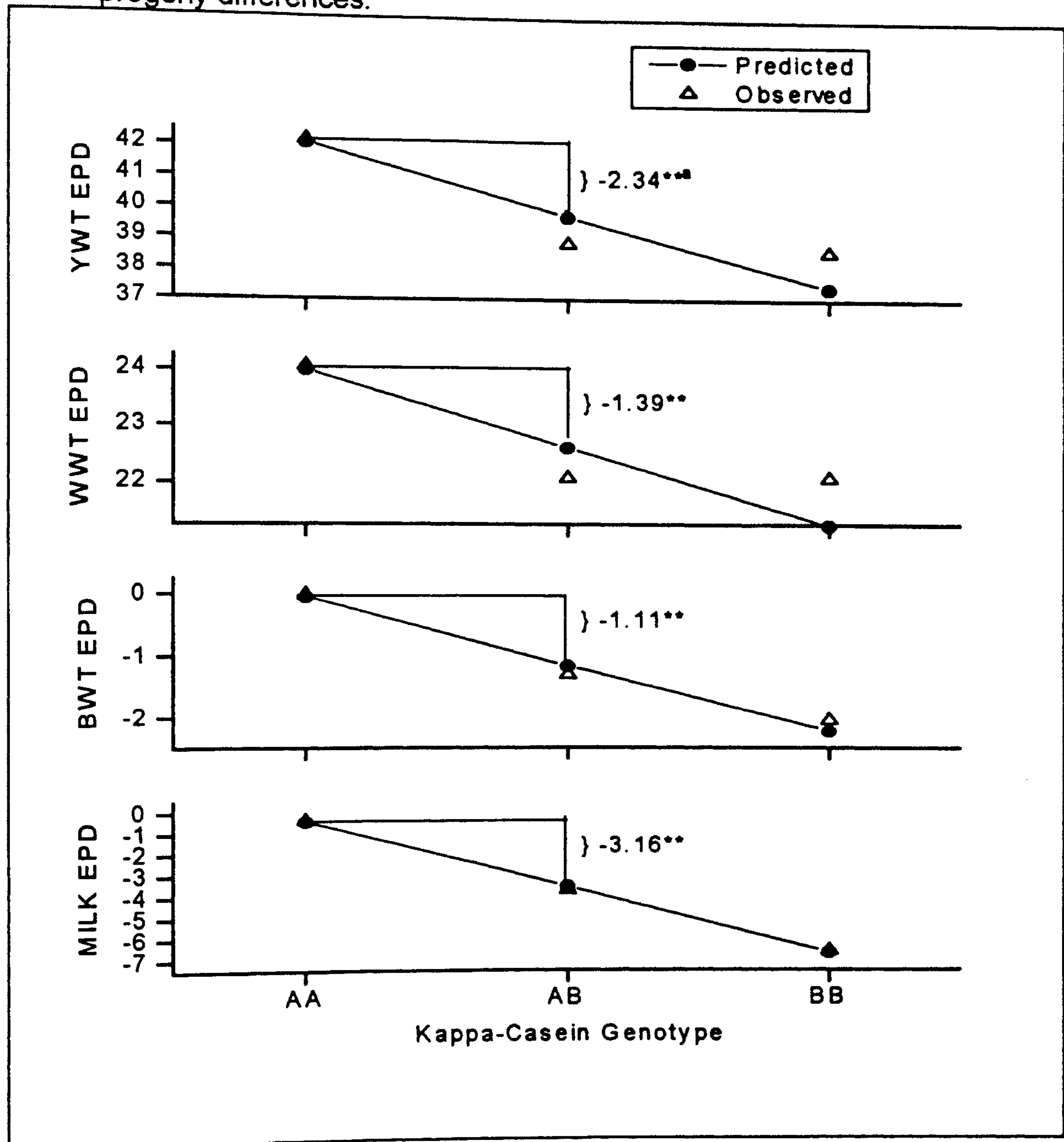


Undigested and AluI digested GHR PCR products visualized after gel electrophoresis and ethidium bromide staining are shown. Lane 1 is the GHR PCR product of ~1945 bp. Lanes 2, 3 and 4 are AluI digested GHR PCR products from cattle of AA, AB, and BB genotype, respectively, characterized by banding patterns of 785, 670, 375 and 115 bp (AA genotype); 785, 670, 475, 375, 195 and 115 bp (AB genotype); and 785, 475, 375, 195 and 115 bp (BB genotype) (The 115 bp band is faint). Faint monomorphic bands of 1175 and 580 bp are also visible. Lane 5 is a size marker (Boehringer Mannheim DNA Molecular Weight Marker VI (Indianapolis, IN); sizes: 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 220 and 154 bp.)

APPENDIX 10

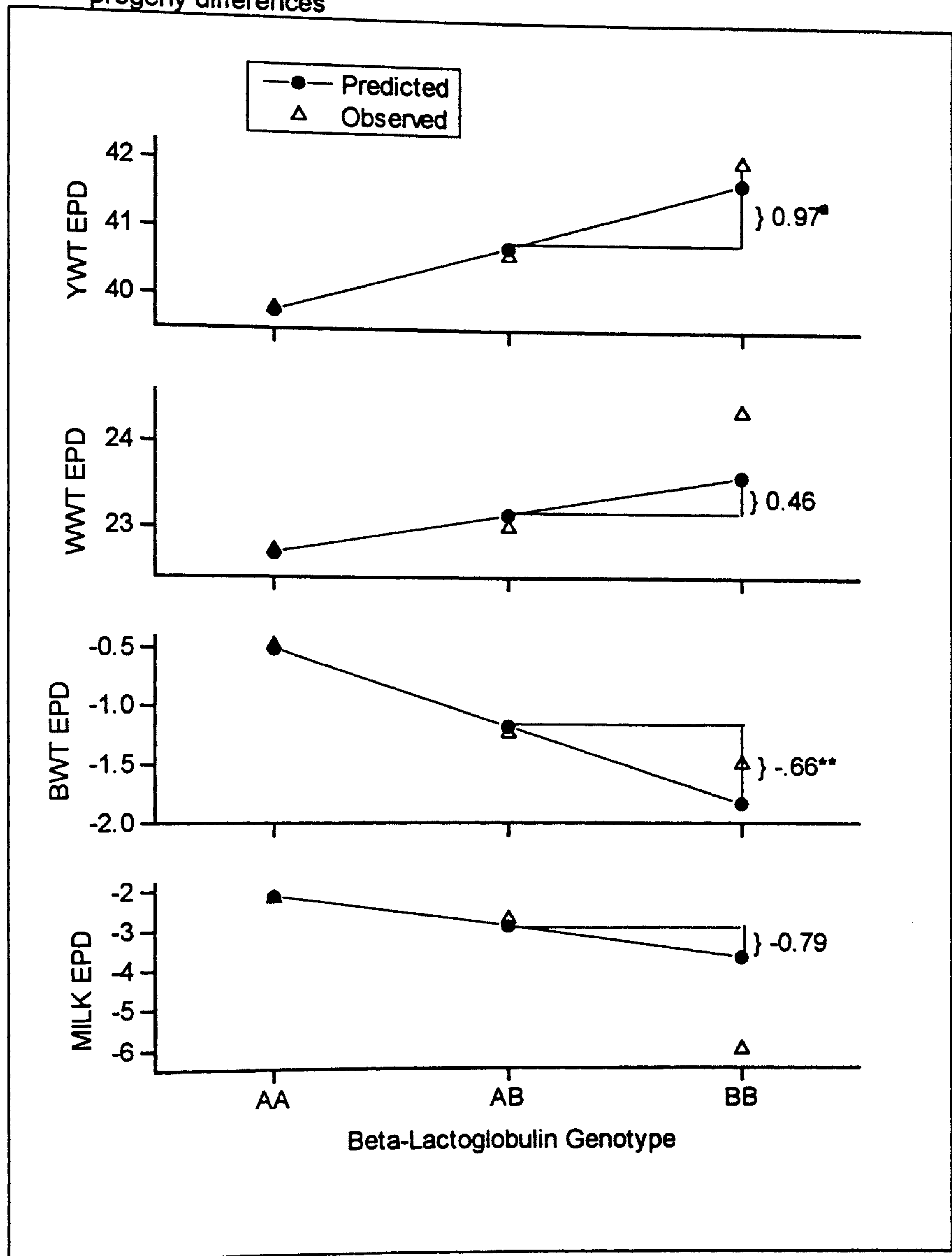
Results from EPD Regression Analyses

Figure 1: Regression analyses to estimate the effect of K-Cas allele substitution on yearling weight, weaning weight, birth weight and milk expected progeny differences.



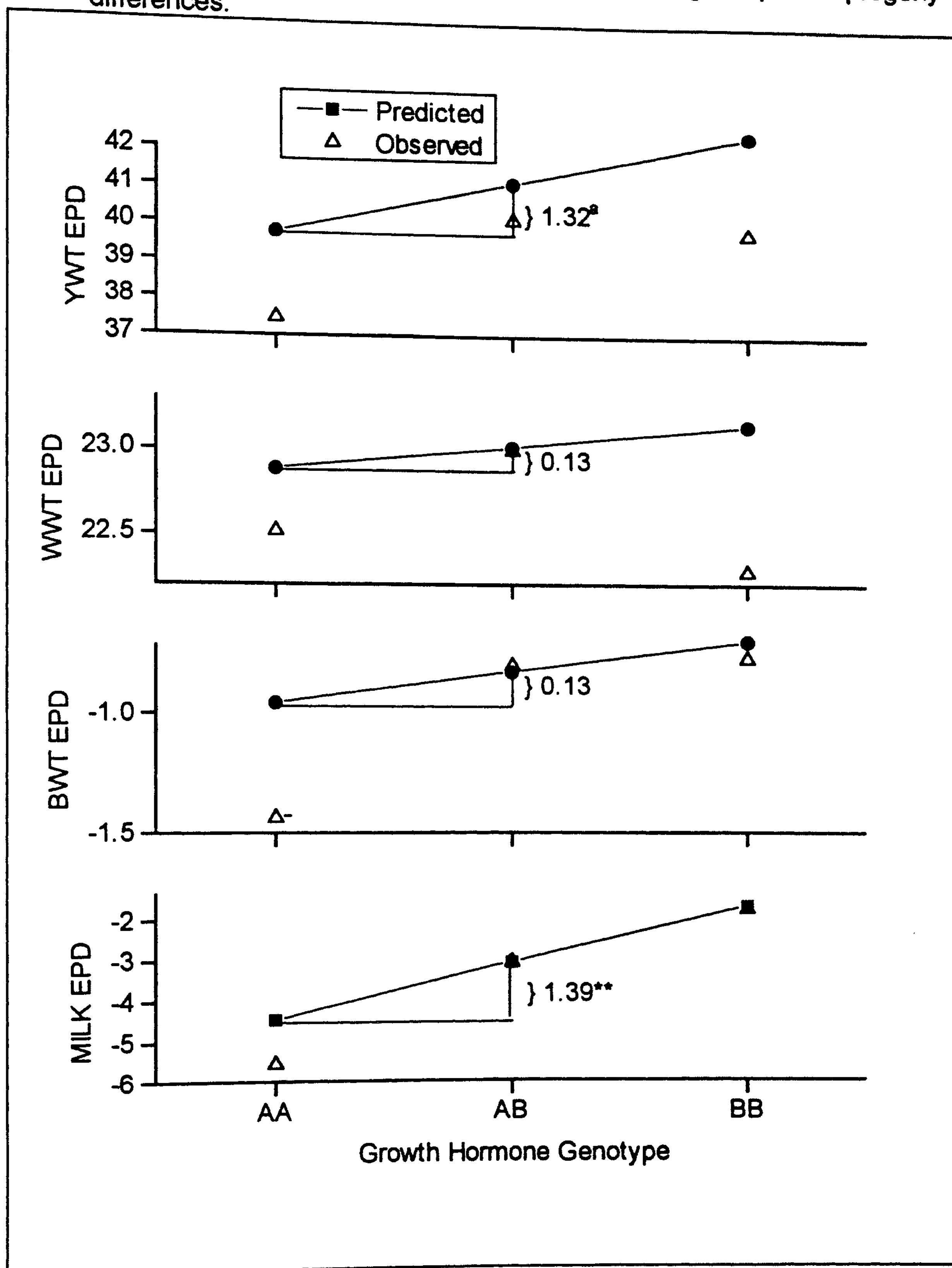
[a] α, the average effect of allele substitution (pounds)
**P<.01, *P<.05, +P<.10

Figure 2: Regression analyses to estimate the effect of B-Lac allele substitution on birth weight, weaning weight, yearling weight and milk expected progeny differences



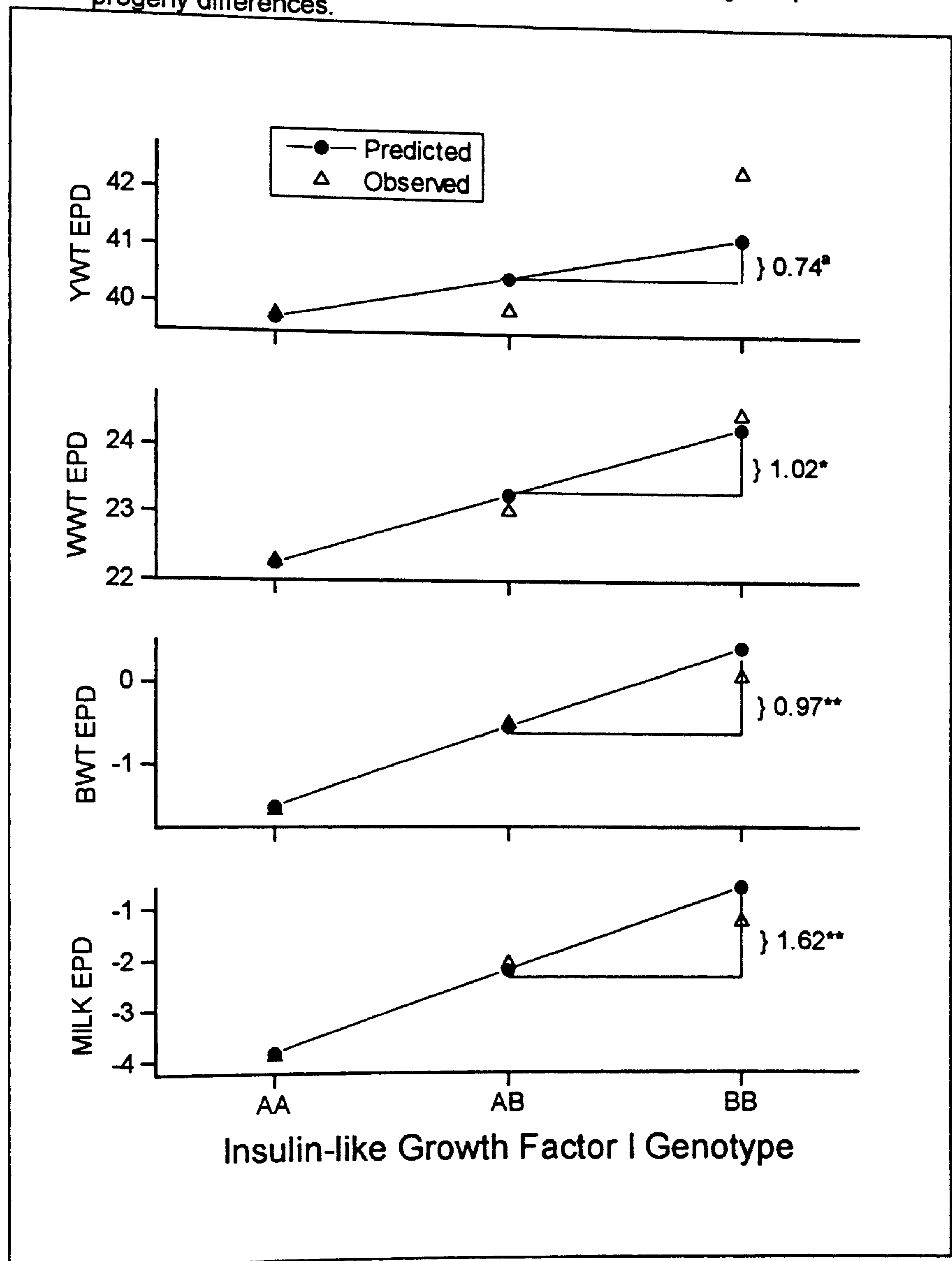
[a] α, the average effect of allele substitution (pounds)
**P<.01, *P<.05, +P<.10

Figure 3: Regression analyses to estimate the effect of GH allele substitution on milk, birth weight, weaning weight and yearling weight expected progeny differences.



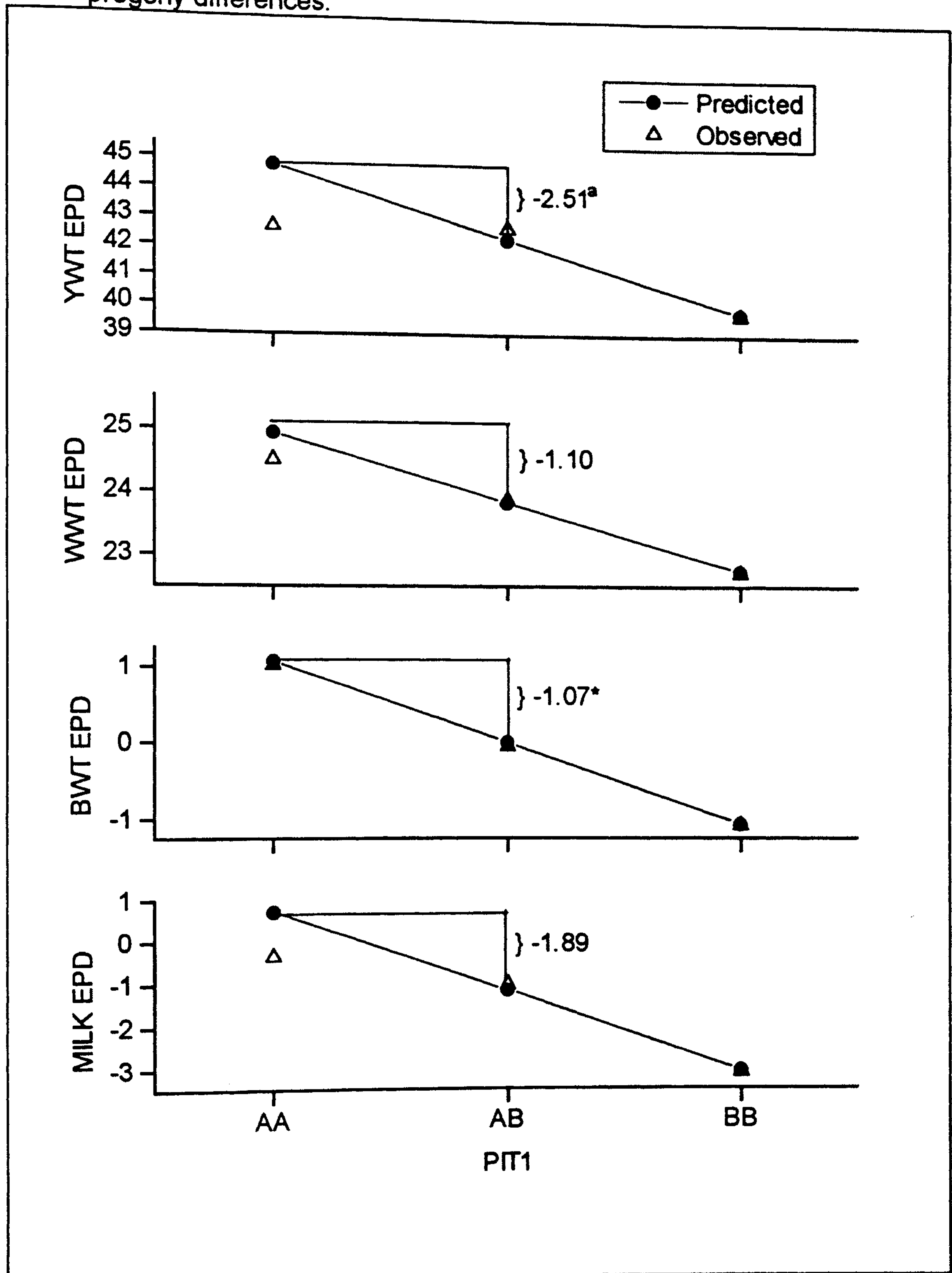
a α, the average effect of allele substitution (pounds)
**P<.01, *P<.05, +P<.10

Figure 4: Regression analyses to estimate the effect of IGF-I allele substitution on milk, birth weight, weaning weight and yearling weight expected progeny differences.



[a] α, the average effect of allele substitution (pounds)
**P<.01, *P<.05, +P<.10

Figure 5: Regression analyses to estimate the effect of Pit-1 allele substitution on milk, birth weight, weaning weight and yearling weight expected progeny differences.



[a] α, the average effect of allele substitution (pounds)
**P<.01, *P<.05, ⁺P<.10

VITA

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Master of Science

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