LOCALIZATION OF MAJOR QUANTITATIVE TRAIT LOCI (QTL) CONTROLLING BODY WEIGHT AND FAT PERCENTAGE ON MOUSE CHROMOSOMES 2 AND 15

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NOMENCLATURE

A	Agouti Locus
ANOVA	Analysis of Variance
CAST	*Mus musculus castaneus* (CAST/Ei)
CIM	Composite Interval Mapping
DAY12	12 Day Body Weight
FAT(P)	Total Fat Pad Weight = SCF + GOF; (FAT/12 Week Weight)
GAIN1	12 Day to 3 Week Weight Gain
GAIN2	3 Week to 6 Week Weight Gain
GAIN3	6 Week to 9 Week Weight Gain
GAIN4	9 Week to 12 Week Weight Gain
GHR	Growth Hormone Receptor
GHRH	Growth Hormone Releasing Factor
GOF(P)	Gonadal Fat Pad Weight; (GOF/12 Week Weight)
HRT(P)	Heart Weight; (HRT/12 Week Weight)
IM	Interval Mapping
KID(P)	Kidney Weight; (KID/12 Week Weight)
LIV(P)	Liver Weight; (LIV/12 Week Weight)
M16i	Selection Line for Increased 3 to 6 Weeks Gain; Inbred
MAS	Marker Assisted Selection
MR	Marker-Regression QTL Mapping
MSR	Mean Square Residual
MSREG	Mean Square Regression
PCR	Polymerase Chain Reaction
PPAR α	Peroxisome Proliferator Activated Receptor
QTL	Quantitative Trait Loci
RFLP	Restriction Fragment Length Polymorphism
SCF(P)	Subcutaneous Fat Pad Weight; (SCF/12 Week Weight)
SPL(P)	Spleen Weight; (SPL/12 Week Weight)
SSLP	Simple Sequence Length Polymorphism
TES(P)	Testis Weight; (TES/12 Week Weight)
WK12	12 Week Body Weight
WK3	3 Week Body Weight
WK6	6 Week Body Weight
WK9	9 Week Body Weight

CHAPTER I

INTRODUCTION

Animal Breeding has changed immensely over the past years because of the recent advancements in molecular biology techniques. The main goal to producers has remained the same even though the technology has changed, to identify animals with desirable genes and increase the population frequency of those genes. Currently, research conducted in hopes of improving livestock is incorporating quantitative and molecular genetic techniques. DNA molecular markers are being used in an attempt to locate quantitative trait loci (QTL) that control economically important traits. Once QTL are located, the producer will be able to use phenotypic as well as genotypic data in a marker assisted selection program.

Knowledge gained about the genetic mechanisms causing the variation observed in body composition and growth rate between animals is very beneficial. This would allow the livestock industry to produce a more appealing animal for the consumer. In addition, the producer would be able to raise livestock in a more efficient manner, resulting in a more profitable enterprise. The overproduction of fat in livestock is widely prevalent and has serious consequences for the industry. Health conscious consumers will continue to reduce the amount of red meat and poultry purchased. There will be an increase in labor

and processing costs for the meat packers. Inevitably, animals will cost more to produce because of the relative inefficiency of fat growth compared with lean growth. Nevertheless, the problem of excess fat is of primary concern for humans since obesity is considered to be a risk factor in the development of cardiovascular disease, diabetes, hypertension, and cancer.

These problems may be avoided by identifying the chromosomal location of the genes that control body weight and fat percentage. Researchers will be able to accomplish this because of the growing information about the genome maps for mice, humans, cattle, swine, sheep, and poultry. Due to comparative mapping, research can be conducted in model specimens that are cost effective, easy to maintain, and reproduce quickly and then the data can be transferred to other organisms.

An initial experiment at Oklahoma State University (OSU) looked at identifying QTL that play significant roles in the control and regulation of body composition and growth in mice (Pomp et al., 1994). An initial cross between lines M16i (based on 27 generations of selection for increased postweaning gain (Hanrahan et al., 1973) followed by 15 generations of inbreeding) and CAST/Ei (an inbred line of wild origin (*Mus musculus castaneus*) denoted as CAST) produced an F_1 which was backcrossed to M16i. A large population (n=424) was created to determine linkage between DNA marker loci and QTL affecting 12 week adult body weight (WK12) and gonadal fat pad weight/WK12 (GOFP). A whole genome screening procedure was utilized with three evenly-spaced microsatellite markers for each of the 19 murine autosomes.

Based on the results obtained from least-squares procedures by Pomp et al. (1994), evidence for a region harboring a QTL, with very large effects, was observed on distal chromosome 2, with differences of 3.9 g WK12 (10% of the mean; P<.0001) and 0.31% GOFP (37% of the mean; P<.0001) between M16i/M16i and M16i/CAST backcross mice. In addition to chromosome 2, a QTL appeared on chromosome 15 with deviations between the homozygous and heterozygous genotypes of 1.9 g WK12 (5% of the mean; P<.0001) and 0.13% GOFP (16% of the mean; P=.0017). The indication of possible QTL associated with body weight and fat percentage on these chromosomes formed the foundation of this experimental study.

The primary objective of this research project was to pinpoint the chromosomal location of the previously mentioned QTL for body weight and fat percentage on chromosomes 2 and 15 in the mouse. This was achieved by statistically analyzing any significant correlations between an individual's phenotype and genotype in this specially designed segregating population. Markers or genes will be identified which have an influence on body compositional traits, such as 12 week adult body weight and gonadal fat pad weight. A second objective was the development of primers for PCR amplification of candidate genes, followed by the mapping of these genes in our population. A final goal was to compare simplified statistical analyses using least-squares procedures with the more complicated composite interval mapping method (Zeng, 1994).

Accomplishment of these research goals will help in the understanding of the mechanisms involved in growth and obesity, along with the identification of superior animals with favorable characteristics. Any genes or markers found to be linked to growth

or obesity in this research project could be used in comparative gene mapping strategies. Identified QTL in mice may expose candidate chromosomal regions harboring homologous QTL in livestock species and humans. Our present knowledge of QTL mapping may also be enhanced with simpler statistical analyses for QTL placement.

CHAPTER II

LITERATURE REVIEW

Introduction

Over the years, progressive advancements for specific selected-traits, such as body weight and lean carcasses in livestock, have been very time consuming because these processes depend on designated matings between selected dams and sires to bring together favorable alleles. For instance, without biotechnology Lasslo et al. (1985), practiced 16 years of selection for 120-day weight in Targhee sheep to yield an 18.1 lb (23% of the mean) increase. Also, a decrease in backfat thickness of 2.6 cm (68% of the initial mean) in Duroc swine required 10 generations of selection (Hetzer and Harvey, 1967). However, if genes affecting growth and obesity were identified, the selection for a more desirable product could be achieved much quicker. This can be accomplished by using a process of making selection decisions based on an animal's phenotypic and genotypic information (marker assisted selection). Maker assisted selection should accelerate genetic gain by increasing selection accuracy, reducing generation interval, and increasing selection differentials. This will be advantageous to the producer by reducing production costs.

Backfat thickness and 120-day weight, along with nearly all economically important traits in livestock, are polygenic or quantitative in nature in that phenotypes are influenced by a number of different genes. Quantitative trait loci (QTL) are those loci with relatively small effects on phenotype. Falconer (1965) described quantitative traits as having continuous measurements. Examples are average daily gain, fat thickness, and body weight. The polygenic model of inheritance assumes an animal's breeding value is the sum of small, additive effects of many genes (Hoeschele, 1988). Innovations in quantitative and molecular genetics, which are now at the forefront of science, have allowed a detailed study of the genetic basis of quantitative traits. In the future, detailed genetic maps will allow desirable genes affecting economically important traits to be isolated, multiplied, and transferred into the germline of the same or different species. Thus, significant amounts of genetic improvement can occur in fewer generations compared to traditional selection methods.

Use of Mice as Animal and Human Models

If given sufficient time, funding, and facilities, animal breeders could do research exclusively with the class of livestock in which genetic improvement is desired. However, this approach is ineffecient when answers can be obtained from research with cheaper, more rapidly reproducing organisms. This is possible because of the common base between physiology and genetic principles in different species and statistics available for quantitative estimates of biological parameters. Chapman (1961) explained the value of

laboratory animals in breeding research as checking theoretical prediction against outcome and development of further theory and research tools. The coefficients of inbreeding and relationship were developed by Wright (1922) from crosses in guinea pigs. The effectiveness of different methods and intensities of selection in producing genetic improvement were studied in laboratory animals and later applied to livestock. A few examples include selection for body size, lactation, and litter size in mice (Falconer, 1955), 13-week individual body weight in the rat (Craig and Chapman, 1953), and trait relationships opposite to those predicted by genetic correlation in mice (Cochrem, 1959).

Of all laboratory mammals, the mouse has probably contributed the most to the advancement of experimental genetic research in animal breeding. This is due primarily to its short gestation period, large litter sizes, ease of maintenance, convenient size, the ability to control matings, and the availability of inbred strains. Also, the recent progress in molecular genetics has caused the rapid development of comparative maps between mice, humans, sheep, cattle, and pigs (O'Brien et al., 1993). This allows for the detection of genes in other species once the gene or area of interest has been mapped in the mouse, based on homology. The annual use of 50 million mice worldwide affirms the importance of the mouse in genetic analysis (Foster et al., 1981).

Inbred strains of mice have been model systems for research for well over a half of a century (Festing, 1979). The advantage of using inbred mice, compared to mice of unknown genetic composition, is that individuals of the same inbred strain are genetically identical. Therefore, the one important variable in a study is under control of the experimenter. Inbreeding is the mating of individuals which are related to one another by

having one or more common ancestors. This can be quantified in terms of the coefficient of inbreeding (F), which is the probability that two genes at any locus are identical by descent (Falconer, 1989). The degree of inbreeding depends on the degree of relationship between the individuals. With mammals, the closest relationship possible is full brother x sister (full-sib) mating. According to the 1952 Committee on Standardized Genetic Nomenclature for Mice, a strain is sufficiently inbred for use in research projects after 20 generations of full-sib mating when F=98.6% (Festing, 1979).

A final reason the mouse is an ideal model organism for animal breeding experiments is the presence of specially designed selection lines. Nonreplicated selection studies may receive biased results from genetic drift (Eisen, 1992). The use of replicated selection lines in livestock is not feasible mainly because of the high production costs. Eisen (1989) reviewed several mice selection experiments for body compositional traits which have been conducted: 12-day litter weight (Eisen et al., 1970), 3 to 6-week postweaning gain (Hanrahan et al., 1973), 6-week body weight (Falconer, 1973), 12-week hind-carcass weight/body weight (Eisen, 1987), 12-week hind-leg muscle weight (McLellan and Frahm, 1973), 10-week body weight (Sharp et al., 1984), 12-week epididymal fat pad weight/body weight (Eisen, 1987), and 10-week epididymal fat pad weight/body weight (Sharp et al., 1984). These and other replicated selection index experiments provide valuable lines for studying the genetic mechanisms controlling energy utilization and maintenance requirements.

In addition to the mouse being a beneficial model for livestock improvement, the mouse is used for human disease, immunological, drug abuse, alcohol abuse, and

behavioral studies. Cancer research demonstrated that the incidence of tumors varied among different families of mice (Morse, 1978). T-cell leukemias or lymphomas are present in nearly 100% of several useful mice strains (Lilly, 1978). Congenic lines are available for 35 minor histocompatibility loci (Graff, 1978). Genetic models for alcohol and drug abuse have been applied to the investigation of sensitivity to initial or adverse drug effects; neuroadaptation underlying chronic tolerance or sensitization, withdrawal, and dependence; and drug aversion (Crabbe et al., 1994a).

Differences were noted among several inbred strains of mice in preferential drinking of alcohol (McClearn and Rodgers, 1959). The following are some selection lines produced to aide in genetic studies: duration of righting reflex suppression by ethanol and a number of benzodiazepines, barbiturates, and gaseous anesthetics (McClearn et al., 1981); sensitivity to morphine or morphine-like opioids (Moskowitz et al., 1985); sensitivity to nicotine (Marks et al., 1989); and sensitivity to ethanol, diazepam, phenobarbitol, and nitrous oxide withdrawal (Crabbe et al., 1985). Behavorial (eating/hoarding food, drinking, food preferences, and fighting) differences between genetic stocks have also been studied extensively (Wimer and Fuller, 1975).

Genetic Linkage Maps

Mouse gene mapping began in the first part of this century, mainly based on the genetic linkage analysis of phenotypic variants. As a result, the knowledge initially gained in this area was slow. An early experiment discovered that two coat color mutations,

pink-eyed dilution and albino, were linked (Haldane et al., 1915). The number of defined loci totaled 11 on five linkage groups by 1935, 27 on 10 linkage groups by 1945, and greater than 400 by 1979 (Foster et al., 1981). The discovery of genes appears to be growing exponentially, with the total number of loci growing to over 1300 by the end of 1993 (Copeland et al., 1993). Currently, the total number of mapped loci is an amazing 6132 (Mouse Genome Database; The Jackson Laboratory, 1995).

The recent surge in gene mapping was due to the finding and mapping of DNA polymorphisms using recombinant DNA techniques. Thoday (1961) used genetic markers on chromosomes to follow inheritance. Quantitative genetic differences in sternopleural chaeta number in *Drosophila melanogaster* were analyzed. This was accomplished by identifying individuals with a chromosome known to give a higher value for the trait than the homologous chromosome marked with recessive major genes. These individuals were test-crossed to a stock homozygous for the recessive major genes, producing a large population of recombinant chromosomes. Progeny-testing was utilized in the classifiation of each into two or three recombinant classes, depending on the number of loci. This research, as well as others at this time, was limited by the insufficiency of beneficial genetic markers.

Associations between gene markers and QTL are made by examining progeny from parents heterozygous at both the marker and QTL (Soller, 1990). To be the most beneficial, the initial populations need to be widely segregating for the QTL of interest. Minor variations in DNA sequences provide genetic markers which are inherited as simple Mendelian factors: one or more individual bases could differ to result in the loss or

formation of a cleavage site, as well as the insertion or deletion of blocks of DNA within a fragment (Botstein et al., 1980). The basic strategy, linkage analysis, allows for the inheritance of markers to be followed, plotting their positions in relation to one another onto a chromosome (White and Lalouel, 1988). These markers were typed initially by restriction fragment length polymorphisms (RFLP) on Southern DNA blots (Botstein et al., 1980), and most recently by the polymerase chain reaction (PCR; Saiki et al., 1988). The first method uses enzymes to cleave DNA fragments which are resolved by agarose gel electrophoresis. Fragments containing specific sequences are detected by Southern blot hybridization with radioactive probes.

The lattermethod employs simple sequence length polymorphism (SSLP) or microsatellite markers defined by PCR. These are DNA segments within the genome that possess a very short, simple sequence of nucleotides, such as poly(G), poly(A), poly(TC), poly(CAC), poly(GATA), poly(CA), and poly(GT), which are densely interspersed within the eukaryotic genome (Beckmann and Soller, 1990). The human and mouse genomes contain at least 100,000 sequences with $(CA)_n$ repeats (Hamada et al., 1982). These markers were initially typed by probing DNA restriction digests within radiolabeled repeats (Weber and May, 1989). However, recent techniques have allowed for the use of primers flanking the repeats to amplify a specific microsatellite by PCR, which is then run on agarose gels to determine size variations among various strains.

A few microsatellites, mainly in the range of 9-30 repeat units, were first discovered by Weber and May (1989) for use in human mapping. A total of 44 SSLP were first found for use in mouse mapping (Love et al., 1990). An important break-through in 1992

by Dietrich and his colleagues greatly increased the number of useful markers (Dietrich et al., 1992). A genetic linkage map of the mouse was constructed with 317 SSLP at an average spacing of 4.3 cM. These highly variable SSLP markers are extremely valuable for genome mapping because they are highly polymorphic, can be rapidly typed by PCR, and almost all loci identified will differ among strains and populations.

Another cause for the escalation in gene mapping was the development of new crosses. Gene assignments in the mouse depend on meiotic mapping which involves recombinational analysis. In the beginning, mouse matings relied on two- and three-point crosses between marked inbred strains or laboratory recombinant inbred (RI) strains (Taylor, 1989). Problems with these approaches included the difficulty in finding allelic differences between different subspecies strains; and RI maintain loci which are closely linked, so the distances around known loci are usually small. By the mid-1980's, interspecific crosses were made between laboratory strains and a species of *Mus* to take advantage of innate diversity among wild species (Avner et al., 1988). Many genes can be mapped from a single cross since most genes and DNA sequences are polymorphic in an interspecific cross. Many laboratories are mating the wild *Mus musculus castaneus* with a different subspecies of laboratory mouse because both sexes are fertile in the F_1 and a very high degree of polymorphism is realized (Copeland et al., 1993).

There have been numerous genetic linkage maps of the mouse genome compiled by various laboratories (Buchberg et al., 1989; Kingsley et al., 1989; Siracusa et al., 1990; Bahary et al., 1991; Chapman, 1991). A map generated from more than 75 years of cumulative effort of researchers shows the chromosomal location of 965 loci, representing

phenotypic and biochemical variants, cloned genes, and polymorphic anonymous DNA markers (Davisson and Roderick, 1989). The first comprehensive molecular genetic linkage map utilized an interspecific backcross between C57BL/6J and *Mus spretus*, positioning more than 600 loci (Copeland and Jenkins, 1991). To accomplish this, the inheritance of cloned DNA probes were followed by Southern blot and RFLP analyses in the backcross progeny.

Additional maps include the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research (WI/MIT-CGR) SSLP map and the 1993 Mouse Chromosome Committee Reports map (Copeland et al., 1993). The WI/MIT-CGR map is a result of using nearly all $(CA)_n$ repeat markers. The inheritance patterns were analyzed by the computer program MAPMAKER (Lander et al., 1987) in a (C57BL/6J-ob and CAST/Ei)F_2 intersubspecific cross. In 1993, this SSLP map contained over 2000 loci. As of 1994, the WI/MIT-CGR SSLP map had grown to 4006 defined loci (Dietrich et al., 1994). At the present time, this SSLP map represents an astonishing 6132 defined loci (Mouse Genome Database; The Jackson Laboratory). The chromosome committee map has loci mapped from many different crosses, so the positions of many loci can only be inferred.

Published murine maps provide known SSLP markers which can be used in the genetic analysis of crosses, and known genes which can suggest likely candidates for mutations, indicating correspondence to a genetic map of the human genome (Donis-Keller et al., 1987). This linkage map of the 23 human chromosomes is based on the pattern of inheritance of 403 polymorphic loci from 21 three-generation families.

Comparative mapping between the mouse and human genomes identified 917 homologs (Copeland et al., 1993). These loci mark 101 segments of conserved linkage homology. Nadeau and Taylor (1984) defined 83 homologous loci, marking 13 conserved segments. Linkage conservation can be found between a region of mouse chromosome 2 and human chromosome 20, as well as mouse chromosome 11 and human chromosome 17. Through the use of conserved loci as reference points, linkage information can be transferred across mammalian genomes (O'Brien et al., 1993). This would benefit livestock species in which genetic maps are not well-saturated with markers and loci.

Until recently, the identification of genetic markers linked to QTL in livestock was limited because of poorly developed linkage maps. Now, genetic maps have been published for ovine (Crawford et al., 1994), bovine (Barendse et al., 1994; Bishop et al., 1994), porcine (Archibald et al., 1995; Rohrer et al., 1994), and poultry (Bumstead and Palyga, 1992). The ovine map, after typing large parental half-sib families, exhibits 19 linkage groups containing 52 markers on 12 chromosomes. The linkage groups range in size from 2 markers showing no recombination to 6 markers covering about 30 cM of the sheep genome. The bovine map explained by Barendse et al. (1994) includes 201 loci organized into 35 linkage groups on 29 chromosomes. On the other hand, the map described by Bishop et al. (1994) includes 313 genetic markers arranged into 30 linkage groups on 24 autosomal, the X, and the Y chromosomes.

A microsatellite linkage map of the porcine genome was assigned to 13 autosomes and the X chromsome (Rohrer et al, 1994). This included 383 markers placed into 24 linkage groups, spanning 1997 cM. The most recent pig map described by Archibald et al.

(1995) includes 239 genetic markers of which 81 correspond to known genes. All 18 autosomes and the X chromosome were assigned linkage groups, extending across 18 Morgans. Finally, a linkage map for the chicken (18 autosomes) has 100 loci (Bumstead and Palyga, 1992). A total map length of 585 cM averaged 8.5 cM between loci. Markers identified 18 linkage groups with 22 unlinked loci.

Once there are detailed linkage maps for mammalian genomes, two approaches can be used to identify QTL and estimate their effects: marker locus, and candidate gene approach (Routman and Cheverud, 1994). Both methods correlate genetic variants with a given phenotype. Marker loci are selected for numerous areas of the genome in hopes of finding linkage to a QTL. For the candidate gene method, loci are selected because they are part of physiological pathways known to affect the phenotype of interest. The disadvantages of the candidate locus approach include that no new loci are discovered, and there may not be variants segregating in the population. However, the marker locus method does allow for the discovery of new QTL. The molecular techniques in these two methods to find QTL are RFLP and SSLP as previously described.

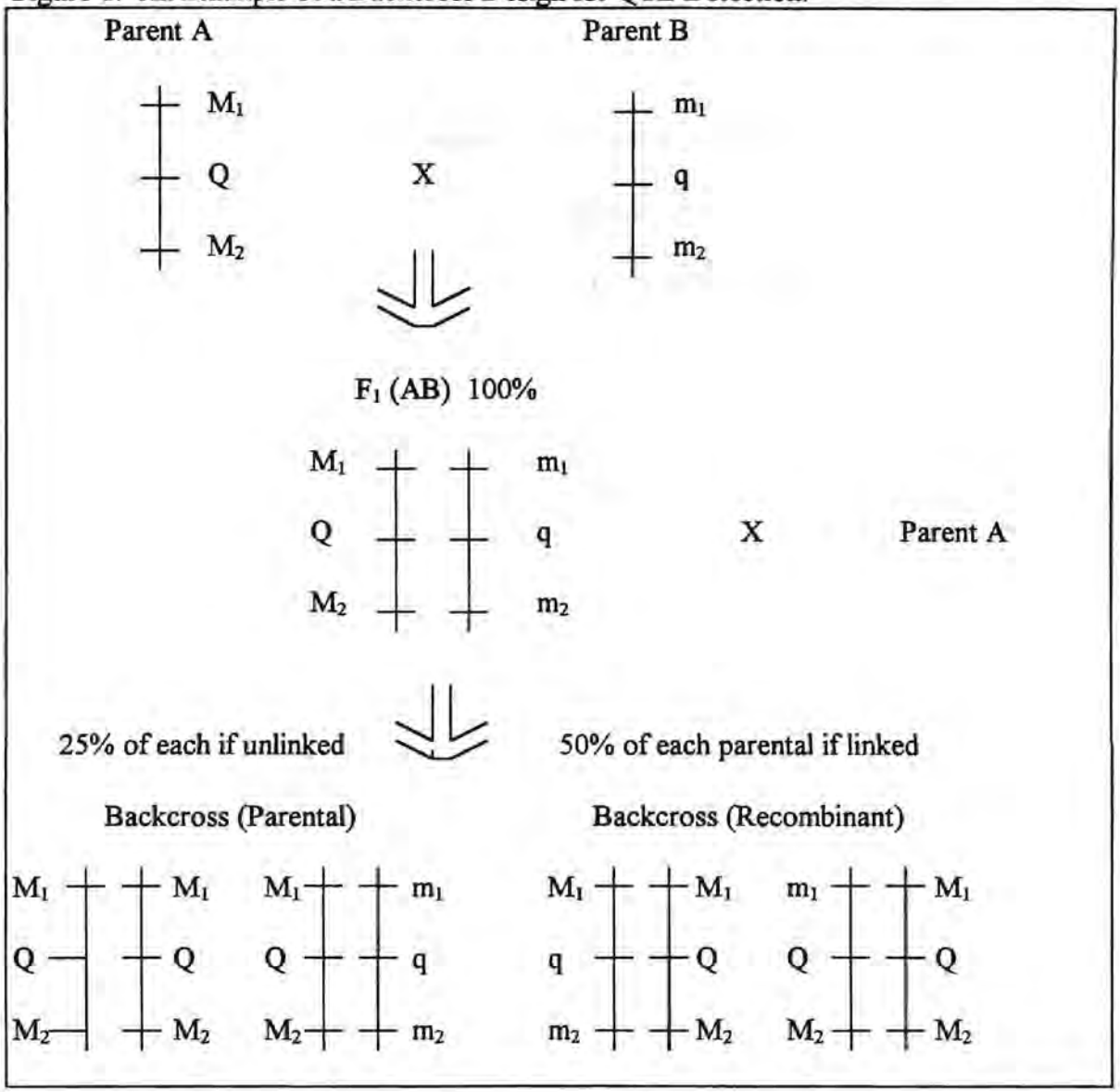
Statistical Detection of Quantitative Trait Loci

Several experimental designs provide an ample population from which data can be collected and analyzed in order to determine the linkage between marker loci and QTL. Examples of these experimental designs include crosses between inbred lines to produce backcross or F_2 populations (Darvasi et al., 1993; Luo and Kearsey, 1988; Soller et al.,

1976), recombinant inbred lines and replicated progenies (Soller and Beckmann, 1990), half-sib families (Weller et al., 1990), and outbred lines (Haley et al., 1994). A backcross design has widespread use in practice and a benefit of analytical simplicity. The optimum spacing of genetic markers for initial studies of marker-QTL linkage are given for the following designs: 30 cM for an F_2 and backcross; and 20 cM for recombinant inbred lines and other relative-pair types (Darvasi and Soller, 1994). For a backcross design, the power of QTL detection was almost the same for marker spacings of 10, 20 and 50 cM (Darvasi et al., 1993). In practice, the number of markers scored per chromosome will be one to three in an initial screening of the genome.

Genotypic information is collected on a given population to detect and measure genetic linkage in a cross in which the alleles at two (or more) loci of interest are segregating. An essential feature of the cross is that at least one of the parents must be heterozygous for both loci. An example in Figure 1 helps illustrate this point. Let M_1 and m_1 represent the alleles at one marker locus and M_2 and m_2 the alleles at the other marker locus. Q and q represent the alleles at a QTL. The parents are mated to produce an F_1 which is then backcrossed onto one of the parents. The genotypes of the offspring will be parental (M_1M_2/M_1M_2 and m_1m_2/M_1M_2) and recombinant (M_1m_2/M_1M_2 and m_1M_2/M_1M_2). Linkage is indicated by the relative frequency of the classes bearing recombinant gametes being significantly lower than one-half (no linkage or independent assortment). A chi-square (χ^2) test determines whether segregation at each of the two loci agrees with expectation and tests for linkage (3 df).

Figure 1. An Example of a Backcross Design for QTL Detection.



As soon as phenotypic and genotypic information are collected on a given population, analytical tools will determine the association between given markers and QTL. The strategies used are classified as either single or flanking marker methods, depending on the number of markers used at one time and their proximity to each other. The power of QTL detection was similar for interval mapping using a likelihood ratio test

and single marker analysis using a *t*-test (Darvasi et al., 1993). Interval mapping is essential only in experiments designed to provide an accurate gene location. The advantage of using single marker analysis over interval mapping with likelihood ratio tests is the simplicity of application. Single marker analysis can be readily applied to the detection of several unlinked QTL using standard software packages for multiple regression.

The single marker method calculates the statistical association between one marker at a time and its phenotype. This traditional method for detecting a QTL near a marker involves the comparison of phenotypic means from progeny with different marker genotypes (Soller et al., 1976). This estimates the phenotypic effect of substituting one allele for the other allele at the QTL. To test the significance of this association, an analysis of variance technique (linear regression) is used to estimate the additive variance. Statistically, this is equivalent to a *t*-test between the observed means.

As stated earlier, this process has a major advantage of being extremely easy to perform. However, there are several drawbacks to this method: the placement of QTL are not well-resolved since tight linkage to a QTL with small effect cannot be distinguished from loose linkage to a QTL with large effect; the number of progeny needed is inflated if the QTL does not lie near the marker locus; the suggested false positive rate (α=0.05; Soller et al., 1976) neglects the fact that many markers are being tested; and the phenotypic effects of QTL are systematically underestimated if the QTL does not lie at the marker locus because of recombination fraction (Lander and Botstein,

1989). The false positive rate should be changed in order to account for the much higher chance of at least one false positive occurring somewhere in the genome.

The standard method used by most geneticists for mapping QTL is the flanking marker method of Lander and Botstein (1989), which is better known as interval mapping. A linear model was used to test for a QTL located between two adjacent markers in backcross or F_2 progeny produced from inbred strains. The evidence for a QTL is measured through the use of a maximum likelihood ratio test statistic (LOD score) at any point in the genome. If the LOD score exceeds a predetermined threshold level, a QTL is placed at the maximum of the interval. A LOD score greater than 3.0 is considered significant in the mouse (Lander and Botstein, 1989).

Interval mapping allows for an accurate estimation of phenotypic effects of QTL, decreases the number of false positives, and efficiently detects and places a QTL in a given chromosome region (Zeng, 1994). On the other hand, the disadvantages to using this form of interval mapping all include the idea of there being an unknown number of QTL on the chromosome. The calculated test statistic is affected by all QTL; so if there is more than one QTL, the identified QTL position and effects will be biased (Knott and Haley, 1992). If a QTL is located at some nearby region on the chromosome outside the interval, the LOD score can significantly exceed the threshold value and wrongly place a QTL in an interval, even though there is no QTL present within a defined interval (Zeng, 1994).

Additional flanking marker models for estimating QTL effects and locations were described for doubled haploid, backcross, recombinant inbred, F_2 , F_3 , and various testcross progeny (Knapp et al., 1990). These models are based on the recombination

frequencies between markers and QTL, and on the QTL genotype means. These parameters are estimated by maximum likelihood methods applying linear, nonlinear, univariate, and multivariate normal distribution models. The problems listed above for Lander and Botstein's (1989) interval mapping also apply to these comparable QTL mapping methods. In addition, the use of only two markers at a time during the test is highly inefficient.

To solve some of these problems, Lander and Botstein (1989) expanded their model to analyze multiple markers for multiple QTL simultaneously. However, this extended search becomes multidimensional, producing problems in estimating parameters and locating QTL. To be more efficient, a process of mapping QTL in a one-dimensional search was developed by Zeng (1994) which combines interval mapping with multiple regression analysis and is known as composite interval mapping. This union can be accomplished since the test statistic is independent of the effects of QTL at other areas on the chromosome.

The following are aspects of multiple regression analysis (Zeng, 1993). The partial regression coefficients of the trait on a marker are unaffected by QTL in other intervals, depending only on those QTL that are located in the interval. This is the basis for an interval test. A test statistic is constructed that is independent of QTL effects in other regions of the chromosome. Next, the statistical power of the test and the efficiency of mapping is increased because the sampling variance is reduced. Even though markers are unlinked, they contain valuable information. Thirdly, the chance of interference of possible multiple linked QTL is reduced, thus increasing the precision of the test and

estimation. There are trade-offs between precision and efficiency by using interval mapping. Finally, markers are usually uncorrelated unless they are next to each other; and even then, they have a very small correlation.

Composite interval mapping fully utilizes all mapping information in an interval test. This method provides a systematic search for QTL along the marker covered genome. The advantages of composite interval mapping include a one-dimensional search when one interval at a time is tested even though there are multiple QTL, estimates of locations and effects of individual QTL are unbiased, the precision is improved since interval tests are on linked markers, simultaneous interval tests on multiple markers applies an increased amount of data, and a QTL likelihood map can be used to present a profile of QTL at various positions along the chromosome. A linkage map provides an anchor location to test for a QTL in the genome and at the same time control the genetic variance in the remaining chromosomes for the interval test (Zeng, 1994).

A simple marker-regression approach, which can be used for populations derived from an F_1 backcross, can locate QTL on a chromosome and estimate their additive and dominance effects (Kearsey and Hyne, 1994). This method involves regressing at a single locus the additive difference between genotypic means against a function of the recombination frequency between a putative QTL and that locus. Regression methods determine a QTL to be the point where the residual mean square is minimized. The reliability of this approach compared to MAPMAKER/QTL (Lander and Botstein, 1989) was tested by computer simulation. The results indicated that the marker-regression estimates of location and gene effects are consistent and as reliable as flanking marker

methods. This method has advantages of being easy to understand and simple to program using standard pc-based statistical software. However, a major disadvantage is that the marker-regression approach can only test for one QTL on a chromosome even if there is more than one linked QTL.

Mapping of Quantitative Trait Loci

Until recently, the genes underlying growth, body composition, litter size, and other economically important traits in livestock have been difficult to identify. As well as the discovery of the actual magnitude of the individual gene effects on a trait of interest. The current mouse, rat, livestock, and human genetic maps are highly saturated with genetic markers. These markers are beneficial only when they are closely linked to genes controlling quantitative traits. Locating molecular markers that are linked to QTL is needed in order to increase genetic improvement in livestock through marker assisted selection. QTL studies are also making progress toward understanding the gentic basis of human diseases, such as hypertension and atherosclerosis. Methods used to identify such genetic markers are the candidate gene approach and the marker locus approach.

In the candidate locus approach, genotypic values are estimated for specific loci chosen because they are part of physiological pathways known to affect the phenotype of interest. Polymorphisms within these genes are analyzed to determine if the candidate genes are involved in regulation of the trait of interest, or if they are closely linked to a QTL. For the marker locus approach (mainly interval mapping), the measured loci are not

expected to be the actual loci affecting a trait. Genetic markers evenly distributed throughout the genome are selected and tested for the presence of a QTL between pairs of flanking markers.

QTL analysis of polygenic traits in animal models pinpoints candidate chromosomal segments or loci that can be tested in livestock and humans. For example, the identification and characterization of the genes controlling obesity in these animal models can then provide clues regarding the genes and the metabolic or developmental pathways that contribute to human obesity or increased fat percentage in livestock. Locating genes involved in obesity will have considerable clinical significance for humans. Also, the overproduction of fat in livestock has serious consequences for the animal industry. Ultimately, there is the possibility of isolating these genes using positional cloning. Several studies will be reviewed where QTL affecting polygenic traits were located: human diseases (atherosclerosis and hypertension), growth rate, body composition, and milk production.

Atherosclerosis, the primary cause of coronary artery disease in humans, is the deposition of fatty substances in the inner walls of the arteries. Chromosomal loci for coronary artery disease risk factors have been identified in both rat and mouse models: diabetes (Todd and Bain, 1992), hypertension (Jacob et al., 1991; Hilbert et al., 1991), lipoprotein levels (Warden et al., 1993), and obesity (Warden et al., 1993; West et al., 1994a; West et al., 1994b). Several experiments that used a mouse model crossed the following inbred strains: AKR/J, SWR/J, C57BL/6J, A/J, and *Mus spretus*. Research identified strains AKR/J and C57BL/6J as having a significantly increased carcass lipid

content, while SWR/J had no or marginal effect on adiposity when fed a condensed milk diet (West et al., 1994b). Also, strains AKR/J and C57BL/6J are more susceptible to the formation of lesions in the aorta on an atherogenic diet (Paigen et al., 1985). The stroke-prone spontaneously hypertensive rat (SHRSP), which exhibits blood pressures much higher than the control line Wistar-Kyoto (WKY), was identified by Yamori (1982). Investigations of genetically hypertensive or obese inbred rodent strains can help determine the causes of many problems in human studies.

A C57BL/6J x *Mus spretus* backcross located QTL for plasma total cholesterol and for the percentage of carcass lipid on chromosome 7 and plasma total cholesterol and a subcutaneous fat pad, the femoral depot, on chromosome 6. The LOD scores for plasma cholesterol were 5.8 and 5.6, respectively (Warden et al., 1993). The position of chromosome 6 and 7 QTL are close to known mutations that cause extreme obesity and diabetes. Chromosome 6 contains the *ob* (obese) gene while chromosome 7 has the *tub* (tubby) and *Ad* (adult obesity and diabetes) genes (Mouse Genome Database; The Jackson Laboratory, 1995).

Single-gene obesity loci are known for the *a* (agouti) locus on chromosome 2 (Bultman et al., 1992), the *tub* (tubby) locus on chromosome 7 (Jones et al., 1992), the *db* (diabetes) locus on chromosome 4 (Bahary et al., 1990), *Ifabp* (intestinal fatty acid binding protein) locus on chromosome 3 (Sweetser et al., 1987), *Ad* (adipose) locus on chromosome 7 (Wallace and MacSwiney, 1979), and the *ob* (obese) locus on chromosome 6 (Friedman et al., 1991). These are just a few examples; a mouse 'fat map' shows the chromosomal locations of genes involved in lipid metabolism and associated mutations

(Lusis and Sparkes, 1989). Dietary obesity has been linked to chromosomes 4, 9, and 15 in the following studies. Two separate studies obtained progeny for genetic evaluation from the intercross and backcross of AKR/J and SWR/J strains.

In the first case, significant genetic linkage on chromosome 4 was observed with the *db* locus at a LOD score of 4.5 for total body adiposity (West et al., 1994b). This QTL is designated as *Do1* (dietary obese 1) and has *glut1* (glucose transporter) as a candidate gene (Bahary et al., 1991). Secondly, two QTL (designated as *Do2* and *Do3*) were found on chromosomes 9 and 15 with significant LOD scores for total adiposity of 4.58 and 3.93, respectively (West et al., 1994a). Candidate genes include *Gnai*-2 (guanine nucleotide binding protein, α-inhibiting-2) for *Do2* (Blatt et al., 1988) and *Ghr* (growth hormone receptor) for *Do3* (Barton et al., 1989).

Yet another study identified a gene that determines atherosclerosis susceptibility and high density lipoprotein (HDL) levels in the mouse. Strains C57BL/6J and A/J were previously characterized as differing at a gene (denoted as *Ath-1*) controlling atherosclerosis susceptibility (Paigen et al., 1987). This locus on chromosome 1 affects plasma HDL cholesterol levels and atherosclerotic lesion formation in mice fed an atherogenic diet. Two years later, a second gene (designated as *Ath-2*) was also found to determine levels of HDL cholesterol on an atherogenic diet (Paigen et al., 1989). This gene was located from a backcross that was segregating resistant and susceptible phenotypes.

Other important quantitative traits are growth rate and litter size. The Quakenbush-Swiss mouse strain was backcrossed with the C57BL/6J line to identify

genetic markers linked to growth and litter size QTL (Collins et al., 1993). The Quakenbush-Swiss line has been selected over the past 55 generations for increased litter size; the strain also has an increased body weight. The C57BL/6J line on a high fat diet exhibits small litter size, low body weight, and an increased carcass lipid content as mentioned earlier. Microsatellite markers were used based on their closeness to growth hormone (*Gh)* and insulin-like growth factor-1 (*Igf-1*). *Gh* and *Igf-1* were mapped to chromosome 11 and 10, respectively (Elliott et al., 1990; Taylor and Grieco, 1991). Two markers (D10MIT12 and D10MIT14) were identified as having an association with growth QTL (Collins et al., 1993). A high growth gene (*hg*), causing a 30-50% increase in 3-6 week gain and mature body size was mapped near *Igf-1* on chromosome 10 (Medrano et al., 1991).

A study by Horvat and Medrano (1995) was designed to characterize the effects of the above mentioned *hg* (high growth) locus on chromosome 10 from a cross between C57BL/6J-*hghg* and CAST/EiJ mice. The *hg* locus had significant LOD score of 24.81 (females; 41.5% of variance) and 9.56 (males; 22.2% of variance) for 14 to 63-day weight gain. Interval mapping using MAPMAKER/QTL 1.1 software (Lincoln et al., 1992) placed the *hg* locus at marker D10Mit41. Comparative mapping shows the distal half of chromsome 10 belonging to a block of homologous genes (including *Dcn*) on human chromosome 12q13-q24 (O'Brien et al., 1993). The decorin gene is involved in human cell proliferation and extracellular matrix assembly (Ruoslahti and Yamaguchi, 1991). Therefore, the mouse *Dcn* gene was postulated as mapping somewhere on murine chromosome 10. Just as hypothesized, the *Dcn* gene mapped distally 3 cM from

D10Mit41 (Horvat and Medrano, 1995). This study will support further fine mapping and cloning of the *hg* locus.

Studies have identified that a small number (2-4) of genes are responsible for the blood pressure variations observed between rat strains (Ely et al., 1990; Lindpaintner et al., 1990; Pravnec et al., 1991). An intercross between rat inbred strains SHRSP x WKY was made to obtain progeny for a linkage study to localize these genes. A locus on chromosome 10 accounted for more than 12-17% and 21-22% of the differences seen in basal blood pressure and blood pressure after NaCl-loading, respectively (Hilbert et al., 1991). This locus was named *BP/SP-1* and is believed to have effects on renal function and volume homeostasis. The candidate gene *ACE* (angiotensin-converting enzyme) on chromosome 10 (Lindpaintner et al., 1992) produces angiotensin II which has been identified as controlling sodium excretion in the kidney (Hall, 1986). Since this was an intercross of F_1 individuals, an analysis of the sex chromosomes was performed. A second locus, *BP/SP-2,* was found on chromosome X which accounted for most of the variability of basal systolic blood pressure in females (Hilbert et al., 1991). No candidate loci were identified at that time.

Another study used the same inbred strains as above in an intercross. The results obtained were very similar to those concluded by Hilbert et al. (1991). A QTL (*Bp1*-blood pressure 1) on chromosome 10, which accounted for 20% of the variation observed, yielded LOD scores of 4.88 and 5.1 for systolic and diastolic blood pressure after sodium loading, respectively (Jacob et al., 1991). A second Qtl (*Bp2*-blood pressure 2) on chromosome 18 had a LOD score of 3.23 with diastolic blood pressure, explaining 13% of

the variation observed. The *ACE* gene is also a candidate for the *Bp1* locus causing hypertension. The angiotensin-converting enzyme plays a major role in blood pressure homeostasis (Dzau, 1988). Comparative gene mapping has revealed considerable conservation of gene order on chromosome 10 (rat), 11 (mouse), and 17 (human) during mammalian evolution (Levan et al., 1991).

These animal models demonstrate the power of marker linkage studies in the indentification of loci involved in the aetiology of obesity and artherosclerosis. The direct mapping of QTL may be difficult in humans since there is likely to be different QTL segregating in different populations or nuclear families. This heterogeneity makes the identification of QTL segregations extremely difficult (Lander and Botstein, 1986). Knowledge gained may ultimately lead to a better understanding of hypertension and obesity in humans, as well as livestock species. This will allow for improved treatment and prevention methods in the future.

The detection of QTL in most studies has been between inbred lines of mice or rats. This is not a very realistic option for most livestock species. However, an analytical method based on least squares procedures has been described for identifying QTL segregating in crosses between divergent outbred lines (Haley et al., 1994). Weller et al. (1990) constructed a likelihood function which estimates parameters related to a linked QTL for daughter designs. Also, the recent progress in livestock genome mapping has allowed for detailed linkage maps based on DNA markers, improving the ability to dissect genetically quantitative traits. The following are some of the experiments being conducted in livestock species.

In an experiment conducted by Andersson et al. (1994), the European wild boar was intercrossed with Large White sows to genetically dissect phenotypic differences between these populations for growth and fat deposition. Evidence for QTL with large effects on growth, length of the small intestine, and fat deposition on chromosome 4 were found. The length of the small intestine indicates the amount of domestication and is positively correlated with growth (Petersson et al., 1979). The QTL had an estimated additive effect of 24 g per day for early growth rate and 5 mm for average backfat depth, explaining 12% and 18% of the residual phenotypic variation, respectively. There were also indications of a QTL with an effect of 14 g per day for early growth (8% of the total variation) on chromosome 13.

A study in cattle looked at the association between weaver disease and milk production QTL. Weaver syndrome is characterized by pelvic limb paresis, ataxia, atrophy of hip and stifle muscles, central nervous system lesions, and atrophied gonads. Brown Swiss weaver carriers produce 26.2 kg more fat and 690 kg more milk on average annually than normal cattle (Hoeschele and Meinert, 1990). A microsatellite locus (TGLA116) was found to be closely linked to the weaver gene on synteny group 13 (Georges et al., 1993). This marker can be used to identify weaver carriers and research the effect this chromosome has on milk production in other breeds of cattle.

Finally, another experiment in dairy cattle utilized progeny testing in combination with interval mapping to map QTL controlling milk production in a Holstein population. A founder sire was determined to be heterozygous for 159 markers, resulting in 104,523 genotypes from informative families (Georges et al., 1995). Evidence for QTL expressing

LOD scores ≥ 3 was seen on chromosomes 1 (U10), 6 (U15), 9 (U2), 10 (U5), and 20(U20). The two QTL mapping to chromosome 6 and 20 caused an increase in milk volume without significant increase in protein and fat yield. The QTL on chromosome 9 increased milk volume without altering fat and protein content. Chromosome 1 and 10 QTL caused an increase in milk production with a differential effect on fat and protein composition. An interval on chromosome 20, bounded by AGLA29/TGLA214 on one side and TGLA126 on the other, is a possible location of the QTL. The QTL effects explained 11 to 52% of the total variance of daughter yield deviations within a half-sib family. Finding QTL segregating for milk production in elite dairy cattle populations will greatly encourage the use of marker assisted selection in the future.

A summary of the QTL researched above in animal models and livestock species is presented in Table 1. A majority of the QTL identified so far have been in the mouse with very few in livestock. However as linkage maps become saturated with markers, more studies will locate QTL in livestock.

In addition, the mouse is a beneficial model for behavioral and drug/alcohol abuse studies which can provide insight into the nature of the genetic influences in human populations. The following studies used the mouse to locate QTL relating to substance (morphine) and alcohol (ethanol) abuse. An F_2 intercross of strains C57BL/6J and DBA/2J was genotyped for extreme values (highest and lowest 7.7%) of morphine consumption at 157 microsatellite polymorphisms (Berrettini et al., 1994). The C57BL/6J inbreds prefer orally-available alcohol (McClearn and Rogers, 1959), opiates (Belknap, 1990), and cocaine (Alexander et al., 1993) to a greater extent than DBA/2J inbreds.

Three loci influencing oral morphine preference were detected on chromosome 10 (LOD score = 20), 6 (LOD score = 15), and 1 (LOD score = 3). The effect of the chromosome 10 and 6 QTL is rather large, as a C57BL/6J allele increases mean F_2 morphine consumption by 60%. The allele for chromosome 1 decreased consumption by a small but detectable amount. Only one candidate loci on chromosome 6 (quinine; denoted as *Qui*) is evident as explaining the LOD score related to morphine consumption. Mapping these loci may reveal important clues to human genetic vulnerability to drug addiction.

A second study used mice from two progenitor inbred strains (C57BL/6J and DBA/2J) and 19 recombinant inbred (RI) BXD strains to identify QTL associated with sensitivity and tolerance to the hypothermic effect of ethanol (Crabbe et al., 1994b). Several doses of ethanol (2, 3, and 4 g/kg) were administered, recording multiple postdrug temperatures. Six traits were analyzed: hypothermic sensitivity (HT2, HT3, and HT4) and tolerance (TOL2, TOL3, and TOL4) to ethanol administration. Results suggest that QTL for all traits analyzed are located on all chromosomes except 6, 10, 12, 15, 18, and X. Candidate loci include *Ahd-1* (aldehyde dehydrogenase) and *Akp-2* (alkaline phosphatase-2 synthesis) on chromosome 4 for HT2 and HT3; *D2dr* (dopamine D_2 receptor) on chromosome 9 for TOL2; *D5dr* (dopamine D_5 receptor) on chromosome 5 for HT3; *D3dr* (dopamine D_3 receptor) on chromosome 16 for HT4 and TOL4; *As-1* (aryl sulfatase) on chromosome 13 and *Qui* (quinine taste sensitivity) on chromosome 6 for HT2; and *Gnat-1* (guanine nucleotide binding protein) on chromosome 9 for HT4. Several chromosomal regions appear to contain loci that contribute measurably to multiple traits related to ethanol-induced hypothermia and tolerance.

Table. 1 Mapped QTL for Growth, Obesity, Hypertension, and Milk Production in Animal Models and Livestock Species.

QTL (Reference)	CHR[1]	LOD[2]	Candidate Genes	Animal
Plasma Cholesterol & Carcass Lipid % (Warden et al., 1993)	7	5.8	*ob* (obese) gene	Mice
Subcutaneous Fat Pad & Plasma Cholesterol (Warden et al., 1993)	6	5.6	*tub* (tubby) gene *Ad* (adult obesity & diabetes)	Mice
Do1 (dietary obese 1); Total Adibosity (Bahary et al., 1991)	4	4.5	*db* (diabetes) *glut1* (glucose transporter)	Mice
Do2 (dietary obese 2); Total Adiposity (West et al., 1994a)	9	4.58	*Gnai-2*(guanine nucleotide binding prot. α-inhibiting-2)	Mice
Do3 (dietary obese 3); Total Adiposity (West et al., 1994a)	15	3.93	*Ghr* (growth hor. receptor)	Mice
Ath-1; Level of HDL Cholesterol and Lesion Formation (Paigen et al., 1987)	1	-	*Apo A*-II	Mice
Marker D10Mit12 & Dd10Mit 14; Body Weight (Collins et al., 1993)	10	-	*Igf-1* (Insulin like growth factor-1) and *hg* (high growth)	Mice
Marker D10Mit41; Weight Gain and Mature Size (Horvat & Medrano, 1995)	10	24.8 & 9.6	*hg* (high growth)	Mice
BP/SP-1 & *BP/SP-2*; Systolic Blood Pressure (Hilbert et al., 1991)	10 X	-	*ACE* (angiotensin-converting enzyme)	Rat
Bp1 (Blood Pressure 1); Systolic & Diastolic BP (Jacob et al., 1991)	10	4.88 & 5.1	ACE	Rat
Bp2 (Blood Pressure 2); Diastolic BP (Jacob et al., 1991)	18	3.23	-	Rat
Early Growth Rate and Backfat Depth (Andersson et al., 1994)	4	-	-	Swine
Marker TGLA116; Milk Production (Georges et al., 1993)	13	-	Linked to Weaver Gene	Dairy Cattle
AGLA29/TGLA214 and TGLA126; Milk Production (Georges et al., 1994)	20	-	Milk Production Gene	Dairy Cattle

[1] Chromosome Number
[2] LOD Score

Marker Assisted Selection

Currently, selection is based on a prediciton of genetic differences between animals, estimated from phenotypic observations. The phenotypes are composed of environmental and genetic components (Meuwissen and Van Arendonk, 1992). Phenotypic information is often a mixture of data from the individual and its ancestors,

siblings, and progeny. With a small family size, the efficiency of marker assisted selection is reduced when phenotypic data is used from relatives. However, large families and common family environmental effects, such as maternal effects on full sib families, will increase the productiveness of selection through the use of molecuar markers (Lande and Thompson, 1990).

These phenotypic measurements provide individual breeding values or EPDs which assist the producer in making the proper matings/cullings to increase production profits. Therefore the phenotypic values are indirectly used to evaluate the animal's genetic makeup. However, if a producer could determine the most beneficial genotype directly, the accuracy in which selection occurs would increase. The likelihood of selecting parents with the best genotype for a given trait will be enhanced. The generation interval could be decreased drastically since some traits would have genotypic information available before phenotypic datum was even collected. Also, benefits of maker assisted selection are based on the producer's ability to select animals not expressing a given trait (Lande and Thompson, 1990).

Problems with using EPD in current selection programs are the following: the reliability of EPD is dependent on the individual and its relatives; information, and measures of genetic merit are indirectly determined from phenotypic information. For the first case, highly reliable estimates require a large number of progeny records from several different environments. Also, many years pass between birth of the animal and the collection of genetic information. For the second issue, traits must be easily measurable and environmental influences can bias genetic estimates.

Once QTL are identified in the mouse, comparative mapping will expedite research in livestock species to find associated genes. The mapping of economically important genes will bring about livestock improvement through the use of marker assisted selection. Marker assisted selection is the process of making selection decisions, based on an animal's phenotypic and genotypic information. Identifiable genetic differences observed at the DNA level are not necessarily the QTL themselves, but are markers linked to these QTL (Soller, 1978). The identification of marker loci linked to QTL will allow for the easy manipulation of major genes, providing a possible route to their ultimate isolation (Haley, 1991). The potential efficiency of marker assisted selection depends on the trait's heritability, the selection method, and the additive genetic variance of the marker loci (Lande and Thompson, 1990). For individual selection, this relative efficiency is greatest for characters with low heritability and when a moderate to large fraction of the additive genetic variance is significantly associated with the marker loci. Further increases in efficiency are possible when selection is practiced on sex-limited traits and young animals before they develop the adult phenotype.

Problems will arise with marker assisted selection from different environments, genetic backgrounds, and selection goals (Pomp, 1994). The environment will affect detection of genes (good and bad) and the amount of genetic advantage expressed in the phenotype. Marker assisted selection, as well as traditional methods, assume selection is practiced in the same type of environment in which animals will be raised. Significant marker effects may only be beneficial in the same conditions in which they were discovered. The breeds used in a marker assisted selection program will also be of

importance. Some livestock breeds or lines of mice may not even be affected by the marker for a given trait. Research will need to be done to evaluate marker effects in various environments and genetic backgrounds.

Several examples of genetic markers have already been associated with QTL in livestock production: the halothane gene in swine (Houde et al., 1993), kappa-casein and beta-lactoglobulin genes in cattle (Medrano and Aguilar-Cordova; 1990a,b), the Booroola fecundity gene in sheep (Montgomery et al., 1993), the callipyge gene in sheep (Muggli-Cockett et al., 1993), and the estrogen receptor gene in swine (Rothschild et al., 1994). The porcine stress syndrome and pale soft exudative pork are more readily seen in pigs with malignant hypothermia than normal animals. These losses to the swine industry have been gentically related to halothane anesthesia (Webb et al., 1982). Houde et al. (1993) identified a mutation in the ryanodine receptor gene as being the cause of malignant hypothermia. This disease is under control in swine breeding companies due to the incorporation of marker assisted selection with the halothane gene.

Two milk protein genes in dairy cattle, kappa-casein and beta-lactoglobulin, significantly influence the composition and physical-chemical properties of milk (Schaar et al., 1985). Also, these protein genetic variants are associated with the quality and quantity of cheese produced from milk. Kappa-casein (Medrano and Aguilar-Cordova, 1990a) and beta-lactoglobulin (Medrano and Aguilar-Cordova, 1990b) polymorphisms result in two possible forms of the protein produced, depending on the animal's alleles. Allelic variants of these genes can be utilized in Holstein cattle sire families to identify chromosome substitution effects for yield traits (Cowan et al., 1992). Maximum likelihood

methodology was used to estimate effects of a marker gene and a linked QTL on quantitative traits in a Dutch dairy cattle population (Bovenhuis et al., 1992). Beta-lactoglobulin had significant effects on fat percent, protein yield, and milk yield; while kappa-casein effected milk yield, protein percentage, and fat yield. These results are consistent with previous conclusions on the same data using a different analysis (Bovenhuis et al., 1992).

The Booroola fecundity gene (Fec^B) results in a marked increase in ovulation and lambing rate in Booroola Merino ewes (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 $\geq$ 5, 3 or 4, and 1 or 2, respectively (Davis et al., 1982). Montgomery et al. (1993) identified linkage between two microsatellite markers (OarAE101 and OarHH55) and the Fec^B gene mutation. These markers could be used in a marker assisted selection program to increase the prolificacy of different breeds of sheep.

A mutation causing muscular hypertrophy was identified as affecting the production efficiency and quality of meat in sheep (Muggli-Cockett et al., 1993). A variable number of tandem repeat marker (LOD score of 8.78) characterized by five codominant alleles was found as being linked to the gene causing this muscle hypertrophy. Results indicated that a single autosomal gene (callipyge; CLPG) was resposible for the muscular hypertrophy condition (Cockett et al., 1994). The marker map of sheep chromosome 18 was used to position the callipyge locus in the interval between CSSM18 and TGLA122. A maximum LOD score of 26.2 was obtained at 3 and 17.5 cM from

these markers, respectively. This potentially advantageous gene lends itself to successful transfer into other meat producing species.

A major gene for litter size, associated with a polymorphism in the estrogen receptor gene, was identified which has a significant effect on total number born and number born alive in swine (Rothschild et al., 1994). Some Chinese breeds (Meishan, Fengjing, and Minzhu) of pigs are extremely prolific. The Meishan pigs were imported and crossed with herds in the United States. The estrogen receptor gene (a steroid binding hormone receptor gene) has one allele, originating from the Meishan, that is significantly associated with higher litter size. Differences among genotypes accounted for an increase of approximately 1.5 pigs per litter born and over 1 pig born alive. Results also suggest that there is no negative pleiotropic effects on growth rate and backfat. This gene may be incorporated into marker assisted selection programs to increase the efficiency of pork production.

The potential improvements in rate of genetic gain from the use of marker assisted selection programs has been predicted in a number of studies. Meuwissen and Van Arendonk (1992) utilized associations between markers and milk production records of daughters of a grandsire by a multiple regression model, determining the value of marker assisted selection. Marker effects were assumed to be due to each QTL having a small effect, forming groups of QTL. Under this assumption, gene frequency changes will be small for each QTL, forcing a slight decrease in genetic variance. For QTL with large effects, marker assisted selection is beneficial in the short term (Saefuddin and Gibson, 1991) while conventional selection is superior in the long term.

Within-family variances of grandoffspring explained by markers amounted to as much as 13.3% (Meuwissen and Van Arendonk, 1992). This variance decreased as the flanking marker distances increased and as the number of daughters analyzed decreased. In progeny testing schemes (the number of young bulls born annually is restricted), the within-family variance was mainly explained by individual or progeny performance data. There was a small increase in genetic gain since marker assisted selection cannot contribute much to the accuracy of selection. However in open and closed nucleus schemes, the genetic gains from marker assisted selection were enhanced substantially by 9.5 to 25.8% and 7.7 to 22.4%, respectively. This was due to increases in accuracy of selection and selection differentials.

Gimelfarb and Lande (1994) studied the amount of selection response seen based on an index which combined both the phenotypic and genotypic data. This computer simulation utilized multiple regression to determine which markers should be included in the index and their relative weight. They showed that selection based on genetic markers is an effective method of selection, especially when the index markers are re-evaluated each generation. Marker assisted selection effectively utilizes the linkage disequilibrium between genetic markers and QTL.

Computer simulations by Zhang and Smith (1992 and 1993) investigated the effects of several factors on selection resoponse in marker assisted selection using linkage disequilibrium (number of marker loci necessary for the existence of significant associations with the QTL). For both studies, selection was on estimates of breeding values based on the marker QTL associations, on the best linear unbiased prediction

(BLUP) of phenotype, or on both. BLUP resulted in greater genetic gains when compared to marker assisted selection (Zhang and Smith, 1992). The detection of markers closer to a QTL would increase the linkage disequilibrium, thereby making marker assisted selection equivalent to selection on the QTL themselves.

Zhang and Smith (1993) concluded that mixed-model estimates produce much larger responses than least-squares procedures since the first method treats QTL effects as random, regressing them back towards zero. The effectiveness of marker assisted selection was greatly increased when the sample size to estimate the marker quantitative trait locus was 1000 instead of 100 individuals. The same results were concluded with the reduction in selection population size. Poorly estimated marker QTL effects added noise to the system and reduced selection response with the use of smaller numbers. A decrease in the value of marker assisted selection in selecting for overall economic merit is caused by some marker QTL effects being favorable for some traits and unfavorable for others. The results by Zhang and Smith (1992, 1993) present limitations in marker assisted selection using linkage disequilibrium until close linkages of QTL and markers are discovered.

Marker assisted selection should accelerate genetic gain by increasing selection accuracy, reducing generation interval, and increasing selection differentials. Currently, advantageous single gene mutations or genetic marker mutations near a gene with major effects are being utilized in various selection programs. More extensive research of markers will have to be conducted before integrating them into a selection program. The surge in discovering new markers, the progress of mice and livestock genome mapping

committees, and specified application goals should incease the plausibility of using marker assisted selection in the future.

CHAPTER III

MATERIALS AND METHODS

Population Design

Stock Population: The mice lines are maintained by Dr. E.J. Eisen at the Mouse Genetics Laboratory at North Carolina State University, Raleigh. The foundation stock (M16), selected on increased postweaning gain (from 3 to 6 weeks), came from a random outbred Institute for Cancer Research (ICR) albino population (Hanrahan et al., 1973). Within full-sib family selection was practiced, standardizing litters at five days of age to four pups of each sex. Pair matings (between eight and ten weeks) were assigned at random, except for avoiding full-sib matings. Throughout 14 generations of selection, M16 compared with control mice had a much faster growth rate, were later maturing, and exhibited moderate obesity (Eisen, 1975). Also, the M16 line exceeds controls in size and number of fat cells in the epididymal fat pad.

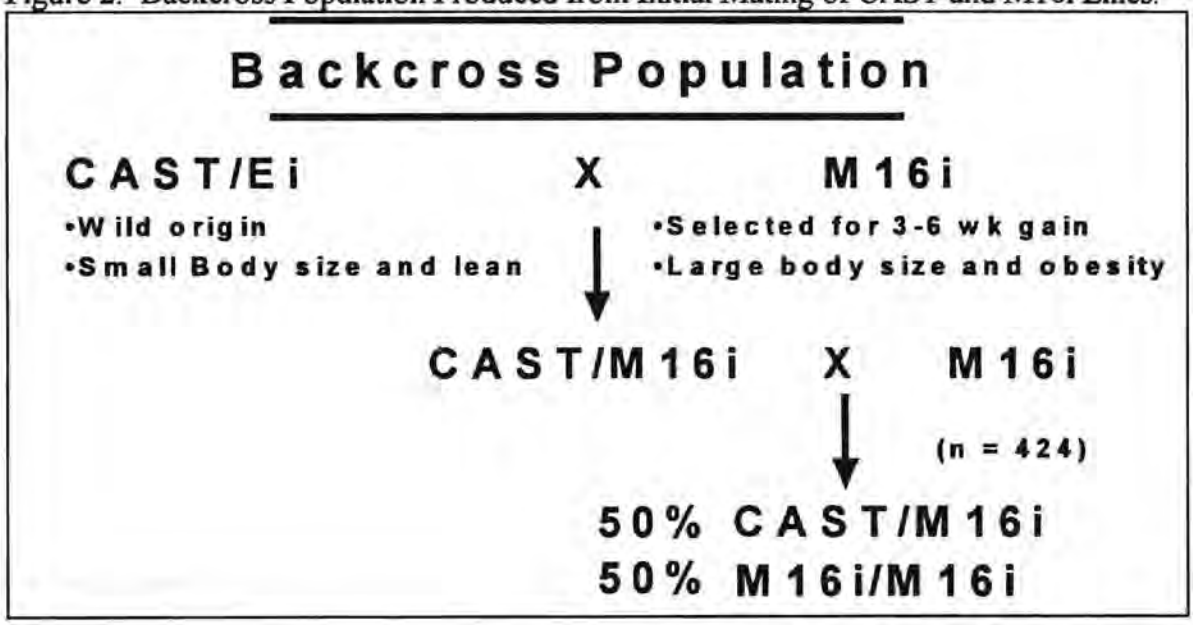
Following long-term selection, inbreeding began in the M16 line to produce a line (M16i) with isogenic mice. Continued full-sib matings were made over the next 15 generations. The M16i line has an inbreeding coefficient (F) of at least 0.95. A mouse strain can only be designated as inbred once F=0.986 which is approximately 20

generations of full-sib mating (Festing, 1979). However, the M16i mice have some degree of inbreeding from the selection on increased postweaning gain.

A second inbred line of wild origin, *Mus musculus castaneus* (CAST/Ei), was purchased from The Jackson Laboratory in Bar Harbor, Maine. For approximately 35 generations, inbreeding of CAST/Ei (CAST) was practiced by full-sib matings (Festing, 1994). CAST individuals display smaller body sizes and lean carcasses as compared to the M16i line.

Mapping Population: Figure 2 illustrates the population design utilized in this research project. A large segregating backcross population was produced. The M16i line females were crossed with males from CAST to produce an F_1. Seven F_1 males were backcrossed to M16i females, producing 54 backcross litters with unique dams. The backcross population contained 421 mice (213 males and 208 females) which had reached an adult age of 12 weeks.

Figure 2. Backcross Population Produced from Initial Mating of CAST and M16i Lines.



Husbandry and Phenotyping

The mice were reared in the N.C. State lab at a temperature of 21°C, 55% relative humidity, and a light:dark cycle of 12 hours each starting at 0700. Pups were weaned at day 21, placing by sex 2-4 pups per cage. Their diet from mating to weaning consisted of ad libitum access to Purina Mouse Chow 5015 (Purina Mills; Richmond, IN) and thereafter Purina Laboratory Chow 5001.

The measured growth traits in the backcross included body weights at day 12 and at 3, 6, 9, and 12 weeks. Growth rates were classified as GAIN1 (day 12 to 3 weeks), GAIN2 (3 to 6 weeks), GAIN3 (6 to 9 weeks), and GAIN4 (9 to 12 weeks). Mice were killed at 12 weeks by cervical dislocation to obtain weights of the right gonadal and hindlimb subcutaneous fat pads, heart, liver, spleen, right kidney, and right testis. All organ and fat pad weights were considered as a percentage of 12 week body weight (WK12). These weight percentages were analyzed instead of the raw organ and fat pad weights. Two traits were selected as the primary candidates for QTL detection: WK12 and gonadal fat pad weight as a percentage of WK12 (GOFP).

WK12 was an adult body weight. The M16 line is larger than controls at weekly ages from birth to 16 weeks: 27% and 60% larger at 3 and 16 weeks of age, respectively (Eisen and Leatherwood, 1978). Therefore, the greatest genetic effects on overall body weight are observed at the later stages of growth. GOFP was chosen as an indicator of overall body fat since the M16 line is known to exceed control mice in size and number of fat cells in the epidiymal fat pad (Eisen and Leatherwood, 1978). Eisen and Coffey (1990)

discovered that the greatest percentage of fat distribution was for gonadal fat in high-fat versus low-fat selection lines when compared to other fat depots. Also, GOFP is an easily dissected discrete fat pad that is highly correlated (r=0.84) with total body fat percentage (Eisen and Leatherwood, 1981). Eisen (1987) found a realized heritability of 0.66 for epididymal fat pad weight/body weight.

Genotyping

DNA extraction: Tail clips were collected at 6 weeks and shipped to Oklahoma State University (OSU) for DNA extraction. Spleens were collected at 12 weeks for a backup tissue source. The method used consisted of an initial overnight digestion with a lysing solution followed by extraction with phenol and chloroform and precipitation with isopropanol (Appendix 1). The concentration of all DNA samples were determined using a spectrophotometer (Appendix 2). Working solutions were then produced at a final concentration of 50 ng/μl for PCR amplification (Appendix 2).

Candidate chromosomes: Initially, Pomp et al., (1994) conducted a whole genome screening procedure with three evenly-spaced SSLP for each of the 19 murine autosomes -- this backcross did not allow for analysis of sex chromosomes. Also, the backcross design only enables the estimation of markers linked to QTL with additive effects. Based on the results obtained by Pomp et al. (1994), chromosomes 2 and 15 appeared to be the most promising for locating QTL.

Chromosome 2 and 15 were selected by comparing all 19 autosomes for the percentage of residual phenotypic variation explained by significant marker effects on both WK12 and GOFP. A major effect on WK12 was identified for chromosome 2 (18.73% variation explained) with the next largest being chromosome 15 (4.41% variation explained). Evidence for a region harboring a QTL, with very large effects, was observed on distal chromosome 2, with differences of at least 3.9 g WK12 (10% of the mean; P<.0001) and 0.31% GOFP (37% of the mean; P<.0001) between M16i/M16i and M16i/CAST backcross mice. In addition to chromosome 2, a QTL appeared on chromosome 15 with deviations between the homozygous and heterozygous genotypes of 1.9 g WK12 (5% of the mean; P<.0001) and 0.13% GOFP (16% of the mean; P=.0017).

To learn more precisely the chromosomal location of these major putative QTL, genotypes were determined at 15 and 6 additional SSLP markers on chromosome 2 (Appendix 3) and 15 (Appendix 4), respectively, as well as for 3 candidate loci (agouti and growth hormone releasing hormone, chromosome 2; and peroxisome proliferator activated receptor, chromosome 15).

Marker detection: A well-saturated microsatellite marker map (Dietrich et al., 1992; Copeland et al., 1993) was used to identify and evaluate possible markers for chromosomes 2 and 15 saturation. The primers (MapPairs; purchased through Research Genetics, Inc., Huntsville, AL) were tested on M16i and CAST DNA for readily identifiable polymorphisms in 4% agarose gels. The size polymorphism between the two parental lines must be large enough in base pairs (about 15 or greater) to classify each genotype. Marker information is presented in Appendix 5. The markers on chromosome

2 were concentrated on the distal half, while the markers on chromosome 15 were localized to the middle region. Marker spacings of approximately 2 to 15 cM provided a reasonable working map of the enitre chromsomes. There was an average marker spacing of approximately 4 cM in the QTL region on both chromosomes 2 and 15.

PCR amplification (Appendix 6) consisted of 15 µl reactions in a 96-well microtiter plate (Falcon 3911; Sigma Chemical Company, St. Louis, MO) using a MJ Research Thermal Controller (Watertown, MA; Model #PTC-100). These reactions contained 50 mM KCl, 10 mM Tris-HCl pH 9, and 0.1% Triton X (Taq buffer; Promega; Madison, WI), 1.5 mM $MgCl_2$, 200 µM each dNTP (New England BioLab, Beverly, MA), 200 nM of each primer in the MapPairs set, 30 ng genomic DNA, and 0.375 U Taq Polymerase (Promega; Madison, WI). Each reaction was overlayed with 50 µl of mineral oil (Sigma Chemical Company; St. Louis, MO) to avoid well contamination, spilling, and condensation. A 3-minute denaturation at 94°C was followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 2 minutes, and extension at 72°C for 2 minutes. The last cycle included an extension step at 72°C for 7 minutes and a cooling step at 4°C until the plate was removed. All markers used these PCR conditions except D15MIT34 which utilized only 30 cycles.

Products were resolved in 4% sieving agarose gels. The gels were made from 2% NuSieve low melting point agarose (FMC BioProducts, Rockland, ME) and 2% Ultrapure Biotechnology Grade high melting point agarose (BioRad Laboratories, Hercules, CA). Separation was acheived by gel electrophoresis at 80 V for 1 to 2 hours, depending on the base pair differences between alleles. Next, the gels were stained with 5 µg/ml ethidium

bromide (Sigma Chemical Company, St. Louis, MO) for 30 minutes followed by destaining in ddH_2O for 30 minutes. A photograph was taken using a Polaroid MP4 Instant Camera System with a Foto Prep I ultraviolet light source (Fotodyne, Inc., Hartland, WI).

QTL Candidate Genes

Identification: Based on preliminary statistical analyses, potential candidate loci were determined for regions near putative QTL. Initially, regions near markers D2MIT49 on chromosome 2 and D15MIT34 on chromosome 15 were investigated since large effects on WK12 and GOFP were discovered by least-squares procedures (Pomp et al., 1994). The Mouse Genome Database (MGD; The Jackson Laboratory, 1995) was utilized to identify known genes with large effects on body weight and obesity. There were several genetic loci near these markers which were possible candidate genes; however, only four were chosen as markers.

Two loci per chromosome were selected as markers to genotype the population: *a* (non-agouti) and *Ghrh* (growth hormone releasing hormone) on chromsome 2 and *Ghr* (growth hormone receptor) and *PPAR-alpha* (peroxisome proliferator activated receptor) on chromosome 15. *PPAR-alpha* was not initially selected as a candidate gene for the observed QTL. *PPAR-alpha* primers were received from Jeff Gimble at the Oklahoma Medical Research Foundation with hopes of mapping the locus in our large backcross population. *PPAR-alpha* was identified on the distal portion of chromosome 15

eventually choosing this gene as a marker. *PPAR-alpha* will be referred to as a candidate gene even though the locus is 30 cM away from *Ghr* on chromosome 15 and it was not actually an original locus selected as a candidate gene.

The agouti locus was initially discovered as controlling the relative amount and distribution of black and yellow pigment in coat hairs. Various agouti locus alleles are associated with embryonic lethality, obesity, diabetes, and the development of tumors in a wide variety of tissues (Eaton and Green, 1963; Wolff, 1965; Odaka et al., 1992 ; Gasser and Fischgrund, 1973).

Somatic cell growth is partially regulated by hormones originating in the hypothalamus which control the amount of growth hormone (GH) secreted from pituitary somatotrophs: Ghrh (stimulates GH synthesis and secretion) and somatostatin (inhibits GH). The secretion of these hormones is regulated by GH feedback and numerous other factors (Frohman and Jansson, 1986). While GH has some direct effects on cellular growth, many of its actions are mediated by insulin-like growth factor type I (IGF-I; Hall and Sara, 1983).

GH binds with high affinity and specificity to a set of related glycoprotein molecules found in membrane and cytosol fractions (Hughes and Friesen, 1985). The membrane-associated forms of the GH receptor (*Ghr*) are assumed to mediate GH actions at the cellular level. Serum *Ghr* increases the stability of circulating GH. The highest concentration of *Ghr* is found in the liver where GH induces the expression and secretion of IGF-I (Posner et al., 1974).

Peroxisome proliferators are chemicals that cause marked proliferation of peroxisomes in selected tissues (liver, brain, heart, kidney, and testis). In addition, there is an increase in the transcription of genes whose products are required for β-oxidation of long-chain fatty acids (Reddy et al., 1986). Peroxisome proliferator activated receptor (*PPAR-alpha*) is a member of the steroid-thyroid superfamily of ligand activated transcription factors (Issemann and Green , 1990). *PPAR-alpha* is involved in regulating aspects of peroxisomal function and cholesterol metabolism. *Ppar* has been isolated in a series of 3 forms: α, β, and γ (Chen et al., 1993). The gene coding for the α form was utilized as a candidate.

Primer Development: In order to use these four candidate genes as markers in the backcross population, polymorphisms needed to be discovered between the two parental lines. A DNA segment for each loci was amplified and then cut with restriction enzymes to receive varying banding patterns for the controls (CAST, M16i, F_1). Initially, primers were designed using the following guidelines: approximately a 50% G/C content; avoid a G or C at 3' end; avoid complementary areas on a primer to prevent folding; avoid primer complementarity; end in a T; and length of 18-25 bp.

A polymorphism is rarely found in exons of the gene because these sequences having funtional properties; therefore, polymorphisms were detected for in introns. Hence, primers were designed in adjacent exon spacings to amplify an intron. The intron spanned by the exon primers was limited to approximately 2 kb to insure consistent PCR amplification of candidate gene segments. Initial intron sizes for *Ghrh* and *Ghr* were between 200 and 400 bp. After testing about 28 enzymes with 4-6 bp recognition sites,

these introns did not produce a polymorphism between the two parental lines, so new primers were constructed to span introns of approximately 2.4 kb.

Mouse candidate gene nucleotide sequences were identified: *a* (Bultman et al., 1992), *Ghrh* (Frohman et al., 1989), *Ghr* (Smith et al., 1989), and *Ppar* α (Gearing et al., 1994). The mouse *a* gene is approximately 18 kb and is composed of 4 exons (72, 170, 65, and 385 bp) and 3 introns (11.5, 2.5, and 2.8 kb). The *Ghrh* gene is at least 7 kb, representing 5 exons (72, 100, 102, 91, and 139) and 4 introns (3.7, 0.23, 2.4, and one of unknown size). The murine *Ghr* gene has two known molecular weight variants for the encoding exon sequences: 1.2 and 2.2 kb. Human estimates were used for *Ghr* gene intron sizes (Godowski et al., 1989). The *Ghr* gene contains 9 exons (72, 66, 75, 216, 150 141, 93, 69, and 981 bp) and 8 introns (14, 27, 6, 5, 24, 3, 4, and 0.4 kb). The mouse *Ppar*α gene has 8 exons (376, 76, 246, 168, 139, 202, 445, and 248 bp) and 7 introns (0.8, at least 5, 11, 1.7, 1.7, at least 5, and 1.8 kb) with an estimated length of at least 30 kb. For all primers, size ranged from 20 to 25 base pairs (bp). The actual sequences and exons listed for each candidate loci are in Appendix 7. Oligonucleotides for primers were obtained from the OSU Biochemistry Core Facility (Stillwater, OK). PCR working solutions (5 μM) were prepared for each set of primers (Appendix 8).

PCR conditions were optimized for each primer pair at various concentrations of $MgCl_2$ (0.75, 1.5, and 2.25 mM), dNTPs (100 and 200 μM each), and primer (0.1, 0.3, and 0.5 μM). The 25 μl reactions contained 50 mM KCl, 10 mM Tris-HCl pH 9, and 0.1% Triton X (10X buffer; Promega, Madison, WI), 50 ng genomic DNA, and 0.875 U

Taq Polymerase (Promega, Madison, WI). Initial optimizations were at 55°C followed by an increase in temperature until a single PCR product was obtained.

The ideal PCR conditions are given in Table 2 for each candidate gene primer. Because the first *Ghr* and *Ghrh* primers did not amplify a DNA segment large enough to produce a polymorphism, a new primer was developed for each (*2Ghr* and *2Ghrh*) to increase the number of base pairs in the segment amplified.

For all primers, the first cycle consisted of a 2-minute denaturation at 95°C, annealing at the proper temperature (see Table 2) for 1-minute, and a 72°C extension period for 2 minutes. The exception was for *2Ghr* which only had a 30-second denaturation due to the large estimated intron size (>2 kb). This cycle was followed by 29 or 34 cycles (see Table 2) of denaturation at 94°C for 1 minute, annealing at the proper temperature (see Table 2) for 1 minute, and extension at 72°C for 1 minute. The exceptions were the following: *2Ghr* had a 15-second denaturation at 95°C; and *Ghr* and *a* which had a 2-minute extension. The last cycle included an extension step at 72°C for 9 minutes and a cooling step at 4°C until the plate was removed.

Table 2. PCR Conditions Used for Amplification of Candidate Gene DNA Segments.

Candidate Gene (Total Cycle #)	$MgCl_2$ (mM)	dNTP (μM)	Primer (μM)	Temperature[a] (°C)
a (30)	1.5	100	0.3	58
Ghrh (35)	1.5	200	0.3	56
2Ghrh (30)	2.25	200	0.3	55
Ghr (30)	1.5	200	0.3	58
2Ghr (35)	1.65	200	0.4	50
PPAR-alpha (35)	1.5	100	0.5	65

[a] PCR Annealing Temperature

Polymorphisms: Restriction fragment length polymorphisms (RFLP) result from a change in nucleotide sequence of one allele that either eliminates or creates a recognition site for a Type II restriction endonuclease. Approximately 28 separate enzymes were randomly tested, with a majority of these having 4-6 bp recognition sites. Polymorphisms between the two parental lines were visualized in 4 % agarose gels. Once an enzyme was determined to generate a polymorphism, no more were tested for that gene. Initial possibilities were tested again to make sure that the resulting polymorphism was reproducible. Finally, the enzyme digestions were tested to make sure that they worked in 96-well PCR plates. *PPAR-alpha* genotyping was conducted in tubes due to the inability to achieve consistent results in the plates.

Known controls (CAST, M16i, and F_1) were used as DNA samples during all candidate gene PCR amplifications and enzyme digestions, testing for a polymorphism. The *a* genotype was produced from digesting an approximately 2100 bp PCR product (11.125 µl) with Dde I (0.125 µl). The *PPAR-alpha* genotype was also discovered from Dde I digestion of the approximately 2100 bp product. The *Ghrh* genotype was determined from digesting an approximately 1800 bp product with Sau96 I. All digestions were processed at 37°C for at least 3 hours and run on gels at 80 volts for 90 minutes. Even with a larger PCR product amplified for *Ghr*, polymorphisms were not found after testing all 28 enzymes.

The *a* genotypes were characterized by the following banding patterns (Figure 3): 445, 380, 298/298, and 270 (M16i/M16i genotype; MM) and 445, 395, 380, 298/298, and 270 (M16i/CAST genotype; MC). When genotyping the backcross population, the MM

and MC individuals were easy to differentiate because of MC extra band at 395 received from CAST. The *Ghrh* genotypes were characterized by the following banding patterns (Figure 4): 1000 and 425 (M16i/M16i genotype; MM) and 1000, 560, 460, and 425 (M16i/CAST genotype; MC). Once again these were easy to genotype due to a difference of two middle bands (560 and 460) for the MC individual. The *PPAR-alpha* genotypes were characterized by the following banding patterns (Figure 5): 650, 560, 500, and 345 (M16i/M16i genotype; MM) and 900, 650, 560, 500, and 345 (M16i/CAST genotype; MC). Finally, the MM and MC individuals were easy to discern because of the extra band (900) for MC.

The following three figures have a 4 % agarose gel with 4 lanes. The first three lanes are the controls (CAST, M16i, and F1), while the last lane is a standard marker (Boehringer Mannheim VI Marker).

Figure 3. Agouti Gene Restriction Fragment Length Polymorphism from Dde I.

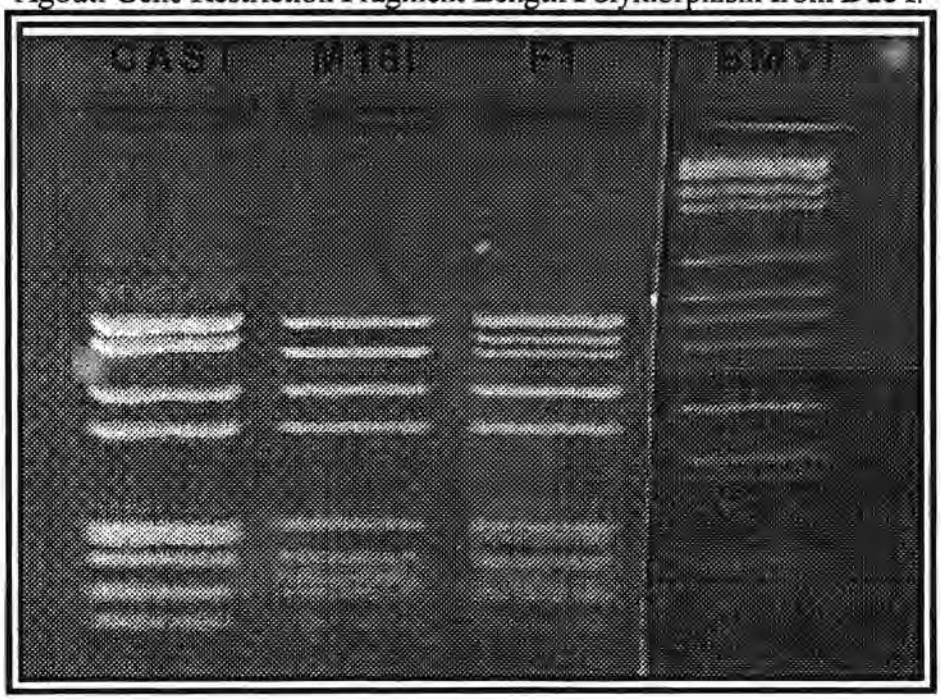


Figure 4. Growth Hormone Releasing Hormone Gene Restriction Fragment Length Polymorphism from Sau96 I.

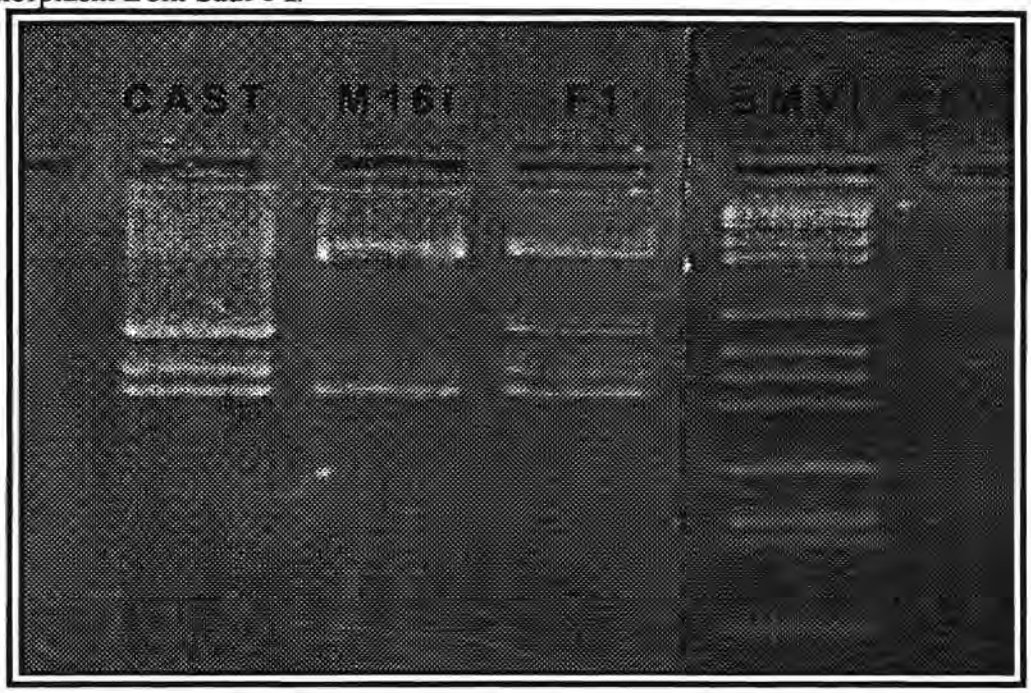
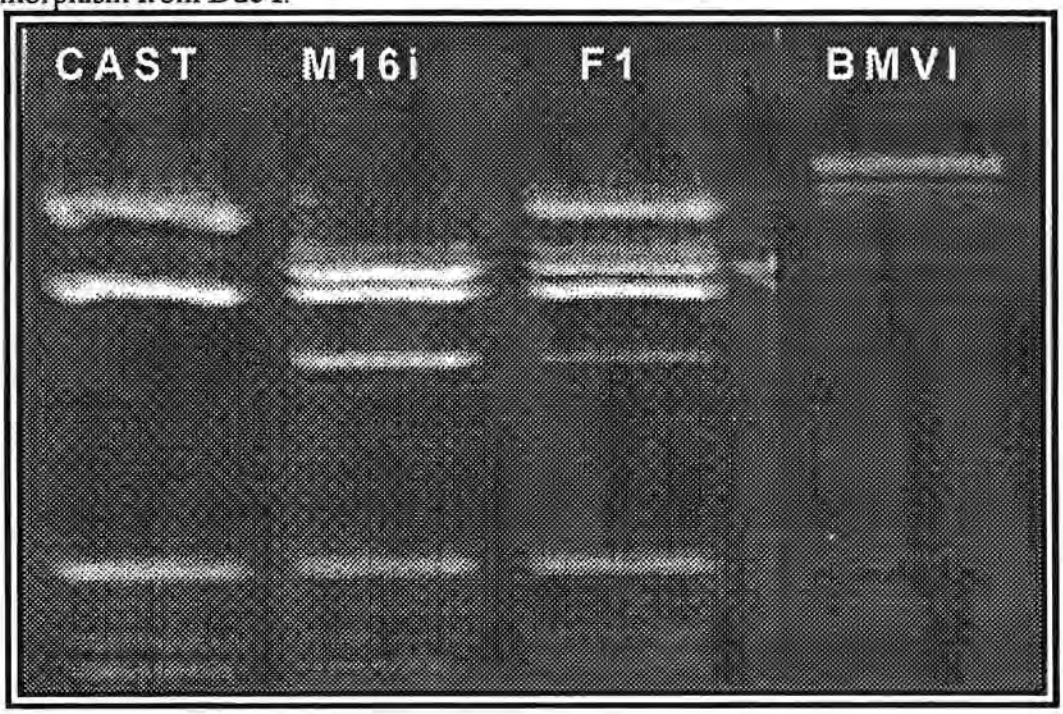


Figure 5. Peroxisome Proliferator Activated Receptor Gene Restriction Fragment Length Polymorphism from Dde I.



Statistical Methods

Descriptive statistics: The phenotypic data for all traits were analyzed for means, standard deviations, coefficients of variation, and ranges. The PROC UNIVARIATE (SAS; 1989) produced these phenotypic specifications. Also, a Shapiro-Wilk statistic was computed in order to test if the backcross population data represented a random sample from a normal distribution. Phenotypic correlations among all traits were computed by a PROC CORR (SAS; 1989). Pearson product-moment correlations were tested using H_o: (correlation = 0) versus a two-tailed alternative to obtain p-values among all traits.

Genotype analysis: Genotypic frequencies at each marker were calculated as the ratio of the number of individuals for each genotypic class (M16i/M16i or M16i/CAST) to the total number of mice genotyped (n = 421). In a backcross, the expected Mendelian ratio is 1:1. A Chi-square test statistic with 1 degree of freedom was used to identify any significant deviations in a marker's genotypic inheritance. The significance level of $P < .05$ results in a confidence interval for genotypic frequencies ranging between 45.14 and 54.48%.

Linkage mapping: Linkage analysis was performed for this backcross population since differences in recombination rate could exist between sexes and populations. The SSLP microsatellite markers were originally mapped for a different cross, so it is important to determine linkages between markers using data from this backcross population. Even though almost all SSLP markers identified will differ among strains and populations, linkage analysis should be conducted. Recombination frequencies for

adjacent marker pairs were determined as the total number of heterozygotes (M16i/M16i at one marker and M16i/CAST at the other) divided by the total number of progeny (n = 421).

In addition, recombination frequencies were calculated in the same way for each sex separately (n = 213 for males and 208 for females). The genetic distance (in cM units) between pairs of markers equals the recombination rate. For chromosome 2 and 15, each most proximal marker was standardized to 2.35 and 5.72 cM, respectively. This is due to the published marker positions for these markers in the WI/MIT-CGR microsatellite marker map acquired from the Mouse Genome Database (The Jackson Laboratory, 1995). A Chi-square test ((total number of markers - 2) degrees of freedom) was performed to test for any significant differences between the male and female marker maps for each chromosome.

The statistical methods employed in this research project dealt with various statistical tests in an attempt to map QTL. The genotypic data collected were analyzed by four different QTL mapping methods: analysis of variance (ANOVA), marker-regression (Kearsey and Hyne, 1994), composite interval mapping (Zeng, 1994; Jiang and Zeng, 1995), and interval mapping (Zeng, 1994; Jiang and Zeng, 1995).

ANOVA procedures: Effect of marker genotype on the measured phenotypic characteristics were tested utilizing PROC GLM (SAS; 1989). The model included the effects of dam (random), sex (fixed), genotype (fixed), and the sex by genotype interaction. Least-squares means were computed for the effects of sex, marker genotype, and sex by marker interaction. To map the QTL, the following values were plotted

against recombination frequency for each trait analyzed: F-values for each marker; genotype least-squares mean differences for single and adjacent markers; and least-squares means for all individuals which possessed the M16i/M16i - M16i/M16i genotype for an adjacent marker pair. In addition, sex by marker interactions were examined for all traits to determine if one sex had a significant advantage over the other due to a QTL only being expressed in a certain sex.

Marker regression: Mean square residuals (MSR) were determined at one cM intervals along chromosomes 2 and 15 by applying linear regression through the origin (Kearsey and Hyne, 1994). This method involves regressing the additive difference between marker genotype means at a locus against a function of the recombination frequency between that locus and a putative QTL. The QTL is located at the position where the MSR is the lowest at that map position. A linear model of gene effect against a function of recombination frequency between the QTL and the marker for the backcross population in this study is the following (Cowen, 1988):

$$1/2(\overline{M_{i1}M_{i1}} - \overline{M_{i1}M_{i2}}) = 1/2(1-2R_i)(d) = \delta_i$$

where $\overline{M_{i1}M_{i1}}$ and $\overline{M_{i1}M_{i2}}$ are the expected mean trait value of all individuals with marker genotype $M_{i1}M_{i1}$ and $M_{i1}M_{i2}$, respectively. The number of markers is i = 1 to k (k was 20 for chromosome 2 and 10 for chromosome 15). The recombination frequency between the QTL and the ith marker is represented as R. The additive (d) effects, defined as the

genetically additive variation by Mather and Jinks (1982), are equal to δ_i (y) if there is complete linkage between the QTL and the marker.

PROC GLM (SAS; 1989) was used to generate least-squares means for all traits at each marker. The difference between the least-squares means for each marker genotype class (M16i/M16i and M16i/CAST) was determined and regressed on 1-2R (x). According to Haldane (1919), the term $(1-2R) = e^{-m}$; where m is the mean chiasma frequency in that interval ($|(X - C_i)/50|$). The putative QTL position (X) and the marker lcous (Ci) are both given in cM. The QTL was moved along the entire length of the chromosome with each calculated mean square being adjusted by multiplying by total number of progeny (n = 420).

The MSR (k-1 degrees of freedom) was divided by an error mean square term (n-(k+1) degrees of freedom; backcross variance - $1/2d^2$) to obtain an F-Value. An interval harboring a QTL is defined by the locations where the adjusted MSR is not significant. The null hypothesis for the test was to determine if the chromosomal positions were acceptable for locating the QTL. A level of .05 was selected to test for non-significant locations for the QTL. For the chromosome 2 F-value degrees of freedom (19, 399), a cutoff value of 1.613 was used at the .05 level in the test. The chromosome 15 F-value degrees of freedom (9, 409) gives a cutoff value of 1.903 at the .05 level.

In addition to the MSR calculated, mean square regression (MSREG) values were computed at one cM intervals along chromosomes 2 and 15. The adjusted MSREG (degrees of freedom = 1) were divided by the adjusted MSR values to obtain a second F-value (F2). A level of .001 was selected to test for significant QTL locations. For

chromosome 2, the degrees of freedom (1, 19) produced a cutoff value of 15.081. For chromosome 15, a cutoff value of 22.857 was obtained from 1, 9 degrees of freedom. These cutoff values were used to test the F2 values at each one cM interval. The cutoff point (cM) determined for F2 was then used to indirectly locate the corresponding MSR value at these map locations. A line was drawn at these MSR values on the marker regression graphs to indicate acceptable locations for identifying a QTL (chromosome points below the line).

Interval mapping: Both interval mapping (Lander and Botstein, 1989) and composite interval mapping (Zeng, 1994; Jiang and Zeng, 1995) analyses were conducted by Dr. Zhao-Bang Zeng at North Carolina State University, Raleigh. A total of 12 phenotypic traits were measured; however, Dr. Zeng only considered two traits in the investigation (WK12 and GOFP). Also, only chromosome 2 data were analyzed by Dr. Zeng by the time this paper was completed; chromosome 15 will be studied at a later date. The following paragraphs are Dr. Zeng's summary for chromosome 2.

A linkage map (Table 9) was constructed for chromosome 2 with MAPMAKER software (Lander et al., 1987) for use in all analyses. The multiple trait analysis (Jiang and Zeng, 1995) and interval mapping (Lander and Botstein, 1989) methods were applied to the mapping of QTL on each trait and each sex separately, on each trait with both sexes jointly, and on both traits with both sexes jointly. This approach tested QTL effects simultaneously or individually and furthermore tested QTL by sex interaction effects on each trait alone or together. This was due to the fact that sex has very significant effects on WK12 and GOFP.

The following model was utilized in the analysis:

$$y_{jik} = \mu_{ik} + b^{*}_{ik}x^{*}_{ji} + \Sigma^{t}_{l} b_{ilk}x_{jil} + e_{jik} \qquad \begin{array}{l} ji = 1 \text{ to } 213 \text{ for } i = 1 \\ \text{and } ji = 1 \text{ to } 208\ i = 2 \end{array}$$

where y_{jik} is the phenotypic value of individual ji on trait k (i = 1 for male and 2 for female), μ_{ik} is the mean model effect for sex i and trait k, b^{*}_{ik} is the putative QTL effect on sex i and trait k, x^{*}_{ji} is the QTL genotype indicator variable (1 for M16i/M16i individuals and 0 for M16i/ CAST individuals), b_{ilk} is the partial regression coefficient of y_{jik} on x_{jil}, x_{jil} is the marker l genotype indicator variable for individual ji, and e_{jik} is the residual effect of individual ji on trait k. The residual effects (error terms) are assumed to be correlated among different traits within the same individual and independent among individuals.

For composite interval mapping, multiple markers were included in the model to block the effects of other possibly linked QTL in the test. Only two linked markers, at least 19 cM away from the testing interval, were fitted into the model. No other markers were implemented into the model since fitting other linked or unlinked markers tended not to have significant effects on QTL mapping in this backcross population. For interval mapping, the model did not include the terms of $\Sigma^{t}_{l} b_{ilk}x_{jil}$.

The following hypothesis tests were executed at one cM intervals along the entire length of chromosomes 2 and 15:

1) Separate mapping on each trait and each sex

$$H_o: b^{*}_{ik} = 0 \qquad H_1: b^{*}_{ik} \neq 0$$

2) Joint mapping on both sexes on each trait

$$H_o: b^{*}_{1k} = 0, b^{*}_{2k} = 0 \qquad H_1: b^{*}_{1k} \neq 0, b^{*}_{2k} \neq 0$$

3) Joint mapping on both sexes and both traits

$$H_o: b^*_{11} = 0, b^*_{21} = 0, b^*_{12} = 0, b^*_{22} = 0$$
$$H_1: b^*_{11} \neq 0, b^*_{21} \neq 0, b^*_{12} \neq 0, b^*_{22} \neq 0$$

4) Testing QTL by sex interactions on each trait

$$H_o: b^*_{1k} = b^*_{2k} \qquad H_1: b^*_{1k} \neq b^*_{2k}$$

5) Testing QTL by sex interactions on both traits

$$H_o: b^*_{11} = b^*_{21}, b^*_{12} = b^*_{22} \qquad H_1: b^*_{11} \neq b^*_{21}, b^*_{12} \neq b^*_{22}$$

These tests were based on the likelihood ratio test procedure which assumes that the residual effects are multivariate normally distributed among individuals. The maximum likelihood estimates can be calculated from use of an expectation/conditional maximization (ECM) algorithm (Meng and Rubin, 1993). The ratio of maximum likelihood estimates is detemined with the log being taken (LOD score). The LOD score is a measure of the likelihood of a QTL being present with a significance level determined by a threshold value based on marker spacing.

CHAPTER IV

RESULTS

Descriptive Statistics

Phenotypic specifications for all traits in the backcross population are provided for male (Appendix 9), female (Appendix 10), and sex-pooled (Appendix 11) groups. Focusing on the coefficients of variation (CV) given for various traits helps to explain the large amount of variation observed in the backcross population. As stated earlier, initial parental populations need to be widely divergent for traits of interest to be the most beneficial. The availabilty and use of selection lines (M16i) has advantageously produced a backcross population which exhibits extreme phenotypic variation.

The CV for the male population compared to the females only changed dramatically for six traits: GAIN3, GAIN4, GOFP, and total fat pad weight percentage (FATP). The sex-pooled CV varied slightly for the body weights (14.51 to 19.96), and organ weights (17.75 to 28.52). However, there was a large change in the CV for growth rates as ontogeny progressed (23.32 to 98.13). Also, the measurements of fat CV's differed, ranging from 53.61 to 79.27. CV's for the early growth rates, GAIN1 (32.92) and GAIN2 (23.32) were much lower than GAIN3 (65.19) and GAIN4 (98.13).

For the sex-pooled population, the only traits that followed a normal distribution were all of the body weight measurements, GAIN1, GAIN2, GAIN4, liver weight percentage (LIVP), and kidney weight percentage (KIDP). However, the opposite was true for 6 week body weight (WK6), GAIN2, and KIDP in each sex which were not normally distributed ($P < .05$). There was a lack of normality for all fat traits, GAIN3, heart weight percentage (HRTP), spleen weight percentage (SPLP), and testis weight percentage (TESP) in the sex-pooled, male, and female poplations ($P < .05$). LIVP was normally distributed for pooled and male populations (approximately $P = .98$); however, LIVP followed a lack of normality pattern for the female population ($P < .01$).

Table 3 displays the phenotypic correlations among all traits in the backcross population. The correlation between WK12 and GOFP was relatively high (0.636; $P < .0001$). The relationship among all body weight traits was significant and positive ($P < .0002$) with the highest being 0.947 for WK12 and 9 week body weight (WK9). The correlations between body weights within each weight group decreased as time between measurements increased.

The association between fat percentage traits ranged from 0.570 (GOFP and SCFP) to 0.946 (GOFP and FATP). The correlation among all growth rates were low on both the positive and negative side, with the highest correlation being 0.353 ($P < .0001$) between GAIN3 and GAIN4. The same was true for the organ weight percentages, with the highest association being 0.345 ($P < .0001$) for SPLP and LIVP.

Table 3. Phenotypic Correlations and Associated P-Values (Sex Pooled) for all Traits in the Backcross Population.

	DAY12	WK3	WK6	WK9	WK12	GAIN1	GAIN2	GAIN3	GAIN4	HRTP	LIVP	SPLP	KIDP	TESP	SCFP	GOFP
WK3	0.791 .0001															
WK6	0.475 .0001	0.551 .0001														
WK9	0.254 .0001	0.290 .0001	0.780 .0001													
WK12	0.183 .0002	0.216 .0001	0.683 .0001	0.947 .0001												
GAIN 1	0.530 .0001	0.938 .0001	0.495 .0001	0.257 .0001	0.196 .0001											
GAIN 2	0.120 .0134	0.093 .0577	0.882 .0001	0.767 .0001	0.692 .0001	0.060 .2186										
GAIN 3	-0.249 .0001	-0.267 .0001	-0.137 .0049	0.514 .0001	0.562 .0001	-0.270 .0001	0.004 .9293									
GAIN 4	-0.112 .0210	-0.105 .0312	0.021 .6605	0.242 .0001	0.541 .0001	-0.082 .0936	0.085 .0816	0.353 .0001								
HRTP	-0.028 .5694	-0.042 .3923	-0.174 .0003	-0.308 .0001	-0.391 .0001	-0.042 .3875	-0.184 .0002	-0.247 .0001	-0.368 .0001							
LIVP	-0.048 .3220	-0.193 .0001	-0.102 .0367	0.039 .4261	0.068 .1647	-0.240 .0001	-0.013 .7949	0.202 .0001	0.102 .0362	0.091 .0630						
SPLP	-0.148 .0024	-0.257 .0001	-0.328 .0001	-0.348 .0001	-0.337 .0001	-0.272 .0001	-0.246 .0001	-0.099 .0435	-0.104 .0326	0.171 .0004	0.345 .0001					
KIDP	0.135 .0055	0.112 .0217	0.190 .0001	0.193 .0001	0.109 .0249	0.079 .1073	0.163 .0008	0.044 .3677	-0.176 .0003	0.213 .0001	0.111 .0232	-0.067 .1716				
TESP	0.108 .1178	0.0002 .9977	-0.150 .0294	-0.314 .0001	-0.374 .0001	-0.059 .3929	-0.181 .0082	-0.204 .0030	-0.193 .0049	0.128 .0632	-.160 .0203	0.045 .5202	0.011 .8734			
SCFP	-0.077 .1179	-0.058 .2376	0.126 .0101	0.285 .0001	0.345 .0001	-0.037 .4543	0.182 .0002	0.276 .0001	0.290 .0001	-0.255 .0001	-.152 .0019	-0.221 .0001	-0.231 .0001	-0.078 .2598		
GOFP	0.054 .2661	0.092 .0586	0.422 .0001	0.651 .0001	0.696 .0001	0.097 .0466	0.454 .0001	0.447 .0001	0.389 .0001	-0.288 .0001	-.060 .2167	-0.380 .0001	0.040 .4172	-0.124 .0724	0.570 .0001	
FATP	0.008 .8775	0.041 .4010	0.350 .0001	0.580 .0001	0.636 .0001	0.053 .2810	0.396 .0001	0.429 .0001	0.392 .0001	-0.308 .0001	-.103 .0350	-0.360 .0001	-0.066 .1754	-0.113 .1021	0.806 .0001	0.946 .0001

Genotype Analysis

The M16i/M16i genotypic frequencies for all markers analyzed are presented in Appendix 5. The frequency for CAST/M16i individuals are simply calculated as 1-Frequency M16i/M16i. All markers for chromosomes 2 and 15 followed the expected 1:1 Mendelian ratio ($P > .05$). The frequency of M16i/M16i, as well as M16i/CAST individuals ranged from approximately 47 to 53 % on chromosomes 2 and 15.

Marker Mapping

The sex-pooled recombination rates between markers are described in Appendix 5. The sex-averaged, female, and male map distances (cM) between markers are illustrated for chromosome 2 (Appendix 3) and 15 (Appendix 4) genetic linkage maps. The male and female recombination rates between markers can be calculated by taking the difference between the marker values given in Appendixes 3 and 4. The smallest interval between adjacent markers was 1.9 cM (D2MIT103 to D2MIT133; D15MIT11 to D15MIT131), while the largest one was 12.83 cM (D2MIT120 to D2MIT157). A Chi-square test revealed no significant differences between the estimated male and female genetic linkage maps for both chromosomes . The test statistics for chromosome 2 (10.63) and 15 (7.06) were much lower than the published table values of 28.87 (18 d.f.) and 15.51 (8 d.f.), respectively ($P < .05$). For chromosome 2, the total female (100.43 cM) and male (97.67

cM) linkage distances differed only slightly, but the chromosome 15 total linkage distance differed by 11.13 cM (female, 47.72; male, 58.85).

The estimated genetic linkage map marker values can be compared to those estimated for the WI/MIT-CGR map previously described (Copeland et al., 1993; The Jackson Laboratory Mouse Genome Database, 1995). All estimated linkage maps in this study have the proximal marker standardized as to be in the same location as that marker on the WI/MIT-CGR map. The estimated marker placements between the WI/MIT-CGR maps and those calculated here by recombination rates (sex-averaged) are very similar. The largest marker disagreements were 7.94 cM (D2MIT120) for chromosome 2 and 8.69 cM (D15MIT3) for chromosome 15. Even with these differences, the entire chromosome lengths for 2 and 15 differed by 0.49 and 4.13 cM, respectively.

QTL Localization - ANOVA

Single marker analysis: Marker genotype effects on phenotypic characteristics (least-squares means and F-values) are presented in Appendixes 12 and 13 for chromosome 2 and 15, respectively. Even though four graphs were plotted for all traits (see materials and methods), only the F-values are presented (Appendixes 14 and 15 for chromosomes 2 and 15, respectively). Because all graphs depicted the same approximate locations for QTL. The following significance levels were utilized for all traits graphed except TESP: $P < .05$ ($F > 3.867$), $P < .01$ ($F > 6.705$), and $P < .001$ ($F > 11.006$). For

TESP, the following levels were used in determining significant individual marker effects: $P < .05$ ($F > 3.901$), $P < .01$ ($F > 6.799$), and $P < .001$ ($F > 11.247$).

A summary for ANOVA results for all traits is presented in Table 4 and 5 for chromosomes 2 and 15, respectively. The number of significant markers at a specific significance level, as well as the marker expressing the largest effect on that phenotypic trait and its location on the chromosome are displayed in Table 4 and 5.

Table 4. Summary of Analysis of Variance Procedures (Significant Marker Quantity) Testing for Linkage of Individual Markers to QTL for Traits on Chromosome 2.

	Marker Number at Significance Level			Largest Effect[a]	
Trait	$P < 0.05$	$P < 0.01$	$P < 0.001$	Marker	Location (cM)
DAY12	17	9	3	D2MIT25	83.82
WK3	16	10	1	D2MIT166	65.53
WK6	11	9	4	D2MIT37	40.36
WK9	20	18	16	D2MIT164	58.41
WK12	18	17	16	D2MIT224	61.02
GAIN1	1	0	0	D2NDS1	49.15
GAIN2	16	15	10	D2MIT37	40.36
GAIN3	16	15	14	D2MIT49	75.03
GAIN4	14	12	8	D2MIT166	65.53
HRTP	12	11	11	D2MIT164	58.41
LIVP	7	3	0	D2MIT164	58.41
SPLP	10	9	4	D2MIT22	68.38
KIDP	17	17	16	D2MIT166	65.53
TESP	14	4	3	D2MIT120	15.18
SCFP	17	16	15	D2MIT49	75.03
GOFP	16	15	15	D2MIT22	68.38
FATP	17	15	15	D2MIT49	75.03

[a] Marker linked to a QTL with the largest effect on each trait.

For chromosome 2, a large number of markers (> 8) were significant at the $P < .001$ level for all fat percentage traits, the later two body weights, the last three growth rate periods, and two organ percentages (HRTP and KIDP). There were only three traits

with more than 8 significant markers at the P < .001 level on chromosome 15: WK9, GOFP, and FATP. A lower number of significant DNA markers for chromosome 15 compared to chromosome 2 can mainly be attributed to the fact that fewer markers were analyzed on 15. HRTP, LIVP, SPLP, and TESP did not have any significant markers at the P < .05 level or lower.

Table 5. Summary of Analysis of Variance Procedures (Significant Marker Quantity) Testing for Linkage of Individual Markers to QTL for Traits on Chromosome 15.

	Marker Number at Significance Level			Largest Effect[a]	
Trait	0.05	0.01	0.001	Marker	Location (cM)
DAY12	1	0	0	D15MIT131	7.64
WK3	3	2	0	D15MIT131	7.64
WK6	9	2	0	D15MIT107	27.83
WK9	10	10	8	D15MIT107	27.83
WK12	10	10	7	D15MIT107	27.83
GAIN1	9	5	2	D15MIT131	7.64
GAIN2	3	1	0	D15MIT107	27.83
GAIN3	5	1	0	D15MIT34	47.72
GAIN4	1	0	0	PPAR-alpha	32.80
KIDP	9	9	0	D15MIT64	22.07
SCFP	10	9	2	D15MIT131	7.64
GOFP	10	10	8	D15MIT64	22.07
FATP	10	10	8	D15MIT64	22.07

[a] Marker linked to a QTL with the largest effect on each trait.

Table 6 and 7 illustrates the significant sex by marker interactions observed for the phentoypic traits analyzed on chromosomes 2 and 15, respectively. Five traits on chromosome 2 had significant sex by marker interactions: GAIN3, LIVP, KIDP, SCFP, and GOFP. For GAIN3, the most proximal 3 markers were significant (P < .05) where there was a clear advantage for males (MM) over females in having higher growth rates. Even though the MM genotypic class had a better growth rate in males, the MC

individuals had the advantage in females. A similar pattern was observed for LIVP; however, markers were significant at $P < .01$ (B and C) and $P < .001$ (A) in addition to $P < .05$ (D). Once again a similar trend was exhibited for KIDP as that identified for GAIN3, with significant sex by marker interactions ($P < .05$).

Table 6. Least-Squares Means and F-Values for all Traits with a Significant Sex X Marker Interaction Using ANOVA on Chromosome 2.

	Male		Female			
Trait	MM[a]	MC[b]	MM	MC	Marker	F-Value[c]
GAIN3	8.483 ± .313	7.077 ± .324	4.155 ± .314	4.318 ± .34	A	5.63[1]
	8.352 ± .323	7.287 ± .315	4.11 ± .311	4.381 ± .347	B	4.13[1]
	8.403 ± .328	7.259 ± .313	4.141 ± .313	4.346 ± .343	C	4.18[1]
LIVP	7.814 ± .068	7.503 ± .071	7.433 ± .068	7.623 ± .074	A	12.07[3]
	7.815 ± .07	7.525 ± .068	7.449 ± .067	7.608 ± .075	B	9.89[2]
	7.812 ± .072	7.532 ± .068	7.455 ± .068	7.601 ± .075	C	8.82[2]
	7.809 ± .073	7.544 ± .066	7.48 ± .071	7.573 ± .072	D	6.18[1]
KIDP	.879 ± .012	.847 ± .012	.761 ± .012	.787 ± .013	A	5.35[1]
	.875 ± .012	.854 ± .012	.76 ± .012	.788 ± .013	C	4.01[1]
SCFP	.578 ± .029	.645 ± .03	.579 ± .03	.506 ± .032	A	5.15[1]
	.573 ± .03	.643 ± .029	.586 ± .029	.496 ± .032	B	6.89[2]
	.585 ± .03	.63 ± .029	.599 ± .029	.481 ± .032	C	6.99[2]
	.609 ± .031	.607 ± .028	.618 ± .03	.469 ± .031	D	5.89[1]
GOFP	1.343 ± .043	1.007 ± .041	.539 ± .042	.383 ± .042	H	4.34[1]
	1.337 ± .041	.997 ± .042	.544 ± .042	.379 ± .041	I	4.23[1]
	1.367 ± .043	.991 ± .04	.542 ± .042	.382 ± .041	J	6.46[1]
	1.37 ± .042	.988 ± .04	.552 ± .041	.375 ± .04	K	5.97[1]
	1.374 ± .042	.985 ± .04	.546 ± .041	.381 ± .04	L	7.21[2]
	1.383 ± .042	.983 ± .039	.571 ± .041	.352 ± .04	M	4.70[1]
	1.386 ± .042	.982 ± .039	.569 ± .041	.359 ± .04	S	5.40[1]
	1.379 ± .041	.977 ± .04	.572 ± .041	.36 ± .039	T	5.29[1]

[a] M16i/M16i individuals
[b] M16i/CAST individuals
[c] Significant F-Values: $P < .05$ (1), $P < .01$ (2), and $P < .001$ (3)

The SCFP sex by marker intraction gave the advantage (lower fat percentage) to these same genotypic classes (M16i/M16i, males; and M16i/CAST; females). For these

genotypic classes, the females compared to the males had leaner carcasses. However for GOFP, there was an advantage for the M16i/CAST individuals (females better than males) since they exhibit a smaller fat percentage. Also, this influence was identified by markers more distally on chromosome 2. Interactions were significant at $P < .05$ and $P < .01$ levels for both fat percentage traits.

For chromosome 15, six traits were influenced by significant sex by marker interactions (Table 7). All interactions were at $P < .05$ except for one marker affecting KIDP ($P < .01$). The M16i/M16i genotypic class compared to M16i/CAST showed an increase in WK3 body weight for both males and females, with the females having a slight advantage (increased body weight) over the males. A similar case was observed for GAIN1; however the two male genotypic classes were almost of equal magnitude. The female M16i/M16i genotypic class possessed higher body weights than the males.

For GAIN2, the M16i/M16i (male) and M16i/CAST (female) individuals expressed the higher growth rates; the males were at an advantage (increased growth rate) over the females. The M16i/CAST genotypic class (male and female) displayed an advantage over their corresponding M16i/M16i individuals for both KIDP and GOFP. The males had the advantage (larger size) for KIDP, but the females possessed leaner carcasses for GOFP. For FATP, results were similar as those for GOFP, where the M16i/M16i individuals (male and female) exhibited a greater total fat percentage than their corresponding M16i/CAST individuals. Overall, the female M16i/CAST individuals had the lean carcass advantage.

Table 7. Least-Squares Means and F-Values for all Traits with a Significant Sex X Marker Interaction Using ANOVA on Chromosome 15.

Trait	Male		Female		Marker	F-Value[c]
	MM[a]	MC[b]	MM	MC		
WK3	12.88 ± .16	12.77 ± .14	13.05 ± .16	12.33 ± .15	B	3.98[1]
	12.89 ± .15	12.76 ± .15	13.07 ± .16	12.32 ± .15	C	4.09[1]
	12.89 ± .15	13.02 ± .16	13.02 ± .17	12.48 ± .16	J	4.18[1]
GAIN1	5.55 ± .112	5.59 ± .11	5.71 ± .113	5.25 ± .116	A	4.79[1]
	5.6 ± .115	5.55 ± .106	5.75 ± .115	5.22 ± .113	B	4.35[1]
	5.6 ± .114	5.55 ± .108	5.76 ± .115	5.22 ± .113	C	4.49[1]
	5.57 ± .117	5.57 ± .108	5.76 ± .115	5.22 ± .113	D	5.34[1]
	5.76 ± .115	5.57 ± .107	5.73 ± .116	5.25 ± .113	E	4.41[1]
	5.55 ± .112	5.6 ± .109	5.74 ± .116	5.24 ± .114	F	5.65[1]
	5.57 ± .113	5.58 ± .11	5.72 ± .116	5.25 ± .114	G	4.29[1]
	5.56 ± .109	5.58 ± .115	5.7 ± .112	5.25 ± .116	H	4.14[1]
	5.63 ± .108	5.7 ± .119	5.73 ± .122	5.33 ± .118	J	3.94[1]
GAIN2	22.33 ± .38	20.61 ± .37	17.27 ± .38	17.45 ± .39	A	6.10[1]
	22.12 ± .39	20.89 ± .36	17.13 ± .39	17.57 ± .38	B	4.69[1]
	22.15 ± .39	20.84 ± .37	17.15 ± .39	17.55 ± .38	C	4.92[1]
	22.15 ± .4	20.86 ± .37	17.22 ± .39	17.46 ± .39	D	3.87[1]
	22.25 ± .39	20.77 ± .36	17.24 ± .39	17.46 ± .38	E	4.88[1]
	22.24 ± .38	20.71 ± .37	17.36 ± .39	17.37 ± .39	F	3.98[1]
	22.37 ± .38	20.59 ± .37	17.41 ± .39	17.34 ± .38	G	4.88[1]
KIDP	.830 ± .012	.896 ± .012	.769 ± .012	.778 ± .012	A	5.45[1]
	.825 ± .012	.897 ± .011	.772 ± .012	.776 ± .012	B	7.98[2]
	.829 ± .012	.895 ± .011	.769 ± .012	.778 ± .012	C	5.58[1]
	.83 ± .012	.893 ± .011	.768 ± .012	.779 ± .012	E	4.59[1]
	.831 ± .012	.895 ± .012	.766 ± .012	.781 ± .012	F	4.07[1]
GOFP	1.3 ± .042	1.04 ± .041	.478 ± .043	.435 ± .043	A	6.22[1]
	1.29 ± .044	1.07 ± .04	.479 ± .044	.433 ± .043	B	3.98[1]
	1.29 ± .043	1.06 ± .041	.485 ± .044	.429 ± .043	C	4.46[1]
	1.31 ± .044	1.05 ± .041	.48 ± .044	.429 ± .042	D	5.91[1]
	1.31 ± .043	1.05 ± .04	.488 ± .044	.427 ± .042	E	5.15[1]
	1.3 ± .042	1.05 ± .041	.502 ± .043	.42 ± .043	G	4.04[1]
FATP	1981 ± 65	1582 ± 64	1046 ± 66	961 ± 67	A	5.64[1]
	1989 ± 68	1595 ± 63	1062 ± 67	940 ± 66	D	4.15[1]

[a] M16i/M16i individuals

[b] M16i/CAST individuals

[c] Significant F-Values: $P < .05$ (1), $P < .01$ (2), and $P < .001$ (3)

The estimated QTL position ($P < .01$) for the various traits analyzed are shown in Tables 8 and 9 for chromosomes 2 and 15, respectively. The QTL location was estimated

by the marker which exhibited the largest effect on the various traits. This was determined by taking the difference between least-squares means for the two genotypic classes. At the $P < .01$ significance level, all traits on chromosome 2 had a QTL except GAIN1. DAY12 GAIN4, HRTP, LIVP, SPLP, and TESP did not have a QTL estimated from the ANOVA procedure for chromosome 15.

In addition, the genotypic class which resulted in an increase in body weight or fat percentage for the traits (referred to as an 'advantage') is given in Tables 8 and 9 for chromosomes 2 and 15, respectively. M16i/CAST individuals illustrated an increase in early body weights and organ weight percentages, the M16i/M16i class had an increase in late body weights, growth rates, and fat percentages for chromosome 2. On the other hand for chromosome 15, M16i/M16i individuals displayed an increase in all phenotypic traits listed except KIDP and SCFP.

For QTL placement using analyis of variance procedures (Appendix 14 and 15 for chromosome 2 and 15, respectively), profiles which display flat, wide significant regions are suspected to contain more than one QTL. This may be the case for WK9, WK12, and HRTP since possible second QTL are located at 75.03, 70.04, and 49.15 cM, respectively on chromosome 2. The second QTL for WK9 and WK12 are most likely the same gene. Because there is a high correlation between the two traits and that they are both late body weight traits. The second QTL for WK9 and WK12 may have pleiotropic action where this locus is the same gene controlling GAIN3, SCFP, FATP, WK3, GAIN4, SPLP, KIDP, and GOFP.

In addition, two traits for chromosome 15 (KIDP and SCFP) may be affected by more than one QTL at 12.45 and 7.62 cM, respectively. As for chromosome 2, these second QTL may have pleiotropic actions on WK3 and GAIN1. However for this mapping approach, the number of QTL cannot be clearly established if the QTL are relatively close on the chromosome.

Table 8. Summary of Analysis of Variance Procedures (QTL Location) Testing for Linkage of Individual Markers to QTL for Traits on Chromosome 2.

Trait	QTL[a] (cM)	Effect[b] (Marker)	Advantage[c]	% Variation[d]
DAY12	83.82	0.27 (D2MIT25)	M16i/CAST	3.52
WK3	65.53	0.43 (D2MIT166)	M16i/CAST	2.04
WK6	40.36	1.65 (D2MIT37)	M16i/M16i	3.71
WK9	58.41	3.25 (D2MIT164)	M16i/M16i	12.24
WK12	61.02	4.08 (D2MIT224)	M16i/M16i	15.36
GAIN2	40.36	1.96 (D2MIT37)	M16i/M16i	6.44
GAIN3	75.03	1.77 (D2MIT49)	M16i/M16i	7.28
GAIN4	65.53	1.11 (D2MIT166)	M16i/M16i	6.60
HRTP	58.41	0.056 (D2MIT164)	M16i/CAST	6.55
LIVP	58.41	0.2 (D2MIT164)	M16i/M16i	2.14
SPLP	68.38	0.058 (D2MIT22)	M16i/CAST	4.74
KIDP	65.53	0.081 (D2MIT166)	M16i/CAST	11.07
TESP	15.18	0.028 (D2MIT120)	M16i/CAST	10.16
SCFP	75.03	0.24 (D2MIT49)	M16i/M16i	15.58
GOFP	68.38	0.31 (D2MIT22)	M16i/M16i	12.37
FATP	75.03	549 (D2MIT49)	M16i/M16i	16.02

[a] Estimated QTL location given in cM for the various traits. Only one QTL was estimated even if it appeared that two were present.

[b] Effects of a marker linked to a QTL for the various traits presented as the difference in the least-squares means between the two genotypic classes (grams for all raw weights or % for all weights taken as a percentage of WK12).

[c] Genotype which gives an increase in body weight or fat percentage traits.

[d] Percentage of residual phenotypic variation explained by significant marker effects.

Body weights: For chromosome 2, there appears to be a QTL for early body weight in the 66 to 84 cM range. Another QTL influencing later body weights was

estimated between 40 and 60 cM. The early body weight gene gives an advantage (higher body weight) to M16i/CAST individuals, but the late body weight QTL was an advantage in the M16i/M16i genotypic class.

For chromosome 15, the QTL for all body weights were advantageous in the M16i/M16i individuals. The QTL effect for WK3 was largest for a proximal marker at approximately 8 cM, but the remaining late body weights had a QTL estimated at a more distal marker (32 cM). For both chromosomes, there appears to be early and late body weight QTL controlling the phenotypes observed for all individuals.

Growth rates: The study of marker effects on growth rates provides a better understanding of the QTL affect throughout ontogeny. Genes have been estimated which influence adult body weight during different periods of development (early and late growth). For chromosome 2, a QTL was estimated around 65 to 75 cM for late growth periods, but a more proximal locus was identified at 40 cM for GAIN2. All the estimated QTL effects are beneficial in M16i/M16i individuals. As stated previously, GAIN1 did not have an estimated QTL from the ANOVA procedure.

Similar results were observed for chromosome 15, where middle to late growth rate QTL were distally located at about 32 to 49 cM. The early growth rate was estimated very proximally at approximately 8 cM. Once again, all the growth rate QTL were advantageous in the M16i/M16i genotypic class.

Organ weight percentages: QTL were estimated for all organs except TESP in the range of 58 to 68 cM on chromosome 2. The estimated QTL for TESP was very

proximally located at 15 cM. The estimated organ weight percentage QTL are all favorable in the M16i/CAST individuals except for LIVP.

Only one organ weight percentage QTL was identified on chromosome 15 (KIDP). This QTL was located at about 24 cM, and was beneficial in the M16i/CAST genotypic class.

Table 9. Summary of Analysis of Variance Procedures (QTL Location) Testing for Linkage of Individual Markers to QTL for Traits on Chromosome 15.

Trait	QTL[a] (cM)	Effect[b] (Marker)	Advantage[c]	% Variation[d]
WK3	7.62	0.44 (D15MIT131)	M16i/M16i	2.11
WK6	32.3	1.3 (D15MIT107)	M16i/M16i	2.37
WK9	32.3	1.89 (D15MIT107)	M16i/M16i	4.38
WK12	32.3	2.23 (D15MIT107)	M16i/M16i	4.81
GAIN1	7.62	0.3 (D15MIT131)	M16i/M16i	1.75
GAIN2	32.3	1.07 (D15MIT107)	M16i/M16i	1.97
GAIN3	48.97	0.91 (D15MIT34)	M16i/M16i	1.70
KIDP	23.99	0.04 (D15MIT64)	M16i/CAST	2.69
SCFP	7.62	0.104 (D15MIT131)	M16i/CAST	3.01
GOFP	23.99	0.181 (D15MIT64)	M16i/M16i	4.32
FATP	23.99	283 (D15MIT64)	M16i/M16i	4.57

[a] Estimated QTL location given in cM for the various traits. Only one QTL was estimated even if it appeared that two were present.

[b] Effects of a marker linked to a QTL for the various traits presented as the difference in the least-squares means between the two genotypic classes (grams for all raw weights or % for all weights taken as a percentage of WK12).

[c] Genotype which gives an increase in body weight or fat percentage traits.

[d] Percentage of residual phenotypic variation explained by significant marker effects.

Fat pad weight percentages: There appears to be a QTL on chromosome 2 for all of the fat traits (SCFP, GOFP, and FATP) at approximately 68 to 75 cM. This QTL is advantageous in the M16i/M16i individuals.

For chromosome 15, a QTL for SCFP was estimated around 8 cM and a second QTL was identified for GOFP and FATP at 24 cM. The SCFP QTL was favorable in

M16i/CAST individuals, while the QTL for the other two traits was beneficial in the M16i/M16i genotypic class. Due to the large phenotypic correlations for body weights and fat pad weight percentages, the QTL observed for these traits may have a pleiotropic effect in which the same gene may actually be controlling both late body weights and fat pad weight percentages.

For chromosome 2, the percent of residual phenotypic variation explained by significant DNA markers was between 2 and 15.5 % for growth, 2 and 11.1 % for organ weights, and 12 and 16.1 % for fat percentage. The body weight QTL effects increased greatly for chromosome 2, depending on the time at which they were expressed. The QTL effects on early body weights were rather small; however, 9 and 12 week body weights had QTL effects which explained a much larger portion of the phenotypic variation. A large portion of the phenotypic variation was explained by significant markers for fat percentage on chromosome 2 (15 %).

For chromosome 15, markers explained between 1.7 and 4.9 % for all traits listed. An increase in the residual phenotypic variation explained by significant markers was also exhibited for chromosome 15, but the relative increase was not as drastic between early and late body weights. A much smaller portion of the phenotypic variation was explained on chromosome 15 (4 %) compared to that seen for chromosome 2.

Appendixes 16 and 17 show the least-squares means for the above listed traits calculated with marker intervals on chromosome 2 and 15, respectively. Interval markers were defined as adjacent marker pairs used in the ANOVA procedure. This was done starting at the most proximal marker and moving toward the most distal marker on each

chromosome. As stated earlier, the least-squares mean differences for adjacent markers, as well as the actual least-squares mean for all individuals which possessed the M16i/M16i - M16i/M16i genotype for a marker pair were graphed for all traits. However, these charts are not included since they are very similar to plotting the F-values against recombination frequency.

QTL Localization - Marker Regression

To estimate QTL location for each trait, mean square residuals (MSR) were plotted against map distance for chromosomes 2 and 15 (Appendix 18 and 19, respectively). A QTL is moved along the entire length of the chromosome with the QTL location representing the point where the mean square residual is minimized. The QTL was then declared significant if the mean square regression F-value was significant. The cutoff level for these points was indirectly placed on the graphs by locating the corresponding MSR value. A disadvantage of this approach is that it only maps one QTL per trait.

The estimated QTL locations with the nearest markers are summarized in Table 10 and 11 for chromosomes 2 and 15, respectively. All traits evaluated had estimated QTL on chromosome 2. This was different than the results obtained from the ANOVA procedures. The triat not displaying a QTL for ANOVA (GAIN1) had an estimated QTL at 57 cM; the distal 95 cM of the chromosome was significant.

For chromosome 15, all traits studied had an estimated QTL except HRTP. This was the same for ANOVA; however in addition, DAY12, GAIN4, LIVP, SPLP, and TESP did not have QTL identified from the ANOVA procedure. For these traits not estimating a QTL for ANOVA, marker regression methods resulted in 75 to 100 % of the chromosome having significant points for locating the QTL. Several traits for chromosome 15 had a wide region (1 to 5 cM) where the mean square residuals were minimized.

Table 10. Summary of QTL Location on Chromosome 2 Using Marker-Regression QTL Mapping Method.

Trait	QTL[a] (cM)	Marker[b]	Trait	QTL[a] (cM)	Marker[b]
DAY12	85	D2MIT25	HRTP	60	D2MIT224
WK3	64	D2MIT166	LIVP	56	D2MIT133
WK6	46	D2NDS1	SPLP	69	D2MIT22
WK9	59	D2MIT164	KIDP	73	GHRH
WK12	64	D2MIT166	TESP	17	D2MIT120
GAIN1	57	D2MIT164	SCFP	73	GHRH
GAIN2	51	D2NDS1	GOFP	72	GHRH
GAIN3	68 - 69	D2MIT22	FATP	72	GHRH
GAIN4	72	GHRH			

[a] QTL location indicated by the map position which yields the lowest mean square residual value.

[b] DNA marker nearest to the estimated QTL location.

Table 11. Summary of QTL Location on Chromosome 15 Using Marker-Regression QTL Mapping Method.

Trait	QTL[a] (cM)	Marker[b]	Trait	QTL[a] (cM)	Marker[b]
DAY12	1 - 5	D15MIT11	GAIN4	40	PPAR-alpha
WK3	1 - 5	D15MIT11	LIVP	1 - 5	D15MIT11
WK6	30	D15MIT29	SPLP	48	D15MIT34
WK9	31	D15MIT107	KIDP	16	D15MIT86
WK12	39	PPAR-alpha	TESP	1 - 5	D15MIT11
GAIN1	9	D15MIT131	SCFP	17	D15MIT121
GAIN2	33	D15MIT107	GOFP	25	D15MIT64
GAIN3	47	D15MIT34	FATP	24	D15MIT64

[a] QTL location indicated by the map position which yields the lowest mean square residual value.

[b] DNA marker nearest to the estimated QTL location.

Body weights: For chromosome 2, the suggested idea from ANOVA procedures of an early and late body weight QTL works well for the marker regression approach. These QTL were estimated in the same ranges (66 to 84 cM for early and 40 to 60 cM for late body weights) as those for ANOVA, except that WK12 was located at the same position as WK3 which was not the case for ANOVA.

For chromosome 15, early body weights were located in approximately the same area (1 to 8 cM) as those estimated for ANOVA. As previously mentioned, DAY12 was not positioned for ANOVA procedures and was located at 1 to 5 cM for marker regression. The later body weights were all in a similar estimated region (30 to 39 cM).

Growth rates: For chromosome 2, the later growth periods were approximated in the same range as that for ANOVA procedures (65 to 75 cM). The marker regression approach located early growth rate QTL in a range of 51 to 57 cM. ANOVA positioned on one early growth rate QTL (GAIN2) at about 40 cM. This is a difference of about 10 cM between the two methods. For chromosome 15, the exact same regions for both early and late QTL were identified from the ANOVA and marker regression methods.

Organ weight percentages: The QTL locations on chromosome 2 determined by marker regression only differ by 1 to 2 cM as those estimated from ANOVA. The lone exception is KIDP which differed between the two methods by 8 cM.

The marker regression approach located QTL on chromosome 15 for LIVP, SPLP, and TESP which were not estimated from ANOVA procedures. The LIVP and TESP QTL were proximally identified around 1 to 5 cM. The SPLP QTL was more

distally located at approximately 48 cM. KIDP QTL placement differed by 7 cM between the two methods.

Fat pad weight percentages: For chromosome 2, the estimates for all the fat pad weight percentages were in the same range as those estimated from ANOVA (68 to 75 cM).

For chromosome 15, the SCFP QTL estimate differed by 10 cM between the two methods. Marker regression located the QTL at 17 cM, but it was positioned at 7 cM from ANOVA procedures. The GOFP and FATP location were exactly the same or only off by one cM between marker regression and ANOVA strategies.

QTL Localization - Interval Mapping

A marker linkage map in Table 12 was estimated for all markers on chromosome 2 utilizing the MAPMAKER computer program (Lander and Botstein, 1989). This genetic linkage map (analysis by Dr. Zeng) can be compared to the map in Appendix 3 which was predicted by recombination frequency.

Table 12. Estimated Genetic Linkage Map for Chromosome 2 Markers by MAPMAKER.

Marker	Location (cM)	Marker	Location (cM)
D2MIT1	2.35	D2MIT224	63.45
D2MIT79	14.15	D2MIT166	67.75
D2MIT120	16.35	D2MIT22	70.15
D2MIT157	30.85	AGOUTI	70.85
D2MIT61	34.65	GHRH	71.85
D2MIT37	43.95	D2MIT49	75.55
D2NDS1	53.65	D2MIT25	85.25
D2MIT103	58.65	D2MIT147	88.95
D2MIT133	59.65	D2MIT174	95.85
D2MIT164	61.35	D2MIT200	100.85

The marker estimates are very similar between the two maps. Two candidate genes, agouti and growth hormone releasing factor loci (see materials and methods) were genotyped and mapped to the region between markers D2MIT22 and D2MIT49.

Interval mapping (MAPMAKER): The interval map for WK12, GOFP, and multiple trait effects are illustrated in Figures 6, 7, and 8, respectively. For WK12, there is little QTL by sex interaction (not significant). However, for GOFP and multiple trait analysis, there is a considerable amount of QTL by sex interaction between markers D2MIT133 (60 cM) and D2MIT22 (70 cM). The largest interaction effect was seen at markers D2MIT164 (61 cM; LOD = 2.8) and D2MIT224 (63 cM; LOD = 3.1) for GOFP and combined trait analysis, respectively.

Figure 6. Interval Mapping of 12 Week Body Weight (WK12).

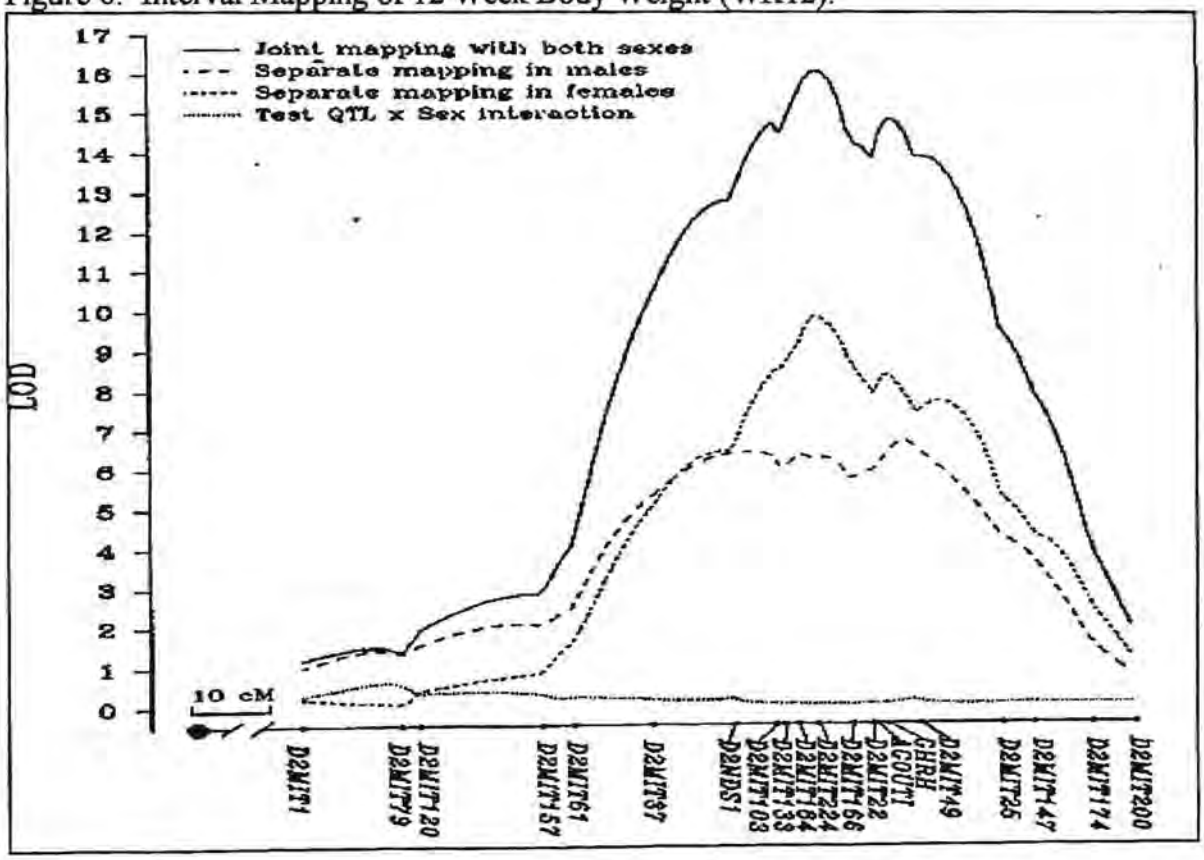
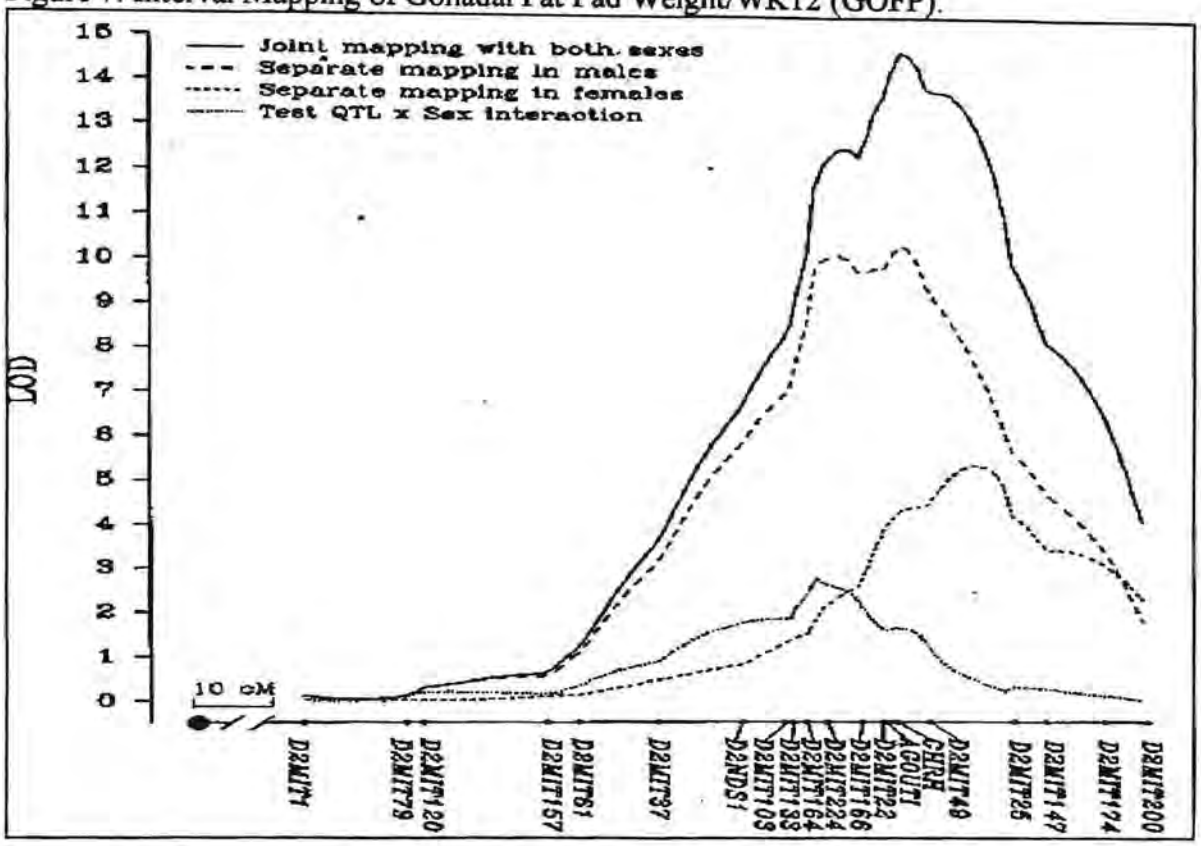


Figure 7. Interval Mapping of Gonadal Fat Pad Weight/WK12 (GOFP).

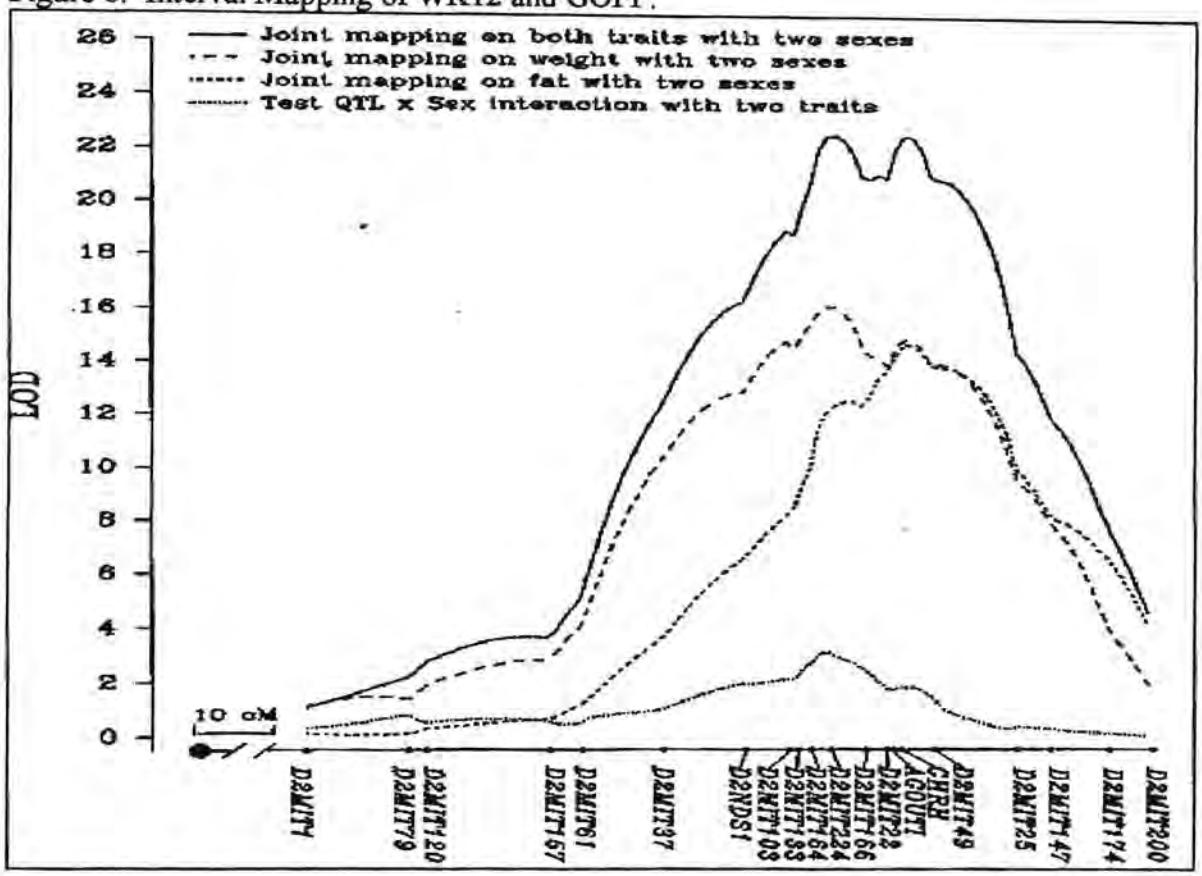


Similar likelihood profile patterns were exhibited for separate and joint sex mapping on WK12, with a majority of the chromosome displaying significant effects (LOD > 3.0). Largest genetic effects were at markers D2MIT224 (63 cM) for female (LOD = 9.9) and joint (LOD = 16.0) mapping, and D2MIT49 (76 cM) for male (LOD = 6.7) mapping. All estimated QTL for WK12 were located in a very wide range between markers D2NDS1 (54 cM) and D2MIT25 (85 cM).

For interval mapping on GOFP and combined trait analysis, a wide region (not as large as the interval displayed by WK12) of the chromosome exhibited significant genetic effects. Joint (male and female) and male mapping strategies produced similar graphs, while separate mapping on females gave a comparable but shifted profile of about 9 cM.

For GOFP, marker D2MIT22 (70 cM) created the greatest genetic effect for male (LOD = 10.2) and joint sexes (LOD = 14.6) mapping. The estimated QTL region was between markers D2MIT133 (60 cM) and D2MIT25 (85 cM). For separate mapping on females, this effect was between markers D2MIT49 (76 cM) and D2MIT25 (85 cM); LOD = 5.3.

Figure 8. Interval Mapping of WK12 and GOFP.



For WK12 and GOFP, joint mapping (both traits and sexes) showed the largest effect at D2MIT224 (63 cM), and between D2MIT22 (70 cM) and D2MIT49 (76 cM); LOD = 22.4. Joint mapping on WK12 with two sexes had the same profile as both traits and sexes; however, only D2MIT224 (63 cM; LOD = 16) produced the greatest effect. For joint mapping on GOFP with both sexes, this effect was now between markers

D2MIT22 (70 cM) and D2MIT49 (76 cM); LOD = 14.7. Estimated QTL for joint mapping with both sexes on two traits and WK12 are in an area between markers D2NDS1 (54 cM) and D2MIT25 (85 cM). This region for joint mapping on fat with both sexes is between markers D2MIT164 (61 cM) and D2MIT25 (85 cM).

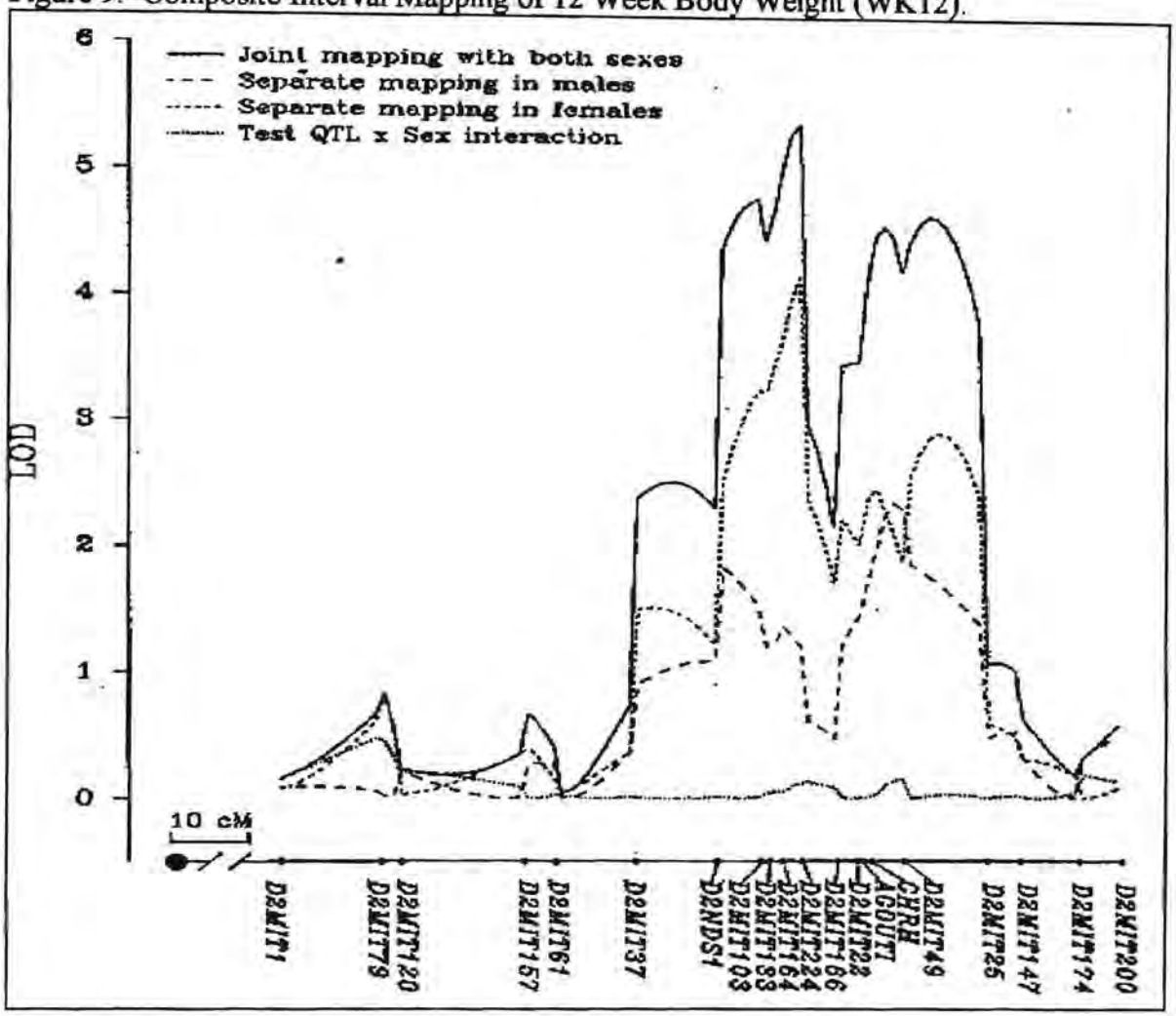
Composite interval mapping: The composite interval map for WK12, GOFP, and combined effects are illustrated in Figures 9, 10, and 11, respectively. For 12 week body weight, there was little QTL by sex interaction (not significant), as seen previously with interval mapping. However, for GOFP and multiple trait analysis, there was a considerable amount of QTL by sex interaction between markers D2NDS1 (54 cM) and D2MIT166 (68 cM). The largest interaction effect was seen at marker D2MIT224 (63 cM).

Similar patterns were observed for the likelihood profiles of WK12 when mapping the sexes separately and jointly. Two distinct peaks (QTL evidence) were located between markers D2NDS1 (54 cM) and D2MIT224 (63 cM); and D2MIT22 (70 cM) to D2MIT25 (85 cM). The LOD scores were lowest for male mapping (1.9, D2NDS1; and 2.3, D2MIT49) and largest for the joint mapping on both sexes (5.3, D2MIT224; and 4.6, D2MIT49/D2MIT25).

On the other hand, there was only an indication of one QTL for female and joint mapping of GOFP. This was in the region of markers D2MIT49 (76 cM) and D2MIT25 (85 cM). The LOD scores were larger than that for body weight: separate mapping on females (4.7) and joint mapping on both sexes (8.3). The male profile generated did not

follow the previous two, with a plateau being reached (LOD = 4.6) between markers D2MIT133 (60 cM) and D2MIT49 (76 cM).

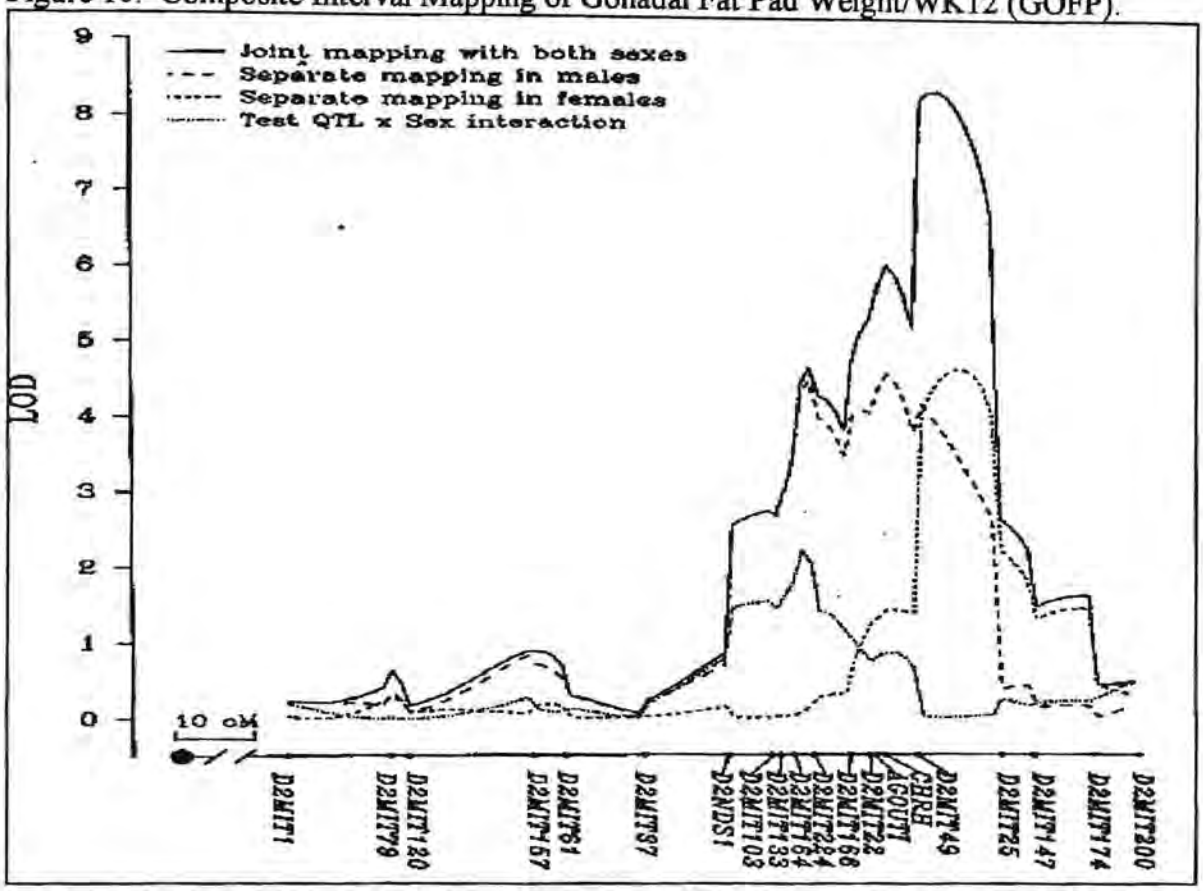
Figure 9. Composite Interval Mapping of 12 Week Body Weight (WK12).



For multiple trait analysis, the profiles for all mapping strategies displayed the same pattern. The same peaks and marker regions observed for WK12 were depicted in this graph. LOD scores ranged from 4.7 (joint mapping on fat) to 9 (joint mapping on both traits) for the proximal QTL and from 4.7 (joint mapping on weight) to 9.9 (joint mapping on both traits) for the distal QTL. The estimated QTL were between markers D2NDS1

(54 cM) and D2MIT166 (68cM) for the proximal QTL, and between markers D2MIT22 (70 cM) and D2MIT25 (85 cM) for the distal QTL.

Figure 10. Composite Interval Mapping of Gonadal Fat Pad Weight/WK12 (GOFP).



All graphs for composite interval mapping procedures resulted in more distinct peaks and regions for QTL compared to those detected by interval mapping methods. QTL effects were estimated by multiple regression (Table 13) at the closest marker near the identified QTL. As stated earlier, there was a large QTL by sex interaction (+ 0.29% for males) by marker D2MIT224 (63 cM) for GOFP. Also presented in table 13 is the percent of residual phenotypic variance explained by these markers. The variance explained was very high (> 17%) for both traits analyzed on males and the GOFP mapping

on males; however, the variation explained for GOFP on females was much lower (9%). The two QTL regions were not as well defined with the interval mapping procedures.

Figure 11. Composite Interval Mapping of WK12 and GOFP.

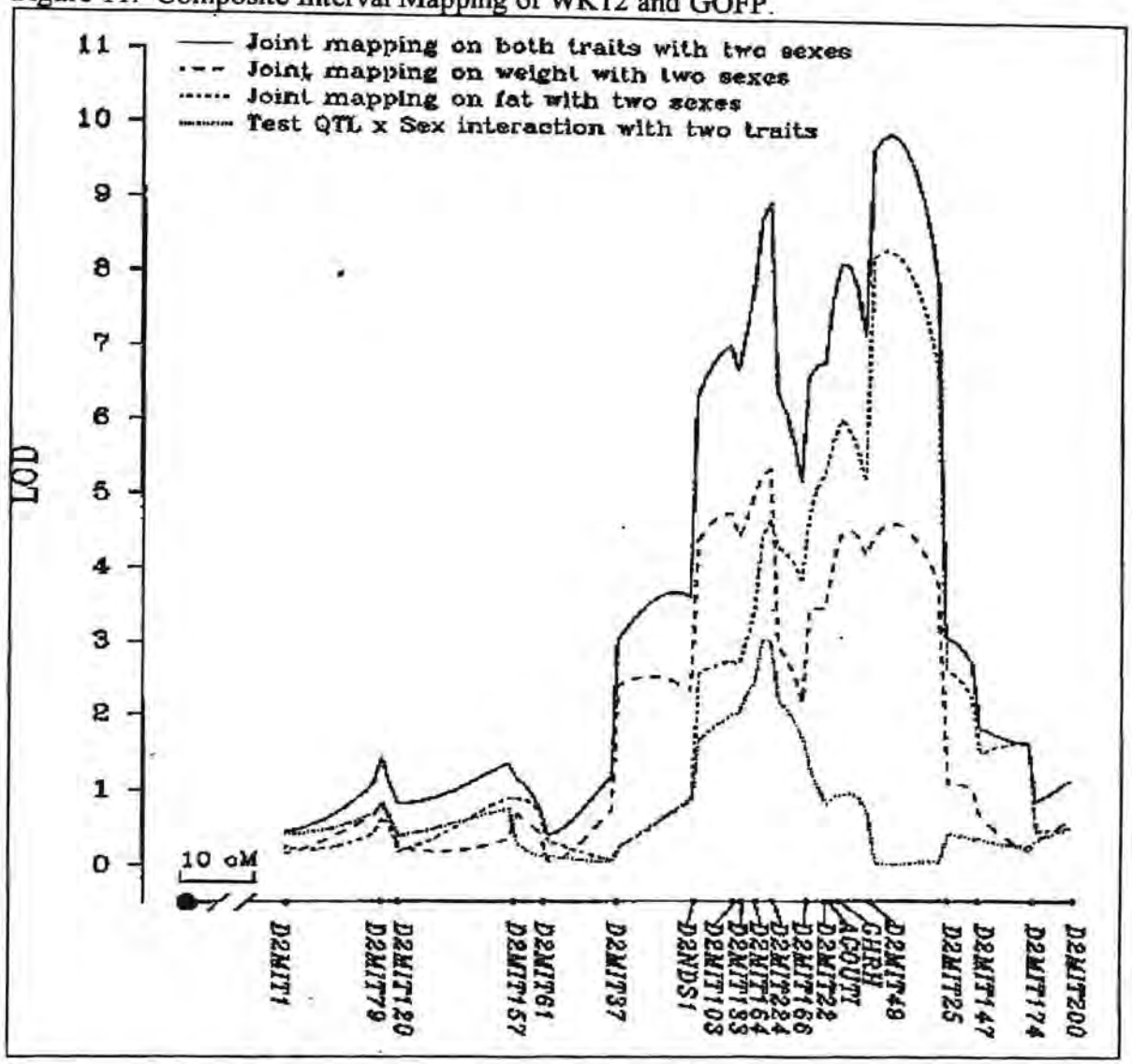


Table 13. Multiple Regression Estimates of QTL Effects for Each Sex & Trait Separately.

Trait	Sex	QTL 1 by D2MIT224	QTL2 by D2MIT49	Variance explained (%)
WK12	Male	2.40	2.63	17
	Female	3.34	1.13	19
GOFP	Male	0.26	0.20	21
	Female	-0.03	0.26	9

Table 14 compares the four mapping methods at locating the QTL for WK12 and GOFP. The ANOVA, MR, and IM procedures produced wide regions for locating all QTL. WK12 sections were always wider than GOFP. Very wide areas observed for WK12 suggest that there are multiple QTL for the trait being analyzed. However, these methods do not have the power to distinguish between one or more estimated QTL for a given trait. On the other hand, CIM was able to determine separate QTL effects by clearly locating two QTL for WK12. All strategies were capable of locating the QTL in almost the same position for WK12 (ranged from 61 to 64 cM). QTL estimates differed by only 9 cM for GOFP between all of the methods. CIM identified a possible second QTL for male GOFP at approximately 63 cM due to the large QTL by sex interaction observed. Finally, the simpler ANOVA and MR procedures located the QTL in almost the same position as the more complicated IM method.

Table 14. Comparison of QTL Mapping Strategies for WK12 and GOFP.

QTL Mapping Method	WK12	GOFP
ANOVA	61	68
Marker Regression (MR)	64	72
Interval Mapping (IM)	63	70
Composite Interval Mapping (CIM)	63 and 78	77

CHAPTER V

DISCUSSION

Currently, research conducted in hopes of genetically improving livestock is incorporating both quantitative and molecular genetic techniques. DNA molecular markers are being used in an attempt to locate QTL that control economically important traits. Knowledge gained about the genetic mechanisms causing the variation observed in body composition and growth rate between animals is very beneficial for both livestock and humans. For QTL mapping studies, the following three aspects need to be present: a widely segregating population for the traits of interest, chromosomal markers (i.e. RFLP, microsatellites), and statistical analyses to map the QTL. Identification of growth and obesity QTL in mice may expose candidate chromosomal regions harboring homologous QTL in livestock species and humans through comparative gene mapping strategies.

Descriptive Statistics of Backcross Population

For QTL mapping, the standard experimental strategy is to type individuals for molecular markers in a cross between two genetically different strains in order to determine the degree of association between regions of the genome and the quantitative

trait value. Informative individuals have, concerning a target trait or gene, a maximal heterogeneity of trait values and/or related genotypes. The power in detecting QTL can be increased by selectively typing extreme individuals for the quantitative trait (Lebowitz et al., 1987). Much greater phenotypic differences between groups containing different marker alleles can be generated by mating widely segregating inbred lines. Interspecific crosses (involving a laboratory strain and a distantly related species of *Mus*) exploit the genetic diversity inherent between wild mouse species and common laboratory strains (Copeland et al., 1993).

The availabilty and use of selection lines (M16i) and wild lines (CAST) has advantageously produced a backcross population which exhibits extreme phenotypic variation. These initial parental populations were easy to distinguish between since they differ in body size and fat percentage. Focusing on the coefficients of variation (CV) given for various traits helps to explain the large amount of variation observed in the this backcross population. There was a large change in the CV for growth periods as ontogeny progressed, suggesting a QTL with larger effects on late compared to early growth. The QTL controlling growth and obesity are being expressed in the genotypic classes (M16i/M16i and M16i/CAST) differently since there is a large degree of variation. Overall, the amount of variation within the population was quite large for all traits analyzed (> 14%). Therefore the first prerequisite stated above has been accomplished in the current QTL mapping study.

The phenotypic correlation between two economically important traits can be influenced by inheritance, environment, or both. The environment has been controlled in

this study, such that all of the animals were kept under the same conditions. When the correlation is mainly genetic, consideration must be taken about the idea of pleiotropy. Pleiotropy is probably the major cause of genetic correlations, although gene linkage may have an effect. Pleiotropy is the process whereby one gene may affect two or more traits at the same time. If pleiotropy is the cause of genetic correlations, the correlated traits would be affected by at least some of the same physiological pathways.

Under the assumption that the phenotypic correlation being comprised of mainly a genetic component, identified QTL may have pleiotropic effects on various traits. The correlations between all body weights were significant and positive. The highest was 0.947 for WK9 and WK12. The association for all fat percentage traits ranged from 0.570 (GOFP and SCFP) to 0.946 (GOFP and FATP). The correlation between WK12 and GOFP was 0.696. Eisen (1987) estimated the genetic correlation between epididymal fat pad percentage and WK12 to be high (0.57 ± 0.05). Due to these very large correlations, the suggested idea of pleiotropy must be taken into consideration for some of the discovered QTL.

Mapping of Markers

This research project developed a well-saturated genetic linkage map with SSLP microsatellite markers and candidate loci for chromosomes 2 and 15 in the mouse. This accomplished the second requirement for a QTL mapping experiment. The mapping population consisted of a large segregating backcross population produced from the initial

mating of M16i and CAST lines. All markers for both chromosomes followed the expected 1:1 Mendelian ratio, according to the Chi-square test. This indicates that no systematically significant errors were made when genotyping the population. Errors could have developed during PCR amplification of the alleles or when visually genotyping the individuals on agarose gels.

Segregation distortion is a possible second reason for not obtaining a 1:1 Mendelian ratio. Transmission ratio distortion, the occurrence of non-Mendelian ratios for some loci, has been observed in plants and animals. Segregation distortion was observed in a backcross population of mice (Biddle, 1987). There was a deficiency of males carrying the intact X chromosome from C3H/HeHa. In an interspecific backcross between C57BL/6J and *Mus spretus*, distortion was reported for chromosomes 2, 4, and 10 (Siracusa et al., 1989; Ceci et al., 1989; Justice et al., 1990). There was consistently an excess of *Mus spretus* alleles inherited. The mechanism responsible for segregation distortion may result from differential embryo survival caused by various combinations of strain alleles (Copeland et al., 1993). Since no deviations from Mendelian ratio were observed in this study, it can be concluded that no segregation distortion occurred in the population.

Genetic linkage maps were estimated by determining recombination frequencies for adjacent marker pairs (male, female, and sex-averaged). This was accomplished by using SAS (1989) to calculate the total number of heterozygotes, dividing this number by the total number of progeny (n = 421). A Chi-square test resulted in no significant differences between the estimated male and female genetic linkage maps for both chromosomes.

Linkage analysis was performed for this backcross population since differences in recombination rate could exist between sexes and populations. Overall the recombination distances appear larger in female than in male meiosis; however, some chromosomal areas have greater male recombination distances (Davisson and Roderick, 1989; White et al., 1985; Donis-Keller et al., 1987).

In mice, a small inversion was found in the proximal region of Mus spretus chromosome 17 (Hammer et al., 1989). The mouse gene-based and SSLP maps described by Copeland et al. (1993) have estimates for total genetic length less than those expected. For the gene-based map, small inversions and other rearrangements between C57BL/6J and *Mus spretus* chromosomes may suppress recombination. There may also be some recombinational suppression in the cross used to obtain the SSLP map. Marked differences in the recombination rates between sexes were identified in the PiGMaP (Archibald et al., 1995). Ellegren et al. (1994) reported that male pigs have a shorter genetic map. The most striking difference was for a region on chromosome 1, lying between markers S0122 and S0313, which had a 45 cM difference between the sexes (Archibald et al., 1995). For the genetic linkage map of the human genome, the autosomal genetic map was about 90 % longer in females than in males (Donis-Keller et al., 1987). Substantial female excess was not observed for chromosomes 14 (none) and 10 (18 %).

The previously explained procedure identified markers in similar locations to those suggested by the WI/MIT-CGR map (Copeland et al., 1993). This was beneficial since markers (and candidate genes) were initially selected from the estimates presented in the WI/MIT-CGR map. The chromosome 2 candidate genes (agouti and growth hormone

releasing hormone) were off by approximately 16 cM between the MAPMAKER (Lander et al., 1987) and WI/MIT-CGR maps. The candidate gene estimates from this research project may provide valuable information for previously published maps. Finally, the simple method of dividing the number of heterozygotes by the progeny number resulted in almost the same estimates for all markers and candidate genes as those estimated by MAPMAKER (Lander et al., 1987). The method of utilizing SAS may be more convenient for some individuals who do not have access to MAPMAKER (Lander et al., 1987).

Comparing the recombination rate (sex-averaged) and WI/MIT-CGR linkage maps, the largest marker disagreements were about 8 and 9 cM for chromosomes 2 and 15, respectively. The largest deviation between MAPMAKER (Lander et al., 1987) and sex-averaged maps was around 4 cM. All of the large deviations for marker locations between the various maps were SSLP markers which were outside of the putative QTL region. This was important since the estimated QTL locations are more reliable for use in future research projects than if the large deviations were near the QTL region. Even with these differences, the enitre chromosome lengths for 2 and 15 differed by no more than 1.2 and 4.1 cM, respectively. The approximate map estimates were similar since both populations utilized the same mating individuals (common laboratory strain and a distantly related species of *Mus*).

Statistical Analyses Comparisons

Body weights: When comparing the ANOVA and marker regression procedures for chromosome 2, all estimated body weight QTL except WK6 were in approximately the same location (± 1 or 3 cM). The WK6 QTL placement differed by only about 6 cM. There appears to be a QTL for early body weight in the 66 to 84 cM range. Another QTL influencing later body weights was estimated between 40 and 60 cM.

WK12 was the only trait which could be compared for all QTL mapping methods. All methods placed the WK12 QTL around 61 to 64 cM. Composite interval mapping was the only process to identify a second QTL for WK12 at approximately 78 cM. The growth hormone releasing hormone was estimated to be at approximately 71.5 cM in this backcross population, making it a very likely gene to be controlling body weight. Also, the agouti locus located at about 70 cM is a second candidate gene for body weight. Obese mice often attain weights of 80 g and approximately 90 % of the excess weight is due to fat (Kandutsch and Coleman, 1975).

For chromosome 15, both ANOVA and marker regression strategies identified early body weight QTL (WK3) on the proximal end at about 8 cM and late body weight QTL (WK6, WK9, and WK12) on the distal end (32 cM). Early body weight estimates (WK3) differed anywhere from about 2.5 to 6.5 cM since marker regression mapping placed the QTL at 5 adjacent postions. At these points, the MSR were all at the same minimum value. The later body weight QTL ranged from 1 to 7 cM, depending on which period was analyzed (greatest deviation for WK12).

For both chromosomes, there appear to be early and late body weight QTL controlling the phenotypes observed for all individuals. The early body weight QTL were

given an advantage in the M16i/CAST individuals and the late body weight QTL were beneficial in the M16i/M16i genotypic class. Several studies have identified M16i mice as being larger than controls at weekly ages from birth to 16 weeks (Eisen et al., 1977; Eisen and Leatherwood, 1978; Eisen, 1986). In addition, at 75 % of mature size, M16i males weighed 49 g at 45 days of age while controls weighed 29 g at 34 days of age (Eisen, 1986). Therefore, M16i mice grow at a much accelerated rate and are later maturing compared to controls.

The strain of wild origin (CAST) needs to grow fast in order to survive in nature; hence the early body weight advantage given to the M16i/CAST individuals. Also, the M16i/M16i genotypic class has an advantage for the later body weights due to them maturing later. This evidence supports the idea of early and late genes controlling body weight. However since this research was conducted for a backcross population, one is unable to measure the dominance effects. If the study was for an F_2 population, the mode of gene action could be determined for the idea of early and late body weight QTL. This project was supposed to be an F_2; however, not enough F_1 males were obtained for the backcross mating.

Growth rates: The estimated QTL for growth periods on chromosome 2 varied from 7 to 11 cM between ANOVA and marker regression strategies. The early growth QTL (GAIN2) was located proximally around 40 to 51 cM. The later growth periods (GAIN3 and GAIN4) were identified in a more distal position approximately at 65 to 75 cM. The marker regression approach identified a QTL for GAIN1 at 57 cM.

For chromosome 15, there was less of a deviation for estimated QTL than that observed for chromosome 2 (0 to 2 cM) between the two methods. GAIN1 was located very proximally at approximately 8 cM. The mid- to late-growth rate QTL were identified distally at about 32 to 49 cM.

The study of marker effects on growth rates provides a better understanding of the QTL effect throughout ontogeny. Genes have been estimated which influence adult body weight during different periods of development: early and late. As previously stated, the fact that M16i mice are larger and later maturing than controls helps to support the early vs. late hypothesis. In addition, the correlations between the various growth rates and their corresponding body weights are all above 0.53. The body weight QTL may have a pleiotropic effect on growth periods.

Organ percentages: For chromosome 2, the QTL estimates between ANOVA and marker regression varied by only about 1 or 2 cM for HRTP, LIVP, SPLP, and TESP. The estimated QTL for TESP was very proximally located at 15 to 17 cM. All other organ QTL were located in the range of 56 to 68 cM. However, the estimated KIDP QTL differed between the two methods by around 7 cM.

The KIDP QTL differed between the two methods by approximately 8 cM for chromosome 15. This QTL was positioned between the range of 16 to 24 cM. The marker regression method located QTL for LIVP (1 - 5 cM), SPLP (48 cM), and TESP (1 - 5 cM). Some of the genes which control body weight may be the same ones that affect various organ weights. All organ weights were standardized, so one cannot conclude that the organs were bigger just because the animals were larger in size. However, some of the

same hormones and chemicals produced in these organs are involved in the biological and physiological mechanisms controlling body weight and fat percentage. Therefore, an increase in body size or lipogenic activity may result in greater production of these hormones and chemicals.

Fat percentages: Both ANOVA and marker regression estimated all the chromosome 2 fat percentage QTL in the same region (± 2 to 4 cM). These estimated QTL are located on the distal end around 68 to 75 cM. The estimates of GOFP QTL for all four mapping methods varied from 2 to 9 cM, placing the QTL anywhere between 68 and 77 cM. There appears to be one QTL controlling both fat percentage traits in males and females. However, a likely second QTL for male GOFP (+ 0.29 % for males) was identified from CIM methods at approximately 63 cM. This QTL may control both SCFP and GOFP. The correlation between SCFP and GOFP was determined to be 0.57. Several studies have associated the agouti gene with obesity (Wolff, 1965; Odaka et al., 1992). The agouti locus was estimated to be at approximately 70 cM in this backcross population, making it a very likely gene to be controlling fat percentage.

For chromosome 15, GOFP QTL estimates only differed by 1 cM and were the same for the FATP QTL when comparing the ANOVA and marker regression strategies. These QTL were identified in an area around 24 to 25 cM. On the other hand, the estimates for the SCFP QTL differed by about 9 cM between the two methods. The SCFP QTL was estimated to be around 8 and 17 cM for ANOVA and marker regression methods, respectively.

In mice, a dietary obesity QTL (LOD score of 3.93) has been linked to markers D15NDS2 and D15MIT22 on chromosome 15 in mice (West et al., 1994a). The *Ghr* was a candidate gene for this QTL which was designated as *Do3*. The *Ghr* locus was mapped to the 10.2 cM point by Barton et al. (1989). This candidate gene may be controlling the SCFP QTL which was identified in a range of 8 to 17 cM. In addition, a second possible candidate locus for the resulting obesity (GOFP QTL; ~ 25 cM) identified on chromosome 15 may be *PPAR-alpha*. The gene was identifed at 32.8 cM in the backcross population utilized in this study. This locus is capable of activating the promoter of the acyl coenzyme A oxidase gene (Dreyer et al., 1992).

Due to the large phenotypic correlations for body weights and fat pad weight percentages, the QTL observed for these traits may have a pleiotropic effect as earlier described. For both chromosomes, the same genes may actually be controlling both body weights and fat pad weights. For chromosome 2, the two distinct peaks obtained from WK12 CIM are approximately in the same areas as the GOFP QTL. This supports the idea of two QTL for both body weight and fat percentage.

QTL mapping profiles for ANOVA, marker regression, and interval mapping were flat and wide, suggesting that there may be more than one QTL for WK12 and GOFP. However, these methods were unable to discern between the two QTL, especially if they were very close together on a chromosome. If only one QTL controlling a specific trait is located on a chromosome, then any of the four procedures should result in locating the QTL correctly. However, if more than one QTL is located on a chromosome, then

composite interval mapping was the only method which clearly identified two very distinct QTL.

A problem is that the researcher does not know if one or more QTL reside on a particular chromosome. The ANOVA procedures are recommended as an initial test for locating QTL on a chromosome. Once a QTL has been identified, the mapping profile should be visualized for width. The flat, wide patterns for the plotted F-values suggest that there are more than one QTL for a trait. The next step would be to use the more complicated method, composite interval mapping, to more precisely locate the QTL.

The marker regression approach identified several organ weight percentages QTL for chromosome 15 which were not estimated by ANOVA procedures. This was possibly because the calculated F-values (MSREG/MSR) were somewhat inflated, resulting in the test for significant QTL locations identifying more points than usual. If the F-values would have been lower, there would not have been QTL estimated for those traits. This occurrence did not affect the body weight or fat percentage traits since they already had very highly significant F-values. Once again, the marker regression approach has the disadvantage of only being able to identify one QTL for a trait even if more than one is present on the chromosome.

Both ANOVA and marker regression strategies are rather simple to conduct and results suggest that both methods locate QTL in approximately the same postion. More complicated QTL mapping methods also located WK12 and GOFP QTL around similar areas identified by ANOVA and marker regression. The four QTL mapping estimates are good enough to obtain a general area for the QTL. The wide array of reliable statistical

analyses fulfills the last criterion for QTL mapping studies. Knowledge is gained as to which significant markers are linked to QTL of interest. All mapping strategies are unable to locate any QTL at exactly the proper position. Usually a range of < 10 cM is reasonable to further QTL mapping in an attempt to pinpoint the chromosomal location of the QTL.

Candidate Gene and SSLP Marker Homology

The agouti (*a*) locus on mouse chromosome 2 at 87 cM (Siracusa et al., 1987) was initially discovered as a regulator of coat color pigmentation. The locus was later found to have some control on embryonic lethality, pronounced obesity, diabetes, and the development of tumors in a wide variety of tissues (Eaton and Green; Wolff, 1965; Odaka et al., 1992; Gasser and Fischgrund, 1973). The diabetic/obesity condition of these mutant mice has similarities to non-insulin-dependent diabetes in humans (Coleman, 1982).

The human homolog of *a* was cloned using an interspecies DNA-hybridization approach (Kwon et al., 1994). The human *a* gene is 85 % identical to the mouse *a* gene. Somatic cell hybrid mapping panels and fluorescence *in situ* hybridization located the human *a* gene to chromosome band 20q11.2. This chromosome region exhibits synteny conservation with the corresponding region of mouse chromosome 2 (Siracusa et al., 1990). This conserved area (about 21 cM on mouse chromosome) has the following genes: Pax1 (paired box gene-1), Pygb (brain glycogen phosphorylase), Hck1

(hemopoietic cell kinase-1), a (agouti), Rpn2 (ribophorin-2), Ada (adenosine deaminase), Ghrh (growth hormone releasing factor), and Gnas (guanine nucleotide binding protein, alpha stimulating). Also, the human *a* gene was found to be expressed in adipose tissue and testis. Suggested ideas have agouti lowering the levels of cAMP within the adipocyte to decrease lipolysis, contributing to adipocyte hypertrophy and obesity (Strålfors et al., 1984).

Growth hormone (*Gh*) plays a central role in normal growth and development in animals and *Gh* deficeincy leads to dwarfism. The major mode of action of *Gh* appears to be the stimulation of insulin-like growth factor I (*Igf1*) production, which in turn elicits a growth response from cartilage and bone (Salmon and Daughaday, 1957). More recent evidence suggests that *Gh* may also stimulate growth of chondrocytes directly (Nilsson et al., 1986). Central to understanding the mechanisms of *Gh* action will be the characterization of specific releasing factors and receptors for this hormone. The chromosome mapping of the loci for growth factors, as well as their releasing factors and receptors, will provide insights into the evolutionary relationships of these genes, suggesting steps leading to oncogenesis.

Growth hormone releasing hormone (*Ghrh*) is a hypothalamic releasing factor that influences the secretion and synthesis of *Gh*. *Ghrh* was mapped to mouse chromosome 2 at 87 cM (Copeland et al., 1993), while this gene was assigned to human chromosome 20q11.2 (Pezzolo et al., 1994). Perez et al. (1994) discovered that *Ghrh* was linked to several markers on human chromosome 20 and located the locus to a region near the centromere between D20S27 (assigned to 20p12.1-p11.23) and D20S16 (assigned to

20q12). The mapping of *Ghrh* is important in humans since gene mutations may result in *Gh* deficiency diseases such as pituitary dwarfism and isolated *Gh* deficiency (Philips, 1983). In addition, the *Ghrh* gene was mapped to bovine chromosome 13 by linkage analysis, using the International Bovine Reference Panel and Cattle Genotypic Database (Moody et al., 1995a).

A second major factor in the proper actions of *Gh* is growth hormone receptors (*Ghr*). *Ghr* acts via specific high-affinity cell surface receptors found in the liver at the highest concentration and in other tissues (Barton et al., 1989). *Ghr* mediate *Gh* actions at the cellular level and serum *Ghr* increases the stability of circulating *Gh* (Barton et al,. 1989). In humans, a form of pituitary dwarfism is characterized by high serum levels of *Gh*, unresponsiveness to *Gh* therapy, and low serum *Igf1* levels (Pertzelan et al., 1968). This disorder is known as Laron dwarfism and appears to result from the complete absence of *Gh* receptor activity. Among the mutations that lead to small body size in the mouse is the miniature locus on chromosome 15. If the miniature mutation were due to a defect in the *Ghr* gene, miniature could be a mouse model for the form of Laron dwarfism caused by *Ghr* deficiency.

The mouse *Ghr* locus maps to the proximal end of chromosome 15 at 10.2 cM, while the human *Ghr* gene is located on the proximal short arm of chromosome 5 in the p13.1-p12 region (Barton et al., 1989). Arden et al. (1990) also found the gene encoding Ghr to be located on the proximal arm of chromosome 5, although the exact location was slightly different from previous results (p13-p14). Moody et al. (1995b) assigned the *Ghr* gene to bovine chromosome 20 using linkage analysis and somatic cell mapping. This

finding provides evidence for homology between bovine chromosome 20 and the region from 5p14-p12 to 5q13 of human chromosome 5. Finally, the *Ghr* gene has been mapped to porcine chromosome 16, using radioactive *in situ* hybridization (Chowdhary et al., 1994).

Peroxisome proliferators are chemicals that cause marked proliferation of peroxisomes in the liver, brain, heart, kidney, and testis. Reddy et al. (1986) discovered that these chemicals caused hepatocelluar carcinoma in mice and rats following long-term administration. Peroxisome proliferators are believed to cause cancer in humans; however, the mechanisms involved have not been elucidated (Sher et al., 1993). Specific steroid hormone receptors (peroxisome proliferator activated receptor; *PPAR-alpha*) are known to mediate the action of peroxisome proliferators in the mouse (Issemann and Green, 1990) and rat (Gottlicher et al., 1992). *PPAR-alpha* are capable of activating the promoter of the acyl coenzyme A oxidase gene which is the key enzyme of peroxisomal fatty acid β-oxidation. Also, *PPAR-alpha* mediates the induction of a cytochrome P450 fatty acid ω-hydroxylase by clofibric acid (Dreyer et al., 1992).

PPAR-alpha has been mapped to a distal region of mouse chromosome 15 at 45 cM (Seldin and Corton, 1994) and to human chromosome 22 slightly telomeric to a linkage group of six genes and genetic markers that are located around 22q12-q13.1 (Sher et al., 1993). The human cDNA exhibited 85 % identity with mouse *PPAR-alpha*. Human *PPAR-alpha* is functional and capable of activating the acyl coenzyme A oxidase gene and inducing the cytochrome P450 fatty acid ω-hydroxylase mentioned previously.

Only one SSLP microsatellite marker used in this research project on both chromosomes was discovered to have any mammalian homolgy. D2MIT1 located very proximally on mouse chromosome 2 has also been mapped in humans to chromosome 11p (D11S14; Glaser et al., 1989). Mouse chromosome 2 shows a 0.8 cM region of conserved synteny with human chromosome 11p (Siracusa et al., 1990). However, this region is located at approximately 58 cM in the mouse, while marker D2MIT1 is positioned around 2.35 cM. Also, SSLP microsatellite marker D2H11S16 is located in this region on chromosome 2 and is designated as D11S16 in humans. Five loci in the centromeric half are homologous to genes on mouse chromosome 2: Fshb (follicle stimulating hormone-beta), D11S16, Cat (catalase-1), D11S14, and Acp2 (acid phosphatase-2,lysosomal).

Other Possible Genes for QTL Observed

In addition to the 4 candidate genes researched, there are many loci which have been identified on chromosomes 2 and 15 that are possibilities for the estimated QTL during this project. These loci are known to play some role in the mechanisms of body weight and obesity. For chromosome 2, three additional genes will be examined which are likely candidates for the QTL identified. One extra candidate loci for chromosome 15 will be reviewed. These include glucagon, vasopressin, and growth differentiation factor 5 for chromosome 2; and insulin I for chromosome 15.

Investigations of the biochemical mechanisms underlying genetic differences in fat percentage utilize various models of obesity. Genetically obese rats and mice are characterized by hyperinsulinaemia, diabetes, hyperglycaemia, increased lipogenesis, and enhanced somatic cell growth (Bray and York, 1971; Herberg and Coleman, 1977; Warbritton et al., 1994). Single-gene mutations, such as the ob/ob and yellow mice (A^{vy}), are known to cause obesity. However, the genetic variation can be studied by comparing the metabolic differences between fat and lean inbred lines which probably resulted from many individual gene variations. Many hormonal and metabolic alterations have been described between these lines, but their relative importance in the development of obesity is still unclear for some.

In contrast to insulin, which promotes energy storage in tissues, glucagon makes energy available to the tissues. Glucagon stimulates the breakdown of stored glycogen, maintains hepatic output of glucose, and promotes hepatic output of ketone bodies from fatty acid precursors (Lefebvre, 1972). Glucagon belongs to the group of lipolytic hormones (Lefebvre, 1972). In several species it enhances the release of glycerol and free fatty acids from pieces of adipose tissue. This effect has been described in rats (Rodbell and Jones, 1966), avians (Prigge and Grande, 1971), and mice (Rudman and Di Girolamo, 1967).

Even though glucagon would not directly cause obesity, a mutation in this gene could possibly be the reason for certain animals to become fatter. The glucagon locus (*Gcg*) was located at 36 cM on chromosome 2 (Lalley et al., 1987). The nearest QTL are identified for late body weight (WK6, WK9, and WK12) on chromosome 2 (around 40 -

60 cM). As stated previously for agouti, the increase in body weight could be due to an increase in fat pad weight percentage.

The hypothalamus plays a pivotal role in integrating the neural and chemical pathways that mediate hunger and satiety. Adrenal steroids seem to contribute to the onset of obesity in the Zucker rat (fa/fa) since adrenalectomy prevents symptoms of the 'fatty' syndrome (Bray and York, 1979). There appears to be abnormal regulation of the hypothalamic-pituitary-adrenal axis in fa/fa individuals (Guillaume at al., 1990). The release of adrenocorticotropic hormone in the anterior pituitary gland is regulated by hypothalamic peptides, such as corticotropin-releasing factor and arginine vasopressin (Antoni, 1986). Basal plasma arginine vasopressin levels were higher in the obese Zucker as compared with lean control rats (Pesonen et al., 1992). The increased basal plasma arginine vasopressin concentrations agree with the results obtained in hyperglycemic rat models (Brooks et al., 1989).

These studies suggest a possible link between arginine vasopressin and obesity in the mouse. The arginine vasopressin (*Avp*) locus has been identified on chromosome 2 at 72 cM (Marini et al., 1993). The nearest QTL identified in this research are the following approximated values from ANOVA procedures: DAY12 (84 cM), WK3 (66 cM), WK9 (58 cM), WK12 (61 cM), SCFP (75 cM), and GOFP (68 cM). The closeness between these QTL and the above mapping position of *Avp* makes this locus a likely candidate gene for the variations in body weight and fat pad weight percentage effects observed in the backcross population.

The TGF-β superfamily contains many multifunctional factors which appear to influence cell fate determination, differentiation, and growth (Kingsley, 1994). One of these is growth differentiation factor 5 (*Gdf5*) which is expressed in mesenchymal condensations that give rise to the limb skeleton in developing mouse embryos. In addition, *Gdf5* was isolated from human embryos, suggesting some function during early development. On the other hand, transcripts have been found in non-skeletal tissues in adult mice: heart, lung, kidney, brain, and adrenal gland. Also, *Gdf5* has been shown to be transcribed in adult human fibroblasts. The importance of *Gdf5* in mice for normal skeletal development was discovered by the brachypodism mutation (Storm et al., 1994). This locus on chromosome 2 was identified between the agouti (87 cM) and rous sarcoma oncogene (91 cM) loci (Storm et al., 1994). The *Gdf5* locus could be a possible candidate gene for the growth QTL identified by ANOVA methods: DAY12 (84 cM), WK3 (66 cM), WK9 (58 cM), WK12 (61 cM). Composite interval mapping located the WK12 QTL at 63 and 78 cM.

Insulin is an important stimulant of lipogenic activity. Insulin has been shown to reduce the concentration of long-chain fatty acyl-CoA esters and activate acetyl-CoA carboxylase, preventing the inhibition of fatty acid biosynthesis in rat adipose tissue (Halestrap and Denton, 1973; McNeillie and Zammit; 1982). Higher insulin concentrations have been reported for the fatter lines when comparing the differences between many grossly obese animals and their lean controls. Plasma insulin concentrations were higher and rose with age for mice in the large, fat VL/fDk line than in the small, lean SWR/fNIMR line (Harrison and Sinnett-Smith, 1990). In addition for

cattle, insulin amounts were greater in obese than in lean heifers (McCann and Reimers, 1985).

Plasma insulin levels in yellow mice increased 5-6 weeks of age before the animals were overtly obese (Frigeri et al., 1983). The fact that obesity is associated with hyperinsulinaemia means that the elevated insulin levels must be primary or secondary to the subsequent obesity. For example, an increase in the number of β cells secreting insulin in young preobese mice would stimulate lipogenesis. This results in excess lipid deposition and insulin resistance, causing severe hyperinsulinaemia. A study by Warbritton et al. (1994) supports this idea which examined the number of pancreatic islet cells in mice lines. In 21-day old mice, the mean number of β cells/pancreas was greater in the preobese yellow mice than in the agouti hybrid mice. Therefore, the increased cell proliferation precedes any genotype-specific increase in pancreatic insulin content or body weight.

All of these studies support an idea that the insulin I (*Ins3*) locus on chromosome 15 (48.3 cM; Meruelo et al., 1987) is a likely candidate gene for the QTL identified. The M16i mice are hyperinsulinaemic. The closest QTL located in the backcross population by ANOVA procedures are those for late body weights and mid- to late- growth rates. All of these QTL are identified in a range anywhere from 32 to 48 cM on chromosome 15. Either mouse *Ins1* or *Ins3* is the active gene for insulin I; the other is probably a pseudogene (Lalley and Chirgwin, 1984). If *Ins3* ("a novel insulin-like gene") is found to be a pseudogene, then it would not be a candidate gene for the discovered QTL in the backcross population.

Besides the major growth and obesity QTL identified on chromosomes 2 and 15, a large effect (10.16 % of the residual phenotypic variation explained by significant marker effects) was discovered for testis percentage on chromosome 2 around 15 cM. Vitamin A is necessary for support of growth, health, reproduction, and survival of animals (McDowell, 1989). The acid form of vitamin A, retinoic acid, as well as retinol acetate are able to reinitiate spermatogenesis in vitamin A-deficient rats (Van Pelt et al., 1992). One of the retinoic acid receptors, retinoid X receptor alpha, plays a role in the process of proliferation and differentiation of A spermatogonia (Van Pelt et al., 1992). The retinoid X receptor alpha locus was mapped to chromosome 2 at 17 cM by Hoopes et al. (1992). This locus may be a possible candidate gene for the increase observed in testis weight.

Past research in many species has shown that lines with above average testes size, libido, and sperm output are also above average in ovulation rate, age at puberty, or litter size. The quantitatively measured expression of male and female libido was found to be genetically correlated in sheep and mice (Land, 1973). Also, the genes controlling reproductive characteristics are common to both males and females. The hormones FSH and LH are intermediates in the physiological control of reproduction. An improvement of female reproductive traits may be acheived by selection for a correlated trait rather than from selection for the desired trait itself.

In cattle, scrotal circumference was correlated with age at puberty in heifers ($r = -0.36$), indicating that as scrotal circumference in bulls increased, their half-sib sisters reached puberty at earlier ages (Brinks et al., 1978). The average correlation of testicular diameter, circumference, length, and volume with the age at first calving, age at first

breeding, and pregnancy rate were -0.66, -0.55 and 0.62, respectively. The values are high enough to suggest that selection for testes size may be effective for altering female reproductive traits.

In mice, the correlation between testes weight and ovulation rate was 0.97 after 12 generations of selection for ovulation rate (Land, 1973). After 22 generations, the realized genetic correlation between testes weight and litter size was 0.60 ± 0.04 (Eisen and Johnson, 1981). The realized partial genetic correlation between testes weight and litter size holding body weight constant was determined to be 0.42. Also, Islam et al. (1976) discovered that a significant positive response occurred in ovulation rate from selection for testes weight. The results from both cattle and mice suggest that selection for testis size might improve female reproductive traits in other species. However, results in swine have shown small and not significant responses in ovulation rate and age at puberty when selecting for testes weight (Johnson et al., 1994).

Other Growth and Obesity QTL Mapping Studies

Until recently, the genes underlying growth, body composition, litter size, and other economically important traits in livestock have been difficult to identify. With the aid of highly saturated genetic marker maps for comparative mapping strategies, genes located in animal models can be applied to livestock. This will improve the livestock industry by decreasing the overproduction of fat while increasing the animal's growth. QTL studies are also making progress toward understanding the genetic basis of human

diseases, such as obesity, hypertension and atherosclerosis. In addition to this research, several studies have been conducted in order to identify QTL affecting lipid metabolism. On the other hand, fewer studies have been attempted for the elucidation of growth rate QTL.

There have been some projects in mice (Collins et al., 1993; Medrano et al., 1991; Horvat and Medrano, 1995) and swine (Andersson et al., 1994) to identify growth rate QTL. Collins et al. (1993) utilized the Quakenbush-Swiss mouse strain (exhibits increased body weight) to locate two markers on chromosome 10 which had associations with growth QTL. A high growth gene (*hg*), causing 30-50 % increase in 3-6 week gain and mature body size, was mapped to mouse chromosome 10 (Medrano et al., 1991). Horvat and Medrano (1995) characterized the effects of the *hg* locus, identifying the gene in a more precise location. Comparative mapping illustrates the distal half of chromosome 10 belonging to a block of homologous genes on human chromosome 12q13-q24 (O'Brien et al., 1993). An experiment conducted by Andersson et al. (1994) discovered QTL with large effects on early growth for swine chromosomes 4 and 13.

The previously mentioned research also located a QTL with large effects on fat deposition on swine chromosome 4 (Andersson et al., 1994). QTL were found which controlled total plasma cholesterol and carcass lipid percentage on mouse chromosome 7 and plasma total cholesterol and subcutaneous fat pad on chromosome 6 (Warden et al., 1993). Significant genetic linkage on mouse chromosome 4 was observed for total body adiposity (West et al., 1994b). In addition, two QTL were found on chromosomes 9 and 15 for total adiposity (West et al., 1994a). A QTL on mouse chromosome 1 was seen to

effect plasma HDL cholesterol levels and atherosclerotic lesion formation during an atherogenic diet (Paigen et al., 1987). A second QTL of unknown chromosome location was also found to determine levels of HDL cholesterol on an atherogenic diet (Paigen et al., 1989).

Single-gene obesity loci in mice are known for the *a* (agouti) locus on chromosome 2 (Bultman et al., 1992), the *tub* (tubby) locus on chromosome 7 (Jones et al., 1992), the *db* (diabetes) locus on chromosome 4 (Bahary et al., 1990), the *Ifabp* (intestinal fatty acid binding protein) locus on chromosome 3 (Sweetser et al., 1987), the *Ad* (adipose) locus on chromosome 7 (Wallace and MacSwiney, 1979), and the *ob* (obese) locus on chromosome 6 (Friedman et al., 1991). The genes with the most dramatic effects on obesity are the *a* (A^y allele) and *ob* loci (Herberg and Coleman, 1977). The *a* locus results in the development of obesity due to fat cell hypertrophy. The *ob* locus causes increased lipogenesis, decreased lipolysis, and marked obesity. These are just a few examples; a mouse 'fat map' shows the chromosomal locations of genes involved in lipid metabolism, as well as mutations which are associated with lipid metabolism disarrangement (Lusis and Sparkes, 1989).

QTL Identification and Characterization

Initially, a whole genome screening procedure was utilized where each of the 19 autosomes contained three evenly spaced SSLP microsatellite markers (Pomp et al., 1994). A major finding was the discovery of a distal marker on chromosome 2 being

linked to a QTL with very large effects on body weight and fat percentage. An additional marker on distal chromosome 15 was found to be linked to a similar QTL with smaller effects. Pomp et al. (1994) localized the QTL for body weight and fat percentage to chromosomes 2 and 15. The current research project has now narrowed in on the QTL location to be between microsatellite markers about 10 cM apart. Chromosomes 2 and 15 were saturated with 20 and 10 markers (microsatellites and candidate genes). The next step will be to further characterize and ultimately identify the actual gene responsible for body weight and fat percentage in the mouse.

To accomplish these goals, congenic lines will need to be produced which differ only in the small chromosomal segment known to harbor the QTL. Congenic lines are a strain of animals developed from an inbred (isogenic) line by repeated matings with animals from another stock that have a foreign gene. The final congenic strain then presumably differs from the original inbred strain only by the presence of this gene region. These lines will be produced by repeated backcrossing of an obese inbred selection line (M16i) and a standard inbred line (C57). Microsatellite markers flanking QTL will be analyzed as to maintain the desired QTL region. The QTL will be fixed after approximately eight generations by intermating heterozygous mice for the QTL and selecting progeny that are homozygous for these flanking markers.

Once the QTL is fixed in the population, an in depth phenotypic analysis will be performed on growth and body composition to obtain a better characterization of the QTL overall effects. For obesity studies, genes are known to have an effect on fat percentage; however, the individual's diet also controls the amount of fat present (Lusis and Sparkes,

1989). Therefore, phenotypic effects should be analyzed under both low and high fat diets. The overall effect of the QTL on body weight and fat percentage should then be realized. To pinpoint the QTL location, additional markers within the QTL region will be genotyped to identify recombinant breakpoints. The ultimate classification of the QTL will be to physically map a much smaller region for candidate genes and use transgenics to prove a cause and effect. Hopefully, further research will provide a better understanding of the mechanisms involved with specific genes controlling growth and obesity. This will improve human health, as well as benefit the various livestock production industries.

Implications

The murine body weight and fat percentage QTL identified in this study will expedite research in livestock species, finding associated genes through comparative mapping. The mapping of these economically important genes will bring about livestock improvement from the utilization of marker assisted selection (MAS). MAS is the process of making selection decisions based on an animal's phenotypic and genotypic information. The potential efficiency of MAS depends on the trait's heritability, the selection method, and the additive genetic variance of the marker loci (Lande and Thompson, 1990). The identification of marker loci linked to QTL will allow for the easy manipulation of major genes, providing a possible route to their ultimate isolation (Haley, 1991).

Several examples of genetic markers have already been associated with QTL in livestock production: the halothane gene in swine (Houde et al., 1993), kappa-casein and

beta-lactoglobulin genes in cattle (Medrano and Aguilar-Cordova; 1990a,b), the Booroola fecundity gene in sheep (Montgomery et al., 1993), the callipyge gene in sheep (Muggli-Cockett et al., 1993), and the estrogen receptor gene in swine (Rothschild et al., 1994). Currently, these advantageous single gene mutations or genetic marker mutations near a gene with major effects are being utilized in various selection programs. MAS should accelerate genetic gain by increasing selection accuracy, reducing generation interval, and increasing selection differentials. The surge in discovering new markers, the progress of mice and livestock genome mapping committees, and specified application goals should increase the plausibility of using MAS in the future.

In addition, the QTL located in mice can be comparatively mapped in humans to help combat obesity and growth disorders. As stated previously, mutations in the *Ghrh* locus may result in *Gh* deficiency diseases, such as pituitary dwarfism (Philips, 1983). Laron dwarfism appears to be the result of a complete absence of *Ghr* activity (Pertzelan et al., 1968). Locating genes involved in obesity will have considerable clinical significance for humans. Atherosclerosis, the primary cause of coronary artery disease in humans, is the deposition of fatty substances in the inner walls of the arteries. A better understanding of a gene's ability to control obesity will be extremely important in order to improve human health and quality of life.

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APPENDIX 1--DNA EXTRACTION FROM MOUSE TAILS

1) Begin by setting the incubator to 50°C and assemble a sufficient number of 1.5 ml microcentrifuge tubes with lids closed.

2) Prepare the lysing solution; count on using 840 µl of lysing solution per tail plus a few extra to allow for pipetting errors. Take 5-10 tail sections from the -80°C freezer at a time and place them in labeled digestion tubes.

3) Incubate with 120 rpm shaking at 50°C overnight. Digests can incubate up to 24 hours with no problems.

4) Label 3 tubes for each tail digest. Add 500 µl phenol/chloroform to each tail digest, shake vigorously, and centrifuge at 3500 rpm for 10 min at room temperature.

5) Transfer aqueous layer to a new tube. Add 500 µl phenol to tube, shake vigorously, and centrifuge at 3500 rpm for 5 min.

6) Transfer aqueous layer to a new tube. Add 500 µl chloroform to tube, shake vigorously, and centrifuge at 3500 rpm for 5 min.

7) While the tubes are spinning from step 6, add 0.6 volume (around 480 µl) cold isopropanol to each tube.

8) Transfer aqueous layer from the chloroform extraction directly to the corresponding isopropanol tube. Shake to precipitate DNA. Tubes may be stored at -20°C or -80°C for 20 minutes to several hours if a stopping point is required.

9) Centrifuge DNA tubes at 12000 rpm for 15 min at room temperature in the IEC tabletop centrifuge.

10) Decant supernatant carefully, making sure to keep an eye on the pellet. Rinse the pellet and decant carefully.

11) Allow the pellet to dry overnight on the bench covered with Cling Wrap.

12) Rehydrate DNA pellet in 150 µl of TE pH 8 overnight at room temperature. Mix well with wide-orifice tips before taking a spectrophotometer reading.

APPENDIX 2--DETERMINATION OF DNA CONCENTRATION USING THE LAMBDA 3B SPECTROPHOTOMETER

1) Turn the spectrophotometer on and allow plenty of time for the machine to warm up. This will take around 15 minutes (the wavelength display should read 850.0 nm and the right hand display needs to stop fluctuating wildly).

2) Select the volume of cuvette needed. Make a blank by putting in the following volumes of ddH_20. The regular size needs 2 ml solution, the mini 1 ml, and the micro 100 µl. Generally, a mini cuvette is chosen and a 100:1 dilution is made of the sample (10 µl of DNA sample and 990 µl ddH_20).

3) Place the blank cuvette into the spectrophotometer, and have the machine goto 260 nm wavelength UV light. Auto zero the machine so the display reads 0.0000.

4) Now prepare a sample cuvette (10 µl of DNA sample and 990 µl ddH_20). Mix the sample well with a wide tip pipette being careful not to introduce air bubbles into the solution. Insert the cuvette into the spectrophotometer and close the lid. Record the absorbance reading at the 260 nm UV wavelength. If the absorbance reading does not stabilize and is fluctuating widely, the sample may need to be remixed.

5) After recording a 260 nm absorbance reading, then change the UV wavelength to 280 nm. Record the new sample absorbance at this wavelength.

6) This DNA sample can now be discarded, and the cuvette should be rinsed out using the cuvette washer. Rinse the cuvette twice with ddH_20 and once with acetone (Sigma Chemical Company, St. Louis, MO).

7) Continue steps 2-6 until all DNA samples have a 260 and 280 nm absorbance reading. The spectrophotometer should be rezeroed every 5 to 10 samples, using a blank cuvette.

8) An Abs260/Abs280 ratio is determined to give an idea of the degree of impurities left behind from the extraction procedure. A ratio of 1.6 to 1.9 is considered satisfactory. A lower ratio implies protein impurity and the higher ratio implies either RNA or solvent impurity.

9) The DNA samples concentration is calculated from the following equation:

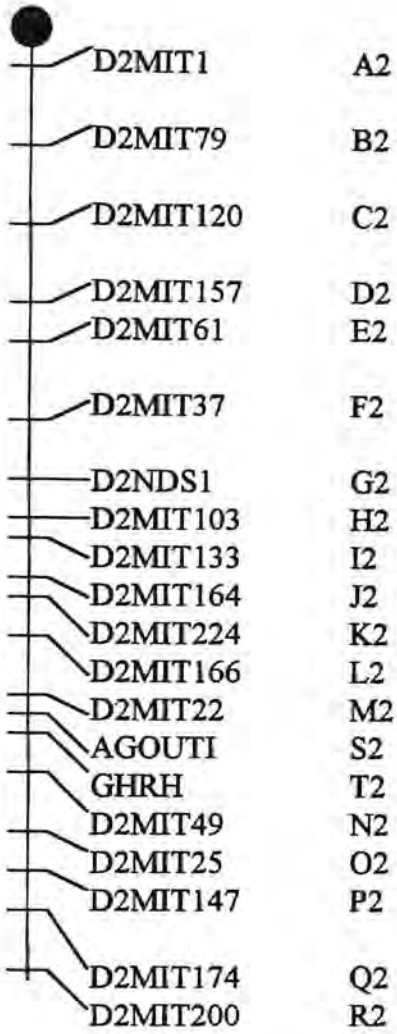
Abs260 X Dilution Factor X 50 ng/µl = DNA concentration in ng/µl

10) A 200 µl of a 50 ng/µl working solution can be made. The final concentration of the working solution should be 10000 ng of DNA.

10000 ng / concentration of DNA sample in ng/µl = µl of DNA to add
200 µl - µl of DNA to add = µl of 1X TE buffer to add

11) Mix the PCR working solutions well and store at 4°C, then store the stock samples at either -20°C or -80°C. Make sure to mark the tubes on both the side and cap.

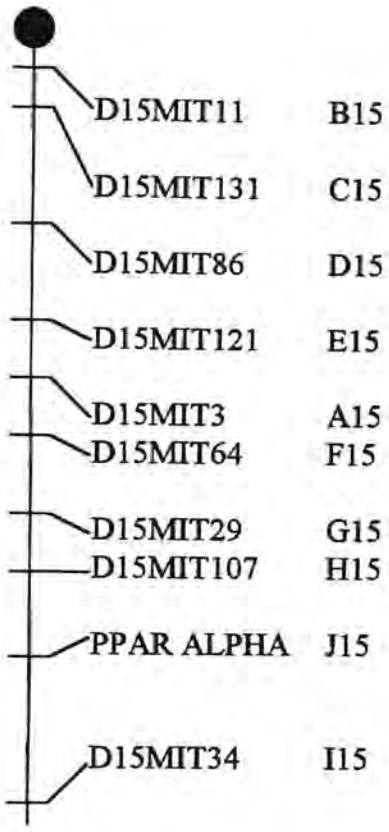
APPENDIX 3--GENETIC LINKAGE MAP FOR CHROMOSOME 2



Marker	Sex-Averaged	Female	Male	Marker	Sex-Averaged	Female	Male
A2	2.35	2.35	2.35	K2	61.02	59.56	62.46
B2	12.80	13.89	11.74	L2	65.53	63.41	67.62
C2	15.18	15.81	14.56	M2	68.38	67.74	69.03
D2	28.01	27.83	28.18	S2	70.04	70.14	69.97
E2	31.57	31.20	31.94	T2	71.47	71.58	71.38
F2	40.36	41.30	39.45	N2	75.03	74.46	75.61
G2	49.15	50.43	47.9	O2	83.82	84.56	83.12
H2	53.90	56.20	51.66	P2	87.62	87.93	87.35
I2	55.80	56.68	54.95	Q2	94.27	95.62	92.98
J2	58.41	57.16	59.64	R2	99.02	100.43	97.67

Note: All distances are given in cM.

APPENDIX 4--GENETIC LINKAGE MAP FOR CHROMOSOME 15



Marker	Female	Male	Sex-Averaged
B15	5.72	5.72	5.72
C15	7.64	7.6	7.62
D15	12.45	15.11	13.8
E15	15.82	20.74	18.31
A15	20.15	28.25	21.61
F15	22.07	34.35	23.99
G15	24.95	39.04	27.79
H15	27.83	45.14	32.3
J15	32.8	48.19	36.27
I15	47.72	58.85	48.97

Note: All distances are presented in cM.

APPENDIX 5--CHARACTERISTICS OF MICROSATELLITE MARKERS USED FOR GENOTYPING CHROMOSOMES 2 AND 15

Marker	Code[a]	PCR Type[b]	Allele Size[c]	Allele Order[d]	Frequency M16i/M16i[e]	Recomb. Rate[f]	MIT Location[g]
D2MIT1	A2	1	140	C > M	52.7	-	2.35
D2MIT79	B2	1	264	C > M	52.3	10.45	12.42
D2MIT120	C2	1	180	C > M	51.3	2.38	23.12
D2MIT157	D2	1	156	C > M	48.5	12.83	33.18
D2MIT61	E2	1	158	C > M	47.3	3.56	37.71
D2MIT37	F2	1	196	C > M	50.4	8.79	46.66
D2NDS1	G2	1	154	M > C	50.1	8.79	52.24
D2MIT103	H2	1	160	C > M	48.7	4.75	55.58
D2MIT133	I2	1	231	C > M	49.6	1.9	57.8
D2MIT164	J2	1	149	C > M	48	2.61	60.43
D2MIT224	K2	1	143	C > M	47.7	2.61	63.4
D2MIT166	L2	1	157	C > M	48	4.51	67.8
D2MIT22	M2	1	182	C > M	48	2.85	73.4
D2MIT49	N2	1	134	M > C	48.9	5.7	81.67
D2MIT25	O2	1	138	C > M	48.2	8.79	85.71
D2MIT147	P2	1	91	M > C	48.2	3.8	89.71
D2MIT174	Q2	1	119	M > C	49.6	6.65	92.59
D2MIT200	R2	1	158	C > M	49.2	4.75	98.53
D15MIT11	B15	1	126	C > M	48	-	5.72
D15MIT131	C15	1	161	C > M	48.5	1.9	11.3
D15MIT86	D15	1	220	C > M	48	6.18	18
D15MIT121	E15	1	130	C > M	47.7	4.51	24.7
D15MIT3	A15	1	152	C > M	50.4	3.33	30.3
D15MIT64	F15	1	128	C > M	49.2	2.38	29.2
D15MIT29	G15	1	184	C > M	49.2	3.8	33.7
D15MIT107	H15	1	187	C > M	52.7	4.51	40.5
D15MIT34	I15	2	124	M > C	48.7	11.16	53.1

[a] OSU Laboratory Identification

[b] PCR conditions (1 = 40 cycles, 55°C; 2 = 30 cycles, 55°C)

[c] CAST/Ei allele size Dietrich et al. (1994)

[d] Parental genotypes (C = CAST/Ei; M = M16i) allele size order

[e] M16i/M16i genotypic frequency; CAST/Ei genotypic frequency = (1-M16i/M16i Frequency

[f] Recombination rate between adjacent markers (sex-averaged); OSU marker estimates (cM) for genetic linkage map; proximal marker standardized to MIT map

[g] WI/MIT-CGR microsatellite linkage map; marker locations (cM) are based on The Jackson Laboratory Mouse Genome Database, 1995

APPENDIX 6--PCR IN 96-WELL MICROTITER PLATE FORMAT

1) Determine the total number of 15 µl reactions needed for each primer. This amount has 1 extra reaction per plate row added onto it since part of the mixture is lost when pipetting. This ensures that there will be enough primer in the end.

2) The total number determined is then multiplied by the base formula for one reaction to calculate the amount needed in the master mix:

PCR H_2O	10X Buffer	10X $MgCl_2$	dNTPs	Primer	DNA	*Taq*	Total
9.78 µl	1.5 µl	1.5 µl	0.3 µl	1.25 µl	0.6 µl	0.075 µl	15 µl

3) Fill out a PCR reaction data sheet which contains the reaction number, who did the PCR, the date, the primers used, the total volume of reagents used, the DNA order in the plate, the number of cycles in the PCR machine, file number, and associated PCR temperatures.

4) Make sure to wear gloves at all times and sterilize the lab bench, pipetters, and ice bucket. The titer plates should be properly labeled with reaction number, who did the PCR, the number of cycles to be ran, and date.

5) Remove the 10X buffer (KCl, Tris-HCl pH 9, and Triton X; Promega, Madison, WI) , 10X $MgCl_2$, dNTPs (New England BioLab, Beverly, MA), and primers from the freezer to thaw at room temperature, placing them on ice. Also place the PCR water, PCR oil (Sigma Chemical Company, St. Louis, MO), DNA, and PCR plates (Falcon 3911) on ice. Make sure to vortex and pulse spin all reagents and DNA tubes before mixing.

6) An extra plate is used for adding the master mix to the plates. Cut out the number of rows which corresponds to the total number of primers. Separate master mixes are made for each primer in 1.5 ml microcentrifuge tubes.

7) Add to the appropriate tubes PCR H_2O, 10X buffer, 10X $MgCl_2$, dNTPs, primer, and *Taq* polymerase (Promega, Madison, WI), holding the later in a benchtop cooler and adding last as to return it to the freezer when done) Vortex and pulse-spin tubes. Add an equal amount of master mix to one row in the extra plate. Use a multichannel pipetter to add 14.4 µl of mix to each well (column A to H).

8) Add 0.6 µl of 50 ng/µl DNA to each well by a single tip pipetter. Finally add 50 µl of PCR oil to each well by using the multichannel pipetter. Place the first two plates in the PCR machine right away and the other plates may be stored at -20°C until they are run.

APPENDIX 6A--WORKING SOLUTIONS

1X Lysing Buffer
25 ml of 1 M Tris-HCL pH 8.0
15 ml of 5M NaCl
10 ml of 0.5 M EDTA
50 ml of 10% SDS
400 ml of ddH_20

5M Sodium Perchlorate
70.25 g of Sodium Perchlorate
ddH_20 to make volume 100 ml

Phenol at pH 7-7.8 (TE Saturated)
100 g Phenol at -20°C
1 M Tris-HCL pH 8
1X TE pH 8
0.1 g 8-Hydroxyquinoline

1X TE
1.576 g Tris-HCL, pH 8
0.37224 g EDTA
ddH_20 to make 1 L

Phenol/Chloroform/Isoamyl Alcohol 25:24:1
25 ml of 24:1 Chloroform/Isoamyl Alcohol
25 ml Phenol
1X TE pH 8

Chloroform/Isoamyl Alcohol 24:1
480 ml Chloroform
20 ml Isoamyl Alcohol

Lysing Solution 4% Agarose Gels
4 Parts 1X Lysing Buffer
1 Part 5 M Sodium Perchlorate
1% β-Mercaptoethanol
100 µg/ml Proteinase K

100 ml 1X TBE
2 g BioRad Agarose
2 g NuSieve Agarose

1% Agarose Gels
100 ml 1X TBE
1 g BioRad Agarose

1X TBE
0.09 M Tris-Borate
0.002 M EDTA

Chloroform, EDTA, 8-Hydroxyquinoline, Isoamyl Alcohol, β-Mercaptoethanol, Phenol, Proteinase K, SDS, Sodium Perchlorate, Tris, and Tris-Borate: Fisher Scientific, Fair Lawn, NJ
NaCl: Sigma Chemical Company, St. Louis, MO
BioRad Agarose: BioRad Laboratories, Hercules, CA
NuSieve Agarose: FMC BioProducts, Rockland, ME

APPENDIX 7--PRIMER SEQUENCE OF CANDIDATE GENES

Agouti (*a*)

5'-Primer	Exon 2	CCTA GTGA GCTT CCTG TGCT TCTT	(24 bp)
3'-Primer	Exon 3	CTTC TCGG CTTC TTTT CTGC	(20 bp)

Growth Hormone Releasing Hormone (*Ghrh*)

1)	5'-Primer	Exon 2	TGAA GGAT GCTG CTCT GGGT	(20 bp)
	3'-Primer	Exon 3	TGAT GTCC TGGA TCAC TTTC	(20 bp)
2)	5'-Primer	Exon 3	GGAA AGTG ATCC AGGA CATC A	(21 bp)
	3'-Primer	Exon 4	CTTG TCCT CTGT CCAC ATGC T	(21 bp)

Peroxisome Proliferator Activated Receptor (*Ppar alpha*)

5'-Primer	Exon 5	CGAC AAGT GTGA TCGG AGCT GCAA G	(25 bp)
3'-Primer	Exon 6	GTTG AAGT TCTT CAGG TAGG CTTC	(24 bp)

Growth Hormone Receptor (*Ghr*)

1)	5'-Primer	Exon 9	CCCA GTCC CAGT TCCA AAGA TT	(22 bp)
	3'-Primer	Exon 10	CTCA TCCA CATC TGCT TCAT C	(21 bp)
2)	5'-Primer	Exon 7	CCTC AGAC GAAC ATAT TGGA	(20 bp)
	3'-Primer	Exon 8	GCAT GACT GCTA CTCC AAAT	(20 bp)

Note: Both the 5'- and 3'-primer sequences are listed in order of 5' to 3'.

APPENDIX 8--PCR PRIMER WORKING SOLUTIONS FROM LYOPHILIZED OLIGONUCLEOTIDES

Dissolve Oligonucleotides

1) The tubes received from the processing core have 100 µl of PCR water added, leaving them undisturbed overnight.

2) The tubes containing primer are vortexed, pulse-spun, and combined into one 1.5 ml microcentrifuge tube. This tube is vortexed and pulse- spun, becoming the stock primer solution.

Measure Absorbance

1) A 1 ml cuvette has 998 µl of water added to zero the spectrophotometer at a wavelength of 260 nm.

2) Add 2 µl of primer stock solution to the cuvette, mix well, and record the absorbance at a wavelength of 260 nm.

3) There is 398 µl of primer stock solution remaining and this was a 1:500 dilution.

Dilution

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

   A) A primer information sheet from the processing core should have come with the primers. From this sheet obtain the µg/OD and molecular weight values.

   B) Calculate the µM concentration of the primer stock solution by using the following equations:

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

   C) The total volume for a 50 µM primer stock solution needed is determined and diluted from use of the following equations:

      Total Volume (µl) = [(µM stock)(µl of stock remaining)] / 50 µM
      µl to Add = Total Volume (µl) - µl of stock remaining

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

2) Once the appropriate amount of PCR water is added to dilute the primer stock solution, it is stored at -20°C.

3) A 5 μM primer working solution is prepared by adding 30 μl of both the forward and reverse primer 50 μM stock and 240 μl of PCR water to make a total of 300 μl.

4) These working solutions may also be stored at -20°C and are ready for use in PCR.

APPENDIX 9--MALE PHENOTYPIC SPECIFICATIONS FOR EACH TRAIT

Trait	Mean	Standard Deviation	Coefficient of Variation	Range	Normality[c]
12 d wt (day12)	7.26	1.06	14.64	4.13 - 10.1	P = .17
3 wk wt (wk3)	12.96	2.7	20.83	6.1 - 20.2	P = .2
6 wk wt (wk6)	34.35	5.47	15.93	11.9 - 45.8	P < .01
9 wk wt(wk9)	42.07	5.34	12.7	21.1 - 58.6	P = .97
12 wk wt (wk12)	45.27	5.86	12.96	18.1 - 67.1	P = .95
day12-wk3 gain	5.7	1.95	34.18	.5 - 11.22	P = .45
wk3-wk6 gain	21.39	4.51	21.11	2.1 - 30.6	P < .001
wk6-wk9 gain	7.72	4.01	51.98	-3.1 - 21.6	P < .001
wk9-wk12 gain	3.2	2.44	76.19	-7.8 - 12.1	P = .98
heart(hrt) wt	258.52	36.91	14.28	176 - 398	P = .46
liver(liv) wt	3472.5	543.7	15.66	1922 - 5072	P = .83
spleen(spl) wt	214.04	55.01	25.7	42 - 534	P < .001
kidney(kid) wt	389.64	70.96	18.21	195 - 610	P = .78
hrt wt/wk12	.573	.074	12.85	.394 - .827	P < .05
liv wt/wk12	7.65	.8	10.48	4.35 - 10.47	P = .99
spl wt/wk12	.474	.119	25.04	.088 - 1.148	P < .001
kid wt/wk12	.861	.135	15.65	.525 - 1.22	P < .001
testis(tes) wt	95.62	19.47	20.37	51 - 208	P = .001
tes/wk12	.213	.046	21.43	.104 - .407	P < .05
scf[a] wt	280.5	160.22	57.12	59 - 1127	P = 0
gof[b] wt	545.22	260.95	47.86	49 - 1802	P < .001
scf+gof (fat) wt	825.72	400.88	48.55	108 - 2929	P < .001
scf wt/wk12	.606	.308	50.75	.205 - 2.131	P = 0
gof wt/wk12	1.17	.47	40.37	.17 - 2.69	P < .001
fat wt/wk12	1779.7	731.9	41.12	375 - 4555	P < .001

[a] scf=subcutaneous fat pad
[b] gof=gonadal fat pad
[c] Normal distribution test (Shapiro-Wilk statistic)

APPENDIX 10--FEMALE PHENOTYPIC SPECIFICATIONS FOR EACH TRAIT

Trait	Mean	Standard Deviation	Coefficient of Variation	Range	Normality[c]
12 d wt (day12)	7.1	1.02	14.3	4.24 - 9.8	P = .39
3 wk wt (wk3)	12.56	2.37	18.88	5.5 - 20.1	P = .85
6 wk wt (wk6)	29.79	4.19	14.06	13.1 - 38.3	P < .001
9 wk wt(wk9)	34.08	4.08	11.97	21.9 - 46.3	P = .83
12 wk wt (wk12)	35.71	4.75	13.3	21 - 50.8	P = .48
day12-wk3 gain	5.46	1.71	31.38	.74 - 10.8	P = .51
wk3-wk6 gain	17.23	3.4	19.75	1.4 - 24.2	P < .001
wk6-wk9 gain	4.29	2.97	69.27	-2.4 - 14.8	P < .001
wk9-wk12 gain	1.63	2.03	124.78	-3.8 - 10	P = .61
heart(hrt) wt	214.69	34.81	16.22	135 - 463	P = 0
liver(liv) wt	2670.2	455.5	17.06	1538 - 4419	P = .27
spleen(spl) wt	210.97	65.94	31.26	101 - 626	P = 0
kidney(kid) wt	273.01	43.84	16.06	141 - 431	P = .45
hrt wt/wk12	.609	.137	22.45	.43 - 2.022	P = 0
liv wt/wk12	7.47	.75	10.01	5.89 - 9.57	P < .01
spl wt/wk12	.593	.174	29.44	.34 - 1.758	P = 0
kid wt/wk12	.768	.107	13.96	.507 - 1.364	P < .01
scf[a] wt	200.14	128.93	64.42	25 - 932	P = 0
gof[b] wt	173.17	162.55	93.87	1 - 1057	P = 0
scf+gof (fat) wt	374.66	264.32	70.55	41 - 1491	P = 0
scf wt/wk12	.542	.305	56.38	.068 - 2.342	P = 0
gof wt/wk12	.461	.391	84.92	.004 - 2.433	P = 0
fat wt/wk12	1005.9	615.6	61.19	150.2 - 3422.1	P = 0

[a] scf=subcutaneous fat pad

[b] gof=gonadal fat pad

[c] Normal distribution test (Shapiro-Wilk statistic)

APPENDIX 11--POOLED (MALE AND FEMALE) PHENOTYPIC SPECIFICATIONS FOR EACH TRAIT

Trait	Mean	Standard Deviation	Coefficient of Variation	Range	Normality[c]
12 d wt (day12)	7.18	1.04	14.51	4.13 - 10.1	P = .09
3 wk wt (wk3)	12.76	2.55	19.96	5.5 - 20.2	P = .14
6 wk wt (wk6)	32.1	5.38	16.76	11.9 - 45.8	P = .3
9 wk wt(wk9)	38.12	6.21	16.29	21.1 - 58.6	P = .27
12 wk wt (wk12)	40.54	7.17	17.68	18.1 - 67.1	P = .64
day12-wk3 gain	5.58	1.84	32.92	.5 - 11.22	P = .2
wk3-wk6 gain	19.33	4.51	23.32	1.4 - 30.6	P = .08
wk6-wk9 gain	6.02	3.93	65.19	-3.1 - 21.6	P = 0
wk9-wk12 gain	2.42	2.38	98.13	-7.8 - 12.1	P = .96
heart(hrt) wt	236.82	42.03	17.75	135 - 463	P < .001
liver(liv) wt	3075.2	642.4	20.89	1538 - 5072	P < .05
spleen(spl) wt	212.52	60.62	28.52	42 - 626	P = 0
kidney(kid) wt	331.88	83.02	25.02	141 - 610	P < .001
hrt wt/wk12	.591	.111	18.78	.394 - 2.022	P = 0
liv wt/wk12	7.57	.78	10.31	4.35 - 10.47	P = .97
spl wt/wk12	.533	.16	30.05	.088 - 1.758	P = 0
kid wt/wk12	.815	.13	15.99	.507 - 1.364	P = .18
testis(tes) wt	95.62	19.47	20.37	51 - 208	P = .001
tes/wk12	.213	.046	21.43	.104 - .407	P < .05
scf[a] wt	240.8	150.89	62.66	25 - 1127	P = 0
gof[b] wt	361.41	286.49	79.27	1 - 1802	P = 0
scf+gof (fat) wt	603.42	408.21	67.65	41 - 2929	P = 0
scf wt/wk12	.574	.308	53.61	.068 - 2.342	P = 0
gof wt/wk12	.822	.562	68.44	.004 - 2.686	P = 0
fat wt/wk12	1398.4	779.3	55.73	150.2 - 4555	P = 0

[a] scf=subcutaneous fat pad

[b] gof=gonadal fat pad

[c] Normal distribution test (Shapiro-Wilk statistic)

APPENDIX 12--LEAST-SQUARES MEANS AND F-VALUES FOR EACH TRAIT ANALYZED WITH CHROMOSOME 2 MARKERS

12 DAY BODY WEIGHT (DAY12)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	7.18 ± .05	7.27 ± .05	1.58	.2102
B2	12.8	7.17 ± .05	7.29 ± .05	2.74	.0987
C2	15.18	7.16 ± .05	7.3 ± .05	3.93	.0483
D2	28.01	7.13 ± .05	7.31 ± .05	6.14	.0137
E2	31.57	7.14 ± .05	7.31 ± .05	5.45	.0201
F2	40.36	7.14 ± .05	7.31 ± .05	5.38	.021
G2	49.15	7.16 ± .05	7.3 ± .05	3.67	.0562
H2	53.9	7.15 ± .05	7.3 ± .05	4.49	.0347
I2	55.8	7.15 ± .05	7.3 ± .05	4.26	.0398
J2	58.41	7.14 ± .05	7.31 ± .05	5.37	.021
K2	61.02	7.13 ± .05	7.31 ± .05	6.25	.0128
L2	65.53	7.11 ± .05	7.33 ± .05	8.8	.0032
M2	68.38	7.12 ± .05	7.32 ± .05	7.88	.0053
S2	70.04	7.11 ± .05	7.33 ± .05	9.51	.0022
T2	71.47	7.11 ± .05	7.32 ± .05	8.3	.0042
N2	75.03	7.11 ± .05	7.34 ± .05	9.91	.0018
O2	83.82	7.08 ± .05	7.35 ± .05	13.58	.0003
P2	97.62	7.1 ± .05	7.33 ± .05	10.33	.0014
Q2	94.27	7.09 ± .05	7.35 ± .05	13.06	.0003
R2	99.02	7.09 ± .05	7.34 ± .05	12.35	.0005

3 WEEK BODY WEIGHT (WK3)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	12.71 ± .1	12.81 ± .11	.42	.5152
B2	12.8	12.72 ± .1	12.79 ± .11	.23	.6318
C2	15.18	12.67 ± .11	12.84 ± .11	1.2	.2742
D2	28.01	12.63 ± .11	12.88 ± .11	2.7	.1013
E2	31.57	12.58 ± .11	12.93 ± .11	4.99	.0261
F2	40.36	12.6 ± .11	12.92 ± .11	4.15	.0423
G2	49.15	12.58 ± .11	12.95 ± .11	5.65	.018
H2	53.9	12.58 ± .11	12.93 ± .11	5.26	.0224
I2	55.8	12.6 ± .11	12.91 ± .11	3.87	.0498
J2	58.41	12.57 ± .11	12.94 ± .11	5.64	.0181
K2	61.02	12.56 ± .11	12.94 ± .11	5.96	.0151
L2	65.53	12.53 ± .11	12.96 ± .11	7.78	.0056
M2	68.38	12.56 ± .11	12.93 ± .11	5.44	.0202
S2	70.04	12.53 ± .11	12.95 ± .1	7.19	.0077
T2	71.47	12.54 ± .11	12.95 ± .11	7.08	.0082
N2	75.03	12.55 ± .11	12.94 ± .11	5.96	.0151
O2	83.82	12.6 ± .11	12.89 ± .11	3.32	.0691
P2	97.62	12.66 ± .11	12.83 ± .11	1.16	.2818
Q2	94.27	12.61 ± .11	12.88 ± .11	3.11	.0788
R2	99.02	12.62 ± .11	12.88 ± .11	2.72	.0998

APPENDIX 12--CONTINUED

6 WEEK BODY WEIGHT (WK6)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	32.37 ± .28	31.93 ± .31	1.05	.3053
B2	12.8	32.47 ± .28	31.83 ± .31	2.3	.1302
C2	15.18	32.53 ± .29	31.76 ± .3	3.31	.0696
D2	28.01	32.73 ± .3	31.63 ± .29	6.92	.0089
E2	31.57	32.78 ± .3	31.59 ± .29	7.8	.0055
F2	40.36	32.96 ± .29	31.31 ± .3	15.25	.0001
G2	49.15	32.79 ± .29	31.5 ± .3	9.03	.0028
H2	53.9	32.91 ± .3	31.44 ± .29	11.89	.0006
I2	55.8	32.96 ± .29	31.37 ± .29	13.99	.0002
J2	58.41	32.92 ± .3	31.45 ± .29	11.93	.0006
K2	61.02	32.85 ± .3	31.53 ± .29	9.54	.0022
L2	65.53	32.55 ± .3	31.81 ± .29	2.97	.0855
M2	68.38	32.57 ± .31	31.78 ± .29	3.39	.0664
S2	70.04	32.58 ± .31	31.8 ± .29	3.33	.069
T2	71.47	32.6 ± .31	31.78 ± .29	3.7	.0552
N2	75.03	32.77 ± .3	31.6 ± .29	7.33	.0071
O2	83.82	32.74 ± .31	31.67 ± .29	6.17	.0135
P2	97.62	32.65 ± .31	31.75 ± .29	4.4	.0366
Q2	94.27	32.33 ± .31	32.03 ± .29	.49	.4857
R2	99.02	32.36 ± .31	31.99 ± .29	.74	.3906

9 WEEK BODY WEIGHT (WK9)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	38.68 ± .3	37.63 ± .32	5.83	.0163
B2	12.8	38.7 ± .3	37.66 ± .32	5.6	.0185
C2	15.18	38.8 ± .3	37.56 ± .32	7.97	.005
D2	28.01	39.05 ± .31	37.41 ± .3	14.44	.0002
E2	31.57	39.18 ± .31	37.32 ± .3	17.83	.0001
F2	40.36	39.59 ± .29	36.7 ± .3	46.45	.0001
G2	49.15	39.7 ± .29	36.64 ± .3	52.56	.0001
H2	53.9	39.83 ± .3	36.62 ± .29	57.77	.0001
I2	55.8	39.8 ± .29	36.58 ± .29	58.28	.0001
J2	58.41	39.88 ± .3	36.63 ± .29	59.85	.0001
K2	61.02	39.84 ± .3	36.67 ± .29	56.71	.0001
L2	65.53	39.55 ± .3	36.94 ± .29	37.12	.0001
M2	68.38	39.61 ± .31	36.9 ± .29	39.59	.0001
S2	70.04	39.73 ± .31	36.83 ± .29	46.12	.0001
T2	71.47	39.7 ± .3	36.83 ± .29	45.26	.0001
N2	75.03	39.71 ± .3	36.77 ± .29	46.69	.0001
O2	83.82	39.5 ± .31	37.05 ± .29	31.59	.0001
P2	97.62	39.4 ± .31	37.13 ± .29	26.79	.0001
Q2	94.27	39.08 ± .31	37.38 ± .3	14.77	.0001
R2	99.02	38.9 ± .32	37.57 ± .3	8.77	.0033

APPENDIX 12--CONTINUED

12 WEEK BODY WEIGHT (WK12)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	41.03 ± .34	40.1 ± .36	3.52	.0613
B2	12.8	41.01 ± .34	40.16 ± .36	2.86	.0916
C2	15.18	41.22 ± .34	39.94 ± .36	6.6	.0106
D2	28.01	41.44 ± .35	39.82 ± .34	10.87	.0011
E2	31.57	41.63 ± .36	39.67 ± .34	15.46	.0001
F2	40.36	42.15 ± .33	38.92 ± .34	45.05	.0001
G2	49.15	42.35 ± .33	38.77 ± .33	56.55	.0001
H2	53.9	42.54 ± .33	38.71 ± .33	64.97	.0001
I2	55.8	42.53 ± .33	38.65 ± .33	67.39	.0001
J2	58.41	42.67 ± .33	38.66 ± .32	73.14	.0001
K2	61.02	42.72 ± .33	38.64 ± .32	76.7	.0001
L2	65.53	42.53 ± .34	38.81 ± .32	62.4	.0001
M2	68.38	42.54 ± .34	38.82 ± .32	61.12	.0001
S2	70.04	42.68 ± .34	38.75 ± .32	69.66	.0001
T2	71.47	42.66 ± .34	38.74 ± .32	69.51	.0001
N2	75.03	42.62 ± .33	38.7 ± .32	68.08	.0001
O2	83.82	42.35 ± .35	39.04 ± .33	46.68	.0001
P2	97.62	42.14 ± .35	39.23 ± .33	35.19	.0001
Q2	94.27	41.79 ± .35	39.5 ± .34	21.32	.0001
R2	99.02	41.5 ± .36	39.8 ± .34	11.35	.0008

DAY12 TO WK3 GAIN (GAIN1)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	5.53 ± .08	5.54 ± .08	.01	.9273
B2	12.8	5.55 ± .08	5.51 ± .08	.15	.6966
C2	15.18	5.52 ± .08	5.54 ± .08	.06	.8125
D2	28.01	5.5 ± .08	5.57 ± .08	.44	.5063
E2	31.57	5.44 ± .08	5.62 ± .08	2.38	.1234
F2	40.36	5.46 ± .08	5.61 ± .08	1.67	.1973
G2	49.15	5.42 ± .08	5.65 ± .08	3.98	.0467
H2	53.9	5.43 ± .08	5.63 ± .08	3.08	.0799
I2	55.8	5.45 ± .08	5.61 ± .08	1.83	.1765
J2	58.41	5.43 ± .08	5.63 ± .08	3.03	.0823
K2	61.02	5.43 ± .08	5.63 ± .08	2.94	.087
L2	65.53	5.42 ± .08	5.64 ± .08	3.58	.0591
M2	68.38	5.45 ± .08	5.61 ± .08	1.92	.1669
S2	70.04	5.43 ± .08	5.62 ± .08	2.81	.0943
T2	71.47	5.42 ± .08	5.63 ± .08	3.15	.0766
N2	75.03	5.45 ± .08	5.6 ± .08	1.74	.1877
O2	83.82	5.52 ± .08	5.54 ± .08	.03	.8608
P2	97.62	5.56 ± .08	5.5 ± .08	.29	.5874
Q2	94.27	5.52 ± .08	5.53 ± .08	.02	.8921
R2	99.02	5.53 ± .08	5.53 ± .08	0	.9631

APPENDIX 12--CONTINUED

WK3 TO WK6 GAIN (GAIN2)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	19.66 ± .26	19.13 ± .28	1.92	.1671
B2	12.8	19.75 ± .26	19.03 ± .28	3.43	.0647
C2	15.18	19.86 ± .26	18.92 ± .28	5.94	.0153
D2	28.01	20.1 ± .27	18.75 ± .26	12.69	.0004
E2	31.57	20.2 ± .27	18.67 ± .26	15.95	.0001
F2	40.36	20.35 ± .26	18.39 ± .27	26.78	.0001
G2	49.15	20.21 ± .26	18.56 ± .27	18.46	.0001
H2	53.9	20.33 ± .27	18.5 ± .26	22.75	.0001
I2	55.8	20.36 ± .26	18.46 ± .27	24.59	.0001
J2	58.41	20.36 ± .27	18.52 ± .26	23.13	.0001
K2	61.02	20.29 ± .27	18.59 ± .26	19.55	.0001
L2	65.53	20.02 ± .28	18.85 ± .26	9.12	.0027
M2	68.38	20.01 ± .28	18.85 ± .26	8.8	.0032
S2	70.04	20.05 ± .28	18.85 ± .26	9.52	.0022
T2	71.47	20.07 ± .28	18.82 ± .26	10.17	.0015
N2	75.03	20.22 ± .27	18.66 ± .26	15.86	.0001
O2	83.82	20.14 ± .28	18.78 ± .26	12.08	.0006
P2	97.62	19.99 ± .28	18.92 ± .26	7.5	.0065
Q2	94.27	19.72 ± .28	19.15 ± .27	2.16	.1429
R2	99.02	19.75 ± .28	19.12 ± .27	2.56	.1103

WK6 TO WK9 GAIN (GAIN3)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	6.32 ± .22	5.7 ± .24	3.71	.055
B2	12.8	6.23 ± .22	5.83 ± .24	1.49	.2237
C2	15.18	6.27 ± .22	5.8 ± .23	2.07	.1511
D2	28.01	6.32 ± .23	5.78 ± .23	2.8	.0951
E2	31.57	6.4 ± .24	5.73 ± .23	4.08	.0442
F2	40.36	6.64 ± .22	5.39 ± .23	14.57	.0002
G2	49.15	6.91 ± .22	5.13 ± .22	30.91	.0001
H2	53.9	6.92 ± .23	5.18 ± .22	28.93	.0001
I2	55.8	6.84 ± .23	5.21 ± .23	25.09	.0001
J2	58.41	6.96 ± .23	5.17 ± .22	30.58	.0001
K2	61.02	6.99 ± .23	5.14 ± .22	33.37	.0001
L2	65.53	7.01 ± .23	5.13 ± .22	34.62	.0001
M2	68.38	7.03 ± .23	5.11 ± .22	35.78	.0001
S2	70.04	7.15 ± .23	5.04 ± .21	44.51	.0001
T2	71.47	7.1 ± .23	5.06 ± .21	41.46	.0001
N2	75.03	6.94 ± .23	5.17 ± .22	29.29	.0001
O2	83.82	6.76 ± .24	5.38 ± .22	17.64	.0001
P2	97.62	6.74 ± .24	5.38 ± .22	17.26	.0001
Q2	94.27	6.75 ± .23	5.35 ± .22	18.39	.0001
R2	99.02	6.53 ± .24	5.58 ± .22	8.26	.0043

WK9 TO WK12 GAIN (GAIN4)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	2.35 ± .15	2.47 ± .16	.3	.585
B2	12.8	2.31 ± .15	2.5 ± .16	.74	.39
C2	15.18	2.42 ± .15	2.38 ± .16	.03	.8518
D2	28.01	2.39 ± .16	2.41 ± .16	.01	.9371
E2	31.57	2.45 ± .16	2.35 ± .16	.23	.631
F2	40.36	2.56 ± .16	2.22 ± .16	2.25	.1342
G2	49.15	2.65 ± .16	2.13 ± .16	5.24	.0226
H2	53.9	2.71 ± .16	2.1 ± .16	7.27	.0074
I2	55.8	2.73 ± .16	2.07 ± .16	8.47	.0038
J2	58.41	2.79 ± .16	2.03 ± .15	11.26	.0009
K2	61.02	2.88 ± .16	1.96 ± .15	16.51	.0001
L2	65.53	2.98 ± .16	1.87 ± .15	25.41	.0001
M2	68.38	2.93 ± .16	1.92 ± .15	20.37	.0001
S2	70.04	2.95 ± .16	1.92 ± .15	21.23	.0001
T2	71.47	2.95 ± .16	1.91 ± .15	22.15	.0001
N2	75.03	2.91 ± .16	1.93 ± .15	18.91	.0001
O2	83.82	2.86 ± .16	1.99 ± .15	14.66	.0002
P2	97.62	2.74 ± .16	2.1 ± .15	8.07	.0048
Q2	94.27	2.71 ± .16	2.12 ± .15	6.9	.009
R2	99.02	2.6 ± .16	2.23 ± .15	2.72	.0998

HRT/WK12 (HRTP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.594 ± .008	.587 ± .008	.32	.5734
B2	12.8	.59 ± .008	.591 ± .008	.02	.8975
C2	15.18	.587 ± .008	.594 ± .008	.43	.5146
D2	28.01	.584 ± .008	.597 ± .008	1.41	.2363
E2	31.57	.581 ± .008	.599 ± .008	2.42	.121
F2	40.36	.566 ± .008	.618 ± .008	22.16	.0001
G2	49.15	.564 ± .008	.619 ± .008	24.6	.0001
H2	53.9	.564 ± .008	.617 ± .008	23.15	.0001
I2	55.8	.565 ± .008	.617 ± .008	22.02	.0001
J2	58.41	.562 ± .008	.618 ± .008	25.63	.0001
K2	61.02	.562 ± .008	.617 ± .008	24.89	.0001
L2	65.53	.564 ± .008	.615 ± .008	20.92	.0001
M2	68.38	.565 ± .008	.614 ± .008	19.08	.0001
S2	70.04	.564 ± .008	.615 ± .008	20.93	.0001
T2	71.47	.563 ± .008	.615 ± .008	21.44	.0001
N2	75.03	.565 ± .008	.614 ± .008	18.69	.0001
O2	83.82	.576 ± .008	.604 ± .008	5.59	.0186
P2	97.62	.583 ± .008	.599 ± .008	1.91	.1683
Q2	94.27	.583 ± .008	.599 ± .008	1.84	.1756
R2	99.02	.588 ± .008	.594 ± .008	.21	.6479

APPENDIX 12--CONTINUED

LIV/WK12 (LIVP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	7.62 ± .05	7.56 ± .05	.75	.3886
B2	12.8	7.63 ± .05	7.57 ± .05	.87	.3523
C2	15.18	7.63 ± .05	7.57 ± .05	.89	.3464
D2	28.01	7.64 ± .05	7.56 ± .05	1.47	.2268
E2	31.57	7.66 ± .05	7.54 ± .05	2.47	.1169
F2	40.36	7.67 ± .05	7.52 ± .05	4.35	.0377
G2	49.15	7.68 ± .05	7.51 ± .05	5.26	.0224
H2	53.9	7.69 ± .05	7.5 ± .05	6.9	.0090
I2	55.8	7.69 ± .05	7.5 ± .05	7.25	.0074
J2	58.41	7.7 ± .05	7.5 ± .05	8	.0049
K2	61.02	7.69 ± .05	7.5 ± .05	6.67	.0102
L2	65.53	7.68 ± .05	7.51 ± .05	5.43	.0203
M2	68.38	7.66 ± .05	7.53 ± .05	3.05	.0814
S2	70.04	7.66 ± .05	7.54 ± .05	2.75	.0983
T2	71.47	7.66 ± .05	7.54 ± .05	2.9	.0896
N2	75.03	7.66 ± .05	7.53 ± .05	2.93	.0878
O2	83.82	7.65 ± .05	7.54 ± .05	2.29	.1315
P2	97.62	7.64 ± .05	7.55 ± .05	1.41	.2355
Q2	94.27	7.62 ± .05	7.57 ± .05	.49	.4863
R2	99.02	7.61 ± .05	7.58 ± .05	.23	.629

SPL/WK12 (SPLP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.542 ± .009	.532 ± .01	.59	.4423
B2	12.8	.539 ± .009	.536 ± .01	.06	.8114
C2	15.18	.54 ± .009	.535 ± .01	.12	.7312
D2	28.01	.538 ± .01	.537 ± .009	.01	.942
E2	31.57	.542 ± .01	.533 ± .009	.42	.5185
F2	40.36	.533 ± .009	.541 ± .01	.32	.5695
G2	49.15	.524 ± .01	.55 ± .01	3.6	.0586
H2	53.9	.517 ± .01	.556 ± .009	7.85	.0054
I2	55.8	.518 ± .01	.557 ± .01	7.8	.0055
J2	58.41	.516 ± .01	.557 ± .009	8.61	.0036
K2	61.02	.514 ± .01	.559 ± .009	10.63	.0012
L2	65.53	.511 ± .01	.562 ± .009	14.17	.0002
M2	68.38	.507 ± .01	.565 ± .009	17.74	.0001
S2	70.04	.508 ± .01	.563 ± .009	16.41	.0001
T2	71.47	.511 ± .01	.561 ± .009	13.04	.0003
N2	75.03	.515 ± .01	.557 ± .009	9.32	.0024
O2	83.82	.519 ± .01	.553 ± .009	5.86	.016
P2	97.62	.525 ± .01	.547 ± .009	2.57	.1096
Q2	94.27	.527 ± .01	.545 ± .009	1.71	.192
R2	99.02	.529 ± .01	.543 ± .009	1.03	.3107

KID/WK12 (KIDP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.82 ± .008	.817 ± .009	.06	.8088
B2	12.8	.82 ± .008	.818 ± .009	.03	.8544
C2	15.18	.818 ± .008	.821 ± .009	.06	.8064
D2	28.01	.801 ± .009	.836 ± .008	8.52	.0037
E2	31.57	.795 ± .009	.84 ± .008	13.39	.0003
F2	40.36	.792 ± .008	.846 ± .009	19.73	.0001
G2	49.15	.788 ± .008	.85 ± .008	25.67	.0001
H2	53.9	.786 ± .008	.85 ± .008	28.12	.0001
I2	55.8	.788 ± .008	.849 ± .008	25.88	.0001
J2	58.41	.784 ± .008	.851 ± .008	30.58	.0001
K2	61.02	.781 ± .008	.853 ± .008	37.08	.0001
L2	65.53	.776 ± .008	.857 ± .008	48.46	.0001
M2	68.38	.779 ± .008	.854 ± .008	39.11	.0001
S2	70.04	.778 ± .008	.854 ± .008	42.19	.0001
T2	71.47	.779 ± .008	.854 ± .008	39.69	.0001
N2	75.03	.782 ± .008	.853 ± .008	34.78	.0001
O2	83.82	.782 ± .009	.851 ± .008	33.13	.0001
P2	97.62	.783 ± .009	.851 ± .008	32.06	.0001
Q2	94.27	.784 ± .008	.851 ± .008	30.93	.0001
R2	99.02	.789 ± .009	.845 ± .008	21.27	.0001

TES/WK12 (TESP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.202 ± .004	.225 ± .005	12.88	.0004
B2	12.8	.199 ± .004	.226 ± .004	17.62	.0001
C2	15.18	.198 ± .005	.226 ± .004	18.27	.0001
D2	28.01	.203 ± .005	.221 ± .004	7.69	.0062
E2	31.57	.205 ± .005	.219 ± .004	4.12	.0441
F2	40.36	.205 ± .005	.221 ± .005	5.14	.0247
G2	49.15	.204 ± .005	.221 ± .005	5.49	.0204
H2	53.9	.205 ± .005	.221 ± .005	4.37	.0382
I2	55.8	.205 ± .005	.221 ± .005	4.24	.0412
J2	58.41	.204 ± .005	.221 ± .005	5.84	.0168
K2	61.02	.203 ± .005	.222 ± .005	6.34	.0128
L2	65.53	.207 ± .005	.218 ± .005	2.19	.141
M2	68.38	.206 ± .005	.219 ± .005	2.61	.1082
S2	70.04	.204 ± .005	.22 ± .005	4.74	.031
T2	71.47	.205 ± .005	.221 ± .005	4.48	.0358
N2	75.03	.205 ± .005	.221 ± .005	4.8	.03
O2	83.82	.207 ± .005	.218 ± .005	2.07	.1519
P2	97.62	.209 ± .005	.217 ± .005	1.06	.305
Q2	94.27	.211 ± .005	.215 ± .005	.21	.645
R2	99.02	.211 ± .005	.215 ± .005	.4	.5263

APPENDIX 12--CONTINUED

SCF/WK12 (SCFP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.578 ± .02	.575 ± .022	.01	.9183
B2	12.8	.58 ± .02	.569 ± .022	.12	.7306
C2	15.18	.592 ± .021	.556 ± .022	1.44	.2302
D2	28.01	.613 ± .021	.538 ± .021	6.29	.0126
E2	31.57	.621 ± .022	.534 ± .021	8.11	.0047
F2	40.36	.652 ± .02	.496 ± .021	27.21	.0001
G2	49.15	.662 ± .02	.488 ± .021	34.09	.0001
H2	53.9	.679 ± .021	.479 ± .02	45.88	.0001
I2	55.8	.675 ± .02	.48 ± .02	44.07	.0001
J2	58.41	.682 ± .021	.479 ± .02	47.65	.0001
K2	61.02	.69 ± .021	.474 ± .02	55.47	.0001
L2	65.53	.692 ± .021	.472 ± .02	57.79	.0001
M2	68.38	.698 ± .02	.468 ± .019	63.67	.0001
S2	70.04	.7 ± .021	.469 ± .019	64.79	.0001
T2	71.47	.699 ± .021	.469 ± .019	63.98	.0001
N2	75.03	.702 ± .02	.462 ± .02	68.96	.0001
O2	83.82	.692 ± .021	.476 ± .02	54.46	.0001
P2	97.62	.679 ± .021	.488 ± .02	41.91	.0001
Q2	94.27	.669 ± .021	.493 ± .02	35.39	.0001
R2	99.02	.647 ± .022	.514 ± .021	18.92	.0001

GOF/WK12 (GOFP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	.816 ± .029	.812 ± .032	.01	.9335
B2	12.8	.81 ± .029	.819 ± .032	.04	.8366
C2	15.18	.826 ± .03	.803 ± .031	.27	.601
D2	28.01	.844 ± .031	.787 ± .03	1.75	.1863
E2	31.57	.86 ± .031	.775 ± .03	3.69	.0554
F2	40.36	.894 ± .030	.73 ± .031	14.34	.0002
G2	49.15	.924 ± .029	.703 ± .03	27.03	.0001
H2	53.9	.941 ± .03	.695 ± .029	33.57	.0001
I2	55.8	.94 ± .029	.688 ± .029	35.57	.0001
J2	58.41	.955 ± .03	.686 ± .028	41.06	.0001
K2	61.02	.961 ± .03	.681 ± .028	45.35	.0001
L2	65.53	.96 ± .029	.683 ± .028	44.94	.0001
M2	68.38	.977 ± .029	.667 ± .028	56.28	.0001
S2	70.04	.977 ± .029	.671 ± .028	55.59	.0001
T2	71.47	.975 ± .029	.669 ± .028	55.55	.0001
N2	75.03	.972 ± .03	.665 ± .028	54.13	.0001
O2	83.82	.944 ± .03	.698 ± .029	33.16	.0001
P2	97.62	.93 ± .031	.711 ± .029	26.03	.0001
Q2	94.27	.917 ± .03	.718 ± .029	21.58	.0001
R2	99.02	.897 ± .031	.74 ± .029	12.85	.0004

FAT/WK12 (FATP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
A2	2.35	1397 ± 45	1388 ± 49	.02	.9015
B2	12.8	1393 ± 45	1389 ± 49	.01	.9436
C2	15.18	1421 ± 46	1358 ± 48	.87	.352
D2	28.01	1460 ± 47	1326 ± 46	4.08	.0441
E2	31.57	1483 ± 48	1310 ± 46	6.58	.0107
F2	40.36	1549 ± 45	1226 ± 47	23.73	.0001
G2	49.15	1589 ± 45	1192 ± 46	37.1	.0001
H2	53.9	1622 ± 45	1174 ± 44	48.02	.0001
I2	55.8	1619 ± 45	1168 ± 45	48.73	.0001
J2	58.41	1640 ± 45	1166 ± 43	54.74	.0001
K2	61.02	1654 ± 45	1156 ± 43	62.09	.0001
L2	65.53	1654 ± 45	1156 ± 43	62.99	.0001
M2	68.38	1677 ± 44	1136 ± 42	75.15	.0001
S2	70.04	1680 ± 45	1140 ± 42	75.12	.0001
T2	71.47	1677 ± 44	1138 ± 42	74.69	.0001
N2	75.03	1677 ± 44	1128 ± 43	76.21	.0001
O2	83.82	1635 ± 46	1177 ± 43	50.43	.0001
P2	97.62	1609 ± 47	1201 ± 44	39.01	.0001
Q2	94.27	1586 ± 46	1213 ± 45	32.48	.0001
R2	99.02	1544 ± 48	1258 ± 45	18.21	.0001

APPENDIX 13--LEAST-SQUARES MEANS AND F-VALUES FOR EACH TRAIT ANALYZED WITH CHROMOSOME 15 MARKERS

12 DAY BODY WEIGHT (DAY12)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	7.29 ± .05	7.16 ± .05	2.99	.0847
C15	7.62	7.3 ± .05	7.15 ± .05	3.88	.0495
D15	13.8	7.27 ± .05	7.18 ± .05	1.27	.2604
E15	18.31	7.25 ± .05	7.2 ± .05	.63	.4280
A15	21.61	7.27 ± .05	7.18 ± .05	1.57	.2110
F15	23.99	7.24 ± .05	7.21 ± .05	.11	.7361
G15	27.79	7.24 ± .05	7.21 ± .05	.2	.6557
H15	32.3	7.24 ± .05	7.21 ± .05	.14	.7085
J15	36.27	7.28 ± .05	7.24 ± .06	.31	.5787
I15	48.97	7.21 ± .05	7.24 ± .05	.19	.6633

3 WEEK BODY WEIGHT (WK3)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	12.97 ± .11	12.55 ± .11	7.27	.0073
C15	7.62	12.98 ± .11	12.54 ± .11	7.95	.0051
D15	13.8	12.93 ± .11	12.58 ± .11	4.88	.0278
E15	18.31	12.91 ± .11	12.61 ± .11	3.66	.0564
A15	21.61	12.9 ± .11	12.6 ± .11	3.63	.0576
F15	23.99	12.88 ± .11	12.63 ± .11	2.52	.1130
G15	27.79	12.89 ± .11	12.62 ± .11	2.85	.0925
H15	32.3	12.87 ± .11	12.63 ± .11	2.4	.1222
J15	36.27	12.96 ± .11	12.75 ± .11	1.6	.2067
I15	48.97	12.84 ± .11	12.67 ± .11	1.27	.2614

6 WEEK BODY WEIGHT (WK6)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	32.59 ± .3	31.78 ± .29	3.63	.0575
C15	7.62	32.63 ± .3	31.74 ± .29	4.3	.0389
D15	13.8	32.62 ± .3	31.74 ± .3	4	.0463
E15	18.31	32.65 ± .3	31.72 ± .29	4.63	.0320
A15	21.61	32.7 ± .29	31.63 ± .3	6.29	.0126
F15	23.99	32.68 ± .3	31.67 ± .29	5.59	.0186
G15	27.79	32.78 ± .3	31.59 ± .29	7.75	.0056
H15	32.3	32.78 ± .29	31.48 ± .3	9.52	.0022
J15	36.27	32.81 ± .3	31.87 ± .31	4.63	.0322
I15	48.97	32.64 ± .3	31.73 ± .29	4.61	.0325

9 WEEK BODY WEIGHT (WK9)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	38.8 ± .32	37.63 ± .31	6.81	.0094
C15	7.62	38.91 ± .31	37.51 ± .31	9.84	.0019
D15	13.8	38.98 ± .32	37.44 ± .31	11.44	.0008
E15	18.31	39.06 ± .32	37.39 ± .3	13.71	.0002
A15	21.61	38.93 ± .31	37.44 ± .31	11.08	.0010
F15	23.99	39.04 ± .31	37.37 ± .31	14.21	.0002
G15	27.79	39.15 ± .31	37.29 ± .3	17.82	.0001
H15	32.3	39.09 ± .3	37.2 ± .31	18.66	.0001
J15	36.27	39.08 ± .32	37.47 ± .33	11.87	.0006
I15	48.97	39.13 ± .31	37.31 ± .3	17.38	.0001

12 WEEK BODY WEIGHT (WK12)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	41.32 ± .36	39.92 ± .35	7.59	.0062
C15	7.62	41.43 ± .36	39.8 ± .35	10.4	.0014
D15	13.8	41.52 ± .36	39.72 ± .35	12.26	.0005
E15	18.31	41.56 ± .36	39.7 ± .34	13.33	.0003
A15	21.61	41.4 ± .35	39.78 ± .35	10.26	.0015
F15	23.99	41.53 ± .35	39.69 ± .35	13.27	.0003
G15	27.79	41.67 ± .35	39.58 ± .34	17.55	.0001
H15	32.3	41.65 ± .34	39.42 ± .35	20.31	.0001
J15	36.27	41.66 ± .36	39.55 ± .37	15.95	.0001
I15	48.97	41.69 ± .35	39.56 ± .34	18.68	.0001

DAY12 TO WK3 GAIN (GAIN1)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	5.68 ± .08	5.39 ± .08	6.47	.0114
C15	7.62	5.68 ± .08	5.38 ± .08	6.53	.0110
D15	13.8	5.66 ± .08	5.4 ± .08	5.18	.0235
E15	18.31	5.65 ± .08	5.41 ± .08	4.35	.0376
A15	21.61	5.63 ± .08	5.42 ± .08	3.18	.0756
F15	23.99	5.64 ± .08	5.42 ± .08	3.76	.0531
G15	27.79	5.65 ± .08	5.41 ± .08	4.02	.0458
H15	32.3	5.63 ± .08	5.42 ± .08	3.47	.0632
J15	36.27	5.68 ± .08	5.51 ± .08	1.86	.1740
I15	48.97	5.63 ± .08	5.43 ± .08	3.25	.0724

WK3 TO WK6 GAIN (GAIN2)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	19.62 ± .28	19.23 ± .27	1.03	.3106
C15	7.62	19.65 ± .28	19.2 ± .27	1.32	.2510
D15	13.8	19.68 ± .28	19.16 ± .27	1.71	.1915
E15	18.31	19.75 ± .28	19.12 ± .27	2.53	.1123
A15	21.61	19.8 ± .27	19.03 ± .27	3.93	.0482
F15	23.99	19.8 ± .27	19.04 ± .27	3.82	.0515
G15	27.79	19.89 ± .27	18.97 ± .27	5.61	.0183
H15	32.3	19.92 ± .26	18.85 ± .28	7.56	.0063
J15	36.27	19.85 ± .28	19.12 ± .29	3.24	.0728
I15	48.97	19.8 ± .27	19.06 ± .27	3.6	.0585

WK6 TO WK9 GAIN (GAIN3)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	6.21 ± .24	5.85 ± .23	1.11	.2920
C15	7.62	6.29 ± .24	5.77 ± .23	2.36	.1256
D15	13.8	6.36 ± .24	5.7 ± .23	3.81	.0518
E15	18.31	6.4 ± .24	5.67 ± .23	4.73	.0303
A15	21.61	6.23 ± .23	5.81 ± .23	1.53	.2174
F15	23.99	6.36 ± .23	5.7 ± .23	3.94	.0480
G15	27.79	6.37 ± .23	5.7 ± .23	4.09	.0438
H15	32.3	6.31 ± .23	5.72 ± .24	3.14	.0772
J15	36.27	6.27 ± .23	5.6 ± .24	3.97	.0473
I15	48.97	6.49 ± .23	5.58 ± .23	7.67	.0059

WK9 TO WK12 GAIN (GAIN4)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	2.52 ± .16	2.3 ± .16	.97	.3244
C15	7.62	2.52 ± .16	2.29 ± .16	1	.3189
D15	13.8	2.54 ± .16	2.27 ± .16	1.28	.2591
E15	18.31	2.5 ± .16	2.31 ± .16	.71	.3996
A15	21.61	2.47 ± .16	2.33 ± .16	.34	.5582
F15	23.99	2.48 ± .16	2.32 ± .16	.49	.4829
G15	27.79	2.52 ± .16	2.29 ± .16	1.02	.3139
H15	32.3	2.56 ± .15	2.22 ± .16	2.23	.1365
J15	36.27	2.58 ± .17	2.08 ± .17	4.25	.0401
I15	48.97	2.56 ± .16	2.25 ± .16	1.91	.1680

HRT/WK12 (HRTP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.592 ± .008	.59 ± .008	.03	.8531
C15	7.62	.592 ± .008	.59 ± .008	.04	.8393
D15	13.8	.589 ± .008	.593 ± .008	.11	.7366
E15	18.31	.59 ± .008	.591 ± .008	.01	.9375
A15	21.61	.591 ± .008	.591 ± .008	0	.9803
F15	23.99	.592 ± .008	.589 ± .008	.06	.8111
G15	27.79	.591 ± .008	.59 ± .008	0	.9581
H15	32.3	.589 ± .008	.592 ± .008	.06	.8010
J15	36.27	.583 ± .006	.594 ± .006	1.61	.2055
I15	48.97	.587 ± .008	.593 ± .008	.28	.6000

LIV/WK12 (LIVP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	7.55 ± .05	7.64 ± .05	1.54	.2153
C15	7.62	7.55 ± .05	7.63 ± .05	1.23	.2683
D15	13.8	7.57 ± .05	7.62 ± .05	.42	.5170
E15	18.31	7.58 ± .05	7.61 ± .05	.15	.6986
A15	21.61	7.56 ± .05	7.63 ± .05	.96	.3286
F15	23.99	7.57 ± .05	7.62 ± .05	.38	.5386
G15	27.79	7.58 ± .05	7.61 ± .05	.11	.7362
H15	32.3	7.6 ± .05	7.59 ± .05	.02	.8989
J15	36.27	7.62 ± .05	7.58 ± .06	.38	.5396
I15	48.97	7.58 ± .05	7.61 ± .05	.22	.6388

SPL/WK12 (SPLP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.535 ± .01	.539 ± .01	.07	.7876
C15	7.62	.535 ± .01	.539 ± .01	.09	.7614
D15	13.8	.534 ± .01	.54 ± .01	.17	.6793
E15	18.31	.538 ± .01	.536 ± .01	.02	.8927
A15	21.61	.54 ± .01	.534 ± .01	.16	.6892
F15	23.99	.54 ± .01	.535 ± .01	.15	.7003
G15	27.79	.539 ± .01	.536 ± .01	.04	.8513
H15	32.3	.54 ± .009	.534 ± .01	.15	.7015
J15	36.27	.539 ± .01	.534 ± .01	.11	.7384
I15	48.97	.541 ± .01	.534 ± .01	.27	.6013

KID/WK12 (KIDP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.799 ± .009	.836 ± .008	9.52	.0022
C15	7.62	.799 ± .009	.836 ± .008	9.2	.0026
D15	13.8	.798 ± .009	.838 ± .009	10.03	.0017
E15	18.31	.799 ± .009	.836 ± .008	8.93	.0030
A15	21.61	.8 ± .008	.837 ± .009	9.3	.0025
F15	23.99	.798 ± .009	.838 ± .008	10.38	.0014
G15	27.79	.8 ± .009	.835 ± .008	8.12	.0046
H15	32.3	.803 ± .008	.836 ± .009	7	.0085
J15	36.27	.803 ± .009	.838 ± .009	7.09	.0081
I15	48.97	.808 ± .009	.829 ± .009	2.91	.0890

TES/WK12 (TESP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.207 ± .005	.218 ± .004	2.42	.1221
C15	7.62	.208 ± .005	.217 ± .005	1.85	.1763
D15	13.8	.209 ± .005	.216 ± .005	.94	.3338
E15	18.31	.21 ± .005	.216 ± .005	.74	.3912
A15	21.61	.209 ± .005	.216 ± .005	1.09	.2979
F15	23.99	.21 ± .005	.216 ± .005	.76	.3848
G15	27.79	.209 ± .005	.216 ± .005	1.14	.2882
H15	32.3	.211 ± .005	.215 ± .005	.22	.6433
J15	36.27	.213 ± .005	.213 ± .005	0	.9785
I15	48.97	.21 ± .005	.216 ± .005	.72	.3976

SCF/WK12 (SCFP)

Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.624 ± .022	.534 ± .021	8.44	.0039
C15	7.62	.631 ± .022	.527 ± .021	11.44	.0008
D15	13.8	.631 ± .022	.526 ± .021	11	.0010
E15	18.31	.631 ± .022	.528 ± .021	10.89	.0011
A15	21.61	.624 ± .021	.53 ± .022	9.22	.0026
F15	23.99	.63 ± .021	.526 ± .021	11.45	.0008
G15	27.79	.627 ± .022	.53 ± .021	9.81	.0019
H15	32.3	.613 ± .021	.537 ± .022	6.11	.0139
J15	36.27	.61 ± .022	.519 ± .023	7.63	.0061
I15	48.97	.623 ± .022	.534 ± .021	8.43	.0039

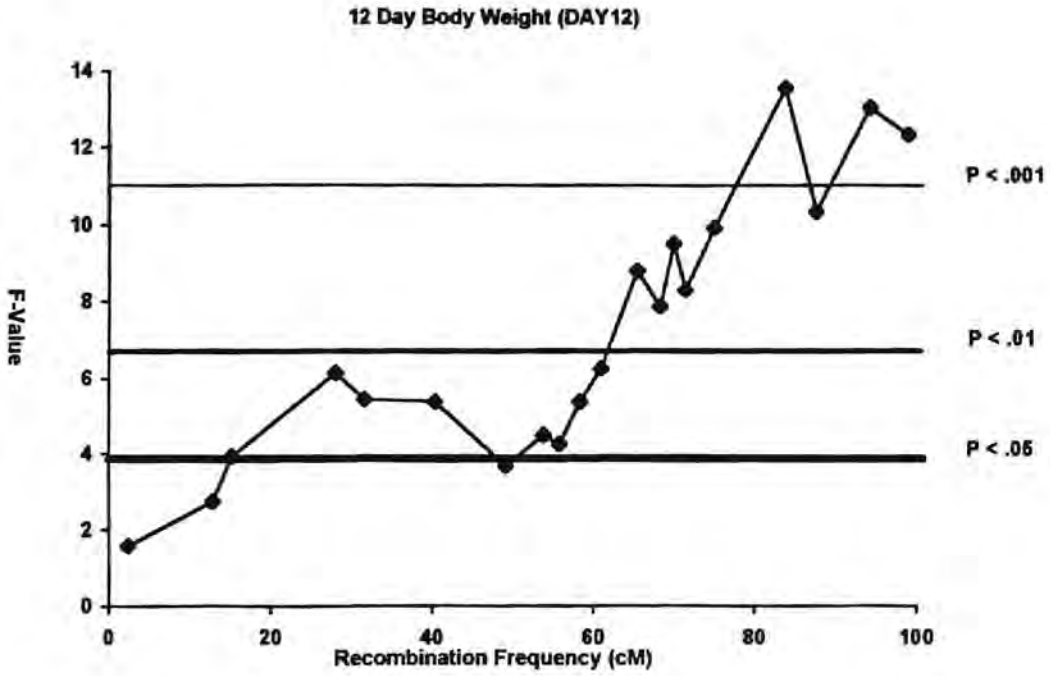
GOF/WK12 (GOFP)

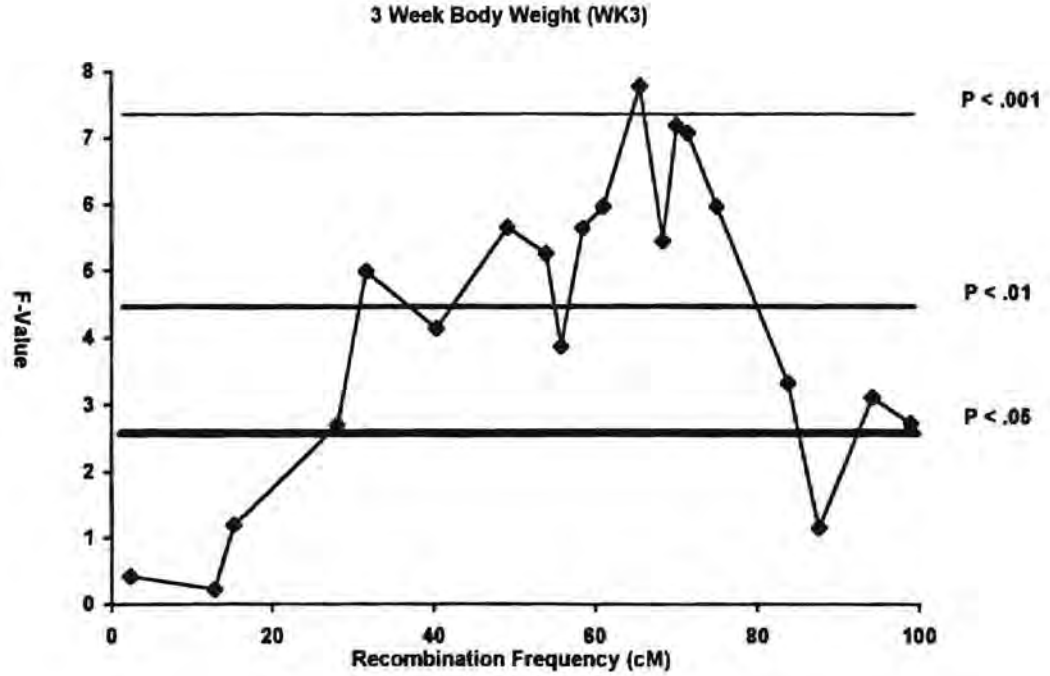
Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	.883 ± .031	.751 ± .03	9.15	.0027
C15	7.62	.89 ± .031	.743 ± .03	11.31	.0009
D15	13.8	.894 ± .031	.738 ± .03	12.42	.0005
E15	18.31	.897 ± .031	.738 ± .03	13.12	.0003
A15	21.61	.889 ± .03	.739 ± .03	12.01	.0006
F15	23.99	.906 ± .03	.725 ± .03	17.46	.0001
G15	27.79	.9 ± .03	.732 ± .03	15.03	.0001
H15	32.3	.882 ± .029	.738 ± .031	11.13	.0009
J15	36.27	.882 ± .03	.724 ± .03	11.41	.0008
I15	48.97	.885 ± .03	.748 ± .03	10.05	.0017

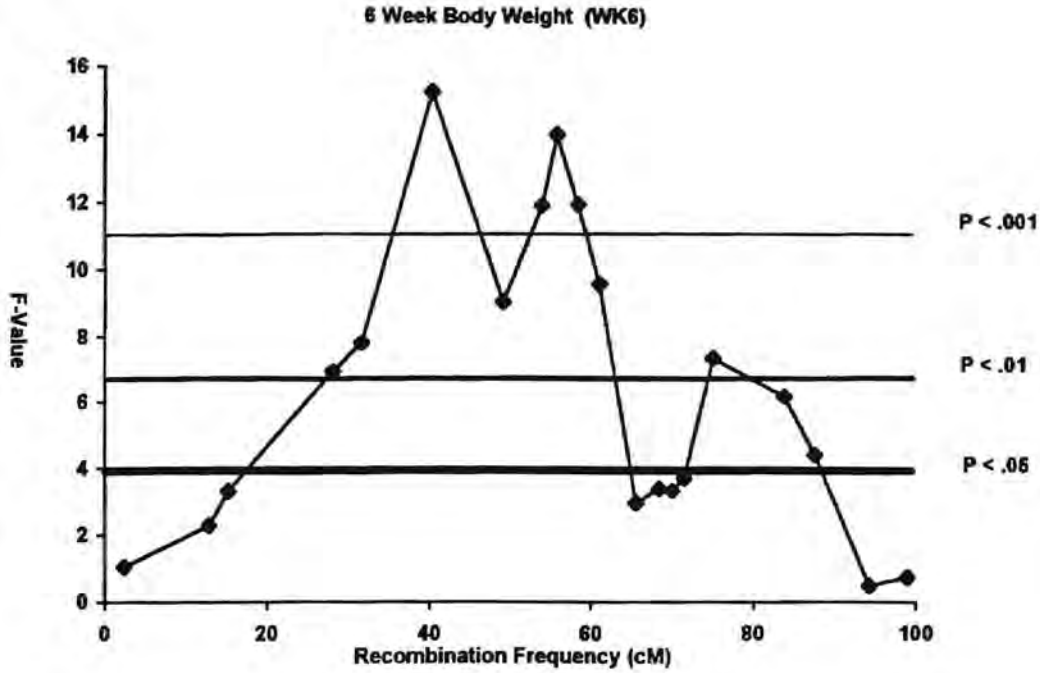
FAT/WK12 (FATP)

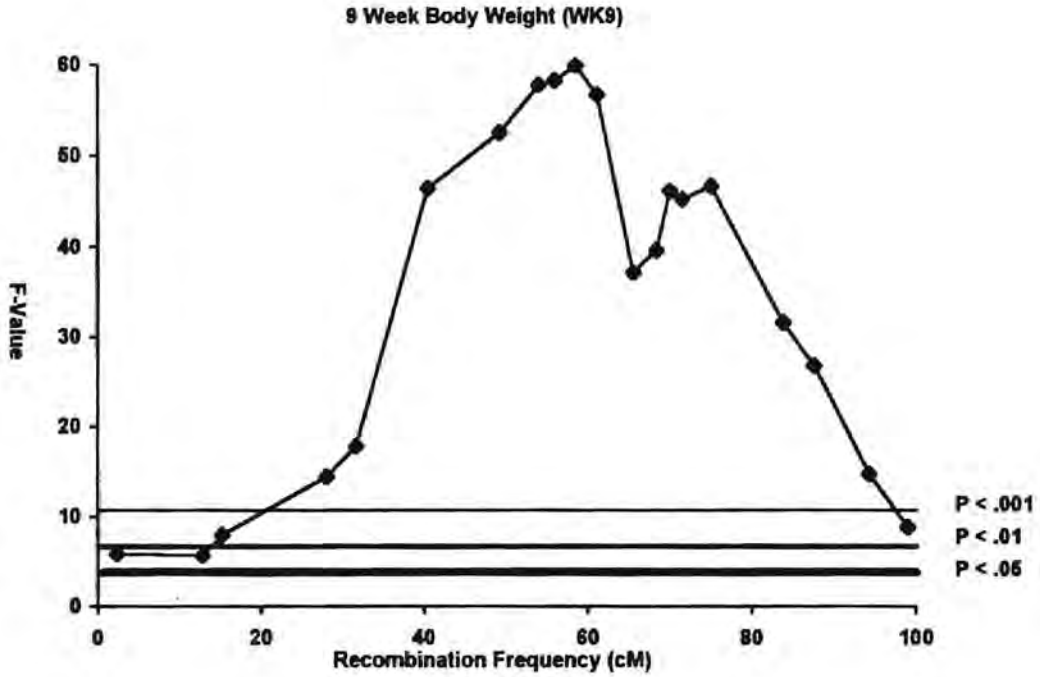
Marker	Location (cM)	M16i/M16i	M16i/CAST	F-Value	P-Value
B15	5.72	1507 ± 47	1287 ± 46	10.74	.0012
C15	7.62	1521 ± 47	1272 ± 46	13.73	.0002
D15	13.8	1526 ± 47	1267 ± 46	14.27	.0002
E15	18.31	1528 ± 48	1269 ± 46	14.7	.0001
A15	21.61	1513 ± 46	1272 ± 47	13.05	.0003
F15	23.99	1536 ± 47	1253 ± 46	17.96	.0001
G15	27.79	1528 ± 47	1265 ± 46	15.41	.0001
H15	32.3	1496 ± 45	1278 ± 48	10.61	.0012
J15	36.27	1494 ± 49	1245 ± 51	11.92	.0006
I15	48.97	1513 ± 47	1281 ± 46	12	.0006

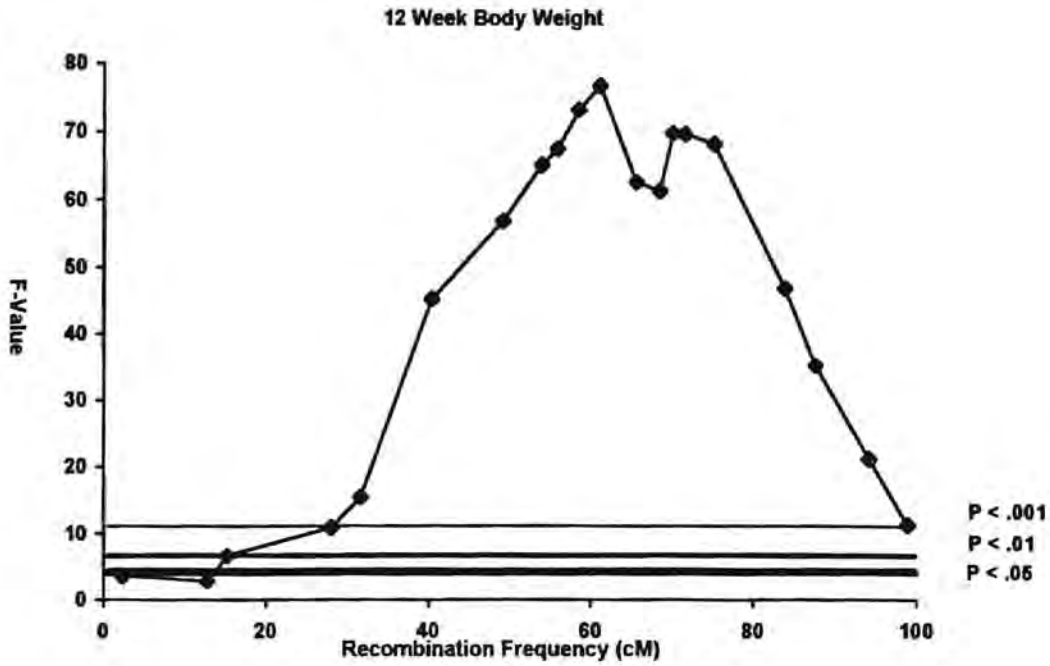
APPENDIX 14--MAPPING OF QTL UTILIZING SINGLE MARKER F-VALUES FOR CHROMOSOME 2

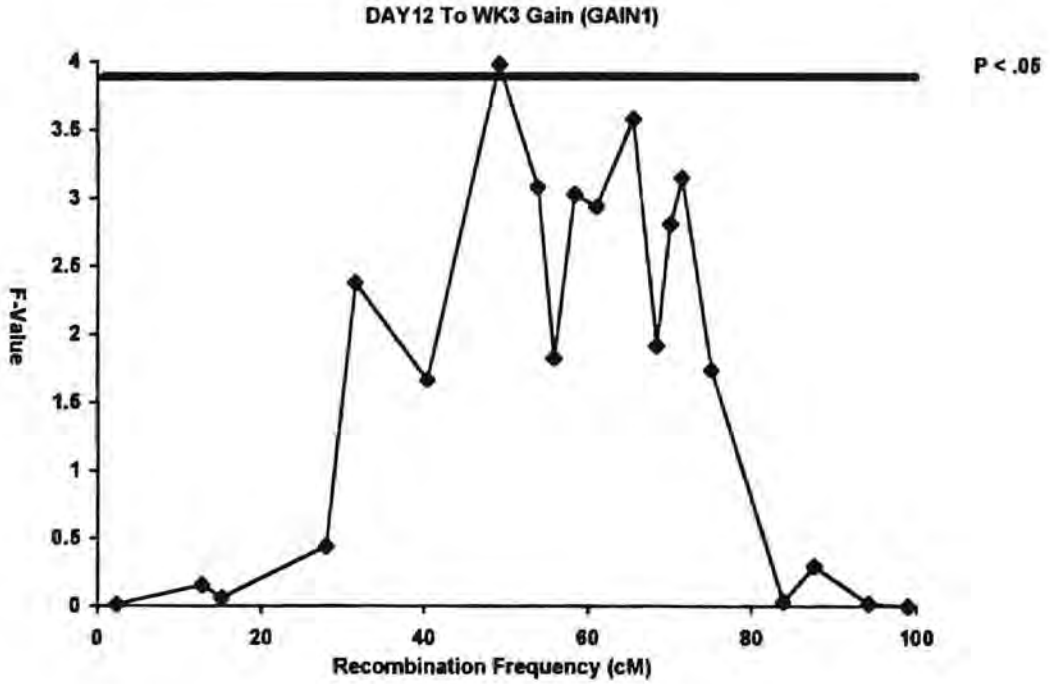


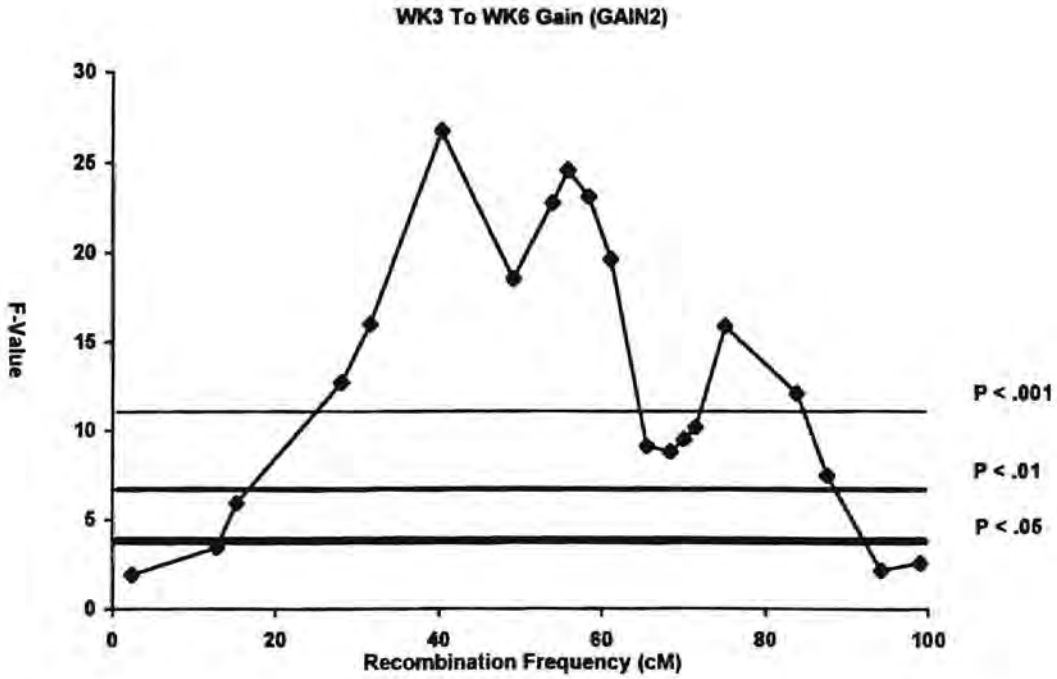


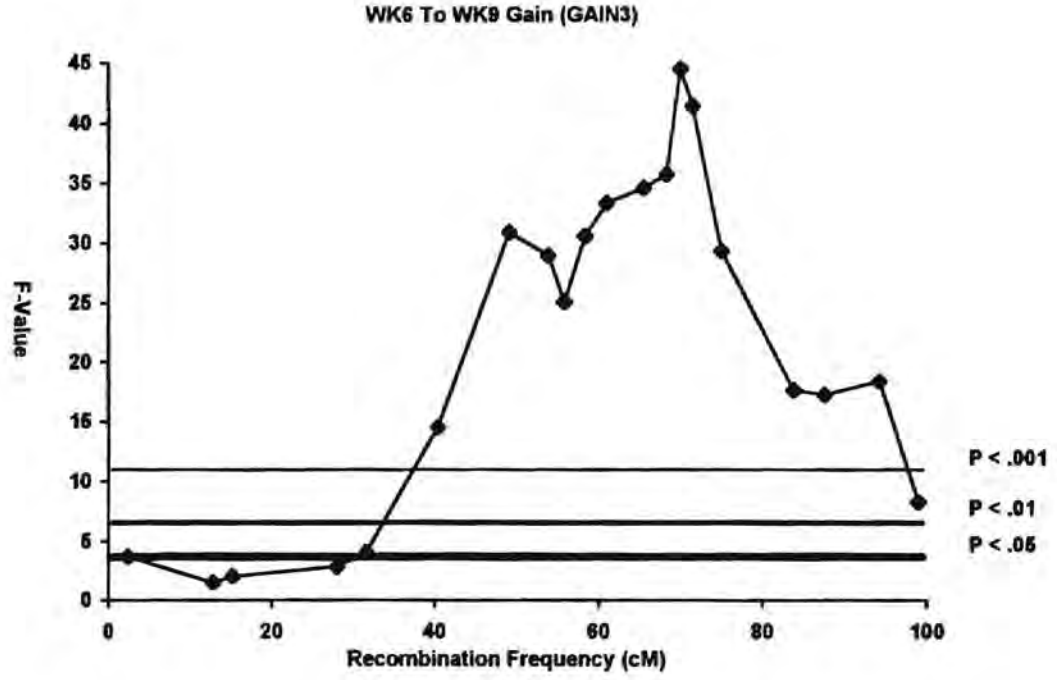

6 Week Body Weight (WK6)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

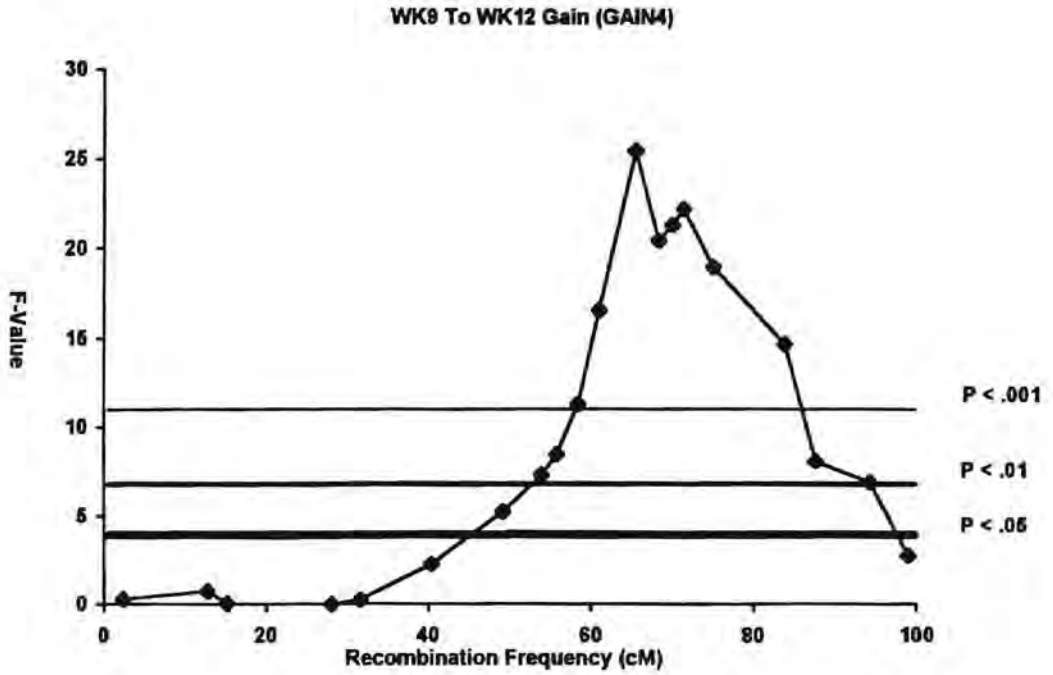

9 Week Body Weight (WK9)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

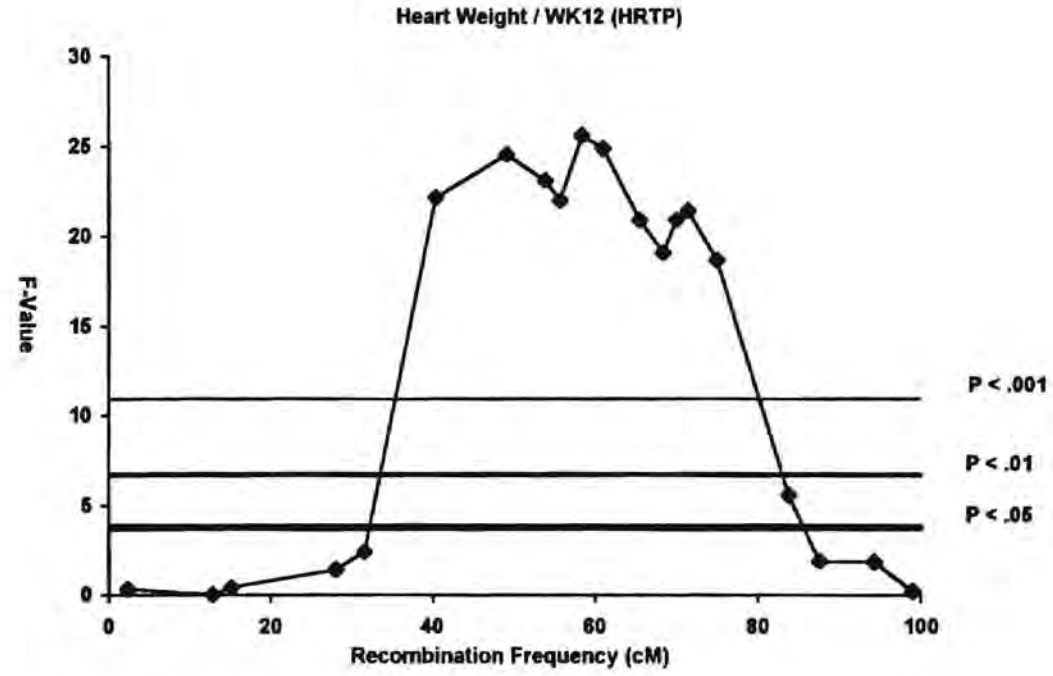

12 Week Body Weight
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

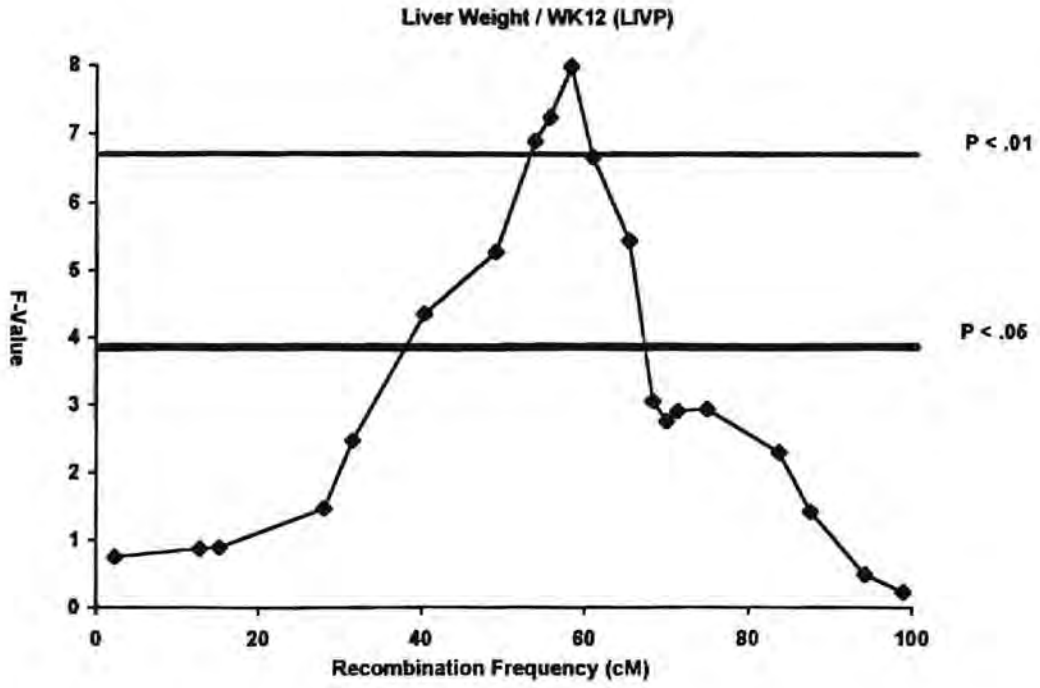

DAY12 To WK3 Gain (GAIN1)
F-Value
Recombination Frequency (cM)
P < .05

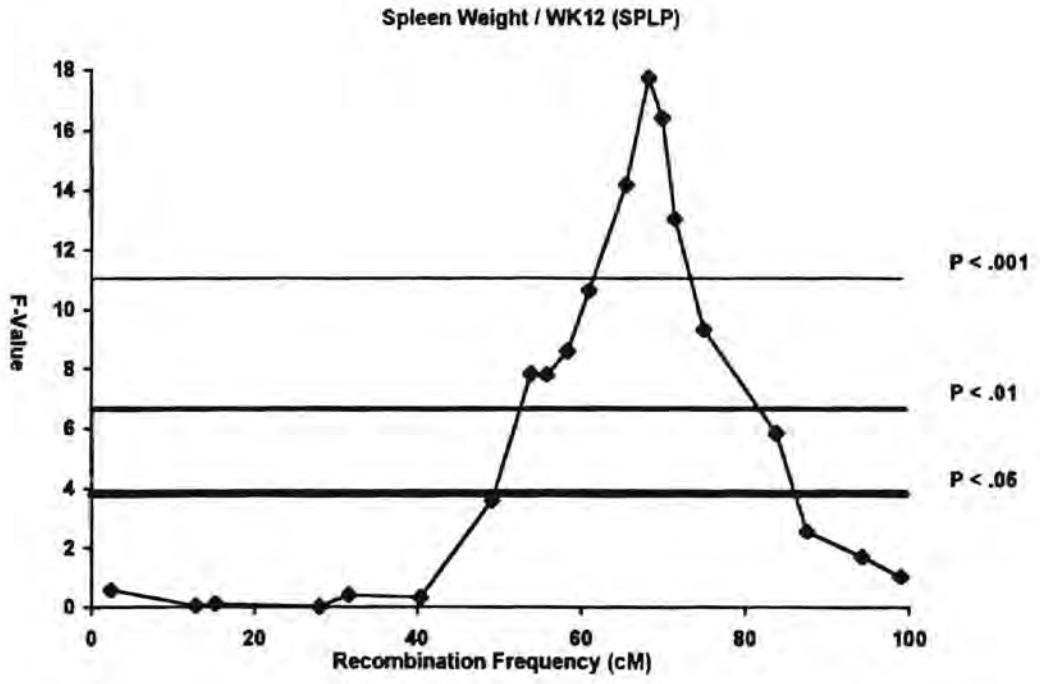

WK3 To WK6 Gain (GAIN2)
F-Value
30
25
20
15
10
5
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

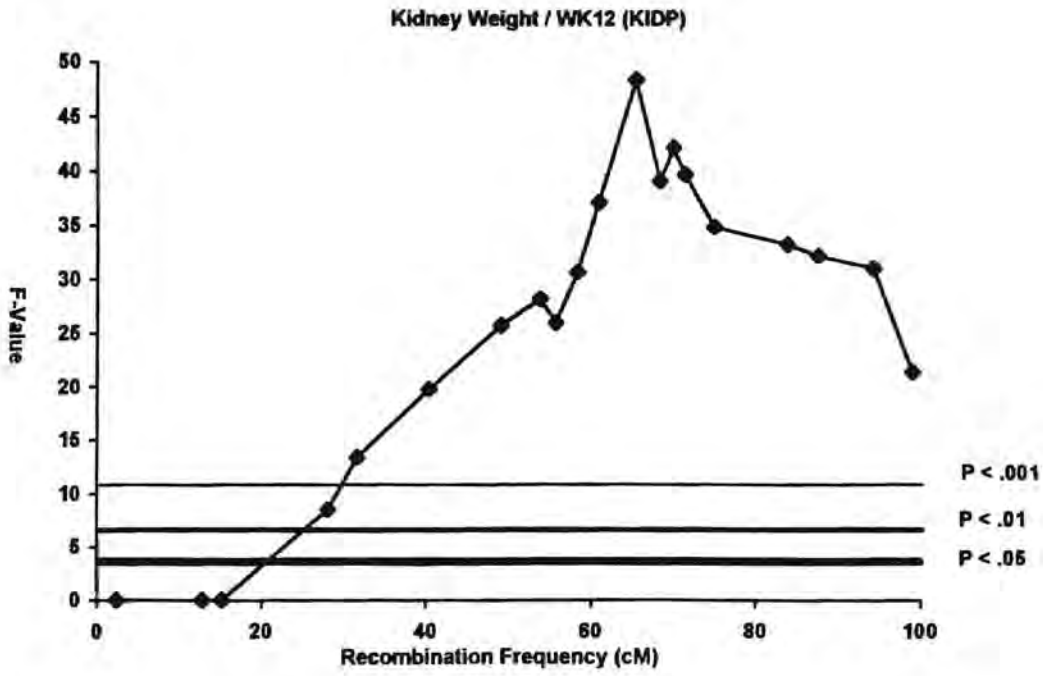

WK6 To WK9 Gain (GAIN3)
F-Value
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

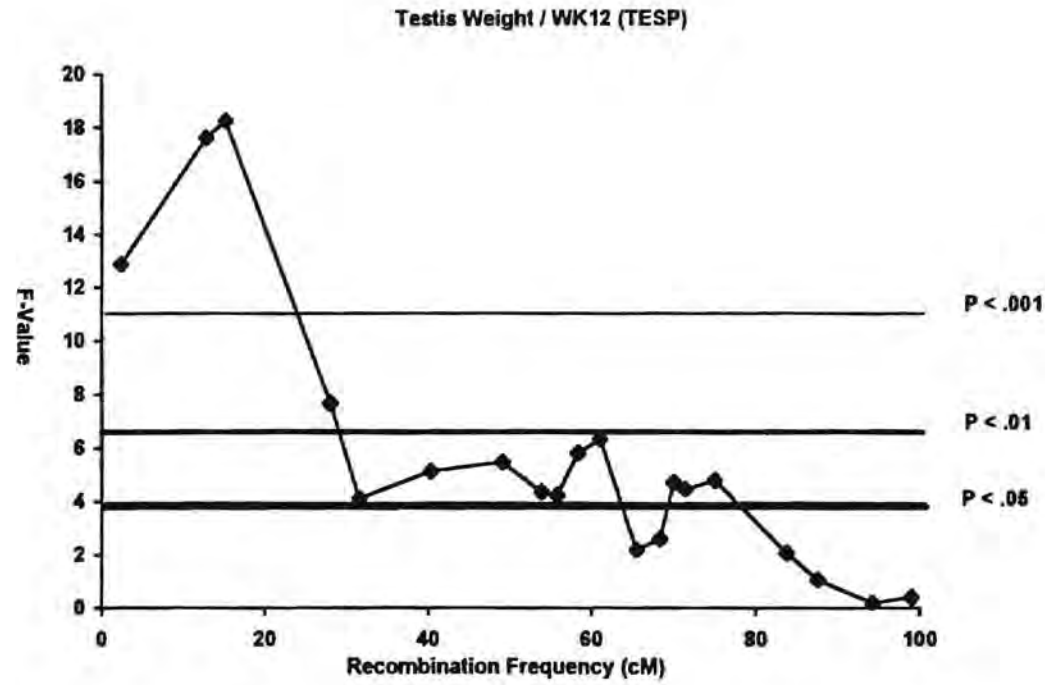

WK9 To WK12 Gain (GAIN4)
F-Value
30
25
20
15
10
5
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

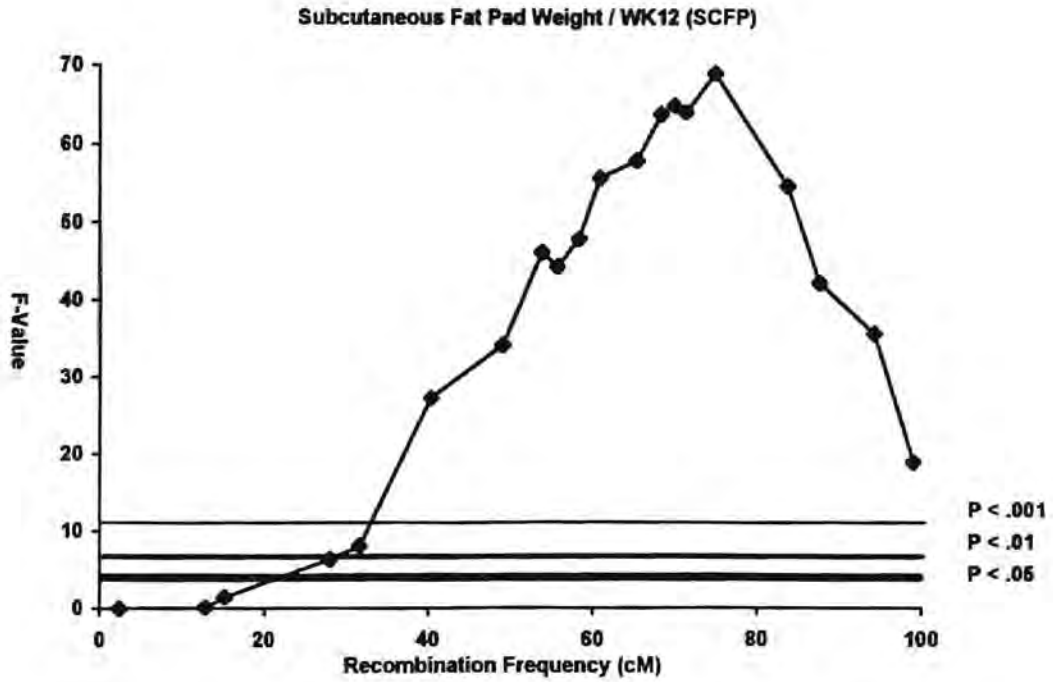

Heart Weight / WK12 (HRTP)
F-Value
30
25
20
15
10
5
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

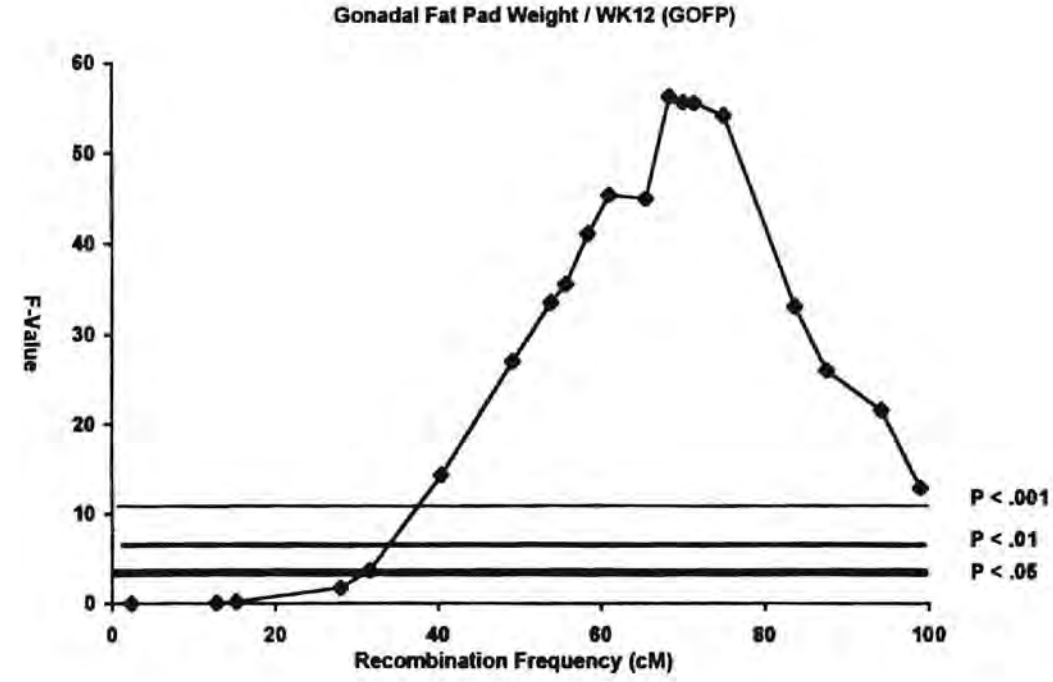

Liver Weight / WK12 (LIVP)
F-Value
Recombination Frequency (cM)
P < .01
P < .06
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100

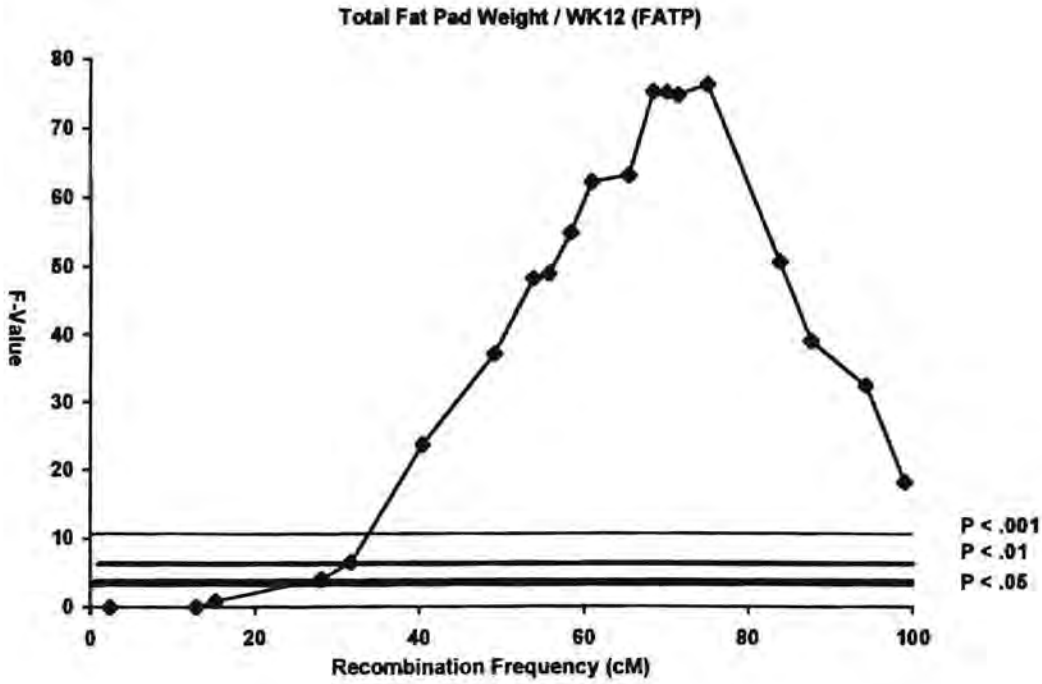

Spleen Weight / WK12 (SPLP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05
0
2
4
6
8
10
12
14
16
18
0
20
40
60
80
100


Kidney Weight / WK12 (KIDP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

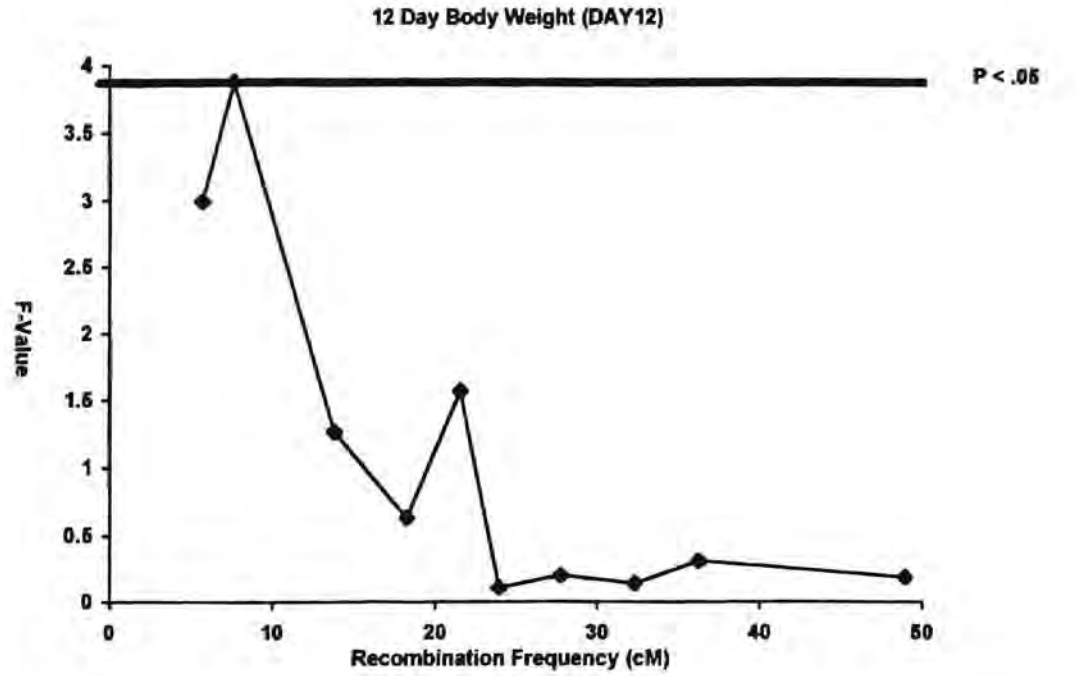

Testis Weight / WK12 (TESP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

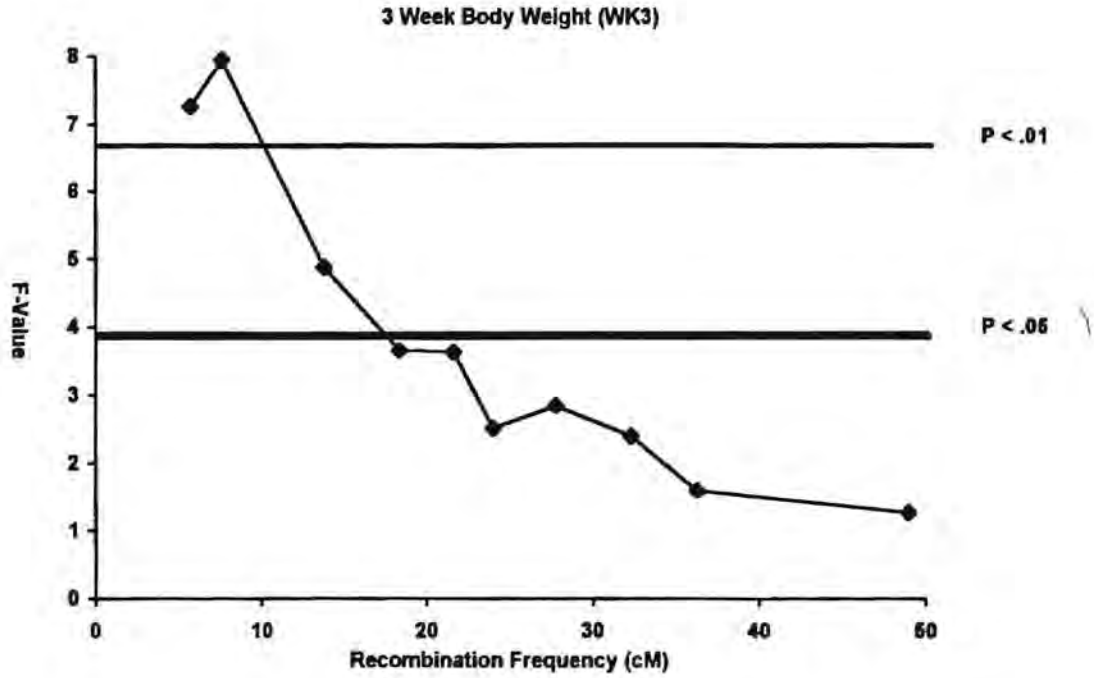

Subcutaneous Fat Pad Weight / WK12 (SCFP)
F-Value
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

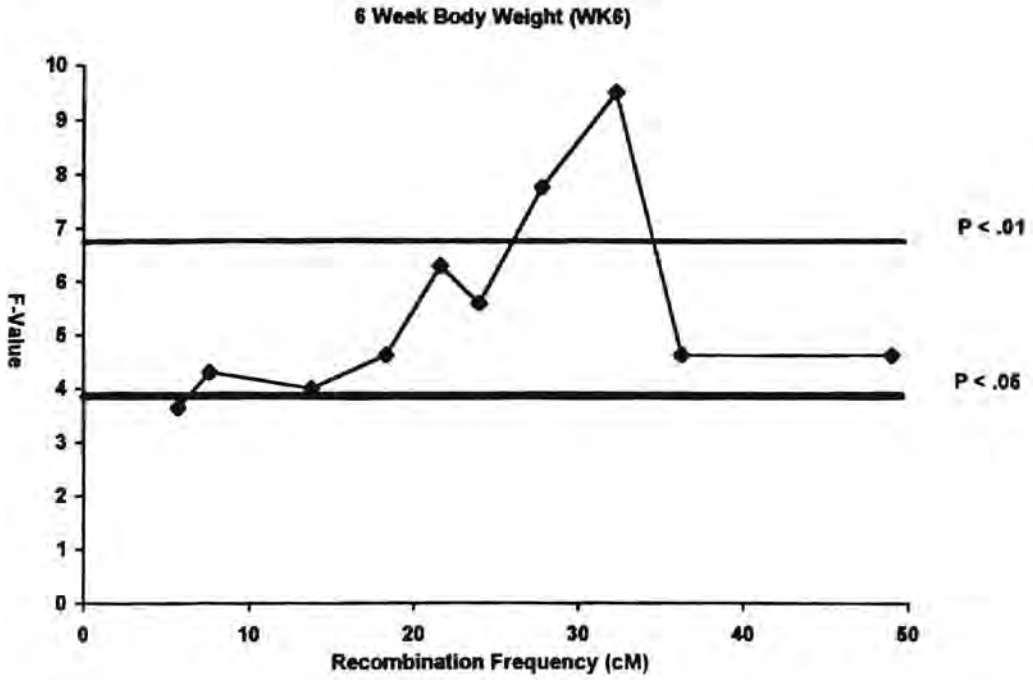

Gonadal Fat Pad Weight / WK12 (GOFP)
F-Value
60
50
40
30
20
10
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

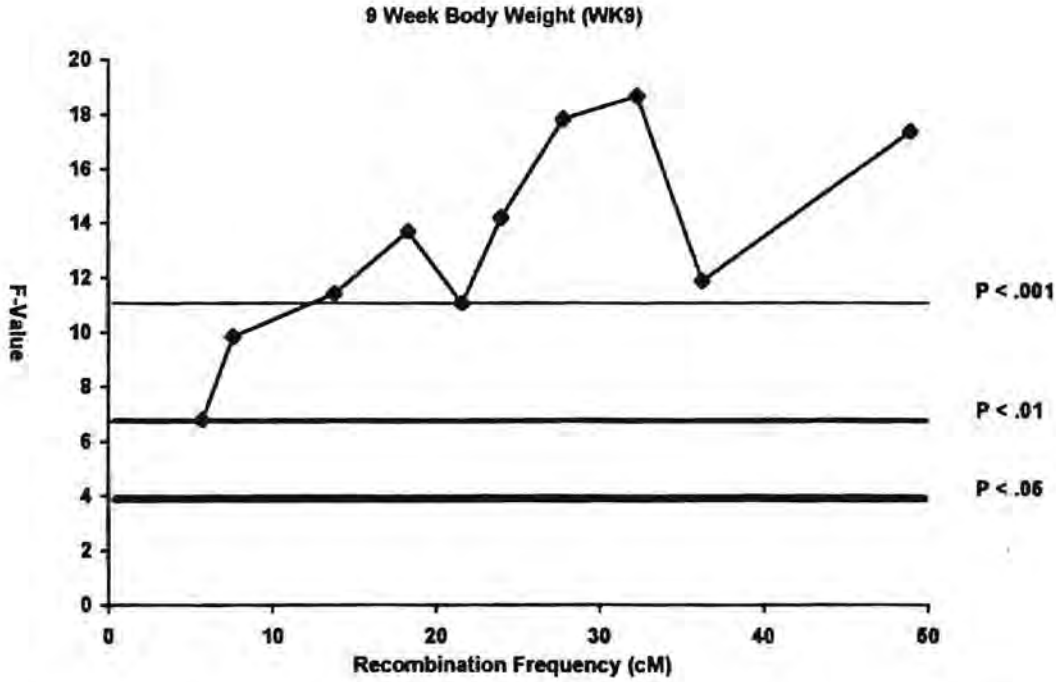

Total Fat Pad Weight / WK12 (FATP)
F-Value
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Recombination Frequency (cM)
P < .001
P < .01
P < .05

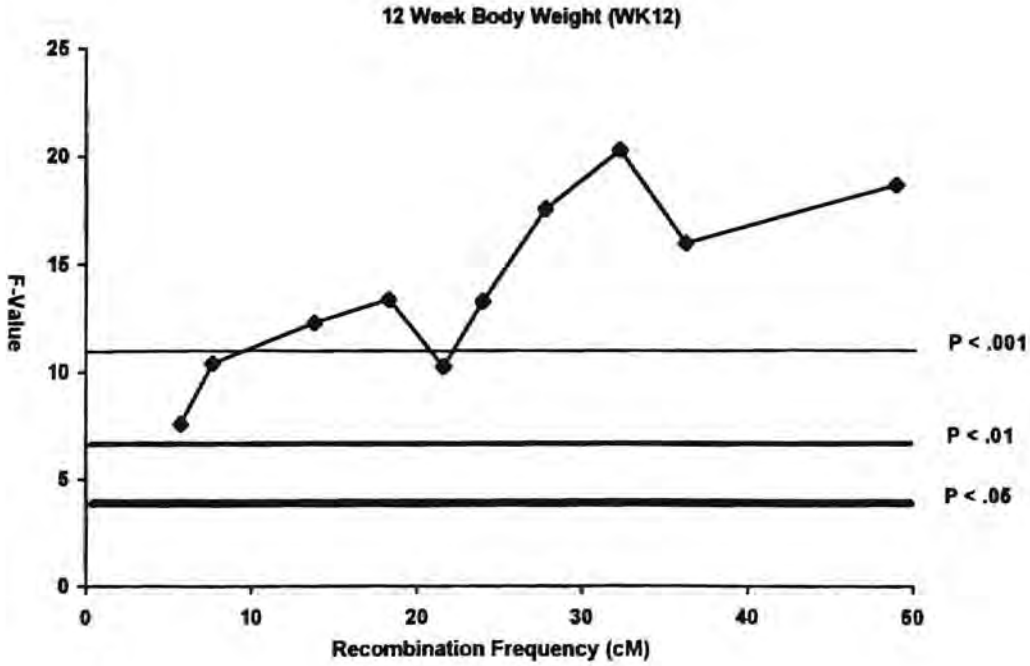
APPENDIX 15--MAPPING OF QTL UTILIZING SINGLE MARKER F-VALUES FOR CHROMOSOME 15

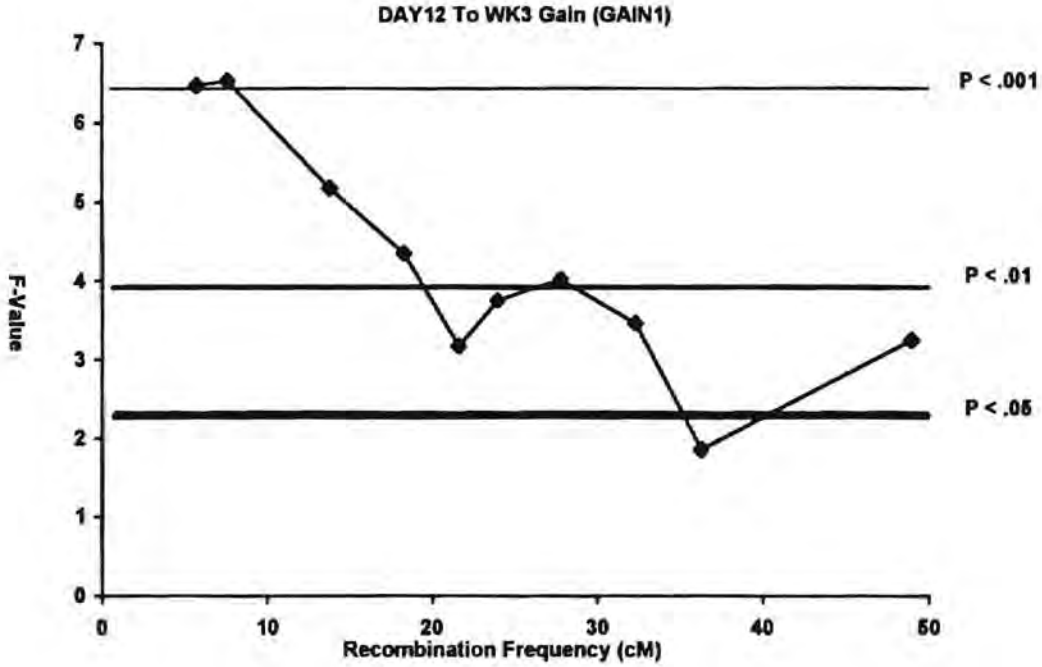


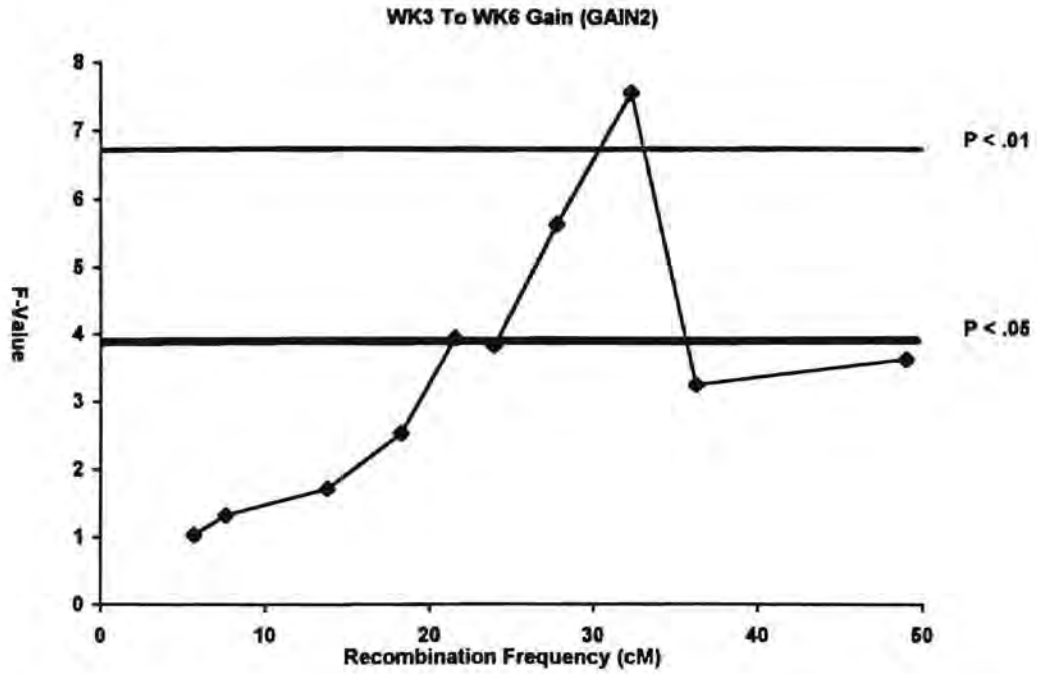


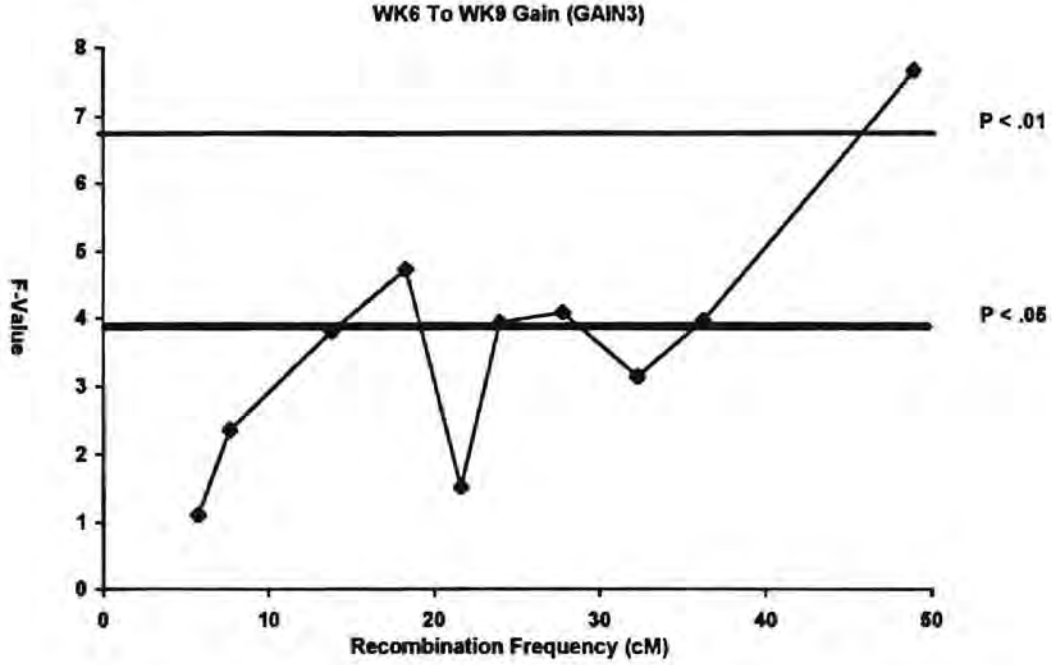

6 Week Body Weight (WK6)
F-Value
Recombination Frequency (cM)
P < .01
P < .05

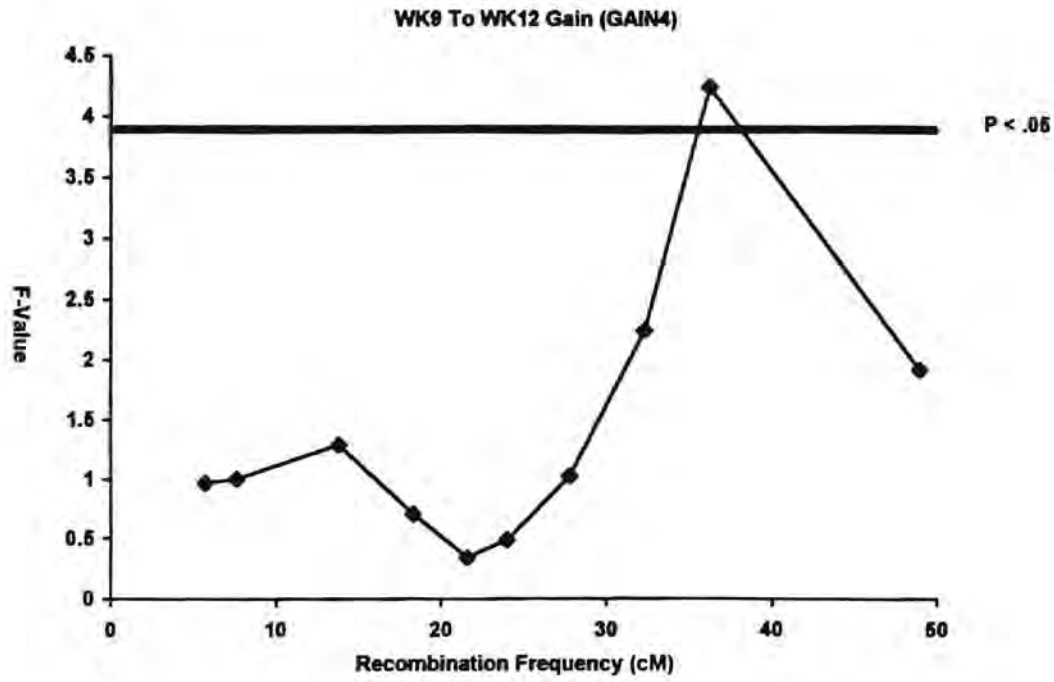

9 Week Body Weight (WK9)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05

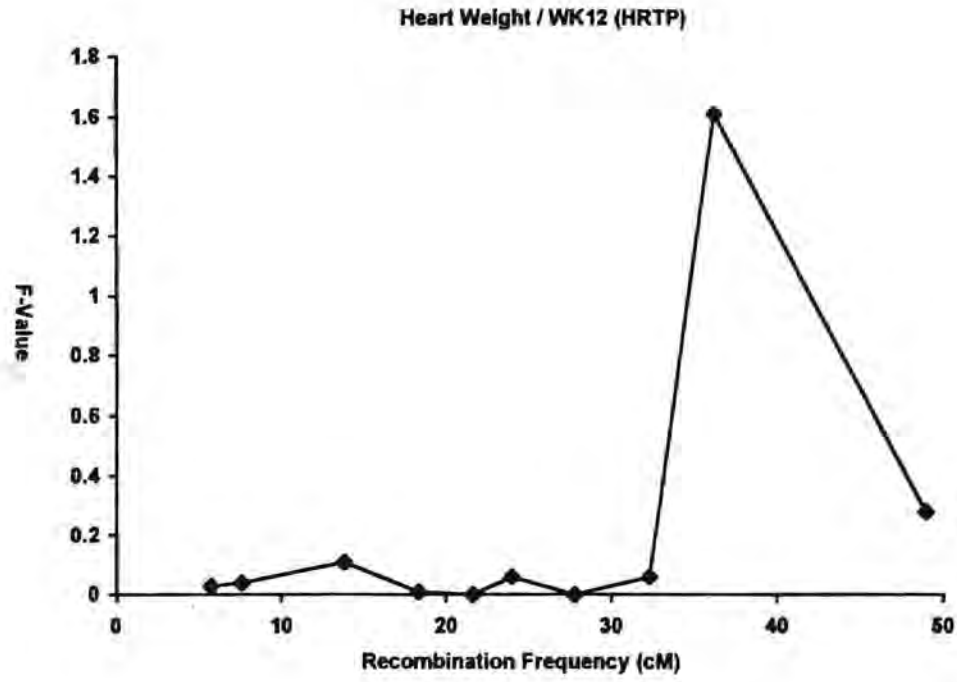

12 Week Body Weight (WK12)
F-Value
25
20
15
10
5
0
0
10
20
30
40
50
Recombination Frequency (cM)
P < .001
P < .01
P < .05

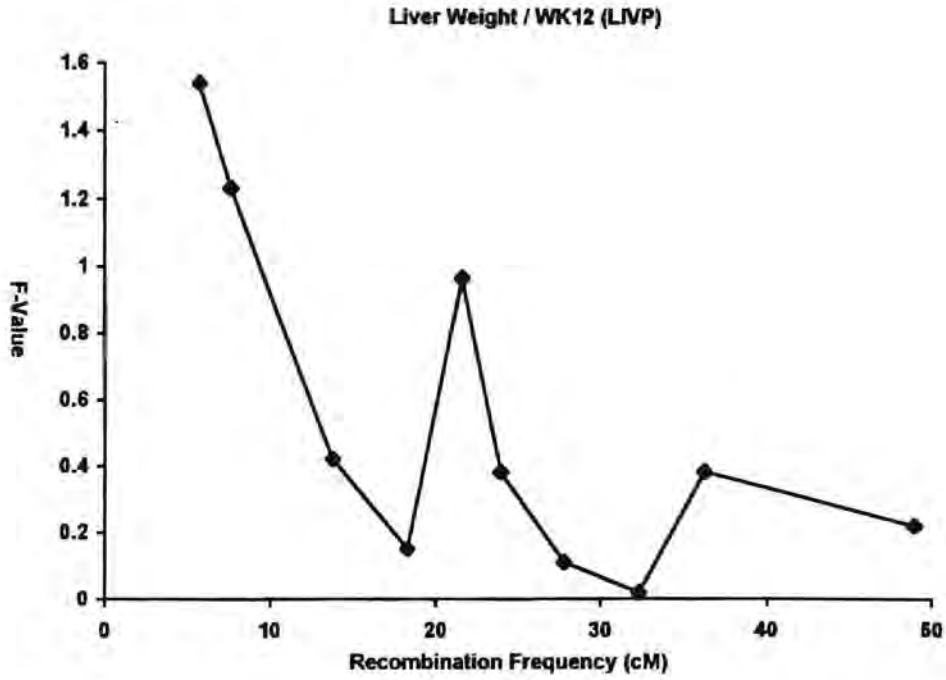

DAY12 To WK3 Gain (GAIN1)
F-Value
7
6
5
4
3
2
1
0
0
10
20
30
40
50
Recombination Frequency (cM)
P < .001
P < .01
P < .05

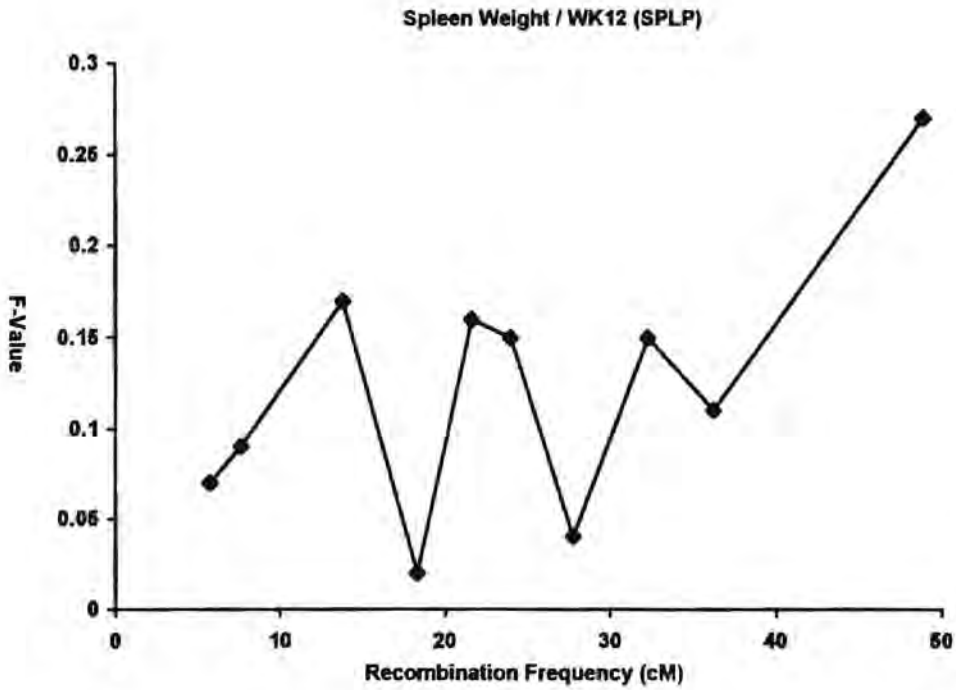

WK3 To WK6 Gain (GAIN2)
F-Value
Recombination Frequency (cM)
P < .01
P < .05
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50

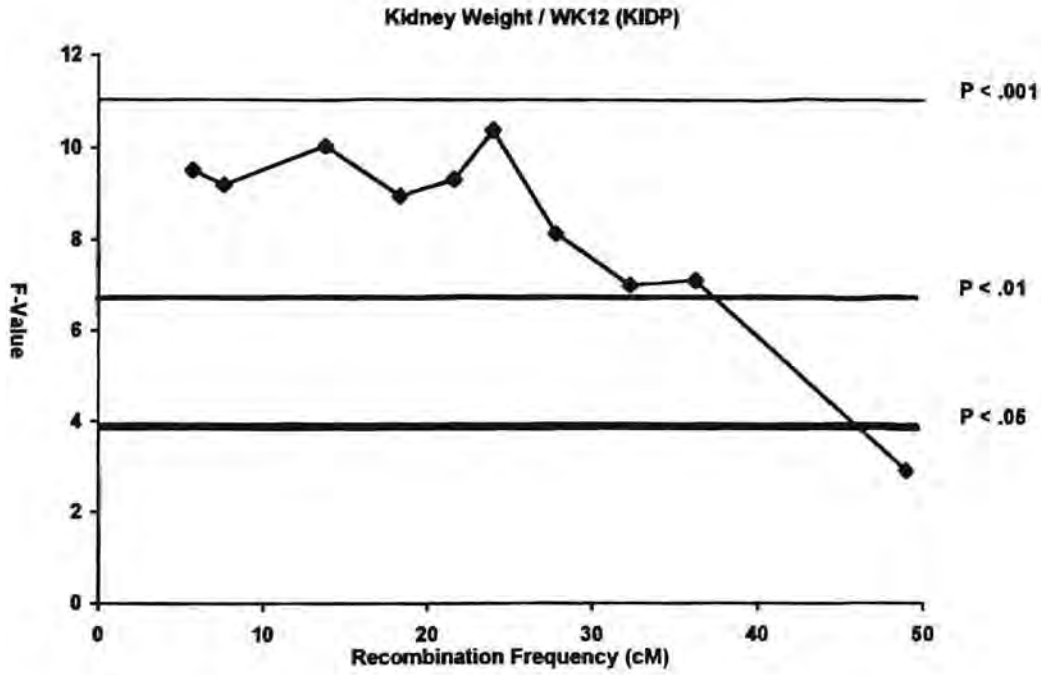

WK6 To WK9 Gain (GAIN3)
F-Value
Recombination Frequency (cM)
P < .01
P < .05
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50

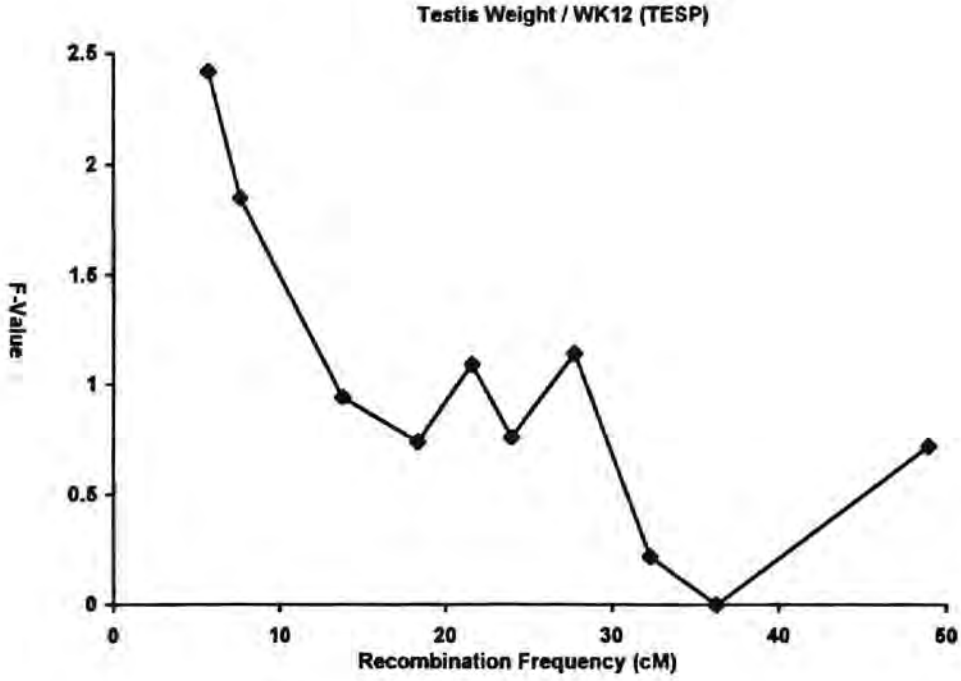

WK9 To WK12 Gain (GAIN4)
P < .05
F-Value
Recombination Frequency (cM)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
10
20
30
40
50

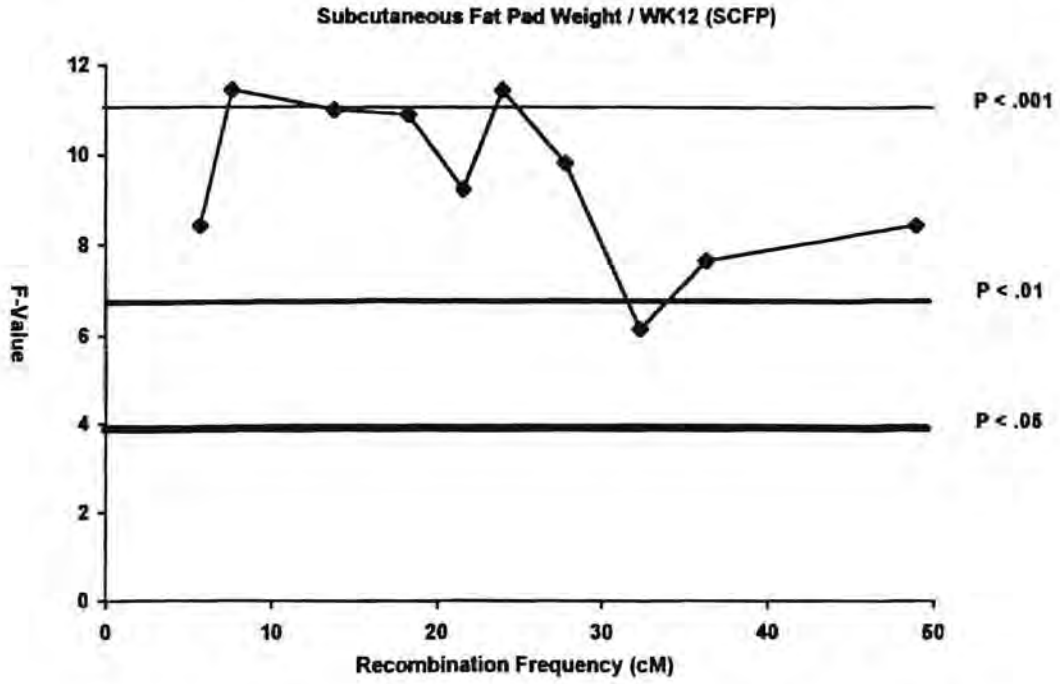

Heart Weight / WK12 (HRTP)
F-Value
Recombination Frequency (cM)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0
10
20
30
40
50

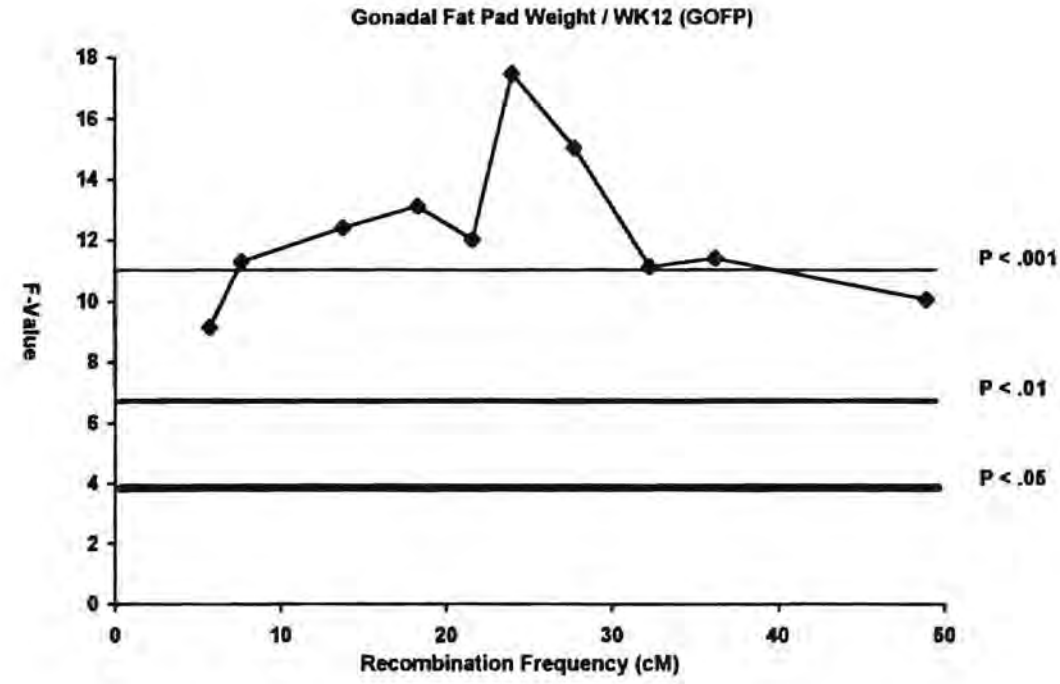

Liver Weight / WK12 (LIVP)
F-Value
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
Recombination Frequency (cM)

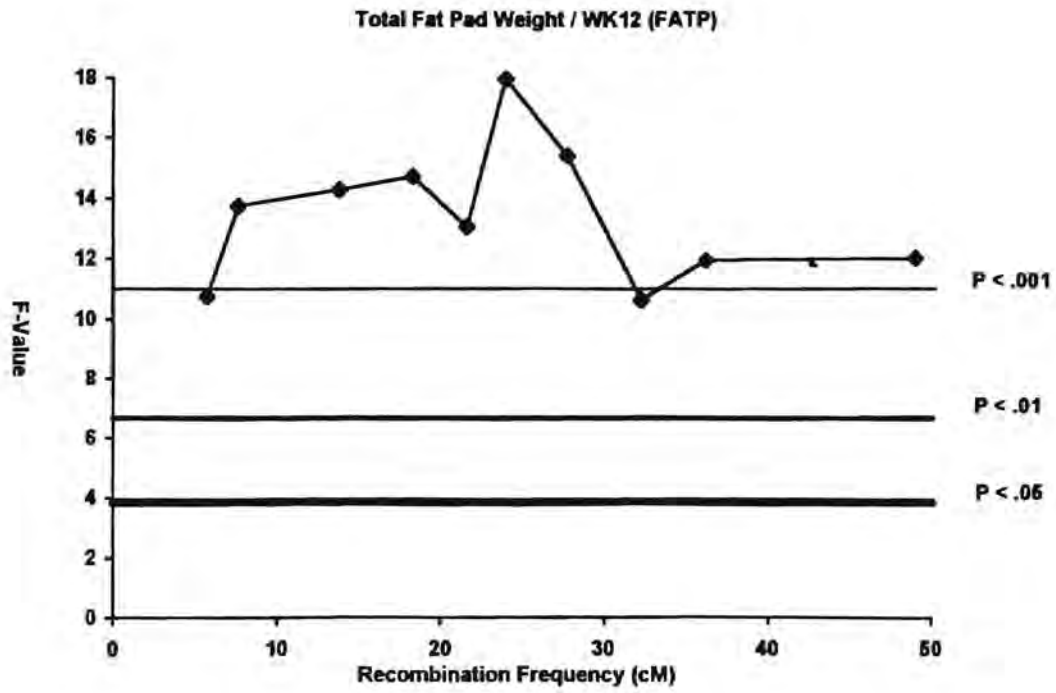

Spleen Weight / WK12 (SPLP)
F-Value
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
Recombination Frequency (cM)


Kidney Weight / WK12 (KIDP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05
0
2
4
6
8
10
12
0
10
20
30
40
50


Testis Weight / WK12 (TESP)
F-Value
Recombination Frequency (cM)
0
0.5
1
1.5
2
2.5
0
10
20
30
40
50


Subcutaneous Fat Pad Weight / WK12 (SCFP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05
0
2
4
6
8
10
12
0
10
20
30
40
50


Gonadal Fat Pad Weight / WK12 (GOFP)
F-Value
Recombination Frequency (cM)
P < .001
P < .01
P < .05
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50


Total Fat Pad Weight / WK12 (FATP)
F-Value
Recombination Frequency (cM)
0
2
4
6
8
10
12
14
16
18
0
10
20
30
40
50
P < .001
P < .01
P < .05

APPENDIX 16--LEAST-SQUARES MEANS FOR EACH TRAIT ANALYZED WITH 19 MARKER INTERVALS ON CHROMOSOME 2

NOTE: MM=M16i/M16i-M16i/M16i MH=M16i/M16i-M16i/CAST
HM=M16i/CAST-M16i/M16i HH=M16i/CAST-M16i/CAST

12 DAY BODY WEIGHT (DAY12)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	7.14 ± .05	7.54 ± .16	7.45 ± .17	7.25 ± .06
B2-C2	12.8-15.18	7.17 ± .05	7.21 ± .29	6.4 ± .43	7.3 ± .05
C2-D2	15.18-28.01	7.11 ± .05	7.42 ± .13	7.35 ± .16	7.29 ± .05
D2-E2	28.01-31.57	7.15 ± .05	6.98 ± .24	6.96 ± .34	7.32 ± .05
E2-F2	31.57-40.36	7.15 ± .05	7 ± .24	7.12 ± .15	7.33 ± .05
F2-G2	40.36-49.15	7.16 ± .05	6.92 ± .17	7.08 ± .17	7.34 ± .05
G2-H2	49.15-53.9	7.15 ± .05	7.28 ± .21	7.13 ± .28	7.3 ± .05
H2-I2	53.9-55.8	7.14 ± .05	7.86 ± .52	7.76 ± .61	7.29 ± .05
I2-J2	55.8-58.41	7.15 ± .05	7 ± .39	6.86 ± .65	7.31 ± .05
J2-K2	58.41-61.02	7.13 ± .05	7.33 ± .3	7 ± .34	7.31 ± .05
K2-L2	61.02-65.53	7.13± .05	7.02 ± .25	6.78 ± .23	7.34 ± .05
L2-M2	65.53-68.38	7.11 ± .05	7.36 ± .31	7.41 ± .34	7.32 ± .05
M2-S2	68.38-70.04	7.1 ± .05	7.97 ± .39	7.88 ± .51	7.32 ± .05
S2-T2	70.04-71.47	7.11 ± .05	7.48 ± .66	7.77 ± .43	7.33 ± .05
T2-N2	71.47-75.03	7.1 ± .05	7.34 ± .33	6.98 ± .25	7.34 ± .05
N2-O2	75.03-83.82	7.09 ± .05	7.29 ± .16	7.06 ± .18	7.36 ± .05
O2-P2	83.82-87.62	7.09 ± .05	6.87 ± .26	7.27 ± .28	7.35 ± .05
P2-Q2	87.62-94.27	7.08 ± .05	7.39 ± .22	7.16 ± .18	7.35 ± .05
Q2-R2	94.27-99.02	7.08 ± .05	7.32 ± .23	7.42 ± .25	7.35 ± .05

3 WEEK BODY WEIGHT (WK3)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	12.65 ± .11	13.18 ± .34	13.37 ± .38	12.74 ± .12
B2-C2	12.8-15.18	12.69 ± .11	13.5 ± .61	11.19 ± .93	12.82 ± .11
C2-D2	15.18-28.01	12.58 ± .11	13.22 ± .28	13.03 ± .35	12.82 ± .12
D2-E2	28.01-31.57	12.63 ± .11	12.74 ± .51	10.78 ± .72	12.94 ± .11
E2-F2	31.57-40.36	12.57 ± .11	12.66 ± .51	12.81 ± .33	12.94 ± .11
F2-G2	40.36-49.15	12.61 ± .11	12.47 ± .37	12.17 ± .37	12.99 ± .12
G2-H2	49.15-53.9	12.57 ± .11	12.59 ± .45	12.75 ± .6	12.96 ± .11
H2-I2	53.9-55.8	12.58 ± .11	12.75 ± 1.12	13.38 ± 1.3	12.91 ± .11
I2-J2	55.8-58.41	12.58 ± .11	12.85 ± .84	11.19 ± 1.4	12.92 ± .11
J2-K2	58.41-61.02	12.55 ± .11	13.03 ± .65	12.82 ± .73	12.94 ± .11
K2-L2	61.02-65.53	12.55 ± .11	12.58 ± .53	12.32 ± .5	12.97 ± .11
L2-M2	65.53-68.38	12.52 ± .11	12.89 ± .67	14.01 ± .74	12.94 ± .11
M2-S2	68.38-70.04	12.54 ± .11	13.98 ± .85	12.02 ± 1.11	12.94 ± .11
S2-T2	70.04-71.47	12.53 ± .11	13.4 ± 1.42	13.21 ± .93	12.96 ± .11
T2-N2	71.47-75.03	12.53 ± .11	12.67 ± .72	12.92 ± .54	12.94 ± .11
N2-O2	75.03-83.82	12.59 ±.12	12.26 ± .36	12.76 ± .38	12.96 ± .11
O2-P2	83.82-87.62	12.63 ± .11	11.86 ± .57	13.21 ± .61	12.87 ± .11
P2-Q2	87.62-94.27	12.63 ± .12	13.14 ± .48	12.3 ± .39	12.87 ± .11
Q2-R2	94.27-99.02	12.59 ± .11	12.97 ± .51	13.19 ± .54	12.87 ± .11

6 WEEK BODY WEIGHT (WK6)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	32.53 ± .3	30.99 ± .93	31.9 ± 1.03	31.95 ± .33
B2-C2	12.8-15.18	32.51 ± .29	30.69 ± 1.68	34.44 ± 2.55	31.79 ± .31
C2-D2	15.18-28.01	32.74 ± .31	31.39 ± .76	32.65 ± .95	31.67 ± .32
D2-E2	28.01-31.57	32.80 ± .31	31.24 ± 1.41	31.88 ± 1.97	31.61 ± .3
E2-F2	31.57-40.36	32.88 ± .31	30.81 ± 1.37	33.47 ± .88	31.33 ± .31
F2-G2	40.36-49.15	33.03 ± .3	32.04 ± .99	29.97 ± 1	31.45 ± .31
G2-H2	49.15-53.9	32.9 ± .3	31.33 ± 1.22	33.18 ± 1.62	31.45 ± .3
H2-I2	53.9-55.8	32.89 ± .3	36 ± 3.02	35.23 ± 3.51	31.32 ± .3
I2-J2	55.8-58.41	32.91 ± .3	33.1 ± 2.3	35.63 ± 3.8	31.35 ± .3
J2-K2	58.41-61.02	32.9 ± .31	33.64 ± 1.76	30.74 ± 1.99	31.47 ± .29
K2-L2	61.02-65.53	32.73 ± .31	35.29 ± 1.45	29 ± 1.36	31.64 ± .29
L2-M2	65.53-68.38	32.57 ± .31	31.92 ± 1.84	32.95 ± 2.04	31.78 ± .3
M2-S2	68.38-70.04	32.58 ± .31	31.26 ± 2.33	32.88 ± 3.07	31.77 ± .29
S2-T2	70.04-71.47	32.61 ± .31	32.18 ± 3.91	33.43 ± 2.56	31.79 ± .29
T2-N2	71.47-75.03	32.68 ± .31	30.07 ± 1.96	34.22 ± 1.48	31.64 ± .3
N2-O2	75.03-83.82	32.89 ± .32	31.92 ± .97	31.16 ± 1.05	31.64 ± .3
O2-P2	83.82-87.62	32.7 ± .31	33.56 ± 1.57	31.42 ± 1.67	31.68 ± .3
P2-Q2	87.62-94.27	32.54 ± .32	34.7 ± 1.3	30 ± 1.07	31.88 ± .3
Q2-R2	94.27-99.02	32.39 ± .32	31.26 ± 1.4	32 ± 1.5	32.04 ± .3

9 WEEK BODY WEIGHT (WK9)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	38.85 ± .31	37.34 ± .97	37.12 ± 1.07	37.71 ± .34
B2-C2	12.8-15.18	38.76 ± .3	36.36 ± 1.74	42.35 ± 2.64	37.6 ± .32
C2-D2	15.18-28.01	39.1 ± .32	37.15 ± .78	38.7 ± .98	37.46 ± .33
D2-E2	28.01-31.57	39.12 ± .32	37.64 ± 1.46	40.96 ± 2.03	37.3 ± .3
E2-F2	31.57-40.36	39.4 ± .31	34.85 ± 1.38	40.97 ± .88	36.8 ± .31
F2-G2	40.36-49.15	39.76 ± .3	37.95 ± .99	38.86 ± 1.01	36.5 ± .31
G2-H2	49.15-53.9	39.84 ± .3	37.83 ± 1.21	39.17 ± 1.6	36.54 ± .3
H2-I2	53.9-55.8	39.81 ± .3	42.96 ± 2.99	42.72 ± 3.48	36.51 ± .29
I2-J2	55.8-58.41	39.86 ± .3	37.61 ± 2.27	43.65 ± 3.75	36.53 ± .29
J2-K2	58.41-61.02	39.89 ± .3	39.42 ± 1.73	38.87 ± 1.96	36.59 ± .29
K2-L2	61.02-65.53	39.8 ± .31	40.69 ± 1.44	34.75 ± 1.35	36.76 ± .29
L2-M2	65.53-68.38	39.66 ± .31	36.37 ± 1.84	38.2 ± 2.04	36.9 ± .3
M2-S2	68.38-70.04	39.72 ± .31	33.28 ± 2.31	40.46 ± 3.04	36.87 ± .29
S2-T2	70.04-71.47	39.75 ± .31	38.91 ± 3.89	38.01 ± 2.55	36.82 ± .29
T2-N2	71.47-75.03	39.72 ± .31	39.3 ± 1.96	38.9 ± 1.48	36.72 ± .3
N2-O2	75.03-83.82	39.8 ± .32	39.33 ± .97	36.33 ± 1.05	36.81 ± .3
O2-P2	83.82-87.62	39.47 ± .32	39.76 ± 1.57	37.02 ± 1.69	37.02 ± .3
P2-Q2	87.62-94.27	39.35 ± .32	40.45 ± 1.33	35.96 ± 1.1	37.21 ± .3
Q2-R2	94.27-99.02	39.01 ± .32	40.33 ± 1.44	36.07 ± 1.54	37.43 ± .31

12 WEEK BODY WEIGHT (WK12)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	41.14 ± .36	40.14 ± 1.11	39.58 ± 1.22	40.17 ± .39
B2-C2	12.8-15.18	41.17 ± .34	35.47 ± 1.97	46.32 ± 2.98	40.07 ± .36
C2-D2	15.18-28.01	41.51 ± .37	39.6 ± .89	40.87 ± 1.12	39.85 ± .38
D2-E2	28.01-31.57	41.53 ± .36	39.64 ± 1.65	45.29 ± 2.31	39.66 ± .35
E2-F2	31.57-40.36	41.89 ± .35	36.57 ± 1.56	44.03 ± 1	39.04 ± .35
F2-G2	40.36-49.15	42.31 ± .34	40.8 ± 1.12	42.72 ± 1.13	38.56 ± .35
G2-H2	49.15-53.9	42.54 ± .34	40.01 ± 1.36	42.35 ± 1.8	38.64 ± .34
H2-I2	53.9-55.8	42.53 ± .33	44.94 ± 3.36	45.82 ± 3.91	38.59 ± .33
I2-J2	55.8-58.41	42.67 ± .33	38.63 ± 2.54	45.74 ± 4.2	38.6 ± .33
J2-K2	58.41-61.02	42.69 ± .33	41.77 ± 1.92	44.53 ± 2.17	38.55 ± .32
K2-L2	61.02-65.53	42.78 ± .34	41.5 ± 1.6	37.55 ± 1.5	38.68 ± .32
L2-M2	65.53-68.38	42.63 ± .34	39.63 ± 2.03	39.76 ± 2.24	38.78 ± .33
M2-S2	68.38-70.04	42.67 ± .34	34.92 ± 2.54	43.06 ± 3.35	38.79 ± .32
S2-T2	70.04-71.47	42.71 ± .34	41.38 ± 4.28	41.11 ± 2.8	38.74 ± .32
T2-N2	71.47-75.03	42.67 ± .34	42.22 ± 2.16	40.84 ± 1.64	38.62 ± .33
N2-O2	75.03-83.82	42.73 ± .35	41.94 ± 1.07	38.34 ± 1.16	38.73 ± .33
O2-P2	83.82-87.62	42.31 ± .35	42.88 ± 1.75	37.38 ± 1.88	39.07 ± .33
P2-Q2	87.62-94.27	42.1 ± .36	43.2 ± 1.49	38.16 ± 1.23	39.29 ± .34
Q2-R2	94.27-99.02	41.67 ± .36	43.79 ± 1.61	37.02 ± 1.72	39.6 ± .35

DAY12 TO WK3 GAIN (GAIN1)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	5.51 ± .08	5.64 ± .25	5.92 ± .28	5.49 ± .09
B2-C2	12.8-15.18	5.52 ± .08	6.29 ± .46	4.8 ± .69	5.52 ± .08
C2-D2	15.18-28.01	5.47 ± .08	5.79 ± .2	5.68 ± .26	5.53 ± .09
D2-E2	28.01-31.57	5.48 ± .08	5.76 ± .38	3.82 ± .53	5.62 ± .08
E2-F2	31.57-40.36	5.43 ± .09	5.67 ± .38	5.69 ± .24	5.61 ± .08
F2-G2	40.36-49.15	5.45 ± .08	5.55 ± .27	5.08 ± .28	5.66 ± .09
G2-H2	49.15-53.9	5.42 ± .08	5.3 ± .33	5.61 ± .44	5.65 ± .08
H2-I2	53.9-55.8	5.43 ± .08	4.89 ± .83	5.62 ± .96	5.62 ± .08
I2-J2	55.8-58.41	5.44 ± .08	5.85 ± .63	4.34 ± 1.04	5.62 ± .08
J2-K2	58.41-61.02	5.42 ± .08	5.7 ± .48	5.82 ± .54	5.63 ± .08
K2-L2	61.02-65.53	5.42 ± .08	5.56 ± .4	5.54 ± .37	5.63 ± .08
L2-M2	65.53-68.38	5.41 ± .08	5.53 ± .49	6.6 ± .55	5.61 ± .08
M2-S2	68.38-70.04	5.44 ± .08	6.01 ± .63	4.15 ± .83	5.62 ± .08
S2-T2	70.04-71.47	5.42 ± .08	5.93 ± 1.06	5.44 ± .69	5.63 ± .08
T2-N2	71.47-75.03	5.42 ± .08	5.33 ± .53	5.94 ± .41	5.61 ± .08
N2-O2	75.03-83.82	5.5 ± .09	4.97 ± .26	5.7 ± .29	5.6 ± .08
O2-P2	83.82-87.62	5.53 ± .09	5 ± .42	5.94 ± .45	5.51 ± .08
P2-Q2	87.62-94.27	5.55 ± .09	5.75 ± .35	5.14 ± .29	5.52 ± .08
Q2-R2	94.27-99.02	5.51 ± .08	5.65 ± .38	5.77 ± .4	5.52 ± .08

WK3 TO WK6 GAIN (GAIN2)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	19.88 ± .28	17.81 ± .85	18.53 ± .94	19.21 ± .3
B2-C2	12.8-15.18	19.82 ± .26	17.19 ± 1.53	23.24 ± 2.32	18.97 ± .28
C2-D2	15.18-28.01	20.16 ± .28	18.17 ± .69	19.62 ± .86	18.85 ± .29
D2-E2	28.01-31.57	20.18 ± .28	18.5 ± 1.28	21.11 ± 1.78	18.67 ± .27
E2-F2	31.57-40.36	20.31 ± .28	18.14 ± 1.24	20.66 ± .8	18.38 ± .28
F2-G2	40.36-49.15	20.41 ± .27	19.57 ± .9	17.8 ± .91	18.45 ± .28
G2-H2	49.15-53.9	20.33 ± .27	18.75 ± 1.1	20.43 ± 1.46	18.49 ± .27
H2-I2	53.9-55.8	20.32 ± .27	23.25 ± 2.72	21.85 ± 3.16	18.41 ± .27
I2-J2	55.8-58.41	20.33 ± .27	20.24 ± 2.06	24.44 ± 3.41	18.42 ± .27
J2-K2	58.41-61.02	20.35 ± .28	20.61 ± 1.59	17.92 ± 1.79	18.53 ± .26
K2-L2	61.02-65.53	20.18 ± .28	22.72 ± 1.31	16.69 ± 1.23	18.67 ± .26
L2-M2	65.53-68.38	20.05 ± .28	19.03 ± 1.67	18.94 ± 1.85	18.84 ± .27
M2-S2	68.38-70.04	20.04 ± .28	17.29 ± 2.11	20.85 ± 2.77	18.83 ± .27
S2-T2	70.04-71.47	20.07 ± .28	18.78 ± 2.32	20.22 ± 2.32	18.83 ± .26
T2-N2	71.47-75.03	20.15 ± .28	17.4 ± 1.78	21.3 ± 1.34	18.69 ± .27
N2-O2	75.03-83.82	20.3 ± .29	19.67 ± .88	18.41 ± .95	18.68 ± .28
O2-P2	83.82-87.62	20.08 ± .28	21.7 ± 1.41	18.21 ± 1.52	18.81 ± .27
P2-Q2	87.62-94.27	19.9 ± .29	21.56 ± 1.19	17.7 ± .98	19.01 ± .27
Q2-R2	94.27-99.02	19.8 ± .29	18.29 ± 1.27	18.81 ± 1.36	19.17 ± .27

WK6 TO WK9 GAIN (GAIN3)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	6.32 ± .23	6.36 ± .72	5.22 ± .79	5.76 ± .25
B2-C2	12.8-15.18	6.25 ± .23	5.67 ± 1.31	7.91 ± 1.98	5.81 ± .24
C2-D2	15.18-28.01	6.37 ± .24	5.76 ± .59	6.04 ± .74	5.78 ± .25
D2-E2	28.01-31.57	6.32 ± .24	6.4 ± 1.1	9.08 ± 1.53	5.69 ± .23
E2-F2	31.57-40.36	6.51 ± .24	4.05 ± 1.06	7.5 ± .68	5.47 ± .24
F2-G2	40.36-49.15	6.74 ± .23	5.91 ± .75	8.89 ± .76	5.05 ± .24
G2-H2	49.15-53.9	6.94 ± .23	6.5 ± .92	5.99 ± 1.23	5.1 ± .23
H2-I2	53.9-55.8	6.92 ± .23	6.96 ± 2.31	7.49 ± 2.69	5.19 ± .23
I2-J2	55.8-58.41	6.95 ± .23	4.51 ± 1.75	8.01 ± 2.89	5.19 ± .23
J2-K2	58.41-61.02	6.99 ± .23	5.78 ± 1.32	8.13 ± 1.49	5.12 ± .22
K2-L2	61.02-65.53	7.07 ± .23	5.39 ± 1.1	5.75 ± 1.03	5.11 ± .22
L2-M2	65.53-68.38	7.09 ± .23	4.45 ± 1.37	5.26 ± 1.51	5.12 ± .22
M2-S2	68.38-70.04	7.14 ± .23	2.02 ± 1.72	7.59 ± 2.26	5.1 ± .22
S2-T2	70.04-71.47	7.14 ± .23	6.73 ± 2.89	4.59 ± 1.89	5.03 ± .22
T2-N2	71.47-75.03	7.04 ± .23	9.23 ± 1.46	4.68 ± 1.11	5.08 ± .22
N2-O2	75.03-83.82	6.91 ± .24	7.4 ± .73	5.16 ± .79	5.17 ± .23
O2-P2	83.82-87.62	6.77 ± .24	6.2 ± 1.19	5.6 ± 1.28	5.34 ± .23
P2-Q2	87.62-94.27	6.81 ± .24	5.75 ± 1	5.96 ± .82	5.33 ± .23
Q2-R2	94.27-99.02	6.62 ± .24	9.07 ± 1.05	4.07 ± 1.13	5.4 ± .23

WK9 TO WK12 GAIN (GAIN4)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	2.29 ± .16	2.8 ± .5	2.46 ± .55	2.46 ± .18
B2-C2	12.8-15.18	2.41 ± .15	-.89 ± .88	3.97 ± 1.34	2.48 ± .16
C2-D2	15.18-28.01	2.41 ± .17	2.45 ± .41	2.17 ± .51	2.4 ± .17
D2-E2	28.01-31.57	2.41 ± .16	2 ± .76	4.33 ± 1.05	2.35 ± .16
E2-F2	31.57-40.36	2.49 ± .17	1.72 ± .74	3.06 ± .48	2.24 ± .17
F2-G2	40.36-49.15	2.55 ± .16	2.85 ± .53	3.87 ± .54	2.06 ± .17
G2-H2	49.15-53.9	2.69 ± .16	2.18 ± .65	3.18 ± .87	2.1 ± .16
H2-I2	53.9-55.8	2.72 ± .16	1.97 ± 1.62	3.1 ± 1.89	2.07 ± .16
I2-J2	55.8-58.41	2.8 ± .16	1.02 ± 1.22	2.09 ± 2.02	2.07 ± .16
J2-K2	58.41-61.02	2.81 ± .16	2.35 ± .92	5.66 ± 1.04	1.95 ± .15
K2-L2	61.02-65.53	2.98 ± .16	.81 ± .76	2.8 ± .71	1.92 ± .15
L2-M2	65.53-68.38	2.97 ± .16	3.26 ± .95	1.56 ± 1.05	1.89 ± .15
M2-S2	68.38-70.04	2.95 ± .16	1.64 ± 1.21	2.6 ± 1.59	1.92 ± .15
S2-T2	70.04-71.47	2.96 ± .16	2.47 ± 2.02	3.1 ± 1.32	1.91 ± .15
T2-N2	71.47-75.03	2.95 ± .16	2.92 ± 1.03	1.94 ± .78	1.91 ± .15
N2-O2	75.03-83.82	2.93 ± .17	2.62 ± .51	2.02 ± .55	1.92 ± .16
O2-P2	83.82-87.62	2.84 ± .16	3.13 ± .81	.36 ± .87	2.05 ± .15
P2-Q2	87.62-94.27	2.75 ± .17	2.76 ± .69	2.2 ± .57	2.08 ± .16
Q2-R2	94.27-99.02	2.66 ± .17	3.46 ± .73	.95 ± .78	2.16 ± .16

HRT/WK12 (HRTP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.594 ± .008	.594 ± .025	.551 ± .028	.591 ± .009
B2-C2	12.8-15.18	.587 ± .008	.677 ± .045	.577 ± .068	.592 ± .008
C2-D2	15.18-28.01	.583 ± .008	.609 ± .02	.588 ± .03	.595 ± .009
D2-E2	28.01-31.57	.582 ± .008	.61 ± .038	.541 ± .053	.599 ± .008
E2-F2	31.57-40.36	.568 ± .008	.801 ± .034	.554 ± .022	.606 ± .008
F2-G2	40.36-49.15	.565 ± .008	.576 ± .026	.56 ± .026	.624 ± .008
G2-H2	49.15-53.9	.563 ± .008	.575 ± .032	.575 ± .042	.62 ± .008
H2-I2	53.9-55.8	.563 ± .008	.574 ± .08	.619 ± .093	.617 ± .008
I2-J2	55.8-58.41	.561 ± .008	.701 ± .06	.514 ± .099	.617 ± .008
J2-K2	58.41-61.02	.561 ± .008	.577 ± .046	.592 ± .052	.619 ± .008
K2-L2	61.02-65.53	.561 ± .008	.574 ± .038	.647 ± .035	.616 ± .008
L2-M2	65.53-68.38	.565 ± .008	.552 ± .048	.562 ± .053	.616 ± .008
M2-S2	68.38-70.04	.564 ± .008	.593 ± .061	.527 ± .08	.615 ± .008
S2-T2	70.04-71.47	.563 ± .008	.601 ± .102	.579 ± .067	.615 ± .008
T2-N2	71.47-75.03	.564 ± .008	.559 ± .052	.611 ± .039	.615 ± .008
N2-O2	75.03-83.82	.566 ± .008	.565 ± .025	.686 ± .027	.608 ± .008
O2-P2	83.82-87.62	.578 ± .008	.59 ± .04	.77 ± .043	.6 ± .008
P2-Q2	87.62-94.27	.584 ± .009	.575 ± .035	.581 ± .029	.6 ± .008
Q2-R2	94.27-99.02	.586 ± .008	.536 ± .037	.653 ± .039	.597 ± .008

LIV/WK12 (LIVP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	7.64 ± .05	7.47 ± .16	7.46 ± .18	7.58 ± .06
B2-C2	12.8-15.18	7.64 ± .05	7.5 ± .28	7.48 ± .43	7.57 ± .05
C2-D2	15.18-28.01	7.66 ± .05	7.51 ± .13	7.58 ± .16	7.57 ± .05
D2-E2	28.01-31.57	7.66 ± .05	7.29 ± .24	7.58 ± .33	7.56 ± .05
E2-F2	31.57-40.36	7.67 ± .05	7.51 ± .24	7.69 ± .15	7.52 ± .05
F2-G2	40.36-49.15	7.68 ± .05	7.52 ± .17	7.59 ± .17	7.51 ± .05
G2-H2	49.15-53.9	7.68 ± .05	7.64 ± .21	7.96 ± .28	7.49 ± .05
H2-I2	53.9-55.8	7.7 ± .05	7.08 ± .52	7.38 ± .6	7.5 ± .05
I2-J2	55.8-58.41	7.7 ± .05	7.31 ± .39	7.91 ± .65	7.49 ± .05
J2-K2	58.41-61.02	7.71 ± .05	7.52 ± .3	7.16 ± .34	7.5 ± .05
K2-L2	61.02-65.53	7.69 ± .05	7.81 ± .25	7.69 ± .23	7.5 ± .05
L2-M2	65.53-68.38	7.68 ± .05	7.81 ± .31	7.03 ± .34	7.52 ± .05
M2-S2	68.38-70.04	7.66 ± .05	7.67 ± .4	7.7 ± .52	7.53 ± .05
S2-T2	70.04-71.47	7.65 ± .05	8.74 ± .66	8.25 ± .43	7.53 ± .05
T2-N2	71.47-75.03	7.66 ± .05	7.47 ± .34	7.51 ± .25	7.54 ± .05
N2-O2	75.03-83.82	7.65 ± .06	7.75 ± .17	7.7 ± .18	7.52 ± .05
O2-P2	83.82-87.62	7.66 ± .05	7.59 ± .27	7.3 ± .29	7.55 ± .05
P2-Q2	87.62-94.27	7.62 ± .05	7.91 ± .22	7.59 ± .18	7.55 ± .05
Q2-R2	94.27-99.02	7.62 ± .05	7.68 ± .24	7.5 ± .25	7.57 ± .05

SPL/WK12 (SPLP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.541 ± .01	.555 ± .03	.525 ± .034	.534 ± .011
B2-C2	12.8-15.18	.539 ± .009	.542 ± .055	.594 ± .083	.535 ± .01
C2-D2	15.18-28.01	.54 ± .01	.539 ± .025	.524 ± .031	.536 ± .01
D2-E2	28.01-31.57	.541 ± .01	.474 ± .046	.584 ± .064	.535 ± .01
E2-F2	31.57-40.36	.541 ± .01	.574 ± .045	.481 ± .029	.54 ± .01
F2-G2	40.36-49.15	.527 ± .01	.589 ± .033	.486 ± .033	.546 ± .01
G2-H2	49.15-53.9	.515 ± .01	.652 ± .039	.598 ± .052	.55 ± .01
H2-I2	53.9-55.8	.517 ± .01	.6 ± .099	.468 ± .115	.556 ± .01
I2-J2	55.8-58.41	.516 ± .01	.465 ± .074	.743 ± .123	.556 ± .01
J2-K2	58.41-61.02	.515 ± .01	.544 ± .057	.455 ± .064	.559 ± .009
K2-L2	61.02-65.53	.51 ± .01	.594 ± .047	.507 ± .044	.561 ± .009
L2-M2	65.53-68.38	.51 ± .01	.512 ± .058	.443 ± .064	.565 ± .009
M2-S2	68.38-70.04	.508 ± .01	.499 ± .074	.504 ± .097	.565 ± .009
S2-T2	70.04-71.47	.508 ± .01	.389 ± .124	.61 ± .081	.561 ± .009
T2-N2	71.47-75.03	.511 ± .01	.509 ± .063	.61 ± .048	.558 ± .009
N2-O2	75.03-83.82	.517 ± .01	.496 ± .031	.54 ± .034	.559 ± .01
O2-P2	83.82-87.62	.521 ± .01	.487 ± .05	.639 ± .054	.55 ± .009
P2-Q2	87.62-94.27	.526 ± .01	.502 ± .042	.547 ± .035	.548 ± .01
Q2-R2	94.27-99.02	.53 ± .01	.487 ± .045	.529 ± .048	.546 ± .01

KID/WK12 (KIDP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.821 ± .009	.814 ± .027	.805 ± .031	.818 ± .01
B2-C2	12.8-15.18	.818 ± .008	.9 ± .049	.792 ± .074	.818 ± .009
C2-D2	15.18-28.01	.807 ± .009	.878 ± .021	.752 ± .027	.828 ± .009
D2-E2	28.01-31.57	.797 ± .009	.857 ± .04	.707 ± .056	.840 ± .008
E2-F2	31.57-40.36	.79 ± .009	.89 ± .039	.81 ± .025	.845 ± .009
F2-G2	40.36-49.15	.791 ± .009	.804 ± .028	.764 ± .029	.854 ± .009
G2-H2	49.15-53.9	.787 ± .009	.811 ± .035	.765 ± .046	.852 ± .009
H2-I2	53.9-55.8	.785 ± .009	.858 ± .086	.777 ± .1	.849 ± .008
I2-J2	55.8-58.41	.784 ± .009	.916 ± .065	.718 ± .108	.85 ± .008
J2-K2	58.41-61.02	.782 ± .009	.868 ± .049	.753 ± .056	.853 ± .008
K2-L2	61.02-65.53	.778 ± .009	.876 ± .04	.792 ± .038	.856 ± .008
L2-M2	65.53-68.38	.777 ± .008	.723 ± .05	.941 ± .055	.857 ± .008
M2-S2	68.38-70.04	.778 ± .009	.827 ± .064	.749 ± .085	.855 ± .008
S2-T2	70.04-71.47	.777 ± .009	.804 ± .108	.865 ± .071	.854 ± .008
T2-N2	71.47-75.03	.779 ± .009	.771 ± .055	.846 ± .041	.854 ± .008
N2-O2	75.03-83.82	.779 ± .009	.806 ± .027	.808 ± .029	.856 ± .008
O2-P2	83.82-87.62	.782 ± .009	.784 ± .043	.803 ± .047	.853 ± .008
P2-Q2	87.62-94.27	.781 ± .009	.805 ± .036	.819 ± .03	.853 ± .008
Q2-R2	94.27-99.02	.786 ± .009	.75 ± .039	.869 ± .042	.85 ± .008

TES/WK12 (TESP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.201 ± .005	.211 ± .014	.178 ± .022	.228 ± .005
B2-C2	12.8-15.18	.198 ± .005	.218 ± .025	.211 ± .036	.226 ± .004
C2-D2	15.18-28.01	.198 ± .005	.198 ± .013	.24 ± .014	.225 ± .005
D2-E2	28.01-31.57	.205 ± .005	.177 ± .02	.243 ± .036	.221 ± .004
E2-F2	31.57-40.36	.206 ± .005	.194 ± .035	.203 ± .014	.221 ± .005
F2-G2	40.36-49.15	.203 ± .005	.224 ± .016	.223 ± .018	.221 ± .005
G2-H2	49.15-53.9	.205 ± .005	.195 ± .023	.212 ± .029	.221 ± .005
H2-I2	53.9-55.8	.205 ± .005	.158 ± .051	.206 ± .021	.221 ± .005
I2-J2	55.8-58.41	.204 ± .005	.213 ± .018	.16 ± .034	.222 ± .005
J2-K2	58.41-61.02	.203 ± .005	.241 ± .029	.23 ± .029	.221 ± .005
K2-L2	61.02-65.53	.204 ± .005	.205 ± .022	.26 ± .02	.219 ± .005
L2-M2	65.53-68.38	.207 ± .005	.214 ± .036	.174 ± .048	.219 ± .005
M2-S2	68.38-70.04	.205 ± .005	.339 ± .05	.152 ± .048	.219 ± .005
S2-T2	70.04-71.47	.204 ± .005	-	.219 ± .03	.221 ± .005
T2-N2	71.47-75.03	.204 ± .005	.216 ± .035	.21 ± .02	.221 ± .005
N2-O2	75.03-83.82	.206 ± .005	.193 ± .018	.226 ± .018	.221 ± .005
O2-P2	83.82-87.62	.207 ± .005	.217 ± .029	.237 ± .02	.217 ± .005
P2-Q2	87.62-94.27	.21 ± .005	.179 ± .023	.219 ± .019	.217 ± .005
Q2-R2	94.27-99.02	.21 ± .005	.243 ± .021	.239 ± .025	.213 ± .005

SCF/WK12 (SCFP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.584 ± .022	.524 ± .067	.542 ± .077	.576 ± .024
B2-C2	12.8-15.18	.587 ± .021	.334 ± .12	.996 ± .181	.563 ± .022
C2-D2	15.18-28.01	.61 ± .022	.49 ± .054	.634 ± .068	.547 ± .023
D2-E2	28.01-31.57	.611 ± .022	.671 ± .1	.980 ± .139	.527 ± .021
E2-F2	31.57-40.36	.633 ± .022	.366 ± .096	.784 ± .062	.498 ± .022
F2-G2	40.36-49.15	.656 ± .021	.603 ± .07	.703 ± .07	.476 ± .022
G2-H2	49.15-53.9	.682 ± .021	.377 ± .084	.618 ± .111	.484 ± .021
H2-I2	53.9-55.8	.68 ± .021	.519 ± .21	.66 ± .244	.479 ± .021
I2-J2	55.8-58.41	.684 ± .021	.569 ± .159	.344 ± .262	.48 ± .02
J2-K2	58.41-61.02	.68 ± .021	.735 ± .118	1.092 ± .133	.466 ± .02
K2-L2	61.02-65.53	.701 ± .021	.467 ± .099	.484 ± .092	.473 ± .02
L2-M2	65.53-68.38	.701 ± .021	.428 ± .123	.588 ± .136	.468 ± .02
M2-S2	68.38-70.04	.703 ± .021	.455 ± .156	.418 ± .205	.468 ± .02
S2-T2	70.04-71.47	.702 ± .021	.596 ± .262	.559 ± .171	.468 ± .019
T2-N2	71.47-75.03	.706 ± .021	.457 ± .131	.592 ± .1	.463 ± .02
N2-O2	75.03-83.82	.704 ± .022	.685 ± .066	.556 ± .07	.455 ± .02
O2-P2	83.82-87.62	.689 ± .021	.743 ± .106	.434 ± .113	.477 ± .02
P2-Q2	87.62-94.27	.689 ± .022	.58 ± .09	.494 ± .074	.487 ± .021
Q2-R2	94.27-99.02	.658 ± .022	.873 ± .096	.379 ± .102	.497 ± .021

GOF/WK12 (GOFP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	.810 ± .031	.872 ± .096	.825 ± .112	.813 ± .034
B2-C2	12.8-15.18	.822 ± .03	.468 ± .173	1.201 ± .262	.814 ± .032
C2-D2	15.18-28.01	.835 ± .032	.775 ± .079	.928 ± .098	.79 ± .033
D2-E2	28.01-31.57	.841 ± .031	.921 ± .143	1.559 ± .2	.767 ± .03
E2-F2	31.57-40.36	.879 ± .032	.451 ± .14	.993 ± .09	.742 ± .031
F2-G2	40.36-49.15	.919 ± .031	.674 ± .1	.99 ± .101	.706 ± .032
G2-H2	49.15-53.9	.943 ± .03	.651 ± .121	.873 ± .161	.697 ± .03
H2-I2	53.9-55.8	.944 ± .03	.584 ± .301	.904 ± .351	.689 ± .03
I2-J2	55.8-58.41	.957 ± .03	.738 ± .226	.557 ± .375	.688 ± .029
J2-K2	58.41-61.02	.951 ± .03	1.055 ± .17	1.418 ± .192	.67 ± .028
K2-L2	61.02-65.53	.974 ± .03	.7 ± .142	.683 ± .133	.681 ± .029
L2-M2	65.53-68.38	.971 ± .03	.661 ± .176	1.096 ± .194	.67 ± .028
M2-S2	68.38-70.04	.981 ± .03	.725 ± .223	.655 ± .294	.668 ± .028
S2-T2	70.04-71.47	.98 ± .03	.632 ± .375	.721 ± .245	.668 ± .028
T2-N2	71.47-75.03	.979 ± .03	.891 ± .189	.873 ± .143	.66 ± .029
N2-O2	75.03-83.82	.967 ± .031	1.056 ± .093	.7 ± .101	.661 ± .029
O2-P2	83.82-87.62	.945 ± .031	.908 ± .154	.572 ± .166	.703 ± .029
P2-Q2	87.62-94.27	.941 ± .032	.753 ± .13	.65 ± .107	.715 ± .03
Q2-R2	94.27-99.02	.91 ± .031	1.019 ± .139	.518 ± .149	.726 ± .03

FAT/WK12 (FATP)

Marker Interval	Location (cM)	MM	MH	HM	HH
A2-B2	2.35-12.8	1396 ± 48	1398 ± 149	1378 ± 172	1388 ± 52
B2-C2	12.8-15.18	1412 ± 46	805 ± 265	2200 ± 402	1377 ± 49
C2-D2	15.18-28.01	1448 ± 50	1270 ± 122	1564 ± 151	1337 ± 51
D2-E2	28.01-31.57	1455 ± 48	1594 ± 220	2540 ± 307	1295 ± 46
E2-F2	31.57-40.36	1515 ± 48	816 ± 213	1779 ± 137	1240 ± 48
F2-G2	40.36-49.15	1580 ± 47	1274 ± 153	1690 ± 155	1183 ± 48
G2-H2	49.15-53.9	1628 ± 46	1028 ± 184	1493 ± 245	1182 ± 46
H2-I2	53.9-55.8	1627 ± 45	1106 ± 459	1570 ± 534	1169 ± 45
I2-J2	55.8-58.41	1643 ± 46	1309 ± 345	901 ± 571	1168 ± 45
J2-K2	58.41-61.02	1634 ± 45	1791 ± 257	2512 ± 290	1137 ± 43
K2-L2	61.02-65.53	1678 ± 46	1169 ± 215	1168 ± 201	1154 ± 44
L2-M2	65.53-68.38	1676 ± 45	1090 ± 266	1682 ± 295	1138 ± 43
M2-S2	68.38-70.04	1687 ± 45	1181 ± 337	1075 ± 445	1137 ± 43
S2-T2	70.04-71.47	1684 ± 45	1226 ± 567	1283 ± 371	1137 ± 42
T2-N2	71.47-75.03	1689 ± 45	1349 ± 285	1468 ± 216	1123 ± 43
N2-O2	75.03-83.82	1671 ± 47	1768 ± 143	1252 ± 152	1117 ± 44
O2-P2	83.82-87.62	1633 ± 47	1651 ± 233	1007 ± 250	1182 ± 44
P2-Q2	87.62-94.27	1626 ± 48	1334 ± 198	1146 ± 163	1205 ± 46
Q2-R2	94.27-99.02	1567 ± 48	1893 ± 211	897 ± 226	1225 ± 46

APPENDIX 17--LEAST-SQUARES MEANS FOR EACH TRAIT ANALYZED WITH 19 MARKER INTERVALS ON CHROMOSOME 15

NOTE: MM=M16i/M16i-M16i/M16i MH=M16i/M16i-M16i/CAST
HM=M16i/CAST-M16i/M16i HH=M16i/CAST-M16i/CAST

12 DAY BODY WEIGHT (DAY12)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	7.29 ± .05	7.45 ± .45	7.71 ± .33	7.15 ± .05
C15-D15	7.62-13.8	7.28 ± .05	7.59 ± .21	6.99 ± .22	7.16 ± .05
D15-E15	13.8-18.31	7.25 ± .05	7.69 ± .24	7.51 ± .26	7.17 ± .05
E15-A15	18.31-21.61	7.25 ± .05	7.32 ± .29	7.42 ± .18	7.18 ± .05
A15-F15	21.61-23.99	7.25 ± .05	6.87 ± .28	7.57 ± .21	7.19 ± .05
F15-G15	23.99-27.79	7.25 ± .05	7.06 ± .27	7.12 ± .27	7.21 ± .05
G15-H15	27.79-32.3	7.24 ± .05	7.77 ± .56	7.2 ± .19	7.21 ± .05
H15-J15	32.3-36.27	7.27 ± .05	7.97 ± .38	7.09 ± .23	7.24 ± .06
J15-I15	36.27-48.97	7.26 ± .06	7.37 ± .14	7.07 ± .18	7.25 ± .06

3 WEEK BODY WEIGHT (WK3)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	12.97 ± .11	13.08 ± .95	13.35 ± .71	12.53 ± .11
C15-D15	7.62-13.8	12.98 ± .11	12.83 ± .45	11.99 ± .47	12.58 ± .11
D15-E15	13.8-18.31	12.91 ± .11	13.33 ± .52	12.78 ± .55	12.57 ± .11
E15-A15	18.31-21.61	12.9 ± .11	13.14 ± .62	12.87 ± .38	12.59 ± .11
A15-F15	21.61-23.99	12.9 ± .11	12.33 ± .61	12.92 ± .44	12.61 ± .11
F15-G15	23.99-27.79	12.89 ± .11	12.64 ± .57	12.77 ± .57	12.62 ± .11
G15-H15	27.79-32.3	12.88 ± .11	14.1 ± 1.2	12.68 ± .4	12.62 ± .11
H15-J15	32.3-36.27	12.94 ± .11	13.43 ± .78	12.68 ± .48	12.75 ± .12
J15-I15	36.27-48.97	12.97 ± .12	12.87 ± .29	12.68 ± .38	12.75 ± .12

6 WEEK BODY WEIGHT (WK6)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	32.61 ± .31	31.71 ± 2.62	33.26 ± 1.95	31.74 ± .3
C15-D15	7.62-13.8	32.75 ± .31	30.72 ± 1.24	30.44 ± 1.28	31.82 ± .31
D15-E15	13.8-18.31	32.72 ± .31	30.61 ± 1.43	31 ± 1.51	31.79 ± .3
E15-A15	18.31-21.61	32.65 ± .31	33.4 ± 1.7	33.34 ± 1.04	31.57 ± .3
A15-F15	21.61-23.99	32.7 ± .3	33.12 ± 1.65	32.79 ± 1.21	31.58 ± .3
F15-G15	23.99-27.79	32.69 ± .31	32.76 ± 1.55	34.69 ± 1.54	31.56 ± .3
G15-H15	27.79-32.3	32.77 ± .3	32.77 ± 3.25	33.33 ± 1.09	31.46 ± .3
H15-J15	32.3-36.27	32.76 ± .3	34.12 ± 2.07	35.24 ± 1.27	31.69 ± .31
J15-I15	36.27-48.97	32.72 ± .32	33.13 ± .76	34.04 ± 1.01	31.7 ± .32

9 WEEK BODY WEIGHT (WK9)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	38.87 ± .32	35.3 ± 2.72	40.92 ± 2.03	37.55 ± .31
C15-D15	7.62-13.8	39.07 ± .33	36.51 ± 1.3	37.54 ± 1.34	37.51 ± .32
D15-E15	13.8-18.31	39.13 ± .32	35.9 ± 1.48	37.04 ± 1.57	37.48 ± .31
E15-A15	18.31-21.61	39.01 ± .32	41.24 ± 1.76	38.03 ± 1.08	37.32 ± .32
A15-F15	21.61-23.99	39.03 ± .32	40.2 ± 1.72	37.47 ± 1.26	37.35 ± .32
F15-G15	23.99-27.79	39.11 ± .32	38.33 ± 1.61	39.98 ± 1.6	37.26 ± .31
G15-H15	27.79-32.3	39.15 ± .31	38.18 ± 3.37	38.79 ± 1.13	37.18 ± .31
H15-J15	32.3-36.27	39.06 ± .32	39.36 ± 2.24	40.05 ± 1.37	37.33 ± .34
J15-I15	36.27-48.97	39.18 ± .34	38.34 ± .82	40.49 ± 1.07	37.22 ± .34

12 WEEK BODY WEIGHT (WK12)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	41.4 ± .36	37.04 ± 3.08	42.87 ± 2.3	39.85 ± .35
C15-D15	7.62-13.8	41.62 ± .37	38.67 ± 1.47	39.96 ± 1.51	39.79 ± .36
D15-E15	13.8-18.31	41.67 ± .37	38.34 ± 1.68	39.12 ± 1.78	39.77 ± .36
E15-A15	18.31-21.61	41.54 ± .36	43.37 ± 2	39.8 ± 1.23	39.66 ± .36
A15-F15	21.61-23.99	41.53 ± .36	42.68 ± 1.95	39.52 ± 1.42	39.68 ± .36
F15-G15	23.99-27.79	41.62 ± .36	40.52 ± 1.82	42.85 ± 1.81	39.56 ± .35
G15-H15	27.79-32.3	41.68 ± .35	39.02 ± 3.81	41.82 ± 1.28	39.42 ± .36
H15-J15	32.3-36.27	41.63 ± .36	42.45 ± 2.54	42.82 ± 1.55	39.38 ± .38
J15-I15	36.27-48.97	41.77 ± .39	40.82 ± .92	43.24 ± 1.21	39.25 ± .39

DAY12 TO WK3 GAIN (GAIN1)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	5.68 ± .08	5.64 ± .7	5.64 ± .52	5.38 ± .08
C15-D15	7.62-13.8	5.7 ± .08	5.24 ± .33	5 ± .34	5.41 ± .08
D15-E15	13.8-18.31	5.67 ± .08	5.64 ± .38	5.27 ± .41	5.4 ± .08
E15-A15	18.31-21.61	5.65 ± .08	5.83 ± .46	5.44 ± .28	5.41 ± .08
A15-F15	21.61-23.99	5.65 ± .08	5.46 ± .45	5.34 ± .33	5.42 ± .08
F15-G15	23.99-27.79	5.65 ± .08	5.59 ± .42	5.65 ± .42	5.41 ± .08
G15-H15	27.79-32.3	5.64 ± .08	6.33 ± .88	5.48 ± .3	5.41 ± .08
H15-J15	32.3-36.27	5.68 ± .08	5.47 ± .57	5.58 ± .35	5.51 ± .09
J15-I15	36.27-48.97	5.71 ± .09	5.5 ± .21	5.61 ± .28	5.5 ± .09

WK3 TO WK6 GAIN (GAIN2)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	19.64 ± .28	18.62 ± 2.39	19.9 ± 1.79	19.21 ± .27
C15-D15	7.62-13.8	19.77 ± .29	17.9 ± 1.14	18.46 ± 1.17	19.24 ± .28
D15-E15	13.8-18.31	19.8 ± .28	17.28 ± 1.3	18.22 ± 1.38	19.22 ± .28
E15-A15	18.31-21.61	19.75 ± .28	20.25 ± 1.55	20.47 ± .95	18.99 ± .28
A15-F15	21.61-23.99	19.8 ± .28	20.79 ± 1.51	19.88 ± 1.1	18.97 ± .28
F15-G15	23.99-27.79	19.79 ± .28	20.12 ± 1.42	21.92 ± 1.41	18.93 ± .27
G15-H15	27.79-32.3	19.89 ± .27	18.67 ± 2.97	20.65 ± .99	18.85 ± .28
H15-J15	32.3-36.27	19.81 ± .28	20.69 ± 1.93	22.56 ± 1.18	18.95 ± .29
J15-I15	36.27-48.97	19.75 ± .3	20.25 ± .71	21.36 ± .94	18.94 ± .3

WK6 TO WK9 GAIN (GAIN3)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	6.26 ± .24	3.6 ± 2.04	7.66 ± 1.52	5.81 ± .23
C15-D15	7.62-13.8	6.32 ± .24	5.79 ± .97	7.09 ± 1	5.69 ± .24
D15-E15	13.8-18.31	6.41 ± .24	5.29 ± 1.11	6.04 ± 1.18	5.7 ± .24
E15-A15	18.31-21.61	6.37 ± .24	7.84 ± 1.32	4.69 ± .81	5.75 ± .24
A15-F15	21.61-23.99	6.33 ± .24	7.08 ± 1.29	4.68 ± .95	5.77 ± .24
F15-G15	23.99-27.79	6.42 ± .24	5.57 ± 1.22	5.29 ± 1.21	5.7 ± .23
G15-H15	27.79-32.3	6.38 ± .24	5.41 ± 2.56	5.46 ± .86	5.72 ± .24
H15-J15	32.3-36.27	6.3 ± .23	5.24 ± 1.63	4.81 ± 1	5.64 ± .25
J15-I15	36.27-48.97	6.46 ± .25	5.22 ± .6	6.45 ± .79	5.53 ± .25

WK9 TO WK12 GAIN (GAIN4)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	2.53 ± .16	1.73 ± 1.4	1.95 ± 1.04	2.3 ± .16
C15-D15	7.62-13.8	2.55 ± .17	2.16 ± .67	2.42 ± .69	2.28 ± .16
D15-E15	13.8-18.31	2.53 ± .17	2.44 ± .76	2.08 ± .81	2.28 ± .16
E15-A15	18.31-21.61	2.52 ± .17	2.13 ± .91	1.77 ± .56	2.34 ± .16
A15-F15	21.61-23.99	2.5 ± .16	2.49 ± .89	2.05 ± .65	2.33 ± .16
F15-G15	23.99-27.79	2.51 ± .16	2.19 ± .84	2.87 ± .83	2.3 ± .16
G15-H15	27.79-32.3	2.53 ± .16	.83 ± 1.75	3.03 ± .58	2.24 ± .16
H15-J15	32.3-36.27	2.57 ± .17	3.09 ± 1.18	2.76 ± .72	2.04 ± .18
J15-I15	36.27-48.97	2.59 ± .18	2.48 ± .43	2.75 ± .57	2.03 ± .18

HRT/WK12 (HRTP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.591 ± .008	.64 ± .07	.627 ± .052	.589 ± .008
C15-D15	7.62-13.8	.59 ± .008	.615 ± .033	.57 ± .035	.591 ± .008
D15-E15	13.8-18.31	.589 ± .008	.592 ± .038	.621 ± .041	.591 ± .008
E15-A15	18.31-21.61	.59 ± .008	.585 ± .046	.588 ± .028	.591 ± .008
A15-F15	21.61-23.99	.592 ± .008	.598 ± .045	.569 ± .033	.591 ± .008
F15-G15	23.99-27.79	.593 ± .008	.585 ± .042	.556 ± .042	.59 ± .008
G15-H15	27.79-32.3	.59 ± .008	.685 ± .088	.56 ± .029	.592 ± .008
H15-J15	32.3-36.27	.584 ± .006	.546 ± .042	.589 ± .026	.595 ± .006
J15-I15	36.27-48.97	.584 ± .006	.583 ± .015	.572 ± .02	.596 ± .006

LIV/WK12 (LIVP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	7.55 ± .05	7.32 ± .45	7.62 ± .33	7.64 ± .05
C15-D15	7.62-13.8	7.55 ± .05	7.63 ± .21	7.97 ± .22	7.61 ± .05
D15-E15	13.8-18.31	7.57 ± .05	7.51 ± .24	7.81 ± .26	7.61 ± .05
E15-A15	18.31-21.61	7.58 ± .05	7.6 ± .29	7.36 ± .18	7.63 ± .05
A15-F15	21.61-23.99	7.56 ± .05	7.93 ± .28	7.58 ± .21	7.62 ± .05
F15-G15	23.99-27.79	7.6 ± .05	7.03 ± .27	7.32 ± .26	7.63 ± .05
G15-H15	27.79-32.3	7.58 ± .05	7.34 ± .56	7.86 ± .19	7.59 ± .05
H15-J15	32.3-36.27	7.62 ± .05	8.03 ± .38	7.56 ± .23	7.58 ± .06
J15-I15	36.27-48.97	7.62 ± .06	7.64 ± .14	7.49 ± .18	7.59 ± .06

APPENDIX 17--CONTINUED

SPL/WK12 (SPLP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.537 ± .01	.443 ± .085	.472 ± .063	.54 ± .01
C15-D15	7.62-13.8	.533 ± .01	.567 ± .04	.565 ± .042	.538 ± .01
D15-E15	13.8-18.31	.536 ± .01	.495 ± .046	.606 ± .049	.538 ± .01
E15-A15	18.31-21.61	.54 ± .01	.497 ± .055	.538 ± .034	.536 ± .01
A15-F15	21.61-23.99	.541 ± .01	.533 ± .054	.53 ± .04	.535 ± .01
F15-G15	23.99-27.79	.54 ± .01	.539 ± .051	.507 ± .051	.536 ± .01
G15-H15	27.79-32.3	.538 ± .01	.579 ± .107	.553 ± .036	.534 ± .01
H15-J15	32.3-36.27	.541 ± .01	.449 ± .071	.542 ± .044	.534 ± .011
J15-I15	36.27-48.97	.545 ± .011	.51 ± .026	.537 ± .035	.534 ± .011

KID/WK12 (KIDP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.797 ± .009	.897 ± .074	.886 ± .055	.835 ± .008
C15-D15	7.62-13.8	.796 ± .009	.841 ± .036	.819 ± .037	.838 ± .009
D15-E15	13.8-18.31	.797 ± .009	.821 ± .041	.858 ± .043	.837 ± .009
E15-A15	18.31-21.61	.799 ± .009	.795 ± .049	.809 ± .03	.839 ± .009
A15-F15	21.61-23.99	.797 ± .009	.8 ± .047	.834 ± .035	.839 ± .009
F15-G15	23.99-27.79	.8 ± .009	.769 ± .045	.821 ± .044	.838 ± .009
G15-H15	27.79-32.3	.799 ± .009	.963 ± .094	.833 ± .031	.835 ± .009
H15-J15	32.3-36.27	.803 ± .009	.818 ± .064	.816 ± .039	.84 ± .01
J15-I15	36.27-48.97	.803 ± .01	.802 ± .024	.848 ± .031	.839 ± .01

TES/WK12 (TESP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.207 ± .005	.182 ± .051	.227 ± .029	.217 ± .005
C15-D15	7.62-13.8	.206 ± .005	.222 ± .019	.245 ± .02	.215 ± .005
D15-E15	13.8-18.31	.207 ± .005	.241 ± .022	.247 ± .021	.214 ± .005
E15-A15	18.31-21.61	.21 ± .005	.197 ± .024	.199 ± .016	.217 ± .005
A15-F15	21.61-23.99	.209 ± .005	.217 ± .024	.206 ± .02	.216 ± .005
F15-G15	23.99-27.79	.209 ± .005	.215 ± .023	.2 ± .023	.217 ± .005
G15-H15	27.79-32.3	.21 ± .005	.163 ± .036	.226 ± .016	.215 ± .005
H15-J15	32.3-36.27	.213 ± .005	.193 ± .038	.17 ± .025	.215 ± .006
J15-I15	36.27-48.97	.212 ± .005	.222 ± .013	.186 ± .024	.214 ± .006

SCF/WK12 (SCFP)

Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.629 ± .022	.323 ± .187	.713 ± .14	.529 ± .021
C15-D15	7.62-13.8	.64 ± .022	.494 ± .089	.476 ± .092	.53 ± .022
D15-E15	13.8-18.31	.64 ± .022	.413 ± .102	.399 ± .108	.534 ± .022
E15-A15	18.31-21.61	.634 ± .022	.599 ± .121	.501 ± .075	.528 ± .022
A15-F15	21.61-23.99	.629 ± .022	.751 ± .118	.543 ± .086	.523 ± .022
F15-G15	23.99-27.79	.623 ± .022	.797 ± .111	.668 ± .111	.52 ± .021
G15-H15	27.79-32.3	.625 ± .022	.766 ± .235	.502 ± .079	.534 ± .022
H15-J15	32.3-36.27	.605 ± .023	.789 ± .158	.647 ± .097	.512 ± .024
J15-I15	36.27-48.97	.619 ± .025	.553 ± .058	.611 ± .078	.51 ± .025

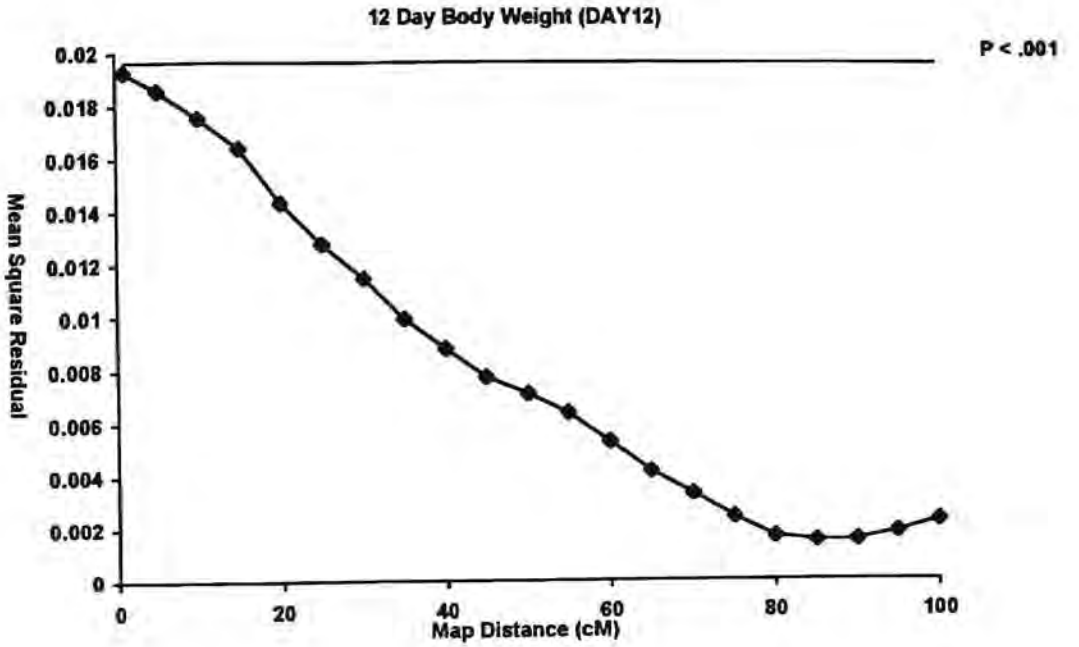
GOF/WK12 (GOFP)

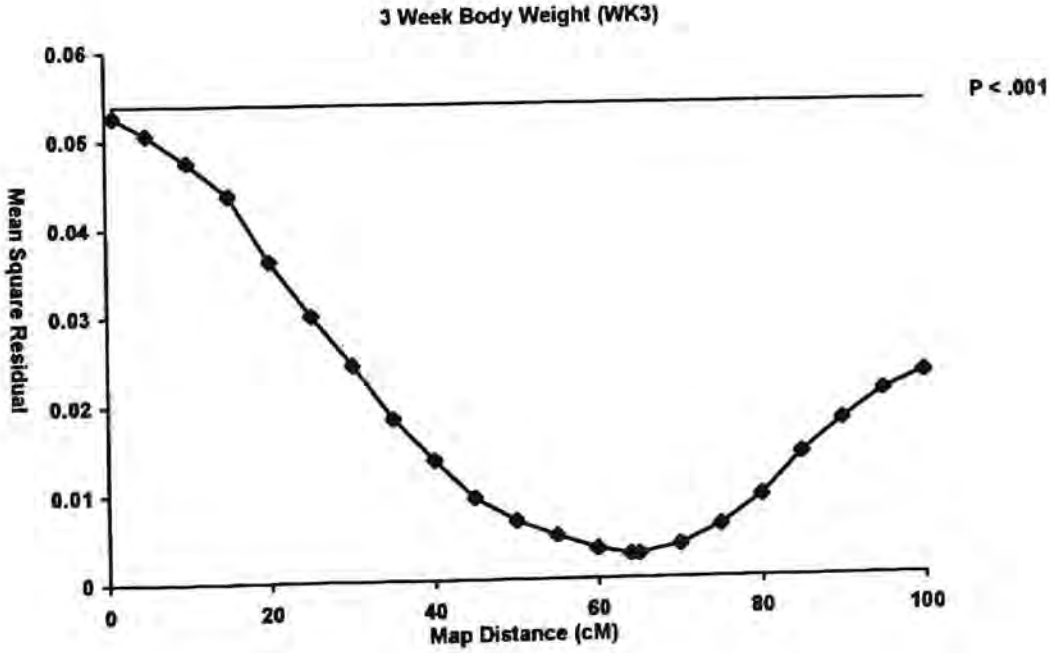
Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	.894 ± .031	.294 ± .264	.791 ± .197	.747 ± .03
C15-D15	7.62-13.8	.902 ± .032	.733 ± .126	.762 ± .13	.74 ± .031
D15-E15	13.8-18.31	.906 ± .031	.642 ± .144	.697 ± .152	.742 ± .031
E15-A15	18.31-21.61	.902 ± .031	.807 ± .172	.757 ± .105	.735 ± .031
A15-F15	21.61-23.99	.902 ± .031	1.161 ± .165	.702 ± .121	.724 ± .03
F15-G15	23.99-27.79	.897 ± .031	1.127 ± .156	.908 ± .156	.717 ± .03
G15-H15	27.79-32.3	.9 ± .031	.909 ± .331	.719 ± .111	.735 ± .031
H15-J15	32.3-36.27	.883 ± .032	.822 ± .225	.782 ± .138	.722 ± .034
J15-I15	36.27-48.97	.901 ± .035	.779 ± .082	.793 ± .109	.722 ± .035

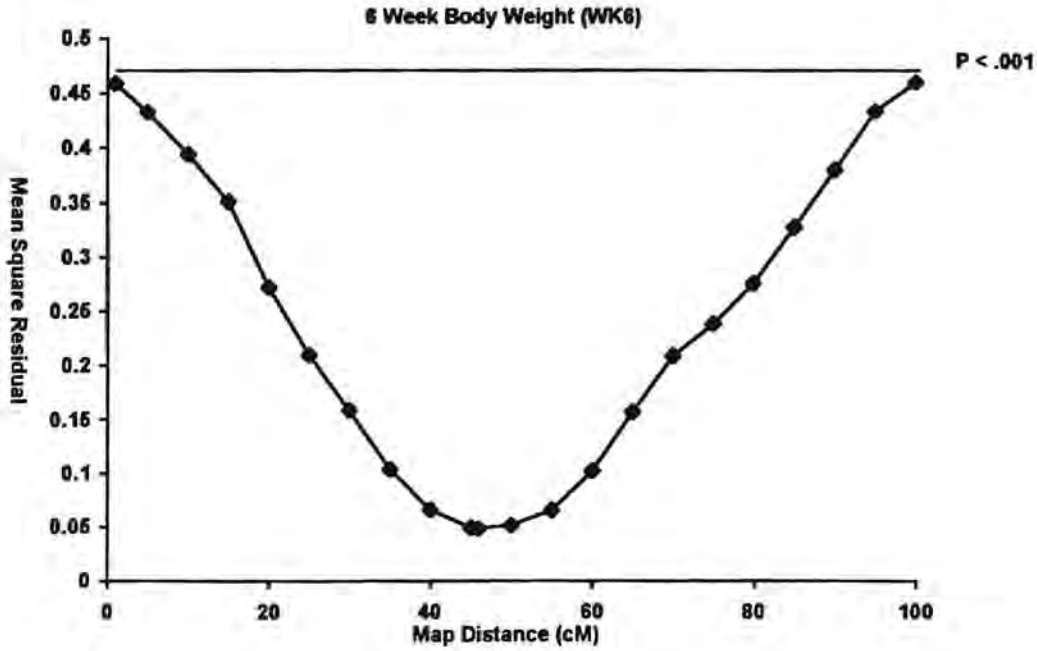
FAT/WK12 (FATP)

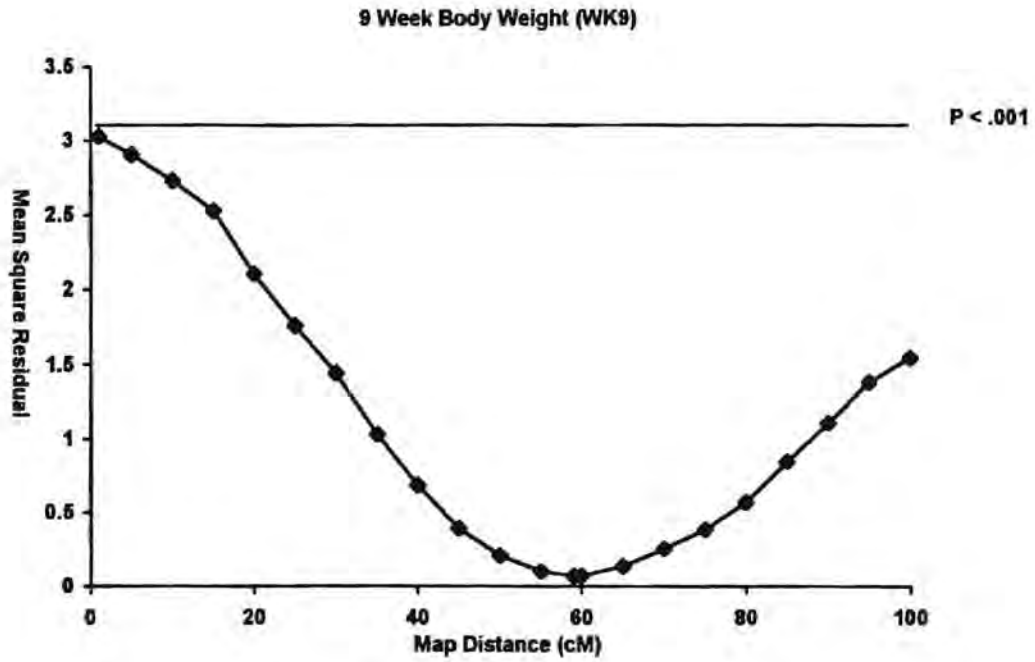
Marker Interval	Location (cM)	MM	MH	HM	HH
B15-C15	5.72-7.62	1525 ± 48	611 ± 407	1486 ± 304	1279 ± 46
C15-D15	7.62-13.8	1543 ± 49	1229 ± 194	1240 ± 200	1272 ± 48
D15-E15	13.8-18.31	1547 ± 48	1057 ± 221	1099 ± 235	1278 ± 47
E15-A15	18.31-21.61	1536 ± 48	1409 ± 265	1260 ± 162	1266 ± 48
A15-F15	21.61-23.99	1532 ± 47	1916 ± 255	1247 ± 187	1250 ± 47
F15-G15	23.99-27.79	1521 ± 48	1926 ± 241	1579 ± 240	1240 ± 47
G15-H15	27.79-32.3	1527 ± 47	1677 ± 510	1219 ± 171	1271 ± 48
H15-J15	32.3-36.27	1490 ± 50	1612 ± 346	1430 ± 212	1236 ± 52
J15-I15	36.27-48.97	1522 ± 53	1330 ± 127	1430 ± 170	1232 ± 53

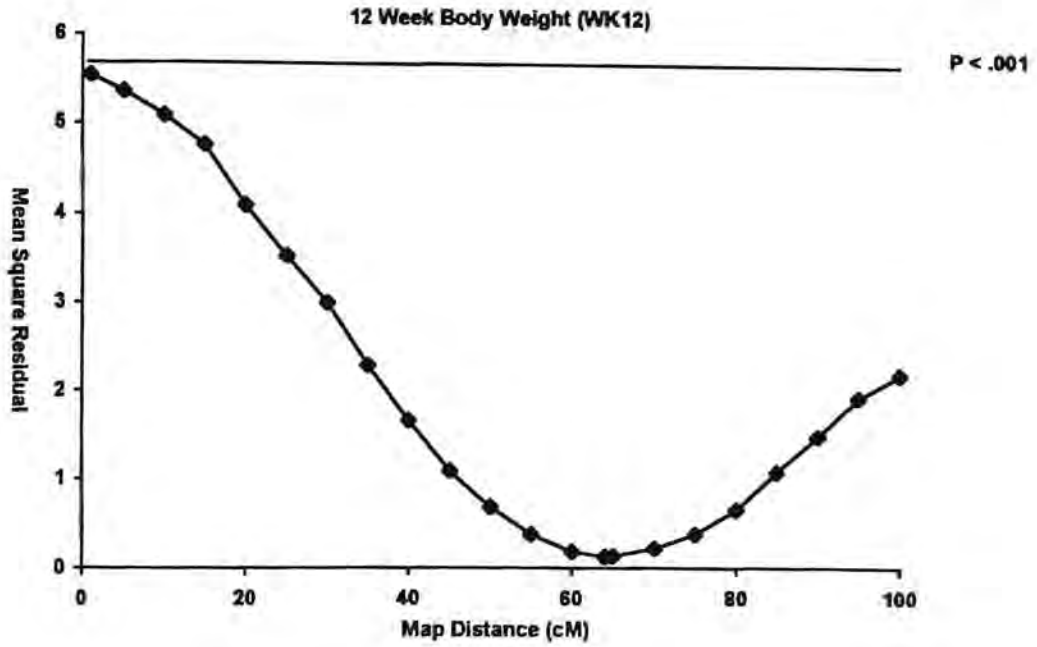
APPENDIX 18--MARKER-REGRESSION QTL MAPPING OF CHROMOSOME 2

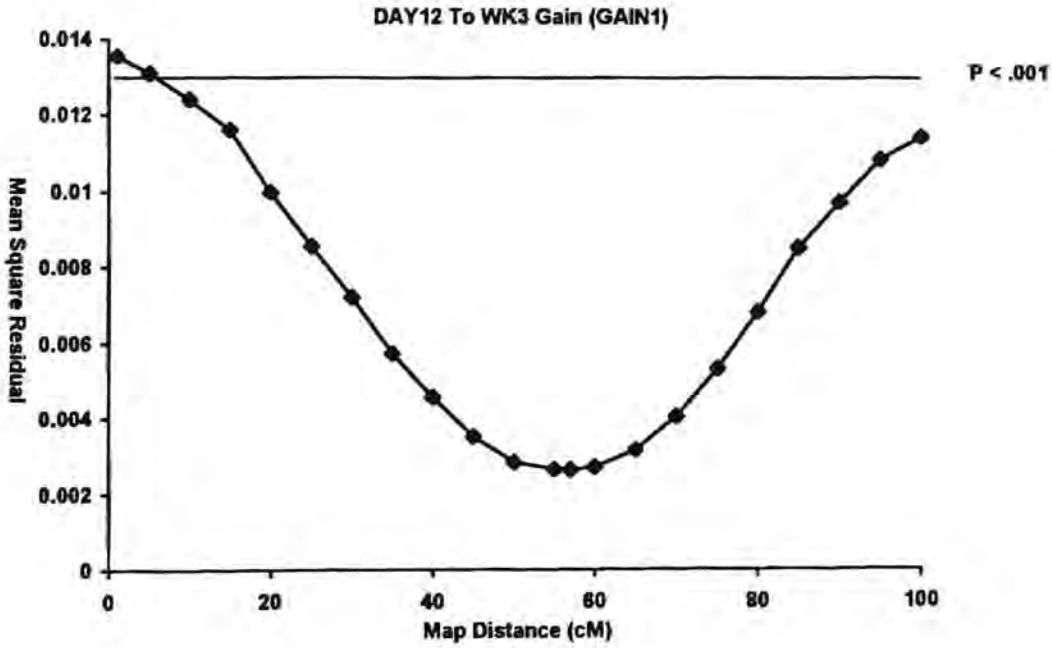


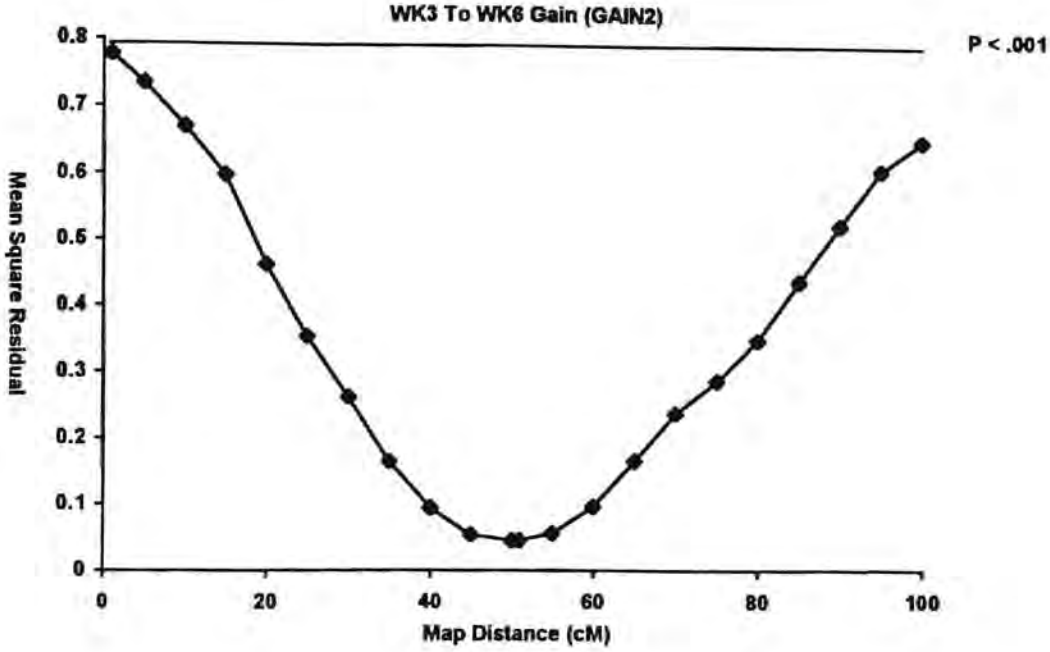


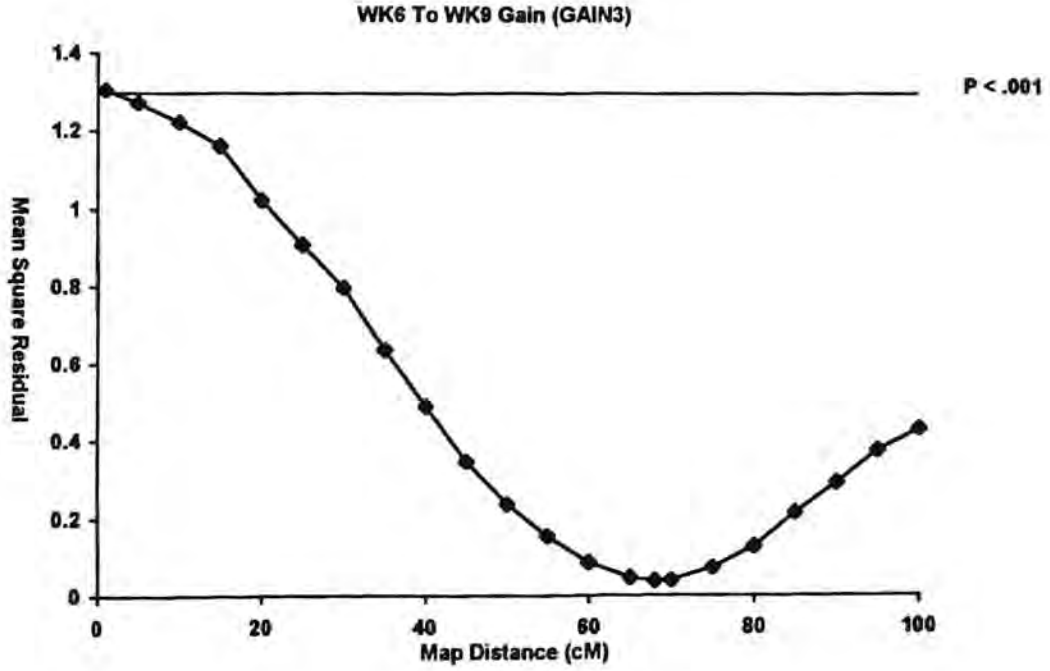

6 Week Body Weight (WK6)
P < .001
Mean Square Residual
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
80
100
Map Distance (cM)

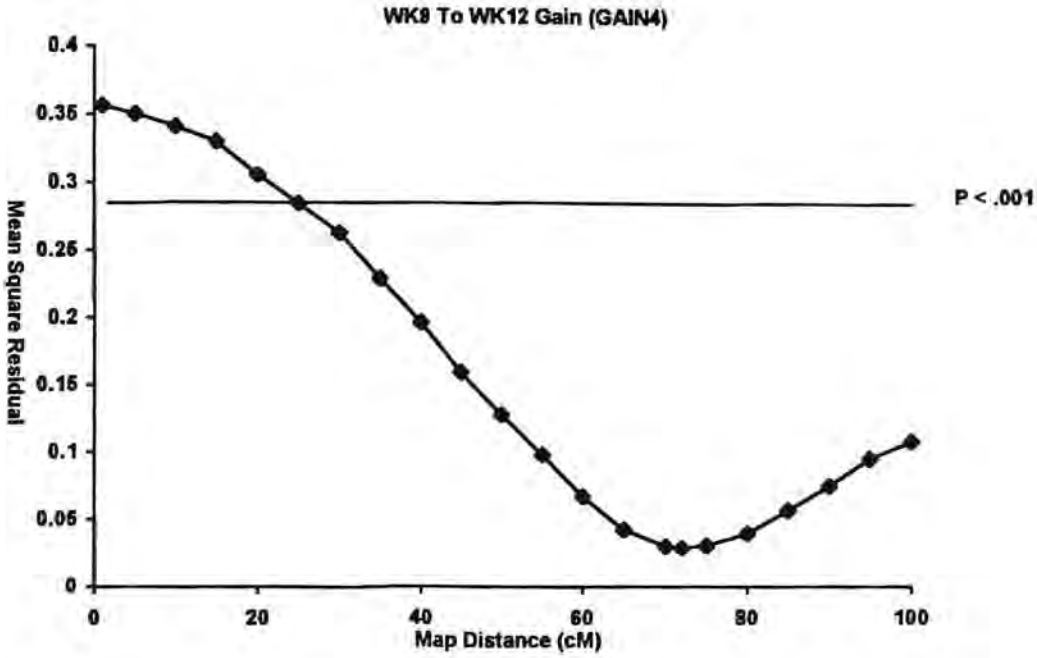

9 Week Body Weight (WK9)
P < .001
Mean Square Residual
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
Map Distance (cM)

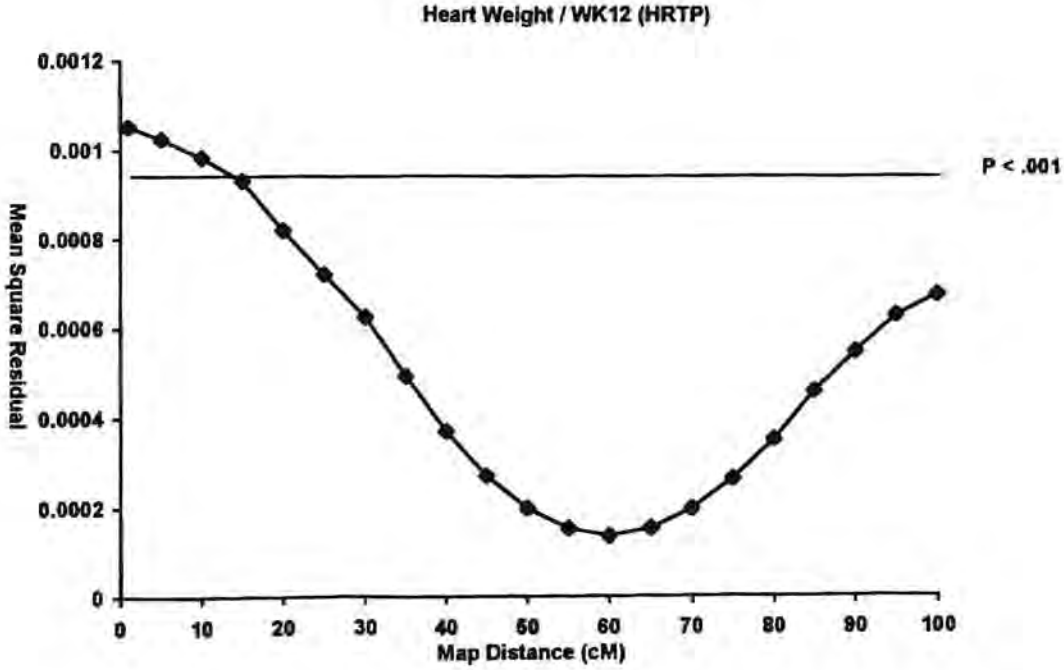

12 Week Body Weight (WK12)
P < .001
Mean Square Residual
0
1
2
3
4
5
6
0
20
40
60
80
100
Map Distance (cM)

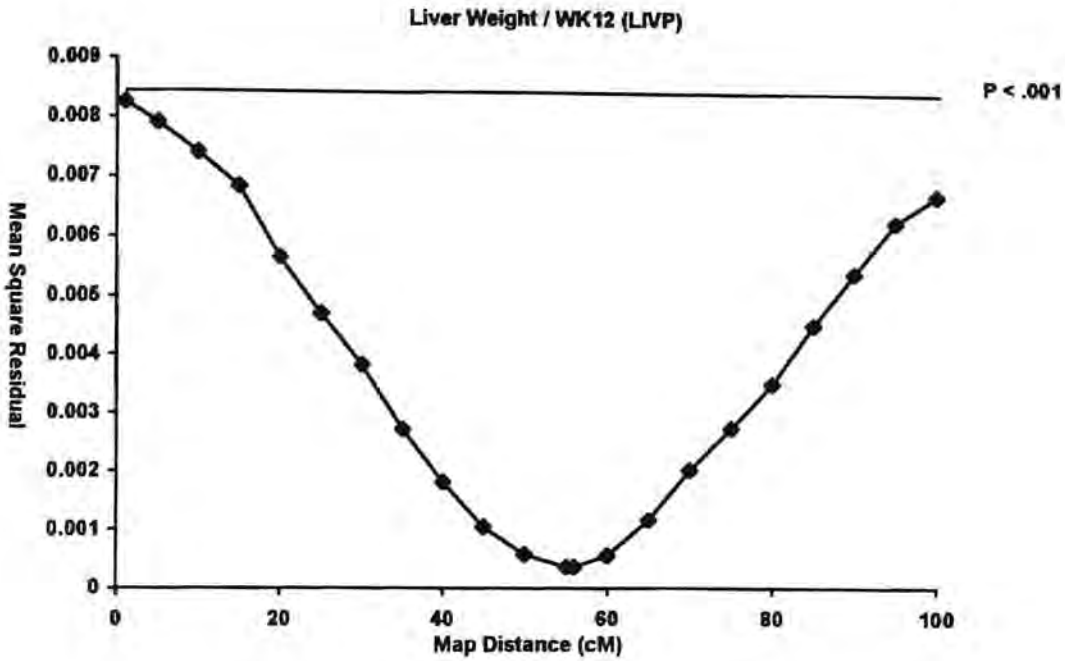

DAY12 To WK3 Gain (GAIN1)
P < .001
Mean Square Residual
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0
20
40
60
80
100
Map Distance (cM)

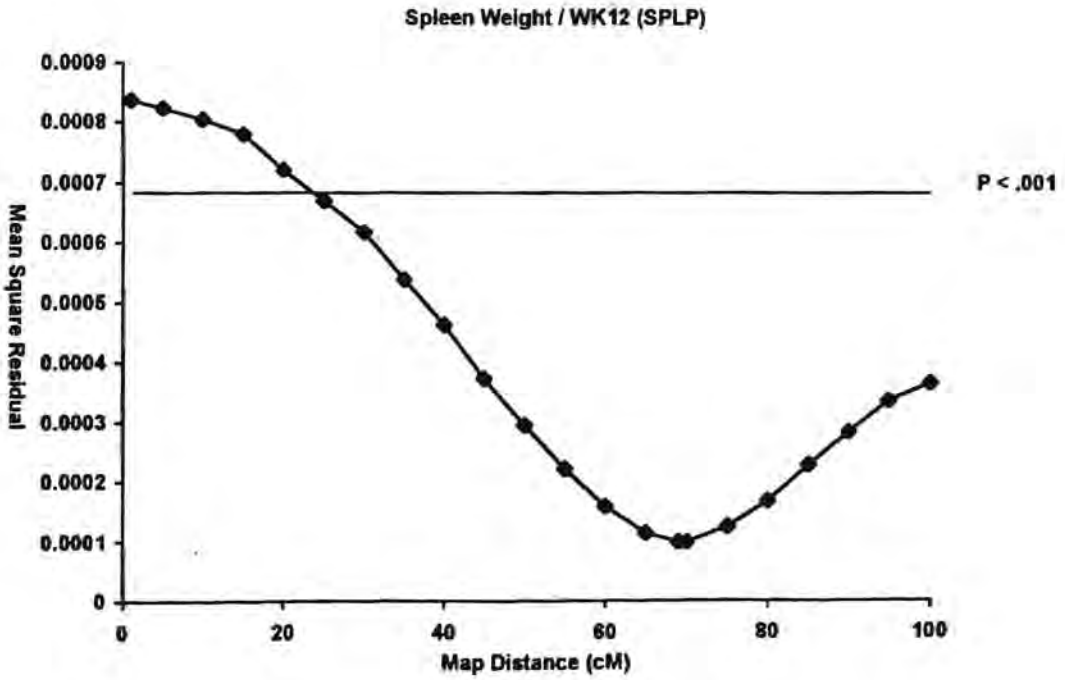

WK3 To WK6 Gain (GAIN2)
P < .001
Mean Square Residual
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
Map Distance (cM)

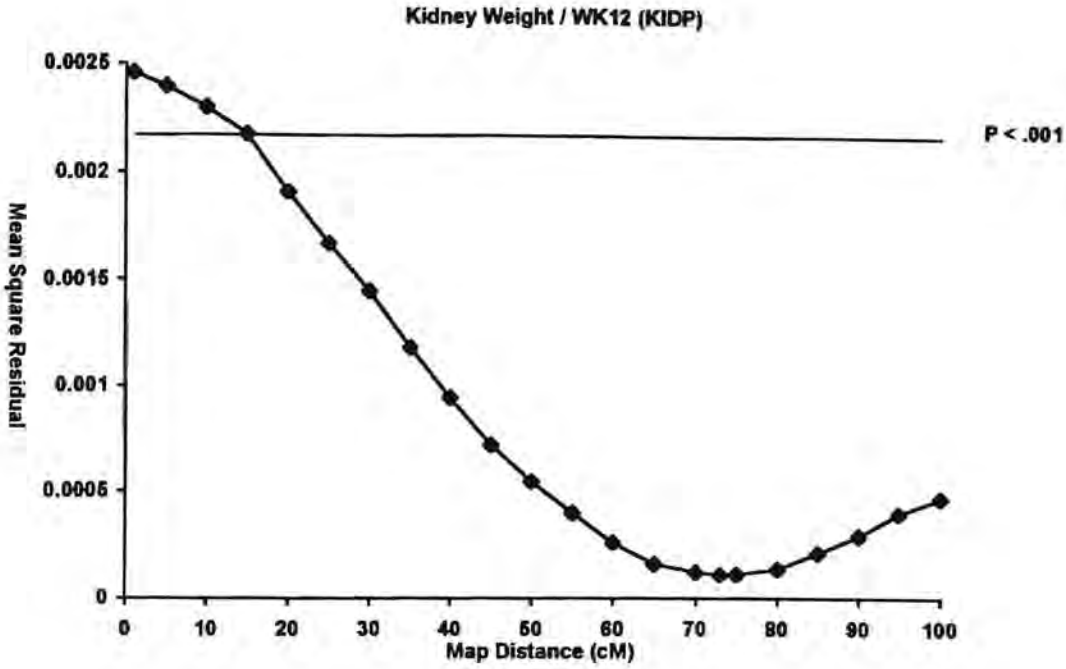

WK6 To WK9 Gain (GAIN3)
P < .001
Mean Square Residual
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
Map Distance (cM)

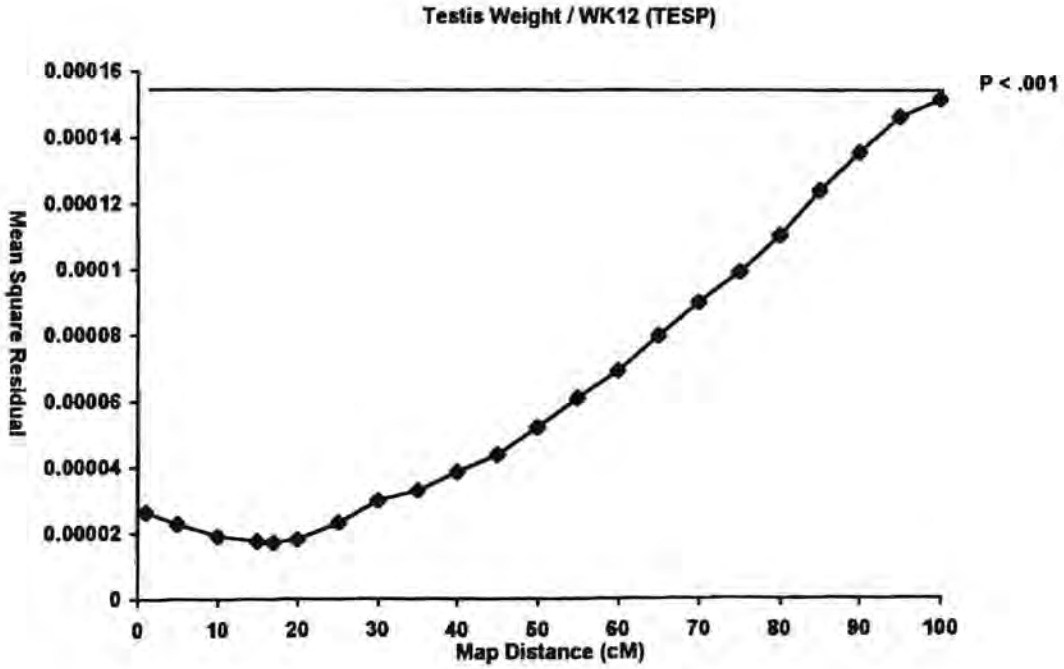

WK8 To WK12 Gain (GAIN4)
Mean Square Residual
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
P < .001
0
20
40
60
80
100
Map Distance (cM)

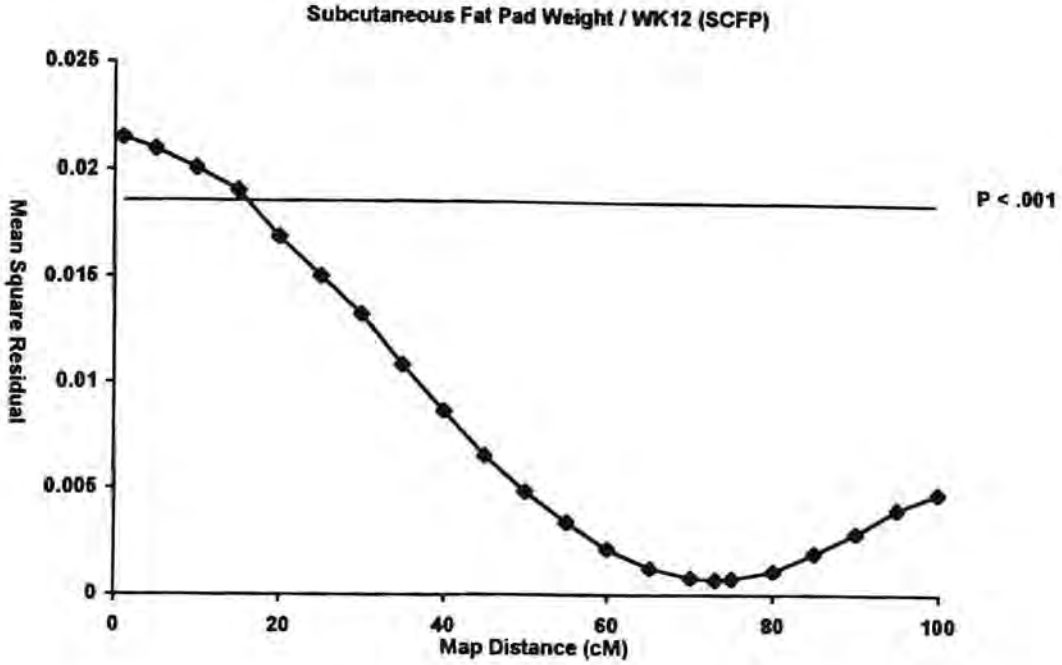

Heart Weight / WK12 (HRTP)
Mean Square Residual
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
P < .001
0
10
20
30
40
50
60
70
80
90
100
Map Distance (cM)

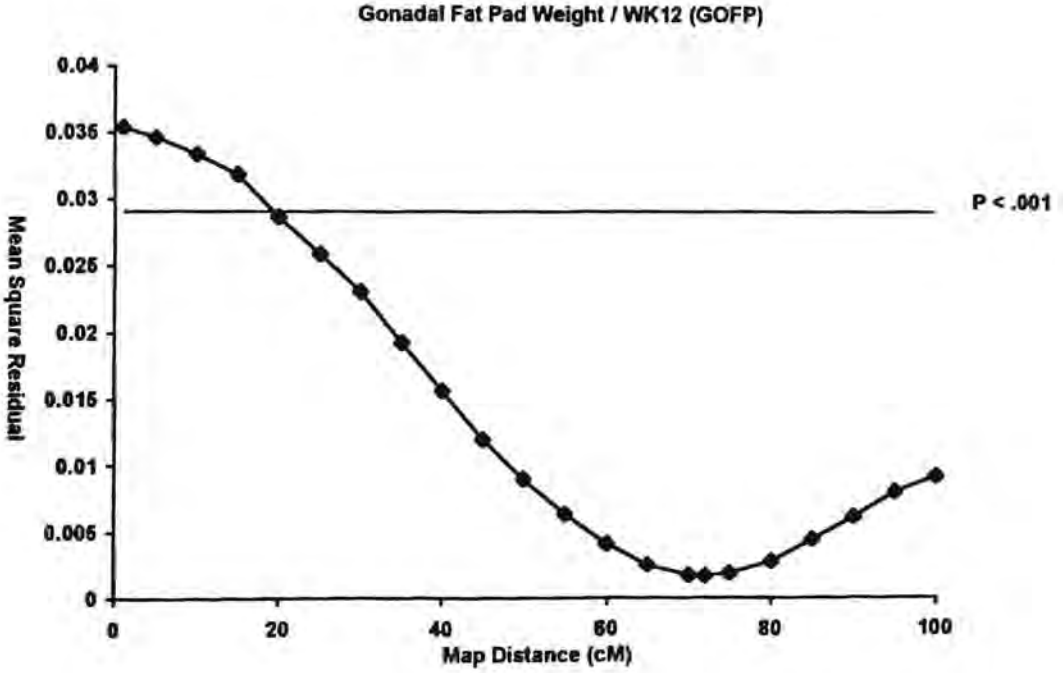

Liver Weight / WK12 (LIVP)
P < .001
Mean Square Residual
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
20
40
60
80
100
Map Distance (cM)

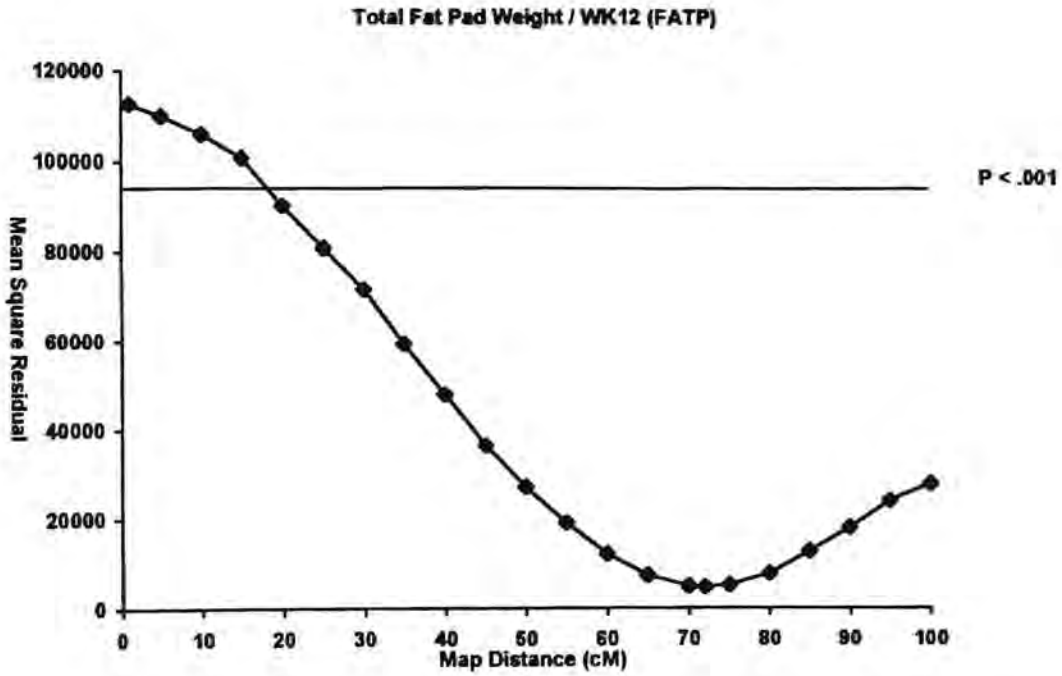

Spleen Weight / WK12 (SPLP)
P < .001
Mean Square Residual
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0
20
40
60
80
100
Map Distance (cM)


Kidney Weight / WK12 (KIDP)
P < .001
Mean Square Residual
0.0025
0.002
0.0015
0.001
0.0005
0
0
10
20
30
40
50
60
70
80
90
100
Map Distance (cM)

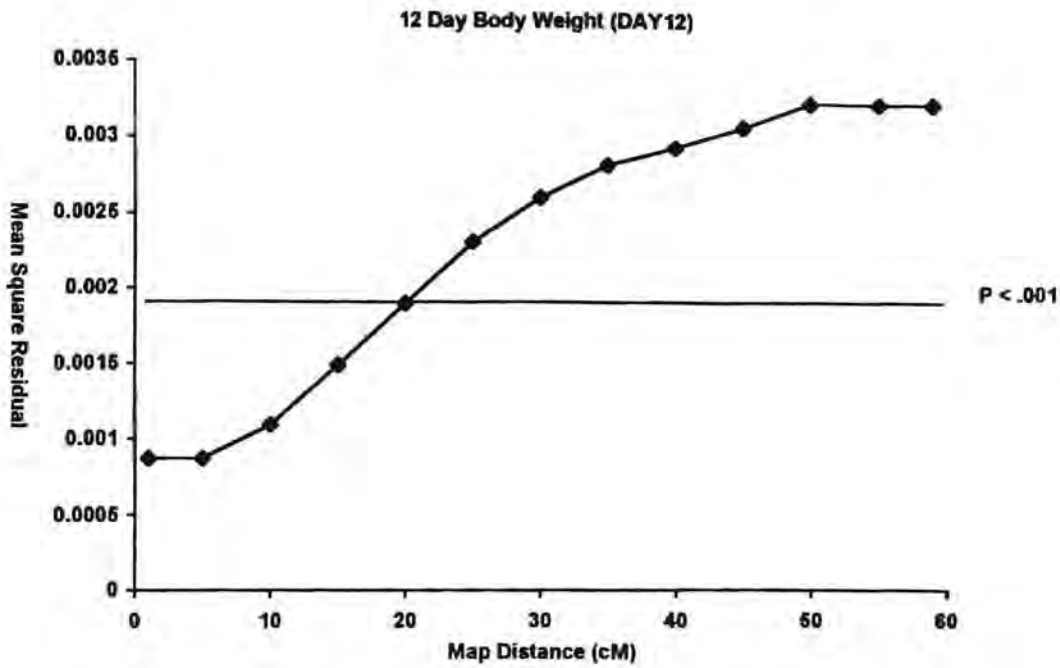

Testis Weight / WK12 (TESP)
P < .001
Mean Square Residual
0.00016
0.00014
0.00012
0.0001
0.00008
0.00006
0.00004
0.00002
0
0
10
20
30
40
50
60
70
80
90
100
Map Distance (cM)

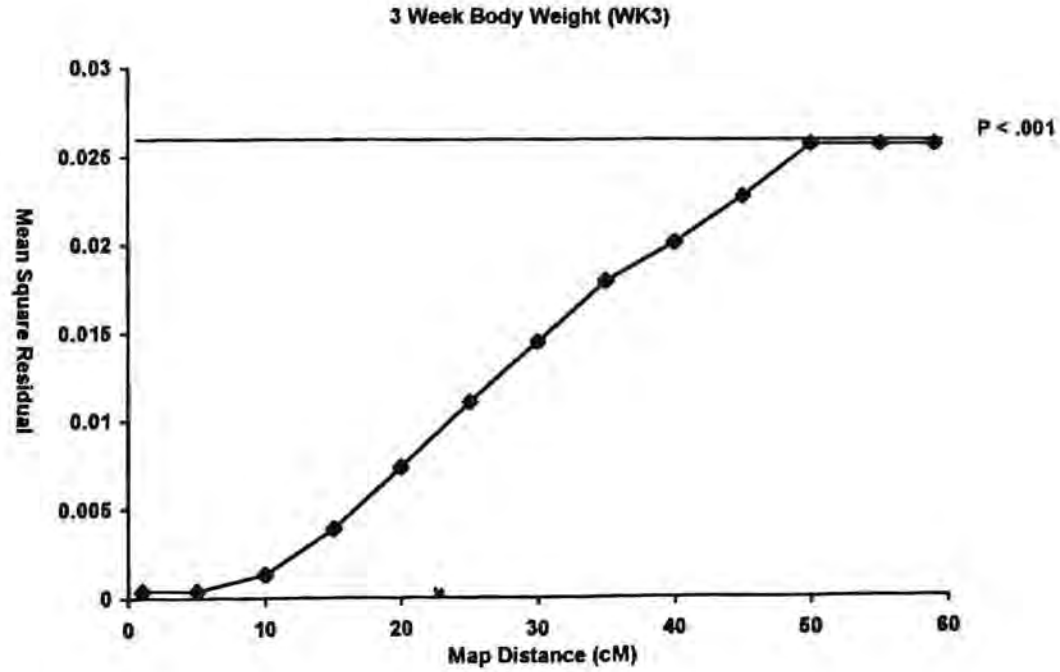

Subcutaneous Fat Pad Weight / WK12 (SCFP)
Mean Square Residual
0.025
0.02
0.015
0.01
0.005
0
0
20
40
60
80
100
Map Distance (cM)
P < .001

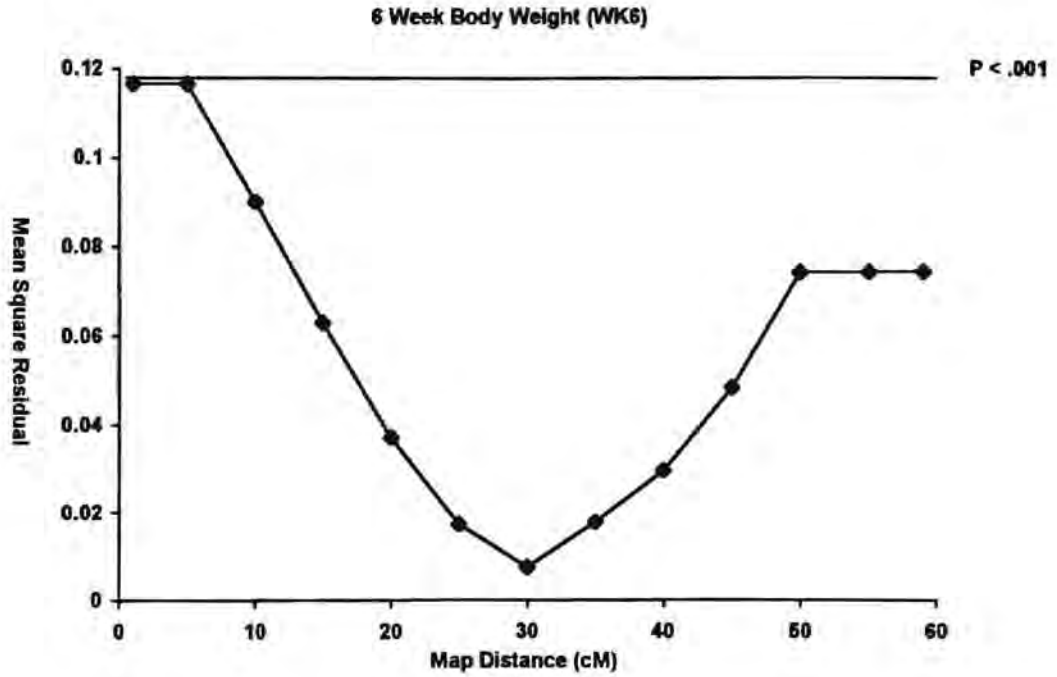

Gonadal Fat Pad Weight / WK12 (GOFP)
Mean Square Residual
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
20
40
60
80
100
Map Distance (cM)
P < .001

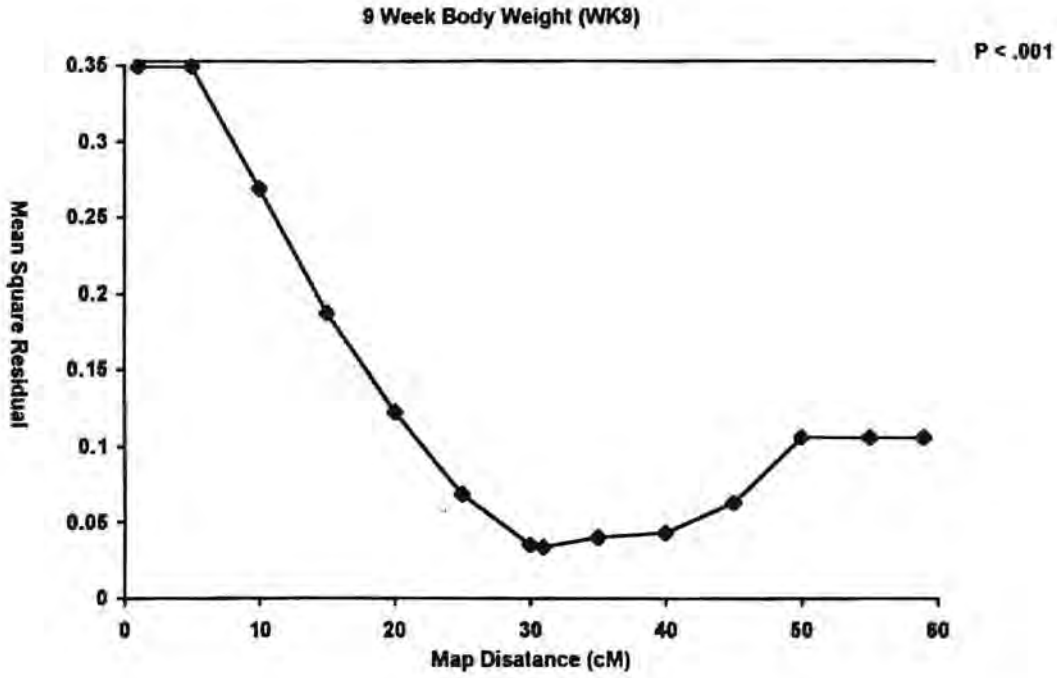

Total Fat Pad Weight / WK12 (FATP)
120000
100000
80000
60000
40000
20000
0
Mean Square Residual
P < .001
0
10
20
30
40
50
60
70
80
90
100
Map Distance (cM)

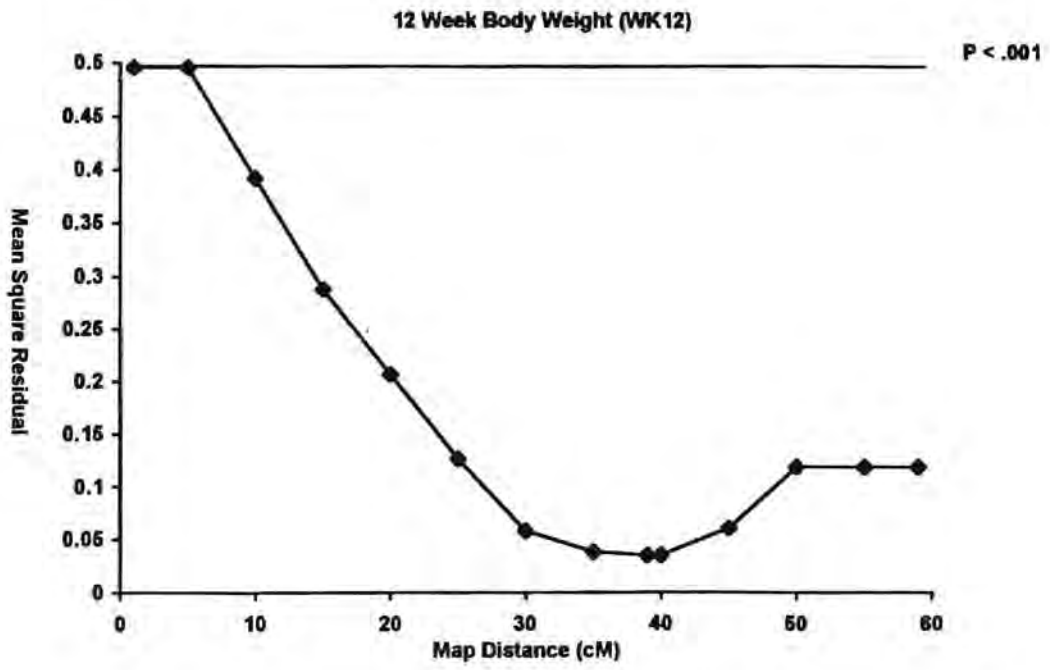
APPENDIX 19--MARKER-REGRESSION QTL MAPPING OF CHROMOSOME 15

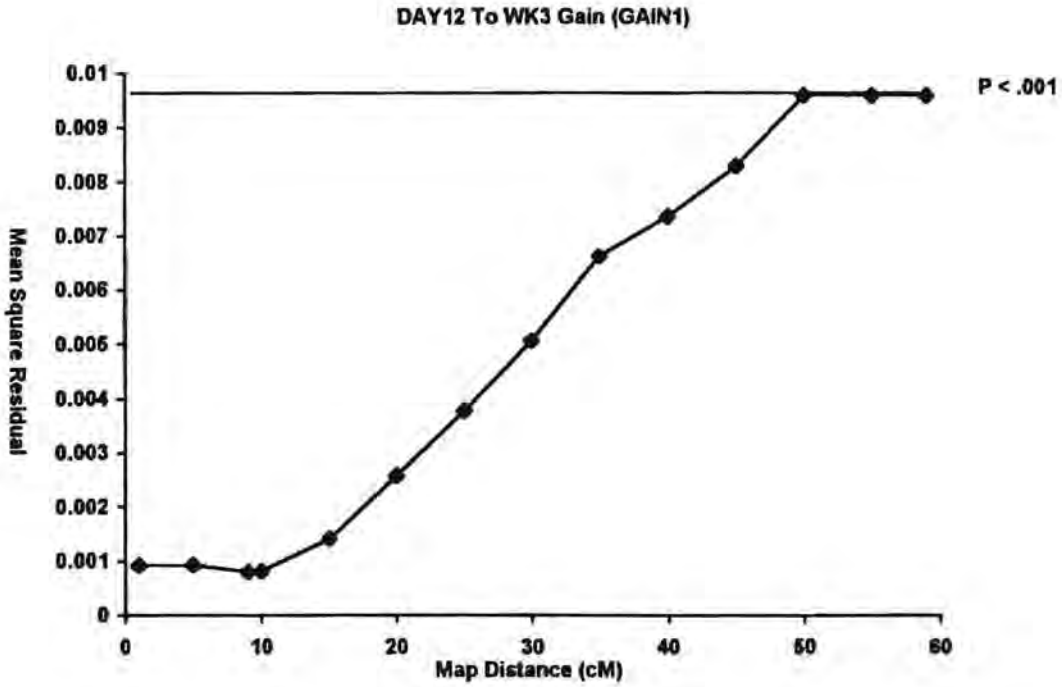


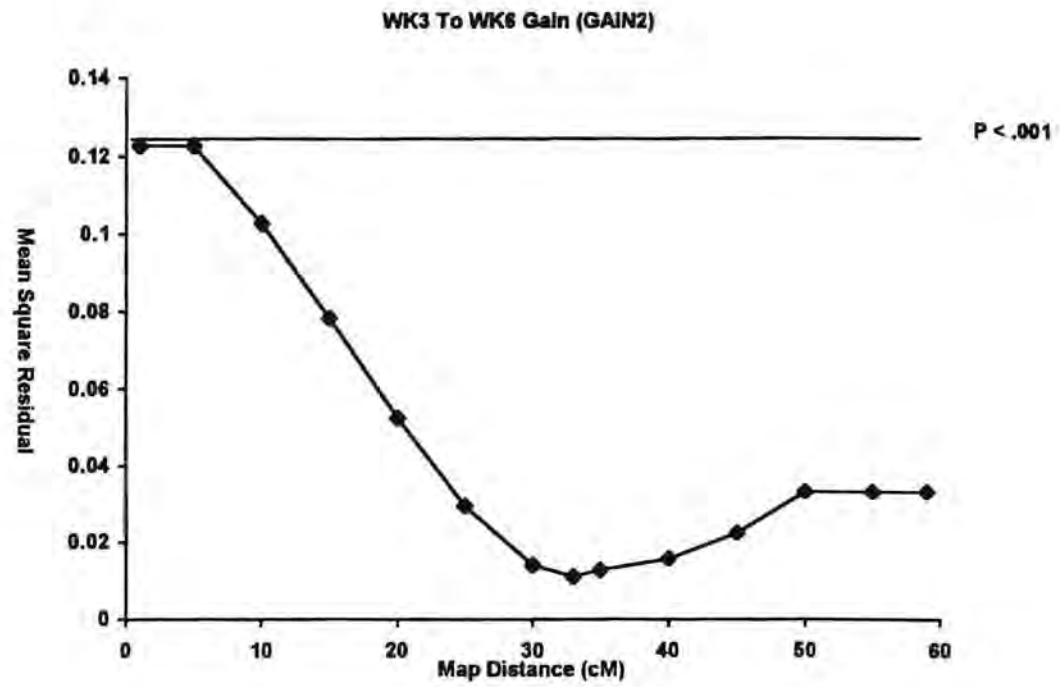


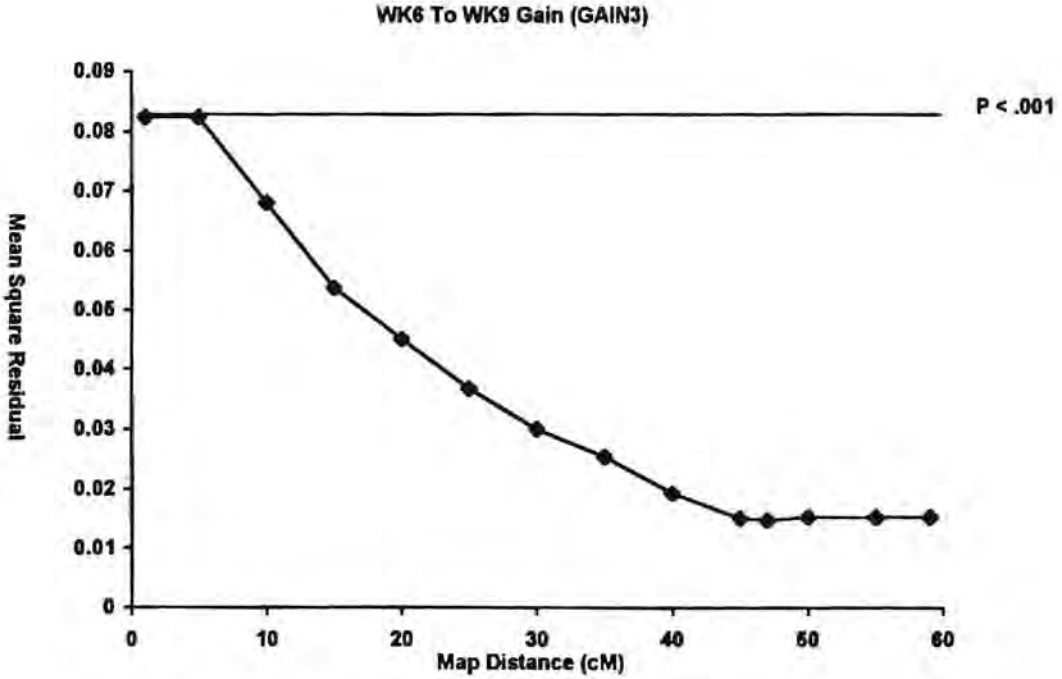

6 Week Body Weight (WK6)
P < .001
Mean Square Residual
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
Map Distance (cM)

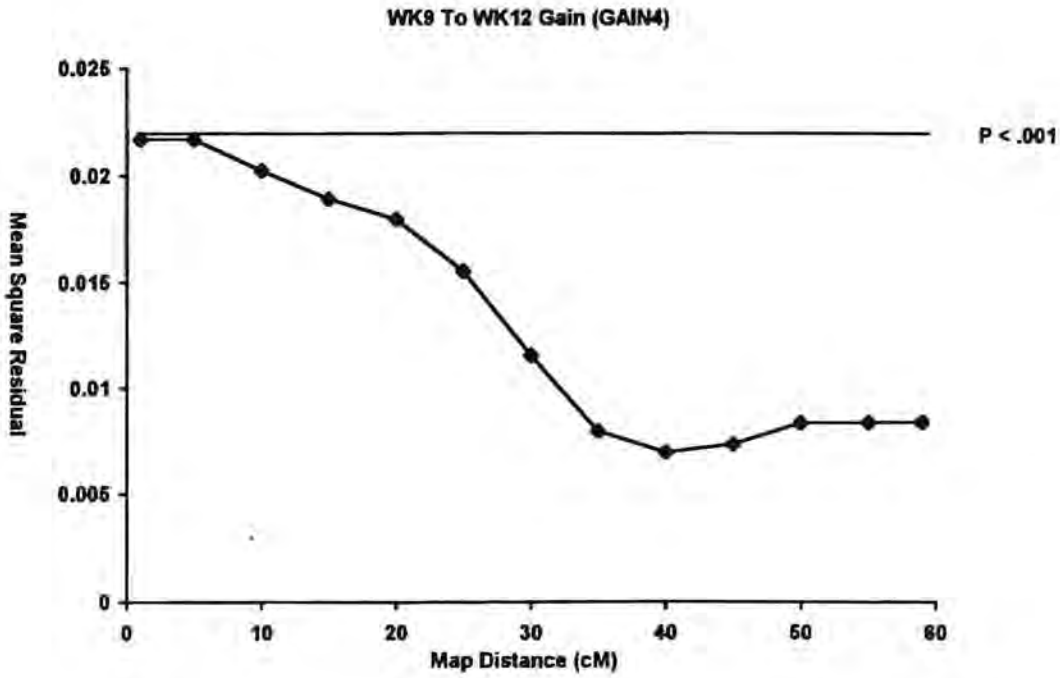

9 Week Body Weight (WK9)
P < .001
Mean Square Residual
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
Map Disatance (cM)

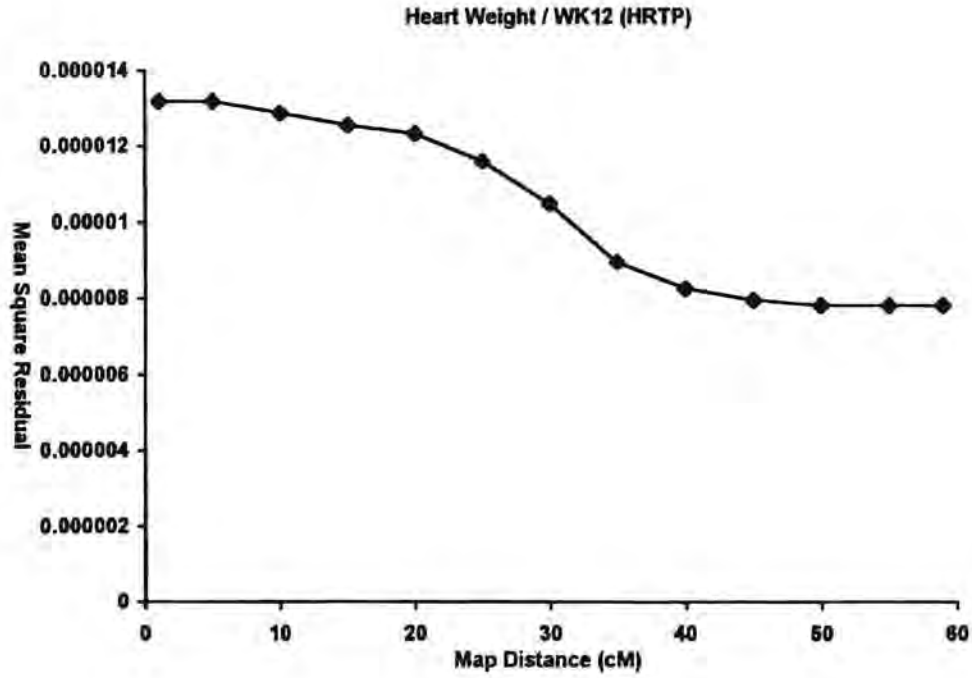

12 Week Body Weight (WK12)
P < .001
Mean Square Residual
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
Map Distance (cM)

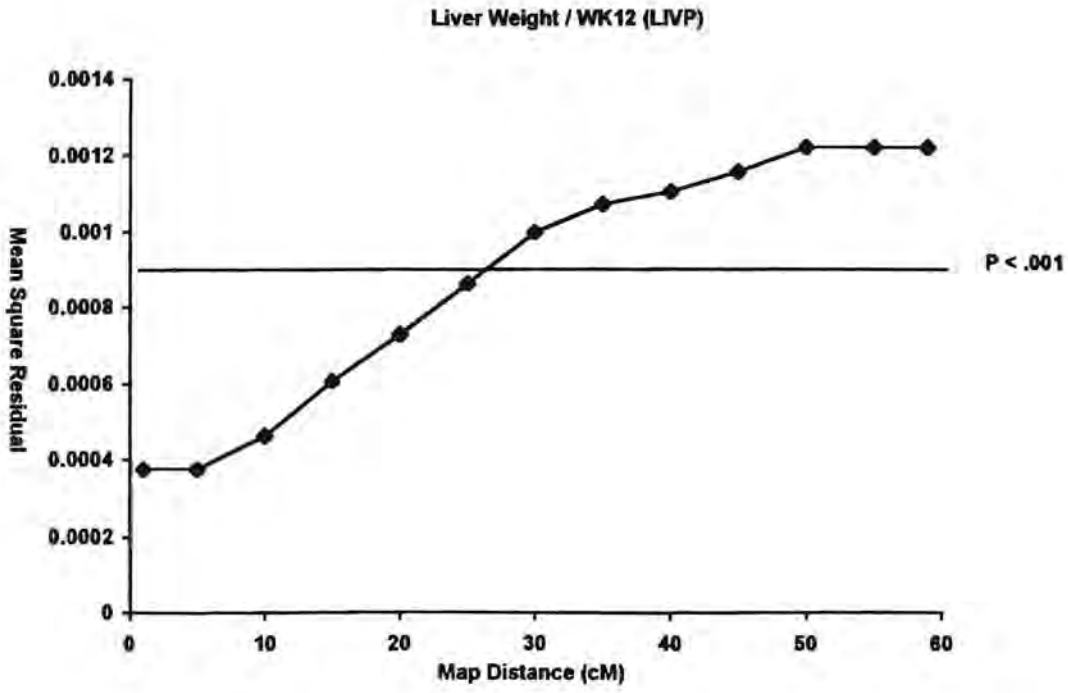

DAY12 To WK3 Gain (GAIN1)
P < .001
Mean Square Residual
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
10
20
30
40
50
60
Map Distance (cM)

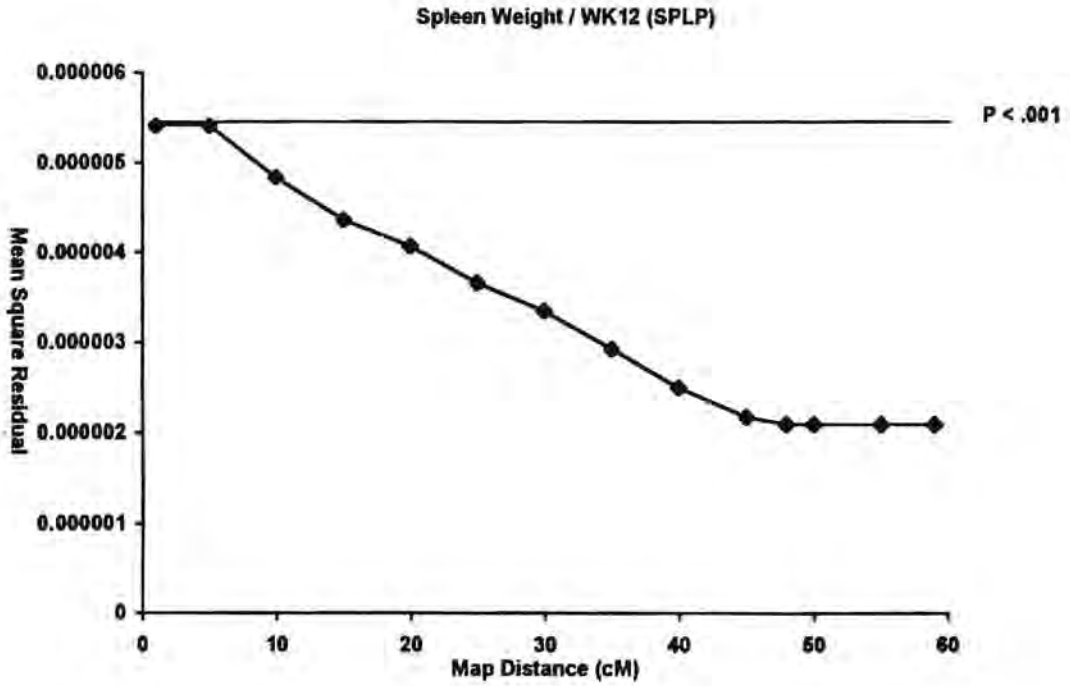

WK3 To WK6 Gain (GAIN2)
P < .001
Mean Square Residual
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
40
50
60
Map Distance (cM)

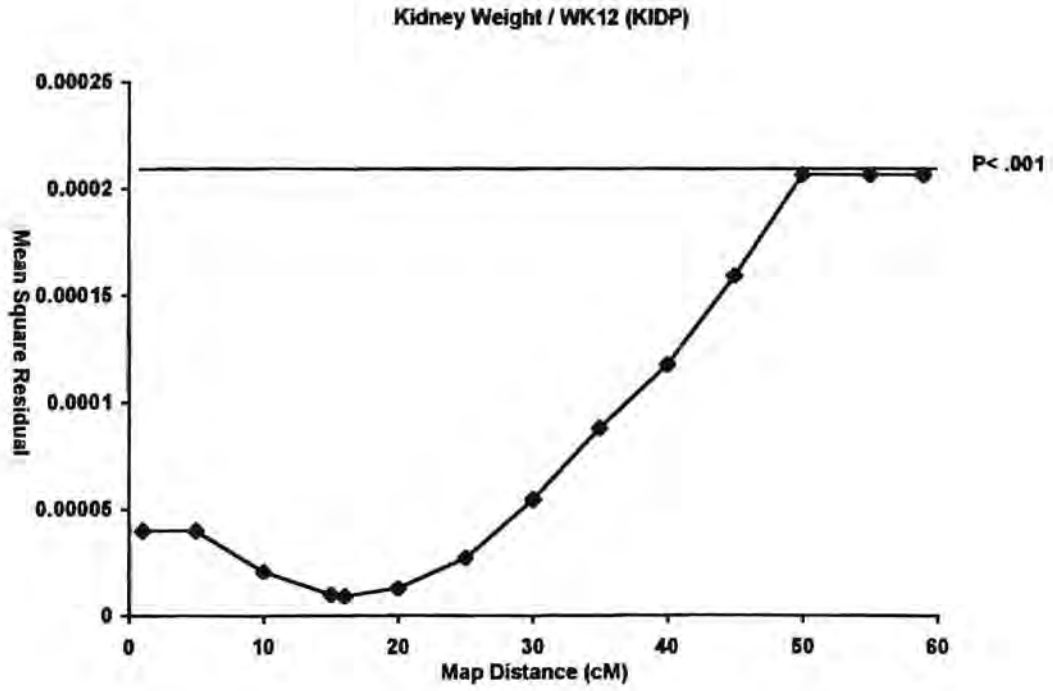

WK6 To WK9 Gain (GAIN3)
P < .001
Mean Square Residual
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
10
20
30
40
50
60
Map Distance (cM)

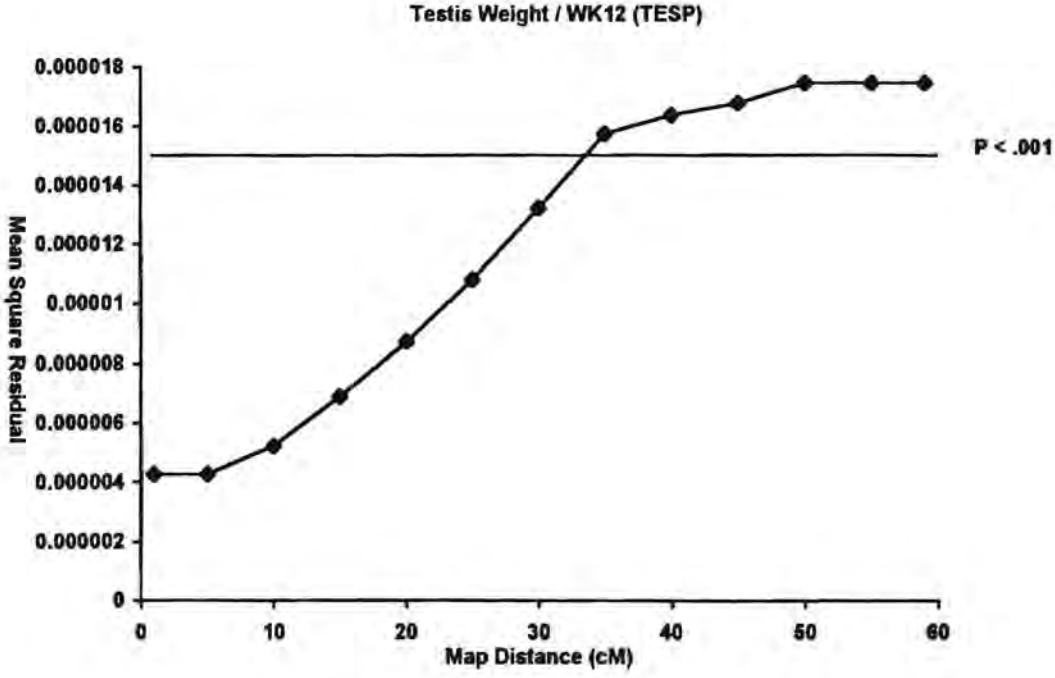

WK9 To WK12 Gain (GAIN4)
P < .001
Mean Square Residual
0.025
0.02
0.015
0.01
0.005
0
0
10
20
30
40
50
60
Map Distance (cM)

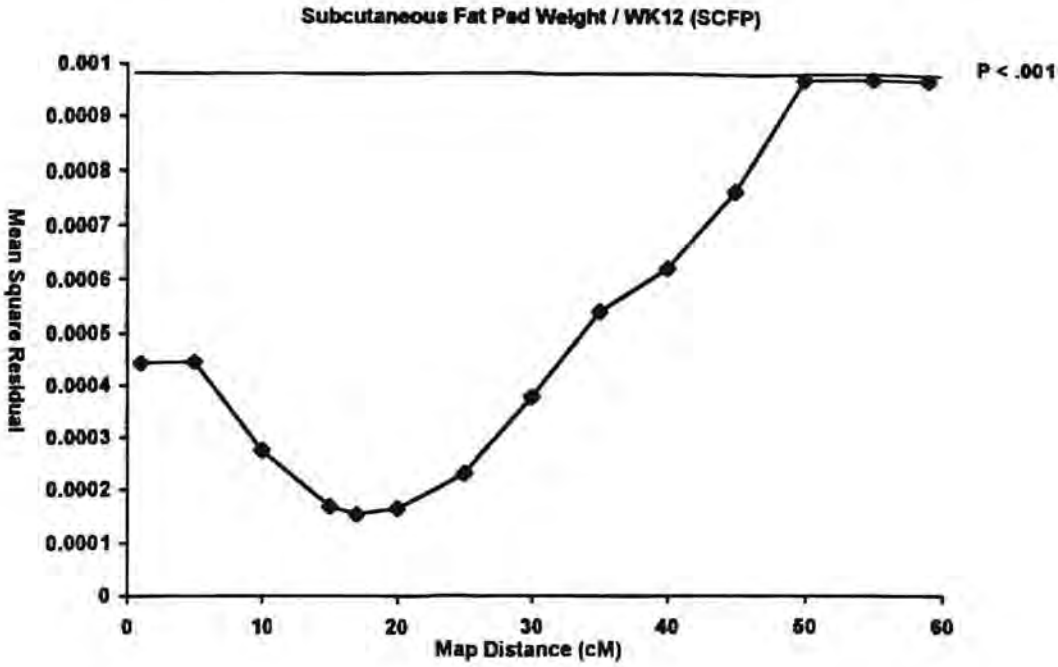

Heart Weight / WK12 (HRTP)
Mean Square Residual
0.000014
0.000012
0.00001
0.000008
0.000006
0.000004
0.000002
0
0
10
20
30
40
50
60
Map Distance (cM)

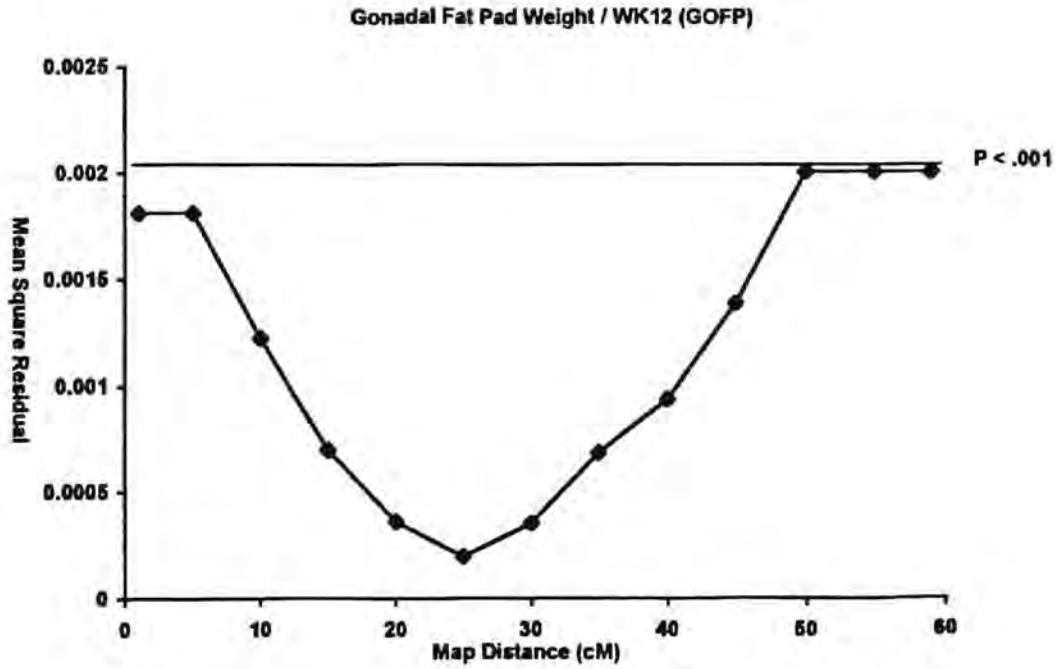

Liver Weight / WK12 (LIVP)
Mean Square Residual
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
0
10
20
30
40
50
60
Map Distance (cM)
P < .001

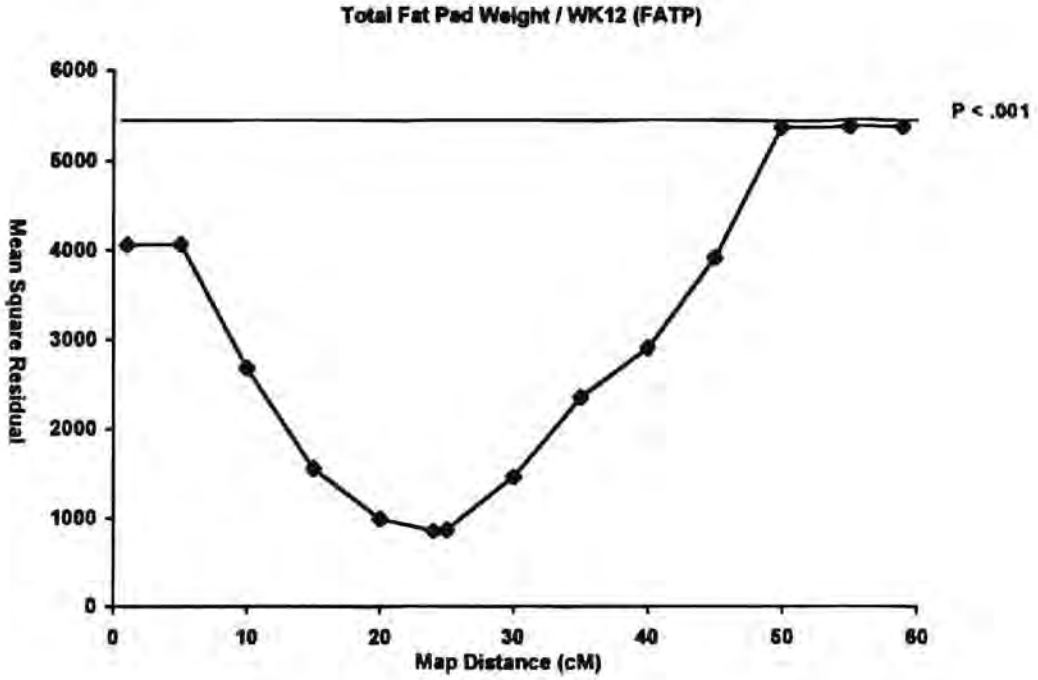

Spleen Weight / WK12 (SPLP)
Mean Square Residual
0.000006
0.000005
0.000004
0.000003
0.000002
0.000001
0
0
10
20
30
40
50
60
Map Distance (cM)
P < .001


Kidney Weight / WK12 (KIDP)
Mean Square Residual
0.00025
0.0002
0.00015
0.0001
0.00005
0
0
10
20
30
40
50
60
Map Distance (cM)
P< .001


Testis Weight / WK12 (TESP)
Mean Square Residual
0.000018
0.000016
0.000014
0.000012
0.00001
0.000008
0.000006
0.000004
0.000002
0
0
10
20
30
40
50
60
Map Distance (cM)
P < .001


Subcutaneous Fat Pad Weight / WK12 (SCFP)
P < .001
Mean Square Residual
0.001
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0
10
20
30
40
50
60
Map Distance (cM)


Gonadal Fat Pad Weight / WK12 (GOFP)
P < .001
Mean Square Residual
0.0025
0.002
0.0015
0.001
0.0005
0
0
10
20
30
40
50
60
Map Distance (cM)


Total Fat Pad Weight / WK12 (FATP)
P < .001
Mean Square Residual
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
Map Distance (cM)

VITA

Denise Kay Drudik

Candidate for the Degree of

Master of Science

Thesis: LOCALIZATION OF MAJOR QUANTITATIVE TRAIT LOCI (QTL) CONTROLLING BODY WEIGHT AND FAT PERCENTAGE ON MOUSE CHROMOSOMES 2 AND 15

Major Field: Animal Science

Biographical:

Personal Data: Born in Fremont, Nebraska, on June 8, 1971, the daughter of Thomas and Elizabeth Drudik. Raised on a livestock (mainly sheep) farm in Grand Island, Nebraska.

Education: Graduated from Grand Island Northwest High School, Grand Island, Nebraska, in May 1989; received Bachelor of Science Degree in Animal Science from the University of Nebraska, Lincoln, Nebraska, in May 1993. Completed the requirements for the Master of Science Degree in Animal Breeding and Genetics at Oklahoma State University, Stillwater, Oklahoma.

Experience: Employed by Dr. Rodger Johnson as a undergraduate research assistant to collect swine data in 1992 at the University of Nebraska, Lincoln, Nebraska. Employed by Barry Anderson as a corn field scout the summers of 1991-1993 at the Aurora Cooperative Association, Aurora, Nebraska. Employed by Dale Clark as a farmyard worker with sheep and swine from 1985-1989 at the Stuhr Museum, Grand Island, Nebraska.

Professional Memberships: Alpha Lambda Delta, American Society of Animal Science, Gamma Sigma Delta, Golden Key, National Honors Society, and Phi Eta Sigma