Scant information is available on the levels of gene expression in the digestive system of cattle. A study was conducted to characterize transcript profiles in rumen, large intestine, small intestine and reference samples. The absolute intensities obtained from cDNA microarrays that included 7653 cattle and control sequences were used as indicators of the transcript levels. The experimental design included dye-swaps totaling six arrays and sequences were duplicated within array. Data normalization included a LOWESS fit to remove dependencies between tissue effect and average expression level. The remaining variation was analyzed using a linear mixed effects model including the effects of array, dye, gene, and gene by tissue. A total of 218 sequences were significant at $P < 10$ to the -6 power, of which 28 were significant at $P < 10$ to the -9 power. The 99.9% bootstrap confidence interval limits of tissue contrasts indicated that 625 genes were expressed at different levels between large and small intestines, 448 were different between the large intestine and rumen, and 401 were different between the small intestine and rumen. Multiple sequences associated with fatty acid metabolism were over expressed in the rumen with respect to the small and large intestines. These results augment the understanding of the gastrointestinal tract development, differentiation, and function and can be consistent with the high fatty acid absorbance power was obtained using 4 arrays with 4 on-chip replication and 10 arrays without gene replication. Results indicate that on-chip replication is a cost effective way to increase power.

Key Words: Microarray, Power, Replicate

630 Normalization, replication, and significance tests in cDNA microarray experiments. G. J. M. Rosa*, R. J. Tempelman, S. Suchyta, S. A. Madsen, J. L. Barton, and P. M. Coussens, Michigan State University, East Lansing, MI.

Spotted cDNA microarray experiments are being increasingly used in animal science to compare gene expression of tissues under different biological states, such as different environmental stress conditions or a time course. These experiments generate large, complex, and noisy data sets, which must be appropriately analyzed for satisfactory mining of important biological information. Several procedures have been proposed for normalizing the data regarding different kinds of biases and sources of systematic variation, e.g. intensity- or spatially-dependent dye biases. Also, a variety of statistical approaches have been suggested for the determination of significant differences between mean expression signals. We apply and contrast some of these methodologies, using robust local regression technique, ANOVA models and mixed model approaches. Four microarray experiments are used to illustrate these methods and to discuss their advantages and drawbacks. The experiments were conducted at the Center for Animal Functional Genomics at Michigan State University, using a bovine-specific cDNA microarray system containing 3,888 total spots representing 709 bovine EST clone inserts, 345 amplicons of known genes derived from bovine sequence, and numerous blank and control gene spots. The first dataset derives from a self-self hybridization trial where the same tissue sample was arrayed with two fluorescent dyes, in a reverse labeling experiment. A second loop design experiment was used to monitor gene expression profile changes in blood neutrophils collected from cows multiple times as they proceeded through parturition. The other two experiments compare gene expression profiles of peripheral blood cells from control and Johne’s disease positive cows. Special attention in the statistical analyses is given to spatial variability, the use of control genes for data normalization, biological replication, multiple testing and the false positive rate. Some suggestions for further research on the statistical treatment of microarray data are outlined, including the use of mixtures and thick-tailed processes, and different alternatives for modeling heterogeneity of variances across genes and slides.

Key Words: Microarray, Normalization, Significance test

631 Accounting for genotyping errors in QTL analyses. G. J. M. Rosa*, Michigan State University, East Lansing, MI.

Construction of genetic maps and the identification of QTL should involve genetic data of high fidelity. However, the rate of mistyping is considerable in most genotypic data, substantially reducing statistical power on detection of linkage between loci and associations between markers and phenotypic traits. Checks for genotyping errors are then crucially important prior to gene mapping analysis based on traditional statistical methods. Common strategies include comparison of duplicate samples, independent calling of alleles, and Mendelian-inheritance error checking. These strategies, however, are not able to detect all errors. A statistical approach that simultaneously infers upon genotyping error rates and allows for the possibility of miscoded genotypes in QTL analyses is presented. The methodology treats observed marker genotypes as phenotypes with a penetrance function that links these variables (which include errors) to the actual (unknown) genotypes. The model includes an additional parameter, which describes the probability of genotype miscoding. A Bayesian approach based on Markov chain Monte Carlo methods is adopted. Backcross data sets with 150 or 300 individuals, genotyped for 5 loci (including some missing data), and with recombination rates between adjacent loci ranging from 0.01 to 0.15 were simulated. Miscoding probabilities were 0, 1, 3 and 5%. Analyses were conducted ignoring or contemplating miscoding in the model.
Results indicate that our methodology provides more precise inferences, especially regarding QTL locations. An analysis of Brassica napus is presented to illustrate how the procedure works in practice.

Key Words: QTL analysis, Genotyping error, Bayesian inference

632 Power to detect loci linked to common diseases of dairy cattle using identical-by-descent based methods of half-sib pair linkage analysis. R. Vallega*, Department of Dairy and Animal Science, Penn State University.

The utility of sib pairs for quantitative trait locus (QTL) mapping is well established and is based on the use of identical-by-descent (IBD) relationships among genotypes. These model-free methods of sib-pair linkage analysis are more robust for the analysis of complex traits, especially regarding QTL locations. An analysis of Brassica napus is presented to illustrate how the procedure works in practice.

Key Words: QTL analysis, Genotyping error, Bayesian inference

633 Combining breed and family information to detect QTL in crosses of outbred populations. S. K. Musani* and G. B. Jansen, Department of Animal Science, Penn State University.

Crosses of outbred populations pose a unique challenge to QTL mapping due to the existence of linkage disequilibrium between breeds and within families. The present study uses simulation to examine a combined model that uses both sources of information. Two backcross populations (BC1 and BC2) were obtained by backcrossing F1 A × B dams to sires of breed A and F1 B × A dams to sires of breed B. Eight sires and 256 dams each of breed A and B and vice versa were used in the matings, leading to a total of 512 backcross progeny. Each backcross consisted of a set of eight half-sib families with 32 progeny per sire. A finite locus model was simulated with eleven independently segregating QTLs, ten small and one large. The large QTL had three alleles with non-additive effects and explained 24 percent of the total genetic variance of a trait with narrow and broad sense heritabilities of 33 and 45 percent, respectively. Thirteen markers were simulated at 10 cM intervals surrounding the large QTL. Single marker regression analysis was applied. Progeny phenotype was regressed on the probability that the maternal allele came from breed A, in the BC analyses, or the probability that the progeny inherited the first sire allele, in the HS analyses. Statistical power was computed separately for BC1 and BC2, and then as a double BC (BC1+BC2). Similar analysis was done for the HS design. For a marker positioned at 0.1 cM from the QTL, theoretical power was 0.21 and 0.37 for single and double BC analyses, and 0.29 and 0.44 for single and double HS analyses, respectively. Double BC and double HS were combined into one design by multiple regression. When choosing the single marker, out of 13, with the largest test statistic, power of QTL detection was determined empirically. Empirical estimates of power for double BC and double HS analyses were 0.20 and 0.33, respectively, and increased to 0.42 for the combined analysis. These results support the use of a combined approach for the detection of QTL in designed experiments of crosses between outbred populations.

Key Words: QTL analysis, Outbred populations


Sufficient variation in production traits exists in commercial populations of livestock to exploit allelic variation of superior animals to increase production efficiency and improve the quality of livestock products. Identification of predictive markers by constructing dense comparative maps with human and mouse genomes will allow identification of genomic regions that impact production traits in swine. Several quantitative trait loci (QTL) for important reproductive traits (age of puberty, AP; ovulation rate, OR; nipple number, NN; and plasma FSH, FSH) have been identified on the long arm of porcine chromosome 10, which by bi-directional chromosome painting has been shown to be homologous to human chromosome 10p. Because few anchored markers have been placed on SSC10, we wanted to increase the density of known genes that map to this region of the porcine genome. A total of 20 genes on human chromosome 10p were mapped to pig chromosome 10q and 7 genes from human 10q mapped to pig chromosome 14. Genes from human 10p represent 36 megabases (Mb) that correspond to 53 centimorgans (cM) of pig chromosome 10q with an average marker distance of 2.9 cM (2 Mb of human DNA). Gene order was highly conserved within these markers from centromere to telomere of porcine chromosome 10q, as compared to human chromosome 10p, with 1 large rearrangement along the center of the region. The large gap was 16 cM (104-120 cM on the pig map) corresponding to human 10p14 (8-11 Mb), a region which is very gene-poor in the human. The breakpoint for pig chromosomes 10 and 14 was at the centromere of human chromosome 10. Positional candidate genes were identified for AP (aldo-keto reductase, AKR1C), OR (AMP regulatory element modulator, CREM), FSH (mammone receptor C1, MRC1) and NN (enhancer of polycomb, EPC1). Nucleotide variation in AKR1C, MRC1 and EPC1 is currently being evaluated in the multi-generation reciprocal backcross resource population as markers for quantitative traits.

Key Words: Genetic markers, Quantitative trait loci, Mapping

635 QTL mapping in extended half-sib families. N. Vukasinovic1 and M. L. Martinez2, 1Monsanto Animal Genomics, 2Embrapa - CNPGL.

QTL mapping in dairy species is usually conducted in presumably unrelated half-sib families, often resulting in imprecise estimates of QTL parameters. Including relationships among sires can improve precision of QTL mapping. In this study we compare the power of QTL mapping analysis that assumes unrelated sire families with a general pedigree approach that extends half-sib families by considering relationships among sires. Two base individuals were simulated and mated to produce 3 sons. The sons were randomly mated to 4 dams each to produce one male offspring per mating. These 12 sires were then mated to 25 dams each to produce one daughter per mating. The terminal generation included 300 individuals in 12 half-sib families. A 60cM chromosomal segment with 5 equally distributed polymorphic markers was simulated. A 5-allelic QTL was simulated at 20cM or 40cM. The QTL heritability was 0.25. Only marker genotypes on all sires and daughters and phenotypes on daughters were assumed available for analysis. QTL mapping was performed using the random model approach in which phenotypic (co)variances between related individuals are functions of the proportion of alleles identical-by-descent (IBD) shared at a putative QTL. The IBD proportions within families in the standard half-sib analysis were inferred from marker genotypes at flanking markers. In the general pedigree analysis, the IBD proportions within and between families were obtained by a recursive deterministic method using the closest informative marker bracket. Maximum likelihood techniques were used to estimate QTL parameters. In the half-sib analysis, the 95% confidence intervals for QTL position were 14.6 to 41.4cM and 27.9 to 58.7cM for QTL located at 20cM and 40cM, respectively. In the general pedigree analysis, the corresponding 95% confidence intervals were 16.9 to 25.1cM and 41.6 to 48.4cM. The QTL heritability, as estimated in model F, was computed in likelihood ratio profiles showing posterior odds, peaks and p-values, whereas the general pedigree analysis produced profiles with a clearly defined peak. The estimates of QTL heritability...
were 0.29 and 0.55 from the general pedigree and halfsib analyses, respectively. Considering relationships among sires is an efficient way to improve results of QTL mapping without considerable cost increase.

Key Words: QTL mapping, Pedigree, Simulation

636 Comparison of statistical methods used to analyze marker data from daughter design with selective genotyping. Y. Pan1,2, N. Caron1, G. B. Jansen1, E. B. Burnside1,2, and J. P. Chesnais1,2. 1The Semex Alliance, Saint-Hyacinthe, Quebec, Canada, 2L'Alliance Boviteq, Saint-Hyacinthe, Quebec, Canada, 3University of Guelph, Guelph, Ontario, Canada.

A daughter design (DD) was used to identify linkage between markers and QTLs. Selective genotyping can considerably reduce the cost of genotyping for a DD; however, it can result in biased estimates of allele substitution effects when simple regression methods are used. A simulation was carried out to compare three methods. They were maximum likelihood (ML: Lander and Botstein, 1989), mean difference between two marker genotypes (MD: Darvasi and Soller, 1992) and logistic regression (LR: Henshall and Goddard, 1999). Phenotypic measurements were simulated for a typical trait in dairy cattle (h² = 0.46, σp = 41.6 kg). Ten marker loci (3 to 10 alleles at each locus) and a QTL (2 alleles) located on one chromosome were simulated. The allele substitution effect was 0.0, 0.2, or 0.5 σp. Selective genotyping with equal and unequal proportions of daughters from each tail was used in a DD. Equal selection proportions were 0.50, 0.25 and 0.05 from each tail and unequal selection proportions were 0.30 (top) vs. 0.20 (bottom) and 0.06 (top) vs. 0.04 (bottom). The accuracy of estimation of allele substitution effects was evaluated as the deviation between estimated and true values. All three statistical methods (ML, MD and LR) provided similar means and standard errors of allele substitution effects when equal proportions were selected from each tail. Estimation errors ranged from 0.002 to 0.017 σp in all cases. However, ML and LR methods performed slightly better than MD when unequal proportions of animals were selected. Compared with LR using a SAS standard procedure, ML required much more computing resources. All three methods were suitable for analysis of marker data from selective genotyping in a DD.

Key Words: Statistical methods, Selective genotyping, Daughter design

637 Superiority of QTL-Assisted Selection in Dairy Cattle Populations with Nucleus Herds. G. A. Abdel-Azim1 and A. E. Freeman1, Iowa State University.

Two-stage selection of dairy sires, the conventional method currently in use, was applied to the simulated data as the reference or base-line scheme. As reported by several simulation studies, QTL-Assisted Selection (QAS) has been most useful in nucleus herds. However, stochastic simulation studies that investigated the superiority of QAS in nucleus herds often simulated small closed nucleus herds with simple selection and mating plans. In the current study, a juvenile hybrid nucleus herd scheme with a hierarchical mating design was simulated. The nucleus herd partially contributed to the group of young bulls tested in the population every year. Twenty years of selection were simulated with overlapping generations and with population and model parameters proportional to the U.S. Holsteins. A moderately heritable quantitative trait (h² = 0.3) affected by 40 bi-allelic loci and one QTL with a major effect was simulated. The favorable QTL allele started at a frequency of 0.1. A general trend across all pathways was observed: low superiority in early years of selection that increased to a plateau in later years and then decreased. Superiority at plateau for selection pathways are listed in the table below. In addition to the percentage of superiority, response to selection attributed to each of the QTL and the polygenes was addressed. Further, the effect of the rate at which the favorable allele approached fixation and the accuracy of predicting breeding values on the percentage of superiority were studied. Two major conclusions can be drawn from the study. The contribution of nucleus herds to QAS was positive, and superiority trends in schemes with nucleus herds were more developed relative to the base-line scheme.

<table>
<thead>
<tr>
<th>Two-Stage</th>
<th>Nucleus Herds</th>
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<tbody>
<tr>
<td>Active Sires</td>
<td>7.0</td>
</tr>
<tr>
<td>Young Bulls</td>
<td>8.1</td>
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<tr>
<td>Bull Dams</td>
<td>13.1</td>
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<tr>
<td>Donor Females</td>
<td>-</td>
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<tr>
<td>First-Lactation Cows</td>
<td>2.7</td>
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</tbody>
</table>

QAS Superiority at plateau computed as percentage difference from QTL-Free Selection.

Key Words: QTL-assisted selection, Nucleus herd, Dairy cattle


The multiple chromosome genome scan approach to detecting QTL for production and conformation traits in dairy cattle has been gaining popularity as the number of documented microsatellite markers increases. This study reports on the use of microsatellite markers covering six chromosomes in a granddaughter design to detect QTL for production and conformation traits in 25 Holstein sire families. A total of 1,835 sons of 25 sires were genotyped for 54 microsatellite markers distributed across chromosomes BTA1 (12 markers), BTA3 (10), BTA9 (9), BTA10 (8), BTA14 (8) and BTA20 (7). The performance data for this study were the USDA production genetic evaluations (PTA) and the Holstein USA conformation genetic evaluations (STA) released in February 2002. Weighted least squares interval mapping was performed across and within sire families using a modified version of the software developed by S. Knott and C. Haley. Analyses were performed at one cM intervals separately for each trait. Permutation testing and false discovery rate were used to control type I error. The information content of the marker genotyped in this study ranged from 0.57 to 0.89 with most between 0.75 and 0.85. Six significant QTL effects were evident from the multiple size analyses for production traits. Evidence for a QTL for both PTA milk and PTA fat% was found on BTA14 (0 cM) and a QTL at 81 cM for PTA protein%. On BTA3 and BTA 20, there was evidence for QTL associated with PTA protein% at 26 and 41 cM respectively. Three significant QTL effects were evident from the multiple sire analyses for conformation traits. All three QTL were found on BTA10; a QTL associated with STA Strength was located at 43 cM, a QTL associated with STA Body Depth was located at 45 cM and a QTL associated with STA Dairy Form was located at 49 cM.

Key Words: QTL, Production, Conformation

639 Putative quantitative trait loci affecting perinatal survival in eleven Holstein families. P. J. Berger1, J. Koltes1, M. H. Healey1, M. S. Ashwell2, R. D. Shanks3, H. Schlesser3, and H. A. Levin3. 1Iowa State University, Ames, IA, 2USDA-ARS-GEML, Beltsville, MD, 3University of Illinois, Urbana, IL.

Perinatal survival (PS) is a categorial trait expressed as the proportion of calves alive 48 hr after birth. Recent estimates of predicted transmitting ability (PTA) for PS for elite Holstein sire families were used to identify putative quantitative trait loci (QTL) for PS. From 55 sire families with 50+ sons, 17 families were shown to have a bimodal distribution for PTA-PS; 11 of the 17 families had genotypic data. Full genome-wide scans were available for two of the 11 families. A total of 56 markers were from the USDA linkage map; 18 markers on BTA 6 and 16 markers on BTA 27. Data were analyzed using ANOVA in the granddaughter design, to identify significant marker-PS associations. Number of informative sons ranged from 38 to 285. Mean number of informative sons across all markers was 131; 115 for BTA 6 and 151 for BTA 27. Six markers, three each on BTA 6 and 27, had significant (P < 0.02) associations with PS. Suggestive of a major genetic component for PS, estimates of effects ranged from -0.45 to 0.28 % PS. Markers on BTA 9, 12, 14, 17, and 18 also exhibited significant associations with PS (P < 0.02), although this data was limited to two families. Estimates of effects for these markers ranged from -0.31 to 0.56 % PS. Distribution of the number of sires with alleles for significant markers was similar to the original bimodal distribution of PTA-PS. Evidence presented implies the existence of QTL linked to major genes affecting PS. Upon validation and fine-mapping, sires can be selected for PS based on the existence of specific marker information.

Key Words: Perinatal survival, Quantitative trait loci, Holstein dairy cattle
A genome scan for chromosomal regions of bovine chromosome one (BTA1) influencing weaning weight (WT6), yearling weight (WT12) and postweaning average daily gain (PWADG) was performed using 112 half-sib progeny of 4 Japanese Black (Wagyu) sires and 98 microsatellite DNA markers. Identity-By-Descent (IBD) probabilities at specific chromosomal locations from multiple marker data were determined and a linear model containing the fixed effects of sex, parity and season of birth as well as age as a covariate, was fitted to the IBD coefficients and phenotypic data. Data were analysed by generating an F-statistic by the regression of phenotype on the IBD probabilities of inheriting an allele from the sire. Permutation tests at chromosome-wide significance thresholds were carried out over 1,000 iterations at 1cM intervals while the bootstrap with resampling procedure was followed to estimate confidence intervals and average QTL locations. All these procedures were implemented in the QTL Express Computer programme with a web-based user interface (available at: http://qtl.cap.ed.ac.uk/). A significant QTL (P chromosome-wise threshold = 0.05) for PWADG was identified in Sires 2 and 3 located at 27cM and 28cM (95% confidence intervals of the QTL locations being 0-132cM and 0-125cM) respectively. Another QTL for WT12 was identified at 13cM in Sire 2. No significant effect of QTL was detected in any of the sires. Selection indices that include QTL with accurately estimated effects on carcass characteristics could reduce the amount of lengthy and costly data collection by providing a means of genetic evaluation early in the life cycle. Since PWADG is positively correlated with WT6 and WT12 in beef cattle, the identification of these QTL in Japanese Black Cattle holds a high prospect for the implementation of marker-assisted selection for the early attainment of slaughter weight in this breed.

Key Words: QTL, Japanese Black, Growth

Extension Education: Extension education and evaluation programs

The mission of the USDA Animal Improvements Program Laboratory (AIPL) is to foster genetic improvement in dairy cattle. Practical improvement in production and profitability is achieved through the distribution of genetic evaluations used by the dairy industry to guide breeding decisions. Since 1997, evaluations have been distributed via the Internet through the AIPL website (http://aipl.ars.usda.gov) and FTP site (ftp://aipl.ars.usda.gov). Data used to calculate evaluations is received via the FTP site from dairy record processing centers (DRPC), breed associations, and other industry cooperators. Between quarterly evaluations, 11.2 million individual animal updates, and 150,000 pedigree updates come from DRPC and breed organizations, respectively. Over 80 interactive tools assist cooperators and AIPL staff with data quality control, and access is customized by user group. Genetic evaluations are also available to the public via the website through 22 interactive queries. More than 20 quarterly or yearly reports are also available. Complete documentation of evaluation procedures is stored in the AIPL website. The user-accessible directory includes 377 Mb of data and information in 12,000 files. A full function search engine assists with site navigation. File metadata also facilitates indexing by outside engines. In 1997, the National Agricultural Statistics Service reported that 20% of all farms with over $100,000 annual sales had internet access. In 2001 that figure was up to 25%. In the second half of 2002, over 170,000 requests for bull evaluations and 67,000 requests for cow evaluations were submitted to the AIPL website. Evaluation access quadruples during the week following evaluation release. Links accessed within the AIPL website account for 74% of all website requests. Outside requests are from links on other sites 12% of the time, others coming from browser history. Almost 80% of the requests are from the top 1% of requesters. Recent website enhancements include Spanish language availability and improved indexing. Planned improvements include more-dynamic database query tools, and user account control for industry cooperators.

Key Words: Internet, Information technology, Dairy genetics

Effectiveness of presenting a national beef breeding management educational program via the Internet

A number of publications based on various studies strongly suggest the existence of several putative quantitative trait loci (QTL) on the bovine chromosome 6 (BTA6) for the milk traits (Bovenhius and Schroten 2002). Further, they partly suggest equal QTL locations for QTL for several traits. For this reason, we applied a multiltrait (co)variance component based QTL mapping method (Sorensen et al. 2003) to a data set involving five granddaughter families with 298 genotyped sons from the German Holstein cattle population. The marker map contained 16 microsatellite markers (according to Khn et al. 1999, extended), distributed across BTA6. The trait values (DYD and EBV) were provided by the VIT Verden. The multiltrait-QTL approach (MTMQTL) is part of the DMI package, developed by the Danish Institute of Agricultural Sciences (DIAS) and allows analysing several traits multivariately, and specifying five different genetic submodels. A chromosome-wise significance threshold was used, because BTA6 is known to harbor QTL for several milk traits. We received significant QTL findings for milk yield (between markers BM1329 and FBN12), for yields of protein and fat (FBN9...FBN13), and QTL for contents of fat and protein (BMI329...FBN12). The multivariate analysis resulted in a significant pleiotropic QTL finding for fat yield and protein yield. The estimates of variance contribution due to the QTL were 20% and 25%, respectively. For fat yield and fat content, a pleiotropic QTL seems to be likely, between FBN12 and TGLA37, but these results were not fully significant. Negatively correlated milk traits are likely affected by trait specific closely linked QTL on BTA6, e.g. 24 cM apart for protein yield and fat content, according to significant results. The confidence interval CI (95%) was computed as suggested by Darvasi and Soller (1997) and ranged from 8 to 13 cM, depending on the model, in significant cases.

Key Words: Milk traits, Multivariate QTL analysis, Pleiotropic QTL

Different images of putative quantitative trait loci on BTA6 for correlated milk traits

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Key Words: QTL, Japanese Black, Growth

Using the Internet for exchange of dairy genetic evaluations and research information for the dairy industry

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