EFFECTS OF INDIVIDUAL LOCI ON PERFORMANCE DURING LACTATION

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SUMMARY

Evidence from studies on estimating the effects of single loci other than milk proteins has been accumulating. Studies have suffered from lack of statistical design, diversity of assumptions, low power to detect differences, multiple testing using high probability of chance associations, and inadequate analyses. Loci selected here were confirmed in at least two independent samples to improve confidence in results. Evidence for major genes segregating within highly selected populations is presented.

INTRODUCTION

Single loci with large beneficial effects (Economic trait loci, ETL) have been sought since the original understanding of genetics. Appeal of genotypes that could be known with certainty and direct selection of valuable alleles has fueled the search in spite of considerable difficulties. Loci with little polymorphism, difficult and expensive genotyping, and lack of consistent results in trait estimation slowed this research. Development of informative molecular markers with nearly complete genome coverage (Fries et al., 1993) has reawakened interest. Most marker-assisted selection schemes have advocated use of genotypes at single loci in distinguishing between young animals of equivalent or identical pedigrees (Smith and Simpson, 1986). Livestock schemes with hierarchical and centralized breeding structures, such as dairy cattle, could make maximum use of any ETL detected (Soller and Beckmann, 1983).

PROBLEMS WITH DETECTION OF SINGLE LOCUS EFFECTS

Literature on single locus effects presents a confusing, contradictory, and unconvincing picture. Statistical models have varied from simplistic to highly sophisticated. Techniques to genotype animals at single loci have improved markedly and standardization of genotype nomenclature has been implemented. Unfortunately, improvements in statistical and genetic techniques cannot address the most important difficulties of this search. Dairy cattle research has rarely been able to set up intentional experimental designs to isolate effects of single genes. Most studies are retrospective using pedigree structures already present and depend on assumptions about linkage (dis)equilibrium, allele frequencies, number of loci, and size of effects. These assumptions are rarely tested and vary between studies of the same populations.

Associations of single loci and lactation performance can result from linkage disequilibrium continuously induced by selection or drift, or rare alleles in slowly decaying disequilibrium due to migration or mutation (Dentine, 1990). These

associations may not be predictive in other data sets and may not indicate physical linkage with ETL. Additionally, many studies utilize a large number of loci and traits resulting in Type I statistical errors based on multiple comparisons. Use of more conservative probability levels for judging significance has been advocated (Kashi et al., 1986) but many studies already have low power of detection due to cost and availability of genotyping. Useful variation associated with single loci may be present, but only large effects will likely be detected with size of most studies. Some authors have suggested more saturated genetic maps will make search for ETL easier, but several studies have shown that 10-20 cM maps may be sufficient for detecting even moderate effects (Dekkers and Dentine, 1991; Knapp et al., 1990). Closer marker spacing may not contribute to locating ETL since "resolving power" is more dependent on number of observations than on spacing of markers (Darvasi et al., 1993).

Although arguments have been made that single loci of large effect would have been noticed if present, low allele frequencies can obscure even large effects (Table 1). Even for effects as large as one genetic standard deviation, contributions to population variance can be small (Table 2). Variance due to single loci contributes more in offspring of heterozygotes and thus leads to models that contrast offspring within heterozygous sires. Additionally, these offspring can help isolate effects of single loci and block against disequilibrium effects of other unlinked loci (Dentine and Cowan, 1990).

Table 1. Polygenic and single locus variance in populations.

	Underlying polygenic variation	single locus with additive effect of ETL = 1 σ_g
Total variance	σ^2_g	2pq
Between sires	$.25\sigma^2_g$	.5pq
Within sires	$.75\sigma^2_g$	1.5 pq
within AA or BB		pq
within AB		.25 + pq

Table 2. Variance from segregation of single ETL(substitution effect = 1 σ_g) expressed relative to all genetic variance as a function of allele frequency.

	Percent combined genetic variance due to single locus		
	p=.1	p=.05	p=.01
Across populations	15.3	8.7	1.9
Between sires	15.3	8.7	1.9
Within sires			
within AA or BB	10.7	6.0	1.2
within AB	31.2	28.4	25.1

Although most studies expressed effects as allele substitution effects of single loci, current molecular and statistical approaches cannot distinguish between several genetic models. Significant segregation effects tracked by alleles at a single locus can be direct results of a major gene at that locus or at a linked locus, the concerted effect of

several loci in a linked cluster (Geldermann et al., 1985), or even polygenic small loci on a chromosome segment tracked by a marker (Dekkers and Dentine, 1991). Models that utilize multiple marker loci to detect recombination events can have more power (Jansen, 1993; Lander and Botstein, 1989), but ambiguities about location, size and number of ETL remain (Kennedy et al., 1992; McMillan and Robertson, 1974).

Three approaches have been suggested to avoid difficulties with multiple comparisons, power considerations and costs. Confidence in results can be increased without large increases in size by using sequential testing of several small samples (Soller and Beckmann, 1990). Secondly, loci and traits can be measured in a limited population. Associations judged significant at a liberal Type I error rate (for instance, $\alpha = .1$) can be retested in additional individuals. Multigeneration segregation studies are another method to effect independent contrasts. Genotyping costs can be decreased by utilizing a grandsire model (Weller et al. 1990) with many granddaughters contributing phenotypic trait information, because genotyping need only be done on the generation of sons used to sire these cows. Independent sampling can be combined with the multigenerational approach in a variety of 4 generation pedigrees for increased confirmation (Cowan et al., 1992). Genotyping of extreme animals of phenotypic distributions also increases power (Lebowitz et al., 1987), but results in some difficulties in testing of hypotheses (Lander and Botstein, 1989; Weller and Wyler, 1992). If more than one trait is under study, extreme animals for all traits would require genotyping.

This review will report only those loci that meet certain criteria. Loci associated with milk proteins, likely candidates for associations with lactation traits, are covered by another speaker at this conference. Loci not associated with milk proteins will be considered only if conditions for decreasing Type I errors are met: at least two independent studies have confirmed association in separate populations or independent contrasts from the same population. These requirements will almost surely exclude some loci with effects that could contribute to marker-assisted selection (Type II errors).

LOCI

A number of loci exhibit significant effects on lactation which do not require large or well-designed studies. These loci include all lethal and many sub-lethal genetic defects. Dwarfism, bovine leukocyte adhesion deficiency (BLAD, Shuster et al. 1992) and deficiency of uridine monophosphate synthase (DUMPS, Shanks et al., 1987) are examples of loci with profound effects on lactational performance associated with recessive alleles. These loci are often dismissed in discussions of major loci, but are undoubtedly one end of a continuum of genetic effects. Alternative alleles to all genetic defects are positive ETL for performance. Selection policies designed to eliminate genetic defects are part of genetic improvement of lactation yields and cannot be separated from other loci with potential for marker-assisted selection.

Amylase

Amylase-1, a plasma protein, has two prevalent alleles in dairy cattle, B and C. Geldermann et al. (1985) reported a substitution effect in 199 offspring of a

heterozygous German Friesian bull with a superiority for fat content (%) of .10 for offspring receiving the B allele rather than the C allele from the sire with a second sire family showing a -.06 (non-significant) effect. More recently, Andersson-Eklund and Rendel (1993) reported on estimates in Swedish Red and White. One study looking at main effects of amylase alleles detected a highly significant interaction by sires (Andersson-Eklund et al. 1990) indicating a linkage relationship of ETL for fat % to the amylase-1 locus. A segregation analysis was made in a further data set using 14 heterozygous sires with 293 sons with estimated breeding values based on progeny test. Including a separate estimate of segregation within each sire contributed 9% of the variance in breeding values across sons. A non-normal distribution of estimated sire effects showed 7 heterozygous sires with estimates near zero and 7 with effects corresponding to ~±.20% of fat content. Four sons showed superiority for the C allele; 3 sons showed similar estimates for superiority for B. A test of within family variance showed highly significant heterogeneity. These results are consistent with linked ETL in various phase relationships with B and C alleles of amylase-1. Interestingly, this data is also consistent with a single ETL or group of linked ETL with moderate frequencies still segregating within a highly selected population.

B blood antigen system

In the B system of blood antigens, high polymorphism information content allow use of haplotypes as alleles for paternity and identity testing. Because records are kept for parentage, the B system has been investigated for a number of years although allele nomenclature has changed over time. Two studies reported significant effects of one allele, BO_1YD', in different breeds of dairy cattle. Rendel (1961) reported a favorable effect of this allele on fat content (.20% in offspring of 205 Swedish Red and White bulls and .18% in daughters of 75 Swedish Friesians). Neimann-Sørensen and Robertson (1961) also found a positive effect of this allele (+.06%) in 108 progeny groups of Red Danish cattle (n=1409). In a within-sire segregation analysis with 290 cows in seven sire lines, Conneally and Stone (1965) confirmed a positive effect in North American Holsteins and estimated effect on fat content at +.33%. These estimates of effects in 4 different populations of dairy cattle are consistent in sign and very convincing. The size of estimates would depend on allele frequencies in various populations and could be expected to differ. Thirty years after the initial Swedish Red and White study, Andersson-Eklund et al. (1990) reestimated effect of this allele and confirmed a positive effect (+.04%) on fat content in 823 bulls. By this time, protein testing was also available and a significant favorable effect was estimated at +.03% for protein. This one allele is remarkable, given size of estimates and consistent evidence across populations.

Other studies have looked at B system as a set of alleles and found significant effects for including B alleles in analyses. Hines (1990) summarized previous studies and indicated that more than 40% of studies found significant effects on fat percentage of milk using B blood groups. Brum et al. (1968) found a highly significant effect of B alleles on fat % accounting for 2.3% of variance (>3,000 Holstein cows). Rausch et al. (1968) showed 27% decreases in sire variation for fat content when polymorphisms for B and L blood systems were included in analyses of 1582 Holstein bulls. Although individual allele estimates were not consistent, there has been more significance of B alleles as markers for fat percentage than likely due to chance alone.

M antigen system

The M blood group has been used as a marker although polymorphism information content is lower than B system. Haenlein et al. (1987) found M alleles associated with fat and solids-not-fat percentages in Guernseys. Andersson-Eklund et al. (1990) found M alleles associated with fat and protein percentages in Swedish Red and White. A direct positive effect of M' allele was estimated at .02% fat content and a significant sire by marker interaction was detected for both fat and protein content. This interaction was interpreted as evidence of linkage to ETL that differed in phase by sire. Geldermann et al. (1985) estimated the segregation effect in one German Friesian sire at .03% increase. This allele has been associated with increased incidence of mastitis in Red Danish cattle (Larsen et al., 1985) and that relationship was confirmed in a study of 1171 Swedish Red and White sires (Andersson-Eklund and Danell, 1993). M blood system has been mapped to the same chromosome as major histocompatibility locus (BoLA) and prolactin (Fries et al., 1993); M alleles may be acting as markers for these loci.

J antigen system

The J blood system of alleles has been found to have significant associations with milk, fat, and protein yields in Swedish Red and White (Andersson-Eklund et. al. 1990) with significant effects of interaction by sire for all traits. Estimated main effects were large, ranging from .77 to 1.3% of phenotypic mean. Total reduction in residual variance associated with including main effects and sire interaction was 8 to 10% of breeding values of sons. This relationship has not been consistently seen in other studies, although significant interactions of J and other blood antigens have been reported (Rendel, 1961; Gonyon et al. 1987). Since significant interaction with sires suggests tracking of a linked ETL, one possible explanation may be that mapping has placed the J locus on the same chromosome as β-lactoglobulin (Fries et al., 1993).

Major histocompatibility complex

Bovine lymphocyte antigens (BoLA) also provide a highly polymorphic marker system. Few confirmed associations with milk yields or milk components have been reported, but significant associations with diseases of dairy cattle have been detected. Østergård et al. (1989) found multiple studies showing allelic differences in resistance to bovine leukosis, mastitis and parasite resistance. Batra et al. (1989) found significant effects for cost of disease treatment in Canadian Holsteins; Oddgeirsson et al. (1988) showed significant effects on mastitis and somatic cell counts in Icelandic cattle; Weigel et al. (1990) found significant effects of alleles on total health costs and mastitis indicators in U.S. Holstein cattle. Lundén et al. (1990) found a large effect of a single allele, DQ^{1A}, accounting for ~8% increase in mastitis incidence in first lactation Swedish Red and White. Repeated studies have also shown a relationship between DRB2 alleles and persistent lymphocytosis in dairy cattle which apparently results in milk yield losses (Da et al., 1993). High linkage disequilibrium in this region may complicate interpretation of results.

Maternally inherited DNA

Maternally inherited non-nuclear genotypes have been associated with variation in lactation yields and component percentages by use of maternal lines determined by

pedigree tracing (Bell et al., 1985). More recently, genotyping of U.S. Holstein lines by sequencing has shown significant effects of particular substitution effects in mitochondrial D-loop (Schutz et al., 1993). Significant effects were concentrated on fat percentages and energy content of milk which is consistent with mitochondrial functions. Work on Israeli Holsteins (Ron et al., 1992) also showed a non-random distribution of mitochondrial genotypes across cattle of various production levels. Subsequent work using selective genotyping (Ron et al., 1993) failed to confirm substitution effects detected by Schutz et al. (1993) as being fixed in high or low producing lines. Questions remain about probability of fixation for non-segregating genotypes and the possibility that drift effects may be present in estimates (Kennedy, 1986) in spite of efforts to use appropriate statistical tools.

Prolactin

Candidate genes have been used in addition to milk proteins in segregation studies. Use of a prolactin cDNA as a probe revealed a polymorphism with two alleles segregating within U.S. Holsteins (Cowan et al., 1990). Within one sire family using the grandsire design, a segregation analysis indicated significant substitution effects on milk yield (566 kg) within 26 sons. This family was explored further using independent sets of later generation offspring (Cowan et al., 1992). Segregations of 73 granddaughters within one heterozygous son resulted in estimated effects of 416 kg. Daughters of the original bull were mated to a second (unrelated, but heterozygous) bull resulting in 37 sons. Genotypes for several linked loci were used to separate haplotypes of the sire and maternal grandsire in 3/4 brothers to estimate an effect of 586 kg for the original bull. Segregations within the daughters of the son would be subject to one additional recombination possibility and resulted in a lower estimate. Segregation estimate from the unrelated bull was close to zero. These results were consistent with linkage to one or more ETL rather than a direct effect of the locus.

Genetic defects

One further evidence for major loci should be mentioned. Several genetic defects in cattle have been used as markers for ETL investigations. At least two have shown segregation effects on lactation: bovine progressive degenerative myeloencephalopathy (Hoeschele and Meinert, 1990, multiple generations of Brown Swiss cattle) and deficiency of uridine monophosphate synthase (independent estimates in Holstein cattle, Shanks and Greiner, 1992; Kuhn and Shanks, 1994) . These effects may be pleiotropic from the disease locus, or , more likely, effects of linkage where the defect has hitchhiked into the population along with favorable ETL. If this second phenomena is present, care will be needed to separate ETL from defect in order to salvage beneficial alleles that would otherwise be eliminated with the defect.

CONCLUSIONS

In summarizing estimates of single loci, several trends emerge. Many studies that have been done had little hope of detecting ETL due to small experimental size and lack of statistical power. Effects that have been found are large or are in studies with more complete statistical designs, especially use of the grandsire model. Percentages of

components dominate earlier studies, perhaps because genes of large effect may still be segregating due to primary selection on yields. Indeed one study has shown major ETL segregating for fat content can be detected without use of any markers (Boichard et al., 1990). In many cases, estimates vary in sign across sire segregation analyses or population indicating that linkage to ETL is more likely than pleiotropy. Without question single loci or clusters of ETL are segregating even in highly selected dairy cattle populations. Some of these loci are in limited use in selection. Questions to be answered include whether the size of effects and allele frequencies warrant use in marker-assisted selection, whether benefits will overcome costs of detection and genotyping (Beckmann and Soller, 1983; Brascamp et al., 1993), and whether methods to incorporate marker information can be improved (Hoeschele and Van Raden, 1993).

REFERENCES

ANDERSSON-EKLUND, L., and DANELL, B. (1993) J. Dairy Sci. 76:3785-3791.

ANDERSSON-EKLUND, L., and RENDEL, J. (1993) Animal Genetics 24:101-103.

ANDERSSON-EKLUND, L., DANELL, B., and RENDEL, J. (1990) Animal Genetics 21:361-376.

BATRA, T.R., LEE, A.J., GAVORA, J.S. and STEAR, M.J. (1989) J. Dairy Sci. 72:2115-2124.

BECKMANN, J.S. and SOLLER, M. (1983) Theor. Appl. Genet. 67:35-43.

BELL, B.R., MCDANIEL, B.T. and ROBISON, O.W. (1985) J. Dairy Sci. 68:2038-2051.

BOICHARD, D., ELSEN, J.M., LE ROY, P. and BONAITI, B. (1990) Proc. 4th World Congr. Genet. Appl. Livestock Prod. 14:167-170.

BRASCAMP, E.W., VAN ARENDONK, J.A.M., and GROEN, A.F. (1993) J. Dairy Sci. 76:1204-1213.

BRUM, E.W., RAUSCH, W. H., HINES, H.C., and LUDWICK, T.M. (1968) J. Dairy Sci. : 51:1031-1038.

CONNEALLY, P.M. and STONE, W.H. (1965) Nature, 206:115.

COWAN, C. M., COYLE, T., DUNCKEL, M.A. and DENTINE, M.R. (1992) J. Dairy Sci. 75 (Suppl. 1): 286.

COWAN, C.M., DENTINE, M.R., AX, R.L. and SCHULER, L.A. (1990) Theor. Appl. Genet. 79:577-582.

DA, Y., SHANKS, R.D., STEWART, J.A. and LEWIN, H.A. (1993) Proc. Natl. Acad. Sci, USA 90:6538-6541.

DARVASI, A., WEINREB, A., MINKE, V., WELLER, J. I. and SOLLER, M. (1993) Genetics 134:943-951.

DEKKERS, J.C.M. and DENTINE, M.R. (1991) Theor. Appl. Genet. 81:212-220.

DENTINE, M.R. (1990) Proc. 4th World Congr. Genet. Appl. Livestock Prod. 14:35-44.

DENTINE, M.R. and COWAN, C.M. (1990) Theor. Appl. Genet. 79:775-780.

FRIES, R., EGGEN, A. and WOMACK, J.E. (1993) Mammalian Genome 4:405-428.

GELDERMANN, H., PIEPER U. and ROTH, B. (1985) Theor. Appl. Genet. 70:138-146.

GONYON, D.S., MATHER, R.E., HINES, H.C., HAENLEIN, G.F.W., ARAVE, C.W., and GAUNT, S.N. (1987) J. Dairy Sci. 70:2585-2598.

HAENLEIN, G.F. W., GONYON, D.S., MATHER, R.E. and HINES, H.C. (1987) J. Dairy Sci. 70:2599-2609.

HINES, H.C. (1990) Proc. 4th World Congr. Genet. Appl. Livestock Prod. 13:121-124.
HOESCHELE, I. and MEINERT, T.R. (1990) J. Dairy Sci. 73:2503-2515.
HOESCHELE, I. and VANRADEN, P.M. (1993) Theor. Appl. Genet. 85:946-952.
JANSEN, R. (1993) Genetics 135:205-211.
KASHI, Y., SOLLER, M., HALLERMAN, E. and BECKMANN, J.S. (1986) Proc. 3rd World Congr. Genet. Appl. Livestock Prod. 12:57-63.
KENNEDY, B. W. (1986) J. Dairy Sci. 69:3100.
KENNEDY, B. W., QUINTON, M., and VAN ARENDONK, J. A. M. (1992) J. Anim. Sci. 70:2000-2012.
KNAPP, S.J., BRIDGES, W.C. and BIRKES, D. (1990) Theor. Appl. Genet. 79:583-592.
KUHN, M.T. and SHANKS, R.D. (1994) J. Dairy Sci. 77:589-597.
LANDER, E. and BOTSTEIN, D. (1989) Genetics 121:185-199.
LARSEN, B., JENSEN, N.E., MADSEN, P., NIELSEN, S.M., KLASTRUP, O. and MADSEN, P.S. (1985) Anim. Bl. Groups Biochem. Genet. 16:165-173.
LEBOWITZ, R. J., SOLLER, M. and BECKMANN, J.S. (1987). Theor. Appl. Genet. 73:556-562.
LUNDEN, A., SIGURDARDOTTIR, S., EDFORS-LILJA, I., DANELL, B., RENDEL, J. and ANDERSSON, L. (1990) Animal Genetics 21:221-232.
McMILLAN, I. and ROBERTSON, A. (1974) Heredity 32:349-356.
NEIMANN-SØRENSEN,A. and ROBERTSON,A. (1961) Acta. Agric. Scand. 11:163-196.
ØSTERGÅRD, H. KRISTENSEN, B. and ANDERSEN, S. (1989) Livestock Prod. Sci. 22:49-67.
ODDGEIRSSON, O., SIMPSON, S.P., MORGAN, A.L.G., ROSS, D.S. and SPOONER, R.L. (1988) Animal Genetics 19:11-16.
RAUSCH, W. H., BRUM, E.W., and LUDWICK, T.M. (1968) J. Dairy Sci. 51:445-451.
RENDEL, J. (1961) Nature, 189:408.
RON, M., GENIS, I. EZRA, E. YOFFE, O., WELLER, J.I., and SHANI, M. (1992) Animal Biotechnology 3:201-219.
RON, M., YOFFE, O., and WELLER, J.I. (1993) Anim. Genetics 24:183-186.
SCHUTZ, M.M., FREEMAN, A.E., LINDBERG, G.L. and BEITZ, D.C. (1993) J. Dairy Sci. 76:621-629.
SHANKS, R. D. and GREINER, M.M. (1992) J. Dairy Sci. 75:2023-2029.
SHANKS, R.D., BRAGG, D. ST. A., and ROBINSON, J.L. (1987) J. Dairy Sci. 70:1893-1897.
SHUSTER, D.E., KEHRLI, M.E., ACKERMANN, M.R., and GILBERT, R.O. (1992) Proc. Nat. Acad. Sci. USA 89: 9225-9229.
SMITH, C., and SIMPSON, S.P. (1986) J. Anim. Breedg. Genet. 103:205-217.
SOLLER, M. and BECKMANN, J.S. (1983) Theor. Appl. Genet. 67:25-33.
SOLLER, M. and BECKMANN, J.S. (1990) Theor. Appl. Genet. 80:205-208.
WEIGEL, K.A., FREEMAN, A.E., KEHRLI, M.E. Jr., STEAR, M.J. and KELLEY, D.H. (1990) J. Dairy Sci. 73: 2538-2546.
WELLER, J. E., KASHI, Y. and SOLLER, M. (1990) J. Dairy Sci. 73:2525-2537.
WELLER, J.I., and WYLER, A. (1992) Theor. Appl. Genet. 83:582-588.