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Structural variation around prolactin gene linked to quantitative traits in an elite Holstein sire family

C. M. Cowan [1,*], M. R. Dentine [1], R. L. Ax [1] and L. A. Schuler [2]

[1] Department of Dairy Science, University of Wisconsin, Madison, WI 53706, USA
[2] Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706, USA

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Summary. Digestion of genomic DNA with the restriction endonuclease AvaII disclosed a probable insertion/deletion of approximately 200 base pairs (bp) near the prolactin gene. Two alleles were apparent as three distinct hybridization patterns. These alleles were statistically associated with quantitative trait loci among sons of one elite Holstein sire family. The favorable genotype was correlated with the presence of a 1.15-kb hybridization band inherited from the sire when genomic DNA was probed with a full-length cDNA for prolactin. Pedigree estimates of genetic merit among genotypes were similar, differing by only 19.3 kg for milk in ancestor merit. Comparisons of genetic estimates for quantitative yield traits in offspring of this heterozygous sire showed significant ($P<0.05$) differences between homozygous genotypes for predicted difference milk (PDM), predicted difference dollars (PD$), cheese yield dollars, and protein dollars. The estimated differences between homozygous genotypes for USDA Transmitting Abilities of PDM, PD$, Cheese Yield $ and Protein $ were 282.93 kg, $74.35, $48.58 and $53.67, respectively. However, the estimated breeding values from progeny ranged over 900 kg in transmitting ability for milk. Frequency of the favorable marker allele was estimated to be 0.231 in the elite cow population used as dams of sons. These results demonstrate the potential of molecular biological techniques to discriminate between individuals within a family and to predict breeding values for selection schemes.

Key words: Genetic marker – RFLP – Quantitative traits

Introduction

Linkages of genetic markers with quantitative trait loci (QTL) in breeding populations have been studied since the 1950s. Early studies dealt with blood group antigens and polymorphic criteria among body fluids. Although major loci affecting quantitative traits in farm animals have been found, linkage relationships among variants were rarely confirmed in subsequent studies. More recently, polymorphic markers in tomatoes (Tanksley et al. 1982), chromosomal markers for milk and blood protein variants in dairy cattle (Geldermann et al. 1985), marked chromosome sections affecting measured body characteristics in mice (Kluge and Geldermann 1982), and selection for immune responsiveness or disease resistance (Gavora and Spencer 1983) have been used to locate QTL.

Insertion/deletion or substitution events that alter the length of DNA fragments after restriction enzyme digestion have provided researchers with a large number of potential genetic markers to study linkage relationships with QTL. These restriction fragment length polymorphisms (RFLPs) may reflect differences in hormone products or simply provide a marker for another gene or group of genes on the same chromosome. RFLPs have been extensively used in human disease diagnosis and studies of modes of inheritance within families. As a result, a number of markers have been identified and applied in human linkage studies. The number of RFLPs linked to human diseases has increased tenfold between 1983 and 1987 (Watkins 1988) with an excess of 3000 markers being identified.

Utilization of marker genes within a breeding scheme requires linkage relationships of favorable chromosomal segments with detectable markers (Roberts and Smith 1982). The intentional crossing of lines homozygous at differing loci has been utilized to explore linkage relationships in the F_2 offspring (Beckmann and Soller 1988). Although this method is well-adapted to plants and laboratory animals, it is impractical in field populations such as dairy cattle.

* To whom correspondence should be addressed

Theoretical strategies to utilize major genes and marker-assisted selection in plants and animals have been extensively developed (Beckmann and Soller 1983; Smith and Simpson 1986; Stam 1986; Soller and Beckmann 1988). The potential value of marker-assisted selection depends on the proportion of genetic variance associated with the marker loci, gene frequencies in the population, and the costs of testing programs (Roberts and Smith 1982; Geldermann et al. 1985). Since conveyance of chromosomes must be traceable in offspring, application of this method to dairy cattle breeding schemes must employ unique markers in parents or be planned to utilize only that proportion of matings where transfer of the marker is unambiguous in progeny (Beckmann and Soller 1988). The overall effects of unknown genes on the same chromosome as the marker may be neutral for a quantitative trait if animals of several families are involved in the mating scheme, since alleles may be linked differently in the various families. Geldermann (1975) and Stam (1986) have suggested the use of markers within families of a heterozygous parent as a way to follow passage of favorable or unfavorable chromosome segments. In addition, offspring from a heterozygous sire for a chosen marker would not be influenced by linkage disequilibria in the population, but would measure the effects of allele transfers on quantitative traits arising from substitution effects of paternal homologous chromosomes (Geldermann 1975).

Genes associated with mammary growth, development, and function are excellent candidates for linkage relationships with QTL. Using cDNA clones for bovine growth hormone and prolactin, four and three RFLP patterns, respectively, were detected within familial lines of Holstein bulls (Cowan et al. 1989). Development of statistical methods utilizing mixed-model methodology to detect major gene effects (Famula 1986; Hoeschele 1988a, b) has provided estimators of major gene effects in the presence of polygenic variation and unknown major genotypes and hypothesis testing procedures for these data. In the present study, sperm genomic DNA was digested with the restriction endonuclease AvaII, and the hybridization patterns detected with a full-length bovine prolactin cDNA clone were examined for linkage relationships with milk production traits within an elite Holstein sire family.

Materials and methods

This study examined the structural variation around the prolactin gene of 26 sons from one elite Holstein sire. In addition to sharing the same sire, several bulls also had the same maternal grandsire. One grandsire was common in the pedigrees of ten bulls, another sire produced three grandsons, and the remaining three pairs were represented by three additional grandsires. Seven of the half sibs had no other common ancestry. Estimated breeding values were obtained from United States Department of Agriculture Sire Summary January 1989, and represented yield information on a total of 1,650 granddaughters. The average USDA Repeatability was 71.2% for all genotypes.

Genomic DNA was extracted from spermatozoa as described by Camper et al. (1984). Ten micrograms of genomic DNA was digested with AvaII in accordance with the manufacturer's (New England BioLabs, Beverly/MA) recommendations. DNA fragments were separated by electrophoresis in 0.8–1.0% agarose gels and then blotted onto nylon membranes using the procedure described by Southern (1975). Filters were prehybridized in 5 × SSPE (1 × SSPE – 180 m*M* NaCl, 5 m*M* sodium phosphate, pH 7.4, and 0.5 m*M* EDTA), 0.4% SDS, 50% deionized formamide, 5 × Denhardts (1 × = 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone), and denatured herring sperm (50 μg/ml) at 42 °C for 6 h.

Bovine prolactin (pBPRL72, Sasavage et al. 1982) cDNA radiolabeled by nick-translation (Rigby et al. 1977) was added with fresh hybridization solution, and the incubation continued for 36 h. Filters were washed twice with 2 × SSC (1 × SSC – 150 m*M* NaCl and 15 m*M* Na citrate, pH 7.0) at 65 °C for 15 min, followed by 2 × SSC and 0.1% SDS at 65 °C for 30 min, then once with 0.1 × SSC at 65 °C for 10 min. Filters were exposed to Kodak XAR-5 film with intensifying screens for 5 days at −80 °C.

In a preliminary study of 14 sons of one elite Holstein sire, DNA samples from each bull were digested with each of 11 restriction enzymes and hybridized with a cDNA clone for bovine prolactin. Digests from four restriction enzymes – AvaII, MspI, RsaI, and SacI – revealed variant hybridization patterns (Cowan et al. 1989). Digestion of DNA isolated from the sire of sons with each of the 11 restriction enzymes separately revealed an AvaII polymorphic pattern with two bands representing alternative alleles. Consequently, the structural variations around the prolactin gene within this sire family at these alleles were tested for linkage with QTL. Preliminary findings indicated a linkage relationship favoring those sons receiving one of the fragments from the sire. Subsequently, semen samples from additional sons were obtained and the DNA was examined. Included among the additional sons surveyed were those surviving progeny testing programs and thus this may have been a biased sample of all sons. This bias would create an underestimate of any gene substitution effects, since the sons with lowest genetic merit would have been eliminated.

Statistical analysis

The model proposed and tested included the genetic effects of major loci linked to marked chromosome fragments added to an underlying additive variance due to other genes (Dentine and Cowan 1990). The estimated variance within the homozygous classes showed a positive relationship between the mean and variance. Data were transformed using natural logarithms to stabilize the variance within the homozygous classes of offspring.

Estimates of D were obtained by using a specialized case of the general model for major loci presented by Hoeschele (1988a).

$$Y_{i(j)} = \mu + \lambda_j(D) + g_i + e_{i(j)},$$

where $Y_{i(j)}$ = yield trait based on first lactations of daughters of son i within RFLP genotype j (j = 1 to 3); μ = overall mean; λ_j = design matrix element representing the probability of a favorable linkage relationship; $\lambda = 1$ for sons characterized as RFLP genotype AA ($j = 1$); $\lambda = 0$ for sons characterized as RFLP genotype BB ($j = 2$); λ = maximum likelihood (ML) esti-

Table 1. Summary of economic traits for initial group of sons of one heterozygous sire

Genotype of sons	Arithmetic means					
	PDM (kg)	PD ($)	PDF (kg)	Cheese yield ($)	PDP (kg)	Protein ($)
AA[a]	572.12	124.00	14.54	108.66	15.00	115.67
AB	447.99	123.30	19.22	121.14	13.04	120.00
BB	246.59	64.70	9.64	62.75	7.16	63.25
Estimated gene substitution effect[b]	334.52*	63.14	6.32	51.98	8.52	57.04
t-value for gene substitution effect	2.49	2.01	0.96	1.48	1.77	1.76

[a] $n_{AA}=3$, $n_{AB}=7$, $n_{BB}=4$
[b] Difference between *AA* and *BB* genotypes
* Significant marker effect at $P \leq 0.05$

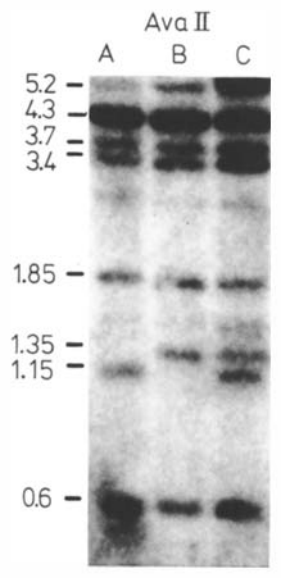

Fig. 1. Ten micrograms of genomic DNA isolated from spermatozoa was digested with *Ava*II, transferred to filters, and hybridized with a full-length cDNA for bovine prolactin. *Lane A* illustrates the *AA* genotypes (1.15-kb band only), *lane B* the *BB* genotypes (1.35-kb band only), and *lane C* the *AB* genotype (both 1.15- and 1.35-kb band)

mate gene frequency of the *B* allele, $q(B)$, in the cow population for sons characterized as RFLP AB ($j=3$). It can be shown that the ML estimator of $q(B)$ = number of *BB* sons in a random sample divided by the sum of the number of *AA* and *BB* sons (Dentine and Cowan 1990). D = sum of gene substitution effects of the favorably linked genes deviated from the less favorable linkage group; g_i = quantitative breeding value (random effect) including all unlinked loci; $e_{i(j)}$ = unexplained random residual elements.

Because the residuals of the observations were neither identical nor independently distributed, generalized least-squares was used instead of an ordinary least-squares. A variance-covariance matrix of the observations (V) was constructed using three factors:

(1) The within-grandsire family residuals were transformed to be identically and independently distributed for homozygous individuals that were otherwise unrelated. Logarithmic transformations were done to stabilize the variance with a constant added to the raw data if necessary to achieve equal variance within the homozygous subclasses. After transformation, the estimated variance in one homozygous class was no more than 1.1 times the estimated variance of the alternate homozygote. Differences in accuracy of the progeny information were accounted for by using the USDA Repeatability (Wiggans et al. 1984) of the published proofs to adjust the diagonals of V.

(2) The correlations between observations within RFLP genotype due to all known additive genetic relationships were incorporated using the usual relationship matrix (Henderson 1976) assuming heritability (h^2) equal to 0.25. This was the assumed heritability after removal of variation due to marked chromosome effects and thus was reduced from 0.30 for all additive genetic effects.

(3) The variance of the heterozygote observations around the weighted heterozygote mean was increased by an amount $q(B)[1-q(B)]D^2$ (Dentine and Cowan 1990).

Since the estimate of D depended on the variance-covariance matrix which included a function of D, an iterative solution was necessary. No more than five iterations were required to achieve convergence based on no more than 0.1% change in $p\,q\,D^2/\sigma_w^2$ in subsequent rounds. The favorable linkage relationship with the RFLP genotypes was tested, using the generalized least-squares *t*-test of the estimated parameter (D) for the difference in genetic value for the RFLP gene substitution.

Results

DNA digested with AvaII and hybridized to a full-length cDNA clone for bovine prolactin produced three hybridization patterns. As shown in Fig. 1, the AvaII polymorphism produced hybridization bands of approximately 1.15 and 1.35 kilobases (kb) in length. Of the 14 preliminary sons tested, 3 demonstrated the pattern found in lane A, 4 revealed the pattern seen in lane B, and 7 showed the hybridizing bands of lane C. Subsequently, 12 additional sons were added to the data set resulting in 6 new individuals showing patterns similar to lane B and 6 illustrating those of lane C. DNA from the sire of sons was subsequently analyzed and found to exhibit the pattern shown in lane C. The intensities of the bands at 1.15 kb or 1.35 kb (lane A or lane B, respectively) are

Table 2. Summary of economic traits for all sons tested of one heterozygous sire

Genotype of sons	Arithmetic means						
	PDM (kg)	PD ($)	PDF (kg)	Cheese yield ($)	PDP (kg)	Protein ($)	Average RPT (%)
AA[a]	572.00	124.0	14.00	108.70	15.00	115.66	69.3
AB	428.00	124.2	19.00	120.80	13.00	120.38	71.7
BB	236.00	67.9	12.00	77.90	8.00	74.50	72.6
Estimated difference[b]	282.93*	74.35**	7.12	48.58*	6.45	53.67*	
R^2[c]	0.227	0.318	0.140	0.168	0.148	0.207	
t-value for gene substitution effect	2.652	3.345	1.979	2.204	2.039	2.504	

[a] $n_{AA}=3$, $n_{AB}=13$, $n_{BB}=10$
[b] Difference between *AA* and *BB* genotypes when the heterozygous genotype *AB* is included in model
[c] Coefficient of determination for model including the marker information above that explained by the mean
* Significant marker effect at $P \leq 0.05$
** Significant marker effect at $P \leq 0.01$

consistent with patterns representing homozygotes (coded *AA* and *BB*), whereas lane C is representative of heterozygotes (*AB*).

If this genetic model were correct, the frequency of offspring of each genotype from a heterozygous parent would be expected to be $0.5\,p$ (*AA*), 0.5 (*AB*) and $0.5\,q$ (*BB*), where p is the frequency of *A* in the population of mates. The frequencies of the observed patterns were consistent with a dam population frequency of $p=0.231\pm0.058$, with exactly 50% of the sons exhibiting the heterozygote pattern as expected. The RFLP genotypes for all tested sons of this sire were found to have genotypic frequencies consistent with single-locus Mendelian segregation when tested using χ^2 statistics. Seven grandsons of one heterozygous son were also distributed in genotypes consistent with the proposed genetic model ($\chi^2_{df=1}=0.143$, $P \geq 0.05$). The pedigree estimates of transmitting abilities, USDA ancester merit (Wiggans et al. 1984), were nearly equal for all genotypic classes. Pedigree estimates for milk for those individuals coded as *AA*, *AB*, or *BB* were 360.3 ± 72, 379.6 ± 35.9, and 363.9 ± 55 kg (means $\pm$ SD), respectively.

USDA estimates of transmitting abilities based on yield data from over 700 granddaughters of the initial 14 sons were significantly linked to quantitative loci (Table 1). The difference in the two marker alleles was estimated to be 338 kg mature-equivalent milk yield in the transmitting ability of the sons ($p \leq 0.05$), depending on which chromosome segment was inherited from the heterozygous sire. The other milk yield traits were not significantly different ($p \geq 0.05$) but favored the *AA* genotype.

Table 2 shows the relationship with RFLP genotype for predicted differences for milk, dollars, fat, protein, and cheese and protein dollars using the generalized least-squares test for the estimated parameter D for the RFLP gene substitution when additional sons were added. The difference in the two marker alleles was estimated to be 283 kg mature-equivalent milk yield, 74.35 dollars, 53.67 and 48.58 for protein and cheese dollars, respectively, in the transmitting ability of sons ($p<0.05$), depending on which chromosome segment was inherited from the heterozygous sire.

Discussion

In this study, the sire of sons was determined to be a heterozygote for the prolactin AvaII polymorphism. Apparently, the 1.15-kb AvaII fragment is linked to a favorable set of genes for milk yield traits and, as such, is the preferred allele within this family. The larger 1.35-kb AvaII hybridization fragment was found to be the less favorable marker allele within this family. This polymorphism segregates in the population, and linkage relationships within other families could vary. Ten sons of two other sire families were examined and did not show the patterns of AvaII polymorphism found here.

The extent of linkage and the number of loci involved cannot be determined from these data. Genetic markers that are closely linked to major gene effects are less likely to disassociate during recombination. As more markers become available, localization of major gene effects would be possible. In addition to being useful for selection schemes, identification of single alleles linked to differences in performance could yield insight into the biochemical control of lactation.

Breeding values used in animal selection schemes are theoretically the sum of many genes, each having only a small effect. However, the existence of "major gene" effects cannot be excluded (Roberts and Smith 1982). Discovery of major gene-marker allele linkage relation-

ships within families would supplement current selection programs by increasing the accuracy of genetic merit estimates (Smith and Simpson 1986; Stam 1986). A statistical approach for discriminating between purely polygenic and mixed major gene and polygenic inheritance among animals with known or unknown qualitative genotype has been proposed (Hoeschele 1988 b). Modifications of that approach for marker gene linkages have been used in this study and have detected significant chromosomal substitution effects within an elite family. Genetic markers due to modification of the length of restriction fragments linked to traits of interest could further characterize an individual's true breeding value and differentiate among individuals with similar pedigrees.

In this study, the ancestor merits among genotypes within the sire family were very similar, differing by only 19.3 kg, but the estimated breeding values based on progeny ranged over 900 kg in transmitting ability for milk. The marker model accounted for a substantial discrimination among individuals within a family ($R^2 = 0.318$ for PD$) of otherwise equal pedigree merit. Since RFLP genotype could have been determined prior to progeny testing, selection against the *BB* genotype could have lowered the cost of progeny testing for sons of this bull.

Genes contributing very large effects may already be fixed in elite populations, although variability might be detectable between breeds selected for different production traits. Salmon et al. (1988) have reported that four RFLPs, detected when employing a cDNA probe for mouse growth hormone, became fixed in mice under selection for high body weight. Three of the polymorphisms appeared to be in the 5′ flanking region and one within the gene itself. These structural variations were fixed after 69 generations of selection, but evidence would suggest that these genes were segregating at an approximate gene frequency of 0.75 in the original population. Early biochemical identification of these major loci and use of this information in the breeding program could have accelerated the fixation, although normal quantitative selection methods eventually produced the same results.

To be of use in domestic animal selection, linkage of marker genes must be naturally occurring in the elite populations from which the parents would normally be selected. The size of the major loci effects would not be sufficient to replace the selection intensity possible from quantitative selection methods but should be used as a supplement to the usual pedigree selection (Smith and Simpson 1986). Genes of moderate effect within breed could be useful for selection if detectable (Hoeschele 1988 b). In our data, the frequency of the favorable allele was estimated to be 0.23 in the elite cow population used as dams of sons. Given this frequency and assuming a Hardy-Weinberg equilibrium in the population of mates, 59% of all sons would be born to *BB* dams where this method could be used to distinguish sons as heterozygotes with higher mean genetic value. Because the frequency of the marker allele is relatively low, several generations of selection could occur before the favorable marker allele would become fixed in the population.

The magnitude of the gene substitution effect on milk yield and dollar values observed in this study is on the order of one genetic standard deviation. Comparison of the Holstein Association's highest Type Production Index (TPI) for January 1989 supports the utility of a major gene effect in this sire family, which contributed 31 of the top 100 bulls listed. These data may have underestimated the differences between homozygous groups because several of the animals added to the initial data set had already been selected based on progeny test data.

In this study, identification of genetically superior sons was detected through progeny testing. Progeny testing is a lengthy and capital-intensive program, which typically returns only one in seven or one in eight young sires examined for use in the general dairy population. Marker-assisted selection of those individuals that had received favorable alleles could effectively pre-screen the population of test animals. In sets of offspring resulting from superovulation and embryo transfer, this procedure would provide the only genetic tool for differentiating between animals of identical pedigree merit prior to progeny testing. This would facilitate the selection process and reduce the number of animals tested to obtain an equivalent number of proven sires.

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References

Beckmann JS, Soller M (1983) Restriction fragment length polymorphisms in genetic improvement: methodologies, mapping and costs. Theor Appl Genet 67:35–43

Beckmann JS, Soller M (1988) Detection of linkage between marker loci and loci affecting quantitative traits in crosses between segregating populations. Theor Appl Genet 76:228–236

Camper SA, Luck DN, Yao Y, Woychik RP, Goodwin RG, Lyons RH, Rottman FM (1984) Characterization of the bovine prolactin gene. DNA 3:237–249

Cowan CM, Dentine MR, Ax RL, Schuler LA (1989) Restriction fragment length polymorphisms associated with growth hormone and prolactin genes in Holstein bulls: evidence for a novel growth hormone allele. Anim Genet 20:157–165

Dentine MR, Cowan CM (1990) An analytical model for the estimation of chromosome substitution effects in the offspring of individuals heterozygous at a segregating marker locus. Theor Appl Genet (in press)

Famula TR (1986) Identifying single genes of large effect in quantitative traits using best linear unbiased prediction. J Anim Sci 63:68–76

Gavora JS, Spencer JL (1983) Breeding for immune responsiveness and disease resistance. Anim Blood Groups Biochem Genet 14:159–180

Geldermann H (1975) Investigations on inheritance of quantitative characters in animals by gene markers. 1. Methods. Theor Appl Genet 46:319–330

Geldermann H, Pieper U, Roth B (1985) Effects of marked chromosome sections on milk performance in cattle. Theor Appl Genet 70:138–146

Henderson CR (1976) A simple method for computing the inverse of a numerator relationship matrix used in prediction of breeding values. Biometrics 32:69–84

Hoeschele I (1988a) Genetic evaluation with data presenting evidence of mixed major gene and polygenic inheritance. Theor Appl Genet 76:81–92

Hoeschele I (1988b) Statistical techniques for detection of major genes in animal breeding data. Theor Appl Genet 76:311–319

Kluge R, Geldermann H (1982) Effects of marked chromosome sections on quantitative traits in the mouse. Theor Appl Genet 62:1–4

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977) Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Roberts RC, Smith C (1982) Genes with large effects – theoretical aspects in livestock breeding. In: Proc 2nd World Congr Genet Appl Livestock Prod, vol VI. Publicaciones Agrarias, Madrid, pp 420–438

Salmon RK, Berg RT, Yeh FC, Hodgetts RB (1988) Identification of a variant growth hormone haplotype in mice selected for high body weight. Genet Res Camb 52:7–15

Sasavage NL, Nilson JH, Horowitz S, Rottman FM (1982) Nucleotide sequence of bovine prolactin messenger RNA. Evidence for sequence polymorphism. J Biol Chem 257:678–681

Smith C, Simpson SP (1986) The use of genetic polymorphisms in livestock improvement. J Anim Breed Genet 103:205–217

Soller M, Beckmann JS (1988) Genomic genetics and the utilization for breeding purposes of genetic variation between populations. In: Proc 2nd Int Conf Quant Genet. Sinauer Associates, Sunderland/MA, pp 161–188

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Stam P (1986) The use of marker loci in selection for quantitative characters. In: Smith C, King JWB, McKay JC (eds) Exploiting new technologies in animal breeding. Genetic developments. Oxford University Press, pp 170–182

Tanksley SD, Medina-Filho H, Rick CM (1982) Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25

Watkins PC (1988) Restriction fragment length polymorphism (RFLP): applications in human chromosome mapping and genetic disease research. Biotechniques 6:310–323

Wiggans GR, Norman HD, Powell RL (1984) Changes in genetic evaluation procedures for January 1984. Dairy Herd Improv Lett 60:1–10