Institut für Tierzucht und Haustiergenetik, Justus-Liebig-Universität Gießen, Germany\(^1\), Roslin Institute Edinburgh, Scotland\(^2\), Institute of Zootechnics, Catholic University of S. Cuore Piacenza, Italy\(^3\), Lab. Genetica Bioquimica y Grupos Sanguineos Zaragoza, Spain\(^4\), Department of Zootechnics University Ankara, Turkey\(^5\)

OLIVER JANN\(^1\), EVA-MARIA PRINZENBERG\(^1\), HORST BRANDT\(^1\), JOHN L. WILLIAMS\(^2\), PAOLO AJMONE-MARSAN\(^3\), PILAR ZARAGOZA\(^4\), CEYHAN ÖZBEYAZ\(^5\) and GEORG ERHARDT\(^1\)

**Intragenic haplotypes at the bovine CSN1S1 locus**

* Dedicated to Prof. Drs. h. c. Franz Pirchner PhD, on the occasion of his 75th birthday

**Summary**

A new alternative genotyping method based on PCR-SSCP was developed for direct differentiation of the CSN1S1 alleles B and C in the coding region. In addition a PCR-RFLP test based on a *Mae*III restriction site in the promoter region of CSN1S1, reported in the literature as an alternative test for the differentiation of CSN1S1*\^B* and C was used and the alleles named b and c. Genotyping of 649 animals belonging to 17 European and Turkish cattle breeds showed differences in occurrence and frequency of the alleles. CSN1S1*\^B* occurred in all breeds with frequencies varying from 0.50 in Anatolian Blackup to 1.0 in e.g. Ayrshire. CSN1S1*b* on the other hand varied from 0.63 in Jersey, 0.97 in Ayrshire to 1.0 in e.g. Angler.

Comparison of the results from both typing methods and positions in the gene showed that both mutations do not always occur together. From the resulting four intragenic haplotypes (B-b, B-c, C-c and C-b) B-b is predominant in all breeds with frequencies varying from 0.50 in Anatolian Black to 1.0 in Angler and Scottish Highland. The number of haplotypes varied from only one in Angler and Scottish Highland, two in Ayrshire, three in Asturian Valley and Turkish Grey Steppe to all four in the other 12 breeds. Correlation between allele frequencies and the geographic origin of the breeds was significant for the *Mae*III promoter polymorphism.

**Key Words:** casein, haplotype, SSCP, cattle
Introduction
Variants of different milk protein genes in cattle are discussed in the context of studies regarding quantitative and qualitative traits and are used within evolutionary and diversity studies. For αs1-casein (CSN1S1) 9 alleles (A, B, C, D, EYak, EBali, F, G, H) have been described within different cattle breeds, with CSN1S1*B being the predominant allele in Bos taurus and CSN1S1*C in Bos indicus and Bos grunniens breeds (Eigel et al., 1984; Formaggioni et al., 1999). The other alleles are rare in other breeds that have been studied. It has been postulated that CSN1S1*B has positive effects on milk yield (Lin et al., 1986), whereas higher milk protein content is found in animal heterozygous for CSN1S1*BC compared to BB homozygous animals (Ng-Kwai-Hang et al., 1990; Aalendri et al., 1990, Boovenhuis et al., 1992). In Nordic cattle breeds Liën et al. (1999) identified an allele frequency gradient with low frequency of CSN1S1*B in native breeds to high frequency of CSN1S1*B and loss of CSN1S1*C in high selected dairy cattle. This is in agreement with the reported very high frequencies of CSN1S1*B in breeds selected for milk production, up to fixation or nearly-fixation in Ayrshire, Angler, or Holstein Friesian (Erhardt, 1993; Ikonen et al., 1996).

Development of several DNA tests for genotyping milk protein genes offered the possibility to type animals independent from age, sex, and lactation (Levéziel et al., 1988). Schleep & Rottmann (1992) and David & Deutch (1992) developed allele specific PCR tests (ASPCR) for differentiation of CSN1S1*B and C, while Liën et al. (1993) used an amplification created restriction site (ACRS) for HphI to discriminate between CSN1S1*B and CSN1S1*C. The latter test detects the causal nucleotide substitution inside exon 17 of the gene. Koczán et al. (1993) described a polymorphism in the promoter of the CSN1S1 gene affecting a MaeIII restriction site. The resulting PCR-RFLP test was suggested as an alternative to the ASPCR test for the differentiation of CSN1S1*B and C. However, Turecková et al. (2001) recently reported that in Czech and German Red Cattle the MaeIII promoter polymorphism is not always linked to the HphI ACRS in exon 17.

Single strand conformation polymorphism (SSCP) analysis is a rapid and sensitive screening technique, that allows mutation identification without restriction enzyme digests or special primers (Orita et al., 1989). PCR-SSCP analysis for milk protein genotyping identified new alleles in both - endangered and production breeds – which were subsequently characterized by DNA sequencing (Prinzenberg et al., 1999; Caroli et al., 2001).

The aim of this study was to determine the occurrence of the two CSN1S1 polymorphisms described in different cattle breeds and to define intragenic haplotypes as genetic markers using a PCR-SSCP based DNA test for typing the causal nucleotide substitution in exon 17.

Materials and Methods
Sample collection: Blood samples of 649 animals belonging to 17 cattle breeds (Anatolian Black n=13, Jersey n=43, Chianina n=32, Casta Navarra n=34, Turkish Grey Steppe n=17, Maremmana n=39, Aberdeen Angus n=36, Piemontese n=42, Fighting Bull n=36, Asturian Valley n=42, Hereford n=46, Pezzata Rossa Italiana n=47, Charolais n=51, British Friesian n=38, Ayrshire n=48, Angler n=46, Scottish Highland n=39) were collected and DNA was extracted by the method of Montgomery and Sise (1990).
DNA test (PCR-RFLP) for CSN1S1 promoter polymorphism: A 310 bp fragment containing the first 274 bp of the promoter and parts of exon 1 was amplified, digested with MaeIII and separated on an agarose gel as described by KOCZAN et al. (1993). Uncut fragments of the 310 bp fragment (indicating G in nucleotide position 1957) were named \( c \), fragments cut by MaeIII (indicating A in nucleotide position 1957) were named \( b \).

PCR-SSCP test for CSN1S1*B and C: For differentiation of the CSN1S1 alleles \( B \) and \( C \) a SSCP-based DNA test was developed using 58 DNA samples from animals with known CSN1S1 genotypes as standard samples. Genomic DNA was amplified by PCR to give a 223 bp-fragment containing exon 17 of the CSN1S1 gene (Position 17644-17867) of GenBank Acc. No. 59856). PCR was in a final volume of 25 \( \mu l \), containing 100 ng genomic DNA, 15 pmol of each primer (\( \text{CSN1S1-5: 5\` CAC TGT TGC TTT TTC AAT GGT C 3\`} \), \( \text{CSN1S1-3: 5\` AAG GCA ACA ATA TGC AGT CAT TT 3\`} \)), 1 U Taq-polymerase (Peqlab Biotechnologie GmbH, Erlangen), 200 \( \mu M \) dNTP, 1.5 mM MgCl\(_2\), 10 mM Tris-HCl pH 8.8, 50 mM KCl with an initial denaturation step at 94°C for 5 min, followed by 30 cycles with a denaturation at 94°C for 1 min, annealing of 56°C for 1 min, elongation of 72°C for 1 min and a final elongation of 72°C for 5 min. Four microlitres of the PCR product were mixed with 6 \( \mu l \) formamide buffer (95% formamide, 0.025% bromphenolblue, 0.025% xylene cyanol FF, 20 mM EDTA), denatured at 91°C for 3 min and immediately chilled on ice. Samples were run 3 h at 200 V at 10°C on a 10% acrylamide:bisacrylamide gel (37:1) with 2% glycerol. DNA fragments were visualised by silver staining (BASSAM et al., 1991).

Haplotype definition: Haplotypes were determined based on the results of PCR-RFLP analyses determining nucleotide position (nt) 1957 (\( b = \text{nt1957: A, c=nt1957: G} \)) and exon 17 PCR-SSCP discriminating alleles \( B \) and \( C \) (Table 1).

<table>
<thead>
<tr>
<th>Sequence position</th>
<th>Haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide position 17807</td>
<td>CSN1S1*B-b</td>
</tr>
<tr>
<td>GAA</td>
<td>GAA</td>
</tr>
<tr>
<td>Amino acid position 192</td>
<td>Glu</td>
</tr>
<tr>
<td>Nucleotide position 1957</td>
<td>A</td>
</tr>
</tbody>
</table>

Data analysis: Allele frequencies, observed and expected genotype frequencies, and deviations from Hardy-Weinberg equilibrium were evaluated by GENEPOP Software (RAYMOND and ROUSSET, 1995). Expected and observed haplotype frequencies were calculated and compared (\( \chi^2 \)-values) with EH software (XIE and OTT, 1993). Correlations between degree of latitude and allele frequencies and linear regressions of allele frequencies on geographic latitude were calculated.

Results

PCR-SSCP analysis for CSN1S1 exon 17 showed two distinct fragment patterns for alleles \( B \) and \( C \) which was in agreement for all 58 DNA samples of known genotypes (Figure 1).
Fig. 1: PCR-SSCP based DNA test for CSN1S1*B and CSN1S1*C. Position of corresponding bands are marked by dots (PCR-SSCP DNA-Test für CSN1S1*B und CSN1S1*C. Die Lage der entsprechenden Banden ist durch Punkte gekennzeichnet)

Genotype frequencies

The observed and expected genotype frequencies and the probability-values for Hardy-Weinberg equilibrium are shown in Table 2. All populations were in Hardy-Weinberg equilibrium at CSN1S1 locus for position 17807 and position 1957 except Pezzata Rossa and Piemontese for the first locus, where an excess of homozygous genotypes was observed. All of the expected six genotypes at the CSN1S1 locus were observed in eight of the 17 breeds analysed while in Scottish Highland and Angler only two genotypes were found.

Table 2

| Breed         | CSN1S1 Position 17807 |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|---------------|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|               | BB        | BC      | CC      | p   | BB        | BC      | CC      | p   | BB        | BC      | CC      | p   | BB        | BC      | CC      | p   | BB        | BC      | CC      | p   |
| Aber. Angus   | 24        | 23.2    | 10      | 11.4  | 2       | 1.3     | 0.59   |     | 21        | 20.9    | 13      | 13.2  | 2     | 1.9     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Anat. Black  | 2         | 3.1     | 9       | 6.8   | 2       | 3.1     | 0.30   |     | 6         | 6.1     | 6       | 5.8   | 1     | 1.1     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Angler       | 46        | 46.0    | 0       | 0     | 0       | 0.0     | -      |     | 46        | 46.0    | 0       | 0.0   | 0     | 0      | 0.0     |     |     |     |     |     |     |     |     |     |     |
| Astur. Valley| 23        | 25.1    | 19      | 14.9  | 0       | 2.1     | 0.09   |     | 35        | 35.3    | 7       | 6.5   | 0     | 0.3     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Ayrshire     | 48        | 48.0    | 0       | 0     | 0       | 0.0     | -      |     | 45        | 45.0    | 3       | 2.9   | 0     | 0.0     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Brit. Friesian| 35       | 35.0    | 3       | 2.9   | 0       | 0.0     | 1.00   |     | 35        | 35.0    | 3       | 2.9   | 0     | 0.0     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Cas. Navarra | 24        | 23.8    | 9       | 9.4   | 1       | 0.8     | 1.00   |     | 14        | 15.4    | 18      | 15.1  | 2     | 3.5     | 0.43    |     |     |     |     |     |     |     |     |     |     |
| Charolais    | 40        | 40.4    | 11      | 9.9   | 0       | 0.6     | 1.00   |     | 42        | 42.4    | 9       | 8.3   | 0     | 0.4     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Chianina     | 22        | 21.8    | 9       | 9.3   | 1       | 0.9     | 1.00   |     | 18        | 17.2    | 11      | 12.7  | 3     | 2.2     | 0.65    |     |     |     |     |     |     |     |     |     |     |
| Fighting Bull| 31        | 30.2    | 4       | 5.6   | 1       | 0.2     | 0.20   |     | 25        | 24.1    | 9       | 10.8  | 2     | 1.1     | 0.30    |     |     |     |     |     |     |     |     |     |     |
| Hereford     | 34        | 33.0    | 10      | 12.0  | 2       | 1.0     | 0.26   |     | 42        | 42.1    | 4       | 3.9   | 0     | 0.1     | 0.10    |     |     |     |     |     |     |     |     |     |     |
| Jersey       | 15        | 16.8    | 24      | 20.3  | 4       | 5.8     | 0.33   |     | 15        | 16.8    | 24      | 20.3  | 4     | 5.8     | 0.38    |     |     |     |     |     |     |     |     |     |     |
| Maremmana    | 25        | 25.4    | 13      | 12.3  | 1       | 1.4     | 1.00   |     | 25        | 25.5    | 13      | 12.3  | 1     | 1.4     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Pezz. Rossa  | 40        | 38.4    | 5       | 8.2   | 2       | 0.4     | 0.04   |     | 42        | 42.1    | 5       | 4.8   | 0     | 0.1     | 1.00    |     |     |     |     |     |     |     |     |     |     |
| Piemontese   | 32        | 29.9    | 7       | 11.1  | 3       | 0.9     | 0.04   |     | 28        | 26.6    | 11      | 13.7  | 3     | 1.6     | 0.33    |     |     |     |     |     |     |     |     |     |     |
| Sc. Highland | 39        | 39.0    | 0       | 0     | 0       | 0.0     | -      |     | 39        | 39.0    | 0       | 0.0   | 0     | 0.0     | -       |     |     |     |     |     |     |     |     |     |     |
| T. Grey Steppe| 7         | 7.6     | 9       | 7.7   | 1       | 1.7     | 0.61   |     | 13        | 13.2    | 4       | 3.6   | 0     | 0.2     | 1.00    |     |     |     |     |     |     |     |     |     |     |
Allele and haplotype frequencies

Table 3 gives the allele and haplotype frequencies at the CSN1S1 locus and shows differences both in the occurrence and the frequencies of the alleles and the haplotypes in the cattle breeds studied. CSN1S1*B occurred in all breeds with frequencies varying from 0.50 in Anatolian Black to 1.0 in Ayrshire, Scottish Highland, and Angler. CSN1S1*b on the other hand varied from 0.63 in Jersey, 0.97 in Ayrshire to 1.0 in Angler and Scottish Highland.

In most breeds frequencies for CSN1S1*b are higher than for CSN1S1*B. In contrast, in Aberdeen Angus, Ayrshire, Casta Navarra, Chianina, Piemontese, and Turkish Grey Steppe CSN1S1*b occurs in lower frequencies than B.

From the four intragenic haplotypes (B-b, B-c, C-c and C-b) B-b is predominant in all breeds with frequencies varying from 0.3450 in Anatolian Black to 1.0 in Angler and Scottish Highland. Beside these two breeds, where only one haplotype occurs, in Ayrshire two haplotypes (B-b and B-c), and in Asturian Valley and Turkish Grey Steppe breed three haplotypes (B-b, C-c and C-b) are present, while four haplotypes occur in the other breeds. A χ²-test comparing expected and observed haplotype frequencies of position 17807 and position 1957 shows highly significant disequilibrium (p<0.01) in Aberdeen Angus, Asturian Valley, Charolais, Jersey and Piemontese and a significant disequilibrium (p<0.05) in British Friesian.

Table 3
Allele frequencies, expected, and observed haplotype frequencies of CSN1S1 in European cattle populations and corresponding χ²-value (Allelfrequenzen, erwartete und beobachtete Haplotype frequenz von CSN1S1 in europäischen Rinderpopulationen und entsprechende χ²-Werte)

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>allele frequencies</th>
<th>haplotype frequencies</th>
<th>observed</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>C</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>Aber. Angus</td>
<td>36</td>
<td>0.81  0.19  0.76  0.24</td>
<td>0.6156  0.1944  0.0456  0.1444</td>
<td>0.7342  0.0714  0.1648  0.0297</td>
<td>22.33</td>
</tr>
<tr>
<td>Anat. Black</td>
<td>13</td>
<td>0.50  0.50  0.69  0.31</td>
<td>0.3450  0.1550  0.1550  0.3450</td>
<td>0.3450  0.1550  0.1550  0.3450</td>
<td>0.00</td>
</tr>
<tr>
<td>Angler</td>
<td>46</td>
<td>1.00  0.00  1.00  0.00</td>
<td>1.0000  0.0000  0.0000  0.0000</td>
<td>1.0000  0.0000  0.0000  0.0000</td>
<td>-</td>
</tr>
<tr>
<td>Astur. Valley</td>
<td>42</td>
<td>0.77  0.23  0.92  0.08</td>
<td>0.7084  0.0616  0.0184  0.2116</td>
<td>0.7857  0.1429  0.0714  0.0000</td>
<td>12.00</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>48</td>
<td>1.00  0.00  0.97  0.03</td>
<td>0.9700  0.0300  0.0000  0.0000</td>
<td>0.9688  0.0313  0.0000  0.0000</td>
<td>-</td>
</tr>
<tr>
<td>Brit. Friesian</td>
<td>38</td>
<td>0.96  0.04  0.96  0.04</td>
<td>0.9216  0.0384  0.0016  0.0384</td>
<td>0.9472  0.0133  0.0261  0.0133</td>
<td>8.14</td>
</tr>
<tr>
<td>Cas. Navarra</td>
<td>34</td>
<td>0.84  0.16  0.68  0.33</td>
<td>0.5712  0.2772  0.0528  0.1088</td>
<td>0.6397  0.2132  0.1101  0.0368</td>
<td>5.45</td>
</tr>
<tr>
<td>Charolais</td>
<td>51</td>
<td>0.89  0.11  0.91  0.09</td>
<td>0.8099  0.0801  0.0099  0.1001</td>
<td>0.8820  0.0101  0.0781  0.0298</td>
<td>25.96</td>
</tr>
<tr>
<td>Chianina</td>
<td>32</td>
<td>0.83  0.17  0.73  0.27</td>
<td>0.6059  0.2241  0.0459  0.1241</td>
<td>0.5781  0.2969  0.0000  0.1250</td>
<td>4.72</td>
</tr>
<tr>
<td>Fighting Bull</td>
<td>36</td>
<td>0.92  0.08  0.82  0.18</td>
<td>0.7544  0.1656  0.0144  0.0656</td>
<td>0.7723  0.1443  0.0362  0.0471</td>
<td>1.52</td>
</tr>
<tr>
<td>Hereford</td>
<td>46</td>
<td>0.85  0.15  0.96  0.04</td>
<td>0.8160  0.0340  0.0060  0.1440</td>
<td>0.8079  0.0399  0.0036  0.1486</td>
<td>0.06</td>
</tr>
<tr>
<td>Jersey</td>
<td>43</td>
<td>0.63  0.37  0.63  0.37</td>
<td>0.3969  0.2331  0.1369  0.2331</td>
<td>0.5232  0.1513  0.2674  0.0582</td>
<td>12.97</td>
</tr>
<tr>
<td>Maremmana</td>
<td>39</td>
<td>0.83  0.17  0.81  0.19</td>
<td>0.6723  0.1577  0.0323  0.1377</td>
<td>0.7136  0.1085  0.0966  0.0829</td>
<td>7.16</td>
</tr>
<tr>
<td>Pezz. Rossa</td>
<td>47</td>
<td>0.90  0.10  0.95  0.05</td>
<td>0.8550  0.0450  0.0050  0.0950</td>
<td>0.8813  0.0230  0.0302  0.0655</td>
<td>4.81</td>
</tr>
<tr>
<td>Piemontese</td>
<td>42</td>
<td>0.85  0.15  0.80  0.20</td>
<td>0.6800  0.1700  0.0300  0.1200</td>
<td>0.7472  0.0981  0.1043  0.0505</td>
<td>13.50</td>
</tr>
<tr>
<td>Sc. Highland</td>
<td>39</td>
<td>1.00  0.00  1.00  0.00</td>
<td>1.0000  0.0000  0.0000  0.0000</td>
<td>1.0000  0.0000  0.0000  0.0000</td>
<td>-</td>
</tr>
<tr>
<td>T. Grey Steppe</td>
<td>17</td>
<td>0.88  0.12  0.68  0.32</td>
<td>0.5984  0.2816  0.0384  0.0816</td>
<td>0.6765  0.0000  0.1176  0.2059</td>
<td>6.05</td>
</tr>
</tbody>
</table>

(χ²-limit for 1% significance level is 11.34 and for 5% level is 7.81. In breeds marked with “–” no χ²-analysis has been done due to fixation of one or two of the loci.)

Correlations and regression analyses with geographic data

Frequencies for CSN1S1*B and b could be shown to be increasing from southern sampling area (Turkey) to northern Europe (Scotland). Correlation of geographic
latitude in the range of the sampling area (37° - 58°N) with $CSN1S1^B$ allele frequencies was $r=0.417$ (n.s.), and $r=0.556$ ($p<0.05$) for $CSN1S1^b$.

Regression analysis resulted in a regression equation of $y=0.00843*x+0.45308$ for $CSN1S1^B$ and of $y=0.01103*x+0.31914$ for $CSN1S1^b$ allele frequencies and geographic latitude (Figure 2).

![Regression analysis of $CSN1S1^B$ and $CSN1S1^b$ allele frequencies and geographic latitude](image)

Fig. 2: Regression analysis of $CSN1S1^B$ ($y=0.00843*x+0.45308$) and $b$ ($y=0.01103*x+0.31914$) allele frequencies and geographic latitude (Regressionanalyse von $CSN1S1^B$ ($y=0.00843*x+0.45308$) und $b$ ($y=0.01103*x+0.31914$) Allelfrequenzen und geographischer Breite)

**Discussion**

The PCR-SSCP test developed for the differentiation of $CSN1S1^B$ and $C$ offers a rapid and cost-effective alternative to the ASPCR described by SCHLEEE and ROTTMANN (1992) and DAVID and DEUTCH (1992) and the ACRS method described by LIEN et al. (1993). Our genotyping results show, in agreement with TURECKOVÁ et al. (2001), that the promoter polymorphism described by KOCZAN et al. (1993) cannot be used as a reliable genotyping method to infer $CSN1S1^B$ and $C$-genotypes in all breeds. The small number of animals analysed by KOCZAN et al. (1993) belonged to Jersey, Holstein Friesian and German Simmental.

Aberdeen Angus, Ayrshire, British Friesian, Charolais, Turkish Grey Steppe, Pezzata Rossa, and Piemontese show low frequencies for $B$-$c$ and $C$-$b$ haplotypes, while frequencies of same haplotypes are high in Chianina and Anatolian Black.

Our results support the occurrence of different intragenic haplotypes in milk protein genes, that might contribute to variation of different milk production traits (SCHILD and GELDERMANN 1996; EHRMANN et al., 1997).

Expected and observed genotype frequencies at $CSN1S1$ nt 17807 in Piemontese and Pezzata Rossa were not in Hardy-Weinberg equilibrium. Both breeds showed much lower frequencies of heterozygous animals than expected from the allele frequencies.
This may be a sampling artefact, however at position nt 1957 all breeds were in Hardy-Weinberg equilibrium. Despite of close genetic linkage $\chi^2$-test for linkage disequilibrium does not show significant linkage in all populations. Linkage disequilibrium declines with increasing generations (FALCONER, 1984), so the level and extent of disequilibrium diminish in older populations. The British breed Ayrshire is a long established breed, as is the Hereford. Casta Navarra and Fighting Bull have also been maintained over many centuries. Maremmana represents a low-selected historic genotype, and Chianina is regarded to be the oldest Italian breed. Anatolian Black and Turkish Grey Steppe are very heterogenous breeds that remained without specific selection pressure over centuries (PORTER, 1991). Thus the limited linkage disequilibrium in these breeds points to the two mutations being very old. On the other hand Pezzata Rossa is a newly founded population (herdbook established in 1957) originating in crosses of Simmental with Friulana cattle. Simmental was planned to substitute Friulana by backcrossing, however its introgression stopped after a few generations. Haplotypes derived from both parental breeds are likely to be still present in the Pezzata Rossa and may cause the lack of linkage disequilibrium observed.

Frequencies of $\text{CSN1S1}^B$ are in tendency higher and significantly higher for $\text{CSN1S1}^b$ in northern than in southern European breeds, with highest values in dairy breeds of north western European origin. LIEN et al. (1999) reported an apparently contrary frequency gradient in Nordic breeds with high frequencies of $\text{CSN1S1}^C$ in autochtonue breeds of northern Scandinavia and lower frequencies to fixation in dairy breeds originated in southern Scandinavia. This indicates rather a selection gradient than a geographic gradient, supporting the suggestion made by LIN et al. (1986) that occurrence of $\text{CSN1S1}^B$ variant is correlated with selection for improvement in milk production traits. Our study analysed northern European cattle breeds, that include a large proportion of dairy breeds, selection pressure thus is expected to lead to increasing fixation of alleles linked to production traits. Allele frequencies of $\text{CSN1S1}$ show that in Anatolian Black both alleles $B$ and $C$ occur in equal frequency. LOFTUS et al. (1999) described a high admixture proportion of $\text{Bos indicus}$ with 30.6% in Anatolian Black and BAKER & MANWELL (1980) pointed out that $\text{CSN1S1}^C$ occurs in very high frequencies in zebu cattle. Therefore the high frequency of $\text{CSN1S1}^C$ in Anatolian Black may have occurred by introgression of zebu genes. However loss of haplotypes along a south-north gradient leading to fixation or nearly fixation of the $\text{CSN1S1} B-b$ haplotype in northern European cattle populations could be explained by drift. The loss of genetic diversity along a south-north gradient has already been described for a number of different loci. This may reflect distance from the center of domestication of cattle (MEDJUGORAC et al., 1994; TROY et al., 2001).

Acknowledgements
The authors thank Heike Wagner for technical assistance. This work was supported by the European Community (RESGEN-CT98-118).

References
BAKER, A.C.M.; MANWELL, C.: 

BASSAM, B.J.; CAETANO-ANOLLES, G.; GRESSHOFF, P.M.: 

BOVENHUIS, H.; VAN ARENDONK, J.A.M.; KORVER, S.: 
Associations between milk protein polymorphisms and milk production traits. J. Dairy Sci. 75 (1992), 2549-2559

CAROLI, A.; JANN, O.; BUDELLI, E.; BOLLA, E.; JÄGER, S.; ERHARDT, G.: 

DAVID, V.A.; DEUTCH, A.H.: 

EIGEL, W.N.; BUTLER, J.E.; ERNSTROM, C.A.; FRRELL, H.M. jr.; HARWALKAR, V.R.; JENNES, R.; McL. WHITNEY:: 
Nomenclature of Proteins of Cow’s milk: Fifth revision. J. Dairy Sci. 67 (1984), 1599-1631

ERHARDT, G.: 

EHRMANN, S.; BARTENSCHLAGER, H.; GELDERMANN, H.: 

FALCONER, D.S.: 

FORMAGGIONI, P.; SUMMER, A.; MALACARNE, M.; MARIANI, P.: 

IKONEN, T.; RUOTTINEN, O.; ERHARDT, G.; OJALA, M.: 

KOCZAN, D.; HOBOM, G.; SEYFERT, H.M.: 

Identification of the two common alleles of bovine κ-casein locus by the RFLP-technique, using the enzyme HindIII. Génét. Sél. Evol. 20 (1988), 247-254

A simple and powerful method for linkage analysis by amplification of DNA from single sperm cells. Genomics 16 (1993), 41-44

LIEN, S.; KANTANEN, J.; OLSAKER, I.; HOLM, L.E.; EYTHORSDOTTIR, E.; SANDBERG, K.; DALSGAID, B.; ADALSTEINSSON, S.: 

LIN, C.Y.; MCALLISTER, A.J.; NG-KWAI-HANG, K.F.; HAYES, J.F.: 
Effects of milk protein loci on first lactation production in dairy cattle. J. Dairy Sci. 69 (1986), 704-712

LOFTUS, R.T., ERTUGRUL, O., HARBA, A.H., EL-BARODY, M.A., MACHUGH, D.E., PARK, S.D., BRADLEY, D.G.: 

MEDJUGORAC, I.; KUSTERMANN W.; LAZAR, P.; RUSS I.; PIRCHNER, F.: 

MONTGOMERY G.W.; SISE, J.A.: 
Extraction of DNA from sheep white blood cells. N Z J Agric. Res. 33 (1990), 437-441

NG-KWAI-HANG, K.F.; MONARDES, H.G.; HAYES, J.F.: 
Association between genetic polymorphism of milk proteins and production traits during three lactations. J. Dairy Sci. 73 (1990), 3414-3420

ORITA, M.; SUZUKI, Y.; SEKIYA, T.; HAYASHI, K.: 
Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5 (1989), 874-879


SCHILD, T.A.; GELDERMANN, H.: Variants within the 5'-flanking regions of bovine milk-protein-encoding genes. III. Genes encoding the Ca-sensitive caseins $\alpha_s1$, $\alpha_s2$ and $\beta$. Theor. Appl. Genet. 93 (1996), 887-893


Received: 2001-11-14
Accepted: 2001-12-20

Authors’ addresses
Dipl. Ing. agr. OLIVER JANN, Dr. EVA-MARIA PRINZENBERG, apl. Prof. Dr. HORST BRANDT, Prof. Dr. GEORG ERHARDT
Institut für Tierzucht und Haustiergenetik der Justus-Liebig Universität
Ludwigstr. 21B
D-35390 Gießen / Germany
E-Mail: Georg.Erhardt@agrar.uni-giessen.de

Dr. JOHN L. WILLIAMS
Department of Genomics and Bioinformatics
Roslin Institute
Midlothian / Scotland EH25 9PS

Prof. Dr. PAOLO AJMONE-MARSAN
Institute of Zootechnics
Catholic University of S. Cuore
Via Emilia Parmense, 84
I-29100 Piacenza / Italy

Prof. Dr. PÍLAR ZARAGOZA
Lab. Genetica Bioquimica y Grupos Sanguineos
Facultad de Veterinaria
Universidad de Zaragoza
Miguel Servet 177
50013 Zaragoza / Spain

Prof. Dr. CEYHAN ÖZBEYAZ
A.Ü.Veteriner Fakültesi Dergisi
Ankara / Turkey