

Evidence for individual and between-family variability of the recombination rate in cattle

Henner Simianer,¹ J. Szyda,¹ G. Ramon,² S. Lien²

¹Department of Animal Husbandry and Animal Breeding, Animal Genetics Group, University of Hohenheim (470/HG), 70593 Stuttgart, Germany

²Department of Animal Science, Agricultural University of Norway, PO Box 5025, 1432 Ås, Norway

Received: 1 February 1997 / Accepted: 15 June 1997

Abstract. We have conducted a study based on single sperm typing in a family design to assess patterns of variability of the male recombination rate in cattle. 2214 sperm of 37 bulls were typed for 11 loci on bovine Chromosomes (Chrs) 6, 23, and the sex chromosomes. Statistically significant individual variability of the recombination rate was observed for one interval in the pseudoautosomal region (PAR) of the bovine sex chromosomes; one marker interval on bovine Chr 23 exhibited individual variability that was close to significance. Thirty-five of the bulls were members of six paternal halfsib groups, and highly significant variability between families was found for one interval in the PAR. This variability may be due to DNA sequence differences in the PAR or to a genetic control of the recombination activity in this region. It is demonstrated that differences in the recombination rate of the magnitude observed in the present study may have a considerable impact on the power of QTL mapping experiments as well as on the sustainability of marker-assisted selection strategies.

Introduction

Genetic recombination was observed to happen with a variable rather than a constant probability in experimental species in the early days of genetics. Different recombination rates for different inbred lines of *Drosophila melanogaster* revealed between-individual variability of this parameter (Goldschmidt 1917; Sturtevant 1917). In selection experiments for high and low recombination rates in given chromosome segments with *Drosophila melanogaster* and *Tribolium castaneum*, clear selection responses provided evidence for a genetic control of this phenomenon (Dewees 1975; Korol and Iliadi 1994). The relevance of a genetic control of the recombination rate in an evolutionary context is supported by the observed excess of overall recombination activity, assessed by chiasma counts, in domesticated mammalian species relative to their wild living ancestors (Burt and Bell 1987).

The recombination fraction between loci is of central importance in genome mapping, since the measures of genetic distance and locus orders are derived from estimated recombination probabilities. In likelihood-based linkage analyses (Fisher 1948), the recombination fraction is modeled as a constant parameter, eventually within subgroups such as males and females. A substantial amount of variability thus may lead to misleading conclusions or may at least decrease the power of a mapping study. Models have been suggested that allow for a variable recombination rate in a Bayesian context (Risch 1988), but experimental data available, for example in human genetics, usually are not sufficient to estimate the actual magnitude of the variability with an acceptable accuracy.

In experimental species including mouse (see, for example, Reeves et al. 1990), the availability of highly inbred lines, short generation intervals, and the relative low costs of generating large numbers of related individuals permit setting up efficient experiments to study variability of recombination. In turn, the absence of these prerequisites made it almost impossible to conduct similar studies in large mammals, including human.

Sperm typing (Li et al. 1988) overcomes some of these prohibitive factors, in that it allows observation of a conceptually unlimited number of meioses for any male individual. With this technique, empirical evidence for individual variability of male recombination in cattle could be derived indirectly from the obvious discrepancy of recombination rates estimated in two different individuals (Park et al. 1995). In a recent study (Yu et al. 1996), this technique was used to demonstrate variability among five human males in the recombination rate between two markers on human Chr 6, bracketing the human major histocompatibility complex.

We have set up an experiment to systematically study variability of male recombination in cattle. The experiment focused on between-individual and between-family variability; therefore, we preferred to type a limited number (~60) of sperm for a larger number (37) of individuals, belonging to six different families. To allow comparisons of different regions of the genome we included, 11 loci on three different chromosomes, bracketing eight first order marker intervals.

Materials and methods

Choice of chromosomes and marker loci. Variability of recombination was studied on bovine Chrs 6, 23, and the XY chromosome. On each of the two autosomes, four microsatellite markers were genotyped. On the sex chromosomes, two microsatellite markers in the PAR and the sex-specific genes ZFY and ZFX (Aasen and Medrano 1990; Kirkpatrick and Monson 1993) situated in the Y- and X-specific chromosome regions, respectively, were used. The relative positions of the chosen marker loci and intervals are sketched in Fig. 1.

Since crossing-overs on the sex chromosomes are possible only in the PAR, recombinations between the marker loci in this region and the ZFX/ZFY system can be interpreted as recombinations between those loci and the respective boundary of the PAR.

The bovine PAR has been physically mapped to the short arm of the Y Chr at p13-ter. On the X Chr, the PAR has been assigned to the short arm of the X Chr at p12-ter (Barendse et al. 1994), but recent findings indicate that the correct position is at q42-ter on the long arm of the X Chr (Ponce de Léon et al. 1996; Yeh et al. 1996).

Structure of the material. Genotypes were available for 285 bulls of Norwegian Cattle for all microsatellite markers included in the study. From these bulls, six groups, consisting of six (group 3: five) paternal halfbrothers, were selected so that the maximum heterozygosity at all markers was obtained. The sires of groups 1 and 2 and of groups 3 and 4, respectively,

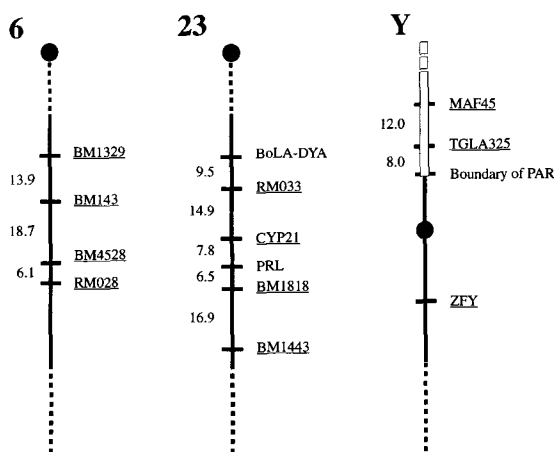


Fig. 1. Published map information on the chromosome regions considered in this study. Distances given in centiMorgan; Chr 6: sex averaged distances from Kappes et al. (1997); Chr 23: male specific distances from Beaver et al. (1996); Y Chr: Barendse et al. (1994); names of loci genotyped in this study are underlined.

were paternal halfbrothers. Sperm of the sires of group 1 and 3 were also genotyped and included in the study on individual variability, but not in the study on variability between families. Among these 37 bulls, 8 were heterozygous at all ten microsatellite marker loci, 19 were heterozygous at nine of them, and 10 were heterozygous at eight loci.

For each of the sons, sperm were picked from two samples of semen, while for the two sires, only one sample was used. The samples used were collected and frozen when the bulls were 15 to 36 months old; 46 sperm of the original 35 sons were typed for the complete set of eleven loci. For 46 sperm of the two sires and an additional sample of on average 14.6 sperm of each of the 35 sons, only a subset of loci, including all loci on the sex chromosomes and markers *CYP21* and *BM1818* on Chr 23, was amplified.

Sperm isolation and lysis. Single sperm sorting and lysis followed the protocol described by Lien and coworkers (1993).

First PCR. Forty cycles of PCR were carried out with a mixture of 11 sets of primers, one for each locus, in a Cetus GeneAmp PCR System 9600. PCR mix contained: 2.5 μ l lysis buffer, 2.5 μ l neutralization buffer, 2.5 μ l 10 \times PCR-buffer (100 mM Tris-HCl, pH = 8.3, 25 mM MgCl₂, 0.01% (wt/vol) gelatin), 0.1 mM of each dNTP, 2.0 pmol of each pair of primers for the first PCR (see Table 1), 0.5 U Taq polymerase, and H₂O to a total volume of 25 μ l. PCR-program was 3 min 94°C, 2 min 60°C, 1 min 72°C for cycle 1, followed by 15 s 95°C, 1 min 58°C, 1 min 72°C for cycles 2–5, and 15 s 95°C, 30 s 58°C, 30 s 72°C for the last 35 cycles.

Second PCR. The PCR products from the first round of PCR were re-amplified in a second PCR in six separate reactions (a–f., see Table 1). All PCR reactions were carried out in 10 mM Tris-HCl, pH = 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 0.001% (wt/vol) gelatin, with the addition of 0.2 mM of each dNTP, 5.0 pmol of each primers, 0.5 U Taq polymerase, 1 μ l product from the first PCR reaction, and H₂O in a total volume of 10 μ l.

The PCR program was generally 3 min 94°C for 1 cycle, followed by 15 s 94°C, 30 s 59–60°C, 30 min 72°C for 30 cycles.

Sex determination. The determination of sex for each sperm cell followed the method described by Kirkpatrick and Monson (1993).

Microsatellite allele determination. One primer for each microsatellite was synthesized with the fluorescent dyes TET, HEX, or FAM and analyzed in an Automated DNA-sequencer Model 373A (Perkin Elmer). A mix of 0.5 μ l PCR product from reaction a, 2.0 μ l product from reaction b, 2.0 μ l from reaction c, 4.0 μ l from reaction d, and 5.0 μ l from reaction e and 50 μ l H₂O was prepared to obtain equal strength of the bands for each satellite. Two μ l of the fluorescently tagged mix was joined with 4 μ l of formamide and 0.5 μ l Genescan 350-TAMRA (Perkin Elmer) and sepa-

rated on a 6% sequencing gel. All ten microsatellites were analyzed on the same gel.

Statistical analysis. Recombination rates were estimated by a two-point maximum likelihood procedure, which also led to the corresponding LOD scores. For individual variability, the Morton test (Morton 1956) was applied. For a given interval, M bulls are double heterozygous at the two flanking marker loci. For bull $i = 1, \dots, M$, K_i recombinant gametes are observed in N_i informative meioses. These observations are assumed to be realizations of a binomial variable. Under the null hypothesis, the recombination rate is assumed to be uniform for all bulls, while under the alternative hypothesis, different individual recombination rates are possible, that is

$$H_0: \theta_i = \theta_j \forall i \neq j \text{ is tested versus } H_1: \theta_i \neq \theta_j \exists i \neq j.$$

The likelihood ratio test statistic is

$$X^2 = -2 \ln \left(\prod_{K_i \neq 0}^M \frac{\hat{\theta}^{K_i} (1 - \hat{\theta})^{N_i - K_i}}{\hat{\theta}_i^{K_i} (1 - \hat{\theta}_i)^{N_i - K_i}} \prod_{K_i = 0}^M (1 - \hat{\theta}_i)^{N_i} \right)$$

where $\hat{\theta} = \sum_{i=1}^M K_i / \sum_{i=1}^M N_i$ and $\hat{\theta}_i = K_i / N_i$.

This test statistic asymptotically follows a χ^2 distribution with $M - 1$ degrees of freedom. More reliable empirical threshold values were derived from 10,000 permutations of the original data (Churchill and Doerge 1994).

The problem of multiple testing was accounted for by applying the sequentially rejective Bonferroni correction suggested by Holm (1979). To perform n tests on the 'multiple level of significance' α (Holm 1979), the resulting n values of the test statistic are ordered ascendingly, and the first (smallest) one is compared with the critical value corresponding to α/n , the second smallest to the critical value corresponding to $\alpha/(n - 1)$, and so forth, the largest value of the test statistic finally being compared with the critical value corresponding to $\alpha/1$. This procedure ensures that the pre-defined overall probability α of a type I error is kept with a considerably smaller loss of power than associated with the classical Bonferroni correction (Miller 1966). In the Results section, type I error probabilities P_i are given for each single test, and significance levels for the overall multiple tests are indicated as * ($P \leq 0.05$) and ** ($P \leq 0.01$).

The test for differences between families was a variation of the test for individual variability, in that all 35 sons were assigned to one of $M' = 6$ families, and $K_{i'}$ and $N_{i'}$ are the numbers of recombinants and informative meioses for halfsib family $i' = 1, \dots, 6$. With these accumulated data, an analogous likelihood ratio test with a permutation-based distribution of the test statistic was carried out. The sequentially rejective Bonferroni correction was applied to account for multiple testing. Empirical type I error probabilities P_F for single tests are reported, together with multiple test significance levels.

Additional, more sophisticated statistical tests (Potthoff and Wittinghill 1966; Risch 1988) were also applied, but did not lead to different conclusions, and therefore the results are not reported here.

Results

Efficiency of sperm typing. In total, 2214 single sperm were collected, that is, 59.8 sperm per bull on average. Amplification of at least one marker locus was obtained for 2174 sperm; the overall technical efficiency of sperm typing thus was above 98%. The proportion of successfully amplified marker alleles per locus ranged from 93.5% for locus *BM1329* to 61.6% for locus *RM028*. Efficiency of amplification was not allele dependent for the microsatellite markers, but it was consistently higher for the gene *ZFX* than for *ZFY*.

Test on individual variability of the recombination rate. In Table 2, the estimated two-point recombination rates for all eight intervals of adjacent markers are given with the corresponding LOD scores. Empirical type I error probabilities (P_i) derived from data permutations for the Morton test on individual variability are also given in Table 2. Taking into account that $n = 8$ simultaneous tests have been performed, individual variability of the recombi-

Table 1. Primers used in the two PCR steps, references (no reference = originally designed for this study), marking dyes and multiplex combinations for the ten microsatellite markers and the ZFX/ZFY system.

Marker	PCR Primers	Marked With	PCR		Multiplex
			1	2	
<i>RM028</i>	5'- cta cag tca tgg gtc tga aag ag -3' ^a	TET	x		a
	5'- atc ttc agc ctg gcc tga gag -3' ^a		x	x	
	5'- gga cca gca aca agc gac ta -3'			x	
<i>BM4528</i>	5'- cag aat cca tac aca tgt caa ca -3' ^b	FAM	x		b
	5'- agg aac agg tat agg aat att gga -3' ^b		x	x	
	5'- tgg tag atg tct aaa aat tta gca -3'			x	
<i>BM143</i>	5'- acc tgg gaa gcc tcc ata tc -3' ^b	HEX	x		c
	5'- ctg cag gca gat tct tta tgc -3' ^b		x	x	
	5'- gac tga gct acc acg gaa gta -3'			x	
<i>BM1329</i>	5'- ttg ttt agg caa gtc caa agt c -3' ^b	HEX	x		d
	5'- aac acc gca gct tca tcc -3' ^b		x	x	
	5'- gcc act gta gct tgg gaa tc -3'			x	
<i>BM1443</i>	5'- aat aaa gag aca tgg tca ccg g -3' ^b	FAM	x		c
	5'- tcg agg tgt ggg agg aag -3' ^b		x	x	
	5'- cca aaa tcc att gca gtt ca -3'			x	
<i>BM1818</i>	5'- agc tgg gaa tat aac caa agg -3' ^b	TET	x		b
	5'- agt gct ttc aag gtc cat gc -3' ^b		x	x	
	5'- tac ctg cac ccc tat gtt ca -3'			x	
<i>CYP21</i>	5'- gga ggg tta cag tcc atg ttg -3' ^c	TET	x		e
	5'- tgc cga tcc aac tcc tcc tga ag -3' ^c		x	x	
	5'- atc tgg gaa atg gag ccc caa t -3'			x	
<i>RM033</i>	5'- gct cat tct cct ggg atg cag a -3' ^d	TET	x		a
	5'- gct cct tta gtt ttc ttg tgg gag -3' ^d		x	x	
	5'- ctt aac gtc cct tcc ctg tgg -3'			x	
<i>MAF45</i>	5'- att gac act tca gta agt taa caa tgg -3' ^e	FAM	x		d
	5'- cag aca caa ctg agc aac tag cgc -3' ^e		x	x	
	5'- cca gga gtg tct ggt tct tca c -3'			x	
<i>TGLA325</i>	5'- ggg cac ttt act ctc tga aca aat c -3' ^e	TET	x		c
	5'- gct gac agt cta ttt cca gaa ggt a -3' ^e		x	x	
	5'- aac aaa tct ata gta ttg ggg att -3' ^f			x	
<i>ZFX/ZFY</i>	5'- ata atc aca tgg aga gcc aca agc t -3' ^f	TET	x		f
	5'- gca ctt ctt tgg tat ctg aga aag t -3' ^f		x	x	
<i>ZFX</i>	5'- gac agc tga aca agg gtt act g -3' ^f	TET		x	f
	5'- aat gtc aca ctt gaa tgg cat c -3' ^f			x	
<i>ZFY</i>	5'- gaa ggc ctt cga atg tga taa c -3' ^f	TET		x	f
	5'- ctg aca aaa ggt ggc gat ttc a -3' ^f			x	

^a Kossarek et al. (1993).^b Bishop et al. (1994).^c Creighton et al. (1992).^d Kossarek et al. (1994).^e Barendse et al. (1994).^f Kirkpatrick and Monson (1993).**Table 2.** Number of double heterozygous bulls (N_h), informative meioses (N_m), maximum likelihood estimates of the recombination rate $\hat{\theta}$, LOD scores (Z), and single test error probabilities for individual (P_I) and between family (P_F) variability of the recombination rate for the eight marker intervals studied. Significance on the multiple test significance level α is indicated by * ($\alpha \leq 0.05$) and ** ($\alpha \leq 0.01$).

Marker Interval	N_h	N_m	$\hat{\theta}$	Z	P_I	P_F
Chromosome 6:						
<i>BM1329-BM143</i>	28	993	0.156	112.1	0.903	0.799
<i>BM143-BM4528</i>	32	1132	0.135	144.7	0.993	0.833
<i>BM4528-RM028</i>	22	657	0.100	104.7	0.365	0.343
Chromosome 23:						
<i>RM033-CYP21</i>	25	851	0.157	95.2	0.386	0.545
<i>CYP21-BM1818</i>	35	1599	0.119	228.2	0.033	0.751
<i>BM1818-BM1443</i>	32	1245	0.161	135.8	0.545	0.647
Sex chromosomes:						
<i>MAF45-TGLA325</i>	28	1430	0.036	333.5	0.019*	0.150
<i>TGLA325-ZFX/ZFY</i>	33	1612	0.059	328.4	0.083	0.003**

nation rate was found to be significant for interval *MAF45-TGLA325* on the sex chromosomes ($\alpha \leq 0.019$) and was close to significant ($\alpha \leq 0.066$) for interval *CYP21-BM1818* on Chr 23.

This result is illustrated by the comparison of the observed frequencies of numbers of recombinant gametes with the respective expected frequencies under the null hypothesis in Fig. 2a and 2b. Expectations are computed from a mixture of binomials with unequal numbers of informative meioses N_i , but constant recombination rate equal to the maximum likelihood estimate of the overall recombination rate $\hat{\theta}$. For interval *MAF45-TGLA325* (Fig. 2a), the observed distribution is broader and flatter than the ex-

pected one, and extreme numbers of recombinants ($K_i = 0, 5$, and 6) are more frequently observed than expected if a uniform recombination rate applies. The range of individual recombination rates goes from $\hat{\theta}_i = 0.0$ (estimated for 7 bulls with 45–58 informative meioses) to $\hat{\theta}_i = 0.102$ for one bull with 59 informative meioses.

A similar pattern is seen in Fig. 2b for interval *CYP21-BM1818*, where again extreme classes ($K_i = 0, 9$, and 12) are overrepresented. For this interval, estimated individual recombination rates range from $\hat{\theta}_i = 0.0$ to $\hat{\theta}_i = 0.250$, estimated from 53 and 48 informative sperm, respectively.

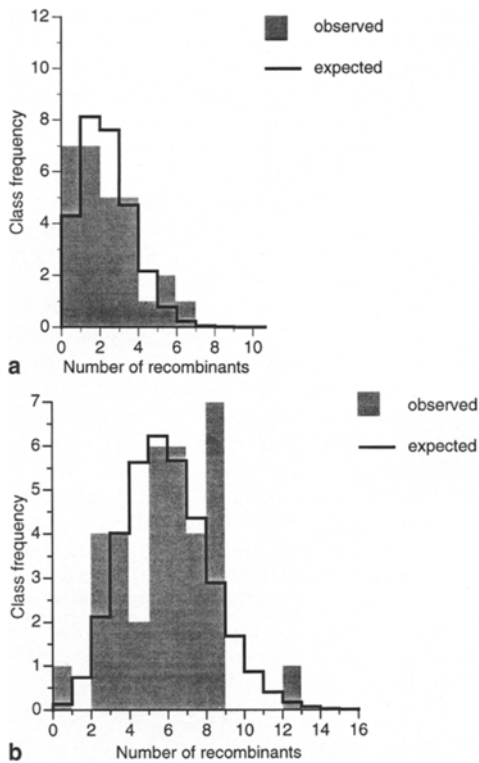


Fig. 2. (a) Observed and expected class frequencies for numbers of recombinant gametes for interval *MAF45-TGLA325* on the sex chromosomes. (b) Observed and expected class frequencies for numbers of recombinant gametes for interval *CYP21-BM1818* on Chr 23.

This result is also supported by the fact that, for all intervals on the sex chromosomes and intervals *CYP21-BM1818* and *RM033-CYP21* on Chr 23, the observed variance of estimated individual recombination rates is larger than its expected value under the assumption of a binomial distribution with a fixed recombination rate. For interval *MAF45-TGLA325*, the observed variance exceeds its expectation by 60.6%, which is significant in the F-test with $P = 0.029$.

Test on variability of the recombination rate between families. The Morton test for variability of recombination between halfsib groups revealed clearly significant variability for the interval *TGLA325-ZFX/ZFY* on the sex chromosomes. The overall level of significance for this result was $\alpha \leq 0.003$. In Fig. 3, the respective estimates of the recombination rate between these two markers are depicted for all informative sons of the six sires. In addition to the evident differences of mean recombination rates for the sire offspring groups, it becomes evident that the recombination rates of sons of some sires (for example, 1, 4, and 5) are clustered around a family-specific level, while the range of values for other offspring groups (esp. of sires 2) covers a major part of the scale. Note that between the two extreme families 3 and 5, there is hardly any overlap of the average recombination rates of the sons belonging to these sire offspring groups.

Discussion

For this study, Chr 6 was chosen as a larger chromosome that has been intensely studied in cattle because it contains the casein genes, which play a major functional role in the regulation of the milk protein synthesis (Threadgill and Womack 1990). On Chr 23, marker intervals were studied that contain parts of the bovine lymphocyte antigen (BoLA) system, the MHC of cattle (Anders-

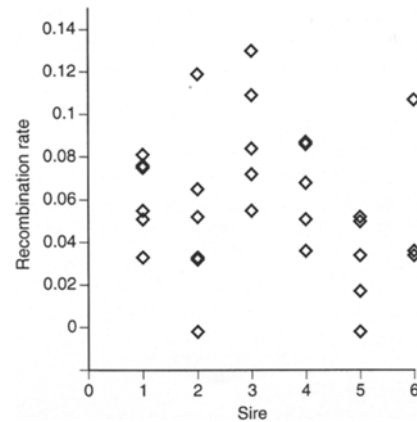


Fig. 3. Estimated individual recombination rates for interval *TGLA325-ZFX/ZFY* for the informative sons of the six sires.

son et al. 1988). For the two loci *BoLA-DYA* and prolactin (*PRL*), significantly different recombination rates were observed for two bulls (Park et al. 1995). The sex chromosomes are interesting candidates, because in the male meiosis recombination is possible only in the pseudoautosomal region (PAR); thus, extreme differences in the recombination rate between male and female meioses are found. For the same pair of microsatellite markers *MAF45-TGLA325* in the PAR, published distances are between 12 centiMorgan (cM) (Barendse et al. 1994) and 2 cM (Yeh et al. 1996).

Individual variability within the breed of Norwegian Cattle has been demonstrated for interval *MAF45-TGLA325* on the sex chromosomes. The factors most frequently reported to cause recombination differences, like sex or age, can be excluded here, because only male meioses were considered, and sperm was sampled from the bulls at a comparable age. However, there may be other unknown, but nongenetic factors like specific environmental effects (for example, food) or the condition of the animal itself (for example, diseases) that may have caused this observed individual variability.

For the interval *DYA-PRL* on bovine Chr 23, a significant difference between the recombination rate estimated for two bulls from two different breeds ($\hat{\theta} = 0.169 \pm .026$ and $0.310 \pm .038$, $P < 0.02$) has been reported (Park et al. 1995). This interval overlaps with the region studied in our experiment (see Fig. 1), including completely interval *RM033-CYP21*, and partly overlapping with interval *CYP21-BM1818*.

This is especially interesting, since these loci lie in the region of the BoLA system, the MHC of cattle. Other than in human and mouse, large genetic distances (~17 cM) have been reported between the class II *DYA* and *DRB3* genes (Andersson et al. 1988), that is, within interval *DYA-PRL*. It was suggested that this may be due to a recombination hot spot (Andersson et al. 1988), but recent results indicate that this observed genetic distance coincides with an adequate physical distance (R. Fries, personal communication).

Individual variation in recombination of two markers (*D6S291*, *D6S109*) bracketing the human MHC region was also found to be highly significant among five human males (Yu et al. 1996). The individual estimates of recombination, estimated from 127 to >1000 sperm per donor, ranged from 0.049 to 0.114. An attempt to verify individual differences for a second marker interval on human Chr 19 failed, however.

Recombination hot spots exist in the MHC regions of mouse (Ling et al. 1993; Heine et al. 1994) and human (Satyanarayana and Strominger 1992; Thomsen et al. 1994). Significant differences between estimated genetic distances for marker intervals in this region have been reported for different interspecific and intersubspecific crosses in mice (Reeves et al. 1990; Himmelbauer and Silver 1993; Heine et al. 1994). These differences in recombination frequency may be caused by prohibitive sequence differ-

ences due to small inversions or deletions (Reeves et al. 1990) or a genetic regulation of recombination at the hot spot (Heine et al. 1994), which has been documented especially for the murine MHC (Shiroishi et al. 1991).

The PAR of the sex chromosomes is also known to exhibit an exceedingly high recombination activity in male meiosis. In the human genome, there are at least two PARs, constituting 2.6 Mb on the short arm (Petit et al. 1988) and 0.32 Mb near the long arm telomeres (Freije et al. 1992). In male meiosis, pairing of X and Y Chrs is only possible in these regions. At least in human and mouse, one chiasma was found to be obligatory for a functional meiosis (de Boer 1986; Shapiro et al. 1989). As a consequence, the genetic length of the human PAR on Xp/Yp is estimated to be 50 cM in the male meiosis, compared with 4–18 cM in female meiosis (Rouyer et al. 1986; Page et al. 1987).

The very few reports on variability of recombination in larger mammals (Park et al. 1995; Yu et al. 1996) are focusing on differences among nonrelated individuals. To our knowledge, the present study is the first to provide evidence on differences among families, the most striking result certainly being the clearly significant variability of recombination rates between sire families in the interval *TGLA325-ZFX/ZFY* in the PAR.

Sons of the same sire share identical Y-specific segments of the Y Chr, while the PAR of paternal halfbrothers may vary owing to eventual recombinations on the sire-son path, as was observed for a total of five bulls in the present study.

Two hypotheses may be used to explain these differences between recombination rates of sire families. It has been demonstrated that recombination occurs with a reduced rate if pairing sequences are different in terms of numbers of copies of repetitive sequences in the respective region (Yoshino et al. 1995). The observed differences between families could be explained if the Y-Chr inherited by, say, sire 5 carried a DNA sequence in the PAR that substantially differs from the 'average' sequence of the PAR of dams, leading to poor homology between PARs of the X and Y Chrs, thus causing a low recombination rate.

A genetic regulation of the recombination activity in the PAR also would explain these findings. Little is known to date about genetic control of recombination in higher organisms. In experimental species like *Drosophila* and *Tribolium*, genetic regulation of recombination has been convincingly demonstrated by selection experiments (Dewees 1975; Korol and Iliadi 1994). Genes involved in the regulation of the mammalian meiosis just start to become known (Baker et al. 1996; Hassold 1996).

The present results certainly would suggest the assumption that the regulatory mechanism responsible for these differences is located on the Y Chr, since this is the only chromosome segment consistently shared by paternal halfbrothers. A better coverage of the PAR by additional markers would be a prerequisite for an attempt to identify and map the respective gene, provided it is located in the PAR, which is not necessarily the case. The number of suitable markers available in this region of the bovine genome, however, is limited, and the power of such a mapping study in the present design would be low.

Individual and between-family variability of the recombination rate seems to be restricted to some chromosomes or chromosome segments, especially the PAR and eventually the MHC-region of bovine Chr 23; this would be in agreement with the reported difference of the recombination rate in this region between two bulls (Park et al. 1995) and the observed individual variability in the human MHC region (Yu et al. 1996). In the latter study, the attempt to find variability between the most extreme individuals on other chromosomes failed, so that the authors concluded that 'it is likely that the variation in recombination is not genomewide'. This conclusion is supported by the results presented here.

Disregarding individual variability of recombination will lead to an underestimation of standard errors and confidence intervals of estimated recombination rates (Yu et al. 1996). In the context of

improvement of livestock, variability of the recombination rate may also have important implications for programs with marker-assisted selection (Park et al. 1995). In general, considering variable recombination rates as fixed will introduce additional stochastic noise, leading to less accurate estimates and reduced experimental power (in, for example, QTL mapping experiments).

Differences in individual or family-specific recombination rates of the magnitude observed in the present study may cause considerable deviations from expected results in gene mapping experiments or in marker-assisted selection schemes, as will be shown below with two examples.

Consider a situation in which a chromosomal region carrying a defect gene is covered with a dense grid of markers with an average recombination rate of 1% between two adjacent markers. In order to identify the smallest interval of markers bracketing the gene, it is necessary to observe at least one recombination in each of the two marker intervals flanking the interval that contains the defective gene.

Let θ_1 and θ_2 be the recombination rate in the marker interval left and right of the putative gene, respectively, and p_{00} , p_{01} , p_{10} , and p_{11} be the probability of observing a recombination in no, only the right, only the left, or both intervals in one meiosis. Assuming no genetic interference, $p_{00} = (1 - \theta_1)(1 - \theta_2)$, $p_{01} = (1 - \theta_1)\theta_2$, $p_{10} = \theta_1(1 - \theta_2)$, and $p_{11} = \theta_1\theta_2$. The probability of observing at least one recombination in each of the two flanking intervals in N meioses can be shown to be

$$1 - (p_{00} + p_{01})^N - (p_{00} + p_{10})^N + (p_{00})^N$$

With $\theta_1 = \theta_2 = 0.01$ and no interference, 366 meioses are required for a $\geq 95\%$ chance to observe at least one recombination in both flanking intervals. If, say, the recombination rate in one family is reduced by 50% and is only $\theta_1 = \theta_2 = 0.005$, the probability of observing at least one recombination in both flanking intervals with 366 meioses is only 65.5 per cent, that is, the probability of failing to observe the event of interest is almost sevenfold. To reach the same probability of success of ≥ 95 per cent, the required sample size in this case would be 734, i.e. more than doubled.

While for mapping experiments high recombination rate is advantageous, low recombination rates are beneficial in marker-assisted selection schemes, when an association between a marker allele and a desired QTL allele is observed in one family and this information is used for selection purposes in subsequent generations.

Let Δ_0 be the phenotypic difference in a quantitative trait between the carriers of two alternative marker alleles in one family (for example, in the offspring of one bull). Due to recombination, this difference will decrease by the factor $(1 - 2\theta)$ per generation, where θ is the recombination rate between the marker and the assumed QTL. After t generations of selection, $\Delta_t = (1 - 2\theta)^t \Delta_0$. Based on this, the 'half-life' of the marker-associated selection differential can be defined as $t_h = \log(0.5)/\log(1 - 2\theta)$.

For $\theta = 0.05$, t_h is 6.58, that is, after seven cycles of selection, half the usable selection differential associated with the marker is lost. If, in one family, the recombination rate in the respective chromosomal region is reduced by 50% to $\theta = 0.025$, t_h is 13.51, thus the sustainability of the marker-associated selection differential is more than doubled in such a family. In a family with a 50% increased recombination rate of $\theta = 0.075$, t_h is only 4.27, i.e. the usable genetic difference is halved two generations earlier.

Acknowledgments. We thank Steve Kappes, Bill Barendse, and Al McGraw for kindly providing us with microsatellite sequences, and the Norwegian Cattle Association (NRF) for the support with bovine sperm straws. The study was financially supported by the Deutsche Forschungsgemeinschaft (DFG).

References

- Aasen E, Medrano JF (1990) Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep and goats. *Bio/Technology* 8, 1279–1281
- Andersson L, Lundén A, Sigurdardóttir S, Davies CJ, Rask L (1988) Linkage relationships in the bovine MHC region: high recombination frequency between class II subregions. *Immunogenetics* 27, 273–280
- Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nature Genet* 13, 336–342
- Barendse W, Armitage SM, Kossarek LM, Shalom A, Kirkpatrick BW, Ryan AM, Clayton D, Li L, Neibergs HL, Zhang N, Grosse WM, Weiss J, Creighton P, McCarthy F, Ron M, Teale AJ, Fries R, McGraw RA, Moore SS, Georges M, Soller M, Womack JE, Hetzel DJS (1994) A genetic linkage map of the bovine genome. *Nature Genet* 6, 227–335
- Beever JE, Lewin HA, Barendse W, Andersson L, Armitage SM, Beattie CW, Burns BM, Davis SK, Kappes SM, Kirkpatrick BW, Ma RZ, McGraw RA, Stone RT, Taylor JF. (1996) Report of the first workshop on the genetic map of bovine chromosome 23. *Anim Genet* 1996 Apr; 27(2): 69–75
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SL, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo J, Beattie CW. (1994) A genetic linkage map for cattle. *Genetics* 136, 619–639
- Burt A, Bell G (1987) Mammalian chiasma frequencies as a test of two theories of recombination. *Nature* 326, 803–805
- Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963–971
- Creighton P, Eggen A, Fries R, Jordan SA, Hetzel J, Cunningham EP, Humphries P (1992) Mapping of bovine markers CYP21, PRL, and BOLA DRBP1 by genetic linkage analysis in reference pedigrees. *Genomics* 14, 526–528
- de Boer P (1986) Chromosomal causes for fertility reduction in mammals. In *Chemical Mutagens*, Vol 10, FJ Serres, ed. (New York: Plenum), pp 427–467
- Deweese AA (1975) Genetic modification of recombination rate in *Tribolium castaneum*. *Genetics* 81, 537–552
- Fisher RA (1948) *Statistical Methods for Research Workers*, 10th ed. (London: Oliver & Boyd)
- Freije D, Helms C, Watson MS, Donis-Keller H (1992) Identification of a second pseudoautosomal region near the Xq and Yq telomeres. *Science* 258, 1784–1787
- Goldschmidt R (1917) Crossing over without chiasmata. *Genetics* 2, 82–95
- Hassold TJ (1996) Mismatch repair goes meiotic. *Nature Genet* 13, 261–262
- Heine D, Khambata S, Wydner KS, Passmore HC (1994) Analysis of recombinational hot spots associated with the *p* haplotype of the mouse MHC. *Genomics* 23, 168–177
- Himmelbauer H, Silver LM (1993) High-resolution comparative mapping of mouse chromosome 17. *Genomics* 17, 110–120
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6, 65–70
- Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopez-Corrales NL, Beattie CW (1997) A second-generation linkage map of the bovine genome. *Genome Research* 7, 235–249
- Kirkpatrick BW, Monson RL (1993) Sensitive sex determination assay applicable to bovine embryos derived from IVM and IVF. *J Reprod Fertil* 98, 335–340
- Korol AB, Iliadi KG (1994) Recombination increase resulting from directional selection for geotaxis in *Drosophila*. *Heredity* 72, 64–68
- Kossarek LM, Grosse WM, Finlay O, Barendse W, Hetzel DJ, McGraw RA (1993) Rapid communication bovine dinucleotide repeat polymorphism: RM028. *Anim Sci* 71, 3177
- Kossarek LM, Grosse WM, Finlay O, Barendse W, Hetzel DJ, McGraw RA, (1993) Rapid communication: bovine dinucleotide repeat polymorphism RM028. *J Anim Sci*. 71:3177. RM033, and RM038. *Anim Genet* 25, 296–297
- Li H, Gyllenstein UB, Cui X, Saiki RK, Erlich HA, Arnheim N (1988) Amplification and analysis of DNA sequences in single human sperm. *Nature* 335, 414–417
- Lien S, Kaminski S, Aleström P, Rogne S (1993) A simple and powerful method for linkage analysis by amplification of DNA from single sperm cells. *Genomics* 16, 41–44
- Ling X, Shenkar R, Sakai D, Arnheim N (1993) The mouse *Eb* meiotic recombination hotspot contains a tissue-specific transcriptional enhancer. *Immunogenetics* 37, 331–336
- Miller RG Jr (1966) *Simultaneous Statistical Inference*. (New York: McGraw-Hill)
- Morton NE (1956) The detection and estimation of linkage between genes for elliptocytosis and the Rh blood type. *Am J Hum Genet* 8, 80–96
- Page DC, Bieker K, Brown LG, Hinton S, Leppert M, Lalouel JM, Lathrop M, Nystrom-Lahti M, de la Chapelle A, White R (1987) Linkage, physical mapping, and DNA sequence analysis of pseudoautosomal loci on the human X and Y chromosomes. *Genomics* 1, 243–256
- Park C, Russ I, Da Y, Lewin HA (1995) Genetic mapping of *F13A* to BTA23 by sperm typing: difference in recombination rate between bulls in the *DYA-PRL* interval. *Genomics* 27, 113–118
- Petit D, Levilliers J, Weissenbach J (1988) Physical mapping of the human pseudoautosomal region; comparison with genetic linkage map. *EMBO J* 7, 2369–2376
- Ponce de Léon FA, Ambady S, Hawkins GA, Kappes SM, Bishop MD, Robl JM, Beattie CW (1996) Development of a bovine X chromosome linkage group and painting probes to assess cattle, sheep, and goat X chromosome segment homologies. *Proc Natl Acad Sci USA* 93, 3450–3454
- Potthoff RF, Wittinghill M (1966) Testing for homogeneity. I. The binomial and multinomial distributions. *Biometrika* 53, 167–182
- Reeves RH, Crowley MR, O'Hara BF, Gearhart JD (1990) Sex, strain, and species differences affect recombination across an evolutionary conserved segment of mouse chromosome 16. *Genomics* 8, 141–148
- Risch NA (1988) A new statistical approach for linkage heterogeneity. *Am J Hum Genet* 42, 353–364
- Rouyer F, Simmler MC, Johnsson C, Vergnaud G, Cooke HJ, Weissenbach J (1986) A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Nature* 319, 291–295
- Satyanarayana K, Strominger JL (1992) DNA sequences near a meiotic recombinational breakpoint within the human HLA-DQ region. *Immunogenetics* 35, 235–240
- Shapiro LJ, Mohandas T, Yen P, Speed R, Chandley A (1989) The pseudoautosomal region of the X chromosome is necessary for sex chromosome pairing. *Am J Hum Genet* 45 [Suppl.], A107
- Shiroishi T, Koide T, Yoshino M, Sagai T, Moriwaki K (1991) Genetic control of sex-dependent meiotic recombination in the major histocompatibility complex of the mouse. *EMBO J* 10, 681–686
- Sturtevant AH (1917) Genetic factors affecting the strength of linkage in *Drosophila*. *Proc Natl Acad Sci USA* 3, 555–558
- Thomsen M, Neugebauer M, Arnaud J, Borot N, Sevin A, Baur M, Cambon-Thomsen A (1994) Recombination fractions in the HLA system based on the data set 'Provinces Françaises': Indications of haplotype-specific recombination rates. *Eur J Immunogenet* 21, 33–43
- Threadgill DW, Womack JE (1990) Genomic analysis of the bovine milk protein genes. *Nucleic Acids Res* 18, 6935–6942
- Yeh CC, Taylor JF, Gallagher DS, Sanders JO, Turner JW, Davis SK (1996) Genetic and physical mapping of the bovine X chromosome. *Genomics* 32, 245–252
- Yoshino M, Sagai T, Fisher Lindahl K, Toyoda Y, Moriwaki K, Shiroishi T (1995) Allele-dependent recombination frequency: Homology requirement in meiotic recombination at the hot spot in the mouse major histocompatibility complex. *Genomics* 27, 298–305
- Yu J, Lazzeroni L, Qin J, Huang M-M, Navidi W, Erlich H, Arnheim N (1996) Individual variation in recombination among human males. *Am J Hum Genet* 59, 1186–1192