Copyright © 1984 by the Genetics Society of America

GENETIC LINKAGE IN THE HORSE. II. DISTRIBUTION OF MALE RECOMBINATION ESTIMATES AND THE INFLUENCE OF AGE, BREED AND SEX ON RECOMBINATION FREQUENCY

LEIF ANDERSSON AND KAJ SANDBERG

Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, s-750 07 Uppsala, Sweden

Manuscript received July 5, 1983
Accepted September 10, 1983

ABSTRACT

In the present study an extensive amount of data, comprising more than 30,000 offspring in total, was analyzed to evaluate the influence of age and sex on the recombination frequency in the *K-PGD* segment of the equine linkage group (LG) I and the influence of age, breed and sex on recombination in the *Al-Es* segment of LG II. A highly significant sex difference is reported for both segments. Male and female recombination values in the *K-PGD* segment were estimated at 25.8 ± 0.8 and 33.3 ± 2.5%, respectively. Similarly, recombination was less frequent in the male (36.6 ± 0.7%) than in the female (46.6 ± 1.2%) in the *Al-Es* segment. Comparison of data from two Swedish horse breeds revealed no significant breed differences in either sex for recombination in the *Al-Es* segment. No evidence of an age effect was found in any segment or sex. The distribution of individual male recombination estimates was also investigated, and a significant heterogeneity among stallions was revealed in the *K-PGD* segment. The results are discussed in relation to previous studies on factors affecting recombination in mammals.

GENETIC recombination is known to be affected by a number of environmental and genetic factors (*cf.* MORGAN, BRIDGES and STURTEVANT 1925; SIMCHEN and STAMBERG 1969). The effect of a given factor may involve the entire genome or be limited to certain chromosomal segments. Most of the knowledge on this subject has been derived from experimental organisms such as Drosophila and Neurospora. In mammals, information on factors influencing recombination is quite limited. Although the biochemical and genetic basis for recombination is likely to be similar among all eukaryotes, we expect the influence of environmental agents (*e.g.*, temperature) to be less important in mammals than in lower eukaryotes due to the higher degree of homeostasis in the former group. Increased knowledge on the most important factors affecting recombination and on the distribution of recombination fractions within populations is of great importance for genetic studies in general and for gene mapping work in particular.

Sex differences in the frequency of recombination in animals are well documented in a number of species, including man and mouse (DUNN and BENNETT

1967; WEITKAMP 1973; CALLAN and PERRY 1977). There is a general tendency for recombination to be less frequent in the heterogametic sex; this effect is extreme in Drosophila males (*XY*) and silk-worm females (*ZO*) in which no recombination is detected. The most extensive information on sex differences in recombination in a mammalian species is provided by data from the mouse; DUNN and BENNETT (1967) tabulated male and female recombination estimates for 53 linkage pairs in this species. For 30 pairs there was no significant difference between sexes, whereas a significantly higher recombination estimate was found for females in 18 cases and for males in five cases. The pairs with a higher female rate were distributed among ten linkage groups. Four of the pairs with a higher male rate were found in one linkage group. Thus, in the mouse there appears to be a region-specific effect of sex on the frequency of recombination. Although based on more limited data, the same conclusion seems to be justified in man (WEITKAMP 1973, 1976).

BRIDGES reported as early as 1915 that crossing over in Drosophila varied with the age of the mother. The effect of parental age on recombination appears to be less obvious in mammalian species. WALLACE, MACSWINEY and EDWARDS (1976) examined data covering 18 segments over eight chromosomes in the mouse and found no consistent age-related trend in either sex when two-point recombination and multiple-point interference ratios were investigated. From their own and previously published work in mice (FISHER 1949; BODMER 1961; REID and PARSONS 1963) they concluded that the number of significant age-related changes observed is small compared with the number of cases in which no significant age effect has been detected. The results suggest that age effects are not common and, if they exist, may be specific to chromosome region, sex and strain. In man, the very limited data published are compatible with these conclusions, because indications of age-related changes have been found in some studies (WEITKAMP 1973) but not in others (RENWICK and SCHULZE 1965; WEITKAMP *et al.* 1973; ELSTON, LANGE and NAMBOODIRI 1976). Most studies on this subject have been based on an assumption of a linear trend with age, but the possibility of a curvilinear relationship has been suggested on the basis of recombination data from the mouse (WALLACE 1957) and also by age trends in chiasma frequencies in various species (MAYO 1974).

Genetic variation with respect to the frequency of recombination has been disclosed in Drosophila and other experimental organisms, because it has been found possible to alter the recombination frequency by artificial selection [see MAYNARD SMITH (1978) and TUCIĆ, AYALA and MARINKOVIĆ (1981) for review]. Genetic variation for recombination has also been postulated to be of common occurrence in natural populations (SIMCHEN and STAMBERG 1969; MAYNARD SMITH 1978). The presence of genes controlling recombination may be reflected in population data as a recombination difference (1) between individuals, (2) between groups of related individuals or (3) between populations or races. Such genes may also be revealed as a difference in recombination associated with a specific marker allele; the specific allele effect may be due to the expression of the allele itself or to a chromosomal rearrangement (*e.g.*, inversion) or some other genetic factor affecting recombination in gametic association with the marker allele. Detecting genetic variation for recombination

from population data is evidently a laborious enterprise and it is not surprising that existing evidence from mammalian species is very sparse. In man, data clearly indicating differences in recombination between populations of blacks and whites from two regions on chromosome *1* and one region on chromosome *6* have been reported (WEITKAMP 1974, 1976). GEDDE-DAHL *et al.* (1975, 1981) found an allele-specific difference of the recombination frequency between the *Pi* and *Gm* loci in man.

The accumulation of data from the routine blood-typing service in our laboratory has created a possibility for an extensive study on genetic linkage in the horse. The first paper in this ongoing study examined the linkage relationships among 15 blood marker loci (SANDBERG and ANDERSSON 1984). Linkage of the loci for serum albumin (*Al*) and serum esterase (*Es*) was demonstrated, and additional data on the previously described linkage (SANDBERG 1974a) between the loci for blood group K (*K*) and 6-phosphogluconate dehydrogenase (*PGD*) were reported; the *K-PGD* linkage and the *Al-Es* linkage belongs to equine linkage groups I and II, respectively. This second paper reports on the influence of age, breed and sex on recombination in the *Al-Es* segment and on the influence of age and sex on recombination in the *K-PGD* segment. The distribution of individual male recombination estimates for these two segments is also analyzed to provide information on the within-population variability of recombination frequencies. The latter analysis is of particular interest for the *K-PGD* segment in which a significant heterogeneity among stallions was previously detected (SANDBERG and ANDERSSON 1984).

MATERIALS AND METHODS

Animals: The horses used in the present study belonged to the Swedish Trotter (ST) breed and the North-Swedish Trotter (NST) breed. The material consisted of all horses blood typed in our laboratory in the period 1970–1979. Altogether 28,652 and 5617 complete families (sire, dam and offspring tested) were available for the ST and NST breed, respectively. The age of parents and offspring was obtained from registration records.

Genetic markers: The *Al, Es* and *PGD* loci all determine simple electrophoretic systems involving multiple codominant alleles. The blood group system K is a simple one-factor (Ka), two-allele system. The methods for the analysis of these genetic markers are given by SANDBERG and ANDERSSON (1984).

Linkage analysis: Paternal and maternal half-sib groups were examined. The genetic contribution of a heterozygous parent with regard to the *Al, Es* and *PGD* loci could be determined in all matings except those in which sire, dam and offspring had the same genotype. However, double intercrosses in the *Al-Es* combination were excluded when sire and dam had the same heterozygous genotype at both loci. On examination of half-sib groups, such matings may introduce a dilocus segregation bias for linked loci (ANDERSSON 1983).

With regard to blood group locus *K*, only matings between parents heterozygous for the blood group factor Ka and parents lacking the factor were included. The heterozygosity of a dam or sire was inferred from the occurrence of at least one offspring lacking the factor. When the family size is small, this selection of parents leads to a segregation bias. The data on recombination in the *K-PGD* segment were, nevertheless, unbiased, because parents were selected through the offspring with regard to only one of the characters under study (*cf.* MORTON 1955).

The linkage phase of stallions was inferred from the segregation data. All stallions with at least 50 informative offspring (*i.e.*, offspring for which the gametic contribution of the sire could be determined) for the *Al-Es* segment and all stallions with at least 15 informative offspring for the *K-PGD* segment were used. These numbers were chosen on the basis of previously reported recombination

estimates (SANDBERG and ANDERSSON 1984) and represent the limits at which the linkage phase of a stallion could be determined with about 95% confidence. This method of selecting families may bias the recombination estimates slightly in the direction of close linkage because the wrong linkage phase will be assumed for those stallions for which the number of recombinants by chance is greater than the number of nonrecombinants. This possible bias is negligible in the present study because for the majority of stallions the number of offspring by far exceeded the limits set to determine the linkage phase with 95% confidence. The data on female recombination were based on all mares for which the linkage phase could be determined from the genotype of their parents.

After the linkage phase of the parents had been determined, each informative offspring could be classified as recombinant or nonrecombinant. The possible occurrence of double or multiple crossovers in the material should be noted.

Statistical analysis: Heterogeneity of recombination fractions in any comparison was tested by conventional contingency chi-square analyses.

Curvilinear regression was applied to examine the influence of age on recombination, as we had no *a priori* reason to expect a linear relationship. For this analysis, recombination fractions were transformed using the angular transformation and weighted by the sample size (HARVEY 1982). The curvilinear regression was performed as described by SOKAL and ROHLF (1981, p. 671) in a stepwise manner by fitting consecutively first a linear regression to the data and then adding higher order terms, the procedure initially being continued up to adding the cubic power of age. In the final analysis, higher order terms were dropped if their effect was insignificant compared with the effect of lower order terms. The General Linear Model procedure of the Statistical Analysis System (GOODNIGHT, SALL and SARLE 1982) was utilized for the calculations.

The possible effect of stallion and age on recombination was analyzed simultaneously using a log-linear model as described by BISHOP, FIENBERG and HOLLAND (1975). This method is designed to be used for tests of interactions in multiway tables of attribute data; age was treated as a qualitative variable in the analysis. In the present study it was of interest to test for three-factor interactions between stallion, age and recombination and to test for two-factor interactions between stallion and recombination, and between age and recombination. The test for two-factor interactions was made within each level of the third factor, *i.e.*, conditional independence was tested for. The computer program BMDP4F (BROWN 1981) was utilized for the calculations of the *G*-statistic (log likelihood ratio test) which is distributed as approximately chi-square.

RESULTS

Influence of breed and sex: Male and female recombination estimates are given for each breed, separately, in Table 1. No data on the *K-PGD* segment were available in the NST breed. The pooled male estimates are identical with those reported by SANDBERG and ANDERSSON (1984). There were no significant differences between breeds for the recombination fraction between *Al* and *Es* loci in either sex (χ^2_1 for males = 0.43, P = 0.51; χ^2_1 for females = 0.10, P = 0.75), and it is obvious that the major heterogeneity in recombination lies between sexes. Thus, recombination estimates were pooled within sex over breeds. The female recombination estimates were significantly higher in both the *Al-Es* segment (χ^2_1 = 54.10, P < 0.001) and the *K-PGD* segment (χ^2_1 = 9.21, P < 0.005). The ratios of female to male recombination were 1.27 and 1.29 for the *Al-Es* and the *K-PGD* segment, respectively.

Influence of age: The possible influence of the age of the parent on recombination was evaluated within each sex and breed separately. Since the age records were complete, the data set used in the age analysis is the same as that summarized in Table 1. An extensive amount of data were, thus, available from the ST breed for all combinations of segment and sex, except for female recombination in the *K-PGD* segment. For this breed, recombination estimates obtained by pooling data within age classes over parents are given in Table 2. The data on male

TABLE 1

Male and female recombination fractions for two linkage pairs in the horse

Segment	Sex	Breed	No. of parents	No. of offspring	No. of recombinants	% Recombination ± SE
Al-Es	Male	NST	5	340	130	38.2 ± 2.6
		ST	33	4013	1463	36.5 ± 0.8
		Pooled	38	4353	1593	36.6 ± 0.7
	Female	NST	104	174	79	45.4 ± 3.8
		ST	773	1686	787	46.7 ± 1.2
		Pooled	877	1860	866	46.6 ± 1.2
K-PGD	Male	ST	43	2718	702	25.8 ± 0.8
	Female	ST	177	363	121	33.3 ± 2.5

TABLE 2

Recombination fractions in the ST breed according to the age of the heterozygous parent at conception

	% Recombination ± SE (n/n_s)		% Recombination ± SE (n)
Age (yr)	*Al-Es*, males	*K-PGD*, males	*Al-Es*, females
2			50.0 ± 17.7 (8)
3	35.3 ± 11.6 (17/3)		59.5 ± 7.6 (42)
4	12.5 ± 8.3 (16/6)	0.0 ± 0.0 (4/3)	45.0 ± 4.7 (111)
5	50.0 ± 10.2 (24/8)	43.8 ± 12.4 (16/5)	50.0 ± 3.7 (186)
6	40.4 ± 5.2 (89/14)	28.6 ± 4.7 (91/13)	49.3 ± 3.5 (201)
7	32.5 ± 4.4 (114/14)	32.0 ± 4.2 (122/16)	50.7 ± 3.4 (219)
8	31.4 ± 3.7 (156/15)	25.2 ± 3.5 (155/16)	45.7 ± 3.4 (221)
9	41.7 ± 3.6 (187/21)	26.1 ± 3.1 (199/26)	44.0 ± 3.9 (159)
10	33.0 ± 2.7 (309/22)	26.2 ± 2.7 (263/30)	39.5 ± 4.3 (129)
11	40.3 ± 3.1 (243/20)	23.9 ± 2.7 (243/27)	45.5 ± 4.7 (112)
12	36.2 ± 3.2 (229/24)	26.4 ± 2.7 (273/25)	35.1 ± 5.5 (74)
13	36.2 ± 2.6 (343/25)	21.8 ± 2.7 (238/28)	50.0 ± 6.1 (68)
14	32.1 ± 2.6 (318/27)	28.4 ± 3.2 (204/29)	45.9 ± 8.2 (37)
15	36.5 ± 2.6 (351/26)	26.3 ± 2.9 (236/27)	54.5 ± 8.7 (33)
16	37.2 ± 2.6 (352/22)	20.0 ± 3.0 (180/19)	42.9 ± 10.8 (21)
17	31.3 ± 3.1 (224/19)	27.2 ± 4.6 (92/14)	36.0 ± 9.6 (25)
18	42.4 ± 3.7 (177/12)	21.1 ± 4.3 (90/10)	43.8 ± 12.4 (16)
19	35.5 ± 4.4 (121/9)	22.9 ± 6.1 (48/10)	50.0 ± 20.4 (6)
20	33.9 ± 4.3 (121/8)	29.5 ± 6.9 (44/5)	57.1 ± 18.7 (7)
21	46.3 ± 4.8 (108/8)	14.7 ± 6.1 (34/5)	75.0 ± 21.7 (4)
22	37.4 ± 4.7 (107/8)	26.7 ± 6.6 (45/5)	66.7 ± 27.2 (3)
23	39.8 ± 4.7 (108/6)	33.3 ± 6.4 (54/6)	
24	32.9 ± 5.1 (85/4)	36.6 ± 7.5 (41/4)	
25	41.3 ± 5.7 (75/4)	28.6 ± 8.5 (28/2)	
26	35.4 ± 6.9 (48/3)	33.3 ± 15.7 (9/1)	
27	45.6 ± 6.6 (57/3)	33.3 ± 15.7 (9/1)	
28	36.0 ± 9.6 (25/1)		
29	22.2 ± 13.9 (9/1)		

n = Total number of offspring pooled over parents. n_s = Total number of stallions represented in the age class.

recombination are based on a limited number of stallions, and the age distribution among stallions is heterogeneous (*cf.* Tables 5 and 6). This means that pooling data over stallions might cause a spurious age effect due to differences in recombination fraction among stallions, especially in those age classes with observations from a few stallions only; the number of stallions contributing to each age class is indicated in Table 2. Nevertheless, the pooled data should disclose whether there is a consistent trend in recombination with increasing age. A similar spurious effect on female recombination is very unlikely as a large number of mares were involved and as no mare had more than a single offspring in any age class.

An inspection of the data given in Table 2 reveals no apparent linear trend with increasing age in any segment or sex. The results of the curvilinear regression analyses applied to the data are compiled in Table 3. The best fit for male recombination in the *Al-Es* segment was obtained for a linear regression on age, but the small positive regression coefficient was nonsignificant. For the other two combinations a quadratic relation, fitting a U-shaped curve, was indicated. Only the effect on male recombination in the *K-PGD* segment was statistically significant. This significance was due to higher recombination fractions at low and high age compared with an intermediate age interval (about 8–22 years, Table 2) in which the recombination fractions appeared to be homogeneous. The age effect was not highly significant and was due to observations in those age classes with sparse data from a limited number of stallions. This implies that a spurious association (due to a stallion effect) cannot be excluded.

For a more complete analysis of the effect of age on male recombination it was necessary to analyze this effect and the possible difference in recombination among stallions simultaneously. Since the mean number of offspring per stallion and age class was small, we decided to pool age classes in order to avoid large numbers of cells with small expected frequencies. The limits of the age intervals were set somewhat arbitrarily by guidance of the result from the pooled data (Tables 2 and 3). The nonsignificant indication of a linear relationship on age for recombination between the *Al* and *Es* loci suggested that three age intervals (3–11, 12–20 and 21–29 years) with an equal number of age classes in each interval would be appropriate. The limits set for the *K-PGD* linkage (4–7, 8–22 and 23–27 years) followed the indication in the pooled data of an increased recombination frequency at low and high age. Additional clumping of data was made in a few cases with small expected frequencies in a particular age interval. The possible interaction between stallion, age and recombination was then analyzed by using three-way log-linear models (results in Table 4). Since there was no indication of a three-factor interaction for any segment, the analysis continued to test two-factor interactions after the three-factor term had been dropped from the model. No significant interaction was revealed, either between stallions and recombination or between age and recombination in the *Al-Es* segment. With regard to the *K-PGD* segment, there was a significant heterogeneity in recombination between stallions ($P = 0.02$; Table 4), but this significance disappeared when the deviating stallion 355 (*cf.* next section) was excluded from the analysis ($P = 0.16$). The analysis revealed no age effect on recombination

TABLE 3

Regression of recombination fractions on age in the ST breed[a]

Interval and sex	Source of variation	d.f.	MS	*F*	P	Regression coefficients
Al-Es, male[b]	Linear regression	1	0.360	1.11	0.30	0.104°
	Unexplained	24	0.323			
K-PGD, male[c]	Explained	2	0.730	4.23	0.03	
	Linear	1	0.016	0.09	0.77	−1.511°
	Quadratic	1	1.444	8.38	0.01	0.050°
	Unexplained	19	0.172			
Al-Es, female[d]	Explained	2	0.519	2.21	0.16	
	Linear	1	0.562	2.39	0.15	−1.549°
	Quadratic	1	0.476	2.03	0.18	0.063°
	Unexplained	10	0.235			

[a] The recombination fractions were transformed by using the angular transformation and weighted by the sample size (*n*).

[b] Age classes 28–29 years were pooled in the analysis.

[c] Age classes 4–5 and 26–27 years were pooled in the analysis.

[d] Age classes 2–3, 14–15 and 16–22 years were pooled in the analysis.

TABLE 4

Analysis of interaction between stallion (S), age (A) and recombination (R) using log-linear models

	Segment *Al-Es*			Segment *K-PGD*		
Interaction	*G*	d.f.	P	*G*	d.f.	P
SAR	20.31	23	0.62	9.31	11	0.59
SR	31.03	32	0.52	64.04	42	0.02
AR	3.00	2	0.22	2.42	2	0.30

such as was indicated in the regression analyses. However, the fact that only a limited number of stallions had offspring in more than one age interval reduced the possibility of detecting a potential age effect by the log-linear analysis.

The data from the NST breed on recombination in the *Al-Es* segment and from the ST breed on female recombination in the *K-PGD* segment were quite limited. For these combinations only a simple test of the age effect on recombination was performed by dividing the pooled data into two classes representing observations above and below the mean age. The analysis revealed no significant heterogeneity, either for the *Al-Es* segment, NST breed (χ^2_1 for males = 1.04, P = 0.31; χ^2_1 for females = 0.00, P = 0.99) or for female recombination in the *K-PGD* segment, ST breed ($\chi^2_1 = 0.09$, P = 0.76).

Distribution of male recombination estimates: Individual male recombination estimates are given in Tables 5 and 6 for the *Al-Es* and the *K-PGD* segment, respectively, together with information on breed origin, paternal half-sib relationships and age interval in service.

TABLE 5

Individual male recombination fractions for the Al-Es *segment, listed in ascending order for 38 stallions*

Identification no. of stallion	Breed	Sire family[a]	Age interval in service[b] (yr)	% Recombination ± SE (*n*)	χ^2[c]
150	ST		9–17	22.9 ± 6.1 (48)	3.9*
7587	ST		9–17	27.3 ± 4.5 (99)	3.8
13307	ST		9–14	28.0 ± 6.3 (50)	1.6
24752	ST		6–9	28.8 ± 5.6 (66)	1.7
2001	ST		3–17	28.8 ± 3.6 (156)	4.1*
22569	NST		5–8	29.8 ± 6.1 (57)	1.1
7766	ST		9–17	29.8 ± 4.1 (124)	2.4
11124	ST		4–14	32.9 ± 5.3 (79)	0.5
513	ST		12–22	33.3 ± 3.3 (201)	0.9
1	ST	B	6–16	34.0 ± 4.6 (106)	0.3
661	ST		16–29	34.7 ± 3.9 (147)	0.2
281	ST	B	5–16	35.4 ± 3.7 (164)	0.1
95	ST		10–18	35.4 ± 5.9 (65)	0
30680	ST	B	15–16	35.8 ± 5.3 (81)	0
172	ST	C	5–17	35.8 ± 4.1 (134)	0
180	ST		4–16	36.2 ± 5.0 (94)	0
146	ST	A	9–19	36.6 ± 4.8 (101)	0
3	ST	C	13–26	37.2 ± 3.2 (226)	0
13883	ST	D	6–11	37.2 ± 3.2 (234)	0
201	ST		8–18	37.4 ± 4.7 (107	0
11448	ST	D	4–11	37.7 ± 5.5 (77)	0
5368	ST	A	11–15	37.9 ± 4.5 (116)	0.1
254	ST		13–23	38.1 ± 4.2 (134)	0.1
276	ST		7–27	38.3 ± 3.0 (264)	0.3
13365	NST		9–14	38.5 ± 5.0 (96)	0.2
2183	ST		13–23	38.6 ± 2.9 (290)	0.5
2163	NST		14–20	38.8 ± 7.0 (49)	0.1
17031	ST		3–10	38.8 ± 6.0 (67)	0.2
8340	ST	B	3–8	38.9 ± 6.6 (54)	0.1
25145	ST	C	11–15	39.4 ± 5.0 (94)	0.3
2166	NST		13–22	40.5 ± 5.7 (74)	0.5
818	ST	A	7–17	40.7 ± 6.7 (54)	0.4
3679	ST		12–22	40.8 ± 7.0 (49)	0.3
1534	ST		9–17	41.3 ± 7.3 (46)	0.5
23616	ST		12–16	42.1 ± 4.3 (133)	1.7
13576	NST		5–12	42.2 ± 6.2 (64)	0.8
347	ST	A	9–18	42.7 ± 3.1 (253)	4.1*
280	ST		12–23	44.0 ± 5.0 (100)	2.4
Total				36.6 ± 0.7 (4353)	33.4[d]

[a] Indications of paternal half-sib relations among stallions; *i.e.*, those with the same letter have a common sire. The same designations of sire families have been used in Tables 5 and 6.

[b] Represents age of the stallion at conception of offspring.

[c] Contribution of each stallion to the total chi-square value in the homogeneity test.

[d] d.f. = 37.

* $P < 0.05$.

TABLE 6

Individual male recombination fractions for the K-PGD *segment, listed in ascending order for 43 stallions*

Identification no. of stallion	Sire family[a]	Age interval in service[b] (yr)	% Recombination ± SE (*n*)	χ^{2c}
355		14–21	0.0 ± 0.0 (25)	8.7**
112		4–13	6.7 ± 6.4 (15)	2.8
177		13–15	11.1 ± 6.0 (27)	3.1
100		13–24	13.3 ± 8.8 (15)	1.2
14039		9–14	15.0 ± 8.0 (20)	1.2
1189		18–24	15.6 ± 5.4 (45)	2.4
818	A	7–17	15.6 ± 6.4 (32)	1.7
101		8–15	16.3 ± 5.3 (49)	2.3
15919		11–14	16.7 ± 5.4 (48)	2.1
1534		9–17	17.5 ± 6.0 (40)	1.5
15308		10–14	17.6 ± 9.2 (17)	0.6
759	A	9–18	18.5 ± 5.3 (54)	1.5
12750		9–16	19.0 ± 8.6 (21)	0.5
632		15–23	20.8 ± 8.3 (24)	0.3
407	A	6–12	20.9 ± 4.3 (91)	1.2
5368	A	9–15	21.1 ± 4.7 (76)	0.9
149	A	7–16	21.2 ± 3.8 (118)	1.3
1137	B	8–19	21.4 ± 7.8 (28)	0.3
10784		6–13	21.4 ± 6.3 (42)	0.4
1916	F	5–15	22.8 ± 3.6 (136)	0.7
18934	A	10–15	24.4 ± 4.9 (78)	0.1
9618	D	4–7	25.0 ± 10.8 (16)	0
24400	C	15–19	25.8 ± 5.6 (62)	0
11124		9–14	26.2 ± 6.8 (42)	0
13883	E	6–11	26.6 ± 3.7 (143)	0
23616	F	12–16	26.8 ± 4.9 (82)	0
30680	B	15–16	27.1 ± 5.8 (59)	0
347	A	9–18	27.2 ± 3.5 (162)	0.1
213	A	8–15	28.2 ± 4.2 (117)	0.4
2183		13–25	28.8 ± 3.3 (191)	0.9
172	C	5–17	29.3 ± 5.3 (75)	0.5
756	A	6–18	29.3 ± 3.7 (150)	0.9
13160	A	9–16	29.5 ± 5.8 (61)	0.4
146	A	9–19	29.7 ± 4.5 (101)	0.8
27372		6–8	30.4 ± 9.6 (23)	0.3
3	C	10–27	32.0 ± 4.2 (122)	2.4
23454	E	6–10	32.5 ± 4.3 (117)	2.7
1590	C	8–11	33.3 ± 11.1 (18)	0.5
13307	D	9–14	34.4 ± 8.4 (32)	1.2
396		6–16	35.7 ± 6.4 (56)	2.8
2476		5–7	40.0 ± 8.3 (35)	3.6
13519		7–10	41.9 ± 8.9 (31)	4.2*
21047	B	10–12	45.5 ± 10.6 (22)	4.4*
Total			25.8 ± 0.8 (2718)	61.4*[d]

[a] Indications of paternal half-sib relations among stallions; *i.e.*, those with the same letter have a common sire. The same designations of sire families have been used in Tables 5 and 6.

[b] Represents age of the stallion at conception of offspring.

[c] Contribution of each stallion to the total chi-square value in the homogeneity test.

[d] d.f. = 42.

* $P < 0.05$; ** $P < 0.01$.

A significant heterogeneity was revealed between stallions for the recombination frequency at the *K-PGD* segment ($\chi^2_{42} = 61.36$, P = 0.03) but not for the *Al-Es* segment ($\chi^2_{37} = 33.38$, P = 0.64). The distribution of recombination estimates for the *K-PGD* segment appeared to follow a normal distribution rather well, and no apparent bimodality was observed (see Table 6); the estimates were in the range 0 to 45.5% recombination. Stallion 355, which had no recombinant offspring among a total of 25 informative offspring, contributed considerably to the heterogeneity among stallions. The chi-square value obtained when this stallion was excluded, however, was still large ($\chi^2_{41} = 52.26$, P = 0.11) indicating a more general heterogeneity.

There were four and six paternal half-sib groups among the stallions informative for the *Al-Es* segment (Table 5) and the *K-PGD* segment (Table 6), respectively. The recombination fractions for stallions involved in any of those half-sib groups were analyzed by a hierarchical contingency chi-square test following the method given by SMOUSE and WARD (1978); the total chi-square value was partitioned into two components testing for differences within and between half-sib groups. No indication of a heterogeneity was obtained at any level, either in the *Al-Es* segment (χ^2_9 for within half-sib groups = 2.13, P = 0.99; χ^2_3 for between half-sib groups = 2.32, P = 0.51; χ^2_{12} for total = 4.47, P = 0.97) or in the *K-PGD* segment (χ^2_{18} for within half-sib groups = 15.95, P = 0.60; χ^2_5 for between half-sib groups = 4.95, P = 0.42; χ^2_{23} for total = 20.92, P = 0.59).

The analysis of a specific allele effect may be performed on all marker loci available but was confined in the present study to the two loci involved in respective linkage. Three alleles were recognized at the *Al* locus (Al^A, Al^I and Al^B), the *Es* locus (Es^F, Es^I and Es^S) and the *PGD* locus (PGD^D, PGD^F and PGD^S) among stallions included in the study. A heterogeneity chi-square test revealed no association between genotype and recombination at any locus. Furthermore, within each dilocus genotype there was no indication of a difference between linkage phases.

Recombination estimates from both segments were available for 13 stallions (identification numbers 3, 146, 172, 347, 818, 1534, 2183, 5368, 11124, 13307, 13883, 23616 and 30680). There was no indication of a correlation between recombination estimates from the two segments.

A tendency of an increased recombination frequency among stallions having large progeny groups was noted in both linkage segments. The recombination estimates for the *Al-Es* segment among stallions having at least 250 offspring and those having fewer than 250 offspring were 37.8 and 35.9%, respectively. The corresponding estimates for the *K-PGD* segment was 27.6 and 24.3%, respectively. The difference was statistically significant for the *K-PGD* segment ($\chi^2_1 = 3.95$, P = 0.05) but not for the *Al-Es* segment ($\chi^2_1 = 1.65$, P = 0.20). However, at present it cannot be excluded that these differences were a spurious effect due to a general heterogeneity among stallions. A more detailed examination of this observation was beyond the scope of the present study.

DISCUSSION

The amount of data on genetic recombination analyzed in the present study is unique for a large mammal. Data on two equine linkage intervals (*K-PGD* and

Al-Es) belonging to different linkage groups (I and II, respectively) have been examined for the detection of factors affecting recombination. It is evident that the sex of the parent is the factor with the most important influence on recombination revealed in the present study. A significantly lower male recombination frequency was observed in both segments (Table 1). The ratio of female to male recombination was estimated at 1.27 and 1.29 for the *Al-Es* and the *K-PGD* segment, respectively. The result is in agreement with the most common observation in other animals (*cf.* DUNN and BENNETT 1967), namely, that when a significant difference is found, recombination is less frequent in the heterogametic sex. It also indicates that the statement by RAO *et al.* (1978), that sex is the major cause of a variable recombination for linked loci in man, may apply also to the horse. The importance of reporting linkage data on males and females separately is evident. An indication of a sex difference in the recombination frequency between the equine *Al* and *Es* loci has previously been reported by WEITKAMP, GUTTORMSEN and COSTELLO-LEARY (1982).

When examining the influence of age on recombination, our most striking observation was the lack of evidence of any effect of this factor. The regression of female recombination on age in the *Al-Es* segment was insignificant but indicated a curvilinear relationship (Table 3); the high female recombination frequency (46.6%) in this segment suggested that it is likely that the sensitivity in this test was reduced by the occurrence of double and multiple crossovers. There was no significant change in male recombination in any segment within a middle age interval (about 8–22 years) comprising the great majority of observations in the present study. In the tails of the age distribution the data were more sparse and based on a small number of stallions. More data from these age intervals are needed to evaluate the indication of a higher recombination frequency at low and high age in the *K-PGD* segment. In conclusion, the results of the present study, together with the conflicting results obtained in other mammalian species (*cf.* ELSTON, LANGE and NAMBOODIRI 1976; WALLACE, MACSWINEY and EDWARDS (1976), may be interpreted as evidence that age *per se* has no unequivocal effect of any significance on recombination frequencies.

The genetic background had no apparent influence on the recombination frequency in the *Al-Es* segment, since there were no significant differences for this character between breeds in either sex (Table 1). The two horse breeds, the ST and the NST, involved in this study are of quite different origin as reflected by the exterior of the horses as well as by the striking allele frequency differences at a number of electrophoretic and blood group loci (SANDBERG 1974b). Our results agree with those of ANDRESEN (1966) who observed no significant difference in recombination estimates between the pig breeds Hampshire and Duroc for one linkage interval studied. The fact that there was no significant heterogeneity in male recombination among genotypes at marker loci or among paternal half-sib groups is further lack of evidence of genetic variation for recombination frequency. With regard to the half-sib analyses, the possibility of detecting genetic variation by such an analysis is reduced for binomial characters such as recombination, compared with characters measured quantitatively, due to the binomial variance within each measured individual for the former character (VALENTIN 1973).

A significant heterogeneity among stallions for the recombination frequency in the *K-PGD* segment was observed in the present study. A large proportion of this heterogeneity was due to the sire 355 which had no recombinant among 25 informative offspring. An inversion or other crossing over suppressor is a plausible explanation for the observation of such a deviating stallion. There was no information available on the recombination frequency for any close relative of this stallion. The karyotype of sire 355 could not be analyzed as he is now dead, but the possibility of a chromosomal change could be investigated in his offspring. Such a study is worthwhile and will be attempted since it might be an opportunity to assign a linkage group to a specific chromosome, which has not yet been done in the horse. With regard to the remaining stallions, the distribution of recombination estimates appeared to be unimodal, suggesting that the data derive from a single population. However, the chi-square value in the contingency test was still large ($P = 0.11$), indicating a more general heterogeneity in recombination for this chromosome segment.

The distribution of recombination values has been studied extensively in some experimental organisms (McCLELLAND and SMITHSON 1968; BROADHEAD and KIDWELL 1975; BUTLER 1977). These studies, based on observations from mosquito flies, Drosophila and tomato plants, respectively, indicate that the variability in recombination often is larger than expected from the binomial variance. Information on the within-population variability of recombination frequencies in mammalian species is very sparse. Experience with the horse suggest that a variability may be common in mammalian species too. A significant heterogeneity among stallions for the frequency of recombination has been observed in the present study for the *K-PGD* linkage and previously for the *Xk-PHI* linkage (ANDERSSON, JUNEJA and SANDBERG 1983). In both of these cases, all recombination estimates were in agreement with linkage, and consequently, the heterogeneity merely reflected a variation in the intensity of linkage. In man, WEITKAMP, GUTTORMSEN and GREENDYKE (1971) reported on a possible heterogeneity between families for the recombination fraction between the *PGD* locus and the blood group locus *Rh*.

A wide variability in the frequency of recombination is, thus, well documented for several plant and invertebrate species and may be common also in mammalian species. This circumstance must be anticipated in studies made with the aim of detecting factors affecting recombination, because the causes of the variability are often unknown and, therefore, uncontrollable. It is recommended that observed differences be interpreted with caution until they have been confirmed by independent data. With regard to the present study, the indication of recombination as being more frequent among stallions having large progeny groups should be further investigated.

We are indebted to Ö. DANELL, B. GAHNE, U. OLSSON, J. RENDEL, N. RYMAN and L. R. WEITKAMP for helpful comments on the paper and to the staff of the Blood Typing Unit at the Department of Animal Breeding and Genetics for valuable assistance. The investigation was supported by grants from the Swedish Racing Board.

LITERATURE CITED

ANDERSSON, L., 1983 Linkage analysis of half-sib groups: a note on the use of double intercrosses. J. Hered. In press.

ANDERSSON, L., R. K. JUNEJA and K. SANDBERG, 1983 Genetic linkage between the loci for phosphohexose isomerase (PHI) and a serum protein (Xk) in horses. Anim. Blood Groups Biochem. Genet. **14:** 45–50.

ANDRESEN, E., 1966 Linkage between the *K* blood-group locus and the *Hp* locus for hematin-binding globulins in pigs. Genetics **54:** 805–812.

BISHOP, Y. M. M., S. E. FIENBERG and P. W. HOLLAND, 1975 *Discrete Multivariate Analysis: Theory and Practice.* MIT Press, Cambridge, Massachusetts.

BODMER, W. F., 1961 Viability effects and recombination differences in a linkage test with pallid and fidget in the house mouse. Heredity **16:** 485–495.

BRIDGES, C. B., 1915 A linkage variation in *Drosophila.* J. Exp. Zool. **19:** 1–21.

BROADHEAD, R. S., and J. F. KIDWELL, 1975 A note on the distribution of the recombination fraction in *Drosophila melanogaster.* J. Hered. **66:** 309–310.

BROWN, M. B., 1981 P4F: Two-way and multiway frequency tables: measures of association and the log-linear model. pp. 143–196. In: *BMDP Statistical Software 1981,* Edited by W. J. DIXON. University of California Press, Berkeley.

BUTLER, L., 1977 Recombination values and their errors. Can. J. Genet. Cytol. **19:** 521–529.

CALLAN, H. G. and P. E. PERRY, 1977 Recombination in male and female meiocytes contrasted. Philos. Trans. R. Soc. Lond. **277:** 227–233.

DUNN, L. C. and D. BENNETT, 1967 Sex differences in recombination of linked genes in animals. Genet. Res. **9:** 211–220.

ELSTON, R. C., K. LANGE and K. K. NAMBOODIRI, 1976 Age trends in human chiasma frequencies and recombination fractions. II. Method for analyzing recombination fractions and applications to the ABO: nail-patella linkage. Am. J. Hum. Genet. **28:** 69–76.

FISHER, R. A., 1949 A preliminary linkage test with agouti and undulated mice. Heredity **3:** 229–241.

GEDDE-DAHL, T., JR., P. J. L. COOK, M. K. FAGERHOL and J. A. PIERCE, 1975 Improved estimate of the *Gm-Pi* linkage. Ann. Hum. Genet. **39:** 43–50.

GEDDE-DAHL, T., JR., R. R. FRANTS, B. OLAISEN, A. W. ERIKSSON, E. VAN LOGHEM and L. LAMM, 1981 The *Gm-Pi* linkage heterogeneity in view of Pi M subtypes. Ann. Hum. Genet. **45:** 143–153.

GOODNIGHT, J. H., J. P. SALL and W. S. SARLE, 1982 The GLM procedure. pp. 139–199. In: *SAS User's Guide: Statistics, 1982 Edition.* SAS Institute, Cary, North Carolina.

HARVEY, W. R., 1982 Least-squares analysis of discrete data. J. Anim. Sci. **54:** 1067–1071.

MAYNARD SMITH, J., 1978 *The Evolution of Sex.* Cambridge University Press, Cambridge.

MAYO, O., 1974 Effect of age on chiasma number in man. Hum. Hered. **24:** 144–150.

MCCLELLAND, G. A. H. and T. W. SMITHSON, 1968 Linkage of *Gold,* its recessive lethality and sex-related variation in crossing-over in *Culex pipiens* (Diptera: Culicidae). Can. J. Genet. Cytol. **10:** 374–384.

MORGAN, T. H., C. B. BRIDGES and A. H. STURTEVANT, 1925 The genetics of *Drosophila.* Bibliogr. Genet. **2:** 1–262.

MORTON, N. E., 1955 Sequential tests for the detection of linkage. Am. J. Hum. Genet. **7:** 277–318.

RAO, D. C., B. J. B. KEATS, N. E. MORTON, S. YEE and R. LEW, 1978 Variability of human linkage data. Am. J. Hum. Genet. **30:** 516–529.

REID, D. H. and P. A. PARSONS, 1963 Sex of parent and variation of recombination with age in the mouse. Heredity **18:** 107–108.

RENWICK, J. H. and J. SCHULZE, 1965 Male and female recombination fractions for the *nail-patella:ABO* linkage in man. Ann. Hum. Genet. **28:** 379–392.

SANDBERG, K., 1974a Linkage between the K blood group locus and the 6-PGD locus in horses. Anim. Blood Groups Biochem. Genet. **5:** 137–141.

SANDBERG, K., 1974b Blood typing of horses: current status and application to identification problems. pp. 253–265. Proceedings of the 1st World Congress on Genetics Applied to Livestock Production, Madrid, Spain, 1974.

SANDBERG, K. and L. ANDERSSON, 1984 Genetic linkage in the horse. I. Linkage relationships among 15 blood marker loci. Hereditas In press.

SIMCHEN, G. and J. STAMBERG, 1969 Fine and coarse controls of genetic recombination. Nature **222:** 329–332.

SMOUSE, P. E. and R. H. WARD, 1978 A comparison of the genetic infrastructure of the Ye'cuana and the Yanomama: a likelihood analysis of genotypic variation among populations. Genetics **88:** 611–631.

SOKAL, R. R. and F. J. ROHLF, 1981 *Biometry*, Ed. 2. W. H. Freeman, San Francisco.

TUCIĆ, N., F. J. AYALA and D. MARINKOVIĆ, 1981 Correlation between recombination frequency and fitness in *Drosophila melanogaster.* Genetica **56:** 61–69.

VALENTIN, J., 1973 Heritability of recombination frequency. Hereditas **75:** 1–4.

WALLACE, M. E., 1957 A balanced three-point experiment for linkage group V of the house mouse. Heredity **11:** 223–258.

WALLACE, M. E., F. J. MACSWINEY and R. G. EDWARDS, 1976 Parental age and recombination frequency in the house mouse. Genet. Res. **28:** 241–251.

WEITKAMP, L. R., 1973 Human autosomal linkage groups. pp. 445–460. In: *Proceedings of the 4th International Congress on Human Genetics*, Vol. 250. Exerpta Medica International Congress Series.

WEITKAMP, L. R., 1974 Population differences in meiotic recombination frequency between loci on chromosome 1. Cytogenet. Cell Genet. **13:** 179–182.

WEITKAMP, L. R., 1976 Linkage of GLO with HLA and Bf: effect of population and sex on recombination frequency. Tissue Antigens **7:** 273–279.

WEITKAMP, L. R., S. A. GUTTORMSEN and P. COSTELLO-LEARY, 1982 Equine gene mapping: a sex difference in recombination frequency for linkage group II. Anim. Blood Groups Biochem. Genet. **13:** 305–306.

WEITKAMP, L. R., S. A. GUTTORMSEN and R. M. GREENDYKE, 1971 Genetic linkage between a locus for 6-PGD and the Rh locus: evaluation of possible heterogeneity in the recombination fraction between sexes and among families. Am. J. Hum. Genet. **23:** 462–470.

WEITKAMP, L. R., J. J. VAN ROOD, E. THORSBY, W. BIAS, M. FOTINO, S. D. LAWLER, J. DAUSSET, W. R. MAYR, J. BODMER, F. E. WARD, J. SEIGNALET, R. PAYNE, F. KISSMEYER-NIELSEN, R. A. GATTI, J. A. SACHS and L. U. LAMM, 1973 The relation of parental sex and age to recombination in the HL-A system. Hum. Hered. **23:** 197–205.

Corresponding editor: D. BENNETT