Genet. Res., Camb. (1994), **64**, *pp.* 161–166 *With 5 text-figures*

Sex differences in chiasma distribution along two marked mouse chromosomes: differences in chiasma distribution as a reason for sex differences in recombination frequency

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(*Received 19 May 1994 and in revised form 28 July 1994*)

Summary

Chiasma distributions along bivalents 1 and 14 in female and male mice were studied. It was shown that the average chiasma number in both chromosomes show no sex difference. There are however, significant sex differences in chiasma distribution along 1 and 14 chromosomes. In males there are two terminal chiasma peaks in chromosome 1 and one subtelomeric peak of chiasmata in chromosome 14. In females chiasma distributions are more even. According to genetic data, females produce more recombinants between loci of chromosome 1 than males do. By means of a computer simulation it was demonstrated that the differences in the average recombination frequency result from differences in chiasma distribution.

1. Introduction

Sex differences in recombination frequency have been shown since long ago. Haldane (1922) was the first who suggested that crossing over should be less frequent in the heterogametic sex. Later on Huxley (1928) came to the same conclusion.

Two approaches are used to investigate sex differences in recombination level: (1) a genetic analysis – a comparison of frequencies of recombinants produced by males and females, and (2) a cytogenetic analysis – a comparison of chiasma frequencies in male and female meiosis.

Cytogenetic analysis shows that in many species chiasma frequency is higher in females than in males (Polani 1972; Traut, 1977). Some species have been described in which chiasma frequencies in male and female meiosis were equal (Hayman *et al.*, 1990). In some species chiasma frequency is higher in male meiosis (Bennett *et al.*, 1986).

It should be noted that in all the above mentioned papers males and females were compared by average number of chiasmata per meiotic cell. However, sex differences in both average chiasma number and chiasma distribution may differ for different chromosomes. To obtain more precise information on the sex differences in recombination it is necessary to study individual chromosomes.

Meiotic chromosomes of the house mouse are suitable for cytological analysis of crossing over. It is easy to obtain diplotene-diakinetic spreads of good quality, where positions of chiasmata are clearly visible. It has been shown that chiasmata in this species do not terminalize, so their location on the diakinetic bivalent represents the actual position of exchanges (Kanda & Kato, 1980). Some individual chromosomes can be identified in meiotic cells after C-band staining (Forejt, 1973). Having the data on chiasma frequency and distribution along the individual chromosome we can compare them to the data on the frequency of crossing over between the genes located on different segments of these chromosomes. More than 1000 genes have been localized on the mouse genetic map. Recombination frequencies between many loci for males and females are available (Davisson *et al.*, 1989).

This paper presents an analysis of chiasma number and distribution along chromosomes 1 and 14 in male and female mice. It also contains the results of computer analysis of the effects of sex differences in chiasma distribution on the average sex differences in recombination frequency. Sex differences in the distribution of crossover exchanges in other chromosomes are also discussed.

2. Materials and methods

Male and female CBA/Lac mice were used for the analysis of chiasma number and distribution at diakinesis. Male meiotic chromosomes were prepared by the method of Evans *et al.* (1964), female ones – by the method described in Chandley's paper (1987).

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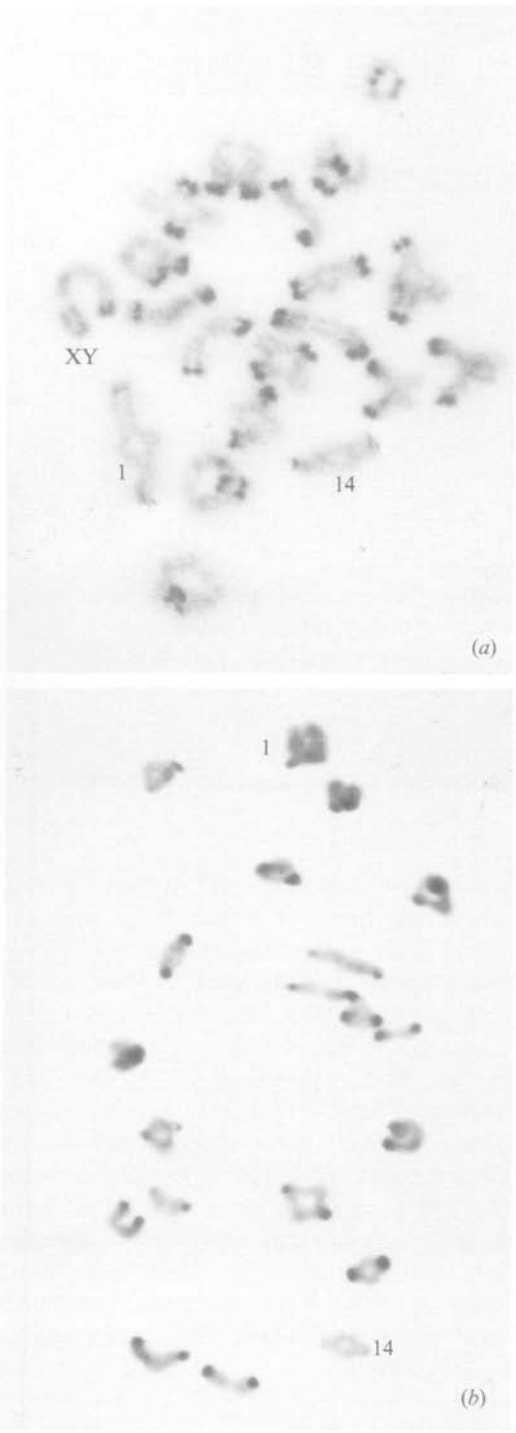

Fig. 1. C-banded male (*a*) and female (*b*) CBA/Lac mouse chromosomes. Bivalents 1 and 14 are marked. On the photo of the male chromosomes a single chiasma in the bivalent 1 is located in the 4th region, in the bivalent 14 – in the 5th region. On the photo of the female chromosomes one can see two chiasmata, located in the regions 2 and 5 of the bivalent 1, and a single chiasma in the 4th region of the bivalent 14.

Slides were C-banded by Sumner's (1972) method. Bivalents 1 and 14 carry a small block of centromeric C-heterochromatin (Forejt, 1973), which allows one to identify those bivalents at diakinesis (Fig. 1; see also Gorlov *et al.*, 1991). A total of 200 diakinesis from four males were studied. Thirty eight 2–4-month-old females were used. 240 oocytes at the diakinetic stage were obtained. Only those diakinetic cells were analysed where chiasma number and position on the bivalents 1 and/or 14 were clearly visible.

Well-spread diakinetic bivalents were traced using a drawing apparatus. Each bivalent of a marked chromosome was subdivided into five equal parts and we determined in what parts of the bivalent chiasmata were located (Fig. 2). The number of chiasmata in each part was estimated (Table 1).

Differences between individual males within genotypes in chiasma distribution were tested according to the methods used by Laurie & Jones (1981). The distribution patterns of chiasmata between genotypes were compared statistically by the chi-square method for two empirical distributions with 4 D.F. (Kendall *et al.*, 1983).

The data on recombination frequencies between loci of chromosomes 1 and 14, and also other chromosomes in male and female mouse tabulated by Davisson *et al.* (1989) were used.

3. Results and discussion

(i) *Sex differences in chiasma distribution along chromosome 1*

Individual differences between males in average chiasma number were not significant and data from separate males were pooled. An average chiasma number in chromosome 1 in males at the diakinetic stage is $1{\cdot}62 \pm 0{\cdot}03$. In females the chiasma number in chromosome 1 is $1{\cdot}67 \pm 0{\cdot}06$. Sex difference in chiasma number is not significant ($t = 0{\cdot}75$).

Since males and females do not differ in the average chiasma number per bivalent, one could expect that the level of genetic recombination must be the same in both sexes.

There are estimates of recombination frequency for 18 pairs of chromosome 1 in males and females separately (Davisson *et al.*, 1989). In general, females produce more recombinants than males do. If recombination frequency in males is assumed as 100%, then the relative recombination frequency in females is 125%.

To test the significance of a sex difference in recombination frequency (Θ_f–Θ_m) we determined if it differs from zero (here Θ_f is a recombination frequency between two specific loci in females, Θ_m is a recombination frequency between the same loci in males). An average difference between females and males for one pair of loci is $3{\cdot}7 \pm 1{\cdot}3\%$. This is significantly higher than zero ($t = 2{\cdot}76$, $P < 0{\cdot}02$). Thus although the males are equal to the females in the average number of chiasmata per bivalent 1, the females produce more recombinants than the males.

We supposed that this disagreement arises on account of a sex difference in chiasma distribution along chromosome 1 (Fig. 3*a*). The chiasma dis-

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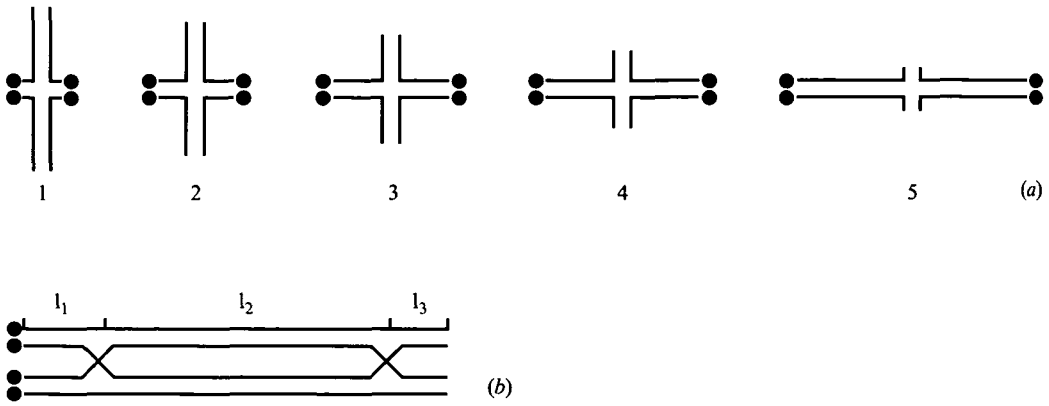

Fig. 2. Morphology of mouse meiotic bivalents; (*a*) – bivalent with a single chiasma, 1–5 – bivalents with chiasma in the regions 1–5; (*b*) – bivalent with two chiasmata. A relative position for the proximal chiasma was counted as $l_1/(l_1+l_2+l_3)$, for the two distal chiasma as $(l_1+l_2)/(l_1+l_2+l_3)$.

Table 1. *Number of observed chiasmata in each of the 5 regions in 1st and 14th bivalents in male and female CBA/Lac mice*

Sex	Bivalent	Number of bivalents analysed	Number of chiasmata in each of the 5 regions 1	2	3	4	5
Male	1	200	106	32	24	38	124
	14	200	38	24	22	32	86
Female	1	58	21	15	12	25	24
	14	72	12	5	18	22	18

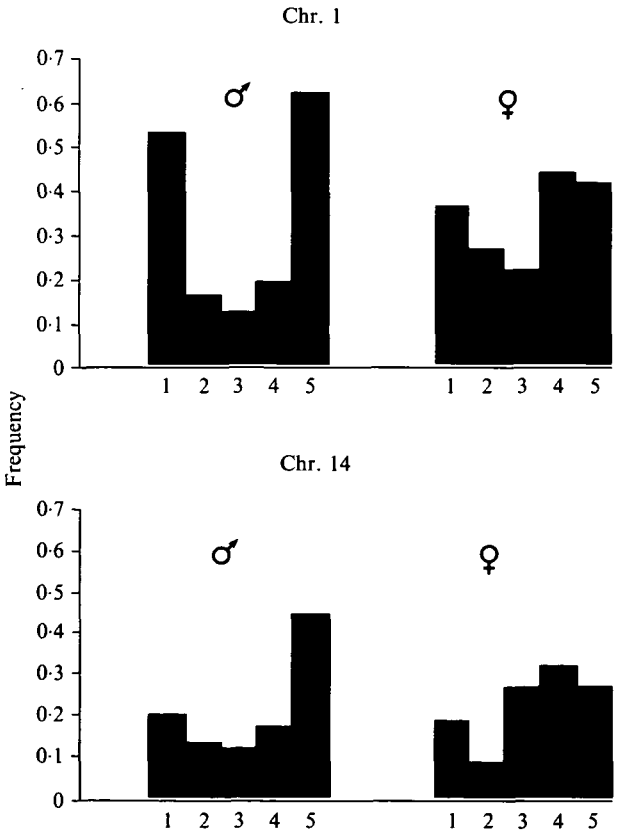

Fig. 3. Chiasma distribution in males and females along bivalent 1 (*a*) and 14 (*b*). Numbering of regions begins from the centromere.

tribution along bivalent 1 in males differs from an even distribution ($\chi^2 = 133{\cdot}6$, $P < 0{\cdot}001$); chiasmata are often formed in terminal parts of bivalent 1 and rarely in the middle part. In general the distribution is U-like.

There are no terminal peaks in the female chiasma distribution. Chiasma distribution in females does not differ from an even distribution ($\chi^2 = 5{\cdot}39$).

Chiasma distribution along bivalent 1 in males rather significantly differs from that in females ($\chi^2 = 22{\cdot}4$, $P < 0{\cdot}01$).

To examine the idea that the difference in chiasma distribution may lead to a sex difference in recombination frequency we used the following computer simulation: (1) in each cycle the computer program "produced" a pair of loci, with locations of these loci on the chromosome 1 being determined by a random number generator; (2) using chiasma distributions found in our experiment, recombination frequencies between these loci were calculated for females (Θ_f) and males (Θ_m) separately (according to their location at the chromosome and chiasma density in the intercept), and (3) the difference between females and males in recombination frequency (Θ_f–Θ_m) was counted.

There were 1000 such cycles in the program. Data obtained were statistically treated: mean value and standard deviation of the sex recombination difference (Θ_f–Θ_m) were calculated. A difference between females

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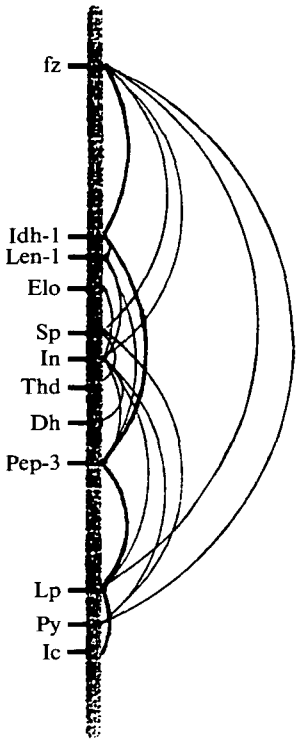

Fig. 4. A fragment of the genetic map of mouse chromosome 1. Lines join the loci recombination frequencies between which are known in males and females. Bold lines join non-overlapping segments of the map.

and males in the recombination frequency per one pair of loci – 6·0 ± 0·2 % – does not differ from the real difference – 3·7 ± 1·3 % – but does differ from zero ($t = 30·0$, $P < 0·001$). Thus the sex difference in chiasma distribution along chromosome 1 is sufficient to cause the observed sex differences in recombination frequency even in the absence of sex difference in chiasma number.

When performing linkage analysis an experimenter usually does not know the location of genes on a chromosome. From this point of view, loci are chosen randomly, and our computer model adequately reflects the situation. If a peak of crossover exchanges is situated in the middle of a chromosome, then two randomly chosen loci will be separated by the peak more often than in the case that the peak is terminal. That is why the sex difference in chiasma distribution may cause the difference in recombination frequency.

Since the total chiasma number in chromosome 1 in females and males is the same, one would expect that the total lengths of genetic maps of chromosome 1 would also be equal. Fig. 4 shows the genetic map of chromosome 1. The loci recombination frequencies between which are known in males and females are joined by lines. There is a considerable overlapping between the regions. If we examine only non-overlapping regions, covering a map segment from *fz* to the *ic* (a total of four regions), the sex difference is 11% and not 25% as it derives from calculations including all the 18 pairs of loci, i.e., in the case of random choice of loci. The 11 % sex difference may be accounted for by the non-completeness of genetic map – absence of terminal markers (in terminal regions level of recombination in males would be higher than in females). To obtain accurate estimates of sex differences in recombination frequency on the basis of genetic data, one should use non-overlapping regions of a chromosome.

(ii) *Sex differences in chiasma distributions along chromosome 14*

As in the previous case, we found no differences between females and males in the average chiasma number per bivalent 14 (1·04 ± 0·02 in females, 1·01 ± 0·01 in males). However, chiasma distributions showed a significant difference (Fig. 3*b*) ($\chi^2 = 20·13$, $P < 0·01$). The difference is similar to that found for the chromosome 1. There is a subtelomeric (but not subcentromeric, as it was in bivalent 1) chiasma peak in males, whereas in females chiasmata are distributed rather evenly along the bivalent.

Unfortunately, in chromosome 14 the recombination frequency was determined both in males and females for six pairs of loci only (Davisson *et al.*, 1989). Although recombination frequency in females is somewhat higher than in males (an average difference for one pair of loci is 1·57 ± 1·99 %), the data are too incomplete for reliable comparison with chiasma data to be made.

(iii) *Sex differences in the recombination level in other chromosomes*

Are there analogous differences in chiasma distribution along other chromosomes? According to our data, an average chiasma number per autosomal bivalent in male mouse of CBA strain is 1·23 ± 0·02; an average chiasma number bivalent in females is 1·21 ± 0·02. The sex difference is not significant. Our estimates of the average chiasma number per bivalent in males and females coincide with those made for this strain by Speed (1977) – 1·19 in 2–12 month old females, 1·26 – in 2 month old females and 1·26 in males. Thus, cytogenetic data show that males and females do not differ in the average chiasma number. However, they may differ in chiasma distributions. The analysis of chiasma distribution along bivalents 1 and 14 shows that the distribution in females is more even than in males. Peaks of exchanges in males are located on terminal parts of the bivalents. For this reason, one would expect that in proximal and distal regions of all the bivalents chiasma frequencies between loci are higher in males than in females.

Table 2 shows the frequencies of recombination in proximal and distal regions of autosomes. We took into account regions which are located the most proximally and distally on the genetic map and for which male and female recombination data are both available. Recombination frequency in the distal pairs

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Table 2. *Frequencies of recombination in the most proximal and distal regions of mouse autosomes*

Chromosomes	Proximal regions					Distal regions				
	Pair of loci		Θ_f	Θ_m	$\Theta_f-\Theta_m$	Pair of loci		Θ_f	Θ_m	$\Theta_f-\Theta_m$
1	*fz*	*Idh-1*	21·40	21·30	0·10	*Lp*	*ic*	2·97	4·60	−1·63
2	*Sd*	*stb*	10·18	11·30	−1·12	*a*	*Ra*	20·62	23·66	−3·04
3	**my*	*ma*	21·32	15·62	5·70	*cdm*	*Va*	8·05	10·53	−2·48
4	*cy*	*b*	21·47	24·11	−2·64	*Pan1*	*Gpd1*	7·32	6·82	0·50
5	*Hm*	*W*	21·20	23·11	1·91	**rd*	*Gus*	7·60	15·35	−7·75
6	*Sig*	*hop*	21·95	13·66	8·29	**mi*	*me*	21·30	25·40	−4·10
7	*Akv1*	*Gpi1*	12·80	13·03	−0·23	**sh1*	*fr*	15·44	16·39	−0·95
8	*Gr1*	*Es1*	15·48	38·24	−22·76	**Es2*	*Prt2*	3·92	12·50	−8·58
9	*Rb163H*	*se*	37·74	37·50	0·24	**Mod1*	*Trf*	8·27	9·36	−1·09
10	**gl*	*Sl*	31·05	17·65	13·40	*Sl*	*cat*	19·21	22·63	−3·42
11	*Tcn2*	*Hba*	18·63	10·50	8·13	*Re*	*tn*	22·83	15·71	7·12
13	*cr*	*Xt*	1·85	0·62	1·23	**mu*	*pe*	29·41	23·62	5·79
14	**pn*	*s*	27·63	24·39	3·24	*slt*	*Rn*	3·03	1·95	1·08
15	**uw*	*Bld*	41·67	16·67	25·00	*Ve*	*N*	0·83	1·92	−1·09
16	*Hc16*	*nd*	8·57	4·08	4·49	—	—	—	—	—
17	*Rb7Bnr*	*T*	5·81	1·96	3·85	**Upg1*	*thf*	6·06	7·27	−1·21
19	**Rb163H*	*bm*	19·63	22·5	−2·87	*ep*	*ru*	1·27	2·18	−0·91
Total			338·38	296·24	42·14	—	—	178·13	199·89	−21·76
Total without pairs marked by asterisks			197·08	199·1	−2·33	—	—	86·13	90·00	−3·87

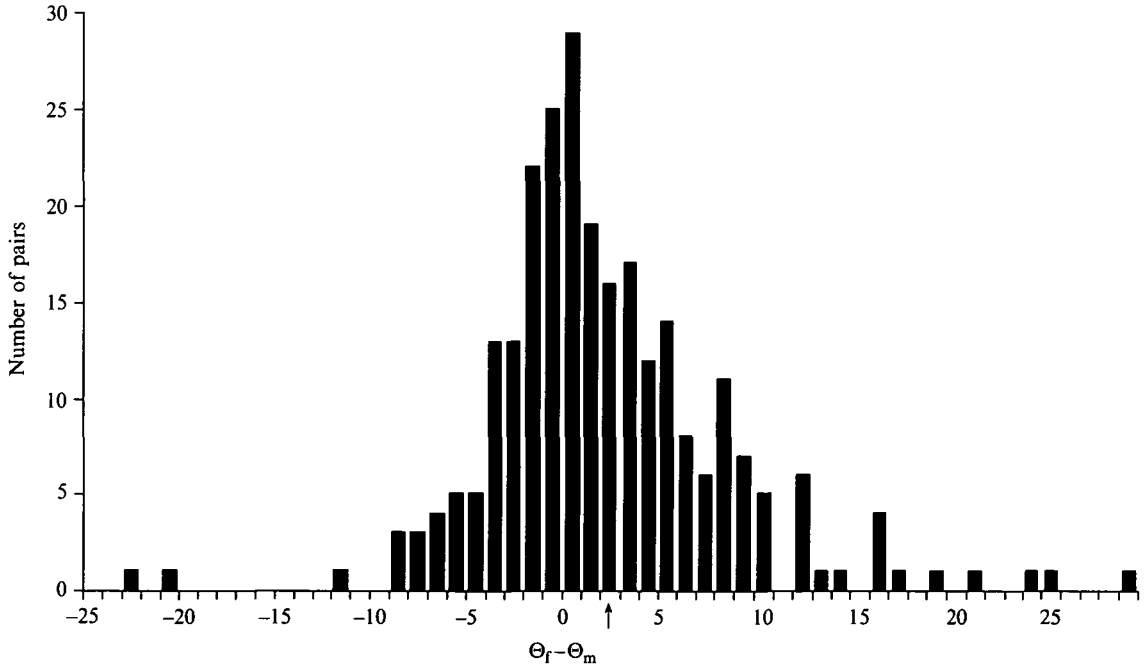

Fig. 5. Distribution of the difference between females and males in recombination frequency. Scale unit is equal to 1% of recombinants. Mean value is indicated by an arrow.

of loci is somewhat higher in males than in females. There is a tendency for long chromosomes (1–10) to show higher recombination frequency in the proximal regions in males and for short chromosomes (11–19) – in females. In short chromosomes, only one chiasma is usually formed. In this case in males there is only one peak of chiasmata near the telomeres (as is observed in chromosome 14) (Gorlov *et al.*, 1987), but not two peaks (subcentromeric and subtelomeric), as in chromosome 1. (In females the chiasmata distributions are more even.)

It is clear that in some cases the most terminal pairs of loci on the genetic map may be in fact not at the ends of the chromosomes (Lyon & Kirby, 1993). These pairs of loci are indicated by asterisks (see Table 2). Ignoring such pairs, recombination frequency in proximal regions, like in distal ones, is also somewhat higher in males than in females.

In total, in the mouse genome recombination frequency in females is significantly higher than in males. On Fig. 5 one can see a distribution of differences between females and males in recombination fre-

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quency (Θ_f–Θ_m) in all regions for which corresponding data are available. A mean value of the distribution is $2{\cdot}22 \pm 0{\cdot}39\%$, which is significantly higher than zero ($t = 5{\cdot}67$, $P < 0{\cdot}001$).

Thus the difference in the frequency of recombinants produced by females and males in some cases may be deduced from the difference in chiasma distributions only. However, it is not the only cause of sex difference in recombination. It has been shown that in some strains of the house mouse chiasma number in females is somewhat higher than in males (Polani, 1972; Speed, 1977). Nevertheless, in those strains there are also sex differences in chiasma distribution (Polani, 1972): a proportion of bivalents with distal chiasmata in males is approximately two times as high as in females. Since the different strains are used for genetic mapping, the sex differences in recombination in some of them may be due to differences in chiasma distribution only, while in other strains the differences are caused by differences both in chiasma number and chiasma distribution.

We thank Dr Pavel M. Borodin and B. M. Wallace for the critical review of the manuscript and for useful discussion. This work was supported by the National Genetic Research Fund.

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