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Research Articles

Distribution of sister chromatid exchanges in different types of chromatin in the X chromosome of *Microtus cabrerae*

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Abstract. We used the X chromosomes of *Microtus cabrerae* as a model to analyze the distribution of sister chromatid exchanges (SCEs) on different types of chromatin, because of the marked heterogeneity of the heterochromatin in the entire short arm and a portion of the long arm of this chromosome. Computer-simulated distributions, according to an algorithm that makes it possible to modify the distribution on the basis of any possible hypothesis, were compared with real distributions by log-linear models. We found that the frequency of SCEs in different types of heterochromatin was higher than that expected for a random distribution, and located an SCE hot-spot at the junction between euchromatin and heterochromatin. The possible relationship between the distribution of SCEs and base composition or chromatin accessibility are discussed.
Key words. Sister chromatid exchanges (SCEs); *Microtus cabrerae*; X chromosome; chromatin structure.

The giant X chromosome of *Microtus cabrerae* shows a large heterochromatic segment[1] in which four different types of heterochromatin have been distinguished on the basis of their response to different banding techniques[2,3]. This heterogeneity is related to the timing of replication[4], and the X chromosome of this species has been used as a model to study the effects of in situ restriction and in situ nick translation on different types of chromatin[5].

Observations concerning the distribution pattern of sister chromatid exchanges (SCEs) and its relationship with different types of chromatin have been contradictory, and as a consequence, such a relationship has not been yet established[6].

Because the X chromosome of *Microtus cabrerae* contains five different classes of chromatin, four of which are heterochromatic and located in the same chromosomal environment, we used this chromosome to study the frequency of SCEs in different types of chromatin under the same experimental conditions. The results were analyzed with computer simulations and log-linear models according to a previously proposed method[7,8].

Materials and methods

Culture conditions. Fibroblast cell cultures from a *Microtus cabrerae* female were grown in Eagle's basal medium at 37 °C. After the addition of 10 μg/ml BrdU and 0.05 μg/ml FdU, cell cultures were incubated in the dark for 48 h. Chromosome preparations were made as previously described[9]. Differential staining of chromatids was done as described elsewhere[10].

Scoring of the SCEs. The X chromosomes of *Microtus cabrerae* show considerable heterogeneity within the heterochromatic block in banding properties and base composition[2,3]. Four different types of heterochromatin were found in this chromosome, in which the G-banding pattern consists of a symmetric region about the centromere, and a tandem repeat of several bands and interbands. On the basis of these findings, and in order to score the SCEs on the X chromosome, we considered five well-defined regions (fig. 1): a) The **P** region, consisting of the tandem repeat in the heterochromatic block, b) the **S** region, consisting of the pericentromeric symmetric bands, c) the **Q** region, consisting of the euchromatin, d) the **PS** region, located at the boundary between **P** and **S**, and e) the **SQ** region, located at the boundary between **S** and **Q**.

The extent of the different regions was determined as follows: Measurements were taken on magnified photographs of 30 G-banded X chromosomes. To minimize the effect of the different degree of chromosomal contraction in different metaphase plates, we divided the length of the different regions by total chromosome length. To further simplify computer analysis 1000 arbitrary units were assigned to the length of the p arm. Accordingly, the total chromosome length was 2360 units. SCEs which could not be unambiguously located on the **P**, **S** or **Q** regions were considered to lie on **PS** or **SQ**. For this purpose, these regions were considered to have a length of 200 units, ie, 100 units on either side

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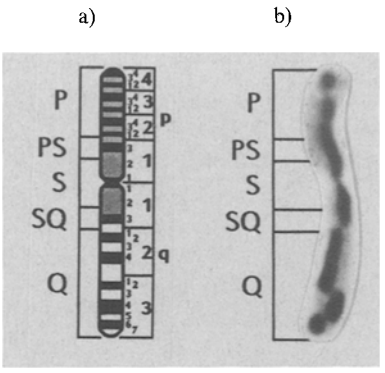

Figure 1. *a*) Regions into which we divided the X chromosome of *Microtus cabrerae* to score the sister chromatid exchanges. *b*) An example of sister chromatid exchange score, showing one SCE in the **P** region, one SCE in the **SQ** region, and two SCEs in the **Q** region.

of the boundary between **S** and **P**, or between **S** and **Q**. In accordance with these considerations, the **S** region was considered to be 344 units long, the **PS** and **SQ** regions 200 units long, and the rest of the p and q arms were considered to be the **P** and **Q** regions, measuring 628 and 988 units, respectively (fig. 1).

Statistical methodology. The number of SCEs observed on each region was scored in individual X chromosomes, and the frequency distribution of SCEs per X chromosome and per chromosome region were determined.

Computer simulations. The method used to analyze these data was inspired by studies performed by Vercauteren et al. on human chromosomes[7,8]. These authors attempted to determine the real distribution of SCEs in human lymphocytes by generating computer-simulated SCEs scores and using log-linear models to compare them with the observed distribution.

For computer simulation we programmed a simple algorithm which made it possible to modify the distribution of SCEs by altering the probability that a given SCE would lie within a defined region (fig. 2). The number of SCEs located on each simulated X chromosome was computed from the observed cumulative distribution of SCEs per X chromosome[7,8]. Each previously chosen SCE was thus located on a specific chromosome region. The region was assigned in accordance with the cumulative frequency distribution of the relative length of the different regions. Nevertheless, we introduced some modifications in the previously described method[7,8] which made it easier to determine the rules that govern the distribution of SCEs. Assuming a random distribution, the number of SCEs on a given chromosome region will be proportional to its length, irrespective of the location of the SCE on the chromosome. If we modify the relative length of a given region, we change the probability of an SCE falling within this region, thus increasing the frequency of SCEs per unit length. We included in the algorithm a so-called "initial probability factors" (IPFn) for each region, and multiplied the length of each region by its IPF before its relative length was calculated. For example, to obtain the simulated distribution of SCEs under the hypothesis that the frequency of SCEs per unit length is twice as high in the **P** region as in the rest of the X chromosome, we defined the following values: IPFp = 2,

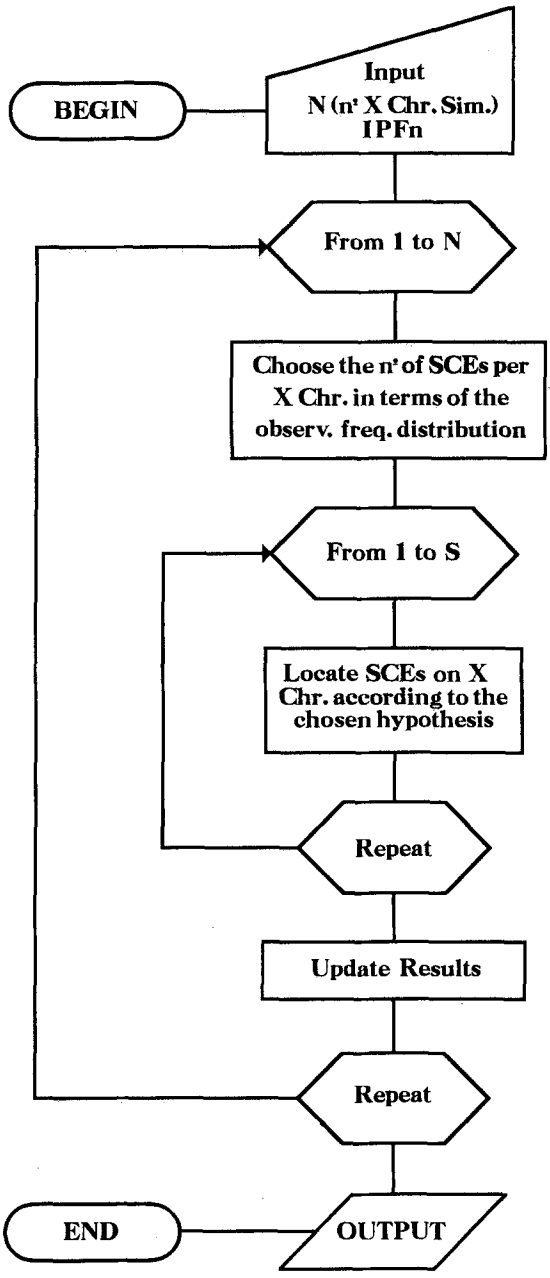

Figure 2. Flow chart of the algorithm used in the simulation to generate sister chromatid exchange scores according to hypothetical distributions of SCEs in metaphasic X chromosomes. (N) indicates the number of X chromosomes simulated. For each X chromosome simulated, a randomly chosen number (S) determines the total SCEs that the chromosome will have. The hypothesis that determines the final location of the SCEs chosen (S) depends on the value assigned to the IPFn.

Table 1. Distribution of sister chromatid exchanges per chromosomal region.

Region	SCE			
	0	1	2	3
P	549	164	13	0
PS	664	61	1	0
S	599	119	7	1
SQ	643	78	5	0
Q	553	151	22	0

IPFps = IPFs = IPFsq = IPFq = 1. The values of these factors were estimated with a binomial distribution, according to the following formula:

$$N_j(n) = \sum_{c=n}^{\text{Max}} \frac{c!}{n!(c-n)!} p^n (1-p)^{c-n} \text{SCE}c$$

where N is the number of a particular region j with n SCEs, p is the probability that this region will contain an SCE, and SCEc is the number of scored X chromosomes with c SCEs.

This formula made it possible to calculate the number of regions which fall within a particular SCE category assuming a random distribution; these data make it possible to directly calculate the IPFn to be used in a simulation: the frequency of SCEs per region for both scored and theoretical distributions may be calculated by dividing the number of SCEs (scored or calculated) in a particular region by the total number of SCEs. The quotient given by F(scored)/F(calculated) for each region provides factors which, after being normalized by dividing by the smaller of them, are equal to the IPFn to be used in the simulation. The IPFn may also be estimated from the relative density of SCEs per chromosomal region, which is calculated by dividing the mean number of SCEs in that region by its length. These relative densities are normalized by dividing them by the lowest density, which gives values similar to those found with the binomial estimation method.

Analysis by log-linear models. Finally, the simulated and observed frequency distributions of SCEs for each chromosomal region were compared with log-linear models[7,8] applied to three-way contingency tables[11].

Because we wished to obtain a hypothesis that generated simulated frequencies proportional to the scored ones, the data in the contingency table had to be independent of whether they were scored or simulated. Thus, the interaction terms of the model that includes the effect of the experimental term had to be eliminated from the saturated log-linear model. In the hierarchical model shown below (abbreviated E,SR), the terms 'number of SCEs', 'chromosomal region', and 'experimental condition' (scored or simulated) are designated S, R and E respectively:

$$\ln F_{ijk} = \mu + S_i + R_j + E_k + SR_{ij}$$

Results

A total of 726 *M. cabrerae* X chromosomes were analyzed, 268 with no SCEs, 294 with a single SCE, 126 with two SCEs, 30 with three SCEs, 6 with four SCES and 2 with 6 SCEs. The scored SCEs were distributed on the different chromosome regions as shown in table 1.

Simulations. A total of 10^6 X chromosomes were simulated; this number of samples gave differences of less than 0.02 between the exceedance probabilities of the E,SR model for ten different simulations.

Random distribution hypothesis. We initially assumed that SCEs were randomly distributed throughout the length of the X chromosome. To test this hypothesis, the IPF associated with each region was given a value of 1. The simulated data were then placed in a three-way table together with the scored data. Analysis of these data with the 4F program for the analysis of multiway tables by means of log-linear models from the BMDP package[12] demonstrated the absence of fit for the E,SR model, showing that SCEs were not randomly distributed (maximum likelihood Chi-square $G = 67.5$, $p < 0.001$).

Real distribution

Binomial estimation of IPFn. The results obtained with the formulas deduced from the statistical methodology (see Materials and methods) are summarized in table 2. These data, together with the scored data of table 1, allow us to estimate the IPFn as shown in table 3. The estimated IPF values were used in a new simulation, and as expected (if the model is correct) the fit was excellent ($G = 5.24$, $p = 0.9822$).

Relative density of SCEs. A second method of estimating the IPF values was tested. The means of SCEs were calculated for each region by dividing the total number of SCEs scored in the region by the total number of regions analyzed (equal to the total number of X chromosomes analyzed: 726). This gave values of 0.262 SCEs for the **P** region, 0.087 for the **PS** region, 0.187 for the **S** region, 0.121 for the **SQ** region and 0.269 for the **Q** region. Then the relative density of SCEs was

Table 2. Calculated random distribution of sister chromatid exchanges per chromosome region according to the binomial method.

Region	SCEs						
	0	1	2	3	4	5	6
P	565.54	143.55	15.59	1.20	0.11	0.01	0.00
PS	671.02	53.06	1.86	0.05	0.00	0.00	0.00
S	633.76	86.78	5.21	0.23	0.01	0.00	0.00
SQ	671.02	53.06	1.86	0.05	0.00	0.00	0.00
Q	488.16	199.46	33.99	3.80	0.50	0.09	0.01

Table 3. Steps followed to calculate the IPFn by the binomial method.

		Regions				
		P	PS	S	SQ	Q
Scored frequency	No. SCEs	190	63	136	88	195
	Total			/672 total SCEs		
	F scored	0.282738	0.093750	0.202381	0.130952	0.290179
Calculated frequency	No. SCEs	178.82	56.93	97.93	56.93	281.35
	Total			/671.96 total SCEs		
	F calcul.	0.266117	0.084722	0.145738	0.084722	0.418701
IPF	Fs/Fc	1.062458	1.106556	1.388664	1.545665	0.693046
	lowest value			/0.693046		
	IPF	1.533028	1.596658	2.003713	2.230251	1.000000

calculated by dividing each mean by the length of the region involved; these values represented the number of SCEs per unit length (**P**: 4.171×10^{-4}, **PS**: 4.350×10^{-4}, **S**: 5.436×10^{-4}, **SQ**: 6.050×10^{-4}, **Q**: 2.723×10^{-4}). When these values were normalized by dividing by the smallest they gave an estimation of the IPF values for each region (**P**: 1.532, **PS**: 1.598, **S**: 1.997, **SQ**: 2.222, **Q**: 1.000). The IPFn values obtained with this method were similar to those obtained with binomial estimation. Thus, the use of these IPFn in a new computer simulation and subsequent analysis with the program 4F of the BMDP package gave similar results.

Discussion

The distribution of SCEs has been extensively studied in several species, but no general pattern of intrachromosomal distribution is recognizable[6]. The results obtained thus far can be divided into two groups: a) results that shows no general correlation between SCEs and constitutive heterochromatin, despite occasional nonrandom distributions[13–15]; and b) results that describe a low frequency of SCEs located on the heterochromatin[16–22], and often a cluster of SCEs located at the boundaries between euchromatin and heterochromatin[16–18]. Attempts to explain these contradictory results should take into account interspecific differences in base composition, as well as structural differences in the heterochromatin.

The X chromosomes of *Microtus agrestis* contains large heterochromatic segments[23], which are late-replicating[24] and are rich in GC base pairs[25]. Large, late-replicating heterochromatic segments have also been found on the X chromosome of *Microtus cabrerae*[2,4], but these segments are rich in AT base pairs[2]. Despite their different base composition, both *M. agrestis*[26] and *M. cabrerae* (this paper) X chromosome heterochromatin have show increased frequency of SCEs. In contrast, fewer SCEs than expected were found in the AT-rich pericentromeric heterochromatin of *Mus musculus*[27]. Hence, differences in base composition seem not to significantly influence the distribution of SCEs.

On the other hand, constitutive heterochromatin is highly accessible in *M. agrestis*[28] as well as in *M. cabrerae*[5], as it is sensitive to digestion with DNAaseI, whereas the pericentromeric heterochromatin of *Mus musculus* is clearly DNAaseI-insensitive[28]. This finding is further evidence of the association between frequency of SCEs and sensitivity to DNAaseI. This association suggests that differences in structural organization of the heterochromatin are responsibles for changes in the frequency of SCEs. This is not a surprising finding, as increased accessibility of the chromatin implies greater susceptibility to breaks in the DNA, which may in turn give rise to SCEs when repaired. This hypothesis is also consistent with the increased frequency of SCEs in G-interbands of human chromosomes[29], as transcriptional activity in these regions may generate a more accessible structure. According to this hypothesis, the higher frequency of SCEs in the S region of the *M. cabrerae* X chromosome may be related to the previously demonstrated spontaneous switch to early replication and variable methylation levels in this region, two factors which may be associated with transcriptional activity[4,5].

The hot-spot at the junction between euchromatin and heterochromatin on the X chromosome in *M. cabrerae* may be explained by the replication model for SCE formation proposed by Painter[30]. According to this author, junctions between replicons are unstable sites where SCEs are likely to occur. Thus, delays in replication of this region – the last one to replicate in the X chromosome of this species[4] – may influence the frequency of SCEs.

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1 Díaz de la Guardia, R., Pascual, L., and Orozco, J. C. Experientia *35* (1979) 741.
2 Burgos, M., Jiménez R., Olmos, D. M., and Díaz de la Guardia, R., Cytogenet. Cell Genet. *47* (1988) 75.
3 Burgos, M., Olmos, D. M., Jiménez, R., Sánchez, A., and Díaz de la Guardia, R., Genetica *81* (1990) 11.
4 Burgos, M., Jiménez, R., Sánchez, A., and Díaz de la Guardia, R., Experientia *48* (1992) 1151.
5 Burgos, M., Jiménez, R., Sánchez, A., and Díaz de la Guardia, R., Exp. Cell Res. *202* (1992) 545.
6 Schubert, I., and Rieger, R. Hum. Genet. *57* (1981) 119.
7 Vercauteren, P., Meulepas, E., Vlietinck, R., Cassiman, J.J. and Van Den Berghe H., Hum. Genet. *67* (1984) 56.
8 Vercauteren, P., Meulepas, E., Vlietinck, R., Cassiman, J. J., and Van Den Berghe, H., Chromosoma *93* (1986) 197.
9 Burgos, M., Jiménez, R., and Díaz de la Guardia, R., Stain Technology *61* (5)(1986) 257.
10 Perry, P., and Wolff, S. Nature *251* (1974) 156.
11 Sokal, R., and Rohlf, F. J., in: Biometry. The Principles and Practice of Statistics in Biological Research, pp. 697–778. V. H. Freeman and Company, New York.
12 Dixon, W. J., Brown, M. B., Engelman, L., and Jennrich, R. I. (Eds). BMDP Statistical Software Manual, University of California Press, Berkeley 1990.
13 Vosa, C. G., Sister chromatid exchange bias in *Vicia faba* chromosomes, in: Current Chromosome Research, pp. 105–114. Eds K. Jones and P. E. Brandman. Elsevier/North-Holland Biomedical Press, Amsterdam, 1976.
14 Schubert, I., Künzel, G., Bretschneider H., Rieger, R., and Nicoloff H., Theor. Appl. Genet. *56* (1981) 1.
15 Ockey, C. H., Cytogenet. Cell Genet. *26* (1980) 223.
16 Carrano, A. V., and Wolff, S., Chromosoma *53* (1975) 361.
17 Bostock, C. J., and Christie, S., Chromosoma *56* (1976) 275.
18 Hsu, T. C., and Pathack, S., Chromosoma *58* (1976) 169.
19 Schwartzman, J. B., and Cortés, F., Chromosoma *62* (1977) 119.
20 Kato, H., Chromosoma *74* (1979) 307.
21 Schneider, N. R., Chaganti, R. S. K., and German, J. Chromosoma *77* (1980) 379.
22 Latt, S. A. Localization of sister chromatid exchanges in human chromosomes. Science *185* (1974) 74.
23 Cooper, J. E., and Hsu, T. C., Cytogenetics *11* (1972) 295.
24 Sperling, K., and Rao, P. N., Chromosoma *45* (1974) 121.
25 Arrighi, F. E., Hsu, T. C., Saunders, P., and Saunders, G. F., Chromosoma *32* (1970) 224.
26 Natarajan, A. T. and Klasterska I., Hereditas *79* (1975) 150.
27 Sperling, K., Kerem, B., Goitein, R., Kottusch V., Cedar, H., and Marcus M., Chromosoma *93* (1985) 38.
28 Painter, R. B., Mutat. Res. *70* (1980) 337.