Theor Appl Genet (1987) 73:469–475

Sister chromatid exchange points in the heterochromatin and euchromatin regions of Chinese hedgehog chromosomes

Yan Yongshan and Shun Ficin

Institute of Genetics, Academia Sinica, Beijing, China

Received July 10, 1986; Accepted September 17, 1986
Communicated by Hu Han

Summary. The Chinese hedgehog has a diploid chromosome number of 48 in which there are eleven pairs of telo- or subtelocentric autosomes, twelve pairs of meta- or submetacentric autosomes, a metacentric X chromosome and a telocentric Y chromosome. The heterochromatin is almost completely distributed in five large distal segments of chromosomes nos. 9 to 12 and no. 18. There is no positive C-band in the centromeres of the chromosomes except for the X chromosome which has a small, weakly stained C-band in the centromere. In Chinese hedgehog cells 52.1% of SCEs are found at the junction between the euchromatin and the heterochromatin, 39.5% in the heterochromatin and 8.4% in the auchromatin. The SCE number per unit C-band is double the SCE number per unit euchromatin. The SCE rate in the heterochromatin or euchromatin regions is not proportional to their chromosome length and can be quite different between different pairs of the chromosomes. Our results indicate that there is a non-uniform distribution of the SCEs in the Chinese hedgehog cells.

Key words: Chinese hedgehog – Chromosome G- and C-bands – SCE distribution – Heterochromatin regions – Euchromatin regions

Introduction

Sister chromatid exchange (SCE) reflects an interchange between DNA molecules at homologous loci within a replicating chromosome and presumably involves DNA breakage and reunion. The precise localization of SCE points to particular regions of individual chromosomes is of very high interest. Heterochromatin, stained intensely by the chromosome C-band technique has been shown to be major sites of highly repeated or satellite DNA sequences in many species.

Studies on the SCE points in the heterochromatin and euchromatin regions of chromosomes are of significance to the proposed mechanisms of satellite DNA evolution by unequal cross-overs (Smith 1976) while SCE points in the heterochromatin regions could play an important role in the evolution of species.

It has been shown that SCE occurs more frequently in euchromatin segments than in heterochromatin regions (Hoo et al. 1979; Carrano et al. 1975, 1977; Bostock et al. 1976; Hsu et al. 1976; Morgan et al. 1977; Schneider et al. 1980). However, Natarajan et al. (1975) and Gatti et al. (1979) have found that the heterochromatin regions have a higher SCE rate than the euchromatin regions. The highest SCE frequency was found at the junction between C-band and non-C band regions (Bostock et al. 1976; Kato 1979; Carrano et al. 1975, 1977; Hsu et al. 1976). It was further demonstrated that all SCE points occurred in the chromosome G-negative band or at the band junction interface (Morgan et al. 1977; Latt 1974). However, Pathak et al. (1975) obtained data indicating that the positive G-bands may also be involved in SCE. Distribution of SCEs in the euchromatin of the chromosomes was found to be proportional to its metaphase length in hamster and Wallaby (Kato 1979). However, a non-uniform distribution of SCEs was observed in human lymphocytes (Vercauteren et al. 1986).

It is thus of interest to test whether SCE number is proportional to the relative chromosome length in the euchromatin of other species, to examine the relationship between SCE rate and C-band length, and to look at the distribution of the SCE points at the heterochromatin, euchromatin and junction between them in other species. In the Chinese hedgehog reported here the heterochromatin is almost completely to be found in the five large distal segments of the chromosomes. There are no positive C-bands in the centromeres of the chromosomes except for the X chromosome which has a small, very weakly stained C-band in the centromere.

In the present paper we report on the chromosome G-band and C-band of the Chinese hedgehog, SCE distribution at the heterochromatin, euchromatin and the junction between them, and the relationship between SCE rate and relative length of both chromosome C-band and non-C band regions in individual chromosomes.

Materials and methods

Cell treatment and chromosome preparation

1 Whole blood cell culture. Six adults of the Chinese hedgehog *Erinaceus dealbatus* Swinhoe were collected from the suburb of Beijing. Five ml of blood from the hedgehogs were added into each of ten culture flasks, each of which contained 3 ml of RPMI 1640 medium supplemented with 20% new born calf serum, one drop (20 unit) of heparin and 0.2 ml of PHA(Difco). The blood cultures were inoculated at 37 °C for 24 h, and then BrdU(Sigma) was added to a final concentration of 25 μg/ml. The cultures were then continuously inoculated at 37 °C for another 48 h under dark conditions. Colcemid(Sigma) was added to a final concentration of 0.01 μg/ml 2 h before cell harvest. The cells were treated with 0.075 M KCl at room temperature for 20 min, and then fixed with a mixture of methanol and glacial acetic acid (3 : 1) three times. The chromosome preparation was performed according to the air-dry method. Before the cell fixation, all procedures were carried out under a safelight.

2 Lung cell culture. Small pieces of lung tissue from healthy animals were trypsinized with 0.25% trypsin(Difco) at 37 °C for 10–15 min. After centrifugation, the cells were inoculated in several culture flasks containing RPMI 1640 medium or Eagle essential medium(MEM) supplemented with 20% new born calf serum. The cells were subcultured for several passages, and then treated for both SCE test and chromosome G- and C-banding.

3 Chromosome preparation from bone marrow cells following short term inoculation with colcemid. Fresh bone marrow cells were inoculated in the RPMI 1640 medium containing 0.02 μg/ml colcemid at 37 °C for 4–5 h. The cells were harvested and treated for chromosome preparation.

Chromosome analysis

1 Karyotype. Ten well-defined metaphases were photographed. The chromosomes in each cell were ordered from larger meta- and submetacentric chromosomes to subtelo- and telocentric chromosomes, smaller metacentric chromosomes and sex chromosomes (Fig. 1). The length of both the short arms and long arms in each pair of the chromosomes were measured.

2 Chromosome G-banding. One- to three-day old slides were treated with 0.25% trypsin at 37 °C for 20–30 s and stained with 1% Giemsa solution after a brief rinse in distilled water. Nine of the G-banded metaphases were photographed. The position of each G-band in each pair of the chromosomes were measured. According to the average of the G-band position, a diagram of the chromosome G-band was drawn.

3 Chromosome C-banding. Three- to six-day old slides were immersed in 0.01 N HCl at room temperature for 30 to 40 min, rinsed in distilled water, treated with a saturated $Ba(OH)_2$ solution at 60 °C, covered with coverslips for one min, rinsed in distilled water, and then incubated in 3XSSC at 60 °C for 2 h. The slides were stained with 10% of Giemsa solution for 15 min. The C-band length and the chromosome length were measured, and a diagram of the C-band was then drawn.

SCE treatment and observation

The one- to six-day old slides were immersed in 0.14 M KCl, 0.014 M KCl and 1/15 M Sorensens buffer (pH 6.8) for 5 min, respectively, and in 0.1 μg/ml of Hoechst 33258 for 20 min, and then rinsed in the buffer twice (5 min each). The stained slides were exposed to a fluorescent light for 2 h in a 3XSSC solution at 60 °C. After a brief rinse in distilled water, the slides were stained with 1% Giemsa solution for 3 min.

Sixty-two metaphases with clear SCE patterns were photographed and enlarged. The SCE number in each cell was scored. According to their size and centromere position, chromosomes 8–12 and no. 18 were marked in the photographs. The position of the SCE points in the chromosomes were measured. All SCEs in the five chromosomes were classified into three groups, i.e., at the heterochromatin, the euchromatin regions and the junctions between them, according to the position of both SCE points and C-bands. C-band relative length for each pair of chromosomes was obtained by chromosome relative length times percentage of C-band length.

Results

Karyotype of the Chinese hedgehog

The Chinese hedgehog has a diploid chromosome number of 48 in which there are eleven pairs of telo- or subtelocentric autosomes (nos. 8 to 18), twelve pairs of meta- or submetacentric autosomes (nos. 1 to 7 and nos. 19 to 23), a metacentric X chromosome and a telocentric Y chromosome (Fig. 1).

The G-band pattern of the hedgehog is shown in Figs. 1 and 3. There is a distinct differential band pattern for each pair of chromosomes. In general, the larger chromosomes have more G-bands except for chromosomes 9 to 12. In the latter, most parts of the long arms are negative to the G-band. Chromosomes 22, 23, the Y chromosome and most parts of the long arms of chromosomes 17 and 18 are also negative to the G-band.

As shown in Figs. 2 and 3, the diploid cell of the Chinese hedgehog has five autosomes (nos. 9 to 12 and no. 18) with large distal segments intensely stained by the chromosome C-band technique. The X chromosome has a small, weakly stained C-band in its centromere. There is no positive C-band in other chromosomes. In other words, the heterochromatin is almost completely to be found in the large distal segments of these five chromosomes. The C-band relative length is 46.6% of the total relative length of the five chromosomes. The relative length of the C-bands for different pairs of chromosomes is shown in Table 3. In Chinese

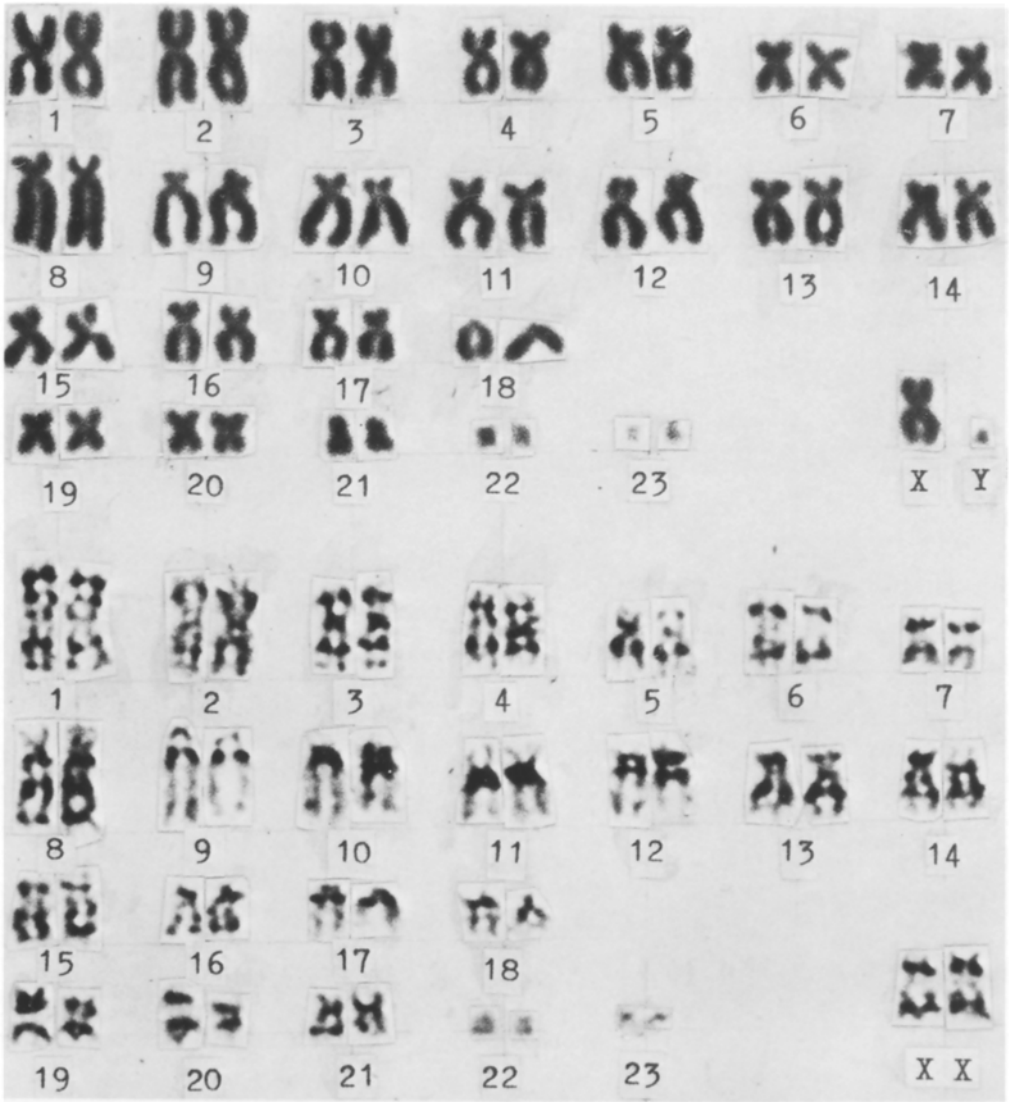
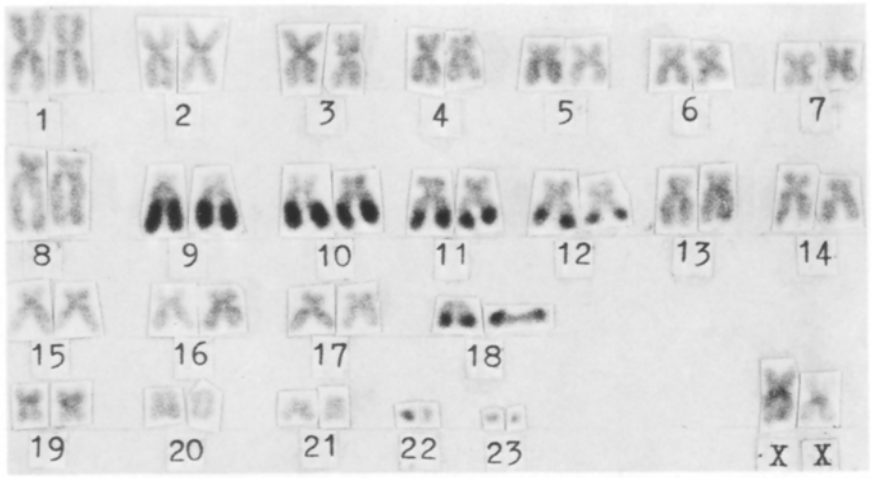


Fig. 1. Karyotype (*upper*) and chromosomal G-banding pattern (*bottom*) of the Chinese hedgehog

Table 1. SCE frequency in chromosomes of the Chinese hedgehog

Item	Chromosome no.						Other 18 pairs of the chromosomes	
	no. 8	no. 9	no. 10	no. 11	no. 12	no. 18	Total	Average for a pair
Total no. of SCE[a]	36	44	23	18	20	14	120	6.7
Percentage of SCE frequency (%)	0.290	16.0	8.4	6.5	7.3	5.1	43.6	2.4
SCE/chromosome	0.290	0.355	0.185	0.145	0.161	0.113	–	0.054
Relative length of chromosomes	6.55	5.98	5.23	4.84	4.57	2.86	69.97	3.89
SCE per unit of chromosome length ($\times 10^{-2}$)	4.43	5.94	3.54	3.00	3.52	3.95	–	1.39

[a] SCE numbers were calculated from photographs of 62 cells



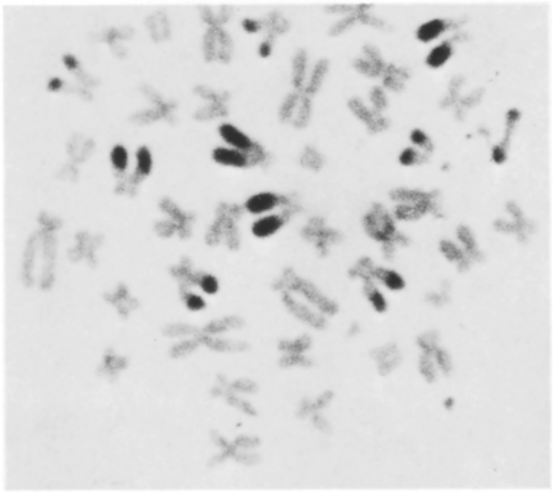


Fig. 2. The chromosome C-banding pattern of the Chinese hedgehog

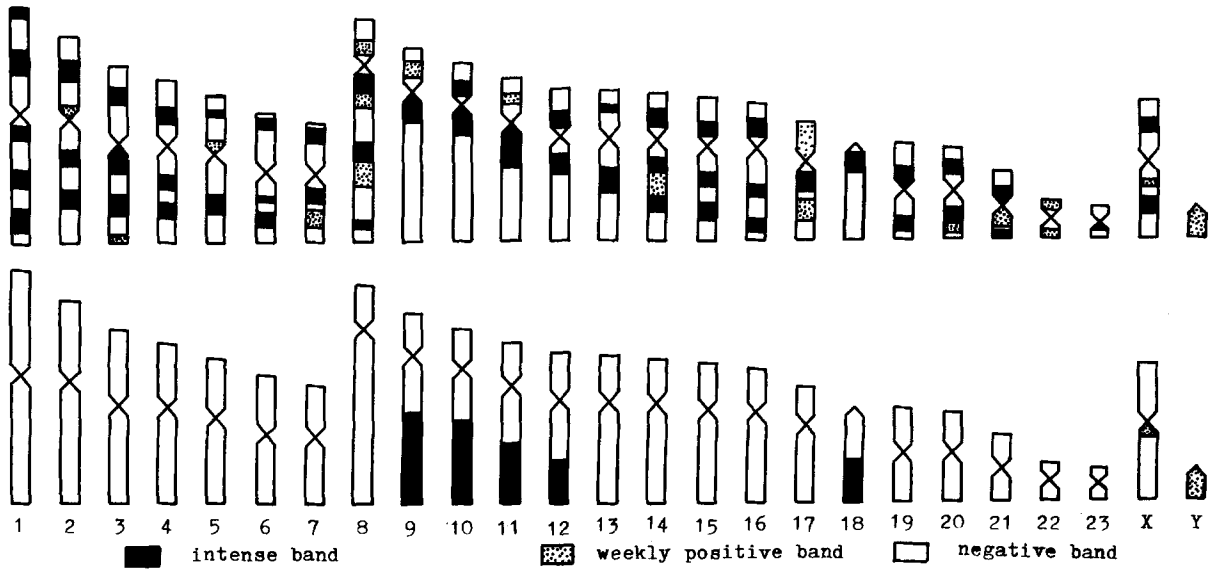


Fig. 3. Diagrams of the G-banding (*upper*) and the C-banding (*bottom*) patterns of the Chinese hedgehog chromosomes

hedgehog cells, the C-bands occupy about 10.97% of the whole set of the chromosomes in size (Table 3), i. e., about 89.03% of the chromosome regions are occupied by euchromatin.

SCE patterns of the hedgehog cells were obtained from whole blood cell cultures (Figs. 4 and 5). SCE frequency in the chromosomes are shown in Table 1. The average SCE per chromosome in the 18 pairs of the chromosomes, which have no positive C-band, is 0.054, but for chromosomes 8 to 12 and no. 18, it is twice to six times higher than in the other 18 pairs of chromosomes (Table 3). For example, the SCE number of chromosomes 8 and 9 are 0.290 and 0.355, respectively, five and six times higher than that found in the other 18 pairs of the chromosomes (0.054). The SCE per unit chromosome length for chromosomes 8 to 12 and no. 18 is twice to four times higher that found than in the 18 pairs of chromosomes: for chromosomes 8 and 9 it is 4.43×10^{-2} and 5.94×10^{-2} respectively, i.e., three and four times higher than in the 18 pairs of the chromosomes (1.39×10^{-2}). In chromosomes 9 to 12 and no. 18 52.1% of the SCEs are found at the junctions, 39.5% in the heterochromatin regions, and only 8.4% in the euchromatin (Table 2). However, there are some differences among these five pairs of the chromosomes; about 38.6% of the SCEs occur at the junctions in chromosome no. 9, 78.6% in chromosome no. 18.

The SCEs distribute within the C-bands of chromosomes 9 to 12 and no. 18 with a quite different rate. For example, the SCE per unit C-band in chromosome no. 9 (6.43×10^{-2}) is twelve times higher than in chromosome no. 18, double that found in chromosome no. 10, and three times higher than in both chromosomes 11 and 12 (Table 3). It seems like that the higher the percentage of the SCEs found at the junctions (Table 2), the lower the SCE per unit of C-band (Table 3).

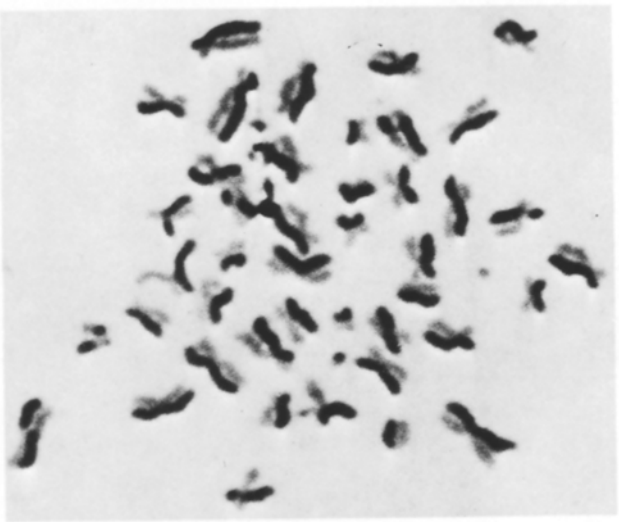


Fig. 4. SCE pattern in the chromosomes of the Chinese hedgehog from the culture of the lymphocytes

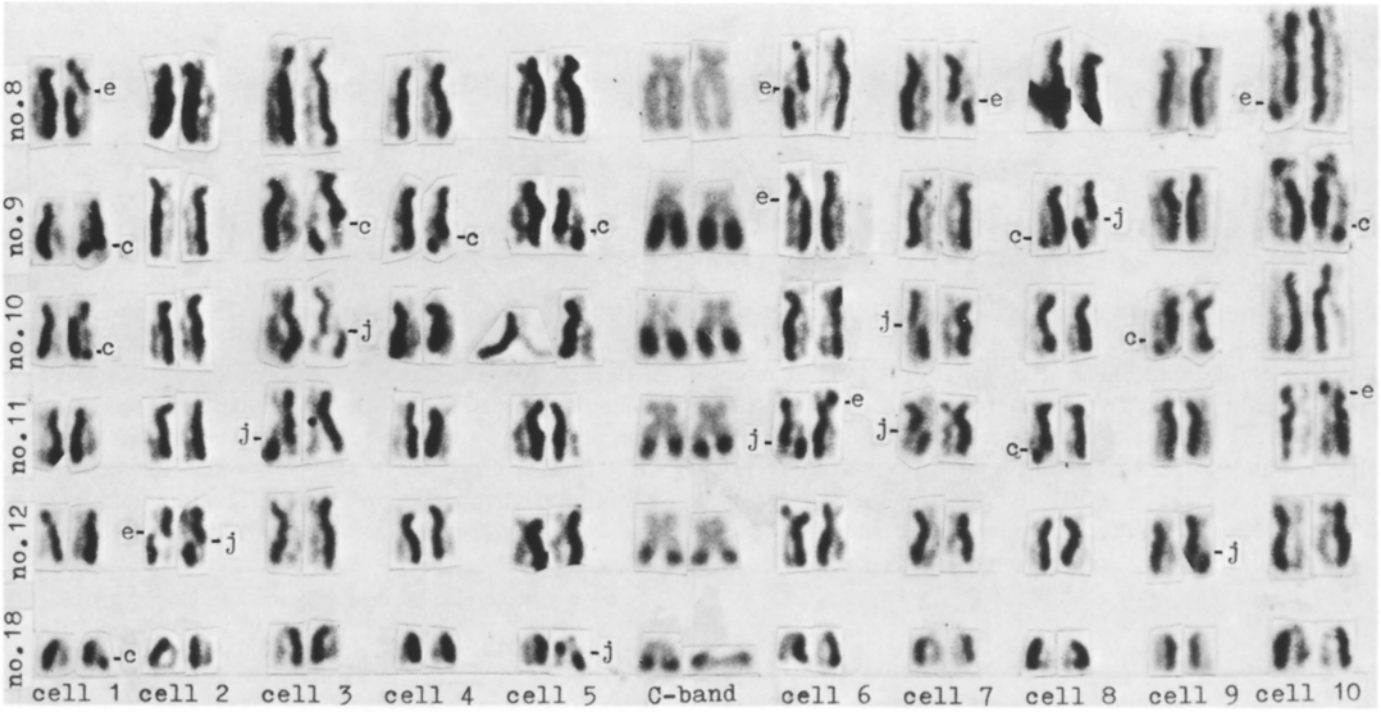


Fig. 5. SCE patterns at the heterochromatin regions (h), euchromatin regions (e) and junction (j) between them of the Chinese hedgehog chromosomes (nos. 8–12 and no. 18)

Table 2. SCE distribution at the hetero- and euchromatin regions of the Chinese hedgehog chromosomes[a]

Item		At heterochromatin regions		At euchromatin regions		At junction between hetero- and euchromatin regions	
		SCE no.	%	SCE no.	%	SCE no.	%
Chromosome no.	no. 8	0	0	36	100	0	0
	no. 9	26	59.1	1	2.3	17	38.6
	no. 10	11	47.8	1	4.3	11	47.8
	no. 11	5	27.8	4	22.2	9	50.0
	no. 12	4	20.0	2	10.0	14	70.0
	no. 18	1	7.1	2	14.3	11	78.6
Total		47	39.5	10	8.4	62	52.1
Other 18 pairs of chromosomes		0	120	100	0	0	0

[a] SCE numbers were calculated from photographs of 62 cells

Table 3. SCE rate per unit chromosome relative length in both hetero- and euchromatin regions

Item	Chromosome no.						Average of other 18 pairs of the chromosomes	In a monoploid
	no. 8	no. 9	no. 10	no. 11	no. 12	no. 18		
At heterochromatin regions								
SCE number	0	0.210	0.089	0.040	0.032	0.008	0	0.380
Percentage of C-band length	0	54.5	53.6	38.6	33.5	52.9	0	–
Relative length of C-band (%)	0	3.26	2.80	1.87	1.53	1.51	0	10.97
SCE per unit of C-band ($\times 10^{-2}$)	0	6.43	3.18	2.14	2.09	0.53	0	3.46
At euchromatin regions								
SCE number	0.290	0.008	0.008	0.032	0.016	0.016	0.054	1.340
Percentage of euchromatin length	100	45.5	46.4	61.4	66.5	47.1	100	–
Relative length of euchromatin (%)	6.55	2.72	2.43	2.97	3.04	1.35	3.89	89.03
SCE per unit of euchromatin ($\times 10^{-2}$)	4.43	0.29	0.33	1.08	0.53	1.19	1.39	1.50

As shown in Table 3, the SCE per unit of euchromatin of chromosomes nos. 9, 10 and 12 is extremely low but for chromosome no. 8 it is 4.43×10^{-2}, four to fifteen times higher than in chromosomes 9 to 12 and no. 18. For the Chinese hedgehog cells, the SCE per unit of C-band is 3.46×10^{-2} but the SCE per unit of euchromatin regions is 1.50×10^{-2}. It is clear that in the hedgehog cells the SCE rate within the C-bands is double that found in the euchromatin regions.

Discussion

The Chinese hedgehog has a diploid chromosome number of 48, similar to that of both the Algerian and European hedgehog (Gropp et al. 1972). However, their karyotypes are quite different: the Algerian hedgehog has 5 subtelo- or telocentric chromosomes while the Chinese hedgehog has eleven. Moreover, the European and Algerian hedgehogs have two and three large blocks of distally located autosomal heterochromatin, respectively (Gropp et al. 1972), while there are five large heterochromatin segments in the Chinese hedgehog.

As is well known, the heterochromatin found in mouse cells is positive to chromosome G-banding but the Chinese hedgehog heterochromatin is negative to G-banding, which is similar to the European and Algerian hedgehog (Gropp et al. 1972).

The SCE per unit of chromosome length in chromosomes 9 to 12 and no. 18, which have large blocks of

heterochromatin, is significantly higher than those other autosomes which have no positive C-bands, except chromosome no. 8. It is clear that the SCE distribution along chromosomes is not proportional to the chromosome length because the SCE number per unit of chromosome length is quite different for many chromosomes (Vercauteren et al. 1986).

In Chinese hedgehog cells, the distribution of SCEs in the euchromatin of the chromosomes is not proportional to chromosome length. SCE number per unit of C-band (3.46×10^{-2}) in hedgehog cells is double the SCE number per unit of euchromatin (1.5×10^{-2}), indicating that SCE distribution in the C-bands is more frequent than in the euchromatin. Our results agree with a previous report by Natarajan et al. (1975) but differ from the observations made in both hamster and Wallaby (Kato 1979). The differences may be due to the different species tested.

In the five chromosomes which have large distal C-bands, there are a few SCEs in the euchromatin, a higher SCE frequency at the junctions (52.1%) and at the heterochromatin (39.5%). This differs from the observation in Indian muntjac which indicated a low SCE frequency in heterochromatin and a high SCE frequency at the junctions (Carrano et al. 1977).

Our results also agree with the non-uniform distribution of SCEs observed in human lymphocytes (Vercauteren et al. 1986).

References

Bostock CJ, Christie S (1976) Analysis of the frequency of sister chromatid exchange in different regions of chromosomes of the kangaroo rat (*Dipodomys ordii*). Chromosoma 56:275–287

Carrano AV, Wolff S (1975) Distribution of sister chromatid exchanges in the euchromatin and heterochromatin of the Indian mutjac. Chromosoma 53:361–369

Carrano AV, Johnston GR (1977) The distribution of mitomycin C-induced sister chromatid exchanges in the euchromatin and heterochromatin of the Indian mutjac. Chromosoma 64:97–107

Gatti M, Santini G, Pimpinelli S, Olovieri G (1979) Lack of spontaneous sister chromatid exchanges in somatic cells of *Drosophila melanogaster*. Genetics 91:255–274

Gropp A, Natarajan AT (1972) Karyotype and heterochromatin pattern of the Algerian hedgehog. Cytogenetics 11: 259–269

Hoo JJ, Parslow MI (1979) Relation between the SCE points and the DNA replication bands. Chromosoma 73:67–74

Hsu TC, Pathaks (1976) Different rates of SCE between euchromatin and heterochromatin. Chromosoma 58:269–273

Kato H (1979) Preferential occurrence of SCE at heterochromatin-euchromatin junctions in the wallaby and hamster chromosomes. Chromosoma 74:307–316

Latt SA (1974) Localization of sister chromatid exchanges in human chromosomes. Science 185:74–76

Morgan WF, Crossen PE (1977) The frequency and distribution of sister chromatid exchange in human chromosomes. Humangenetik 38:271–278

Natarajan AT, Klasterska I (1975) Heterochromatin and SCE in the chromosomes of *Microtus agrestis*. Hereditas 79: 150–154

Pathak S, Stock AD, Lusby A (1975) A combination of sister chromatid differential staining and Giemsa banding. Experientia 31:916–918

Smith G (1976) Evolution of repeated DNA sequences by unequal crossover. Science. 191:528–535

Schneider NR, Chaganti RSK, German J (1980) Analysis of a BrdU-sensitive site in the cactus mouse (*Peromyscus eremicus*) chromosomal breakage and sister chromatid exchange. Chromosoma 77:379–389

Vercauteren P, Meulepas E, Vlietinck R, Cassiman JJ, Van den Berghe H (1986) Non-uniform distribution of SCE in human lymphocytes. Chromosoma 93:197–202