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Chromosome spatial clustering inferred from radiogenic aberrations

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Abstract.
Purpose: Analysing chromosome aberrations induced by low linear energy transfer (LET) radiation in order to characterize systematic spatial clustering among the 22 human autosomes in human lymphocytes and to compare their relative participation in interchanges.
Materials and methods: A multicolour fluorescence *in situ* hybridization (mFISH) data set, specifying colour junctions in metaphases of human peripheral blood lymphocytes 72 h after *in vitro* exposure to low LET radiation, was analysed separately and in combination with previously published results. Monte Carlo computer simulations and mathematical modelling guided data analysis.
Results and conclusions: Statistical tests on aberration data confirmed two clusters of chromosomes, {1, 16, 17, 19, 22} and {13, 14, 15, 21, 22}, as having their members being on average closer to each other than randomness would predict. The first set has been reported previously to be near the centre of the interphase nucleus and to be formed mainly by gene-rich chromosomes, while the second set comprises the nucleolus chromosomes. The results suggest a possible interplay between chromosome positioning and transcription. A number of other clusters suggested in the literature were not confirmed and considerable randomness of chromosome–chromosome juxtapositions was present. In addition, and consistent with previous results, it was found that chromosome participation in interchanges is approximately proportional to the two-thirds power of the DNA content.

1. Introduction

Organization of chromosomes during the cell cycle has been studied for more than a century (Rabl 1885). During interphase, chromosomes are predominantly confined into subnuclear regions called chromosome territories (for a review, see Cremer and Cremer 2001). Positions of these territories are determined at mitosis and at least in some cases do not change drastically during G0/G1 (reviewed in Parada and Misteli 2002, Gerlich *et al.* 2003, Walter *et al.* 2003). Chromosome territories for different chromosomes are believed to have a non-random radial arrangement (Sun *et al.* 2000, Boyle *et al.* 2001, Kozubek *et al.* 2002, Tanabe *et al.* 2002) that is driven mainly by gene density and/or chromosome size. At the same time, a considerable randomness is found when looking at chromosome–chromosome juxtapositions using ionizing radiation as a probe (Cornforth *et al.* 2002).

A number of chromosome clusters for human lymphocytes, which may be responsible for deviations of chromosome juxtapositions from a totally random picture, have been proposed in the literature (Nagele 1999, Alcobia *et al.* 2000, Boyle *et al.* 2001, Cremer *et al.* 2001, Kozubek *et al.* 2002, Parada and Misteli 2002, Roix *et al.* 2003). Some of these chromosome clusters have been associated with other biological phenomena such as gene content (Boyle *et al.* 2001), gene expression (Parada and Misteli 2002) or cancer (Lukasova *et al.* 1997, 1999, Roix *et al.* 2003).

Ionizing radiation induced aberrations are commonly used as probes to study chromosome structure, nuclear architecture and DNA repair/misrepair mechanisms (e.g. Cornforth *et al.* 2002, Vazquez *et al.* 2002, reviewed in Hlatky *et al.* 2002). Sparsely ionizing radiations, such as gamma-rays, create DNA double-strand breaks (DSB) randomly and independently throughout the genome. Chromosome interchanges that involve sufficiently large chromosome segments (more than several Mb) can be detected by multiplex fluorescence *in-situ* hybridization (mFISH; Speicher *et al.* 1996), where heterologous chromosomes are 'painted' different (pseudo-)colours; thus for the autosomes in human cells 22 different colours are involved.

The production of chromosome aberrations is subject to proximity effects (reviewed in Sachs *et al.*

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1997), that is, preferential interactions between break ends formed in spatial as well as temporal proximity as opposed to those that formed far apart in space or time. Consequently, interchange frequencies and spatial proximity are correlated. The present study analysed proximity effects for the 22 human autosomes, omitting the X and Y chromosomes because more extensive data are available for the autosomes. mFISH colour junctions for each of the $22 \times 21/2 = 231$ colour pairs were considered. Our study extends that of Cornforth *et al.* (2002). The method allows combined analysis of simple and complex aberrations, of aberrations at various doses, and of aberrations from different post-irradiation metaphases.

We can confirm statistically two groups of chromosomes whose members undergo interchanges more frequently than randomness would predict, indicating spatial proximity. We also analysed individual chromosome participation and found, consistent with previous results (Cigarrán *et al.* 1998, Wu 2001, Cornforth *et al.* 2002, Durante *et al.* 2002), that participation is approximately proportional to (DNA content)$^{2/3}$.

2. Materials and methods

2.1. *Experimental methods*

Previously published data involved 1587 cells from seven donors (five male, two female) in two different laboratories. At the University of Texas Galveston laboratory, human lymphocytes from two male donors were irradiated *in vitro* at three doses (Loucas *et al.* 2001). Human lymphocytes from five donors (three male, two female) at the Department of Radiation Oncology, Technical University of Munich, were irradiated *in vitro* at 3 Gy (Greulich *et al.* 2000, Cornforth *et al.* 2002).

A new Munich data set is added here for peripheral blood lymphocytes from one male donor exposed to *in vitro* acute doses of 3 Gy (255 cells), 4 Gy (1144 cells) or 5 Gy (599 cells) of 6 MeV photons (2.8 Gy min^{-1}, Siemens MX-2, Munich, Germany). Lymphocyte cultures were set up and metaphases prepared 72 h after radiation exposure. Metaphase spreads were prepared following a standard protocol (Rooney and Czepulkowski 1997). mFISH was applied to metaphases using the SpectraVisionTM system (Vysis, Downers Grove, USA; Applied Imaging, Santa Clara, USA). Scoring used fluorescence microscopy in combination with the appropriate filter sets and image analysis software. Ambiguities (Lee *et al.* 2001) were resolved using inverse 4′,6-diamidino-2-phenylindole (DAPI) patterns. Colour junctions were recorded for each metaphase.

The basic data used was the pairwise yield $f(j, k)$, defined as the number of metaphases in which at least one interchange between autosome j and autosome k is observed, as indicated by one or more junctions between the corresponding colours. Thus, $f(j, k) = f(k, j)$. This pairwise yield $f(j, k)$ is easier to determine experimentally and is more robust than other measures such as the total number of interchanges between the chromosomes. For example, suppose one sees (in mPAINT (multiple protocol for aberration and nomenclature terminology) notation) the dicentric pattern (1′, 2′) and does not see an accompanying acentric fragment. Quite possibly the fragment (1, 2) is present and then there is a second colour junction for these two colours. Our approach avoids the necessity of deciding whether or not the acentric fragment is present. Similar considerations apply to more complex situations when true incompleteness or complicated karyotypes are analysed. The approach involves disregarding some information. However, it minimizes the number of false-positives (since no extra assumptions have to be made for undetected colour junctions) and also facilitates pooling of data. Examples of pairwise yields $f(j, k)$ are shown in table 1.

2.2. *Monte Carlo computer analysis*

We used our chromosome aberration simulator (CAS) Monte Carlo computer software (Chen *et al.* 1997, Hlatky *et al.* 2002) to estimate possible biases in the results due to cell proliferation or to combining different doses (see Section 3.1) and also to estimate chromosome participation (see Section 3.2). CAS implements standard biophysical models of aberration formation using a discrete-time Markov chain with two adjustable parameters, the number of interaction sites (S) and the number of reactive DSB per Gy (δ). The present study used $S = 10$ as found in previous studies (Sachs *et al.* 2000) assuming the breakage-and-reunion aberration formation pathway (Savage *et al.* 1998, Levy *et al.* 2004). Results are independent of the aberration formation pathway assumed (Cornforth *et al.* 2002).

2.3. *Mathematical methods to model lymphocyte proliferation*

Cells observed 72 h after irradiation are known to be distributed among several post-irradiation metaphases and to be under selection effects related to the transmission of dicentric and acentric fragments (Carrano 1973a, b, Bedford *et al.* 1978, Braselmann *et al.* 1986, Pala *et al.* 2001). The model

Table 1. Yields.

Chr	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Sum	Lin	2/3
1	44	38	42	29	26	29	18	39	29	25	18	15	18	34	31	22	12	14	22	9	27	541	623.5	520.6
2		43	37	32	30	24	25	29	16	24	30	29	9	26	8	24	8	7	12	13	15	485	565.2	485.2
3			21	31	32	24	21	26	23	25	23	21	18	18	19	21	11	17	11	12	10	465	511.5	456.0
4				23	27	28	24	26	20	13	19	23	22	20	16	18	11	6	12	10	7	425	480.5	435.2
5					17	31	26	25	24	30	25	25	15	19	8	19	13	7	16	7	4	426	465.3	426.2
6						18	22	21	31	13	30	18	15	19	14	15	13	10	9	8	7	395	446.2	413.3
7							20	20	17	28	25	13	18	8	18	23	11	9	19	6	7	396	405.6	390.0
8								13	12	24	11	25	15	16	12	16	17	4	9	7	8	345	383.9	377.7
9									21	25	7	23	23	27	20	15	22	8	9	7	10	416	362.3	360.1
10										18	21	14	14	10	19	14	9	5	11	7	3	338	353.8	353.3
11											25	5	15	16	19	15	8	10	12	3	11	364	369.3	364.0
12												9	16	9	12	16	8	13	10	5	5	337	367.1	363.2
13													29	10	10	7	16	5	6	7	9	319	294.2	311.8
14														22	13	6	10	2	6	13	11	310	266.1	293.7
15															22	13	9	7	11	7	9	332	266.2	294.9
16																12	15	12	20	8	13	321	268.1	296.1
17																	5	4	11	5	10	291	226.3	262.9
18																		2	11	9	3	223	223.3	263.7
19																			6	0	8	156	184.2	229.4
20																				7	10	240	167.3	215.0
21																					6	156	118.1	169.3
22																						193	125.6	177.9

Each entry $f(j, k)$ is the pairwise yield corresponding to row j and column k, giving the number of cells that contain at least one colour junction between chromosome j and chromosome k in the combined data. Pairwise yields obey $f(j, k)=f(k, j)$. The column labelled 'Sum' shows individual chromosome yields $f(j)$, obtained by summing a row together with its corresponding column. The last two columns show theoretical individual chromosome yields assuming that the distribution of reactive breaks is either linear with chromosome content (Lin) or follows a two-thirds power law (2/3).

of Carrano and Heddle (1973) as implemented by Braselmann *et al.* (1986) was incorporated into CAS by Vazquez *et al.* (2002). Six adjustable parameters are needed to model cell proliferation and selection. The first three parameters correspond to the percentage of cells in each of the first three post-irradiation metaphases (M1|M2|M3). The other three parameters ($W|P|T$) model cell selection, where W is the dicentric survival parameter, where $1-W$ is the probability that a simple dicentric will lead to the death of both daughter cells, P is the acentric survival parameter, where $1-P$ is the probability that an acentric is lethal for both daughter cells assuming that no other aberration affecting survival is present, and T is the acentric transmissibility parameter such that $2T$ is the probability for a cell with an acentric to transmit one or two copies of the acentric to one daughter but not both.

2.4. *Mathematical methods to measure chromosome participation*

While the main goal was analysing chromosome clustering, the data also give information on individual chromosome radiosensitivity. Chromosome participation (i.e. the number of reactive DSB per chromosome) was estimated from pairwise yields as follows. The individual chromosome yield for the jth autosome, denoted by $f(j)$, was defined as $\Sigma f(j, k)$, where Σ is the sum over k for $k \neq j$ and $f(j, k)$ is the pairwise yield defined above. To estimate whether chromosome participation is proportional to the DNA content or DNA content to the two-thirds power, experimental values of $f(j)$ were compared with values generated by CAS. CAS is needed because even if the average number of reactive DSB in a chromosome is linearly proportional to DNA content, chromosome yields $f(j)$ will deviate somewhat from linearity. CAS accounts for the various correction terms. For example, consider a hypothetical genome with only two homologue pairs and assume one pair is much larger than the other. According to our assay, both colours would have the same individual yield $f(j)$, despite the discrepancy in DNA content. The larger chromosomes would have many more intrachanges and homologue–homologue interchanges, but these are not included in our pairwise yield $f(j, k)$ or our chromosome yield $f(j)$ because they do not produce colour junctions. In the actual human genome there are also similar, but smaller, discrepancies between participation and individual yield $f(j)$. CAS can translate from chromosome yield $f(j)$ to chromosome participation and vice versa.

2.5. *Mathematical methods to confirm statistical significance of candidate clusters*

Spatial proximity among chromosomes was estimated from the pairwise yields $f(j, k)$. For randomness of chromosomal interchanges, one would have, apart from statistical fluctuations, a product form $f(j, k) = g(j)g(k)$ for some appropriate factors $g(j)$. We investigated deviations from this product form, with $g(j)$ being estimated from the data as described in the appendix.

Overall deviations from randomness were computed by the statistic $\xi^2 = \Sigma\Delta^2(j, k)$, where $\Delta(j, k) = [f(j, k) - g(j)g(k)]/(g(j)g(k))^{1/2}$ and Σ is the sum over all 231 autosome pairs. We computed p values by comparing the experimentally observed ξ^2 with values generated by Monte Carlo computer simulations. In the computer simulations, each $f(j, k)$ was given by a Poisson distribution of mean $g(j)g(k)$. To analyse which clusters alter the overall statistic from that given by a random model, we used the additional one-sided test statistic $\Sigma[f(j, k) - g(j)g(k)]/[g(j)g(k)]^{1/2}$, where Σ is a sum over all pairs that could be formed among chromosomes in a candidate cluster. Various clusters suggested in the literature were tested. These statistical methods for confirming or rejecting candidate clusters do not apply directly to data mining for new clusters, which would require more extensive calculations.

3. Results

3.1. *Pairwise yields*

Since the statistics are improved by combining the new data with previous data, and for brevity, we emphasize the combined results rather than just the new data. Results for the new data set by itself can be inferred from the combined data presented herein and the data previously of Cornforth *et al.* (2002).

We first show that the data can be pooled. Cornforth *et al.* (2002) showed by a Kruskal–Wallis test that nine data sets involving different genders, doses and distributions across post-irradiation metaphases could be pooled together. Here we take another approach and show by modelling that in a geometrically random situation the $g(i)$, apart from one overall normalization, are independent of the dose and of the distribution of cells in different post-irradiation metaphases. It is geometric proximity, not dose or selection during proliferation, that determines the statistics considered here. The modelling was done using CAS. Figure 1 shows an example comparing $g(i)$ for different distributions of cells in various post-irradiation metaphases. The diagonal behaviour shows that the values obtained are independent of the distribution of cells over metaphases. Similar results comparing other proliferation parameters or different doses (figure 1) allowed pooling of all the data.

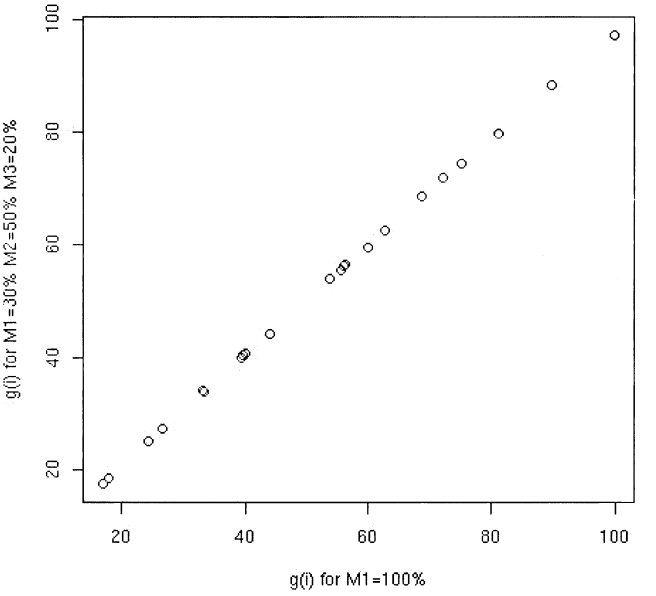

Figure 1. Calculations relevant to data pooling. A calculated scatter plot is shown that compares $g(i)$ values for metaphase distributions given by Hoffmann *et al.* (1999) (M1|M2|M3) = (0.3|0.5|0.2) (y-axis) versus (M1|M2|M3) = (1|0|0) (y-axis); selection parameters $(W|P|T) = (0.42|1|0.41)$ (as determined by Bauchinger *et al.* 1986) and a dose of 3 Gy with $\delta = 2.63$ DSB Gy^{-1} are assumed. An overall renormalization was used to make the sums $\Sigma g(k)$ the same in both calculations. The diagonal clearly shows that the values obtained are independent of the distribution of cells over metaphases. Additional, similar calculations were done for all pairwise combinations of (M1|M2|M3) = (100|0|0), (M1|M2|M3) = (0|100|0) and (M1|M2|M3) = (0|0|100) with selection parameters $(W|P|T) = (0.4|1|0.4)$ and (M1|M2|M3) = (0.3|0.5|0.2) with selection parameters (0|0|0) (full lethality) or (1|1|0.5) (unrestricted transmissibility). Results for every pair of calculations showed independence. Similarly, dose-independence of $g(i)$ was observed apart from an overall scale factor by comparing calculated results for 1, 2, 3 and 4 Gy.

The basic data therefore are the pairwise yields $f(j, k)$ for all 231 autosome pairs for the combined data (table 1). Note that with one exception, all entries are different from zero, indicating considerable variability in chromosome neighbourhoods—enough that every autosome pair but one was close enough for interchange production in some cells.

3.2. *Chromosome participation*

The chromosome yield for chromosome j, denoted by $f(j)$, was computed by summing $f(j, k)$ for fixed

j (i.e. adding a row and its corresponding column in table 1). The results are given in the column labelled Sum. To get a theoretical estimate of chromosome yields as a function of DNA content, we used CAS (see the Materials and methods) with $S=10$ and $\delta \sim 2.6$. Parameter δ was obtained according to the data itself.

Computer simulations were done for two cases: (1) the number of reactive DSB for a given chromosome is proportional to its DNA content; or (2) this number is proportional to (DNA content)$^{2/3}$. Results are shown in the last two columns of table 1 and in figure 2. It was found that the two-thirds law was superior to the linear model ($p<0.01$ in a χ^2-test).

3.3. *Chromosome interchanges and clustering*

We tested candidate chromosome clusters as suggested in the literature. For those studies where several clusters have been proposed, only those that ranked best are reported herein. Deviation from randomness for interchange reactions between different chromosomes was quantified as explained in the Materials and methods. Using the values obtained in table 1, entries $\Delta(j, k)$ for table 2 were generated. Using Monte Carlo computer simulations, the p value for the overall statistic ξ^2 was computed. The observed values differed significantly from randomness ($p<0.05$) for both the new data (not shown) and the combined data (table 2).

To investigate further this deviation from randomness, we computed the one-sided test described in the Materials and methods and assigned p values to candidate clusters of chromosomes (table 3). For the combined data, statistical significance ($p<0.05$) was confirmed in two of the 11 cases.

4. Discussion

The present paper has investigated both the sensitivity of different chromosomes to ionizing radiation and the statistically significant deviations from randomness in chromosome neighbourhoods.

The results on sensitivity of individual chromosomes have some similarities to results recently found by Braselmann *et al.* (2003). When comparing with a model where the number of breakpoints is linear with content, Braselmann *et al.* found that chromosomes 2 and 3 are underrepresented, while chromosomes 15–17 are over-represented, consistent with the results in figure 2 and table 1. We also found chromosome 9 to be more sensitive than expected, in agreement with Knehr *et al.* (1996).

Our overall results for the new and combined data are also in agreement with Cornforth *et al.* (2002), who reported that the number of reactive DSB for a given chromosome was approximately proportional to (DNA content)$^{2/3}$ (figure 2). This implies that larger chromosomes have fewer reactive DSB and small chromosomes have more reactive DSB than what would be expected by a random distribution of DSB over the whole genome. A possible interpretation of this result is that chromosome interchanges preferentially involve the periphery of the chromosomes (Cigarrán *et al.* 1998, Wu 2001).

We showed that there are large fluctuations in chromosome neighbourhoods but two groups of chromosomes have a statistically significant tendency to cluster more often than randomness would predict. Clustering for the set of chromosomes that form the nucleolus {13, 14, 15, 21, 22} gave $p=0.079$ for Cornforth *et al.* (2002), $p=0.002$ for the new data set and $p=0.002$ for the combined data set. The set of chromosomes previously reported to be in the centre of the nucleus by Boyle *et al.* (2001) {1, 16, 17, 19, 22}, gave $p=0.001$ for Cornforth *et al.* (2002), $p=0.09$ for the new data set and $p=0.001$ for the combined data set. The other candidate clusters were not confirmed ($p>0.05$).

There is increasing evidence for the role of higher-order chromosome geometry and nuclear architecture in basic cellular processes such as gene expression (Gasser 2001, reviewed by Parada and Misteli 2002). The present results suggest that intensive transcription may be coupled with positioning of chromosomes during interphase. In particular, the nucleolus is an extreme case of high transcription for ribosomal RNA. The set of chromosomes proposed by Boyle *et al.* (2001) as being in the centre of the nucleus and gene rich also suggests a high transcriptional activity. There are other chromosomes that may cluster due to other transcriptional structures such as the Oct1/PTF/transcription (OPT) domain associated with chromosomes 6 and 7 (review Parada and Misteli 2002). Nevertheless, we did not find statistical significance for such associations. In general, lack of statistical significance for a candidate cluster might indicate that the proximity of chromosomes in the cluster is weak, or is highly variable from cell to cell, or involves comparatively small subregions of the chromosomes as compared with the two confirmed clusters. An alternate explanation of time fluctuations within each cell is rendered doubtful by direct data on variations over time during interphase in any one cell (Gerlich *et al.* 2003, Walter *et al.* 2003).

To summarize, the large-scale organization of chromosomes in an interphase nucleus is determined by an interplay between systematic biology and randomness.

Table 2. Pairwise correlations for the combined data.

Chr	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.88	0.19	1.47	−0.79	−0.89	−0.37	−1.71	1.10	0.53	−0.64	−1.60	−1.96	−1.23	1.61	1.22	−0.10	−1.23	0.63	0.84	−0.82	3.21
2		1.79	1.33	0.40	0.47	−0.69	0.23	−0.02	−1.52	−0.25	1.39	1.50	−2.67	0.64	−3.00	0.90	−1.84	−1.08	−1.08	0.77	0.53
3			−1.40	0.47	1.11	−0.46	−0.38	−0.34	0.14	0.18	0.16	0.00	−0.53	−0.84	−0.47	0.44	−0.92	2.19	−1.17	0.62	−0.70
4				−0.57	0.63	0.82	0.72	0.15	−0.06	−1.91	−0.27	0.89	0.81	0.02	−0.73	0.16	−0.60	−1.03	−0.58	0.29	−1.29
5					−1.42	1.42	1.15	−0.06	0.81	1.71	1.05	1.34	−0.83	−0.21	−2.57	0.39	−0.05	−0.71	0.47	−0.71	−2.19
6						−0.89	0.64	−0.49	2.82	−1.62	2.61	0.08	−0.52	0.14	−0.90	−0.26	0.23	0.54	−1.14	−0.15	−1.08
7							0.18	−0.71	−0.42	1.70	1.44	−1.12	0.20	−2.44	0.04	1.72	−0.35	0.18	1.60	−0.85	−1.09
8								−1.61	−1.06	1.53	−1.30	2.47	0.04	0.01	−0.87	0.56	1.98	−1.23	−0.71	−0.12	−0.37
9									0.26	0.77	−2.87	1.00	1.15	1.71	0.28	−0.47	2.55	−0.31	−1.31	−0.64	−0.33
10										0.19	1.29	−0.26	−0.14	−1.42	1.01	0.10	−0.42	−0.81	−0.05	−0.06	−1.98
11											1.89	−2.78	−0.18	−0.22	0.67	0.07	−0.95	0.81	−0.02	−1.71	0.44
12												−1.54	0.39	−1.67	−0.78	0.66	−0.72	2.19	−0.33	−0.80	−1.30
13													4.14	−1.23	−1.11	−1.62	2.01	−0.67	−1.39	0.10	0.21
14														2.04	−0.21	−1.83	0.18	−1.78	−1.31	2.52	1.00
15															1.87	−0.10	−0.36	−0.01	0.02	−0.01	0.09
16																−0.25	1.66	2.00	2.90	0.47	1.57
17																	−1.29	−0.86	0.47	−0.46	0.86
18																		−1.23	1.40	2.02	−1.16
19																			0.44	−1.80	1.99
20																				0.89	1.51
21																					0.99

The values of $\Delta(j, k)$ are shown Values estimate deviations from spatial randomness for pairwise chromosome–chromosome geometric associations. A value of zero suggests randomness, while positive or negative values, respectively, suggest spatial proximity or extra separation.

Table 3. *p* values for candidate clusters.

Candidate cluster	Data		
	Old	New	All
A {1,16,17,19,22}	**0.001**	0.09	**0.001**
B {13,14,15,21,22}	0.079	**0.002**	**0.002**
C {17,19,20}	0.67	0.28	0.47
D {13,21}	0.68	0.38	0.51
E {14,22}	0.25	0.34	0.19
F {6,7}	0.93	0.53	0.83
G {9,22}	0.90	0.35	0.66
H {15,17}	0.33	0.8	0.57
I {8,14}	0.34	0.74	0.52
J {14,11}	1.0	1.0	1.0
K {14,18}	0.06	0.98	0.47

Entries are for chromosome associations proposed in the literature: (A) Boyle *et al.* (2001); (B) i.e. nucleolus, reviewed in Parada and Misteli (2002); (C) Cremer *et al.* (2001); (D, E) Alcobia *et al.* (2000); (F–H) reviewed in Parada and Misteli (1999); (I, J) Roix *et al.* (2003); (K) Lukasova *et al.* (1999). Under data, the first column corresponds to data previously published (Cornforth *et al.* 2002), the second corresponds to the new data and the third corresponds to the combined data (see table 2). Significant values ($p<0.05$) are in **bold**.

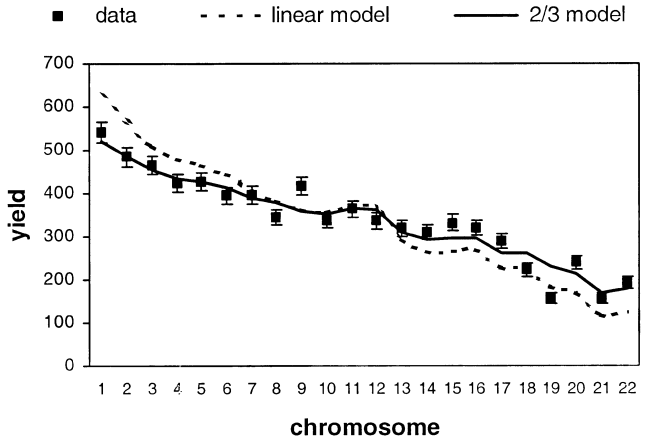

Figure 2. Chromosome participation. The *x*-axis represents chromosome number and the *y*-axis the individual chromosome yield $f(j)$ for $j=1, \ldots, 22$. Chromosome yields are compared with simulated results. Error bars represent the standard deviation of the mean, computed assuming Poisson distributed values. Two models were tested against the experimental data. The first assumes a linear relation between DNA content and participation; the second uses the two-thirds power of DNA content instead.

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Appendix

Let $f(j, k)$ be the pairwise yield and $f(j)$ be the one-chromosome yield. Table 1 gives the definitions and examples of these quantities. This appendix will show how to estimate $g(j)$ in terms of $f(j, k)$. For full randomness, one would have the equation:

$$f(j, k)=g(j)g(k). \tag{1}$$

The estimate of $g(j)$ cannot directly use equation (1), which need not hold if proximity effects are important, so an averaged form of equation (1), obtained by summing over chromosome partners, is used instead (Cornforth *et al.* 2002). The method is standard except that in the present case, one must do a little extra work because the diagonal terms $f(j, j)$ correspond to exchanges that do not produce a colour junction and are omitted in the analysis.

Specifically, summing equation (1) over k with $k\neq j$ gives:

$$f(j)=g(j)[-g(j)+\Sigma g(k)]. \tag{2}$$

The sum on the right-hand side goes over all k, including j.

$\Sigma g(k)$ is independent of j, and it is denoted by β. Thus, equation (2) can be rewritten as:

$$f(j)=\beta g(j)-g(j)^2. \tag{3}$$

Treating β as known temporarily, one can solve equation (3) for each $g(j)$ in terms of β and $f(j)$. Substituting this result into equation (2) and summing over all j then gives an equation that determines β. Inserting β back into equation (3) then determines each $g(j)$.

Since $g(j)$ is small with respect to $\beta=\Sigma g(k)$, one can implement this procedure by standard perturbation methods, solving the following equation using power series in the perturbation parameter λ and then setting $\lambda=1$ at the end of the calculation:

$$f(j)=\beta g(j)-\lambda g(j)^2. \tag{4}$$

Here

$$g(j)=g_0(j)+\lambda g_1(j)+\lambda^2 g_2(j)+\ldots,$$
$$\beta=\beta_0+\lambda\beta_1+\lambda^2\beta_2+\ldots, \tag{5}$$

and β_i is the sum of $g_i(j)$ over all j.

By equating powers of λ on both sides of the equation, one obtains successively more accurate approximations for β and for $g(j)$. To indicate how this works, we will show how to obtain the first two approximations.

For order 0 in λ, equation (4) reads:

$$f(j)=\beta_0 g_0(j). \tag{6}$$

Summing equation (6) over all j gives $\Sigma f(j)=\beta_0{}^2$.

In other words:

$$\beta_0 = [\Sigma f(j,k)]^{1/2},\ g_0(j) = f(j) \Big/ [\Sigma f(j,k)]^{1/2}, \quad (7)$$

where Σ is the sum over k with $k \neq j$ followed by the sum over all j.

For order 1 in λ, equation (4) reads:

$$0 = \beta_0 g_1(j) - g_0(j)^2 + \beta_1 g_0(j). \quad (8)$$

We can solve for β_1 by summing over all j, which gives $0 = 2\beta_0\beta_1 - \Sigma g_0(j)^2$, i.e. $\beta_1 = \Sigma g_0(j)^2 / 2\beta_0$, where the right-hand side is known from equation (7). Substituting β_1 back into equation (8) now gives $g_1(j)$. Higher order corrections are found similarly, and by going to a third order adequately accurate approximations to $g(j)$ were obtained from equation (5).

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