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GENETICS

Arrangement of Chromosomes in Human Cells. I. Associations in Metaphase Figures

by

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Presented by H. KOWARZYK on March 12, 1965

To our knowledge the arrangement of human chromosomes has not been the object of systematic investigation, the "satellite association" [2] and the association of acrocentric chromosomes with the centromere region of chromosome No. 1 [6] excepted.

Preparations from short-term cultures of peripheral blood cells were obtained with the use of polyvinyl alcohol to facilitate the isolation of leukocytes, phytohem--agglutinin [5], colcemide and hypotonic shocking in 0.8 per cent solution of sodium citrate. Our procedure [7] enabled us to obtain several hundreds of mitoses from 1 ml. of blood, many of which were perfectly suitable for chromosome analysis.

Two male and two female patients without recognizable chromosome anomalies were subjected to detailed examination. Sets of 25 cells each were collected from the individual cell cultures.

We reduced our study to the arrangement of centromeres, these being the only points defined biologically and geometrically without ambiguity. The procedure can be followed in the Figure 1.

The first step consists in pricking with a pin, on the 3200 times magnified photo of a single cell (Figure 1, left), through all the 46 centromeres. The result is a set of 46 geometric points on the back of the photo. As second step we connect by arrows every point of the set with its nearest neighbour (Figure 1, centre). In some cases the point next to P is Q, and the point next to Q is P ("reciprocal neighbourhood"). There are, however, other cases: Q is next to P but the point next to Q is R, different from P ("one-way neighbourhood"); double, respectively single darts indicate the corresponding arrows. This procedure yields eventually a geometrical configuration known as a "dendrite" [3]. The advantage is the reduction of more than 1000 distances between the 46 centromeres to at most 40 single or double arrows on our diagrams. In most cases the dendrites are split into separate branches. The number of branches is rarely less than 11 and rarely surpasses 15. The third step is to provide the points (centromeres) of the dendrites with letters A, B, ..., G X, Y given by *Denver classification* [1]. They are to be read on the reverse of our

dendrites. As a normal set of chromosomes includes 7 groups, the 46 points of our dendrites fall into 7 groups denoted by the letters A (6 points), B (4 points) C (14 points), D (6 points), E (6 points), F (4 points), G (4 points) and $X+X$ or $X+Y$ (2 points) (Figure 1, right). The collection of such dendrites, provided with letters, constitutes the stock of empirical data to be analyzed statistically as follows.

Let us call H_0 (= zero hypothesis) the assumption that the distribution of the Denver letters over the vertices of a dendrite is purely random; admitting H_0 we can easily compute the expected number of arrows, say $\overrightarrow{AB}$ and $\overrightarrow{BA}$, in a set of 25 pictures, and compare this value with the observed number which results immediately from a real dendrite provided with Denver letters. This is, roughly speaking, the method we applied to recognize, in sets of 25 cells each, the incompatibilities with the random distribution of centromeres.

TABLE I

Expected numbers

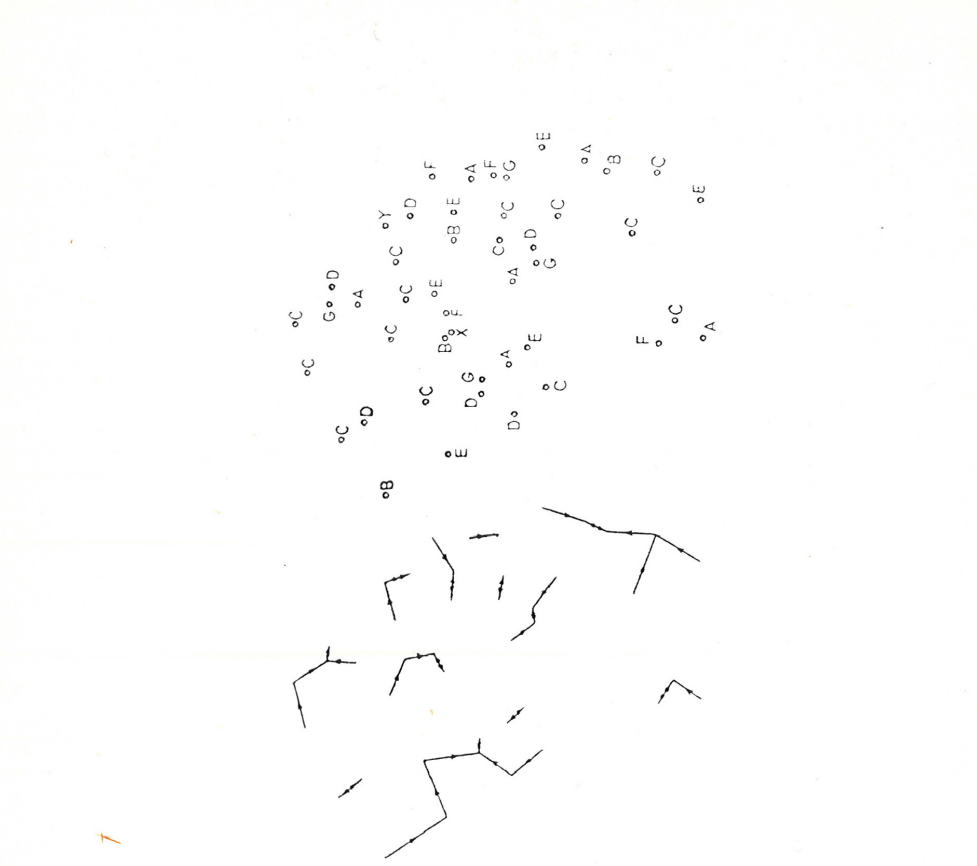
	A	B	C	D	E	F	G	X	Y	
A	16.7	13.3	46.7	20.0	20.0	13.3	13.3	3.3	3.3	150
B	13.3	6.7	31.1	13.3	13.3	8.9	8.9	2.2	2.2	100
C	46.7	31.1	101.1	46.7	46.7	31.1	31.1	7.8	7.8	350
D	20.0	13.3	46.7	16.7	20.0	13.3	13.3	3.3	3.3	150
E	20.0	13.3	46.7	20.0	16.7	13.3	13.3	3.3	3.3	150
F	13.3	8.9	31.1	13.3	13.3	6.7	8.9	2.2	2.2	100
G	13.3	8.9	31.1	13.3	13.3	8.9	6.7	2.2	2.2	100
X	3.3	2.2	7.8	3.3	3.3	2.2	2.2		0.6	25
Y	3.3	2.2	7.8	3.3	3.3	2.2	2.2	0.6		25
	1150	100	350	150	150	100	100	25	25	1150

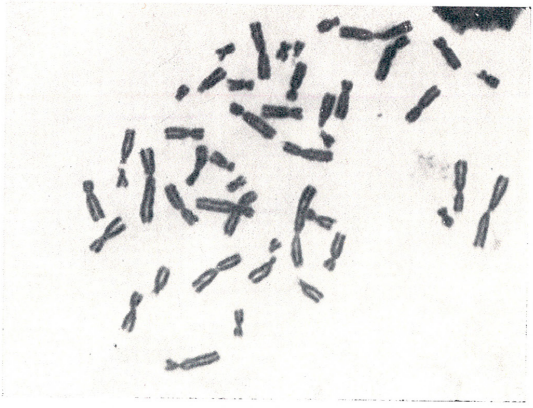
The figures listed in this Table are the expected numbers of arrows connecting letters along the upper border with those along the left one. E.g.: the number 13.3 on the crossing of line A with column B tells the reader that in a total of 25 mitoses we are to expect 13.3 cases in which the point next to A is B. This assertion is a purely mathematical consequence of the hypothesis H_0; the numbers listed in Table I do not depend on observations; they are the result of computation and apply to all normal men in the sense of the *Denver classification*. In the case of women, column and line Y are to be cancelled and the figures in column and line X doubled; 1.1 should be put in the crossing XX.

To exemplify, let us compute, say, the expected number of neighbourhoods AB. Denoting this number by $\exp AB$ we have to apply the formula

$$\exp AB = \frac{a}{46} \cdot \frac{b}{45} \cdot n,$$

where a = number of A-centromeres in a cell = 6, b = number of B-centromeres in a cell = 4, n = total number of centromeres in 25 cells = 25·46. Consequently,





$a/46$ is the probability for a point to be A, $b/45$ the probability for the next point to be B (one A has been discarded!). It follows for the expected number of neighbourhoods AB:

$$\exp AB = 6 \cdot 4 \cdot 25 \cdot 46 : 46 \cdot 45 = 13.33.$$

The computation of $\exp BA$, i.e. of the expected number of neighbourhoods BA, yields the same result: $\exp BA = 13.33$. The formula for AA-neighbourhoods is

$$\exp AA = \frac{a}{46} \cdot \frac{a-1}{45} \cdot n.$$

Other expected numbers can be determined in a similar way. The identity of $\exp AB$ and $\exp BA$ is valid for all pairs of letters; thus in Table I the diagonal $AA-YY$ is its axis of symmetry.

TABLE II

Observed numbers. Case ♂ J.S.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*X*	*Y*
A	22.5	13.5	41.5	20	9.5	13	2.5	1	2
B	14.5	5	30	12.5	13.5	14	4.5	5	0
C	51.5	29	110.3	28	45.5	31	19	8	13
D	21	13	33.3	34	19.5	8	25	2	2
E	12	15.5	44.5	12	24.5	14	11.5	5	2
F	17.5	14	35.8	6.5	14	6	9.5	2	3
G	5	5	37	28	15.5	9	22	2	3
X	3	5	10.5	4	5	3	3		0
Y	3	0	7	5	3	2	3	0	
	150	100	350	150	150	100	100	25	25

Example. To find the proximal neighbourhood $\overrightarrow{AB}$ one must take the crossing of column *A* and line *B*, and for $\overrightarrow{BA}$ – column *B* and line *A*!

Table II presents the numbers of neighbourhoods supplied by 25 mitoses obtained from ♂ J.S. It is striking to see most data of Table II approaching the corresponding data of Table I; some fields, however, exhibit positive or negative differences from the expected numbers. The question arises what are the probabilities to come across such or greater difference if the zero-hypothesis is to be admitted? The answer is furnished by the *chi-square* test.

The *chi-square* values are given by the formula

$$\frac{(\text{observed} - \text{expected})^2}{\text{expected}}.$$

Numbers less than 1 are denoted by 0 without decimals. Void fields pertain to the expected numbers less than 5.

TABLE III

Table of *chi-square*. Case ♂ J.S.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*X*	*Y*
A	2.0	0	0	0	**5.5**	0	**8.8**		
B	0	0	0	0	0	2.9	2.2		
C	0	0	0	**5.9**	0	0	**4.7**	0	3.5
D	0	0	0	**18.0**	0	2.1	**10.2**		
E	3.2	0	0	3.2	3.6	0	0		
F	1.3	2.9	0	3.5	0	0	0		
G	**5.2**	1.7	1.1	**16.1**	0	0	**36.2**		
X			0						
Y			0						

Example 1. Table II shows at the intersection of column *G* and line *A* the observed number 2.5; on the corresponding field on Table I we read 13.3 as the expected number. Consequently, on the corresponding field of Table III we read 8.8 because

$$\frac{(2.5 - 13.3)^2}{13.3} = 8.8.$$

Statistical tables tell us that the probability for the *chi-square* to surpass 8.8 under the condition H_0 is 0.003, [4]. Thus we have to reject H_0 "on the 0.003 significance level" and admit biological causes as responsible for the deviation observed. In other words: we are entitled to suppose a repulsion between G and A chromosomes.

Example 2. Column *B*, line *G* yields *chi-square* = 1.70. The aforesaid statistical tables indicate for *chi-square* = 1.70 a probability of 0.20. The 0.20 significance level does not allow to suppose a nonrandom distribution, such statement being exposed to error in 20 per cent cases.

Statements based on expected numbers less than 5 are uncertain [4]. Table I exhibits such low numbers only for the expected neighbourhoods of *Y* and *Y* chromosomes. This is the reason why the corresponding *chi-square* values are omitted in Table III.

We limit our attention to the entries of Table III yielding at least 3.84 as *chi-square* value. Deviations from the random distribution indicate approaching or keeping back tactics. Both cases are symbolized by marks over the entries of Table III: $\wedge$ means that the observed number of arrows exceeds the expected level, $\vee$ means that this number lies below this level.

Table III shows an essential trend in bringing together chromosomes of the *D*+*G* group. Opposite effects can be seen between *D* and *C*, *G* and *C*, *G* and *A*,

A and *G*; they might be interpreted as a compensatory consequence of the essential trends.

In addition, a keeping away effect has been observed between *E* and *A* in this case. Several minor effects might be suspected.

The method might be simplified by disregarding the one-way neighbourhoods and taking into account exclusively the numbers of reciprocal neighbourhoods (double darted arrows) in the dendrite. This would reduce the considered number of neighbourhoods to almost 37 per cent and make the results of Table III even more convincing.

Let us compute the probability P' of a pair to be $A \rightarrow B$:

$$P' = \frac{a}{46} \cdot \frac{b}{45} \cdot 2 \cdot m,$$

where a = number of centromeres $A = 6$,
b = number of centromeres $B = 4$,
m = number of double-darted arrows found in 25 dendrites.
(The number 2 takes into account the reciprocity of $\overrightarrow{AB}$ and $\overrightarrow{BA}$).

Analogically, the probability P'' of some pair to be $A \rightarrow A$ is given by the formula

$$P'' = \frac{a}{46} \cdot \frac{a-1}{45} \cdot m,$$

where $a = 6$ denotes the number of *A*-centromeres.

The values found in this way are the expected numbers of the reciprocal neighbourhoods. The corresponding *chi-square* values are listed in Table IV. Beneath the diagonal the Table is void as the figures ought to be simply repeated in the opposite fields of the Table.

TABLE IV

Table of *chi-square* pertaining to the reciprocal neighbourhoods. Case ♂ J.S.

	A	*B*	*C*	*D*	*E*	*F*	*G*	*X*	*Y*
A	1.8	1.1	0	1.3	4	0	**6.1**		
B			0	1.1	0	4	0		
C			0	2.3	0	0	0		
D				**12.8**	2	1.1	**2.1**		
E					0	0	0		
F							0		
G									
X									
Y									

Table IV corroborates the close neighbourhood of the centromeres *D* and *G*. Several relations are omitted in the Table IV, as the expected number of double-darted arrows between the points is less than 5. It is only in consideration of this scruple that we prefer not to list the high significance of the reciprocal neighbourhood between the *G*-centromeres. This experiment is, however, even more convincing than the results listed in Table III.

In conclusion: 1. Our statistics corroborate the close association of the acrocentric chromosomes.

2. No regularly occurring associations of the meta- and submetacentromeric chromosomes could be found, although statistically significant effects were noticed several times in sets of cells derived from the same cultures. This might reflect an orderly spatial grouping before metaphase, persisting in spite of the destruction of the spindle treated with colcemide and in spite of the technical process of spreading. These problems will be the subject of a subsequent paper.

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