CHARACTER OF THE ASSOCIATION BETWEEN SIZE OF C SEGMENTS AND CORRESPONDING EUCHROMATIC REGIONS OF HUMAN CHROMOSOMES 1, 9, AND 16 DURING MITOTIC CONDENSATION

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Determination of the size of the C segments of chromosomes 1, 9, and 16 by morphometry can eliminate subjectivism in the assessment of their size, which arises in the case of visual analysis. However, the objective quantitative characterization of the size of C segments is impossible if the variability of their size due to mitotic condensation of the chromosomes is disregarded [8]. Although several methods have been suggested for this purpose [1-6, 9, 11], there is as yet no unified method of evaluation of the size of the C segments of chromosomes 1, 9, and 16. Data in the literature do not enable the advantages of any particular method in overcoming variability of the size of the C segments due to mitotic condensation of chromosomes to be judged. Because of the need to develop such a method, the character of the relationship between the size of the C segments and of the euchromatic regions of chromosomes 1, 9, and 16 was studied during mitotic condensation in the investigation described below.

EXPERIMENTAL METHOD

Preparations of metaphase chromosomes from blood lymphocytes of three healthy women were stained by the C method [10]. From each individual 100 metaphase plates were photographed on "Mikrat-300" film. The negatives were projected on the screen of a "Mikrofot" apparatus and the boundaries of the C segments and euchromatic regions of chromosomes 1, 2, 9, and 16 were marked in accordance with the recommendations in [2]. The results of the measurements were estimated in microns, considering that the total magnification from the original size of the chromosome was 3000 times. Homologous chromosomes were investigated separately. The resulting sample of results of measurements of the C segments (qh) and of the euchromatic regions (p + q) of chromosomes 1, 9, and 16 of each individual was split up into groups, on the basis of the length of chromosome 2 in the metaphase plate: under 5 μ (7-10 metaphases), 5-6.5 μ (8-16 metaphases), 6.5-8.5 μ (27-38 metaphases), 8.5-10 μ (16-21 metaphases), 10-12 μ (10-21 metaphases), and over 12 μ (6-11 metaphases). In each group and for the sample as a whole, the magnitude and statistical significance of the coefficient in the linear regression equation $qh = a + b(p + q)$, the coefficient of correlation ρ of this association, and also the values of the standard deviation σ of the size of the C segments of chromosomes 1, 9, and 16 were determined.

EXPERIMENTAL RESULTS

The results are evidence of linear correlation between the size of the C segments and the size of the euchromatic regions of chromosomes 1, 9, and 16 during their mitotic condensation (Table 1). However, an increase in the values of the coefficients of the regression equation and of correlation in the boundary stages of the process of mitotic condensation of the chromosomes compared with their values in the central stage (Fig. 1) points to the heterogeneous character of this association. Since the tendency observed was characteristic of all chromsomes of the individual studied, it was possible to distinguish three ranges of

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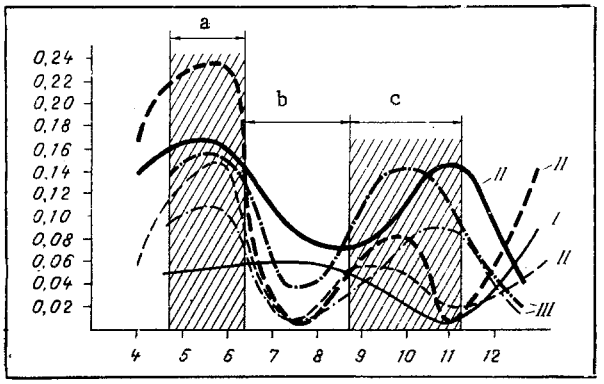

Fig. 1

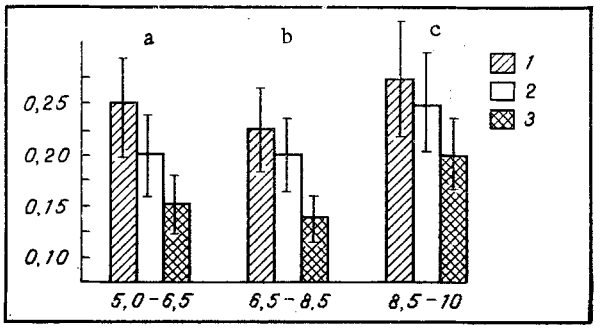

Fig. 2

Fig. 1. Character of linear correlation between size of C segments and of euchromatic regions of chromosome 1 at different stages of its mitotic condensation. Abscissa, length of euchromatic regions of chromosome 1 (in μ); ordinate, values of coefficient in regression equation. I, II, III) Individuals tested (bold and thin lines denote homologous chromosomes 1 of each individual. a, b, c) Ranges 1, 2, and 3, respectively.

Fig. 2. Variability of absolute size of C segments of chromosomes 1, 9, and 16 at individual stages of their mitotic condensation. Abscissa, length of euchromatic regions of chromosome 2 (in μ): a, b, c) ranges 1, 2, and 3 (respectively) of mitotic condensation of chromosome 2, differing in character of linear correlation between sizes of euchromatic and heterochromatic regions of chromosomes 1, 9, and 16; ordinate, standard deviation σ of mean values of absolute sizes of C segments. 1, 2, 3) Chromosomes 1, 9, and 16, respectively.

TABLE 1. Statistical Significance of Linear Correlation Between Size of C Segments and of Corresponding Euchromatic Regions of Chromosomes 1, 9, and 16 in the Course of Their Mitotic Condensation

Chromosome	Magnitude and statistical significance of coefficients of regression equation and of correlation					
	individual I		individual II		individual III	
	b	ρ	b	ρ	b	ρ
1′	0,11***	0,7***	0,09***	0,4***	0,11***	0,6***
1″	0,88***	0,6***	0,07**	0,3**	0,08***	0,4***
9′	0,21***	0,5***	0,2***	0,5***	0,15***	0,4***
9″	0,18***	0,6***	0,12***	0,4***	0,08*	0,3*
16′	0,17***	0,6***	0,11*	0,3*	0,27***	0,6***
16″	0,21***	0,7**	0,13***	0,4***	0,31***	0,7***

Legend. Strokes indicate homologous chromosomes. In all cases sample consisted of 100 chromosomes. Asterisks indicate levels of significance of coefficients of regression equation (b) and correlation (ρ): *$p < 0.025$, **$p < 0.005$, ***$p < 0.001$.

mitotic condensation of chromsomes, differing in the character of linear association between the size of the C segments and of the euchromatic regions of chromosomes 1, 9, and 16 (Table 2).

Using mitotic condensation of chromosome 2 as the guide, the width of the ranges of 1, 2, and 3 was 4-6.5, 6.5-8.5, and 8.5-11 μ, respectively. Differences in the values of the coefficients of the regression equation and of correlation between ranges 2 and 3 were statistically significant in character for all chromosomes studied, whereas between ranges 1 and 2, they were observed only for chromosomes 1 and 9 ($p < 0.05$). A decrease in the values of the coefficients b and ρ compared with ranges 1 and 3 also was observed in metaphase plates in which chromosome 2 was less than 4 μ and over 11 μ in length, in agreement with data in the literature [14] on changes in the linear character of correlation between the values for the euchromatic and heterochromatic regions of the chromosomes at these stages of their mitotic condensation.

In our view, range 2 is of particular interest for the quantitative estimation of size of the C segments, for it was marked by minimal values of the standard deviation of size of

TABLE 2. Stages of Mitotic Condensation of Chromosomes 1, 9, and 16, Differing from Each Other in the Character of Linear Correlation Between the Size of the C Segments and of the Euchromatic Regions of These Chromosomes

Chromosome	Range of individual stages of mitotic condensation of chromosomes (in μ)		
	range 1	range 2	range 3
1	4.0—6.5	6.5—8.5	8.5—11
9	2,5—3.4	3,4—4.3	4.3—4,8
16	1.5—2.6	2,6—2.8	2.8—3,6

the C segments (Fig. 2). This is evidence that variability of size of the C segments is least marked in this range, by comparison with the other stages of mitotic condensation of the chromosomes. Differences in the value of σ between ranges 2 and 3 were statistically significant ($p < 0.05$).

The data obtained are evidence of a change in the character of linear correlation between the sizes of the euchromatic and heterochromatic regions of chromosomes 1, 9, and 16 at individual stages of their mitotic condensation. The use of two methods of estimation of the absolute size of the C segments depending on the degree of mitotic condensation of the chromosomes can thus be recommended. In metaphase plates in which chromosome 2 is between 6.5 and 8.5 μ in length, it is worthwhile averaging the results of measurements of the length of the C segments, which is evidence that this method is correct [7]. Within the range of mitotic condensation of chromosome 2 from 11 to 4 μ, regression correction of their values is indicated [1, 5, 6], the essential condition for which must be determination of the statistical significance of the coefficient of the regression equation. The absence of linear correlation between the sizes of the euchromatic and heterochromatic regions of chromosomes 1, 9, and 16 is an indication for estimation of the absolute size of the C segments by averaging the results of measurements of their length.

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