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Genetics

Base ratio, DNA content, and quinacrine-brightness of human chromosomes

(autoradiography/banding/DNA content/quinacrine stain)

JULIE R. KORENBERG AND WILLIAM R. ENGELS

Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT **Human chromosomes were labeled with base-specific radioactive DNA precursors and examined autoradiographically to measure their DNA content and base ratio (percentage A·T base pairs). The requirement that incorporation of labeled bases be uniform during DNA synthesis was met by the use of inhibitors of *de novo* synthesis of DNA precursors. The genome was subdivided into 75 segments based on quinacrine banding, and the base ratio of each was calculated by a method that corrects for bias due to the scatter of grains about their source. Estimates of base ratio are shown to be sufficiently precise to detect variability among chromosomes and among segments within a chromosome. Analysis of these data and of measurements of the quinacrine fluorescence intensity of segments leads to the following conclusions. Base ratio is positively correlated with brightness, as predicted from independent *in vitro* studies. Larger chromosomes tend to have higher base ratios and to be brighter than smaller ones. The best prediction of the brightness of a segment must take into account not only its base ratio but also its DNA content. To explain these results, we suggest an evolutionary model in which chromosomes containing repeated sequences of A·T-rich DNA tend to grow by means of unequal sister chromatid and meiotic exchanges.**

The human genome consists of 100–200 metaphase bands defined by fluorescent staining. Various studies indicate that these bands are functional units of the chromosome. For example, quinacrine-bright regions replicate their DNA later during S phase and condense earlier in prophase than do dark ones. They also have fewer mitotic chiasmata (1) and x-ray-induced chromosomal breaks (2) than do dark bands. Furthermore, recent work suggests that genes are concentrated in the dark areas (3).

Because each band has an average of 10^6 base pairs, we might expect that, unless highly reiterated short sequences are involved, the average base composition of a band would be similar to that of the whole cell. However, qualitative studies with fluorescent dyes (4), base-specific antibodies (5), and autoradiography (6) suggest that base compositions may vary considerably from one band to the next. Although work with eukaryotic satellite DNAs and A·T- vs G·C-rich fractions of main band DNA has not been able to determine the extent to which fluorescence is due to base composition (7), *in vitro* studies (8) indicate that quinacrine-bright bands may be A·T rich.

We have devised an autoradiographic technique to estimate the base composition of human chromosomes and chromosomal segments with sufficient accuracy to determine that base ratios do vary over long stretches of DNA. The data also provide estimates of the DNA content of each chromosome and segment. We use these estimates to investigate the organization of chromosomes and the relationship of base composition to other chromosome parameters such as size and fluorescence intensity.

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MATERIALS AND METHODS

Cell Culture and Autoradiography. Peripheral lymphocytes from a male donor were grown for 72 hr in medium F10 (Gibco) lacking thymidine in the presence of either [^{3}H]thymidine (New England Nuclear) or [^{3}H]guanine (Schwarz/Mann), with the addition of Colcemid (0.06 μg/ml) for the last 2 hr of culture. The cells were then treated with hypotonic solution (0.075 M KCl), fixed in methanol/acetic acid, 3:1 (vol/vol), dropped on cold wet slides, air dried, stained with quinacrine, and photographed. After photography the slides were treated at 37° for 90 min with RNase (Worthington, 150 μg/ml in 0.30 M NaCl/0.03 M Na citrate, pH 7.0). After autoradiography with Ilford K5 emulsion the cells were stained with orcein and photographed. Both types of photograph were made on Kodak high-contrast copy film and printed at the same magnification, ×3000, so that the two could be superimposed. Cuts were made *through* the banded fluorescent print onto the print of the autoradiograph to delineate the segment borders (Fig. 1). Grains lying within 1 μm of a chromosome were assigned to specific quinacrine segments and counted.

Labeling. DNA was isolated by standard methods on cesium sulfate gradients, dialyzed, digested to mononucleotides with DNase I (Sigma) and snake venom phosphodiesterase, and analyzed by high-voltage electrophoresis (9). To eliminate change in specific activity of DNA precursor pools during S phase, *de novo* synthesis of guanine was inhibited by 16.7 μM mycophenolic acid (10) (Imperial Chemical Industries), which prevents the conversion of inosine monophosphate to xanthine monophosphate. In the presence of this inhibitor, lymphocytes show no uptake of [^{3}H]thymidine during the last 24 hr of culture. However, growth is restored when guanine (67, μM; 0.3 μCi/ml) is also present. Because we found that, in lymphocytes, 30% of the ^{3}H added as [^{3}H]guanine was incorporated into DNA as [^{3}H]adenine, unlabeled adenine (30 μM) was added to decrease the proportion to 10%. The calculations of base ratio and DNA content take this 10% conversion into account. Similarly, to prevent fluctuation of the thymidine precursor pool, *de novo* thymidine synthesis was inhibited with 5-fluorodeoxyuridine at 10 μM (11). After 3 days of growth at this concentration, more than 96% of the thymidine incorporated into DNA derives from the exogenous pool. None of the ^{3}H added as thymidine is found in other DNA nucleotides. In addition to the [^{3}H]thymidine (0.75 μCi/ml, 10 μM), guanine (10 μM), adenine (30 μM), and uridine (10 μM) were added to the medium because they increased the growth rate.

Fluorescence Intensity. Quinacrine-brightness ratings for each section were modified from Kuhn (1). Brightness of each quinacrine band was evaluated in one cell by comparison to a

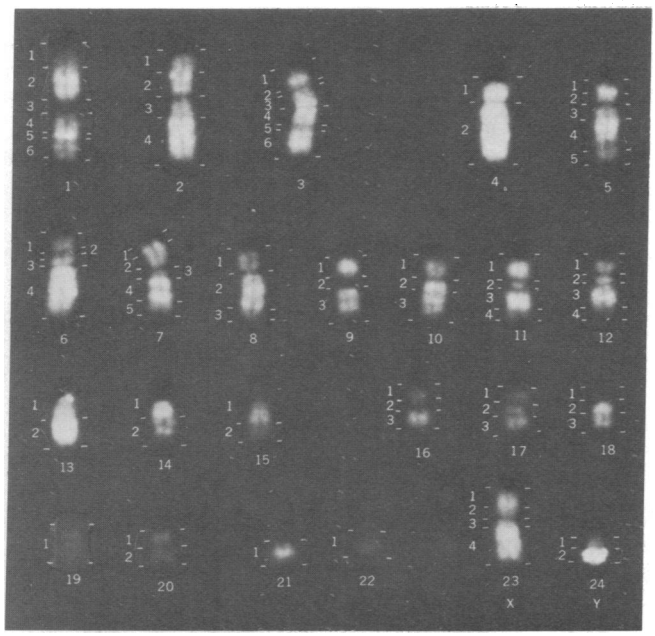

FIG. 1. Quinacrine fluorescence of human chromosomes, delineating the chromosome segments used in this study [after Kuhn (1)]. Relative brightness of chromosomes and sections was determined from this photograph.

standard chart. The cell was selected on the basis of uniform fluorescence of homologues. The brightness of chromosomes is the average value of the brightness of each of the sections, weighted by its DNA content.

THEORY

The DNA content and base ratio of a chromosome or chromosome segment can be estimated from the average numbers of grains found in it. The precision of these estimates is greatest when only A·T base pairs are labeled in one of the two complementary treatments and only G·C base pairs in the other. In general, however, it is only necessary that the average base compositions of the labeled base pairs are not identical in the two categories.

We define DNA content, λ_i, and base ratio, A_i, of the ith chromosome in terms of the (unknown) numbers of A·T and G·C base pairs in that chromosome. If we call these numbers a_i and b_i, respectively, then $\lambda_i = (a_i + b_i)/(a + b)$, and $A_i = a_i/(a_i + b_i)$, in which a and b are the total numbers of A·T and G·C base pairs in the genome. These definitions can be written in terms of the average base ratio of the genome:

$$\lambda_i = \frac{a_i}{a}A + \frac{b_i}{b}(1 - A); \qquad [1]$$

$$A_i = \frac{a_i}{a} \cdot \frac{A}{\lambda_i}, \qquad [2]$$

in which $A = a/(a + b)$. Because the value of A can be determined by independent experiments, it is only necessary to calculate the ratios a_i/a and b_i/b from the autoradiographic data. To do this, two assumptions are needed:

Assumption 1. The number of grains observed over a chromosome is proportional to the number of labeled bases in that chromosome, and this proportionality is the same for all chromosomes. It will be convenient to express the average number of grains in a chromosome as a fraction of the genomic total. Thus, t_i is the fraction of all average grains in the thymine-labeled cells that were found over chromosome i, and g_i is the fraction of grains found over chromosome i in the guanine-labeled cells. Assumption 1 can be expressed mathematically as:

$$t_i = \frac{a_i' + b_i'}{a' + b'}; \qquad g_i = \frac{a_i^* + b_i^*}{a^* + b^*},$$

in which a_i', etc. refer to the *labeled* base pairs in the thymine-labeled cells, and a_i^* etc. refer to the *labeled* bases in the guanine-labeled cells.

Assumption 2. The ratio of labeled to unlabeled bases is the same for all chromosomes. This assumption is valid when the specific activities of the two nucleotide pools are constant throughout the DNA synthesis period. This assumption can be written as:

$$a_i^*/a_i = a^*/a; \quad a_i'/a_i = a'/a; \quad b_i^*/b_i = b^*/b; \quad b_i'/b_i = b'/b.$$

If labeling occurs only in G·C base pairs in one of the two labeling categories and only in A·T base pairs in the other, then assumption 1 reduces to $g_i = b_i^*/b^*$ and $t_i = a_i'/a'$; and, by assumption 2, the ratios a_i/a and b_i/b are simply g_i and t_i, respectively. DNA content and base ratio can then be computed directly from Eqs. 1 and 2. In general, however, when more than one base is labeled by a given treatment, the ratios a_i/a and b_i/b can still be computed from

$$a_i/a = [t_i(1 - A^*) - g_i(1 - A')]/(A' - A^*), \qquad [3]$$

and

$$b_i/b = [g_iA' - t_iA^*]/(A' - A^*), \qquad [4]$$

in which $A' = a'/(a' + b')$ and $A^* = a^*/(a^* + b^*)$; these are the fractions of A·T base pairs among the labeled bases in the two labeling categories and are measured independently. The proof of Eq. 4 is as follows. Use assumption 1 to substitute for t_i and g_i, and replace A' and A^* by their definitions. Then

$$\frac{g_iA' - t_iA^*}{A' - A^*} = \frac{(a_i^* + b_i^*)a' - (a_i' + b_i')a^*}{(a^* + b^*)a' - (a' + b')a^*},$$

$$= \frac{a_i^*a' - a_i'a^* + b_i^*a' - b_i'a^*}{b^*a' - b'a^*}.$$

Using assumption 2 to replace a_i^*, a_i', b_i^*, and b_i' with a^*a_i/a, $a'a_i/a$, b^*b_i/b, and $b'b_i/b$, respectively, leads to Eq. 4. The proof of Eq. 3 is similar. The final formulae for calculating DNA content and base ratio for whole chromosomes are obtained by combining Eqs. 1, 2, 3, and 4 and simplifying, to give

$$\lambda_i = t_i\frac{A - A^*}{A' - A^*} + g_i\frac{A' - A}{A' - A^*}; \qquad [5]$$

$$A_i = A\left[\frac{t_i(1 - A^*) + g_i(A' - 1)}{t_i(A - A^*) + g_i(A' - A)}\right]. \qquad [6]$$

We next consider the problem of calculating DNA content and base ratio in segments of chromosomes when radioactive decays in one segment can result in grains in another. This situation is due to the long mean path length of a ^3H disintegration and causes the grains in a particular segment to reflect the base ratios of neighboring segments. If the photographic emulsions used in the two cell treatments are assumed to be identical, it can be shown that the approximate base ratio calculated from Eq. 6 is a linear combination of the true base ratios of all the segments of that chromosome, with coefficients determined by the geometry of the chromosome and the degree of scatter of grains about their source. (Details of this calculation will be supplied by W. E. on request.) The true base ratios are found by numerically solving the system of simultaneous equations for each of the subdivided chromosomes. This correction is

Table 1. Base ratio, DNA content, and brightness of whole chromosomes and segments

Chromosome or segment*	Base ratio† ±SD	DNA content‡ ±SD	Brightness§	Chromosome or segment*	Base ratio† ±SD	DNA content‡ ±SD	Brightness§
1	60.3 ± 0.8	8.67 ± 0.12	0.54	7.1	61.7 ± 3.8	0.77 ± 0.03	0.51
2	61.3 ± 0.8	8.23 ± 0.12	0.66	7.2	57.6 ± 4.4	1.26 ± 0.04	0.63
3	60.8 ± 0.8	7.03 ± 0.10	0.59	7.3	56.2 ± 7.4	0.53 ± 0.03	0.55
4	59.7 ± 0.9	6.57 ± 0.11	0.79	7.4	60.7 ± 2.4	1.99 ± 0.05	0.76
5	60.5 ± 0.8	6.25 ± 0.10	0.60	7.5	59.9 ± 2.8	1.00 ± 0.04	0.51
6	60.7 ± 0.9	5.92 ± 0.11	0.67	8.1	61.9 ± 1.9	1.58 ± 0.05	0.49
7	59.5 ± 0.9	5.55 ± 0.09	0.63	8.2	60.2 ± 1.7	2.41 ± 0.06	0.72
8	59.9 ± 0.9	4.97 ± 0.09	0.59	8.3	56.3 ± 2.8	0.99 ± 0.04	0.43
9	59.7 ± 0.9	4.69 ± 0.08	0.43	9.1	62.6 ± 2.3	1.44 ± 0.05	0.55
10	61.4 ± 0.9	4.81 ± 0.08	0.52	9.2	53.9 ± 4.5	0.90 ± 0.03	0.12
11	59.8 ± 1.0	4.77 ± 0.08	0.55	9.3	60.2 ± 1.5	2.35 ± 0.06	0.47
12	60.4 ± 0.9	4.64 ± 0.08	0.49	10.1	59.1 ± 2.1	1.51 ± 0.05	0.49
13	60.2 ± 1.1	3.60 ± 0.08	0.77	10.2	69.3 ± 4.0	0.95 ± 0.03	0.64
14	57.9 ± 1.1	3.53 ± 0.07	0.61	10.3	59.6 ± 1.5	2.36 ± 0.06	0.49
15	58.4 ± 1.1	3.40 ± 0.07	0.46	11.1	59.0 ± 2.2	1.57 ± 0.05	0.52
16	59.7 ± 1.0	3.25 ± 0.06	0.41	11.2	61.0 ± 5.3	0.92 ± 0.04	0.48
17	61.5 ± 1.2	3.06 ± 0.07	0.36	11.3	64.0 ± 3.8	1.29 ± 0.04	0.73
18	61.4 ± 1.1	2.84 ± 0.06	0.42	11.4	54.6 ± 3.1	0.99 ± 0.04	0.41
19	56.3 ± 1.4	2.25 ± 0.06	0.20	12.1	62.8 ± 2.5	1.29 ± 0.04	0.40
20	57.6 ± 1.3	2.38 ± 0.06	0.33	12.2	60.1 ± 5.5	0.84 ± 0.03	0.53
21	56.2 ± 1.7	1.70 ± 0.05	0.41	12.3	60.0 ± 3.0	1.49 ± 0.04	0.64
22	58.4 ± 1.6	1.88 ± 0.06	0.19	12.4	58.2 ± 2.7	1.02 ± 0.04	0.35
X	59.3 ± 1.3	5.51 ± 0.13	0.67	13.1	58.0 ± 2.0	1.66 ± 0.05	0.72
Y	62.4 ± 1.8	2.01 ± 0.07	0.73	13.2	62.1 ± 1.6	1.93 ± 0.05	0.82
				14.1	58.8 ± 1.9	1.72 ± 0.05	0.73
1.1	57.8 ± 1.7	2.15 ± 0.06	0.35	14.2	57.0 ± 1.8	1.81 ± 0.05	0.50
1.2	64.4 ± 2.4	1.92 ± 0.06	0.73	15.1	60.2 ± 1.7	1.81 ± 0.05	0.45
1.3	53.3 ± 5.7	0.97 ± 0.04	0.29	15.2	56.4 ± 1.9	1.59 ± 0.05	0.47
1.4	63.7 ± 5.3	1.12 ± 0.04	0.56	16.1	59.7 ± 2.8	1.14 ± 0.04	0.31
1.5	66.1 ± 5.8	0.94 ± 0.04	0.84	16.2	60.2 ± 5.8	0.67 ± 0.03	0.31
1.6	56.9 ± 2.3	1.56 ± 0.05	0.53	16.3	59.4 ± 2.3	1.44 ± 0.04	0.53
2.1	63.4 ± 2.1	1.40 ± 0.05	0.56	17.1	66.4 ± 3.4	0.96 ± 0.04	0.27
2.2	64.2 ± 2.0	1.84 ± 0.05	0.64	17.2	54.4 ± 5.5	0.89 ± 0.03	0.36
2.3	56.7 ± 2.8	1.37 ± 0.04	0.59	17.3	62.8 ± 2.9	1.20 ± 0.04	0.42
2.4	60.7 ± 1.4	3.63 ± 0.08	0.73	18.1	60.1 ± 3.9	0.86 ± 0.03	0.21
3.1	60.6 ± 2.6	1.40 ± 0.05	0.44	18.2	67.2 ± 6.2	0.86 ± 0.03	0.59
3.2	52.0 ± 6.9	0.67 ± 0.03	0.47	18.3	58.0 ± 3.0	1.12 ± 0.04	0.45
3.3	70.2 ± 5.0	1.14 ± 0.04	0.72	19	56.3 ± 1.4	2.25 ± 0.06	0.20
3.4	60.6 ± 4.1	1.30 ± 0.04	0.71	20.1	57.6 ± 2.5	1.07 ± 0.04	0.36
3.5	49.7 ± 6.3	0.76 ± 0.03	0.53	20.2	57.6 ± 2.3	1.30 ± 0.04	0.31
3.6	64.3 ± 2.2	1.77 ± 0.06	0.61	21	56.2 ± 1.7	1.70 ± 0.05	0.41
4.1	57.8 ± 1.8	1.86 ± 0.05	0.64	22	58.4 ± 1.6	1.88 ± 0.06	0.19
4.2	60.5 ± 1.1	4.72 ± 0.10	0.85	X.1	56.0 ± 3.6	1.55 ± 0.07	0.58
5.1	58.6 ± 3.5	0.99 ± 0.04	0.53	X.2	62.1 ± 11.1	0.61 ± 0.04	0.56
5.2	61.9 ± 6.9	0.75 ± 0.03	0.61	X.3	62.4 ± 10.7	0.66 ± 0.04	0.68
5.3	59.0 ± 4.3	1.17 ± 0.04	0.59	X.4	59.6 ± 2.4	2.69 ± 0.10	0.75
5.4	66.0 ± 2.1	2.01 ± 0.06	0.75	Y.1	59.8 ± 3.6	0.92 ± 0.04	0.51
5.5	54.2 ± 2.3	1.34 ± 0.05	0.41	Y.2	64.6 ± 3.6	1.09 ± 0.05	0.92
6.1	62.2 ± 5.1	0.83 ± 0.03	0.48				
6.2	51.9 ± 10.5	0.52 ± 0.03	0.47				
6.3	64.0 ± 8.4	0.85 ± 0.04	0.63				
6.4	61.3 ± 1.5	3.72 ± 0.09	0.75				

* Segment number follows chromosome number after decimal point.
† Percentage A·T.
‡ DNA content as percentage of total DNA in haploid set of autosomes plus X and Y.
§ From Kuhn (1).

especially important for very short segments and for those flanked by segments of dissimilar base ratio.

RESULTS

For each of the 24 human chromosomes, an average of 340 thymidine-labeled and 274 guanine-labeled chromosomes were analyzed. Table 1 lists the DNA contents and base ratios of whole chromosomes and segments, calculated by using the values 0.6, 0.1, and 1.0 for A, A^*, and A', respectively, in Eqs. 5 and 6. Large-sample procedures were used to calculate the standard deviations of the DNA contents and base ratios from the empirical variances of the grain counts. (Details of these calculations will be supplied by W.E. on request.)

Table 2. Tests for uniformity of base ratio

Hypothesis	Degrees of freedom	Test statistic	Observed value
H_1	23	$\sum_i \frac{(A_i - A)^2}{V(A_i)}$	35.8*
H_2	51	$\sum_{ij} \frac{(A_{ij} - A_i)^2}{V(A_{ij})}$	69.8*

A likelihood ratio test for the hypotheses that base ratios are uniform among whole chromosomes (H_1) and that base ratios are uniform within chromosomes (H_2). A_{ij} is the base ratio of section j of chromosome i. A_i and A are the maximum likelihood estimates of the base ratios of chromosome i and the whole genome, respectively. $V()$ indicates variance.
* Significant at $P < 0.05$.

The base ratios of the 24 chromosomes all fell within the range 56–64%. Nevertheless, the precision of these base ratio estimates is sufficient to show that this variability is real and not merely due to statistical error. If all chromosomes have the same base ratio, and our estimates are approximately normally distributed, then the test statistic in Table 2 is χ^2 distributed. The high value of the test statistic indicates that the base ratios do vary among chromosomes. A similar test, also shown in Table 2, shows that the base ratios of individual chromosomes vary along their lengths.

The DNA contents of the chromosomes were approximately what would be expected from their visual lengths. It was not expected, however, that these DNA contents would be correlated with their base ratios. Yet, the correlation in Table 3 shows that large chromosomes tend to have high base ratios.

The quinacrine-brightness value of each chromosome and segment is given in Table 1. Table 3 shows that brightness is positively correlated with both base ratio and DNA content. Plots of base ratio against brightness are given in Figs. 2 and 3. An attempt was made to combine base ratio, DNA content, and other variables in a linear model for predicting brightness of the chromosome segments by using standard multiple regression methods. It was found that, when both DNA content and base ratio were used in a model, each had a significant effect in predicting brightness. After trying several other variables, we found that the model could be improved by subtracting a constant value of 0.12 brightness unit from the predicted brightness of each segment that contained the tip of its chromosome. This model accounted for 39% of the observed variance in brightness. A similar model predicted variation in brightness within chromosomes. The brightness value of each segment was expressed as a deviation from the mean brightness of its chromosome, with appropriate reduction in degrees of freedom. Thus, the final model, which accounts for more than

Table 3. Spearman rank correlations of base ratio, DNA content, and brightness

Correlation	Whole chromosomes (n = 24)	Sections (n = 75)
Base ratio:brightness	0.323 (P = 0.03)*	0.474 ($P < 0.0001$)*
Base ratio:DNA content	0.344 (P = 0.05)	0.041 (P = 0.36)
Brightness:DNA content	0.600 ($P < 0.0001$)	0.281 (P = 0.0075)

* One-tail test.

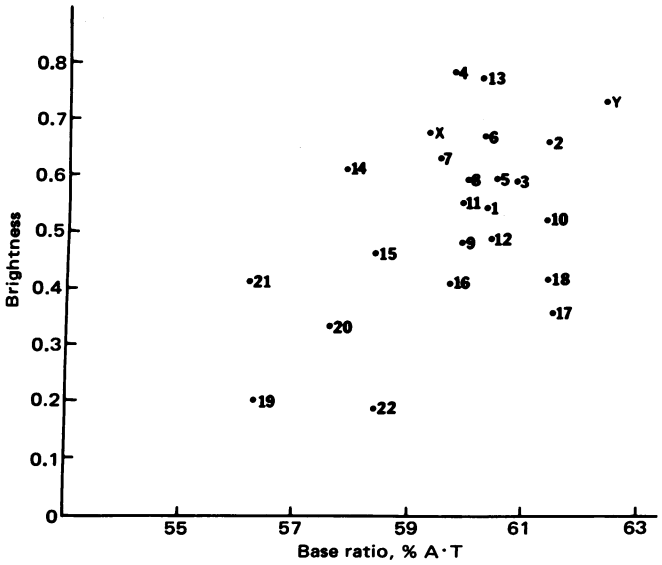

FIG. 2. Plot showing the positive correlation of base ratio with quinacrine brightness for the 24 human chromosomes. Points are labeled by chromosome number.

half of the variance about the chromosomal means, included significant contributions from base ratio, DNA content, and tip effect, all with positive regression coefficients.

DISCUSSION

We have estimated two basic parameters of human chromosomes and metaphase bands—base ratio and DNA content. Our results clearly establish that base ratios are significantly variable both among whole chromosomes and among segments within a chromosome. Although base ratios have not been previously measured in chromosomes, DNA content has been estimated spectrophotometrically (12). These previous estimates and our autoradiographic DNA estimates are in close agreement. Furthermore, although length measurements indicate that chromosome 19 is longer than chromosome 20, both DNA measurements indicate that chromosome 20 contains more DNA. Similarly, the X chromosome has slightly less DNA than does chromosome 7. The close agreement between these two independent sets of DNA measurements supports the validity of both methods.

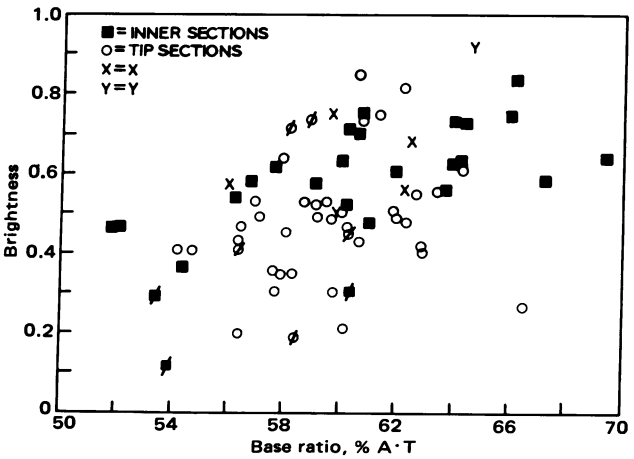

FIG. 3. Plot showing the positive correlation of base ratio (A-T pairs/total) with quinacrine-brightness (1) for chromosomal segments as delineated in Fig. 1. ■, Inner sections; ○, tip sections; X, X chromosome; Y, Y chromosome.

The brightness of a chromosome or segment is related to its function. Our findings show that brightness is also related to both base ratio and DNA content. The positive correlation between base ratio and brightness is especially evident on comparison of the dark chromosomes 19 and 20, both of which have a low base ratio, with the bright, A·T-rich Y chromosome. This relationship is not surprising because *in vitro* experiments relating dye fluorescence to DNA base ratios as well as the observations that bright bands are late replicating (13) and late-replicating DNA in humans is A·T-rich (14) suggest that bright bands are A·T-rich.

More surprising are the positive correlations we observed between brightness and size of both whole chromosomes and chromosomal segments. The lack of correlation between size of segments and their base ratio, along with the multiple regression results, clearly shows that this correlation is not due to a common association with base ratio. The fact that long segments are brighter than short segments of similar base ratio may be an artifact of the placement of segment boundaries which tends to exclude short, dark bands obscured by surrounding bright ones.

However, our finding that large *chromosomes* also have high fluorescence intensity and tend to be A·T-rich suggests an evolutionary model involving repeated sequences of high base ratio and brightness. If these sequences promote unequal crossing over, especially between sister chromatids in mitosis, they could grow in evolutionary time to an equilibrium length determined by the dynamics of the sister chromatid exchange process. These sequences correspond to the bright, A·T-rich segments we observed. Because chromosomes containing many of these sequences would be longer, brighter, and more A·T-rich than other chromosomes, the model predicts our observed correlations among whole chromosomes. It makes no prediction concerning the lengths of segments.

In addition to explaining the present data, this model is consistent with the chromosomal organization found in *Xenopus* (15) in which the 5S rRNA genes are separated by variable numbers of a 15-base-pair repeat unit. It also agrees with the earlier idea (16) that quinacrine-bright segments, which are known to cause relatively light phenotypic effects when trisomic or monosomic (3), contain intercalary heterochromatin and, therefore, few genes.

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