# THE DNA BASE COMPOSITION OF INDIVIDUAL CHROMOSOMES AND CHROMOSOME SEGMENTS FROM *CHIRONOMUS TENTANS*

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ABSTRACT

The base composition of DNA was determined for individual chromosomes from the dipteran *Chironomus tentans* and for each one of six different segments of one of the chromosomes. The isolations were carried out by micromanipulation and the DNA purines were first extracted from the isolated components and afterwards separated by means of microelectrophoresis on a cellulose fiber. It was found that DNA from this material has an unusual composition corresponding to a guanine + cytosine content of about 30%. This composition was not a function of the polytenic condition but was also found for DNA from testis tissue. Furthermore *Drosophila* has a more traditional base composition for the bulk of DNA. Statistically significant variations in base data were found between whole chromosomes as well as between the segments from one of the chromosomes.

## INTRODUCTION

Sedimentation analysis of DNA in neutral cesium chloride gradients has contributed to an understanding of the distribution, in different eukaryotic organisms, of nuclear DNA as determined by content of guanine + cytosine (GC). It is characterized by a main distribution peak and in several instances by satellite peaks (6, 11). Apart from this variation in GC contents between different stretches of DNA from the same organism, there is a variation among the members of the population of the main peak, increasing with evolutionary complexity (10).

Only some of the background to the variation in base composition between different DNA stretches is known or can be inferred. Thus, it is clear that amino acid differences in different proteins correspond to codon differences in respective cistrons. The use of different code words for the same amino acid at different points of the genome would also be a source of variability. Possibly parts of the DNA have other functions than coding for protein and have a composition determined by these unknown purposes.

For an understanding of the organization of nuclear DNA in higher organisms, studies of the composition of DNA as related to genomic localization are of fundamental importance. Electrophoretic base analysis, scaled down to permit analysis of DNA obtained from individually isolated polytene chromosomes and chromosome segments, has been used for this purpose.

## MATERIALS AND METHODS

Late fourth instar larvae of *Chironomus tentans*, cultured as described by Beermann (1), were used. For determination of base composition of DNA in whole, unsegmented chromosomes the salivary glands were fixed in ethanol–acetic acid and dissected as described in an earlier paper (2). For dissection of chromosome segments this fixation was found to be unsuitable, not permitting an accurate delineation of segments. Therefore part of the material was

fixed with formaldehyde as described by Edström and Beermann (5). After fixation, the glands were rinsed in three successive baths of distilled water, each for 10 min. The wet glands were subsequently dissected in the oil chamber, under the phase-contrast microscope.

In ethanol-acetic acid–fixed material, the four chromosomes were isolated and the nucleoli were removed from chromosome II and III. In the formaldehyde-fixed material, only the large first chromosome, which does not carry nucleoli or Balbiani rings, was used. It was sectioned into six segments, shown in Fig. 1. While micromanipulation is normally performed with only one de Fonbrune micromanipulator, capable of holding two microneedles, for dissection of chromosome segments it was advantageous to use two manipulators, one equipped with two needles, the other with a single needle. In this way it was possible to hold the chromosome extended with two needles and to use an interposed needle for separating segments (Fig. 1).

Single unsegmented chromosomes were used for base analysis. The three large chromosomes offered material for two or three base analyses each, the small fourth chromosome offered material for only one analysis. In the case of the segmented first chromosome, segments from four to five chromosomes were usually pooled for a single analysis.

Before the DNA base analyses, RNA was extracted from the isolated samples in the oil chamber. Extraction was performed with nanoliter volumes of 0.4 mg RNase per ml of 0.1 M ammonium acetate solution at pH 7.0 for 60 min at 37°C, followed by two rinses with distilled water. The enzyme used was crystalline pancreatic ribonuclease (Worthington, Freehold, N. J.).

Base analyses were carried out with a previously published microelectrophoretic procedure (3). According to this technique, the purines are extracted directly from the tissue DNA with 1 N hydrochloric acid for 16 hr at 37°C and afterwards separated by electrophoresis on a microscopic cellulose fiber. The quotient between the two purines is determined, and molar proportions are calculated on the assumption that adenine + guanine equals 50 mole per cent. The adenine values so derived are given in this paper. The average of the values from duplicate or triplicate determinations for single chromosomes was used in the statistical treatment. In this way it was possible to reduce the effect of inaccuracies in the electrophoretic and photometric technique for the unsegmented large chromosomes.

## RESULTS

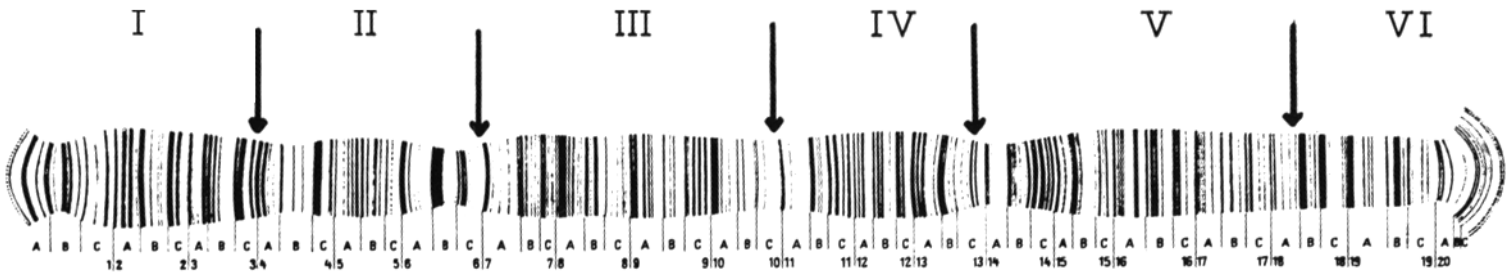
The adenine to guanine ratio as measured by microelectrophoresis was found to be high for all chromosomes, corresponding to an adenine content of 35% (Table I). Chromosome I was investigated both after ethanol-acetic acid treatment and after formaldehyde fixation. It was found that, compared to ethanol-acetic acid fixation, the latter treatment resulted in 1.5% higher adenine values. A similar effect has previously been noted in other material (3). It is not known whether the former treatment gives too low or the latter treatment too high a value.

The high adenine content (or low GC content) is unusual for eukaryotic nuclear DNA. An investigation showed that it is not a characteristic property of polytene chromosomes only. Testis DNA as well as chromosomes of lower levels of polytenization show similar values (Table I). It can also be shown that a low GC content is not a property characteristic of all organisms capable of polytenization, since *Drosophila* displays more traditional values (Table I; references 7, 9).

It was possible to obtain analyses for all four chromosomes from 19 cells. In several cases, data were obtained only for the three large chromosomes, because the small fourth chromosome offered amounts of DNA lying in the lower range of the method. In cases in which the last replication step has not been reached, the amounts are too small to permit analysis.

Data for the three large chromosomes were obtained for 29 cells. These data were used in an analysis of variance. It was found that there is a significant variation ($P < 0.01$) between chromosomes (Table II). Statistical analysis also showed the existence of a significant variation at the $P < 0.01$ level between cells or sets of analyses. There is no reason to attribute this variation to biological causes, since it could represent an error in the technique, giving some variation in results from one set to another. The chromosomes were always analysed in parallel, to eliminate as much of this analytical error as possible. Among the chromosomes the first one differs from all the others in its particularly high adenine (or low GC) content (Table I). Both the second and fourth chromosome are comparatively GC rich.

Analyses of the segments from the first chromosome were carried out so that segments from the same chromosomes were analysed in parallel, for minimizing the analytical error, and a two-sided analysis of variance was afterwards carried out. Many of the sets (one set consisting of one base value from each of the six segments) were incomplete and the complete sets were too few for statisti-



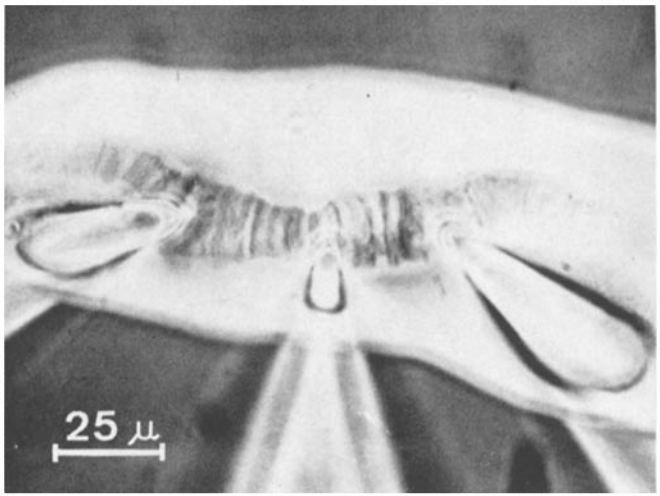




FIGURE 1 Map of the first chromosome from *Chironomus tentans* (from Pelling [8]) showing the delimitations of the different segments analyzed for DNA base composition. The photomicrograph shows how a chromosome is cut between segments II and III in the oil chamber. The two lateral needles are used for holding the chromosome extended, and the middle one is used for cutting.

cal treatment; therefore, sets with one or two missing values were also used in the statistical treatment. Here a modified analysis of variance was performed, since the observations were arranged in a two-way classification with several missing values. The results show the presence of a variation significant at the $P < 0.01$ level between the different segments (Tables III and IV).

TABLE I

*Mole Per Cent Adenine in DNA from Individual Salivary Gland Cell Chromosomes from Chironomus tentans*

| Chromosome | Adenine | n |
|---|---|---|
| | % ± SEM | |
| I | 35.5 ± 0.16 | 36 |
| II | 34.8 ± 0.17 | 31 |
| III | 35.1 ± 0.22 | 29 |
| IV | 34.7 ± 0.18 | 20 |
| Testis tissue | | |
| *Chironomus* | 35.5 | |
| *Drosophila* | 28.3 | |

The analyses, therefore, showed the presence of a significant variation in base composition, not only between different chromosomes but also between different segments on the same chromosome. Since the variation is of a size not larger than one that can just be established with the methods used, it is not possible to compare the precise size of this variation in the two instances. It is clear, however, that the variation is moderate, which is not surprising, considering the large amount of genetic material constituted by the DNA of a chromosome or segments of the size used here. Values for DNA content per chromosome have been given previously (2).

## DISCUSSION

The DNA base composition of *Chironomus tentans* is unusual for a eukaryote, since it has a GC content of about 30%. It was noted previously that the microelectrophoretic technique tends to give somewhat high adenine values in certain cases (3), and that the real GC content might, therefore, be somewhat higher. However, unpublished results of sedimentation of *C. tentans* DNA in a cesium chloride gradient support the present results, indicating a GC content of 27% (Lambert and Cummins, to be published). These results also showed a relatively narrow unimodal distribution in the gradient, although, in view of the possibilities of heterogeneity within the sedimented molecules, they do not exclude the presence of stretches with more traditional GC content.

The base composition of RNA in a cell containing DNA of the *C. tentans* type is naturally of great interest. Unfortunately, only the composition of the bulk of cytoplasmic RNA as well as of nucleolar and chromosomal RNA is now known, and these RNAs may very well represent the

TABLE II

*Analysis of Variation of Base Data for Individual Chromosomes (Chromosomes I, II, and III)*

| Source of variation | Degrees of freedom | Mean square | F* |
|---|---|---|---|
| Between chromosome segments | 2 | 521.83 | 5.11 ($P < 0.01$) |
| Between sets of determinations (= cells) | 28 | 252.26 | 2.47 ($P < 0.01$) |
| Remainder | 56 | 102.17 | |

* Variance ratio.

TABLE III

*Mole Per Cent Adenine in DNA from Segments of the First Chromosome of Chironomus tentans*

| Segment No. | Adenine | n |
|---|---|---|
| | % ± SEM | |
| I | 36.9 ± 0.31 | 29 |
| II | 36.3 ± 0.37 | 26 |
| III | 37.3 ± 0.36 | 27 |
| IV | 37.4 ± 0.30 | 35 |
| V | 37.4 ± 0.23 | 33 |
| VI | 36.9 ± 0.35 | 28 |

TABLE IV

*Analysis of Variation of Base Data for Segments from Chromosome I*

| Source of variation | Degrees of freedom | Mean square | F* |
|---|---|---|---|
| Between chromosomes | 5 | 768.22 | 3.74 ($P < 0.01$) |
| Between sets of determinations (= first chromosomes) | 32 | 541.35 | 2.64 ($P < 0.001$) |
| Remainder | 128 | 205.44 | |

* Variance ratio.

transcribed products of minor stretches of the genome only, unrepresentative of the bulk of DNA. These types of RNA have GC contents in the range of 40–45% (5), very different from what would be expected for copies of the bulk of DNA. The RNA of *C. tentans* is similar in base composition to that of *Drosophila melanogaster* (4), in spite of the large difference in composition between their DNA's.

The finding that there is a variation in DNA base composition between different parts of the genome does not, in itself, mean a revelation of a biologically interesting fact, since at some level of analytical precision such differences have to exist. The results give, however, an indication of the order of the variation, and the fact that characteristics of this kind are open to investigation should be of importance in reaching a better understanding of the arrangement of the genetic material in eukaryotes.

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