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LOCALIZATION OF REPETITIVE DNA IN HOUSE FLY CHROMOSOMES WITH A MODIFIED GIEMSA STAIN

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ABSTRACT

K. Y. Jan and A. P. Shu (1972) *Localization of Repetitive DNA in House Fly Chromosomes with a Modified Giemsa Stain.* Bull. Inst. Zool., Academia Sinica **11**(2): 29-33. Specific Giemsa staining patterns were obtained on house fly chromosomes after denaturation with alkali and renaturation with saline incubation. The centromeric regions of autosomes, the whole Y chromosome and most parts of X chromosomes were darkly stained. These darkly stained areas presumably contain DNA of repetitive sequences. The present status of this staining technique is discussed.

The recent, rapid development in Giemsa staining coupled with denaturation and renaturation have revealed specific banding patterns on chromosomes[1,8,5,7,17,18]. Although the exact mechanism involved in the production of these bands remains to be elucidated, it seems to be a general belief that darkly stained bands reflect the locations for repetitive DNA sequences in the chromosomes.

This technique has been tested on the chromosomes of house fly in the hope of locating the repetitive DNA and understanding the nature of the technique. Here we report some of the preliminary results.

MATERIAL AND METHODS

The culture of house fly (*Musca domestica* L.) has been maintained in the laboratory as previously described[11]. The brains and ventral ganglia of third instar larvae, the testes or ovaries of one-day-old adults were dissected out in distilled water, fixed for 30 minutes in freshly prepared methanol-acetic acid (3:1), 1 minute in 45% acetic acid then squashed with a drop of 45% acetic acid on albuminized slides. The coverslips were removed by using the liquid nitrogen technique. The slides were then dipped in 95% alcohol for 5 minutes and air dried.

The Giemsa staining technique was modified from Dret and Shaw[7], Pera[17] and Schnedl[18]. Slides were treated with the following procedures:

A. 0.07 N sodium hydroxide in 0.112 N sodium chloride (pH 12.0) for 2 minutes.
B. three changes of 70% alcohol and three changes of 95% alcohol then air dried.

C. $2\times SSC$ (17.53 g of sodium chloride and 8.81 g of sodium citrate in 1 liter of distilled water, pH 7.0 adjusted with 0.1 N hydrochloric acid) at 65°C for 16 hours.

D. three changes of 70% alcohol and three changes of 95% alcohol then air dried.

These slides were then stained in diluted Giemsa solution[1] for 5-10 minutes then briefly washed in distilled water. The coverslips were mounted with Euparal. Finally they were examined under a Nikon photomicroscope with blue and green filters and photomicrographs were done by using Kodak Panatomic-X films.

RESULTS

In an attempt to develop a staining technique for best banding pattern, we have tested the alkaline treatment for 30, 60 and 120 seconds, renaturing incubation at 65°C in $2\times SSC$ for 16, 24, 48 and 72 hours and the pH of Giemsa solution at 6.8 and 9.0. The results indicated that the best banding pattern was obtained in

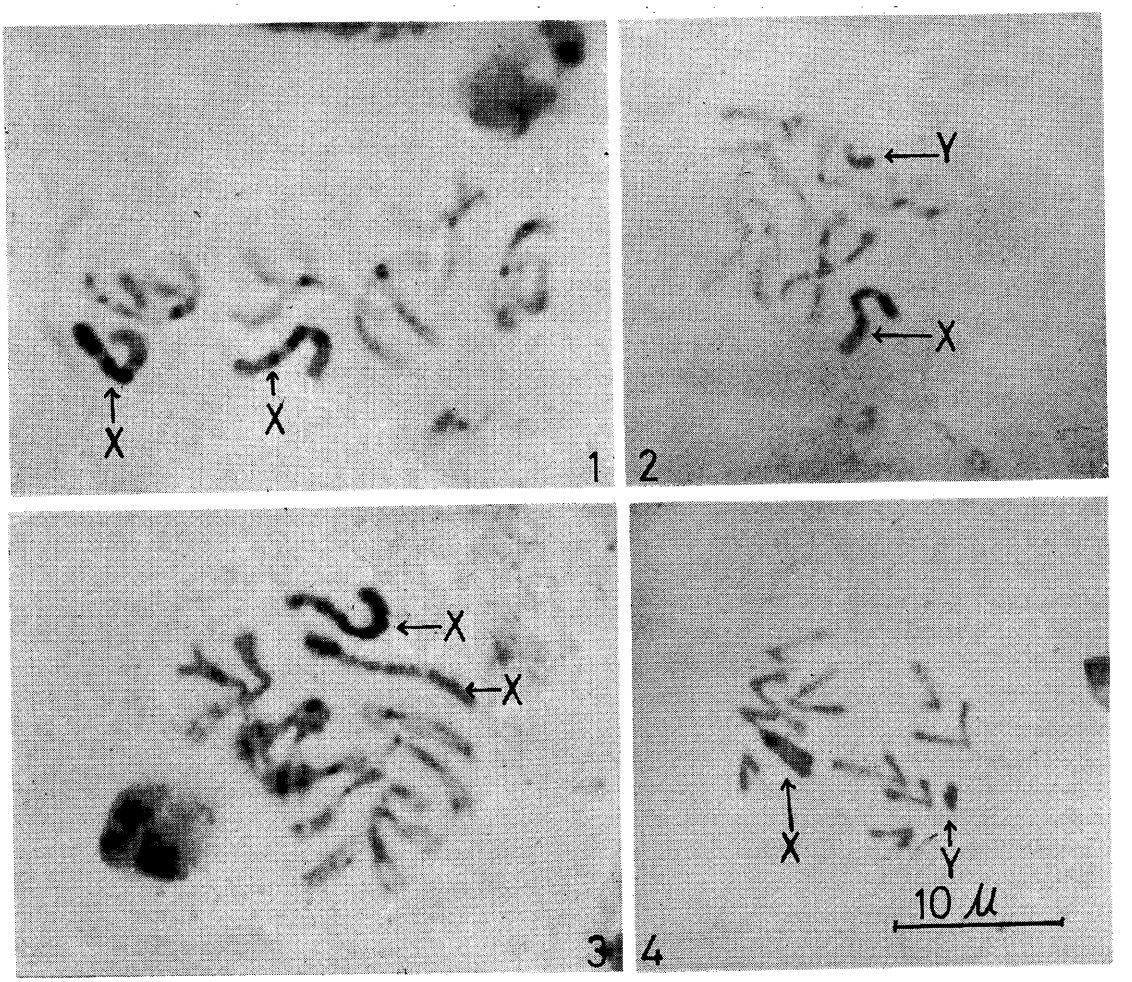

Fig. 1-4. Giemsa stain on house fly chromosomes. 1 & 2, mitotic metaphase from larval brain. 3, mitotic metaphase from adult ovary. 4, meiotic anaphase from adult testis.

slides treated in the alkaline solution for 120 seconds, postincubated in $2\times SSC$ at 65°C for 16 hours and stained in Giemsa solution of pH 6.8.

Although in the larval brain preparation some metaphase chromosomes were evenly stained, in the great majority a specific staining pattern was observed (Figs. 1 & 2). The centromeric regions of all five autosomal pairs, the *Y* chromosomes and most part of *X* chromosomes were darkly stained. Whereas both arms of all five autosomal pairs and one small region proximal to the centromere, on each arm of *X* chromosomes were not stained. This staining pattern is illustrated in Diagram 1. On both *X* chromosomes of the *XX* cells and the single *X* chromosome of the *XY* cells, the two unstained regions were regularly observed and the darkly stained regions were often identical. Nevertheless, in some cases the stained regions of one *X* were darker than those of other *X* and even more, one region was darker than other on the same *X*. The *Y* chromosome was often darkly stained along its length, but occasionally one end which was out of focal plane and gave the image of less darkly stained.

In the ovarian preparation, many mitotic metaphase figures were obtained but not the meiotic metaphase figures. The staining patterns of these ovarian mitotic metaphase figures (Fig. 3) were less distinct but more or less the same in comparison with the staining patterns for the larval brain metaphase chromosomes.

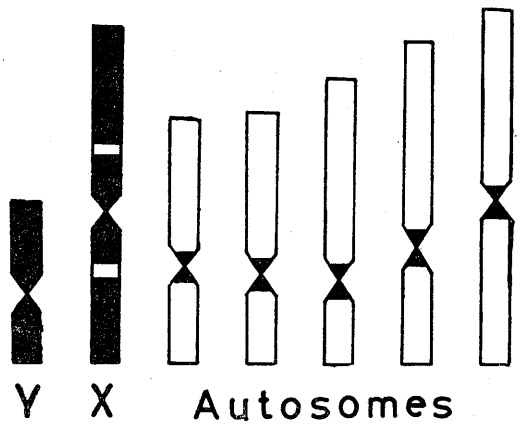

Diagram 1. Illustration of Giemsa stain on the house fly chromosomes

In the testis preparation, a number of meiotic figures were obtained but not the mitotic metaphase figures. These meiotic metaphase chromosomes were all evenly stained and no banding could be revealed. While in some meiotic anaphase chromosomes observed, the *X* and *Y* chromosomes were evenly, darkly stained and the autosomes were less darkly stained (Fig. 4).

DISCUSSION

The repetitive DNA is known to renature much more rapidly than non-repetitive DNA[4]. So it is generally assumed that Giemsa bandings on chromosomes after alkaline denaturation and warm *SSC* renaturation represent the locations for repetitive DNA[1,3,5,7,17,18]. Chromosomes receiving alkaline denaturation but not renaturing incubation do not reveal specific Giemsa banding[1,18]. In mouse chromosomes the centromeric heterochromatin contains repetitive DNA[13] and are preferentially stained with Giemsa[15]. In *Microtus agrestis* the chromosomal sites for constitutive heterochromatin contain repetitive DNA and are also Giemsa positive[2]. In addition, the Giemsa patterns may have some correlation with quinacrine fluorescent patterns[6,8,9,10,16,19] and late replication patterns[6,10] directly or indirectly.

The Giemsa stain on the autosomal centromeric regions, the *Y* and most part of *X* chromosomes of house fly as revealed in the present experiments, may to some extent represent the locations for repetitive DNA. Thus the sex chromosomes may contain a higher proportion of repetitive DNA than autosomes. And it should be interesting to ask whether the repetitive DNA at all centromeric regions consist of the same nucleotide sequences and whether the repetitive DNA at noncentromeric regions consist of nucleotide sequences different from those of centromeric regions? One would also like to know whether the repetitive property is required for centromeric function? The repetitive DNA on the *X* and *Y* chromosomes of the house fly may have some

connections with their late replication and heterochromacity[11]. Nevertheless, the exactness of these relationships await further experimentation.

In most reports, the cultured mammalian cells were used for chromosome preparation and there is no discrepancy in the technique for chromosome fixation. However, the denaturation, renaturation, pH of Giemsa solution and staining time were varied from laboratory to laboratory. By varying the alkaline treatment, different Giemsa patterns may be obtained[18]. DNA denaturation can also be achieved by high temperature treatment[17,20]. Sumner *et al.*[19] reported an ASG technique in which chromosomes were also fixed in methanol-acetic acid but not treated with any denaturing agent before incubation in $2\times SSC$, gave marked Giemsa bandings; whereas, chromosomes not treated with $2\times SSC$ were uniformly stained with Giemsa. Patil *et al.*[16] claimed that the pH of Giemsa solution and the staining time are important. Specific Giemsa banding can be produced by staining the fixed chromosomes in the Giemsa solution at pH 9.0 for 5 minutes without denaturing and renaturing treatments. But with staining time of 15 to 30 minutes, uniform staining with Giemsa was observed. Different Giemsa reactions[19] were recognized on the centromeric regions of human chromosomes by different pretreatments, and those secondary constrictions which are nucleolar organizing regions and contain repetitive DNA of ribosomal RNA in origin were Giemsa negative with the ASG technique. Thus one would like to know the answers of (A) how do the different procedures and different results account for? (B) by what procedure of Giemsa stain, the repetitive DNA could be best revealed? and (C) how much repetitive DNA do these Giemsa bandings stand for?

At the moment, we feel that in Giemsa bandings by denaturing and renaturing pretreatment, the degree of repetition of DNA seems to be involved, but other factors should not be ignored completely. For example, Sumner *et al.*[19] believed that in ASG technique the bandings obtained after $2\times SSC$ treatment could be a result of a loss of affinity for Giemsa in the regions which become pale bands. Kato and Yosida[14] also claimed that electrolytes such as phosphate buffer in diluting Giemsa, might play some important roles in producing bandings. If the degree of repetition of DNA is involved in Giemsa patterns, then the extent of chromosome denaturation and renaturation may affect the banding pattern. And this may in part be accounted for some metaphase which gave no bandings. Alternatively, it would be difficult to assume that the repetitive DNA are present in most of the mitotic metaphase but not in others, nor in the meiotic metaphases from the testes. Perhaps the chromosomes of the meiotic metaphase from the testes have a different organization which require some modifications of the procedure for revealing the repetitive DNA sequences. In contrast to other reports in which cultured cells were used for staining, the freshly isolated insect tissues were used for the present experiments. Thus the staining procedure developed for mitotic metaphase chromosomes in the present system may need some modifications for giving the best banding patterns in other systems.

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用吉氏染色劑探測重複 DNA 在家蠅染色體上的位置

詹 崑 源　　舒 安 平

家蠅染色體經鹼液及生理食鹽水處理後以吉氏染色劑染色，則在 X 染色體之中節、中節兩側、兩臂遠端，Y 染色體及體染色體中節處，染色皆較深。此等部位所含之 DNA 可能有多次之重複。