May 8, 1973

To the Graduate Council:

I am submitting herewith a thesis written by Anthony John Calandra entitled "Quinacrine Staining Patterns of Normal and Aberrant Fourth Chromosomes of Drosophila melanogaster." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

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QUINACRINE STAINING PATTERNS OF NORMAL AND ABERRANT FOURTH CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Anthony John Calandra

June 1973

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ABSTRACT

The quinacrine fluorescence pattern of the wild type (Oregon-R) fourth chromosome of Drosophila melanogaster was compared to that of deficient (Df (4) G and Df (4) M^{63a}), duplicated (ey^D), and translocated (T (2;4) 99) fourth chromosomes of this species and to the purportedly inverted fourth chromosome of its sibling species, D. simulans, as part of a continuing effort in this laboratory to characterize completely this microchromosome.

The wild type stock exhibited intensely fluorescent bands at 101F and 102D, moderately fluorescent bands at 102B and at the chromocenter, weak fluorescence in the remaining bands of the chromosome, and non-fluorescence at the interband regions. The Df (4) G and Df (4) M^{63a} chromosomes displayed identical fluorescence patterns. A similar pattern was observed for the ey^D chromosome, but the intensely fluorescent band at 102D was observed to be thinner and speckled, and an additional band judged to be moderately fluorescent was seen at 102F. The T (2;4) 99 chromosome exhibited a similar fluorescence pattern although the fluorescence at both 102B and 102D were much reduced in intensity. The D. simulans chromosome displayed an entirely different pattern characterized by moderately fluorescent dots, apparently not associated with specific chromosomal

bands, and intense fluorescence near the chromocenter.

These data are discussed in the light of current knowledge of the structure and organization of chromosome 4 and the significance of quinacrine fluorescence patterns. It is hypothesized that the intensely fluorescent regions evident after quinacrine staining represent regions of the chromosome where the proper array of non-histone proteins permit quinacrine binding and that these areas may identify specific control sites of this micro-chromosome.

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I. INTRODUCTION

The fruit fly, Drosophila melanogaster, whose well characterized mutants, short life cycle, and well studied chromosomes make it an ideal organism for genetic study, has been employed in numerous classical, and of late, biochemical investigations. It occupies a position on the evolutionary scale between the prokaryotes, where much information on the nature and mode of action of the genetic material was originally obtained, and the higher eukaryotes which must be studied for a fuller understanding of such processes as cell and tissue differentiation. The fourth chromosome (hereafter referred to as chromosome 4) of the chromosomal complement of D. melanogaster offers an unusual, if not unique, opportunity for genetic study, that of completely characterizing the genetic organization and significance of an entire eukaryotic autosome. Many features of this chromosome make it suitable for this type of study such as its small size (less than 0.3 microns at mitotic metaphase), and its estimated content of 50 or so loci. Finally, and perhaps most important, is the fact that previous mutagenesis experiments, primarily with the mutagen ethyl methanesulfonate (EMS), followed by genetic and cytological mapping of induced mutants have already identified

42 to 44 of the 50 postulated loci. Hence it seems that this chromosome is close to the point of being fully characterized genetically. When this goal is reached, it will allow us to answer some very important genetic questions including how many genes can occupy a given length of a eukaryotic chromosome, how gene size in eukaryotes compares with that of prokaryotes, and whether clustering of structural genes under the control of regulator genes occurs in eukaryotic genomes.

Despite previous success with EMS experiments in this laboratory, it seems that a point of diminishing returns is being reached using this approach. Thus, the complete analysis of chromosome 4 requires that any new tools which might increase the present knowledge of this microchromosome be fully employed. Caspersson, et al., (1967, 1969a, 1969b) developed a fluorescent staining technique using quinacrine, an acridine derivative, for the study of Vicia faba and Chinese hamster chromosomes. Since the inception of this method, many species of plants and animals have been examined in order to obtain fluorescent patterns which might shed light upon the structure and chemical differentiation of eukaryotic chromosomes. To date, quinacrine staining has been employed to study such widely divergent species as the mouse Microtus agrestis (Natarajan, et al., 1971; Mukherjee and Nitowsky, 1972),

Drosophila (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a, 1971b; Barr and Ellison, 1972; Zuffardi, et al., 1971), and the Indian deer, the muntjac (Comings, 1971). Equally prominent in fluorescent studies has been the examination of various plant species including Melandrium dioicum (Kampmeijer, 1972), Tulbaghia (Vosa, 1970a), and Allium cepa and Scilla sibrica (Caspersson, et al., 1969b; Vosa, 1970b), and Trillium erectum (Caspersson, et al., 1969b). If one adds to this list the many reports exploiting quinacrine fluorescence techniques for the detection of human chromosomal aberrations, it can immediately be seen that a substantial amount of research has been done in the very brief time since quinacrine staining procedures were developed.

In addition to the utility of the method for the detection of chromosomal differences which are not readily detectable by morphological criteria alone (Caspersson, et al., 1967, 1969a, 1969b; Ellison and Barr, 1971a), it has also been recognized as a potential tool for karyotyping in vitro cell populations of Drosophila (Zuffardi, et al., 1971) and the Indian muntjac (Comings, 1971). In addition, Ellison and Barr (1971b) have indicated the possibility of employing quinacrine staining in evolutionary and population studies.

The prominent quinacrine fluorescence of chromosome

4 has been reported by several investigators (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a, 1971b; Barr and Ellison, 1972; Zuffardi, et al., 1971) who have studied both ordinary mitotic and polytene chromosomes of D. melanogaster. The present study was undertaken with several goals in mind. The first was to develop a quinacrine fluorescent staining technique for the polytene chromosomes of D. melanogaster which would give repeatable results around which future experiments could be planned. This method could then be employed to establish the quinacrine fluorescence pattern of our laboratory wild type stock of this species. Our findings could next be compared to conflicting reports of other investigators (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a). Once the normal pattern was established, it was desirable to know whether one or more of the four major chromosomal aberrations (duplication, deficiency, translocation, and inversion) could cause a shift in this pattern and thus shed some light on the chemical or physical organization of this chromosome. To this end a wild type stock, two stocks carrying deficient fourth chromosomes, a stock carrying a duplicated fourth chromosome, and a stock with a translocated fourth chromosome of D. melanogaster were examined for their characteristic quinacrine fluorescence patterns. Since an inversion in chromosome 4 does not

exist in D. melanogaster, it was necessary to investigate the fourth chromosome of D. simulans, since the fourth chromosome of this closely related species is believed to have a gene sequence inverted with respect to the D. melanogaster chromosome.

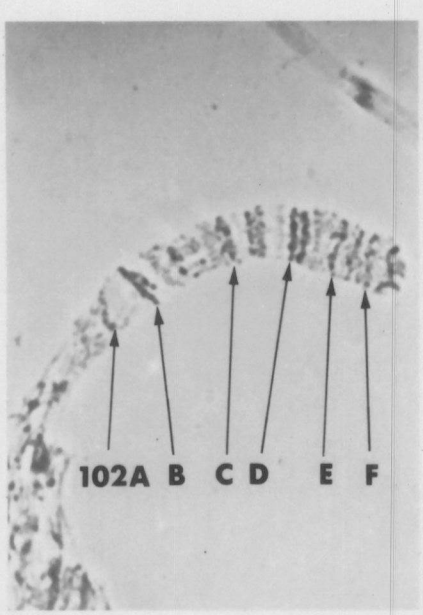
One additional question which could be asked concerned the origin of the chromosomal aberration involved in the visible mutation referred to as eyeless dominant (ey^D). This aberration involves the insertion of a duplication in subdivision 102D of chromosome 4 of D. melanogaster as a reversed repeat. The origin of the duplication is unknown, but Hochman (1971) has postulated that it originated from the left arm of chromosome 4 (4L) based on the similarities of the banding patterns of 4L and the duplication. It was reasoned that it should be possible to observe the fluorescence pattern of 4L and compare it to that of the duplication. If identical fluorescence patterns could be obtained, strong evidence in favor of this hypothesis would be gained. Results other than these would at least give insight as to the chemical or physical organization of this aberration.

II. METHODS

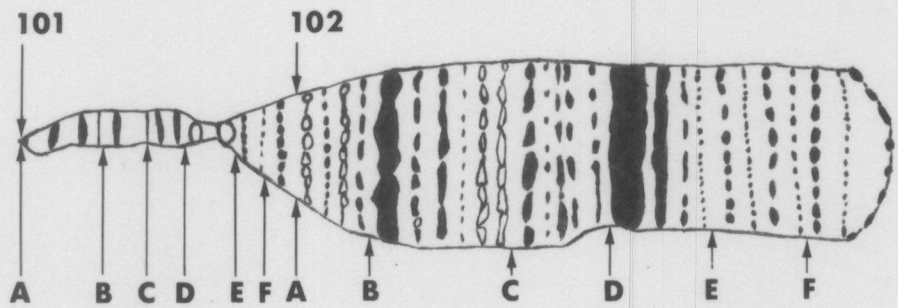
Description of Stocks

Five stocks of D. melanogaster, Oregon-R, Df (4) G/ ci^D, Df (4) M^{63a}/ci^D, ci^D/ey^D, and T (2;4) 99, and one of D. simulans were examined for their characteristic quinacrine fluorescence patterns.

Oregon-R is a commonly studied wild type strain of D. melanogaster which was used to obtain the normal pattern of fourth chromosome fluorescence for this species. In conventionally stained preparations such as Figure 1a, the fourth chromosome of this stock displays the characteristic banding described by Bridges (1935) and illustrated in Figure 1b. It can be seen that the chromosome is divided into divisions designated 101 and 102, each of which is further divided into six lettered subdivisions designated A through F. Within each of these lettered subdivisions each band is individually numbered. The Df (4) G chromosome lacks the distal portion of the right arm of chromosome 4 (4R) from bands 102E2 to the tip of 4R. Eight to nine bands are missing from this segment and have been replaced by X chromosome material which includes subdivision 1A (Hochman, Gloor, and Green, 1964). Df (4) M^{63a}/ci^D is also a deficiency stock. The Df (4) M^{63a} chromosome lacks bands in subdivisions 101F and 102A



a



b

Figure 1. The normal salivary gland fourth chromosome of D. melanogaster.

A. Highly stretched, conventionally stained preparation.[1]

B. Diagrammatic representation of the normal banding pattern.

[1]Photograph courtesy of Dr. B. Hochman.

(Lindsley and Grell, 1968). Hochman (1965) reports that this chromosome is deficient for bands 101F 6,7 and 102A 1,2. The *ey*^{D} chromosome was X-ray induced and carries a duplication of about a dozen bands of unknown origin inserted into subdivision 102D as a reversed repeat (Lindsley and Grell, 1968). *T* (*2;4*) *99* is a translocation having breakpoints at subdivisions 33A and 101A. It is thus a translocation in which nearly all the right arm of chromosome *4* has been translocated onto chromosome *2*. It should be noted that the *Df* (*4*) *G*, *Df* (*4*) *M*^{63a}, and *ey*^{D} chromosomes are carried over a homologous *ci*^{D} chromosome which exhibits the normal fourth chromosome banding pattern described for the Oregon-R stock in conventionally stained preparations.

D. simulans is a close relative of *D. melanogaster* which was shown by Horton (1939) to be inverted for at least bands 102B 1,2 to 102E 1,2 when compared to chromosome *4* of *D. melanogaster*, and to differ in the number of bands at the proximal and distal ends of the chromosome. The stock used in this study was derived from flies trapped in Florida and has been maintained in our laboratory since 1965.

Culture Conditions

For each genotype examined, fifteen females and ten

males were placed into quarter pint culture bottles filled with a culture medium which contained yeast, cornmeal, sucrose, dextrose, agar, and a propionic-phosphoric acid mixture. The cultures were placed in an incubator maintained at 18° C, and the females allowed to lay eggs for a period of two days. The cultures were then enriched with yeast to assure the production of larvae with well developed salivary glands. This yeast enrichment was obtained by adding a few grains of either Fleischman's activated dry yeast, or Brewer's yeast (Nutritional Biochemicals), or by adding a few drops of a yeast-sucrose suspension made by combining two teaspoons of Brewer's yeast with one teaspoon of sucrose and 30 ml of distilled water. After yeast enrichment, the cultures were kept at 18° C until ready for use. The most suitable cytological preparations were obtained with third instar larvae raised under these conditions and harvested on days 10 to 12.

Cytological Procedures

A. Fluorescent staining. At least a triplicate series of slides was made and systematically examined for each genotype analyzed. These preparations were stained with quinacrine hydrochloride dihydrate (Schwartz-Mann, Orangeburg, N.Y.) using a modification of a method derived by Lewis and Craymer (1971) as follows:

1. Third instar larvae were dissected in a drop of 45 percent acetic acid on a siliconized glass slide (five parts Siliclad, Clay-Adams, Incorporated, to one hundred parts water, immersed for five seconds) using a pair of syringes with fine point needles as dissecting instruments.

2. Salivary glands were fixed for two to three minutes in the original drop of 45 percent acetic acid on a siliconized coverglass.

3. A siliconized slide was placed over a coverglass in an inverted position and the coverglass drawn to the slide by means of surface tension. The slide was brought to its normal upright position with a quick movement of the wrist and the coverglass centered.

4. The slides were wrapped in bibulous paper to blot off excess fixative, and care was taken to notice the position of the glands.

5. The cells of the glands were broken by gently tapping over the region of the glands with a small rubber tipped hammer.

6. The tissue was squashed with sufficient pressure to obtain chromosome spreads suitable for accurate cytological examination of chromosome 4.

7. The preparations were checked for fixation of chromosomes and degree of spreading by observation under

a Zeiss phase-contrast Photomicroscope.

8. The slides were frozen on a block of dry ice and the coverglasses removed with a razor blade after the slides were well frosted.

9. The slides were stained in 0.5 percent quinacrine hydrochloride dihydrate in 45 percent acetic acid using the following schedule:

a. 95 percent ethanol - one minute.

b. 100 percent ethanol- one minute.

c. Preparations were air dried by waving for one to one and one-half minutes.

d. Slides were stained for five minutes.

e. Slides were rinsed in 95 percent ethanol for three seconds.

f. Slides were rinsed in 100 percent ethanol for three seconds.

g. Preparations were air dried.

10. The coverslips were mounted using a 1M sucrose solution (made with double distilled water) by inverting the preparation over a drop of the sucrose solution on a coverslip, drawing the coverslip to the slide (as in item 3) and quickly moving the slide to its normal upright position.

11. Excess mounting medium was blotted off by wrapping the preparations in bibulous paper.

12. The coverglasses were centered and sealed with a clear, colorless nail polish.

B. Restaining with lactic-acetic-orcein. After the preparations were examined for their fluorescence patterns, one slide from each series was subsequently restained with 2 percent lactic-acetic-orcein for more accurate determination of banding patterns. The coverglasses were removed by moistening the nail polish with 95 percent ethanol until the coverglasses slid off. The slides were subsequently stained using the following schedule:

1. 95 percent ethanol- one minute.
2. 100 percent ethanol- one minute.
3. Preparations were air dried.
4. Preparations were stained with 2 percent lactic-acetic-orcein for fifteen to twenty minutes.
5. Preparations were rinsed in 95 percent ethanol for ten seconds.
6. Preparations were rinsed in 100 percent ethanol for ten seconds.
7. Preparations were air dried.
8. Coverglasses were mounted in 1M sucrose mounting medium.
9. Coverglasses were sealed with a clear, colorless nail polish.

C. Observations. After finding fields of interest under the Zeiss Photomicroscope, the same fields were examined under a Zeiss fluorescent microscope equipped with a 200 watt mercury vapor lamp and a dark-field condenser. A BG 12 excitation filter and barrier filters 53 and 44 were found to supply the best contrast for the preparations, and were employed in all fluorescent observations.

Photographic Techniques

All fluorescent preparations were photographed on the same day on which they were made to ensure a minimum of artifacts. Photographs were taken using Kodak Tri-X film, a 100X oil objective with its lens diaphragm completely open, and an 8X kpl eye piece going to the camera. A uniform exposure time of 90 seconds was used.

After completion of fluorescent photography, the same fields were photographed without restaining using the phase-contrast system of the Photomicroscope equipped with an automatic exposure device and a built-in camera. Photographs were taken either on Adox KB 14 film or Kodak High Contrast Copy film using a camera setting of 3.2X, a 100X oil objective, and an Optivar setting of 1.25X.

Subsequent to fluorescent and phase-contrast photography, one slide from each series was restained with

lactic-acetic-orcein as described above. The same procedures were used for phase-contrast photography of restained preparations as were used for quinacrine preparations.

With some preparations it was desirable to reverse the negatives obtained from fluorescent photography and subsequently print a negative of the fluorescent image. This allowed observation of more familiar dark banding patterns of the chromosomes and photographs of exactly the same proportions and thus greatly facilitated cytological comparisons. The reversal process involved placing the negative in a slide copier/reverser, covering with a translucent filter, holding the camera seven and one-half inches away from a 75-watt light source in a darkened room, and exposing the film (Kodak Panatomic X) for two seconds.

III. RESULTS

Wild Type Fluorescence Pattern

Figure 2a through 2c depicts the fluorescence pattern of wild type fourth chromosomes of D. melanogaster. It is evident from these figures that different gradations of fluorescence can be discerned. There were arbitrarily classified as intensely fluorescent, moderately fluorescent, weakly fluorescent, and non-fluorescent. Intensely fluorescent bands can be seen in Figure 2a at subdivisions 101F and 102D. A moderately fluorescent band is exemplified by the fluorescence at 102B in Figure 2a. The pale-looking bands which correspond to darkly staining bands in conventionally stained preparations, and the chromocenters in Figure 2a through 2c are considered weakly fluorescent. The interband regions of the chromosomes (Figure 2a through 2c) are dark and exemplify non-fluorescent regions.

The Oregon-R stock exhibited intensely fluorescent bands at subdivisions 81F (in chromosome 3), 101F, and 102D, a moderately fluorescent band at subdivision 102B, weak fluorescence at the remaining bands of chromosome 4 and at the chromocenter, and non-fluorescence in the interbands. Although our attention will be focused on the microchromosome, it should be mentioned that the four grades of fluorescence can be observed in the larger chromosomes also.

Figure 2. The quinacrine staining fluorescence pattern of wild type (Oregon-R) fourth chromosomes of D. melanogaster, seen in dark-field.

A. A highly stretched pair of fourth chromosomes. The letters F and D indicate the intensely fluorescent bands at subdivisions 101F and 102D, respectively. The moderately fluorescent bands at 102B 1,2 are indicated by B. The letters W and N denote two of the weakly fluorescent bands and two non-fluorescent interbands, respectively. C indicates the chromocenter.

B. Fourth chromosomes moderately stretched. No change in fluorescent band pattern is observed. The intensity of fluorescence is seen to vary and may be related in part to the amount of stretching. Symbols as in Figure 2a.

C. Relatively unstretched pair of fourth chromosomes. No change in fluorescent band pattern was observed as in Figure 2b. Symbols as in Figure 2a.

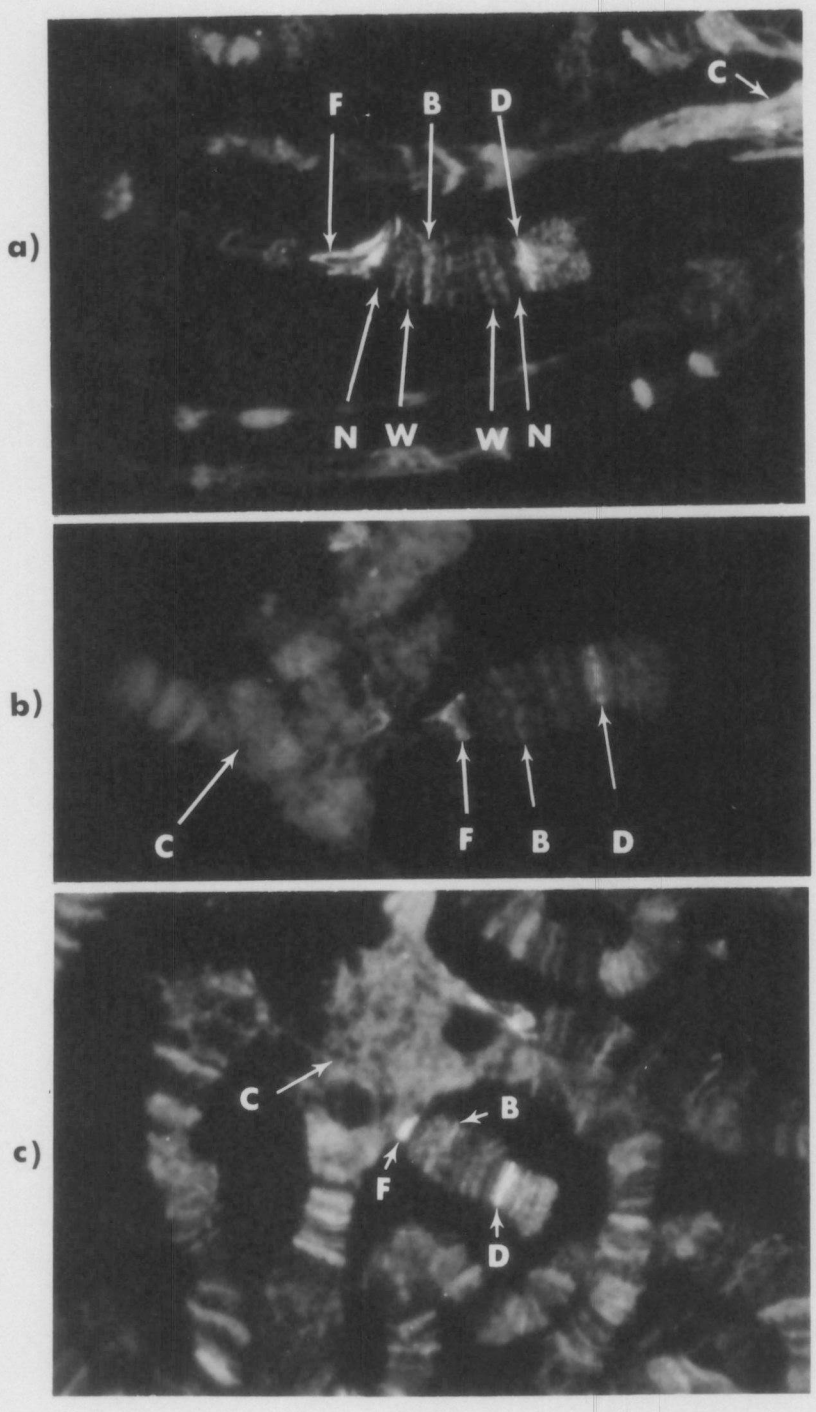


Figure 2

The degree of stretching of chromosome 4 was seen to have little or no effect on the fluorescence pattern obtained. This is readily noted by a comparison of Figure 2a through 2c, in which Figure 2a shows a highly stretched pair of fourth chromosomes, 2b a moderately stretched pair, and 2c a relatively unstretched pair. In all cases the pattern reported above was observed. Some of the photographs in these figures seem to display much more intense fluorescence than others, and reasons for this will be discussed later.

Fluorescence Patterns of Deficient Fourth Chromosomes

The Df (4) G chromosome is shown in Figure 3. A conventionally stained preparation is shown in Figure 3a, while a comparison of the resolution obtainable with the various cytological and photographic procedures employed is provided in 3b through 3e. Clearly, the lactic-acetic-orcein restained preparation (3e) permits the most accurate assignment of fluorescence to particular bands of the chromosome, although the assignments can also be made using the quinacrine preparation examined under phase-contrast (3d). The former, however, was found to be much easier to photograph than the latter in regard to the photographic procedures employed since the lactic-acetic-orcein stained bands facilitated focusing. The negative reversal procedure (3c), though lacking in sharpness,

Figure 3. The Df (4) G chromosome and comparison of cytological and photographic procedures employed.

A. Conventionally stained preparation.[1]

B. Quinacrine stained preparation, dark-field image.

C. Reversed image of field shown in B.

D. Phase-contrast image of field shown in B.

E. Phase-contrast image of field shown in D after lactic-acetic-orcein restaining. F, B, and D denote subdivisions 101F, 102B, and 102D, respectively. X designates X chromosome material associated with this aberration.

[1]Photograph courtesy of Dr. B. Hochman.

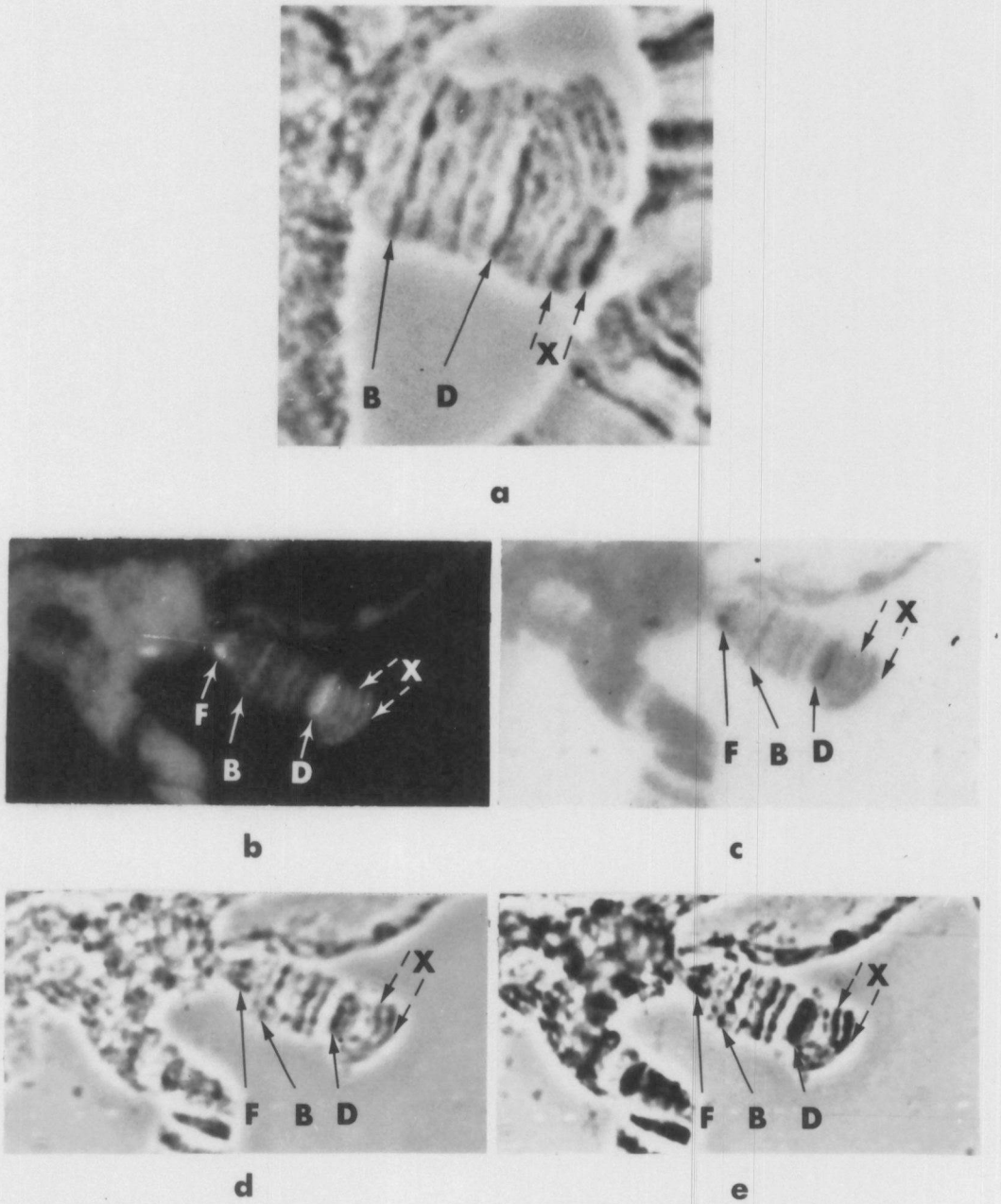


Figure 3

allowed ready determination of fluorescence intensity, and hence was found to be invaluable in the subjective determination of gradations of fluorescence. Thus, when these procedures were used in combination, precise localization of fluorescence could be achieved.

The fluorescence pattern of the Df (4) G chromosome (Figure 3b) was seen to be identical to the wild type pattern. The X chromosome material associated with this aberration exhibited the weak fluorescence typical of most bands in the genome.

The Df (4) M^{63a} chromosome is illustrated in Figure 4 in both dark-field images (4a and 4b) and a phase-contrast image (4c) of quinacrine stained preparations. This chromosome was found to have a fluorescence pattern identical to that of Oregon-R.

Fluorescence Pattern of the Duplicated Fourth Chromosome

The fluorescence pattern of the ey^D chromosome is shown in Figure 5b through 5d. A conventionally stained preparation is provided in 5a as a means of orientation. The fluorescence at 101F and 102D was consistently observed. In Figure 5b the fluorescence at 102B is not particularly evident, but it was repeatedly observed in other preparations, for example, 5d. The fluorescence at 102D, though of equal intensity to that of wild type

Figure 4. The Df (4) M^{63a} chromosome.

A. Quinacrine stained preparation seen in dark-field.

B. Dark-field image of a quinacrine stained preparation. The reduced intensity of fluorescence, as compared to Figures 2, page 16, and 3, page 19, is most likely due to the use of a new mercury vapor bulb and the more rapid quenching it induced.

C. Phase-contrast image of the field shown in Figure 4b. F, B, and D denote subdivisions 101F, 102B, and 102D, respectively.

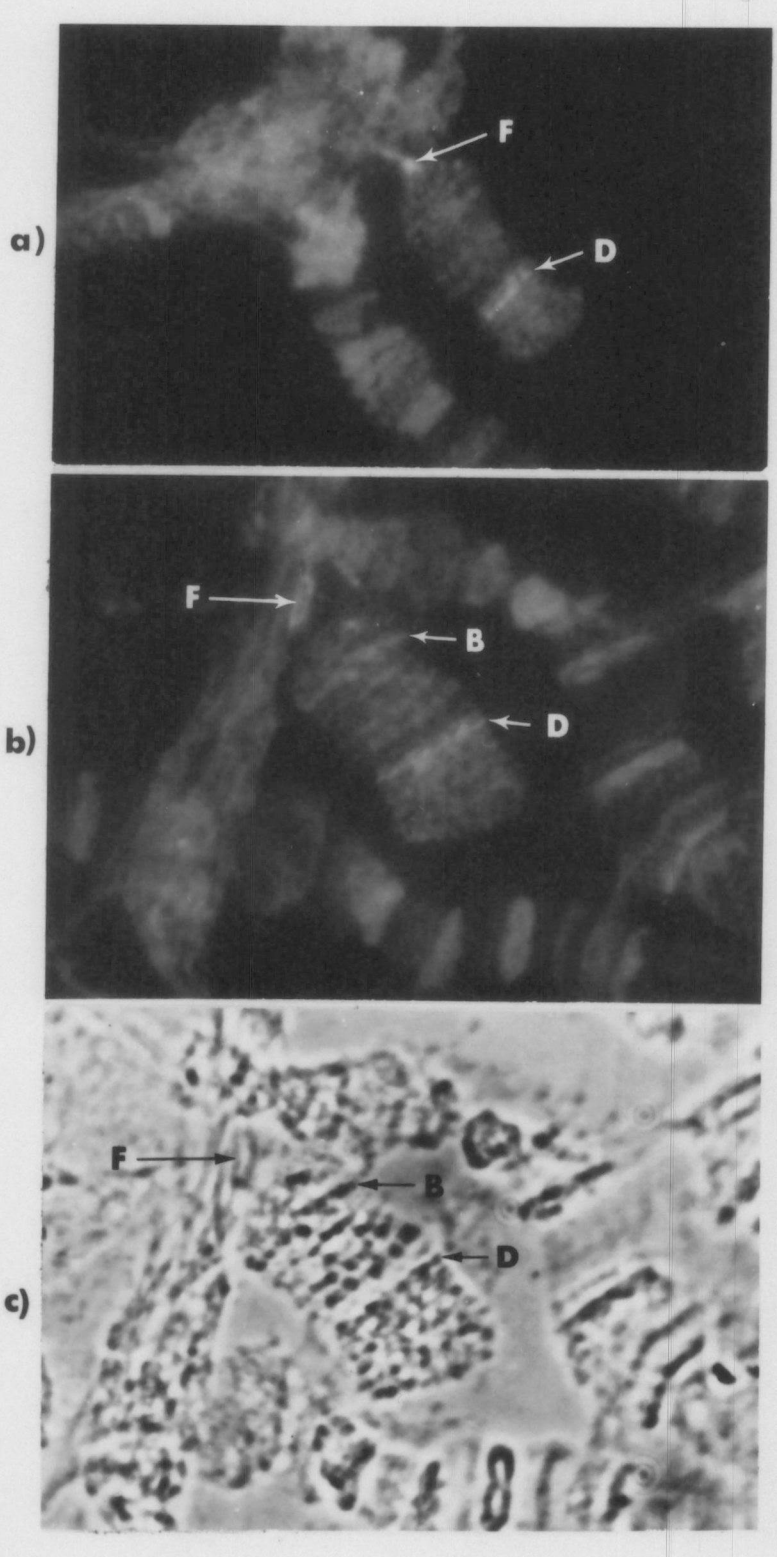


Figure 4

Figure 5. The $\underline{ey}^{D}$ chromosome.

A. Conventionally stained preparation.[1]

B. Quinacrine stained preparation, dark-field image.

C. Reversed image of the field shown in B.

D. Quinacrine stained preparation, dark-field image. F, B, C, D, and 2F denote subdivisions 101F, 102B, 102C, 102D, and 102F, respectively. The dashed arrows indicate the extent of the $\underline{ey}^{D}$ duplication.

[1]Photograph courtesy of Dr. B. Hochman.

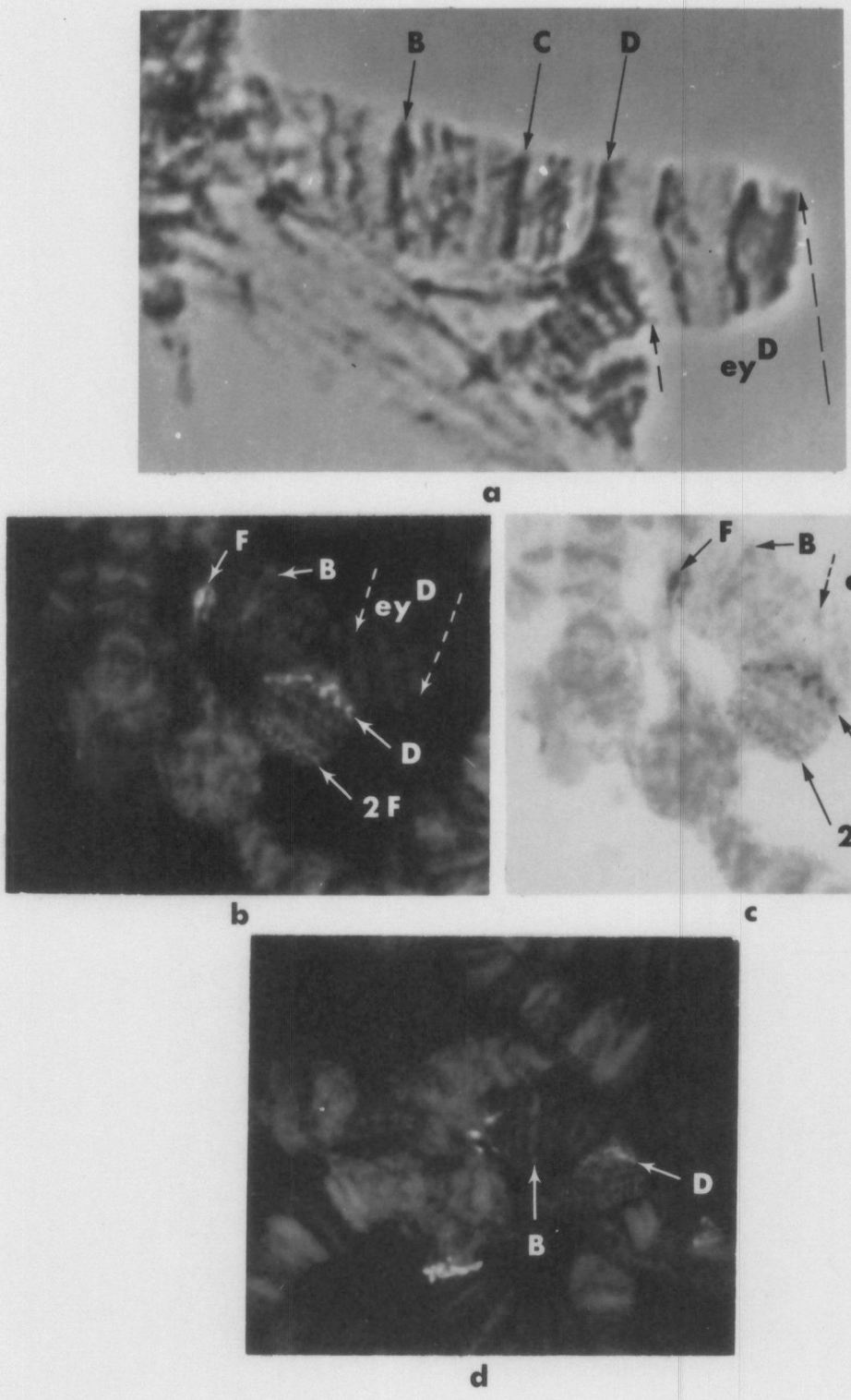


Figure 5

preparations, was highly speckled rather than solid in both homologues. An additional speckled hand was observed at subdivision 102F and was classified as moderately fluorescent. The duplicated material associated with this aberration did not display fluorescence any more brilliant than that characteristic of most bands in the genome.

Fluorescence Pattern of the Translocated Fourth Chromosome

A comparison of a conventionally stained T (2;4) 99 chromosome and quinacrine stained preparations of this aberration is given in Figure 6a through 6c. The fluorescence pattern of this chromosome was again similar to the wild type pattern. The fluorescence at 101F was still observed, while that at 102D seemed much less intense, and the fluorescence at 102B resembled that of ordinary weakly fluorescent bands of wild type. However, care must be taken in interpreting these results since quantitative means for determining fluorescence intensity were not available.

Fluorescence Pattern of the Inverted Fourth Chromosome

The D. simulans fourth chromosome is seen in Figure 7 in which a dark-field image of a quinacrine stained preparation (7a) is compared to a phase-contrast image of the same field (7b). This chromosome exhibited a

Figure 6. The T (2;4) 99 chromosome.

A. Conventionally stained preparation.[1] The two fourth chromosomes (designated by 4) are not synapsed. The structurally normal 4 extends from the chromocenter (C) at the left; the translocated 4 is to the right. The breakpoint in chromosome 2 is adjacent to subdivision 33B (shown in figure).

B. Quinacrine stained preparation seen in dark-field. Note the reduced intensity of fluorescence at 102D.

C. Dark-field image of a quinacrine stained preparation. The figure demonstrates that the breakpoint in 4 is proximal to 101F. F, B, and D denote subdivisions 101F, 102B, and 102D, respectively.

[1]Photograph courtesy of Dr. B. Hochman.

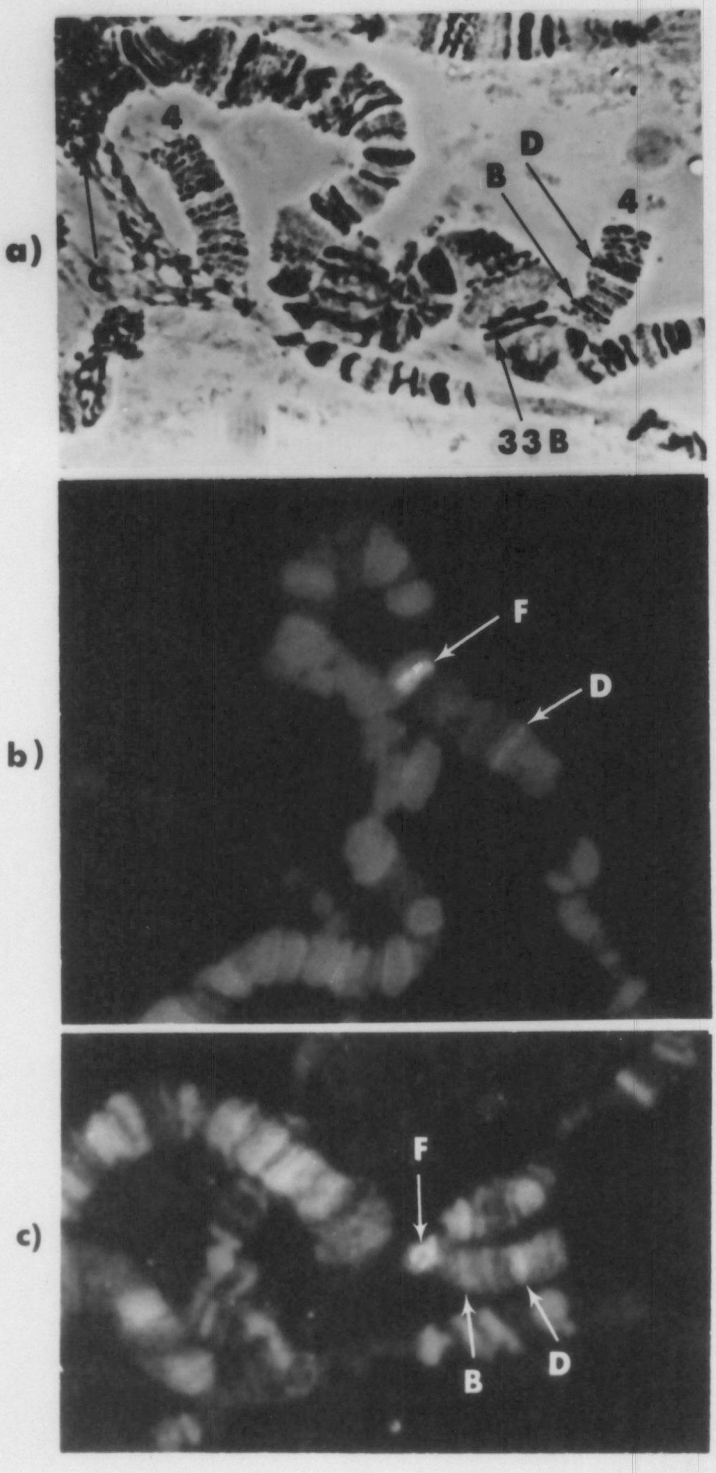


Figure 6

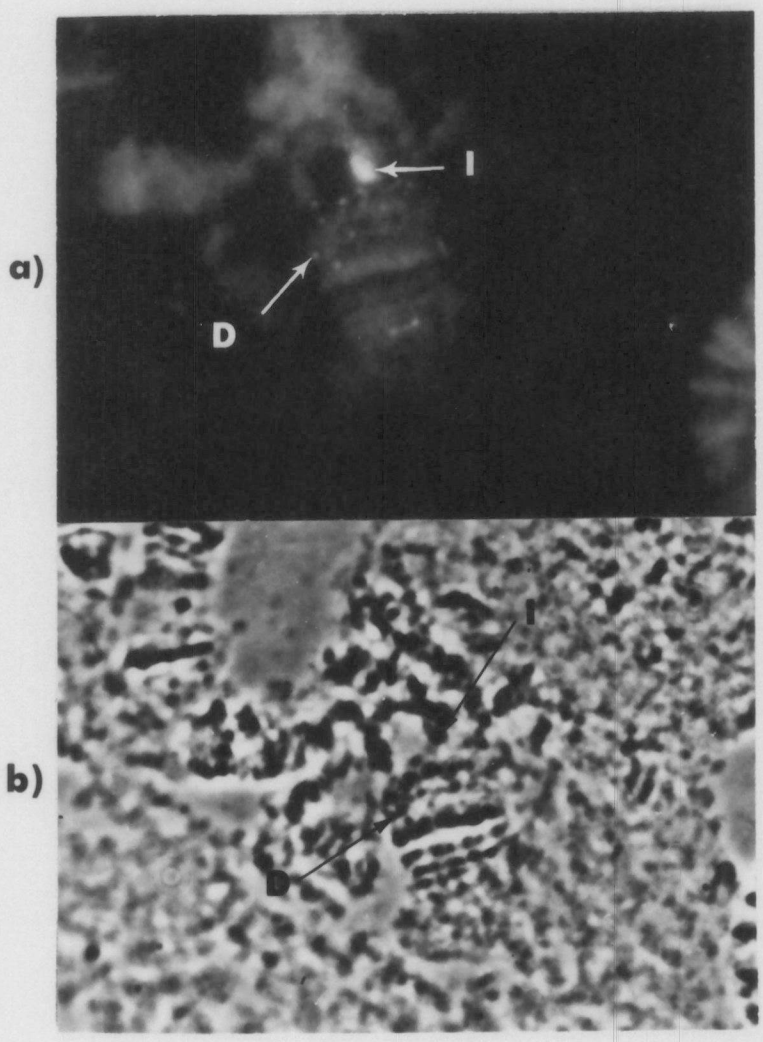


Figure 7. The D. simulans fourth chromosome.

A. Quinacrine stained preparation, dark-field image.

B. Phase-contrast image of field shown in A. I denotes intensely fluorescent material. D indicates moderately fluorescent dots.

fluorescence pattern quite different from that of the wild type D. melanogaster pattern. Intense fluorescence was observed near the chromocenter, while moderately fluorescent dots, apparently not associated with any particular chromosome band, were scattered at random over the chromosome. The bands of the chromosome displayed weak fluorescence, as did the chromocenter. The interbands were non-fluorescent.

IV. DISCUSSION

The fluorescence pattern obtained for the Oregon-R stock of D. melanogaster is essentially the same as that reported by other workers. Adkisson, et al., (1971) observed intensely fluorescent bands at 101F and 102D, and droplets of intense fluorescence at 102E and F in all their preparations except those made from prepupal or near prepupal stages. They also noted moderate fluorescence in other banded regions, and very little or no fluorescence in the interbands of the chromosome. Ellison and Barr (1971) detected intensely fluorescent bands at 101 and 102D 1,2 but could observe dotted fluorescent bands at 102F only in "very large preparations" as the authors term them. Vosa (1970b) reported intensely fluorescent bands at 101F, 102B, and 102F. Examination of Vosa's photographs suggests an explanation for the differences between his data and that obtained in this investigation and by other workers. The poor quality of the photographs in Vosa's paper makes it difficult for the reader to verify the location of the brightly fluorescent bands. His inability to observe the brilliant fluorescence at 102D casts doubt on his interpretation of the sites of fluorescence.

The failure to detect consistently an intensely fluorescent band at 102E or 102F in the present investigation may be due to stock differences or in the failure to obtain very large chromosomes from well-fed larvae for observation. For the most part, however, the similarities of the fourth chromosome fluorescence patterns obtained by independent investigators indicates the reliability of the quinacrine method and its valuable use as a cytogenetic tool.

In presenting the wild type fluorescence pattern (Figure 2, page 16), it was noted that some of the photographs seemed to display a much more intense fluorescence than others. This was not due to variation of staining or photographic procedures employed, but rather can be attributed to several other factors. One cannot avoid the application of unequal squashing pressures in making the preparations, and this is one possible explanation for the different fluorescent intensities observed. An unstretched pair of fourth chromosomes would thus exhibit a more intense fluorescence than a highly stretched pair due to the greater compactness of the chromosome material. Other causes of the variation in fluorescence intensity must, however, be invoked since the highly stretched pair of fourth chromosomes shown in Figure 2a, page 16, display fluorescence intensity equal to that of the unstretched

pair shown in Figure 2c, page 16. Sources of variation were most likely introduced during the photography of the slides. It was necessary to find the same fields of interest under the fluorescent microscope that were found under the Photomicroscope, and in many cases this was more difficult to do than in others. Consequently, some of the fields were exposed to the ultraviolet (UV) irradiation emitted by the mercury lamp for a longer period of time than others. It was noted during the course of this investigation, and by previous workers (Caspersson, et al., 1967, 1969a, 1969b; Adkisson, et al., 1971), that a decrease in or quenching of fluorescence intensity occurred upon continued irradiation of the fields with UV light, and that the chance of this happening was greatly increased the longer the preparations were exposed. Thus, the diverse intensities exhibited by these preparations might reflect various stages of quenching. This was clearly demonstrated upon the replacement of the old bulb of the mercury lamp with a new bulb, after which quenching was seen to occur within much shorter exposure times than previously observed. In addition to the dissimilarity in exposure times attributed to finding the same fields of interest under the two microscopes, one must also include the time factor introduced during the fine focusing of the fluorescent microscope on the different

fields of interest, for in some cases a decision on the level of focus to use for the photomicrograph took longer than in others.

Neither the presence of X chromosome material at its tip nor the absence of its distal portion affected the staining properties of the Df (4) G chromosome or its normal ci^D homologue. Since intense fluorescence was maintained at 101F and 102D, and moderate fluorescence maintained at 102B in both homologues, it can be concluded that this aberration exerts neither a cis nor a trans effect upon the quinacrine fluorescence patterns of the paired fourth chromosomes of the Df (4) G/ci^D stock. A similar conclusion can be reached concerning the deficient region of the Df (4) M^{63a} chromosome since both the structurally normal ci^D homologue and the Df (4) M^{63a} chromosome exhibit the wild type fluorescence pattern. The deleted segment of the Df (4) M^{63a} chromosome has been reported to include bands in 101F and 102A (Lindsley and Grell, 1968), and more specifically lacks bands 101F 6,7 and 102A 1,2 (Hochman, 1965). The failure to observe a loss of fluorescence near the chromocenter in this deficient chromosome suggests that the intensely fluorescent band reported at 101F (Vosa, 1970b; Adkisson, et al., 1971) may actually be proximal to bands 101F 6,7.

The moderately fluorescent band detected at 102F in the ci^D/ey^D preparations might indicate that the insertion

of the duplication has somehow affected both homologues. Alternatively, it may be that the size and quality of the preparations were such to allow observation of fluorescence in this region. This seems more likely in view of the detection of fluorescence in this region by other investigators (Adkisson, et al., 1971; Vosa, 1970b; Ellison and Barr, 1971a).

The observation of a speckled, rather than a solid, fluorescent band at 102D may, however, support the contention that the ey^D duplication has exerted some influence on the structure and chemistry of the aberrant 4. How this influence might be extended to the normal homologue is unclear. Perhaps some segment of the chromosome has been lost, or a band at 102D split due to the insertion of the duplication. The fluorescence seen in subdivision 102D in the ey^D chromosome (Figure 5b through 5d, page 24), when compared to the conventionally stained chromosome in 5a, suggests strongly that bands distal to 102D 1,2 are the fluorescent ones. That is, 102D 1,2, or at least half of it, can clearly be seen proximal to the insertion of the duplication in Figure 5a, but no fluorescence appears immediately proximal to the duplication in 5b. The duplication either has split doublet band 102D 1,2 into two single bands, or the fluorescence in 102D is restricted to the second doublet

band (102D 5,6 in Slizynski's 1944 drawing). Visual evidence favoring the idea that 102D 1,2 has been divided by the duplication comes from a comparison of the size of the first band in 102D in the duplication chromosome with its greater width in the normal 4. As is evident from Figures 1a, page 7, 3a, page 19, and 6a, page 27, the first band in 102D is always thicker than the first band in 102C. Examination of Figure 5a (page 24), however, reveals that in the duplicated chromosome this is not the case, for the first band in 102C is wider than the first band in 102D. On the other hand, the lack of thickness of the first 102D band in the ey^D chromosome might indicate that some material was lost at the time the aberration originated.

Hochman (1971), on the basis of banding pattern comparisons, postulated that the duplication involved in this aberration originated from the left arm of chromosome 4 (4L). It was hoped that a preparation could be obtained which would reveal the fluorescence characteristic of the left arm of chromosome 4. This pattern could then be compared to the ey^D chromosome. The left arm of this chromosome is much thinner than the rest of the chromosome (presumably a result of reduced polyteny) and it is extremely difficult to extract from the chromocenter. Arm 4L has been observed by very few investigators

(Hochman, personal communication). The failure to obtain a fluorescence pattern for the left arm of 4 thus renders data obtained for the ey^D chromosome inconclusive with respect to the origin of the duplication involved in this chromosomal rearrangement.

The data obtained for the T (2;4) 99 chromosome at best merely suggests that the fluorescence intensity at 102D has been modified owing to the translocation of most of the right arm of chromosome 4 onto the second chromosome. Though this data is only suggestive, there are other reports which indicate that fluorescence intensities change upon chromosomal rearrangement. Adkisson, et al., (1971) observed that there was an increase in fluorescence intensity in the band at 101F in a translocation of the fourth chromosome onto the X chromosome in D. melanogaster. Zuffardi, et al., (1971), using mitotic chromosomes, noticed a decreased fluorescence in segments of translocated Y chromosomes of Drosophila. Hence, changes in fluorescence intensities may be common in translocations of Drosophila chromosomes.

The data obtained for D. simulans are less comparable to those of previous reports. Adkisson, et al., (1971) reported intensely fluorescent bands at 101F and 102D as well as a small amount of fluorescent material near the tip of the chromosome. Ellison and Barr (1971a)

detected only one area of bright fluorescence at the base of chromosome 4 in this species, but in another report (Ellison and Barr, 1971b) they noted variation in the fluorescence pattern. Some strains examined had two areas of bright fluorescence, one adjacent to the chromocenter, and the other at the tip of the chromosome, while another stock exhibited the previously reported pattern. Such discrepancies may be due to stock differences, but they may also serve to illustrate that the chromosomes of D. simulans possess a greater degree of variation in their chemical composition than those of D. melanogaster (Ellison and Barr, 1971b).

It is difficult to decide upon the significance of these patterns since despite the overwhelming success of numerous investigators in establishing the fluorescence patterns of various species, relatively little is known about the mode of binding of quinacrine to the chromosome. Neville and Davis (1966) provide X-ray diffraction evidence that acridine dyes bind to DNA by intercalation. Fluorescent studies (Caspersson, et al., 1967, 1969a) also indicate that quinacrine binds to DNA by intercalation of its acridine nucleus or ionic bonding of its diamino side chain to phosphate groups of DNA, and most likely binds to guanine rich regions. Recent evidence from both in vitro studies (Weisblum and De Haseth, 1972) and in situ

hybridization techniques (Barr and Ellison, 1972) suggests that quinacrine binds to regions with a high concentration of adenine-thymine base pairs, i.e., AT rich regions. Early data revealed that quinacrine bound to exclusively heterochromatic regions (Caspersson, et al., 1967, 1969b; Vosa, 1971) but additional data indicated that some well known heterochromatic areas did not fluoresce after quinacrine staining, and that some non-heterochromatic areas did so after this procedure (Vosa, 1970a, 1970b; Adkisson, et al., 1971). Furthermore, in polytene chromosomes of Drosophila heterochromatic areas do not appear as bands, yet all reports indicate that intensely fluorescent bands occur in the genome of this species (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a, 1971b; Barr and Ellison, 1972). Rae (1970) has localized highly repetitious DNA sequences with low guanine and cytosine content near the centromeric region of D. melanogaster polytene chromosomes, while Jones and Robertson (1970) using similar techniques point out that these sequences are not likely to contain many ribosomal RNA cistrons. Kram, et al., (1972) demonstrated that these highly reiterated sequences are interspersed with spacer DNA of enriched GC content, which is likely to be involved with processes requiring specific recognition. Thus, it is possible that quinacrine binds to these spacer DNA

sequences since one band consistently reported in the literature lies in close proximity to the chromocenter (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a, 1971b). Alternatively, quinacrine may be binding to highly repetitious DNA (Adkisson, et al., 1971). Whatever the mode of quinacrine binding, it is definitely not due to an increase in DNA concentration in intensely fluorescent areas as has been shown by ultramicrospectrophotometric measurements (Caspersson, et al., 1967, 1969a, 1969b) and phase-contrast microscopy (Adkisson, et al., 1971).

Equally in dispute is the interpretation of quinacrine fluorescence patterns. If suggestions about the binding of quinacrine to specific nucleotide sequences are correct, then intense fluorescence would indicate the location of AT or GC rich segments in the genomes investigated. Studies testing the effect of DNAase and RNAase treatments or histone removal on fluorescence patterns (Adkisson, et al., 1971; Comings, 1971; Ellison and Barr, 1972) reveal that DNA is required for quinacrine binding to chromosomes, and that non-histone proteins may be responsible for the differential fluorescent banding patterns observed in many species. An alternative interpretation is that particular chromosomal conformations are required for quinacrine binding, and the concomitant

demonstration of such configurations in intensely fluorescent areas (Vosa, 1971; Zuffardi, et al., 1971; Mukherjee and Nitowsky, 1972). A closely related argument is that intensely fluorescent regions of chromosomes may represent areas in which DNA is most accessible for binding of fluorochromes and other exogenous materials (Caspersson, et al., 1967, 1969a; Vosa, 1971). Furthermore, the demonstration of ectopic pairing in some intensity fluorescent areas of D. melanogaster (Barr and Ellison, 1972) and D. simulans (Ellison and Barr, 1971b) polytene chromosomes may mean that highly fluorescent areas depict regions of chemically similar DNA. If spacer DNA with enriched GC content does in fact intersperse highly repetitious DNA with low GC content as Kram, et al., (1972) suggest, then the intensely fluorescent band reported at 101F for D. melanogaster polytene chromosomes (Vosa, 1970b; Adkisson, et al., 1971) might indicate that quinacrine binds to spacer DNA. Jones and Robertson (1970) suggest that highly repetitious DNA in the chromocentric heterochromatin of D. melanogaster may function to bring nonhomologous chromosomes together for metabolic regulation. Hence the intensely fluorescent band at 101F may identify such a control site.

The data from the ey^D chromosome, though inconclusive as to the origin of the duplication involved in this

aberration, may still supply information on the chemical nature of this aberration if the data are considered in the light of present theories of quinacrine binding and the significance of quinacrine fluorescence patterns. Since the duplcation does not fluoresce, it may be presumed to be neither AT nor GC rich. Furthermore, it may be deficient in highly repetitious and spacer DNA. Finally, the duplication may be judged as a region which lacks the proper array of non-histone proteins necessary for quinacrine binding or the proper chromosomal configuration for such binding. If part of 4L is the source of the duplication, then this arm is either not fluorescent or it loses its brilliance when the level of polyteny is increased. Alterations in properties of chromosomal segments, including an increase in polyteny, following their translocation away from the chromocenter have been reported by Roberts (1972).

The data obtained from the study of deficient fourth chromosomes can be interpreted as lending support to observations that quinacrine binds to specific base sequences (Caspersson, et al., 1967, 1969a, 1969b; Weisblum and De Haseth, 1972; Ellison and Barr, 1972). It is equally probable that not enough alteration of genetic material has occurred to cause a configurational change in the chromosome sufficient to upset the normal pattern

of quinacrine binding. The fluorescence pattern obtained in this study for D. simulans and that of previous investigators (Ellison and Barr, 1971a, 1971b) suggests that sufficient disruption of chromosome structure has occurred to alter the normal quinacrine binding pattern. This is to be expected since the inverted segment of the fourth chromosome of D. simulans comprises a major portion of the chromosome. Similarly, the data from the translocation stock, though at best suggestive, indicate that the disruption of chromosome structure which might be expected to occur in such a chromosomal rearrangement could explain the change in fluorescence intensity which was observed.

Comings (1971) suggests that specific patterns of quinacrine binding may be influenced by certain classes of non-histone proteins. Though no direct proof of this contention currently exists, there are data which support it. Other investigators have observed changes in fluorescence intensity upon translocation of Drosophila chromosomes (Adkisson, et al., 1971; Zuffardi, et al., 1971). Enzymatic removal of RNA (Adkisson, et al., 1971; Comings, 1971; Ellison and Barr, 1972) or histones (Comings, 1971) was shown to have no effect on the subsequent fluorescence pattern of the chromosome preparations, thus implicating non-histone proteins in the binding of quinacrine to chromosomes.

All this evidence points to a model in which non-histone proteins play a significant role in the establishment of differential fluorescence patterns, for as Zuffardi, et al., (1971) point out, it is highly unlikely that structural rearrangements of chromosomes would be accompanied by base changes which would result in increased or decreased fluorescence in the affected areas.

A model in which non-histone proteins influence fluorescence patterns is consistent with data obtained from in situ hybridization experiments. Highly reiterated DNA sequences have been localized in the chromocentric heterochromatin of D. melanogaster using such techniques (Rae, 1970; Jones and Robertson, 1970). Kram, et al., (1972) indicate that this satellite DNA is interspersed with spacer DNA which could function in processes requiring specific recognition. The fact that one intensely fluorescent band has been reported in this region by a number of investigators (Vosa, 1970b; Adkisson, et al., 1971; Ellison and Barr, 1971a) indicates that a very limited class of structures is being recognized by quinacrine.

Such a model is also consistent with Crick's (1971) model for the eukaryotic chromosome in which it is postulated that control elements are located within the banded regions of the chromosomes. To date there is no report of intense fluorescence occurring in other than the banded

regions of D. melanogaster polytene chromosomes. The fact that only two or three areas (possibly containing control elements) are stained on chromosome 4 after quinacrine treatment is again explicable by the non-histone protein model since non-histone proteins can be seen to provide specific configurations needed for the binding of quinacrine to the chromosome.

It is thus hypothesized that the intensely fluorescent bands seen on the fourth chromosome of D. melanogaster after quinacrine staining of polytene preparations reflect regions of this chromosome at which non-histone proteins provide the proper configurations necessary for the binding of quinacrine to chromosomal DNA, and that such areas identify a certain class of control elements of the D. melanogaster genome. Much work is needed to test this hypothesis, such as the study of fluorescence patterns of chromosomal aberrations in other species, quantitative measurements of fluorescence intensity in normal and aberrant chromosomes, and the development of a deeper understanding of the structural and functional roles of non-histone proteins.

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