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Fluorescence Patterns of Heterochromatin in Mitotic and Polytene Chromosomes in Seven Members of Three Sub-groups of the *melanogaster* Species Group of *Drosophila*

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Abstract. A comparative study of fluorescence patterns of heterochromatin in mitotic and polytene chromosomes of seven species belonging to 3 sub-groups (*melanogaster* sub-group: *D. melanogaster* and *D. simulans; montium* sub-group: *D. kikkawai* and *D. jambulina; ananassae* sub-group: *D. ananassae, D. malerkotliana* and *D. bipectinata*) of the *melanogaster* species group of *Drosophila* (*Sophophora*) has been made. Hoechst 33258 (H) fluorescence patterns of mitotic chromosomes reveal differences correlated to the taxonomic groupings of these species. The *melanogaster* sub-group species have H-bright regions on heterochromatin of all chromosomes; the *montium* sub-group species have H-bright regions mainly on the 4th and Y-chromosomes; in the *ananassae* sub-group, while *D. ananassae* chromosomes do not show any H-bright regions, *D. malerkotliana* and *D. bipectinata* have small H-bright segments only on their 4th chromosomes. The H- and quinacrine mustard (QM) fluorescence patterns of larval salivary gland polytene chromocentre in these species, however, do not show the same taxonomic correlation. While *D. ananassae* and *D. kikkawai* polytene nuclei lack any H- or QM-bright region in the chromocentre, the remaining species have prominent H- and/or QM-bright region(s). In *D. jambulina,* the QM-bright regions are generally bigger than H-bright regions, while in *D. malerkotliana* and *D. bipectinata* the situation is reversed. Actinomycin D counterstaining prior to H-staining of polytene preparations of each species confirms that the H-bright region/s in the chromocentre are composed of A–T rich sequences. In vivo labelling of salivary gland polytene nuclei with 5-bromodeoxyuridine for 24 to 48 h and subsequent H-staining reveals that in all the species, the H-bright regions do not replicate in 3rd instar stage and presumably represent the non-replicating alpha heterochromatin. Significantly, in all the species (excepting *D. kikkawai* and *D. ananassae*), the size, location and the number of H- and/or QM-bright regions were seen to vary in different polytene nuclei in the same gland. It seems that the organization and the extent of under-replication of alpha heterochromatin varies in different polytene nuclei. Present studies also show that even closely

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related species differ in the content and organization of H-bright heterochromatin. The 81F band at the base of 3R in *D. melanogaster*, but not in *D. simulans*, appears to contain non-replicating H-bright sequences in addition to replicating chromatin.

Introduction

Different species of *Drosophila* carry a significant, but variable, amount of heterochromatin in their mitotic cells. Following the classical studies of Heitz (1934), it is now known (Rudkin, 1965; Gall et al., 1971; Lakhotia, 1974) that a major part of this heterochromatin does not polytenize in larval salivary glands and forms the non-replicating alpha heterochromatin while the remainder constitutes the replicating beta heterochromatin in the common chromocentre. As in other organisms, *Drosophila* heterochromatin is also enriched in satellite and/or other repetitive sequences; the analysis and localization of these sequences in chromosomes of different species of *Drosophila* have provided significant information about the structure, organization and evolution of heterochromatin in related species (Gall et al., 1971; Hennig, 1972a, b; Peacock et al., 1973, 1978; Barnes et al., 1978). In addition, fluorescent stains have been used in studying the distribution and evolution of heterochromatin in mitotic and polytene cells of several species of *Drosophila* (Vosa, 1970; Adkisson et al., 1971; Barr and Ellison, 1971, 1972; Ellison and Barr, 1971; Holmquist, 1975a; Gatti et al., 1976; Pimpinelli et al., 1976; Lemeunier et al., 1978; Kumar and Lakhotia, 1977; Lakhotia and Kumar, 1978).

The *melanogaster* species group in the genus *Drosophila* (sub-genus *Sophophora*) includes a large number of widely distributed and diversified species and these have been further classified into several sub-groups (Bock and Wheeler, 1972). While several species of the *melanogaster* species sub-group have been studied with respect to their satellite and/or repetitive sequences and their cytological localization (Peacock et al., 1973, 1978; Holmquist, 1975a; Gatti et al., 1976; Barnes et al., 1978; Lemeunier et al., 1978), little is known about the members of the other sub-groups. In the present study, we have examined the fluorescence patterns of heterochromatin in 7 species belonging to 3 sub-groups of the *melanogaster* species group i.e., *melanogaster, simulans, kikkawai, jambulina, ananassae, malerkotliana* and *bipectinata*. The first two are sibling species and belong to the *melanogaster* sub-group; the third and fourth species belong to the *montium* sub-group while the last three species are grouped under *ananassae* sub-group (Bock and Wheeler, 1972). We have used Hoechst 33258 (H) and Quinacrine Mustard (QM) fluorescence patterns to obtain a comparative information on the distribution, localization and organization of heterochromatin in mitotic chromosomes and in polytene chromocentre in these related species. We have also used actinomycin D (AMD) counterstaining with H-staining to enhance the A–T specific H-fluorescence (Jorgenson et al., 1978). In addition, H-fluorescence patterns after 5-bromodeoxyuridine (BrdU) incorporation in polytene nuclei have also been examined to ascertain the non-replicating nature of the H-bright material in the polytene chromocentre (Kumar and Lakhotia,

1977). A preliminary account of these studies was presented earlier (Lakhotia, 1978).

Materials and Methods

Wild type strains of the following 7 species were used: *D. melanogaster, D. simulans, D. kikkawai, D. jambulina, D. ananassae, D. malerkotliana* and *D. bipectinata.* The stocks of *D. melanogaster* (Oregon R) and *D. simulans* have been maintained in the laboratory for several years. *D. kikkawai* (Columbia) was obtained from Dr. V. Baimai (Bangkok) while the other four local species were taken from Dr. J.P. Gupta's collection. The flies and larvae were grown on standard agar-cornmeal-brown sugar-yeast food at 24° ± 1° C.

Mitotic chromosome preparations from brain ganglia of healthy late 3rd instar larvae of each species were made by the air-dry technique (Lakhotia and Kumar, 1978) after fixing the ganglia as per the sequential fixation described by Lemeunier et al. (1978). For polytene chromosome preparations, salivary glands from late 3rd instar larvae of each species were dissected out in ringer, fixed briefly in fresh aceto-methanol (1:3) and squashed in 50% acetic acid. After the removal of coverslips, the polytene preparations were dehydrated and dried. Mitotic as well as polytene preparations were stained with an aqueous solution of Hoechst 33,258 (0.05 µg/ml for mitotic and 5 µg/ml for polytene chromosomes) for 15 min (mitotic) or 5 min (polytene). After washing the excess stain with water, the preparations were again dried. A drop of buffer (pH 4.0 for mitotic and pH 7.0 for polytene preparations) was placed on the slide and covered with a coverslip and the sides sealed with DEPEX. Some polytene preparations of each species were treated with Actinomycin D (0.0128 mM in Sørensen's buffer at pH 6.8) for 10 min prior to normal H-staining to enhance the resolution of A–T specific H-fluorescence (Jorgensen et al., 1978).

The polytene preparations of each species were also stained with an aqueous solution of Quinacrine Mustard Dihydrochloride (Sigma, 0.5 mg/ml) for 10 min and after washing, were mounted with a pH 5.5 McIlvaine buffer.

To localize the non-replicating H-bright alpha heterochromatin by fluorescence microscopy (Kumar and Lakhotia, 1977), early to mid-3rd instar larvae of each species were taken out of the normal food in which they were growing and were transferred to smaller vials containing filter papers soaked in 2% sucrose solution supplemented with 40 µg/ml 5-bromodeoxyuridine (Sigma). The larvae were kept in dark at 24° C for 24 to 48 h. In some cases, BrdU was mixed with the standard food at a concentration of 200 µg/g food and the larvae fed on this medium for 24 to 48 h. In either cases, the BrdU-labelling of salivary gland polytene nuclei was satisfactory as detected by a general quenching of H-fluorescence. The in vivo labelled salivary glands were dissected out and processed for fluorescence microscopy after staining with H and mounting with pH 7.0 buffer as above. The fluorescence patterns of mitotic (after H-staining) and polytene nuclei after H- (with or without AMD or BrdU pretreatment) or QM-staining were examined in at least 5 preparations of each kind for each species. A Carl Zeiss (Jena) 'Fluoval' fluorescence microscope with incident illumination was used for the purpose.

Observations

I. Mitotic Chromosomes

The metaphase karyotypes of the different species (see Patterson and Stone, 1952; Clayton and Wheeler, 1975; Singh and Gupta, 1980, for the *D. jambulina* karyotype) studied show differences from each other on the lines of their taxonomic groupings. Each of the species has 4 pairs of chromosomes of which the two pairs of large metacentric autosomes (chromosomes 2 and 3) are common

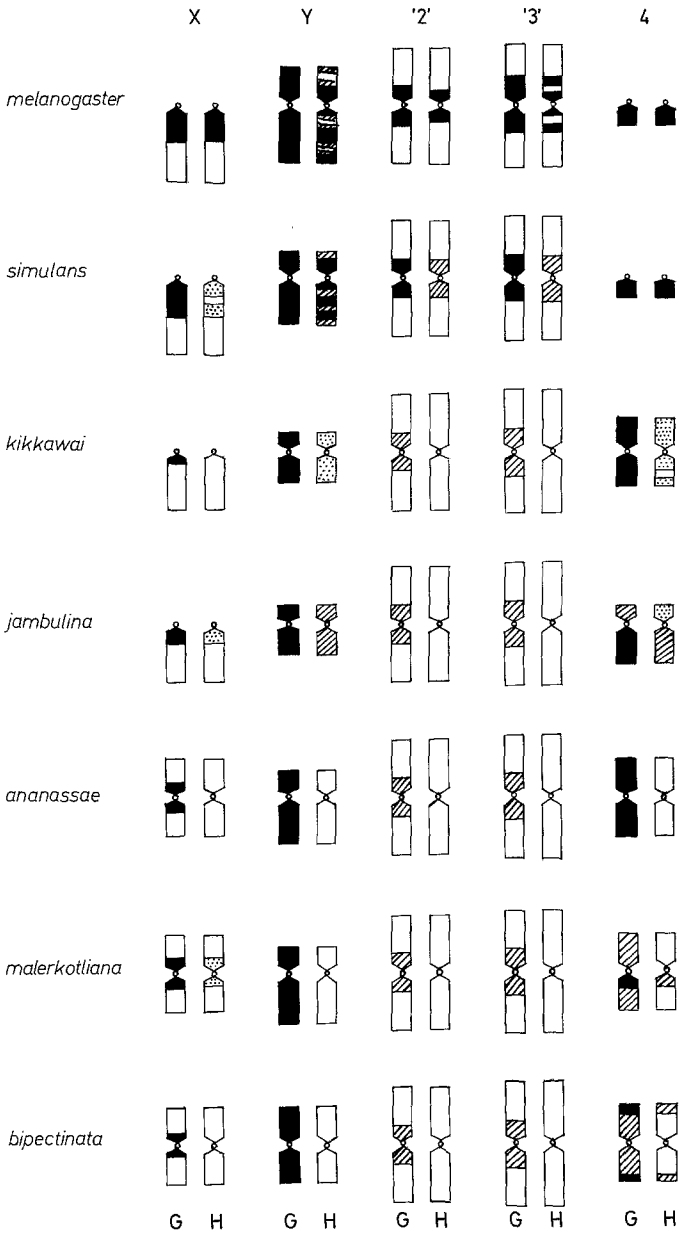

Fig. 1. Diagrammatic summary of metaphase chromosomes in the 7 species examined after Giemsa (*G*) or Hoechst 33258 (*H*) staining. (Data for *D. melanogaster* and *D. simulans* after Holmquist, 1975a, Gatti et al., 1976, and Pimpinelli et al., 1976). In Giemsa stained chromosomes: ▭ normal euchromatic staining, ▨ moderately dark staining and ■ very dark staining, and in H stained chromosomes: ▭ dull, ▩ slightly bright, ▨ moderately bright and ■ very bright fluorescing regions. The relative sizes of different chromosomes are only approximate

to all while the X and 4th chromosomes differ. In the *melanogaster* sub-group species (*D. melanogaster* and *D. simulans*), the X is acrocentric with 1/3 to 1/2 of its proximal part being heterochromatic while the 4th chromosomes are small dots. In *D. kikkawai* and *D. jambulina* (*montium* sub-group), the X is acrocentric with smaller proximal heterochromatic segment while the 4th chromosomes are larger and nearly completely heterochromatic. In the *ananassae* sub-group (*D. ananassae, D. malerkotliana* and *D. bipectinata*), the X as well as the 4th chromosomes are metacentrics and the large 4th chromosomes in all the 3 species are heterochromatic. The heterochromatic Y chromosome in all the 7 species is a large meta- or sub-metacentric element.

The H-fluorescence patterns of mitotic chromosomes of these 7 species are diagrammatically shown in Figure 1. It is obvious that the amount and location of H-bright regions in metaphase chromosomes of closely related species varies considerably. In general, however, it appears that the *melanogaster* sub-group species have more H-bright material than the species belonging to the *montium* or *ananassae* sub-groups. In the *ananassae* sub-group, while *D. ananassae* does not have any H-bright regions, *D. malerkotliana* and *D. bipectinata* have some H-bright material on the 4th chromosomes (Fig. 1).

II. Polytene Chromosomes

In all the species examined, the polytene nuclei show a well defined chromocentre to which the different chromosome arms remain attached. In all cases, the entire chromocentre as well as the dark bands show a bright fluorescence with H or QM in normal preparations while in the AMD-counterstained or BrdU-labelled preparations, the overall H-fluorescence is considerably quenched. In the present study, we have paid particular attention to those regions within or close to the chromocentre whose H or QM fluorescence is brighter than the rest of the chromocentre since these regions are likely to be related to specific types of repetitive/satellite sequences (see Discussion). Only these more brightly fluorescing segments/regions are referred to as H- or QM-bright in the following description. It may be noted that in all the species, the H-fluorescence patterns of chromocentre region after in vivo BrdU incorporation or AMD-counterstaining are the same as with only H-staining, except that the fluorescence of non-H-bright regions is considerably quenched with BrdU or AMD. In general, the H- and QM-fluorescence patterns are also the same except in a few species. In the following description, therefore, only the location of H-bright regions in the chromocentre is described. The differences in the QM patterns, where seen, have been specifically noted.

Polytene nuclei of *D. melanogaster* and *D. simulans* have 5 long arms and a short 4th chromosome arm attached to a well defined chromocentre. In the polytene nuclei of these sibling species, a small very brightly fluorescing body is usually located at the base of the 4th chromosome (Fig. 2). In many nuclei, one or two additional small H- and QM-bright regions are also seen in the chromocentre region. In male polytene nuclei of *D. melanogaster,* occasionally a bright body is also seen at the base of 3L after H or QM staining (Fig. 2a);

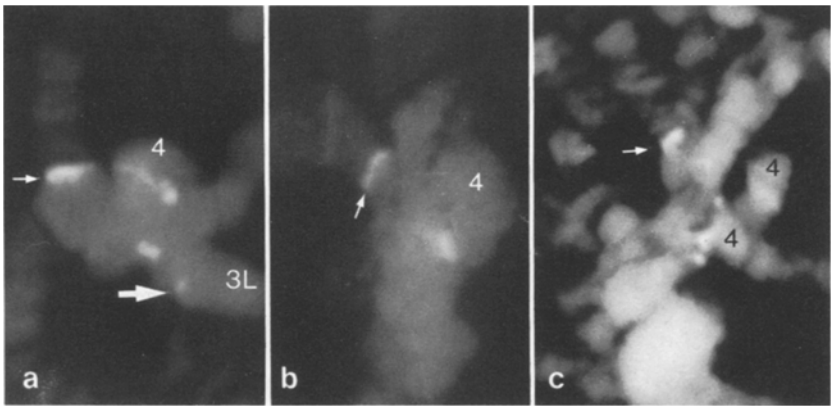

Fig. 2a–c. a QM stained polytene chromocentre in *D. melanogaster* male; **b** BrdU-H stained chromocentre in *D. melanogaster* female; **c** H stained chromocentre in F_1 hybrid of *D. melanogaster* female x *D. simulans* male. Note the bright fluorescence of the 81F band (→) in *melanogaster* 3R. In **a**, the Y chromosome is presumably located at the base of 3L (⟶). Magnification in these and the other figures is ×2,400

a similar body is not seen in female nuclei (Fig. 2b) so that this body may represent the Y-chromosome (see Discussion). The band 81F at the base of 3R in *D. melanogaster* is always brightly fluorescing and often this band appears as a ring or as a figure – of – eight. In *D. simulans*, the band 81F does not fluoresce brightly neither with H nor with QM.

For a closer comparison of the homology of brightly fluorescing regions in the two species, hybrid female larvae were obtained by crossing virgin *melanogaster* females with *simulans* males. In the polytene nuclei of these F_1 interspecific hybrids, the H-bright bodies are distributed at the base of asynapsed 4th chromosomes and at other locations in the chromocentre as in the two parental species. However, the number of H-bright bodies in hybrid nuclei is generally more than in either of the parents (Fig. 2c). The base of 3R remains asynapsed in the hybrids and one of the homologs (*melanogaster*) shows very bright fluorescence of the 81F band while the other homolog (*simulans*) lacks bright fluorescence of 81F band (Fig. 2c).

Polytene nuclei of *D. kikkawai* and *D. jambulina* show a well defined chromocentre from which 5 long and a short (4th chromosome) arms radiate out as in *D. melanogaster* and *D. simulans*. More than 30 polytene preparations of *D. kikkawai*, from as many salivary gland pairs, have been examined and in most of them no distinct H- or QM-bright region could be seen within the chromocentre – the entire chromocentre fluoresces with uniform brightness (Fig. 3a). Only in one H stained preparation, a small slightly H-bright region could be identified within the chromocentre in a few, but not all, polytene nuclei.

In most of the polytene preparations of *D. jambulina*, stained with H, a small bright region (occasionally two regions) is seen within the chromocentre, although in some preparations, none of the nuclei show any distinct H-bright

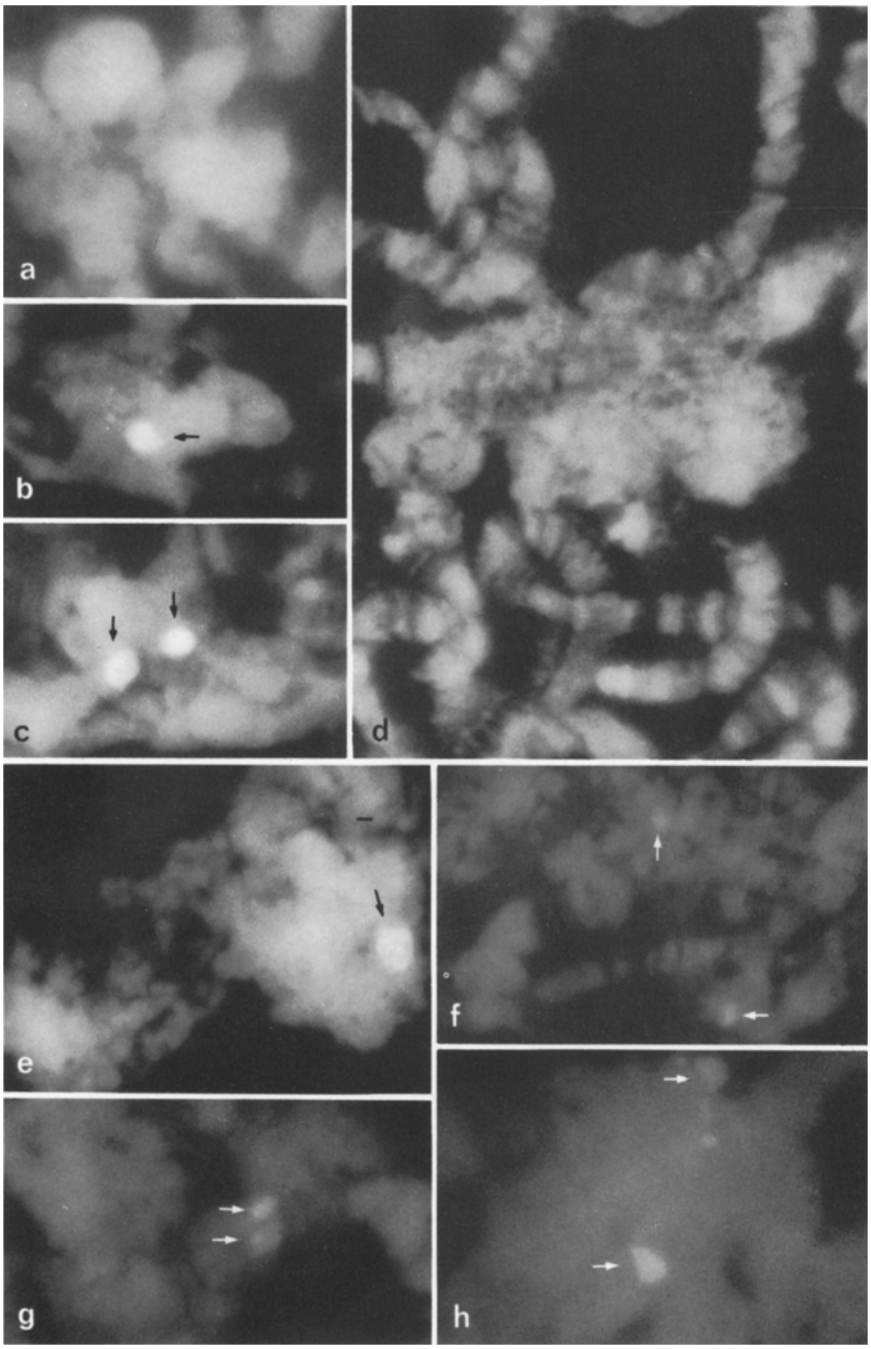

Fig. 3a–h. Polytene chromocentre of *D. kikkawai* (**a** H stained), *D. jambulina* (**b**, **c** QM stained), *D. ananassae* (**d** H stained), *D. malerkotliana* (**e** H stained, **f** QM stained) and *D. bipectinata* (**g** H stained, **h** AMD + H stained)

region in the chromocentre. QM stained polytene nuclei of *D. jambulina* show more prominent one, or occasionally two, brightly fluorescing body (bodies) in the chromocentre (Fig. 3b, c). The size of these QM-bright regions is generally larger than the H-bright region(s). In some QM stained nuclei, however, there is only one smaller bright region. It is significant that these variations in size and number of H- and QM-bright bodies can be seen in different nuclei of the same gland.

The three species belonging to the *ananassae* sub-group share a similar polytene karyotype when stained with Giemsa. All have a large chromocentre with which 6 long arms XL, XR, 2L, 2R, 3L and 3R are attached. No distinct 4th chromosome is seen in any of these species; apparently, the entire large heterochromatic 4th chromosome remains buried in the chromocentre which in these species has a different organization than in the preceding 4 species. In the *ananassae* sub-group species the chromocentre is much larger and granular, more so in *D. ananassae*.

In polytene preparations of *D. ananassae,* the whole of the chromocentre appears uniformly fluorescing with no specific region which is more H- or QM-bright (Fig. 3d). Unlike *D. ananassae,* the chromocentre in *D. malerkotliana* and *D. bipectinata* shows prominent brighter regions. However, although *D. malerkotliana* and *D. bipectinata* can be hybridized, with their hybrid female progeny being fertile upon back crossing to either of the parents (Jha and Rahman, 1973, and our observations), the H- and QM-bright regions in these two species differ. In *D. malerkotliana,* there is usually one large H-bright body (Fig. 3e) in the chromocentre; however, in some nuclei even from the same gland, there are two medium sized bright bodies while in some other nuclei, only one smaller body is seen. With QM staining also, the chromocentre in polytene nuclei of *D. malerkotliana* shows one or two bright bodies but the size of QM-bright regions is always smaller than the H-bright regions (Fig. 3f).

In polytene preparations of *D. bipectinata,* H- or QM-staining usually shows one bright region within the chromocentre; sometimes, a relatively less bright second region is also seen (Fig. 3g, h). Compared to *D. malerkotliana,* the brightness, and occasionally the size also, of H-bright regions in *D. bipectinata* appears less.

The H-fluorescence patterns in the chromocentre in polytene nuclei from F_1 hybrid larvae obtained by reciprocal crossing of *malerkotliana* and *bipectinata* males and females have also been examined. In most of these nuclei, two, and occasionally more, brightly fluorescing regions are seen and interestingly, of the two, one appears smaller than the other. Furthermore, the size of H-bright bodies in hybrid larvae appears smaller than their counterparts in the parental species. It seems, therefore, that each of the parental types of H-bright material condenses separately in the hybrid nuclei.

Discussion

In the present study, we have attempted to characterize and compare the heterochromatin in related species of *Drosophila* with a view to understand i), some

aspects of the evolutionary diversification of sequences associated with heterochromatin and ii) their organization in polytene nuclei. It is now known that in general, the heterochromatic regions which fluoresce brightly with H and/or QM, are relatively rich in A–T base pairs (Comings, 1975; Holmquist, 1975a; Gatti et al., 1976; Lakhotia and Kumar, 1978; Jorgenson et al., 1978). We have also used AMD pretreatment with H-staining to confirm the A–T richness of H-bright regions seen in polytene chromocentre in different species since as shown by Jorgenson et al. (1978), the G-C specific non-fluorescent antibiotic AMD enhances the resolution of A–T specific H-fluorescence. As expected, we have observed that in all the species examined, the H-bright regions in the polytene chromocentre become more distinct when pretreated with AMD. In *D. kikkawai* and *D. ananassae* where no H- or QM-bright regions are generally seen in polytene nuclei, AMD pretreatment also failed to uncover any such region. This shows that all the brightly fluorescing regions observed by us in the other species contain A–T rich sequences. This specificity of H and QM fluorescence permits us to compare the location and the extent of specific class/classes of heterochromatin in the related species of *Drosophila* examined by us. Another point of similarity in the H-bright regions in the polytene chromocentre in the species studied here is that these regions apparently do not replicate during the 3rd instar stage as evidenced by persistent bright fluorescence with H even after prolonged (24 to 48 h) in vivo BrdU incorporation. It is known that Brdu incorporated into replicating DNA quenches H fluorescence (Latt, 1974) and it has been shown earlier (Kumar and Lakhotia, 1977) that the non-replicating alpha heterochromatin in polytene nuclei of *Drosophila* can be conveniently localized in cytological preparations by in vivo BrdU-labelling and H-staining technique.

Phylogenetically, it is believed that within the *melanogaster* species group, an initial evolutionary dichotomy generated the *montium* and *ananassae* subgroups on the one hand and the *melanogaster* and other sub-groups on the other (Bock and Wheeler, 1972). While the *melanogaster* species sub-group has retained the original acrocentric X and dot-like 4th chromosomes, a translocation of the basal heterochromatin of X to the 4th chromosomes is believed to have occurred in the *montium* and *ananassae* sub-groups (Patterson and Stone, 1952; Lemeunier et al., 1978). The metacentric X seen in members of the *ananassae* sub-group has presumably resulted from a pericentric inversion (Patterson and Stone, 1952). The present observations suggest that in addition to these structural changes that have occurred during the evolutionary diversification, there have also been profound changes in the quality of heterochromatin associated with different chromosomes in these related species. The members of the *melanogaster* sub-group seem to have more H-bright material (also see Lemeunier et al., 1978, for localization of QM-bright heterochromatin in metaphase chromosomes of several species of the *melanogaster* sub-group) than the species belonging to the *montium* or *ananassae* sub-groups. In the *ananassae* sub-group, we see, in parallel with the two species complexes (*ananassae* and *bipectinata* complexes) recognised by Bock (1971), that *D. ananassae* lacks any H-bright regions (also see Adkisson et al., 1971), while *D. malerkotliana* and *D. bipectinata* have at least some H-bright material on their largely heterochro-

matic 4th chromosomes. Interestingly, however, these H-bright regions in the chromosomes of *D. bipectinata* and *D. malerkotliana* differ from each other not only in their location on the 4th chromosomes (see Fig. 1) but apparently also in their DNA sequence composition since in the interspecific F_1 hybrids, the H-bright materials of the parental species condensed separately in the chromocentre. It is known that in *Drosophila* and other organisms the heterochromatic regions which share similar satellite/repetitive sequences usually condense together while those which differ, form separate condensed masses (Barr and Ellison, 1972; Mayfield and Ellison, 1975; Schmid et al., 1975; Holmquist, 1975b; Lakhotia and Kumar, 1978). Moreover, it is also known that the satellite sequences associated with heterochromatin in *D. melanogaster* and *D. simulans* have diversified (Peacock et al., 1978) and correspondingly, we observe that the number of H-bright regions in the polytene chromocentre in their F_1 hybrid larvae is greater than in either of the parental species. Thus it seems that as in the case of *D. melanogaster* and *D. simulans* sibling species, the sequences associated with H- and/or QM-bright regions have diversified between *D. malerkotliana* and *D. bipectinata* and these diversified sequences condense separately in the polytene chromocentre.

It seems that the relative content of H- or QM-bright material in the polytene chromocentre of a species is not always correlated to the extent of H-bright segments seen in the mitotic chromosomes. *D. malerkotliana* and *D. bipectinata* have more H-bright material in their polytene chromocentre than is seen in their mitotic chromosomes. In species like *D. melanogaster, D. simulans* and *D. jambulina,* the relative amount of H-bright material in their respective polytene and mitotic nuclei appears comparable. On the other hand, in *D. kikkawai,* the polytene chromocentre has little, if any, H-bright material although the mitotic chromosomes do show some such regions. The comparable amount of H-bright material in mitotic and in most of the polytene cells of *D. melanogaster, D. simulans* and *D. jambulina* is as expected if these regions do not polytenize in polytene nuclei. Our BrdU labelled preparations reveal that these H-bright regions do not replicate at least during the late larval stages. The relative abundance of H-bright material in polytene chromocentre of *D. malerkotliana* and *D. bipectinata* compared to their mitotic chromosomes and to the polytene chromocentre of other species, appears intriguing. In vivo BrdU labelling and subsequent H staining shows that these H-bright regions do not incorporate BrdU and therefore, are not replicating in the late larval stages. This may imply that either the H-bright segments in polytene chromocentre of these two species replicate during early stages of polytene development or there are some other unknown factors which cause an apparent increase in their size in the polytene chromocentre.

In contrast to the above two species, the other member of the *ananassae* sub-group, viz., *D. ananassae,* lacks any H- or QM-bright material in its polytene and mitotic nuclei (also see Adkisson et al., 1971). Apparently, the heterochromatin of *D. ananassae* lacks A–T rich sequences and in view of this, the cytological organization of polytene chromocentre can not be adequately studied by present techniques.

The mitotic chromosomes of *D. kikkawai* show H-bright bands all along

the large 4th chromosomes. In most polytene nuclei, however, no H- or QM-bright region could be seen. AMD counterstaining or BrdU labelling also failed to reveal any H-bright area in *D. kikkawai* polytene chromocentre. The fate of the H-bright bands seen on mitotic 4th chromosomes, in polytene nuclei is not clear. One possibility is that in polytene nuclei, the H-bright DNA sequences remain dispersed either along the length of the polytenized euchromatin part of 4th chromosome or within the chromocentre so that a distinct H- or QM-bright mass is not seen. In this context, it would be interesting to examine the H and QM fluorescence of mitotic and polytene chromosomes of other strains of *D. kikkawai* in which extensive polymorphism of the 4th chromosome has been recorded (Baimai, 1969, 1974).

D. melanogaster, D. simulans, D. jambulina, D. malerkotliana and *D. bipectinata* always show distinct brightly fluorescing region/s in the polytene chromocentre. However, it is significant that within a species, and often within the nuclei from the same salivary gland, the number, size and location of the H- and/or QM-bright regions vary. It seems unlikely that all the variations noted in the location, size and number of the brightly fluorescing bodies in different nuclei of a gland can be attributed to technical factors such as squashing, fluorescent staining etc. In all these 5 species, the H- and QM-bright regions in the chromocentre seem to be related to the non-replicating alpha heterochromatin since as discussed earlier, these regions do not incorporate BrdU and also these regions are expected to be composed of satellite and/or other highly repetitive sequences which are known to be underreplicated in polytene nuclei. If these brightly fluorescing regions were equally underreplicated in all nuclei of a salivary gland, we would expect them to appear of nearly similar size in different nuclei. Even though, we have not measured the precise area of H- and/or QM-bright regions in different nuclei, the observed variations are very obvious. In *D. nasuta,* where the non-replicating alpha heterochromatin forms a single well defined mass in polytene nuclei (Kumar and Lakhotia, 1977), we have measured the area of this region in a large number of nuclei (Lakhotia and M. Kumar, unpublished data) and found that the area of alpha heterochromatin in this species varies atleast two-fold in different nuclei, suggesting a varying degree of underreplication. The variability in the H-bright regions observed in the present study thus suggests that the extent of underreplication and the location of underreplicated regions may not be the same in all nuclei of a salivary gland. Also, the heterochromatic segments of different chromosomes, which contribute to the total alpha heterochromatin, often do not coalesce into one mass. This contrasts with the organization of the alpha heterochromatin in polytene nuclei of *D. virilis* (Gall et al., 1971; Holmquist, 1975a) or *D. nasuta* (Kumar and Lakhotia, 1977) where it forms one compact mass in the chromocentre of all polytene nuclei. Presumably, as discussed earlier, the presence of more than one H- and/or QM-bright regions in the polytene chromocentre reflects the heterogeneity of the satellite/repetitive sequences constituting the alpha heterochromatin.

The behaviour of the 81F band in *D. melanogaster* and *D. simulans* is of particular interest. The persistence of H-bright fluorescence in this band in all BrdU labelled polytene nuclei of *D. melanogaster* could imply that it

is also not replicating in large polytene nuclei. However, using ^{3}H-thymidine autoradiography, we found (data not presented) that the 81 F band is distinctly labelled with ^{3}H-thymidine and therefore, replicating in polytene nuclei of late 3rd instar larvae. Thus, either the bright fluorescence of the 81 F band is due to other non-chromatin material so that BrdU incorporation does not affect its brightness, or the 81 F band in *D. melanogaster* contains replicating as well as non-replicating sequences so that this band shows ^{3}H-thymidine labelling and also its H-fluorescence is not quenched by BrdU incorporation. Peacock et al. (1978) observed that the 1.672 g/cc satellite of *D. melanogaster* hybridizes in situ with a band in secion 81, presumably the band 81 F since this is the only band identifiable in section 81 of Bridges' (1935) map. A few other bands (like 1 A on X and 21 D on 2 L) on the euchromatic arms of polytene chromosomes of *D. melanogaster* have also been seen to hybridize in situ with some of the satellite sequences which are present in the chromocentre as well (see Peacock et al., 1978); however, our BrdU-H fluorescence studies do not provide any evidence for non-replication at these loci. Presumably, not all satellite sequences in polytene nuclei may be subjected to equal underreplication.

Band 81 F in *D. simulans* did not show bright fluorescence, neither with H- nor with QM. In earlier studies Adkisson et al. (1971) reported 81 F of *D. simulans* to be QM-bright, while Ellison and Barr (1971) found this band to be QM-negative. There may be polymorphism in *D. simulans* populations for the 81 F band similar to the already noted polymorphism for the band 83 D in *D. melanogaster* (Barr and Ellison, 1972).

Peacock et al. (1973) and Holmquist (1975a) have suggested that in polytene nuclei of *D. melanogaster,* the non-replicating Y chromosome is located at the base of 2 R. We did not see any H- or QM-bright region at the base of 2 R in male nuclei from *D. melanogaster.* Instead, in many, though not all, male polytene nuclei of *D. melanogaster* we observed a bright region at the base of 3 L. Since we have not seen a similar body in female nuclei in comparable location, we tentatively suggest the bright body seen at the base of 3 L to represent the Y-chromosome. In other species examined by us, we did not see any consistent difference between male and female polytene nuclei.

The H- and QM-fluorescence patterns of chromosomes in the species belonging to the *melanogaster* species group thus reveal that even very closely related species differ considerably in their H- and QM-bright regions, and therefore, in the type and amount of heterochromatin present in their genome. Similar conclusions have been drawn by Peacock et al. (1978), Barnes et al. (1978) and by Lemeunier et al. (1978). The present observations also raise the possibility that the organization of heterochromatin may vary even in different polytene cells of an individual. In this context it may further be noted that the location and size of H-bright regions in the chromocentre do vary depending upon the developmental conditions of the larvae. We have seen (Mishra, 1979 and other unpublished data) that in *D. melanogaster* larvae grown at 10°–12° C, the overall size of the chromocentre is bigger as also is the size of the H-bright bodies in it. In polytene nuclei of *Phryne* also, variations in the morphology of heterochromatin in relation to developmental conditions are known (Wolf and Sokoloff, 1976). Nash and Vyse (1977) have also raised the possibility

of a variable degree of polytenization of the basal heterochromatin of X in *D. melanogaster* polytene nuclei. Similarly, a variable polytenization of telomeric heterochromatin in salivary gland nuclei of *D. melanogaster* has been also suggested (Roberts, 1979). Thus a limited variability in polytenization may be a common property of different heterochromatic regions in *Drosophila* genome.

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References

Adkisson, K.P., Perreault, W.J., Gay, H.: Differential fluorescent staining of Drosophila chromosomes with quinacrine mustard. Chromosoma (Berl.) **34**, 190–205 (1971)

Baimai, V.: Karyotype variation in D. montium. Dros. Inf. Ser. **44**, 115–116 (1969)

Baimai, V.: Cytogenetic studies of certain strains of Drosophila kikkawai. Genetics **74** (Suppl.), 15 (Abstr.) (1974)

Barnes, S.R., Webb, D.A., Dover, G.: The distribution of satellite and main band DNA components in the melanogaster species sub-group of Drosophila. I. Fractionation of DNA in actinomycin D and distamycin A density gradients. Chromosoma (Berl.) **67**, 341–363 (1978)

Barr, H.J., Ellison, J.R.: Quinacrine staining of chromosomes and evolutionary studies in Drosophila. Nature (Lond.) **233**, 190–191 (1971)

Barr, H.J., Ellison, J.R.: Ectopic pairing of chromosome regions containing chemically similar DNA. Chromosoma (Berl.) **39**, 53–61 (1972)

Bock, I.R.: Taxonomy of the Drosophila bipectinata species complex. Univ. Texas Publ. **7103**, 273–280 (1971)

Bock, I.R., Wheeler, M.R.: The Drosophila melanogaster species group. Univ. Texas Publ. **7213**, 1–102 (1972)

Bridges, C.B.: Salivary chromosome maps. J. Hered. **26**, 60–64 (1935)

Clayton, F.E., Wheeler, M.R.: A catalog of Drosophila metaphase chromosome configurations. In: Handbook of genetics (R.C. King, ed.), vol. **3**, pp. 471–512. New York: Plenum Press 1975

Comings, D.E.: Mechanisms of chromosome banding. VIII. Hoechst 33258–DNA interactions. Chromosoma (Berl.) **52**, 229–243 (1975)

Ellison, J.R., Barr, H.J.: Differences in the quinacrine staining of the chromosomes of a pair of sibling species: Drosophila melanogaster and Drosophila simulans. Chromosoma (Berl.) **34**, 424–435 (1971)

Gall, J.G., Cohen, E.H., Polan, M.L.: Repetitive DNA sequences in Drosophila. Chromosoma (Berl.) **33**, 319–344 (1971)

Gatti, M., Pimpinelli, S., Santini, G.: Characterization of Drosophila heterochromatin. I. Staining and decondensation with Hoechst 33,258 and quinacrine. Chromosoma (Berl.) **57**, 351–375 (1976)

Heitz, E.: Über α- und β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei Drosophila. Biol. Zbl. **54**, 588–609 (1934)

Hennig, W.: Highly repetitive DNA sequences in the genome of Drosophila hydei. I. Preferential localization in the X chromosomal heterochromatin. J. molec. Biol. **71**, 407–417 (1972a)

Hennig, W.: Highly repetitive DNA sequences in the genome of Drosophila hydei. II. Occurrence in polytene tissue. J. molec Biol. **71**, 419–431 (1972b)

Hennig, W., Hennig, I., Stein, H.: Repeated sequences in the DNA of Drosophila and their localization in giant chromosomes. Chromosoma (Berl.) **32**, 31–63 (1970)

Holmquist, G.: Hoechst 33258 fluorescent staining of Drosophila chromosomes. Chromosoma (Berl.) **49**, 333–356 (1975a)

Holmquist, G.: Organization and evolution of Drosophila virilis heterochromatin. Nature (Lond.) **257**, 503–506 (1975b)

Jha, A.P., Rahman, S.M.Z.: On crossing between Drosophila bipectinata and Drosophila malerkotliana. Cytologia (Tokyo) **38**, 425–436 (1973)

Jorgenson, K.F., Sande, J.H. van de, Lin, C.C.: The use of base pair specific DNA binding agents as affinity labels for the study of mammalian chromosomes. Chromosoma (Berl.) **68**, 287–302 (1978)

Kumar, M., Lakhotia, S.C.: Localization of non-replicating heterochromatin in polytene cells of Drosophila nasuta by fluorescence microscopy. Chromosoma (Berl.) **59**, 301–305 (1977)

Lakhotia, S.C.: EM autoradiographic studies on polytene nuclei of Drosophila melanogaster. III. Localization of non-replicating chromatin in chromocentre heterochromatin. Chromosoma (Berl.) **46**, 144–159 (1974)

Lakhotia, S.C.: Alpha-heterochromatin in related species of Drosophila. Abstr. Cell Biology Conf. (Jan. 9–11, 1978, Delhi Univ., Delhi), 104 (1978)

Lakhotia, S.C., Kumar, M.: Heterochromatin in mitotic chromosomes of Drosophila nasuta. Cytobios **21**, 79–89 (1978)

Latt, S.A.: Detection of DNA synthesis in interphase nuclei by fluorescence microscopy. J. Cell Biol. **62**, 546–550 (1974)

Lemeunier, F., Dutrillaux, B., Ashburner, M.: Relationships within the melanogaster subgroup species of the genus Drosophila (Sophophora). III. The mitotic chromosomes and quinacrine fluorescent patterns of the polytene chromosomes. Chromosoma (Berl.) **69**, 349–361 (1978)

Mayfield, J.E., Ellsion, J.R.: The organization of interphase chromatin in Drosophilidae. The self-adhesion of chromatin containing the same DNA sequences. Chromosoma (Berl.) **52**, 37–48 (1975)

Mishra, A.: Effect of low temperature on chromocentre organization in polytene nuclei of Drosophila melanogaster. Abstr. IIIrd All India Cell Biology Conf. (Jan. 10–12, 1979, Cancer Research Inst., Bombay) 77 (1979)

Nash, D., Vyse, E.R.: On the polytene morphology of a Drosophila melanogaster chromosomal inversion including the nucleolus organizer. Canad. J. Genet. Cytol., **19**, 637–644 (1977)

Patterson, J.T., Stone, W.S.: Evolution in the genus Drosophila. New York: McMillan (1952)

Peacock, W.J., Brutlag, D., Goldring, E., Appels, R., Hinton, C.W., Lindslay, D.L.: The organization of highly repeated DNA sequences in Drosophila melanogaster chromosomes. Cold Spr. Harb. Symp. quant. Biol. **38**, 405–416 (1973)

Peacock, W.J., Lohe, A.R., Gerlach, W.L., Dunsmuir, P., Dennis, E.S., Appels, R.: Fine structure and evolution of DNA in heterochromatin. Cold Spr. Harb. Symp. quant. Biol., **42**, 1121–1135 (1978)

Pimpinelli, S., Santini, G., Gatti, M.: Characterization of Drosophila heterochromatin. II. C- and N-banding. Chromosoma (Berl.) **57**, 377–386 (1976)

Roberts, P.A.: Rapid change of chromomeric and pairing patterns of polytene chromosome tips in D. melanogaster: migration of polytene – nonpolytene transition zone? Genetics **92**, 861–878 (1979

Rudkin, G.T.: The structure and function of heterochromatin. Genetics Toady (Proc. XI Int. Cong. Genetics, The Hague), vol. **2**, pp. 359–374. London: Pergamon Press 1965

Schmid, M., Vogel, W., Krone, W.: Attraction between centric heterochromatin of human chromosomes. Cytogenet. Cell Genet **15**, 66–80 (1975)

Singh, B.K., Gupta, J.P.: Pattern of chromosomal polymorphism in Drosophila jambulina Parshad and Paika. Jap. J. Genet. **55**, 81–90 (1980)

Vosa, G.: The discriminating fluorescence patterns of the chromosomes of Drosophila melanogaster. Chromosoma (Berl.) **31**, 446–451 (1970)

Wolff, B.E., Sokoloff, S.: Changes in the form of the polytene X-chromosome in Phryne (Sylvicola) cincta – causes and functional significance. Chromosomes today (Proc. Leiden Chromosome Conf., 1974) vol. **5**, 91–108 (1976) New York: John Willey & Sons

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