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CHROMOSOMA

Relationships Within the *Melanogaster* Subgroup Species of the Genus *Drosophila* (*Sophophora*)

III. The Mitotic Chromosomes and Quinacrine Fluorescent Patterns of the Polytene Chromosomes

Françoise Lemeunier[1], Bernard Dutrillaux[2], and Michael Ashburner[3]

[1] Laboratoire de Biologie et Génétique Evolutives C.N.R.S., 91190 Gif-sur-Yvette, France; [2] Institut de Progenèse, 15, rue de l'Ecole de Médecine, 75006 Paris, France; [3] Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH, England

Abstract. Five members of the *melanogaster* species subgroup of the subgenus *Sophophora* have been studied cytologically, their mitotic chromosomes analysed after Giemsa, C-banding and quinacrine staining. In all five species (*D. yakuba, D. teissieri, D. erecta, D. orena and D. mauritiana*) n = 4 and all of the species except *D. orena* have a typical *melanogaster* like mitotic karyotype though there are clear differences between species in the distribution of both C^+ and Q^+ material. *D. orena* has large metacentric X and Y chromosomes due to the accumulation of intensively fluorescing material on these elements with respect to their homologues in *melanogaster*. This extra heterochromatin of *D. orena* correlates with a very high proportion of satellite DNA in its nuclear genome (S. Barnes, unpublished). The polytene chromosomes of these species were studied after quinacrine staining and Q^+ material found to be restricted to the polytene fourth chromosomes, with the exception of *D. orena* which possesses considerable Q^+ material in its chromocentre. These findings are discussed in the light of other studies of karyotype evolution in the genus *Drosophila*.

Introduction

The *melanogaster* species subgroup of the subgenus *Sophophora* is known from a complex of seven morphologically similar species. These species appear to be Afro-Tropical (Crosskey and White, 1977) in origin since five of them are endemic to this zoogeographical region (see Tsacas and Lachaise, 1974 and Tsacas and David, 1978 for a discussion of the problem of the origin of the subgroup). The remaining two species, that is *D. melanogaster* and *D. simulans*, are more or less cosmopolitan in their distribution (Bock and Wheeler, 1972) and are remarkably synanthropic in their associations (Povolny, 1971). In a previous paper (Lemeunier and Ashburner, 1976) we compared the polytene chromosome banding patterns of six species of the *melanogaster* species subgroup (the seventh species, *D. orena*, then not having been discovered) and, on the basis of this information constructed a phylogenetic "tree" indicating their inter-relationships. Study of the polytene chromosomes only allows the analysis

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of part of the genome since, in *Drosophila* at least, major fractions of the genome are proportionately underreplicated during the process of polytenisation (Spear, 1977 for review). The underreplicated parts of the genome include those characterised biochemically as satellite DNAs and cytologically as heterochromatin. In order to study differences in the heterochromatin between species it is necessary to study not polytene but diploid chromosomes. There are several examples within the genus *Drosophila* of species with similar polytene chromosome karyotypes yet with different mitotic karyotypes due to differences in heterochromatic regions (e.g. Clayton, 1969; Ward and Heed, 1970). Indeed even within species polymorphisms for heterochromatic regions, not detectable in the polytene nuclei, are known (e.g., in *D. kikkawai* (Kikkawa, 1936; Baimai, 1974), in *D. disjuncta* (Baimai, 1975) and in *D. recticilia* (Baimai, 1977).

In this paper[1] we discuss the results of a study of the mitotic chromosomes of the seven *melanogaster* species subgroup members and of their polytene chromosomes after quinacrine staining.

Materials and Methods

Strains. The species studied, and the particular strains used, are listed in Table 1. With the exception of *D. orena* the polytene chromosomes of the same set of strains were analysed by Lemeunier and Ashburner (1976). *D. orena* has recently been discovered in West Africa (Tsacas and David, 1978) and a detailed description of its polytene chromosome banding pattern will be published elsewhere (Lemeunier and Ashburner, in preparation). Suffice it to say here that the banding patterns of the polytene chromosomes of *D. orena* place this species close to *D. erecta* – the polytene chromosomes of these two species differ only by a few paracentric inversions.

Table 1. The species and strains used in this study

Species	Strain[a]	Origin
D. erecta	154.1	Lamto, Ivory Coast
D. mauritiana	163.2	Chaland, Mauritius
D. orena	188.1	Bafut Ngemba, Cameroun
D. teissieri	128.2	Mt. Selinda, Zimbabwe
D. yakuba	115	Kounden, Cameroun

[a] Strain numbers of the Gif stock collection

Chromosome Preparations. Mitotic metaphase or late prophase chromosomes were prepared from the brains of third instar larvae. After dissection in a Ringer's solution the tissue was treated with colchicine (at 1 mg per ml) for 1 h and then treated hypotonically with 1 vol. human serum diluted with 2 vol. distilled water. The chromosomes were fixed by sequential exposure to ethanol-chloroform-glacial acetic acid (6:3:1, v/v/v, 30 min) and ethanol:glacial acetic acid (3:1, v/v, 30 min). The chromosomes were stained with 3% Giemsa, with quinacrine mustard (after Caspersson et al., 1968), C-banded by the method or Summer (1972) or R-banded after Dutrillaux (1975).

Results

1. Mitotic Chromosomes

a) D. yakuba. The mitotic karyotype of *D. yakuba* (Fig. 1) consists of two pairs of long metacentric chromosomes, a pair of small dots chromosomes,

[1] Part II of this series is Lemeunier and Ashburner (1976)

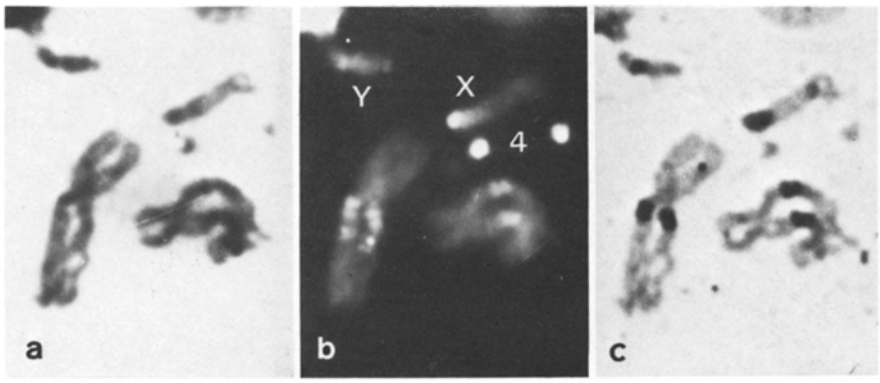

Fig. 1a–c. Mitotic chromosomes of *D. yakuba* male stained with Giemsa (**a**), quinacrine mustard (**b**) and C-banding (**c**)

an acrocentric X chromosome and a submetacentric Y chromosome. The Y chromosome is somewhat smaller than the X. All of the chromosomes, except the fourths, show darkly stained regions after C-banding (that is to say are C^+). On the X chromosome there is one dark C^+ region at the proximal end and another, fainter one, near to the distal end of the chromosome. The Y chromosome also has two C^+ regions, a strong one around the centromere and a faint one distal on the short arm. Each of the metacentric autosomes have C^+ regions adjacent to their centromeres.

After quinacrine staining the pattern of fluorescence is similar, though by no means identical, to that of the C^+ bands. Only the proximal X is quinacrine fluorescent (i.e. Q^+), indeed the proximal X appears to have a very bright region coincident with its C^+ region and a fainter Q^+ region extending distally. The entire long arm of the Y chromosome is faintly Q^+ though two regions of greater fluorescent intensity are seen in this arm. The fourth chromosomes, which are only faintly stained after C-banding, are very intense in their fluorescence after quinacrine staining. Each pair of metacentric autosomes have Q^+ regions located approximately medially. On one metacentric pair there are two Q^+ bands, one on each side of the centromere. On the other pair there are four bands, three on one arm and one on the other.

b) D. teissieri. Except for its Y chromosome the mitotic karyotype of *D. teissieri* (Fig. 2) is similar to that of *D. yakuba*. In the former species the Y chromosome is acrocentric and about equal in length to the X chromosome.

The C-banded mitotic metaphase chromosomes of *D. teissieri* show strong C^+ blocks adjacent to the centromeres of all chromosomes except the fourth pair. The Y chromosome stains strongly after C-banding with three major C^+ blocks.

Neither the X nor the Y chromosomes show any very brightly fluorescent regions after quinacrine staining, although the C^+ regions of both of these chromosomes are weakly fluorescent. The fourth chromosomes are moderately fluorescent. The metacentric autosomes show Q^+ bands near their centromeres.

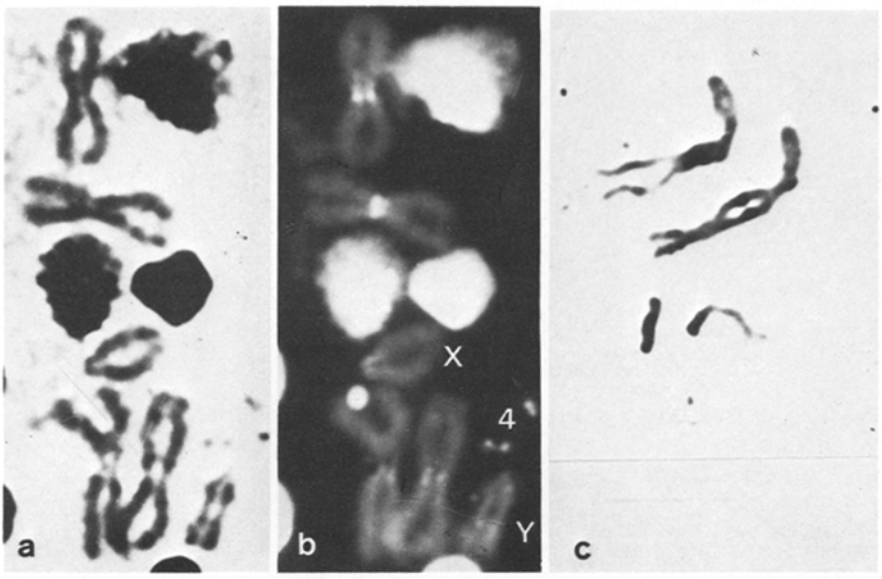

Fig. 2a–c. Mitotic chromosomes of *D. teissieri* male stained with Giemsa (**a**), quinacrine mustard (**b**) and C-banding (**c**)

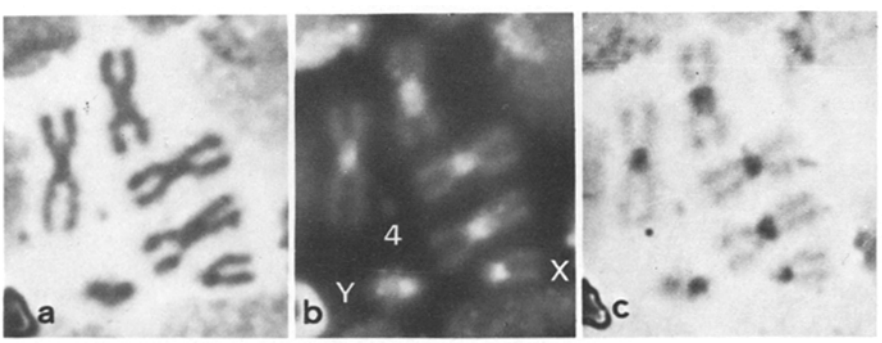

Fig. 3a–c. Mitotic chromosomes of *D. erecta* male stained with Giemsa (**a**), quinacrine mustard (**b**) and C-banding (**c**)

In the case of one metacentric pair the fluorescence is relatively weak with one band on either side of the centromere. The other metacentric pair has much brighter Q^+ bands, with one very intense band on one arm and a weaker one on the other.

c) D. erecta. The mitotic chromosomes of this species (Fig. 3), like those of the two previously discussed, conform to the general *melanogaster* pattern except that the Y chromosome is acrocentric and similar in size to the X chromosome. The strain studied was frequently trisomic for chromosome 4. After C-banding

all of the chromosomes, except chromosome 4, show C^+ blocks in their centromeric regions. In addition much of the Y chromosome is moderately stained.

Q-banding shows the fourth chromosome pair to be Q^-, the X and Y chromosomes to have Q^+ bands coincident with their C^+ regions and the two metacentric autosome pairs also to have Q^+ centromeric regions. One metacentric autosome pair has a second, though weak, Q^+ band on one arm.

d) D. orena. The mitotic metaphase karyotype of *D. orena* (Fig. 4) is strikingly different from those of other members of this complex of species. There are three large pairs of metacentric chromosomes, instead of the typical two, in both males and females. That is to say both the X and the Y chromosomes are metacentric elements approximately equal in size to the major autosomes.

The centromeric regions of all chromosomes (including the fourths) are C^+. In addition one entire arm of the X chromosome is C^+, but only strongly so adjacent to its centromere.

The fluorescence pattern of the mitotic chromosomes of *D. orena* after staining with quinacrine is remarkable. The entire metacentric Y chromosome fluoresces very intensively as does one arm of the X chromosome. The Q^+ arm of the X is slightly longer than the Q^- arm and is the arm which is also C^+. One metacentric autosome pair shows four large, very bright, Q^+ blocks three of which are on one chromosome arm and the fourth on the other arm (that is to say similar to the situation seen for one metacentric autosome pair of *D. yakuba* though the Q^+ blocks of the *D. orena* autosome are brighter and proportionately larger). The C^+ region of this autosome lies between the Q^+ regions, i.e., is Q^-. The other metacentric autosome pair has a long, apparently continuous, Q^+ region extending either side of its centromere. The intensity of fluorescence of this region is considerably less than that of the Q^+ blocks of its heterologues. The chromosomes 4 of *D. orena*, which are short rods rather than dots, are moderately fluorescent after quinacrine staining.

The pattern of fluorescence after R-banding (Fig. 4f) is seen to be the reverse of that after Q-staining. The Y chromosome and the longer arm of the X are hardly fluorescent whilst the shorter, euchromatic, arm of the X is brightly so. The centromeric regions of the major autosomes and the entire fourth chromosomes fluoresce very weakly whilst the distal autosome arms do so strongly.

e) D. mauritiana. The mitotic chromosomes (Fig. 5) of this very close relative to *D. simulans* are typical of those of the subgroup. The X and Y chromosomes are approximately similar in size, the former chromosome is acrocentric and the latter is subacrocentric. Each of the metacentric autosomes have strong C^+ regions in their centromeric regions: on one pair this C^+ region block appears to be continuous across the centromere and on the other pair it is present as two discrete blocks, one on either arm. The X chromosome has a strong C^+ region proximally and the Y chromosome is rather weakly stained after C-banding though three bands, one on Y^S and two on Y^L can be seen. The fourth chromosome pair is C^-.

The quinacrine fluorescent pattern of the chromosomes of *D. mauritiana* is very simple. Only the Y chromosome and the fourths are brightly fluorescent.

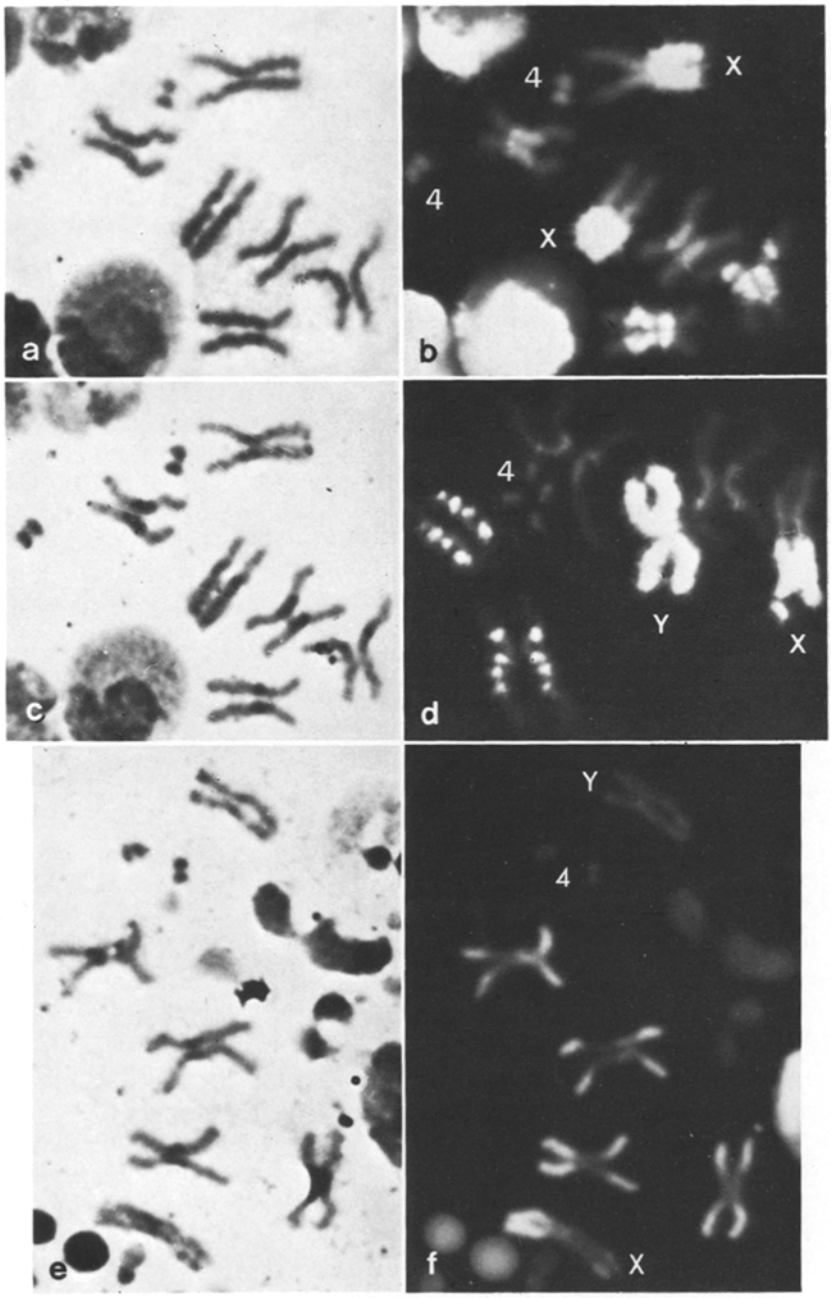

Fig. 4a–c. Mitotic chromosomes of *D. orena* female stained with Giemsa (**a**), quinacrine mustard (**b**) and C-banding (**c**). **d–f** Mitotic chromosomes of *D. orena* male stained with quinacrine mustard (**d**), C-banding (**e**) and R-banding (**f**)

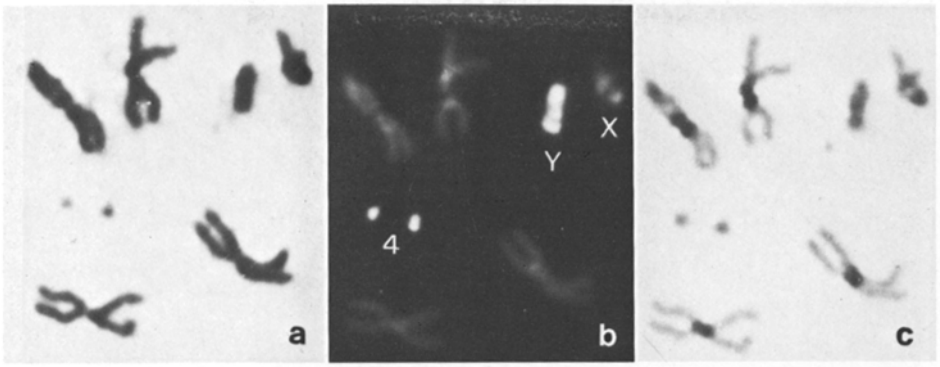

Fig. 5a–c. Mitotic chromosomes of *D. mauritiana* male stained with Giemsa (**a**), quinacrine mustard (**b**) and C-banding (**c**)

Three Q^+ bands are clear even on the condensed metaphase Y chromosome, one on Y^S and two in the C^- region of Y^L. The metacentric autosome pairs are essentially Q^- though a pair of faintly fluorescent bands can be seen, one on each arm, of one chromosome pair.

2. Polytene Chromosomes

The polytene chromosomes of these five species have been stained with quinacrine and the patterns of fluorescence observed are illustrated in Figure 6. In all species the Q^+ regions are limited to the chromocentre and the fourth chromosomes.

Except for a few bright spots the chromocentres of the polytene nuclei in *D. yakuba, D. mauritiana, D. teissieri* and *D. erecta* are non, or only weakly, fluorescent. In the case of *D. orena,* however, there are very large intensively fluorescent granules and "blobs" in the chromocentre standing out against a weakly fluorescent background.

The banding patterns of the polytene fourth chromosomes of these species have not been homologized with those of either *D. melanogaster* or *D. simulans.* It is not possible, therefore, to give the locations of the fluorescent bands seen in the fourth chromosomes of this species in terms of the standard polytene chromosome maps. Nevertheless in all species there is one, or more, intensively fluorescent band on the polytene fourth chromosome. In three of the species, *D. mauritiana, D. yakuba* and *D. teissieri,* there is a very bright Q^+ region right at the base of the polytene fourth and in *D. erecta* there is a similar block located subproximaly. In *D. mauritiana* (in particular) and *D. orena* the tip of the polytene fourth chromosome is Q^+, this region being a discrete band in the former species but broken up into Q^+ dots in the latter. All species except *D. mauritiana* also show, in addition to these very bright Q^+ blocks, more weakly fluorescing bands within the body of the chromosome.

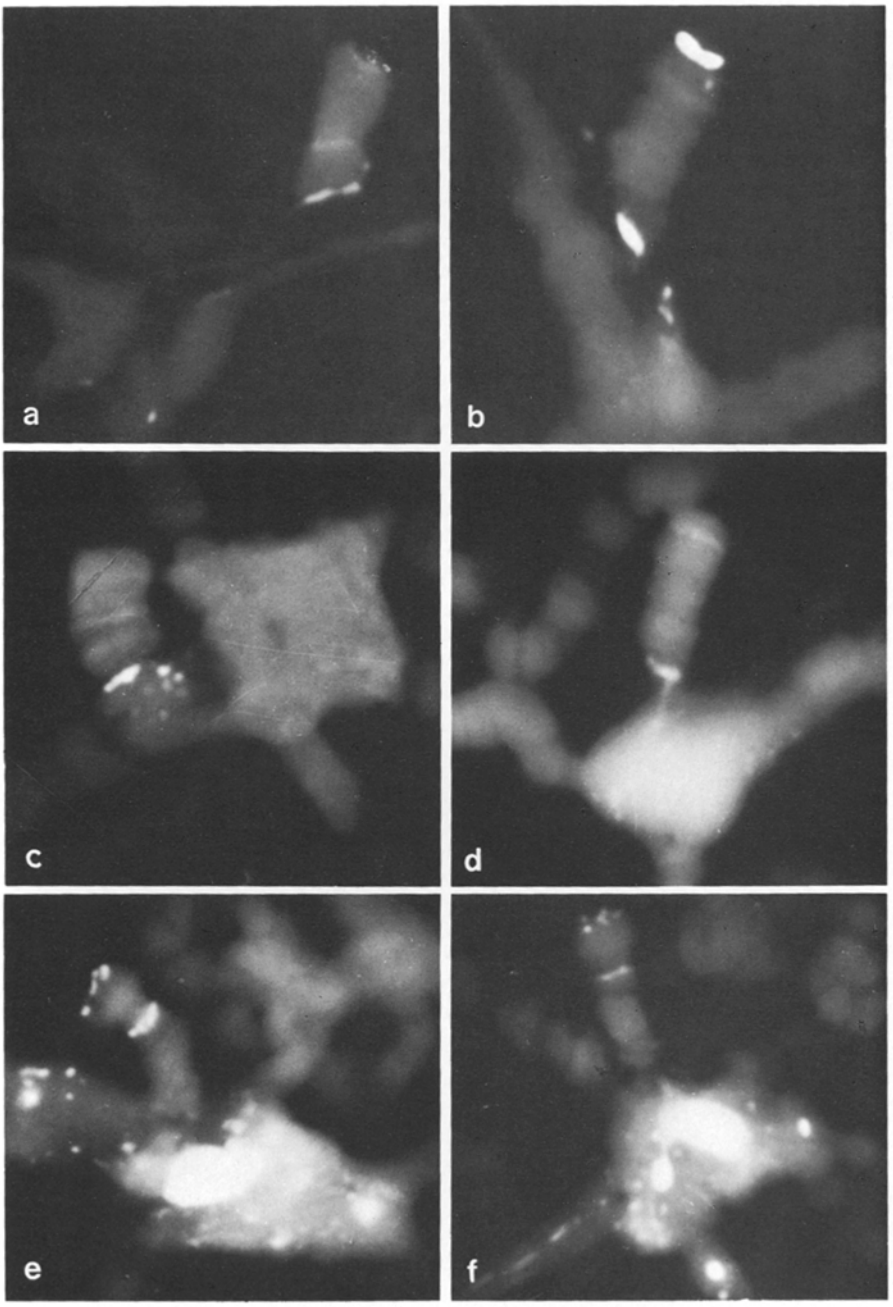

Fig. 6a–f. Quinacrine mustard stained polytene chromosomes of **a** *D. yakuba*, **b** *D. mauritiana*, **c** *D. erecta*, **d** *D. teissieri* and **e, f** *D. orena*

Discussion

The metaphase karyotypes of well over 500 species of the genus *Drosophila* have been described (Clayton and Wheeler, 1975). In over half of these species the haploid chromosome number is six, there typically being five acrocentric chromosomes and one small dot like chromosome (5R, 1D). Within the subgenus *Sophophora* only 3% or so of the studied species retain this "primitive" karyotype, most species having undergone chromosome "fusion" during their evolution so that the modal basic chromosome number of this subgenus is three. In the *melanogaster* species subgroup all known species have n=4 only elements A (the X chromosome) and F (chromosome 4) remaining free. Karyotype variation within this subgroup is restricted to variation in the amount and distribution of heterochromatin.

There are four major classes of mitotic chromosome karyotype within the *melanogaster* species group as a whole. Only the monotypic *elegans* subgroup has retained all of its chromosomes as acrocentrics. Even in this case the absence of a dot chromosome presumably indicates a fusion of element F with some other chromosome. The second and third classes of karyotype are similar, that is to say the 2V, 1R, 1D karyotype characteristic of *melanogaster* and the 2V, 1R karyotype of other species subgroups. The fact that the 2V, 1R karyotype is found in the *montium, suzuki,* and *takahashii* species subgroups might indicate that the fusion of element F with another chromosome needed to derive this configuration from 2V, 1R, 1D has occurred on several independent occasions during evolution. The final class of karyotype, that which characterises the *ananassae* species subgroup, has 4V chromosomes. The genetic evidence indicates that the fusion of elements to produce metacentric from acrocentric chromosomes has been similar in both the *melanogaster* and *ananassae* type of karyotypes. That is to say chromosome 2 of *D. melanogaster* represents a B.C fusion and chromosome 3 a D.E. fusion; in *D. ananassae* chromosome 2 is a D.E fusion and chromosome 3 a B.C fusion (Sturtevant and Novitski, 1941). Whether or not these represent events ancestral to the evolution of the separate species subgroups or are examples of karyotypic orthoselection (White, 1975) is not known.

The basic karyotypes, of course, only reveal part of the history of the evolution of the chromosomes of this species group. There is, for example, clear genetic evidence that in *D. ananassae* a translocation between elements A and F has occured so that the nucleolus organizer, and its genetic marker bobbed, is absent from the X chromosome but, instead, is linked to chromosome 4 (Kikkawa, 1938).

It is worth comparing the evolution of the metaphase karyotypes of the *melanogaster* species group with that of the Hawaiian *Drosophila.* In the endemic Hawaiian fauna the metaphase and polytene karyotypes of over 101 species have been described (Carson and Kaneshiro, 1976, for summary and recent data). In 92 of these species the metaphase karyotypes are very similar, that is 5R, 1D. In the remaining nine species addition of heterochromatin to the dot chromosome (chromosome 6) has resulted in a 6R metaphase karyotype in six species and the other three species have one or more metacentric chromo-

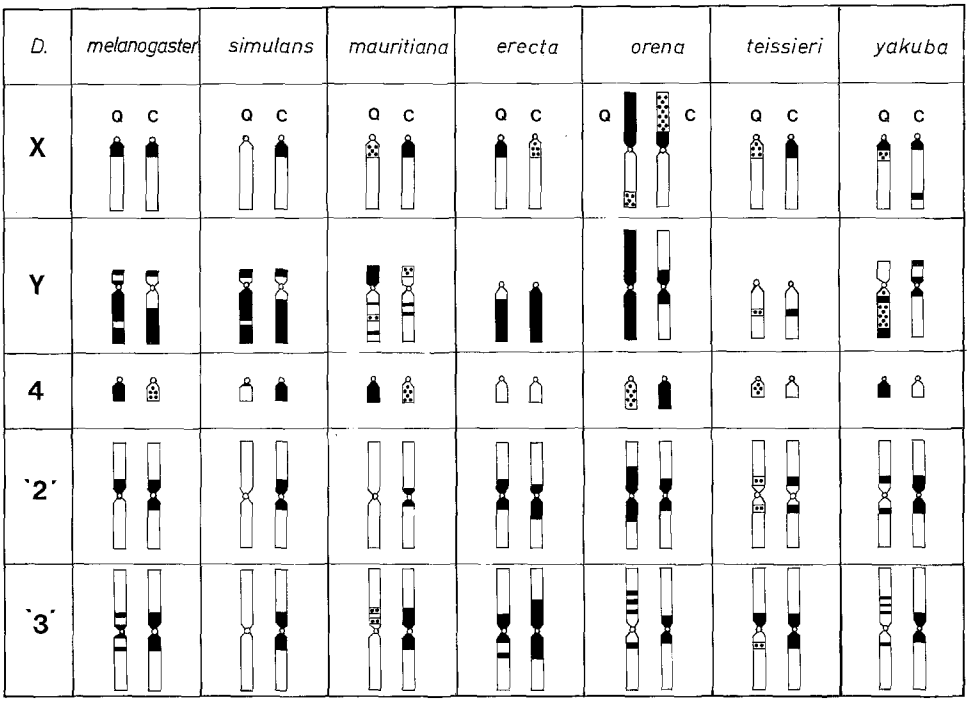

Fig. 7. Diagrammatic summary of the metaphase chromosomes of the *melanogaster* species subgroup members after quinacrine mustard staining and C-banding. Data for *D. melanogaster* and *D. simulans* after Vosa (1970); Adkisson et al., (1971); Ellison and Barr (1971), Holmquist (1975); Gatti et al. (1976) and Pimpinelli et al. (1976). ☐ no staining, ▒ medium staining, ■ intense staining

somes due to the addition of heterochromatin to one (*D. melanocephala* and *D. prostopalpis*) or all (*D. cyrtoloma*) of the rods. Thus the large Hawaiian fauna is characterised by great karyotypic stability. In the *melanogaster* species group, on the other hand, considerable karyotypic divergence has accompanied species speciation, even within the *melanogaster* subgroup one of the seven species has a clearly novel karyotype.

A summary of the mitotic karyotypes of the seven *melanogaster* subgroup species in shown in Figure 7. The karyotypes of *D. melanogaster* and *D. simulans* are taken from the literature (cited in the figure legend). The most remarkable discovery is the massive addition of heterochromatin to the X and Y chromosomes of *D. orena*. This recalls the evolution of the X chromosome in the *hydei* species subgroup, from the acrocentric X of *D. eohydei* to the metacentric X, with one arm completely heterochromatic, of *D. hydei* itself (Wasserman, 1962). At least at the resolution of the metaphase chromosomes all of this "extra" heterochromatin appears to be brightly fluorescent. The amount of Q^+ material on the autosomes of *D. orena* is also greater, relatively speaking, than in the other six species. Indeed a comparison of the fluorescent staining patterns of all seven species indicates that changes in the amount of Q^+ material do not occur on one pair of homologues alone but, rather, coordinately throughout the karyotype.

The differences in the amounts of C^+ and Q^+ material of the mitotic chromosomes of the seven species are not reflected in their polytene karyotypes. Thus even *D. orena* has only five long and one short arms radiating from the chromocentre of polytene nuclei. After staining with quinacrine, however, differences between the species are seen in polytene nuclei, in particular the massive accumulation of Q^+ material in the chromocentre of *D. orena*.

It is probable that the Q^+ material of the chromocentres of the polytene nuclei represents the Q^+ heterochromatin of the mitotic chromosomes that has failed to replicate during the process of polytenization. This cannot be the whole story, however, since the intensively Q^+ regions of the polytene chromosome 4 occur in typically banded regions. It is noticeable that there is little correlation between the intensity of fluorescence after quinacrine staining of the polytene and mitotic fourth chromosomes, compare, for example, the fourth chromosomes of *D. yakuba* and *D. erecta*.

The Q^+ blocks of the polytene fourth chromosomes, with the possible exceptions of those as at the bases of the chromosomes, are not at constrictions. If underreplication of DNA sequences during polytenization results in constrictions (i.e. weak points), as suggested by Ellison and Barr (1971), Barr and Ellison (1972) then perhaps the Q^+ regions of the polytene fourths, unlike those of the centric heterochromatin of other chromosomes and of the Y chromosome, are not underreplicated.

The extent to which the Q^+ regions of the mitotic chromosomes can be equated with satellite DNA sequences is, as yet, uncertain. Certainly a high AT content is a necessary, but perhaps not sufficient, basis for quinacrine fluorescence (see Holmquist, 1975 and Gatti et al., 1976). Recent studies of the satellite DNAs of the seven members of the *melanogaster* species subgroup (Barnes et al., 1978 and S. Barnes, personal communication) show a correspondence between the proportion of nuclear DNA that is satellite and the amount of Q^+ material in the mitotic chromosomes. Thus in *D. orena* about 30% of the nuclear DNA is satellite (in adult flies), a figure to be compared with one of 6% for *D. simulans*. The precise relationship between the different types of heterochromatin that can be identified after C and Q-banding and the different satellite DNAs identifiable on caesium chloride gradients in these species will require study by in situ hybridization of purified satellite DNAs to the mitotic chromosomes.

The most important theoretical question which is raised by the type of study we have done is, of course, to what extent the karyotype differences seen between species played a causative role in speciation and to what extent they arose subsequent to, or as a consequence of, speciation (White, 1978). With respect to the changes in distribution and amount of heterochromatin we surely require a far more detailed knowledge of the molecular basis of the different types of heterochromatin and of the mechanisms that lead to changes in the abundance of different satellite DNA families before we can answer this question. The *melanogaster* species subgroup should provide ideal material for study since there is the possibility for an experimental, rather than solely an historical, analysis with *D. melanogaster* itself.

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