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Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (*Sophophora*)

II. Phylogenetic relationships between six species based upon polytene chromosome banding sequences

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[Plates 40–45]

The *melanogaster* species subgroup of *Drosophila* comprises six sibling species. The interrelationship between these species has been studied by analysis of the banding patterns of their polytene chromosomes. The species fall into two groups: (1) *melanogaster*, *simulans* and *mauritiana* and (2) *erecta*, *teissieri* and *yakuba*. The former group are chromosomally closely related, indeed *simulans* and *mauritiana* are homosequential. The latter group (all African endemic species) are less closely related although they all share eight autosomal inversions of the standard (i.e. *melanogaster*) sequence. From this shared sequence the chromosomes of the three African endemic species have diverged considerably by many paracentric inversions.

Both *D. teissieri* and *D. yakuba* are polymorphic; we describe nine and four inversion sequences in them respectively. *D. erecta* is monomorphic although our sample size is very small (only two populations).

We discuss both the origin of interspecific inversions, especially the problem of inversion breakpoint coincidence, and the light this study throws upon evolutionary relationships within this group of species.

Introduction

'*Drosophila simulans* is a species of unusual interest to the geneticist', noted Sturtevant (1929) 'since it is the one form that can be crossed with *D. melanogaster* ...'. Until comparatively recently this remained true. Extensive collecting of *Drosophila* in Africa in the last few years has, however, added greatly to our knowledge of those species closely related to *D. melanogaster*. Within the last three years three new species have been described. Together with a species

described some twenty years ago the number of members of the *melanogaster* species subgroup (Hsu 1949) is now six. *Drosophila melanogaster* and *D. simulans* have been considered a classic example of sibling species and the four new species must similarly be so considered with them. Females of the six species are difficult to distinguish with reliability while the separation of males must rely upon the characteristics of their genitalia.

In this paper we give an account of the banding patterns of the salivary gland polytene chromosomes of the six members of the *melanogaster* species subgroup and, using well-established arguments, construct a 'family tree' of the group. Like all, or most (Carson 1973), such studies we can offer no evidence, from these data alone, of the direction of evolution within the group. That is to say the cytological evidence is quite neutral with respect to the question of which of these six species is the most 'primitive'. For the sake of convenience alone we discuss the phylogeny of the group as if it had evolved from *D. melanogaster*.

The species

The subgenus *Sophophora* is divided into seven species groups: *willistoni*, *saltans* (both Neotropical), *obscura* (predominantly Holarctic), *populi* (Nearctic) *melanogaster* (predominantly Oriental), *mommai* (Oriental) and *firma* (Ethiopian). The *melanogaster* species group has recently been revised by Bock & Wheeler (1972) who recognized 75 species. Of these 48 were endemic to Southeast Asia. These 75 species have been divided between 12 species subgroups of which the *melanogaster* subgroup is one. Formal descriptions of the *melanogaster* species group and *melanogaster* species subgroup will be found in the paper of Bock & Wheeler cited above. Although *D. melanogaster* is familiar to geneticists and many other biologists the other species may not be and we will provide thumb-nail sketches of them.

(1) *D. melanogaster* Meigen (1830). Described from Europe. A cosmopolitan *Drosophila* though perhaps absent from, and certainly rare in, Southeast Asia. Often associated with man over much of its range yet not a domestic species in, for example, parts of West Africa (Lachaise 1974).

(2) *D. simulans* Sturtevant (1929). First recognized in the United States of America as strains of '*D. melanogaster*' giving unisexual progenies in some crosses. Shown to be a distinct species by Sturtevant who made an extensive comparison of its genetics with those of *D. melanogaster* (summarized in Sturtevant 1929). Also cosmopolitan in its distribution yet absent from Southeast Asia and the Indian subcontinent. Often a domestic species but has been found remote from human habitation (see, for example, Dobzhansky & Pavan 1950).

(3) *D. yakuba* Burla (1954). Discovered in West Africa and now known to be distributed widely in that continent south of the Sahara. Found in similar habitats as the two former species but also, at least in West Africa, in the savanna (Lachaise 1971, 1974). See Lemeunier (1971).

(4) *D. teissieri* Tsacas 1971. A species known from central and western Africa. A species of the gallery forest and fringing savanna in West Africa (Lachaise 1974).

(5) *D. erecta* Tsacas and Lachaise 1974. Known only from the Ivory Coast and Congo in West Africa. This is ecologically an interesting species since it is associated almost exclusively with the fruits of the tree *Pandanus candelabrum* Beauv (Pandanacea) (Lachaise & Tsacas 1974). This is 'species 5' of Bock & Wheeler (1972), p. 12.

(6) *D. mauritiana* Tsacas and David (1974). Discovered on the island of Mauritius by Dr Jean David where it apparently replaces both *D. simulans* and *D. melanogaster*. On Reunion, 160 km distant from Mauritius, both of the cosmopolitan species are present (Tsacas & David 1974; David & Tsacas 1975).

The question of the degree of reproductive isolation between these species will be considered in detail in further publications from the Gif laboratory. Suffice it to say at this stage that *D. mauritiana* behaves in crosses with *D. melanogaster* just like *D. simulans* – that is to say, sterile hybrids, predominantly of the sex of the *melanogaster* parent, are obtained. With *D. simulans*, *D. mauritiana* gives fertile female hybrids in each of the reciprocal crosses and either no males (with *mauritiana* as the female parent) or sterile males (with *simulans* as the female parent) (David, Lemeunier, Tsacas & Bocquet 1974). With the remaining three species, hybrids are either only obtained with great difficulty or not at all.

Materials and methods

Table 1 lists the strains, and their origins, we have used in the present study. With the exception of those noted they were all founded by several wild caught flies and have since been maintained in the laboratory by mass culture. All species grow well at 25 °C on either standard *Drosophila* medium or yeast–glucose medium.

For cytological analysis larvae were grown, at 25 °C, on yeast–glucose medium. Third instar larvae or 'prepupae' were dissected in a *Drosophila* Ringer-type solution and their salivary glands fixed in propionic acid : ethanol (1 : 3). Temporary squash preparations of salivary gland polytene chromosomes were prepared after staining in propionic orcein-carmine.

Most of the chromosome analysis has been done from photographs of the polytene chromosomes. Except for *D. simulans* and *D. mauritiana* the species hybrids have been of little value (see Results). Our practice has been to assemble a large number of photographs of each chromosome region stretched to different degrees and in different stages of the puffing cycles. Using transparent overlays the break points of the chromosomes, with respect to the *D. melanogaster* sequence, have been plotted on a standard series of photographs of the *D. melanogaster* chromosomes. The evaluation of break points, and in certain cases of sequences, is not as objective as one would wish. In the absence of hybrids with fully synapsed chromosomes we are, however, left with no choice but to use this technique. Although we cannot claim that all our break point determinations are accurate we are of the opinion that the conclusions of this study will not be seriously affected by subsequent revision.

TABLE 1. LIST OF STRAINS STUDIED

(The strain numbers refer to the Gif collection.)

species and strain number	collected at	collected by	collection date	notes
melanogaster				
Canton-S	laboratory wild type (Oak Ridge National Laboratory)	.	.	.
simulans				
Berkeley wild type	Department of Zoology, University of California, Berkeley: unknown provenance	.	.	.
mauritiana				
163.1	Riviere Noire, Mauritius	J. David	8/73	type culture
163.2	Chaland, Mauritius	J. David	8/73	.
163.3	Chaland, Mauritius	J. David	8/73	.
erecta				
154.1	Lamto, Ivory Coast	L. Tsacas	6/71	type culture
160.5	Boko, Congo	J. Vouidibio	4/73	.
teissieri				
128.2	Mt Selinda, Rhodesia	H. Patterson	1/70	type culture
131.3	Ipassa, Gabon	J. David	7/70	.
140.5	Lamto, Ivory Coast	L. Tsacas	10/70	.
144.4	Ozom, Cameroun	L. Tsacas	10/70	.
145.1	Zoatoupsi, Cameroun	L. Tsacas	11/70	.
165.1	Mt Nimba, Ivory Coast	D. Lachaise	8/73	.
165.3	Mt Nimba, Ivory Coast	D. Lachaise	8/73	.
yakuba				
115	Kounden, West Cameroun	L. Tsacas	10/67	single female line
131.6	Ipassa, Gabon	J. David	7/70	.
140.2	Lamto, Ivory Coast	L. Tsacas	10/70	.
140.7	Lamto, Ivory Coast	L. Tsacas	10/70	.
141.6	Banco, Abidjan, Ivory Coast	L. Tsacas	11/70	.
142.6	Lamto, Ivory Coast	L. Tsacas	11/70	.
143.3	N'Kolbisson, Cameroun	L. Tsacas	11/70	.
147.2	N'Koemvone, Cameroun	L. Tsacas	11/70	.
160.3	Madibou, Congo	J. Vouidibio	4/73	.
162.1	Kampala, Uganda	A. Tallantire	/73	.
162.2	Kampala, Uganda	A. Tallantire	/73	.
162.3	Kampala, Uganda	A. Tallantire	/73	.
165.4	Mt Nimba, Ivory Coast	D. Lachaise	8/73	.
165.10	Mt Nimba, Ivory Coast	D. Lachaise	8/73	.
168.2	Lamto, Ivory Coast	D. Lachaise	10/73	single female line
172.3	Limbe, Malawi	H.R. Feijen	6/73	.
172.4	Limbe, Malawi	H.R. Feijen	6/73	.

All break points are given with respect to the standard map of the polytene chromosomes of *D. melanogaster* published by Bridges (1935). Only in a few instances have we used the revised maps. All inversions and sequences are given with the *D. melanogaster* sequence as the standard. Merely for convenience, and to avoid excessive circumlocution, we will treat sequences as if they had evolved

from the *melanogaster* sequence. In fact, of course, any of the sequences we have found, or predicted as intermediates, may have been the historical precursor of the other sequences.

Each inversion is given a lower case letter following the designation of the chromosome arm(s) involved. The alphabetical order of such letters is quite arbitrary. A sequence, either extant or hypothetical, is defined by the inversion events required to derive it from the *melanogaster* standard. Their order will be indicated by the order of lower case letters following the chromosome arm symbol. When the relative order of occurrence of two or more inversion events cannot be determined (i.e. in the case of included or independent inversions) the inversion symbols will be bracketed. When a single inversion symbol is bracketed then that inversion could have occurred at any time, relative to the other inversion events, during the evolution of a sequence. For example, sequence (2LRa, 2Lb)g-(jk)l(h) is to be derived from the *melanogaster* 2L by the pericentric inversion 2LRa, the paracentric 2Lb (in either order), then inversion 2Lg then inversions 2Lj and 2Lk (in either order), then 2Ll; inversion 2Lh could have occurred at any time relative to the occurrence of the other six inversion events.

In the diagrams of the sequences we underline in solid the sequence we denote as standard for any one species. This is not necessarily the most 'primitive' sequence but is always the most widespread sequence among the material available to us. Polymorphic sequences are underlined with broken lines in these diagrams.

Polymorphic inversions found within species are given the symbol of the inversion, or inversions, which are necessary to derive them from the standard sequence of that species. For example, (2LRa, 2Lb)g(jk)l(h)n is polymorphic in *D. yakuba*; the standard 2L of *D. yakuba* is (2LRa, 2Lb)g(jk)l(h) and the inversion is simply called 2Ln. When a polymorphic inversion is more primitive (i.e. nearer to *melanogaster*) than our standard for a species we use the minus sign superscript, e.g. the polymorphic (2LRa, 2Lb)g(jk)(h) sequence of *D. yakuba* is called simply 2l^{-}.

Results

Mitotic chromosomes

We have studied mitotic metaphase figures from larval brains for each species. The basic chromosome complement of all species is similar to that well known for *D. melanogaster*. That is to say in females there are two rods, two large metacentric pairs and a pair of dots while in males one of the rods is replaced by a J-shaped element.

Polytene chromosomes

Polytene chromosomes in species hybrids

Within the trio of species *D. melanogaster*, *D. simulans* and *D. mauritiana* we have readily obtained hybrids and their polytene chromosomes show quite high degrees of synapsis of homologues. This reflects, we presume, the close similarity

in the sequences of these homologues (see below). We have also examined hybrids between *D. mauritiana* and *D. yakuba*, *D. erecta* and *D. teissieri*. The polytene chromosomes of these hybrids are almost entirely asynapsed and are, therefore, almost useless for analysis of banding homologies.

Polytene chromosomes in the species in terms of the D. melanogaster *standard*

The X chromosome (figure 1)†

D. simulans and *D. mauritiana*. As noted by Horton (1939) the X chromosome of *D. simulans* differs from that of *D. melanogaster* by two small inversions (1E1.2; 1E3 and 3A1.2; 3A5) and at the chromosome tip. Except for the chromosome tip the X of *D. mauritiana* is identical in its banding sequence to that of *D. simulans*.

D. erecta, *D. teissieri* and *D. yakuba*. The X chromosomes of these three species have been very difficult to analyse. For this reason we will not give any detailed X chromosome phylogeny for them. Tentatively we propose the following sequences

erecta: 1A–5C/11B–7D/14DF–19A/14DF–11B/5C–7D/19A–20. (Xe)

teissieri: 1A–2B/6D–2B/11A–12D/18D.19A–12D/11A–9B/6D–9B/18D.19A–20. (Xt)

yakuba: 1A–2B/6D–2B/11A–12A/8A–6D/9B–11A/12D–18D.19A/12D–12A/8A–9B/18D.19A–20. (Xt, a)

It is clear that the *teissieri* and *yakuba* X chromosomes are closely related, apparently differing by a single inversion (8A; 12A: Xa), and are quite different from the X chromosome of *D. erecta*.

Chromosome 2

D. simulans and *D. mauritiana*. The sequence on the left arm of chromosome 2 in each of these species is the same as in *D. melanogaster*. On the right arm both species are homozygous for a small basal inversion (42D4.5; 42E2.3) first described by Horton (1939) in the former species (figure 7). We are unable to say whether or not this inversion is present in the other three species.

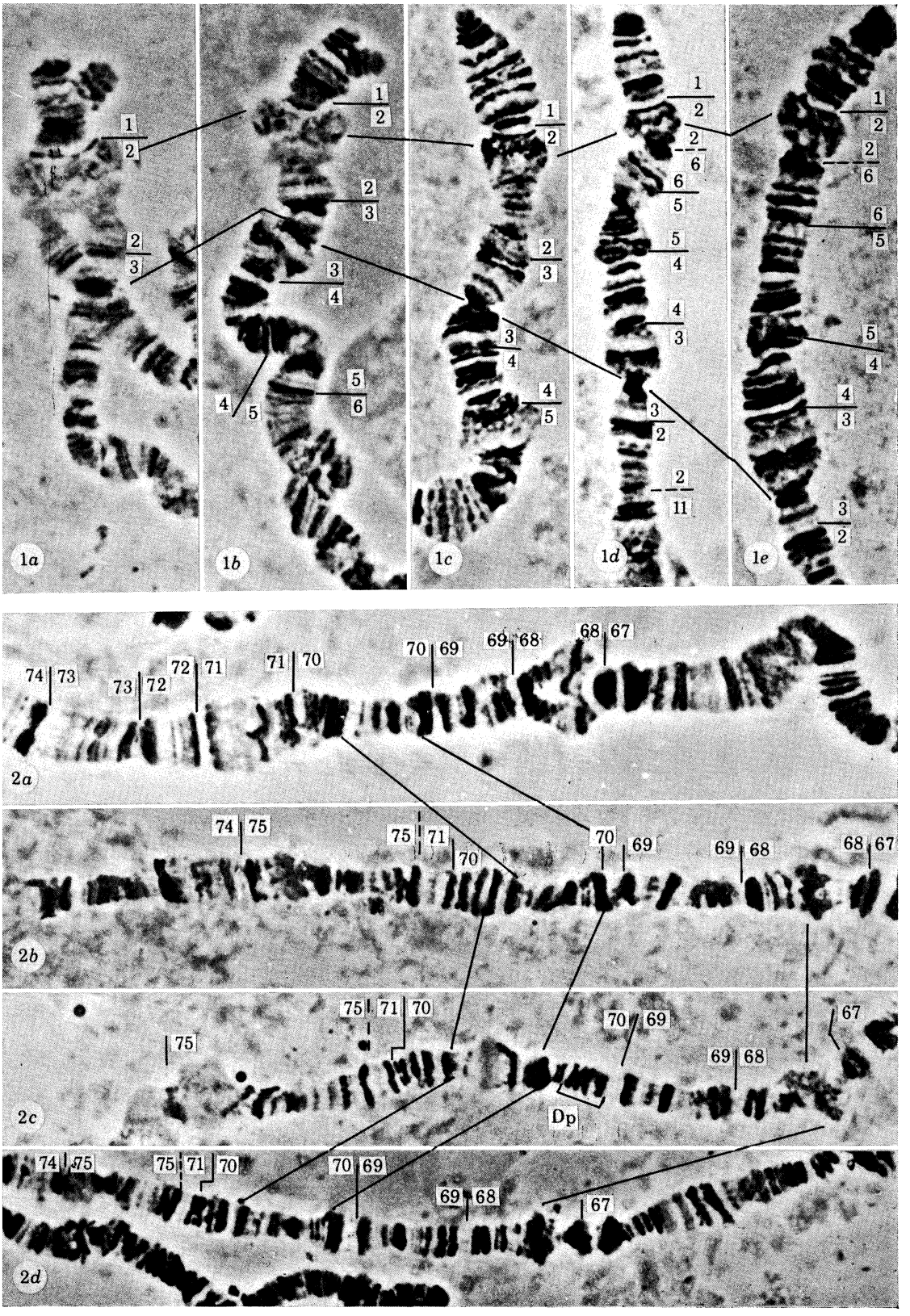
The three remaining species have all undergone a long pericentric inversion on chromosome 2. The precise location of the breakpoints of this inversion is rather difficult since some modifications of the banding pattern, in comparison

† Figures 1–10 appear on plates 40–45.

Description of plate 40

Figure 1. The tip of the X-chromosome of (*a*) *mauritiana* × *melanogaster* F1, (*b*) *mauritiana* × *simulans* F1, (*c*) *erecta*, (*d*) *teissieri* and (*e*) *yakuba*. All from female larvae.

Figure 2. Region 68–70 of chromosome arm 3L of (*a*) *melanogaster* × *simulans* F1, (*b*) *erecta*, (*c*) *teissieri* and (*d*) *yakuba* showing the six-band duplication between 69F1.2 and 70A1.2 of *teissieri*.



FIGURES 1 AND 2. For description see opposite.

(*Facing p.* 280)

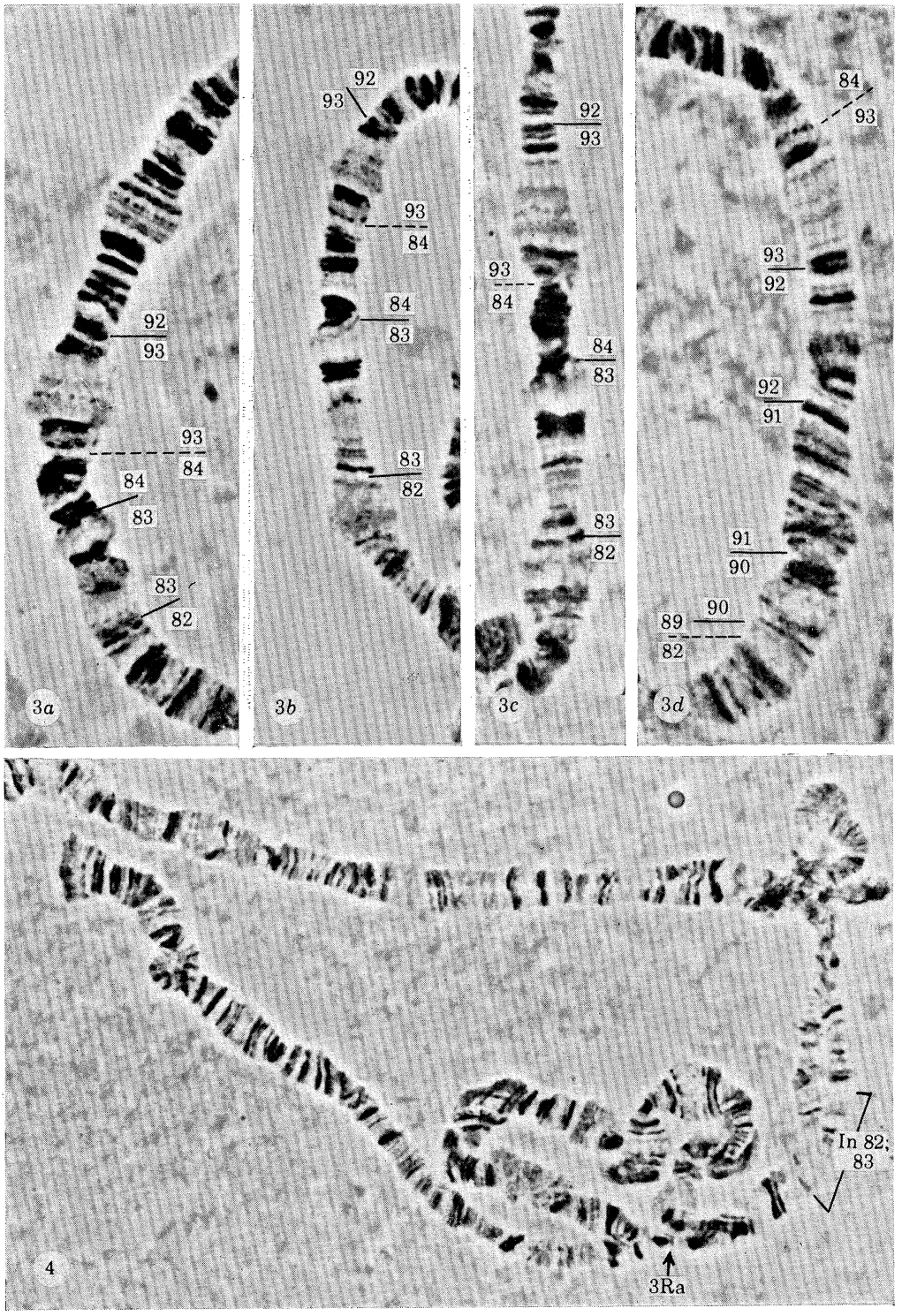


FIGURE 3. Base of chromosome arm 3R of (*a*) *simulans*, (*b*) *erecta*, (*c*) *teissieri* and (*d*) *yakuba*. The arrow indicates the 84F/93F break point of *In3Ra*.

FIGURE 4. Chromosome arm 3R of *mauritiana* × *melanogaster* F1 showing *In3Ra* heterozygous.

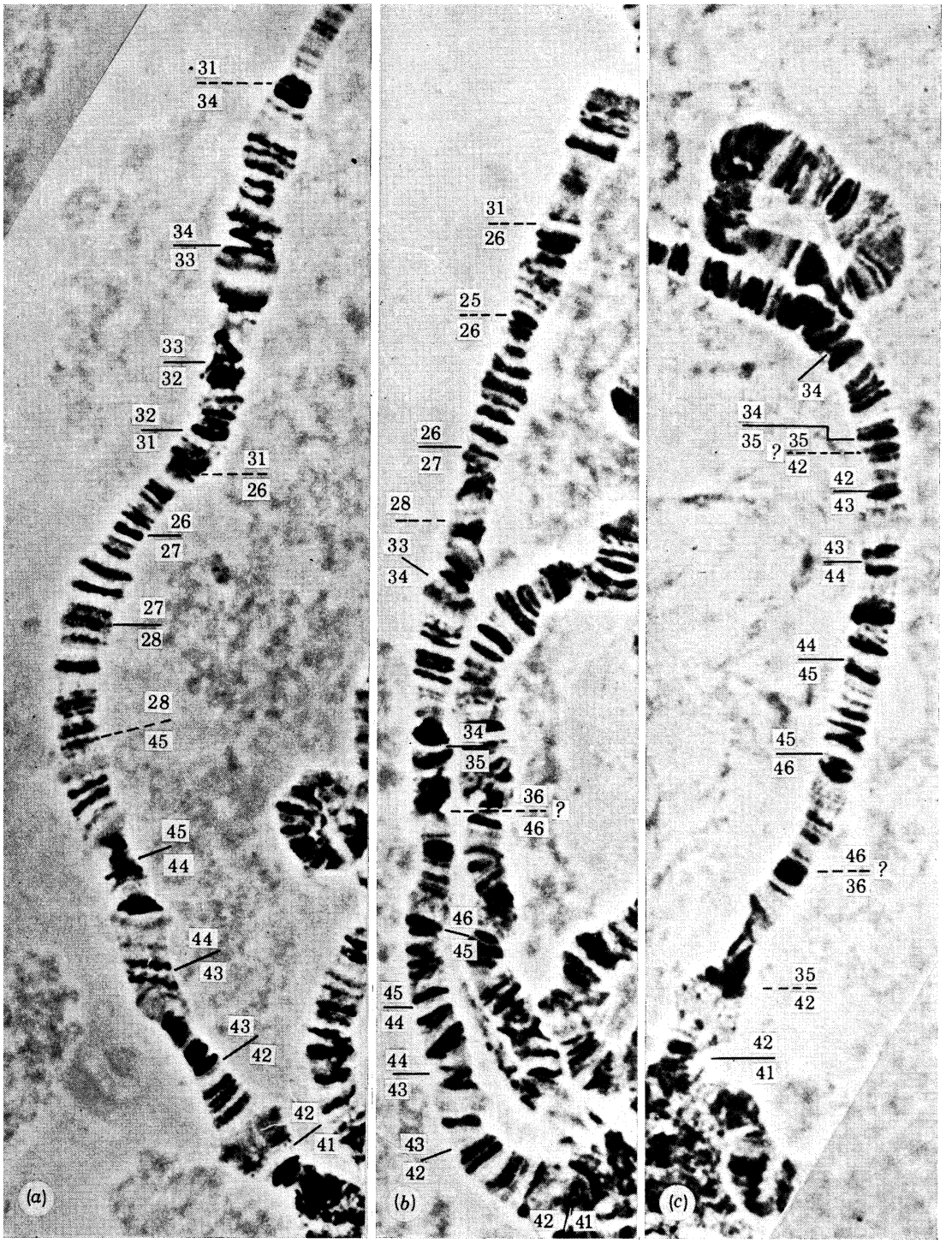
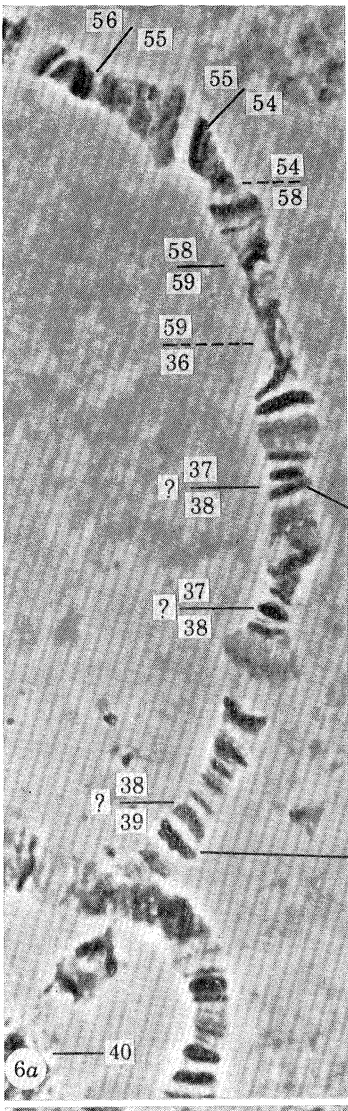
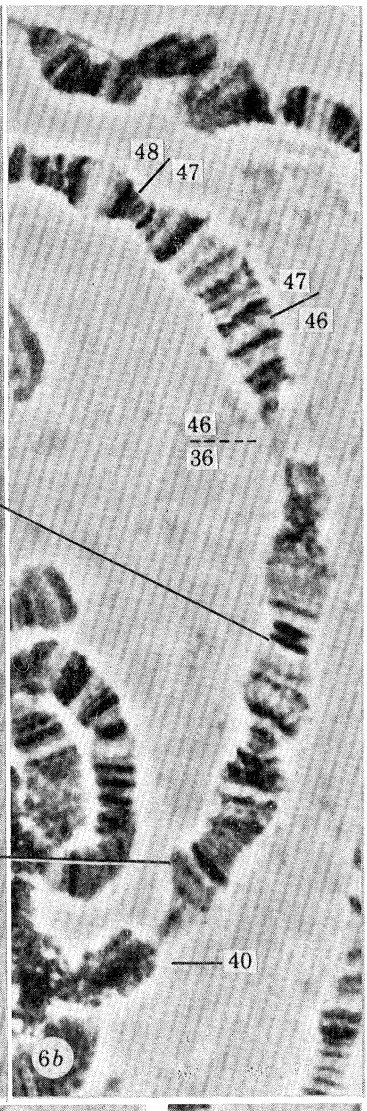
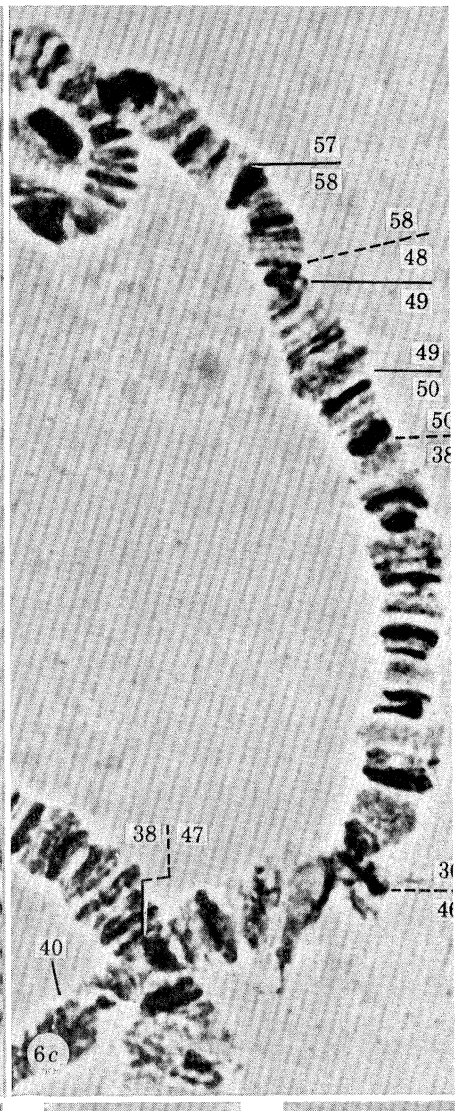
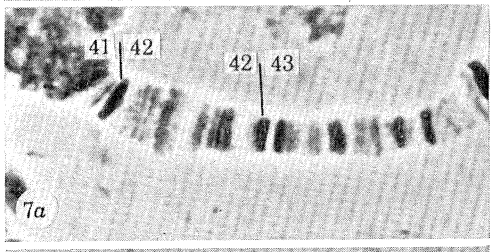


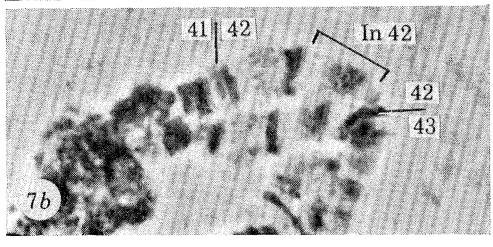
FIGURE 5. Base of chromosome arm 2L or (*a*) *erecta*. (*b*) *teissieri* and (*c*) *yakuba*. The *yakuba* chromosome is heterozygous for *In2l*$^{-}$/+.

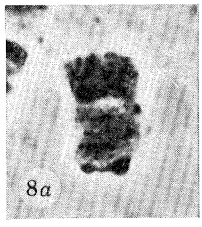


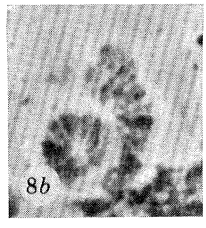


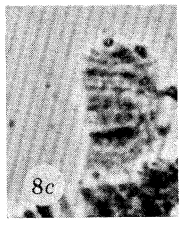


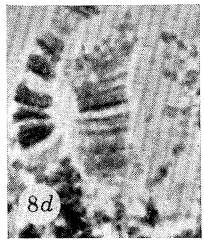


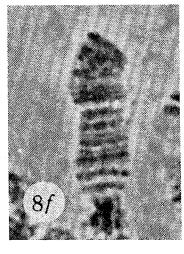












FIGURES 6–8. For description see opposite.

to that of *D. melanogaster*, appear to have occurred near them. Our best estimates of the break points are 2L:36C and 2R:46D. Reference to figures 5 and 6 will bring to the reader's attention the facts that not only are there a group of 3 or 4 bands distal to the 46D breakpoint at the 36C distal/46D proximal junction of unknown origin but that the 2L break point appears to be 35C distally but 36C proximally. The problems arise in part from the fact that the 35–36 region in *D. melanogaster* is, at the best of times, very difficult to analyse.

Chromosome arm 2L (*figure* 5)

D. erecta (scheme 1). The pericentrically inverted 2LRa sequence has undergone three paracentric inversions, 2Lb, 2Lc and 2Ld to give the extant *erecta* 2L sequence (2LRa, 2Lb)cd. Inversion 2Lb is common to both *D. teissieri* and *D. yakuba*. Since 2Lb, c and d all overlap we can unambiguously assign this order to their occurrence.

D. teissieri (scheme 2). The standard 2L of *D. teissieri* is derived from (2LRa, 2Lb) by two overlapping paracentric inversions 2Le and 2Lf. This species is

melanogaster
↓ 2LRa, 2Lb
21A–26A/31E–26A/31E–36C/46D–41
↓ 2Lc
21A–26A/31E–28F(?)/45F–46D/36C–31E/26A–28F(?)/45F–41
↓ 2Ld
(2LRa, 2Lb)cd 21A–26A/34D–36C/46D–45F/28F(?)–31E/34D–31E/26A–28F(?)/45F–41

SCHEME 1. Evolution of the *erecta* 2L.

(2LRa, 2Lb)
↓ 2Le
21A–26A/31E–28D/33A–31E/26A–28D/33E–36C/46D–41
↓ 2Lf
(2LRa, 2LB)ef 21A–25A/?/31E–33A/28D–31E/26A–25A/26A–28D/33E–36C/46D–41
↓ 2Lo
21A–21F/27C–26A/25A–26A/31E–28D/33E–31E/25A–21F/27C–28D/33E–36C/46D–41
↓ 2Lp
(2LRa, 2Lb)ef(op) (=2Lop) 21A–21F/30B–31E/26A–25A/26A–27C/30B–28D/33E–31E/25A–21F/27C–28D/33E–36C/46D–41

SCHEME 2. Evolution of the *teissieri* 2L.

DESCRIPTION OF PLATE 43

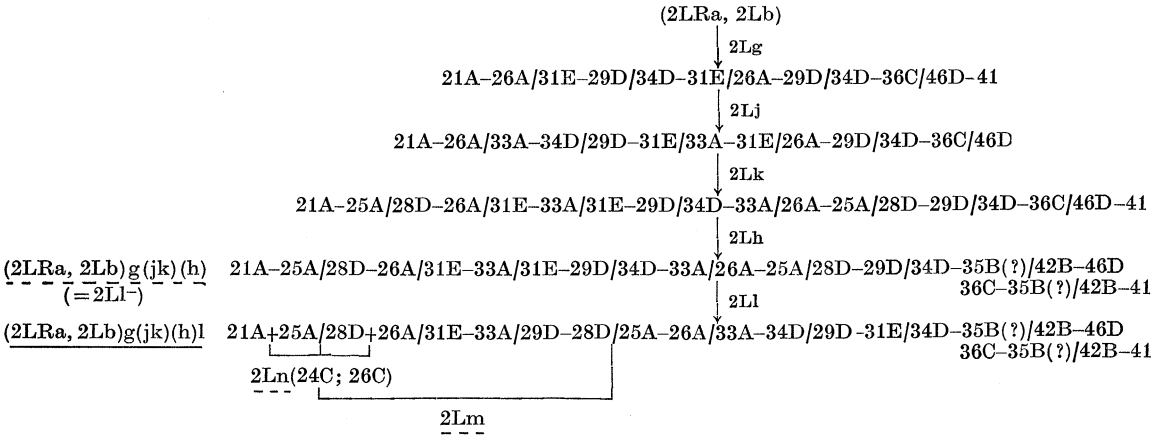
FIGURE 6. Base of chromosome arm 2R of (*a*) *erecta*, (*b*) *teissieri* and (*c*) *yakuba*.

FIGURE 7. Base of chromosome arm 2R of (*a*) *mauritiana* × *simulans* F1 and (*b*) *mauritiana* × *melanogaster* F1 showing the small basal inversion In(2R)42D; 42E of *simulans* and *mauritiana*.

FIGURE 8. Fourth chromosomes of (*a*) *melanogaster*, (*b*) *mauritiana* × *melanogaster* F1 (showing the *mauritiana* homologue looped back to the chromocentre), (*c*) *mauritiana* × *simulans* F1, (*d*) *erecta*, (*e*) *teissieri* and (*f*) *yakuba*.

polymorphic for a single sequence, found in all strains, which is related to the standard by two inversions 2Lo and 2Lp (figure 9*b*). Inversion 2Lp is included within 2Lo, and in fact, shares its distal break point, so that the relative order of these inversions is unknown. Neither has been found alone.

D. yakuba (scheme 3). The *D. yakuba* 2L is also derived from (2LRa, 2Lb). Five inversions are necessary one of which, 2Lh, is quite independent of all the others. It is broken in region 35 and within the '2R' section 42. The other four are 2Lg, j, k and l occurring in the order 2Lg(jk)l. Inversion 2Lj is fully included within 2Lk.

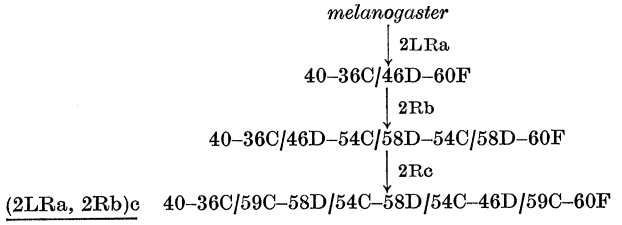


SCHEME 3. Evolution of the *yakuba* 2L.

There are three polymorphic sequences, in addition to standard, on the *D. yakuba* 2L. Two are both simple, two break, inversions of standard (2Lm and 2Ln (figures 10b and 10g respectively)). The third is the sequence (2LRa, 2Lb)g(jk)(h) – that is to say it is an intermediate in the evolution of the 2L *yakuba* standard from the *melanogaster* 2L. This sequence (2Ll⁻ (figure 5*c*)) is relatively common being found in both East and West African strains. Both 2Lm and 2Ln are rarer inversions known only from the Cameroun and Congo respectively (table 2A).

Chromosome arm 2R (*figure* 6)

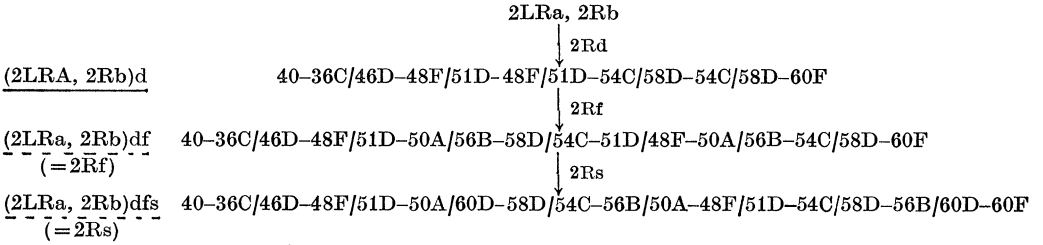
D. erecta (scheme 4). The 2R arm of *D. erecta* is very simply derivable from 2LRa by two independent inversions 2Rb and 2Rc the former not only being fully included within the latter but also being common to the other two species. We prefer, therefore, the order to be (2LRa, 2Rb)c.



SCHEME 4. Evolution of the *erecta* 2R.

D. teissieri (scheme 5). A single inversion, 2Ld, converts (2LRa, 2Rb) into the standard 2R of this species.

There are two polymorphic sequences on 2R in *D. teissieri*. One, 2Rf (figure 9*c*), is a simple inversion of standard while the other requires a further inversion of 2Rf (2Rs, figure 9*e*), with its proximal breakpoint (at 50A) in common with 2Rf. Both are widespread (table 2B).



SCHEME 5. Evolution of the *teissieri* 2R.

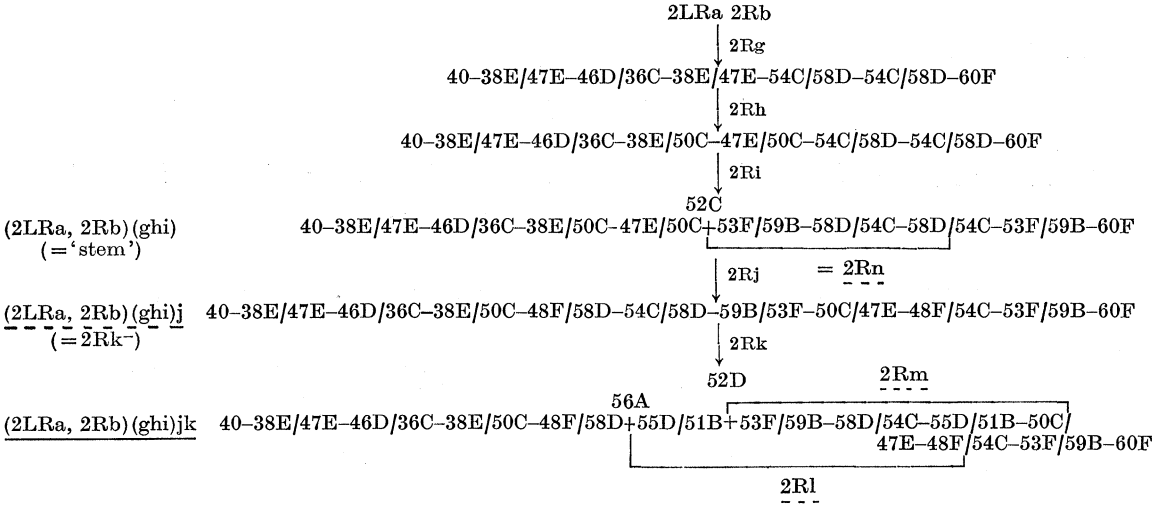
TABLE 2A. DISTRIBUTION OF POLYMORPHIC SEQUENCES IN *D. YAKUBA*

		sequence								
strain	origin	2Ll⁻	2Lm	2Ln	2Rl	2Rk⁻	2Rn	2Rm	3Lj	3Rh
115	Cameroun	+	−	−	−	+	+	−	−	+
143.3	Cameroun	−	+	−	−	+	+	−	−	−
147.2	Cameroun	−	+	−	−	+	+	−	−	+
142.6	Ivory Coast	+	−	−	−	+	+	−	−	−
140.2	Ivory Coast	+	−	−	−	−	+	−	−	−
140.7	Ivory Coast	+	−	−	−	+	+	−	−	−
141.6	Ivory Coast	+	−	−	−	+	+	−	−	−
168.2	Ivory Coast	−	−	−	−	−	+	−	−	−
165.4	Ivory Coast	−	−	−	−	−	+	−	+	−
165.10	Ivory Coast	−	−	−	+	+	−	−	−	−
131.6	Gabon	+	−	−	−	+	+	−	−	−
160.3	Congo	−	−	+	−	+	+	−	−	+
162.1	Uganda	+	−	−	−	−	−	+	−	−
162.2	Uganda	−	−	−	−	+	+	+	−	+
162.3	Uganda	−	−	−	−	−	−	+	−	−
172.3	Malawi	−	−	−	−	−	−	−	−	−
172.4	Malawi	−	−	−	−	+	−	−	−	−

TABLE 2B. DISTRIBUTION OF POLYMORPHIC SEQUENCES IN *D. TEISSIERI*

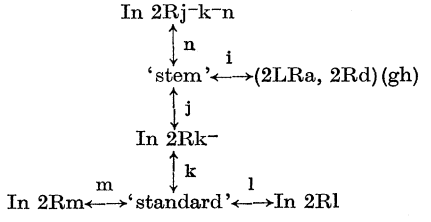
		sequence			
strain	origin	2Lop	2Rf	2Rs	3Lefg
128.2	Rhodesia	+	+	−	+
131.3	Gabon	+	+	+	+
140.5	Ivory Coast	+	+	+	+
165.1	Ivory Coast	+	−	+	+
165.3	Ivory Coast	+	−	+	+
144.4	Cameroun	+	+	−	+
145.1	Cameroun	+	+	+	+

D. yakuba (scheme 6). This arm has been the most complex that we have had to decipher. Three inversions of (2LRa, 2Rb) give rise to the sequence (2LRa, 2Rb) (ghi) which we will consider as the yakuba 2R 'stem' sequence. From it the *D. yakuba* standard 2R, found in all strains studied, is obtained by two further inversions, 2Rj and 2Rk. Although 2Rk is included within 2Rj, and thus the order of their occurrence cannot be certainly determined, for reasons which will become clear below we prefer the order 2Rjk.



SCHEME 6. Evolution of the *yakuba* 2R.

The *D. yakuba* strains studied carried four polymorphic 2R sequences. The first of these is, in fact, the sequence (2LRa, 2Rb) (ghi)j (2Rk⁻, figure 10*e*), the sequence intermediate between 'stem' and 'standard'. From standard two inversions 2Rl and 2Rm give rise to two independent polymorphisms (figures 10*c* and 10*h* respectively). The fourth polymorphic sequence is inversion 2Rn (figure 10*a*). This has the order (2LRa, 2Rb) (ghi)n and, it will be seen, is derived not from standard or 2Rk⁻ but from 'stem'. The stem sequence itself is, so far, unknown. Inversions 2Rk⁻ and 2Rn are common (table 2A) but 2Rl is known only from a single strain from the Ivory Coast and 2Rm from the three Ugandan strains. A summary of the relationships between the 2R inversions of *D. yakuba* is given in scheme 7.



SCHEME 7. Interrelationships between the polymorphic inversions of the *yakuba* 2R.

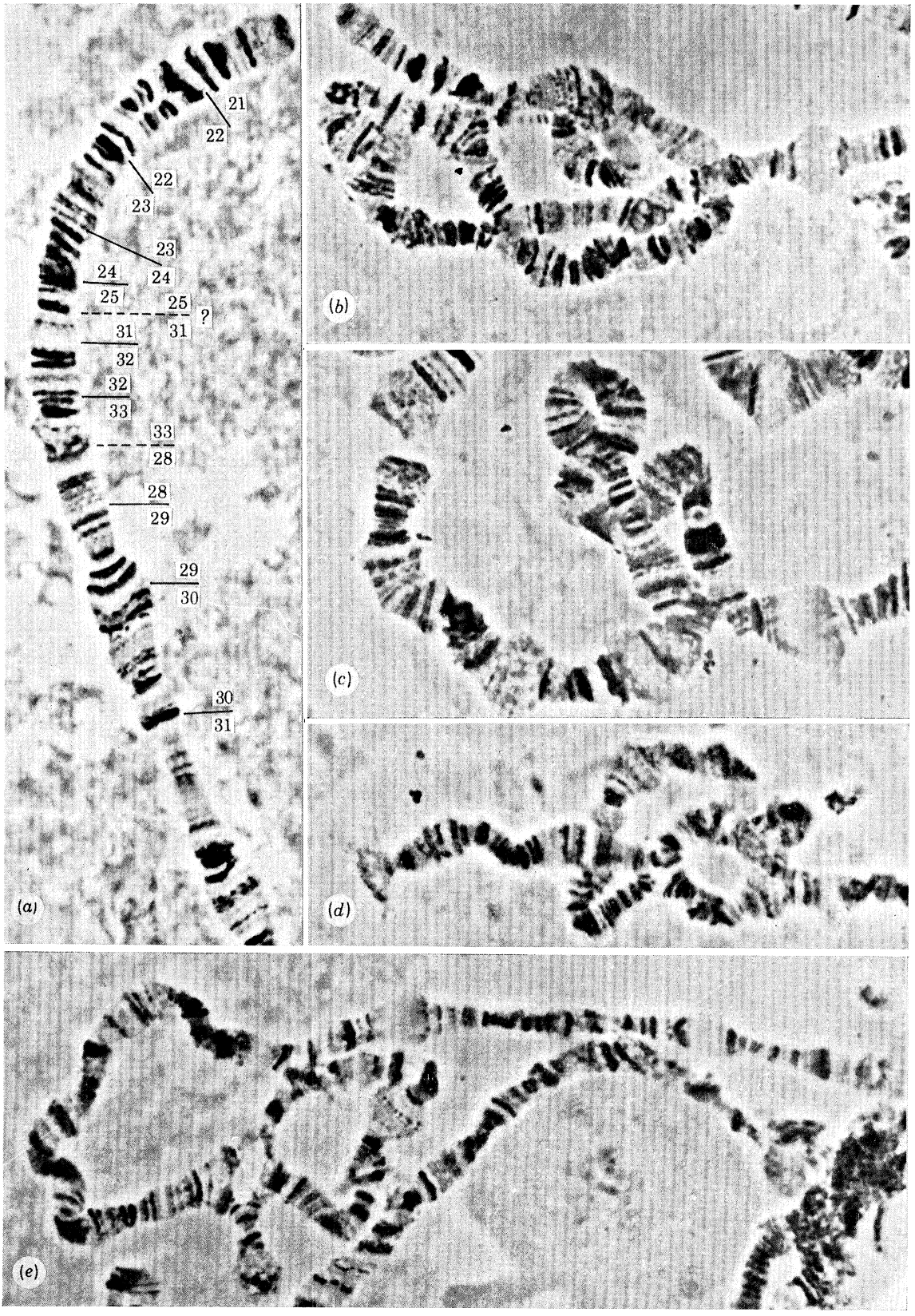


FIGURE 9. Inversions of *teissieri*. (*a*) standard sequence 2L, (*b*) *2Lop*/+, (*c*) *2Rf*/+, (*d*) *3Lefg*/+ and (*e*) *2Rs*/+.

(*Facing p.* 284)

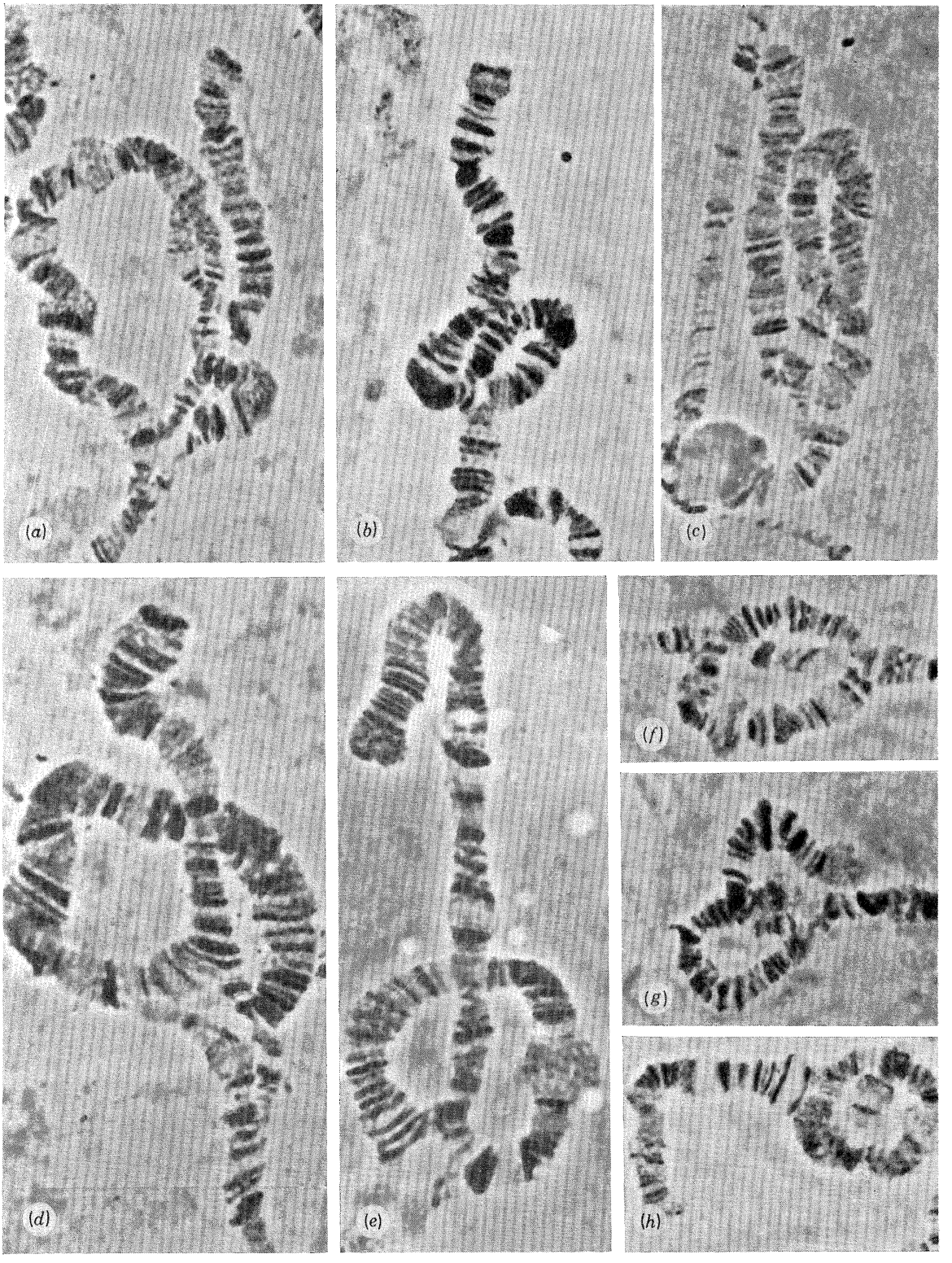


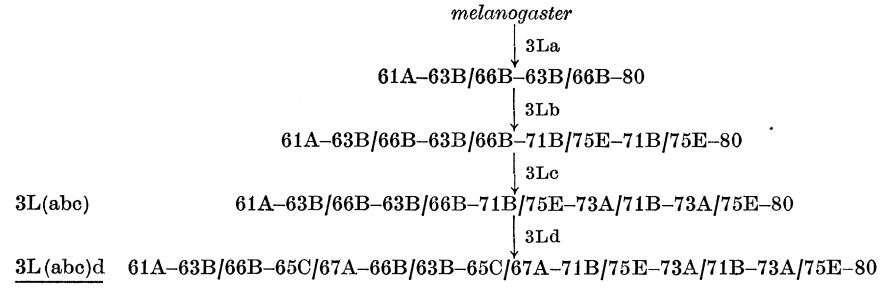
FIGURE 10. Inversions of *yakuba*. (*a*) *2Rn*/+, (*b*) *2Lm*/+, (*c*) *2R1*/+, (*d*) *3Rh*/+, (*e*) *2Rk*$^{-}$/+, (*f*) *3Lj*/+, (*g*) *2Ln*/+ and (*h*) *2Rm*/+.

Chromosome arm 3L (*figure* 2)

D. simulans and *D. mauritiana*. The left arm of chromosome 3 is identical, in these species, to that of *D. melanogaster* excepting some slight differences of chromosome tips.

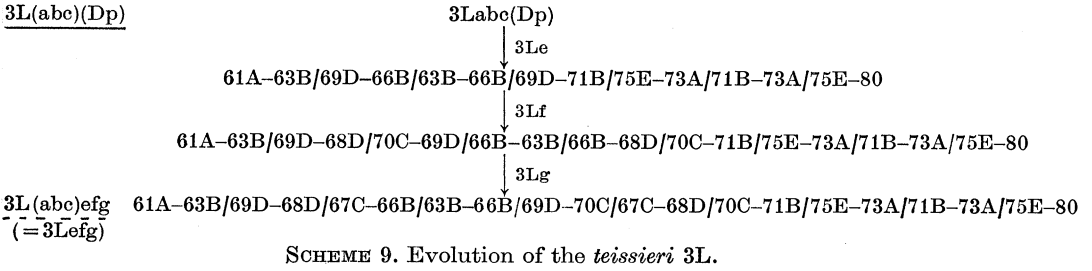
D. erecta (scheme 8). Four inversions of the *D. melanogaster* 3L give the *D. erecta* 3L. The order of the first three is unknown since they fail to overlap while the fourth, 3Ld, only overlaps 3La. Since 3Ld is unique to *D. erecta* while 3La is found in both *D. teissieri* and *D. yakuba* we prefer the order 3L(abc)d.

D. teissieri (scheme 9). The standard 3L of *D. teissieri* is 3L(abc) the penultimate sequence of the *erecta* phylogeny. In a previous paper (Ashburner & Lemeunier 1972) we described, as the sequence of the *D. teissieri* 3L, a much more complex



SCHEME 8. Evolution of the *erecta* 3L.

sequence than this. At that time we were aware that, in the strain we were studying (128.2), a complex rearrangement was segregating. This was, in a few larvae, homozygous. Subsequent study of other strains of *D. teissieri*, and in particular the analysis of the *D. erecta* 3L, made us realize that what we took to be a complex rearrangement of our standard 3L for this species was, in fact, the primitive 3L(abc) sequence and that our published *D. teissieri* 3L sequence was a rearrangement of this.



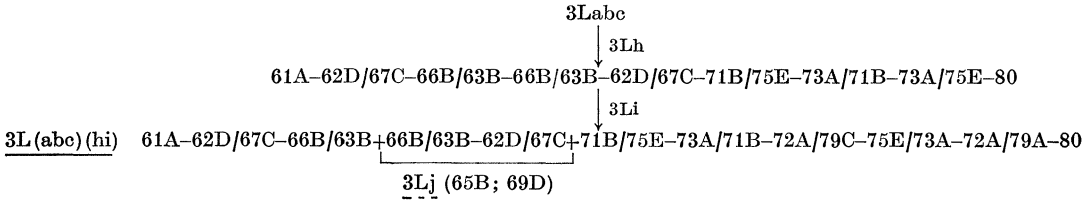
SCHEME 9. Evolution of the *teissieri* 3L.

This complex rearrangement (figure 9*d*) is the only polymorphic 3L known in *D. teissieri*. The sequence given here is a revision of that previously published. It is related to the standard by three overlapping inversions, and has been found in all seven strains analysed.

Our statement that 3L(abc) of *teissieri* is identical to the penultimate *erecta* sequence is not strictly true. This is because in the *D. teissieri* 3L, both standard and the inversion 3Lefg, there is a small 'duplication' of six bands intercalated between 69F1.2 and 70A1.2. The origin of these bands has not been determined. The 'duplication' is absent from the 3L's of either *erecta* or *yakuba* (figure 2).

D. yakuba (scheme 10). In the context of a study of puffing patterns in *D. yakuba* the sequence of the 3L of this species was given in Ashburner & Lemeunier (1972). The sequence published now differs from that previously published only in minor revisions of the break points The *D. yakuba* 3L can be derived from 3L(abc) by two further, non-overlapping, inversions 3Lh and 3Li.

There is a single polymorphic inversion on 3L in *D. yakuba* It (3Lj, figure 10*f*) is a simple two-break inversion of standard, and was only found in one strain, from the Ivory Coast (table 2A).

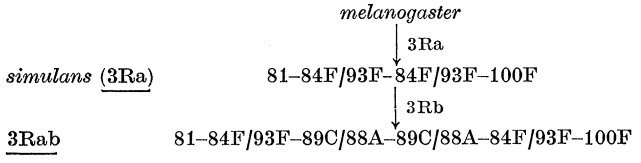


SCHEME 10. Evolution of the *yakuba* 3L.

Chromosome arm 3R (*figure* 3)

D. simulans and *D. mauritiana*. The existence of a long inversion of 3R of *D simulans* has been known for many years (Pätau 1935; Dubinin, Sokolov & Tiniakov 1937; Horton 1939). The break points of this inversion were given as 84B3 and 92C3 by Horton (1939), and 84E and 93F by Dubinin *et al.* (1937); in our opinion (see Ashburner 1969) they are at 84F1 and 93F6–7. In addition the *D. simulans* 3R carries a small basal inversion (82F3; 83B3) (Horton 1939) The 3R of *D. mauritiana* is the same as that of *D. simulans* (figure 4). We are unable to assess the presence or absence of the minor *simulans* inversion (82F; 83B) in the three remaining species.

D. erecta (scheme 11). The 3R of *D. erecta* is simply a single inversion of the *D. simulans* 3R. This inversion (3Rb) is fully included within the long *simulans* inversion.

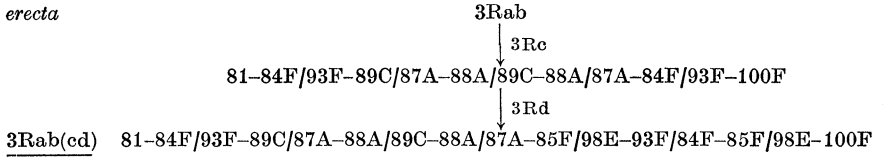


SCHEME 11. Evolution of the *erecta* 3R.

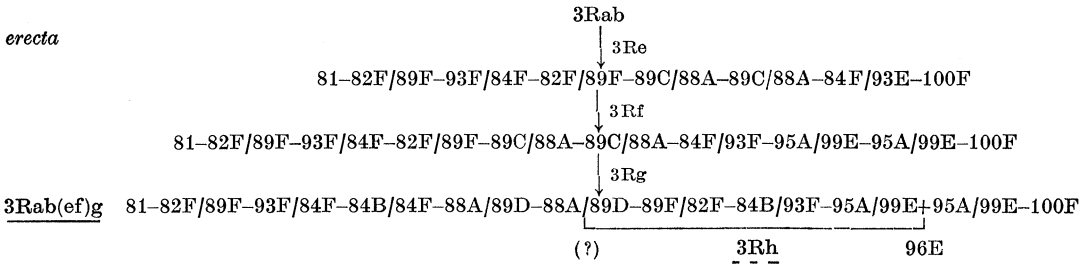
D. teissieri (scheme 12). From the *D. erecta* sequence 3Rab two, non-overlapping, inversions 3Rc and 3Rd, give the *D. teissieri* 3R sequence 3Rab(cd).

D. yakuba (scheme 13). Slightly more complex than the 3R's of the other species but also derived from 3Rab. Following two independent inversions, 3Re and 3Rf, inversion 3Rg (which overlaps 3Re) gives the standard *D. yakuba* 3R sequence.

A single polymorphic sequence is known which is related to the standard by a simple inversion, 3Rh (figure 10*d*). As indicated in table 2A it was found in relatively few strains but those which did carry it included strains from both West and East African collections.



SCHEME 12. Evolution of the *teissieri* 3R.



SCHEME 13. Evolution of the *yakuba* 3R.

Chromosome 4 (*figure* 8)

We have made no great effort to decipher the small fourth chromosome of these species. As can be seen from figure 8 the fourths of all six species are generally similar to each other. In *D. melanogaster*/*D. mauritiana* hybrids the fourth chromosomes fail to synapse and one of them, probably the *mauritiana*, is very often looped back to the chromocentre by its tip (figure 8*b*).

DISCUSSION

(*a*) *On the origin of inversions*

A striking feature of the chromosome phylogenies we have constructed for these six species is the extent to which they demand breaks at coincident places. A convenient way to express this is to calculate the number of breaks that should be identifiable on the basis of the number of inversion events required to derive one sequence from another – assuming all inversions to arise from two break events (see below) – and to divide this number into the number of breaks actually identified. This 'coincidence ratio' should, if no breaks are coincident and all breaks have been identified, be one. In fact it is usually < 1.

Consider, as a typical example, the standard 2L of *D. yakuba*. Our derivation of this sequence from that of *D. melanogaster* demands one pericentric inversion and six paracentrics which should have left their traces as 13 identifiable breaks. In fact we can only see ten breaks – a concidence ratio of 0.77. The distal break of 2Lj occurs at apparently the same place as the distal break of the previous 2Lb while the breaks of 2Ll are apparently identical to those of 2Lj (distally) and 2Lg (proximally).

We stress that the identity of breaks is defined by our inability to resolve them as different. We do not wish to imply that the precise locations of 'coincident' breaks are strictly identical in terms of the chromosome's nucleotide sequence.

Coincidence ratios of less than one are a common feature of polytene chromosome phylogenies. Coincident breaks were recognized in the phylogeny of the polymorphic A chromosome of *D. azteca* by Dobzhansky & Socolov (1939): to derive the *eta* sequence from the *alpha* sequence demands four inversions yet only five break points have been identified – a concidence ratio of 0.625. More recently the phylogenies of the chromosomes of the Hawaiian picture-winged species contain many examples of break point coincidence – consider, for instance, the X chromosome of *D. primaeva* (Carson & Stalker 1969). This chromosome differs from its immediate ancestor (X iko) by seven inversions ($Xj^2k^2i^2h^2\ f^2d^2e^2$). Instead of the expected 14 breaks only 10 have been resolved (a coincidence ratio of 0.7).

TABLE 3. SUMMARY OF NUMBER OF INVERSIONS REQUIRED TO DERIVED CHROMOSOMES OF *MELANOGASTER* SUB-GROUP SPECIES FROM *MELANOGASTER* ('MINOR' *SIMULANS* INVERSIONS OMITTED)

	X	2L	2LR	2R	3L	3R	total
D. simulans	0	0	0	0	0	1	1
D. mauritiana	0	0	0	0	0	1	1
D. erecta	$\geqslant 3$	3	1	2	4	2	$\geqslant 15$
D. teissieri	$\geqslant 3$	3	1	2	3	4	$\geqslant 16$
D. yakuba	$\geqslant 4$	6	1	6	5	5	$\geqslant 27$
total inversions events	$\geqslant 7$	10	1	8	6	7	$\geqslant 37$

We must stress that several studies, with several species of *Drosophila*, have failed to show any similar non-randomness in the distribution of X-ray induced chromosome breaks (Bauer, Demerec & Kaufmann (1939) with *D. melanogaster*, Helfer (1941) and Koller & Ahmed (1942) with *D. pseudoobscura*, Seecof (1957) with *D. ananassae* and Kunze-Mühl (1961) with *D. subobscura*).

Before discussing the problem of break point coincidence and its implications for the origin of inversions we should point out that it is a separate problem from that of the non-random distribution of inversions between different chromosome arms. These problems have sometimes been confounded (see, for example, White 1973). With respect to both fixed and polymorphic inversions the *melanogaster* subgroup does not show any marked departure from a random distribution between chromosome arms (table 3, χ^2_4 2.78, $P > 0.1$ %, using the *D. melanogaster*

polytene lengths for calculating the expected distributions). This is in contrast to the situation found in, for example, the *repleta* group (with 67 of 96 fixed inversions on chromosome 2) or the Hawaiian picture-winged species (with 54 of 123 fixed inversions on the X chromosome).

One special type of relationship between inversions leads to low coincidence ratios – that is inversions which are in tandem. An example from the present study is the pair of inversions 2Rg and 2Rh of *D. yakuba*. These are of the common type ABCDEFGH ⟶ AB.DC.FE.GH. The most dramatic instance of tandem inversions is that found in *D. busckii*, where several inversions are arranged in tandem complexes (Krivshenko 1963). Tandem inversions simulate three break events. There are, however, two lines of evidence that they do originate from two two-break events with a shared break point: (1) One member of a tandem complex may occur independently of the other (as for example on chromosome 2 of *D. busckii* in which inversions 5 and 6 (which are not tandem) occur independently of inversion 7 (which is tandem to both of them) or in *D. subobscura* where inversion o_2 occurs independently of o_{20} (but not vice versa) (Kunze-Mühl & Muller 1958)). (2) Rothfels & Fairlie (1957) pointed out that if 3-break events were common then they would be expected to give rise to inversions of the type ABCDEFGH ⟶ AB.EF.CD.GH as commonly as to tandem inversions. In fact the former type of inversion occurs rarely, if at all.

Considering the problem of break point coincidence Rothfels & Fairlie (1957) reviewed most of the data then available from *Drosophila* and *Chironomus* and pointed out that 'much of the total non-randomness of breaks originates from the tendency to break coincident in successive inversion steps'. Since 1957 extensive chromosome phylogenies have been published for several *Drosophila* groups – in particular *D. subobscura* (Kunze-Mühl & Muller 1958), the *robusta* group (Narayanan 1973) and the picture-winged Hawaiian species (Clayton, Carson & Sato 1972 for references). Analysis of our own data and that of the authors referred to fully substantiates the statement of Rothfels & Fairlie. Whenever two inversions share a break point they almost invariably occur, or could have occurred, in successive steps in the sequence. In contrast, break-point coincidence is rare (though not unknown; see, for example, the *D. subobscura* phylogenies) when we are certain that different inversion events are unrelated. For example, within *D. melanogaster* itself over 50 inversions are known to be polymorphic. With a single exception these are all independent two-break inversions of the standard sequence – there is no case to break point coincidence among them.

Novitski (1946) suggested a hypothesis to account for a break-point coincidence. He pointed out that 'the most obvious situation in which break points of an inversion are differentiated from other sections is that found during prophase of a cell heterozygous for an inversion' since the inverted-loop configuration results in asynapsis at and near the break points. This situation may itself result in an increased frequency of breakage in this region or the frequency of breakage

may be unchanged but the probability of reunions to give novel sequences may be enhanced due to the close proximity of broken ends.

Apart from the concordance of natural inversion phylogenies with Novitski's hypothesis there have been two attempts to test its predictions experimentally. In one, by Novitski himself (Novitski 1961*a*), reversions of the roughest^3 phenotype were selected from the progeny of X-irradiated females heterozygous for *In(1)rst*^{3}. Of 15 reversions 6 were cytologically precise reinversions of the roughest^3 inversion to the standard sequence. In the second experiment Bernstein & Goldschmidt (1961; see Novitski 1961*b*) X-rayed male *D. melanogaster* heterozygous for two natural third chromosome inversions (*In(3L)P* and *In(3R)P*) and isolated aberrations induced on the standard homologue in spermatocytes. Thirty-five new breaks were induced and plotted with respect to the break points of the Payne inversions. Unfortunately the precise break points of the induced aberrations were never reported but the authors do appear to have found some evidence for a clustering of the new break points around those of the Payne inversions.

TABLE 4. NUMBERS OF POLYMORPHIC SEQUENCES IN SPECIES OF *MELANOGASTER* SUBGROUP

	X	2L	2LR	2R	3L	3LR	3R	total
*D. melanogaster**	0	14	6	13	6	0	14	53
*D. simulans**	0	0	0	0	0	0	0	0
D. mauritiana	0	0	0	0	0	0	0	0
D. erecta	0	0	0	0	0	0	0	0
D. teissieri	0	1	0	2	1	0	0	4
D. yakuba	0	3	0	4	1	0	1	9
total	0	18	6	19	8	0	15	66

* From Ashburner & Lemeunier (1976).

(b) The relationship between the species of the melanogaster species subgroup

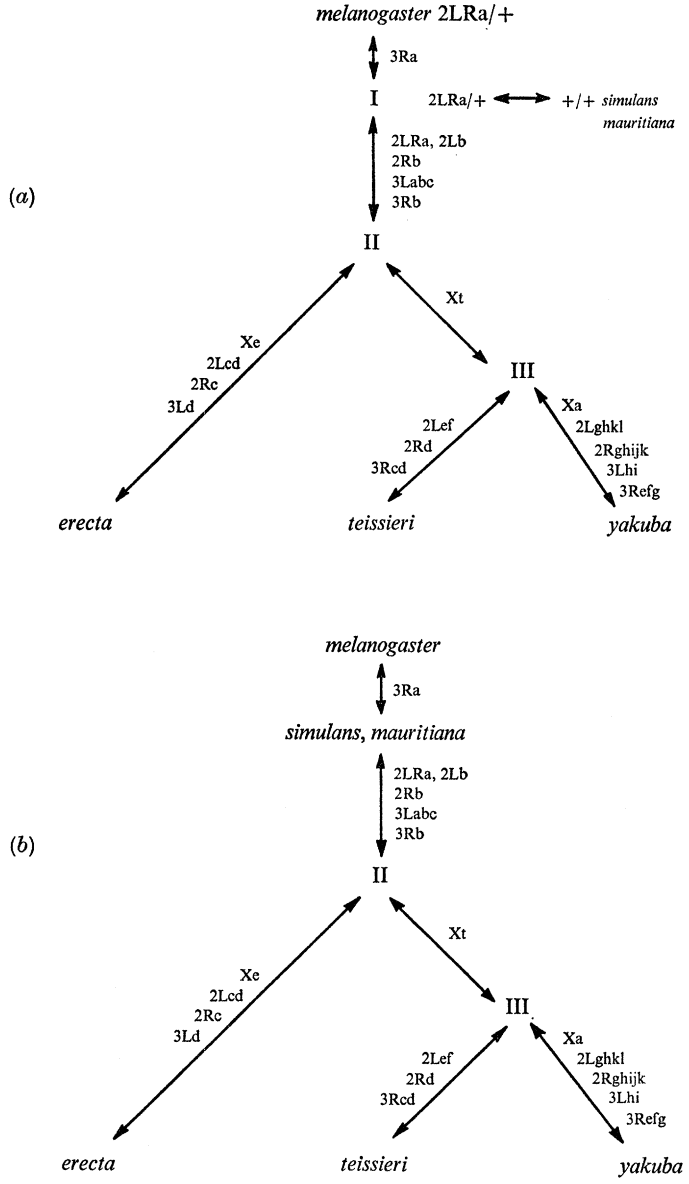
The six members of the *melanogaster* species subgroup whose chromosomes we have analysed may be considered as a complex of six sibling species. Their adult morphologies are very similar and the only reliable guides to their distinction are the male genitalia. The results of the chromosomal analysis confirm the close relationships between these species.

Chromosomally the six species fall into two groups. On the one hand there are *melanogaster*, *simulans* and *mauritiana* whose chromosomes differ by only one major paracentric inversion (3Ra) and a few minor inversions; indeed *simulans* and *mauritiana* are homosequential. On the other hand the three African endemic species, *erecta*, *teissieri* and *yakuba*, share seven inversions of the standard *melanogaster* sequence.

Uncertainty as to the precise relationship of the major chromosome 2 pericentric inversion of the African endemic species with the *In(2LR)36D; 46F*

polymorphic in a West African collection of *D. melanogaster* (Ashburner & Lemeunier 1976) obliges us to present two alternative chromosome phylogenies of this subgroup (scheme 14).

The break points of these two inversions are clearly similar but the changes in banding near the break points of 2LRa that appear to have occurred in the African endemic species makes it very difficult to determine whether or not they are identical inversions.



SCHEME 14. (*a*, *b*). Two possible schemes showing the interrelationship between the six species of the *melanogaster* species subgroup. See text for discussion.

If they are identical then it is necessary to suppose that a population existed in which this inversion was polymorphic and in which 3Ra was fixed. From this hypothetical-I *melanogaster* alone has inverted 3Ra to standard and retained the second chromosome pericentric polymorphism; *simulans* and *mauritiana* fixed the chromosome 2 standard sequence and the African endemics the chromosome 2 inverted sequence.

On the other hand if the *In(2LR)36D; 46F* of *D. melanogaster* and 2LRa are unrelated, no such hypothetical need be considered. Then from the sequence now retained by *simulans* and *mauritiana* seven inversions can give rise to a stem sequence (hypothetical-II) ancestral to all three African endemic species. The sequence of *D. erecta* can be derived from II by four unique autosomal inversions. The fact that the X chromosome sequences of *D. erecta*, on the one hand, and *D. teissieri* and *D. yakuba* on the other, are unrelated would suggest that hypothetical-II would have, if extant, an X sequence similar to standard.

Apart from the inversions characteristic of hypothetical-II the only inversions in common between *D. teissieri* and *D. yakuba* are those of the X chromosome. The X chromosome of *D. teissieri* differs from standard by at least three, and probably more, inversions. The X of *D. yakuba* differs from that of *D. teissieri* by a single identifiable inversion.

These facts imply the existence of a further hypothetical population, III, differing from II only in the nature of its X chromosome sequence. From III the existing sequences of *D. teissieri* require five autosomal paracentrics and those of *D. yakuba* a minimum of 11 autosomal paracentrics and one X chromosome paracentric.

Of the four species studied here for the first time two display intraspecific chromosome polymorphism (table 2). In both of these species, *D. teissieri* and *D. yakuba*, several of the polymorphic sequences are complex, in contrast to the great rarity of such polymorphic sequences in *D. melanogaster* itself (Ashburner & Lemeunier 1976). All four of the polymorphic sequences of *D. teissieri* are widespread in their distribution. In *D. yakuba* only four of the nine polymorphic sequences were found in strains collected far apart. We are naturally hesitant to conclude that the remaining two species, *D. mauritiana* and *D. erecta*, are, like *D. simulans*, chromosomally monomorphic species until many more collections have been analysed.

It is only very rarely (see, for example, Carson 1973) that considerations of inversion phylogenies give any indication of the direction of evolution within a group of related species. We stress again that our practice in regarding *D. melanogaster* as the 'primitive' species of this subgroup is but a literary convenience. We may hope to gain further insights into the relationship between these six species by a comparison of their chromosome banding sequences with those of related subgroups. The centre of the present day distribution of the *melanogaster* species group is Southeast Asia (Bock & Wheeler 1972). In Southeast Asia there are now over 50 endemic species belonging to nine species subgroups. As one

proceeds westwards, first into the Indian subcontinent and then into the Ethiopian region, the *melanogaster* group fauna becomes poorer. In India there are less than 25 *melanogaster* group species (six subgroups) and in the Ethiopian region only 20 or so species, predominantly in two subgroups (*melanogaster* and *montium*). It is natural therefore to enquire whether or not the *montium* subgroup shows any chromosomal relationship to the *melanogaster* subgroup and, if so, which species of these subgroups are most closely related.

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References

Ashburner, M. 1969 Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. III. A comparison of the autosomal puffing patterns of the sibling species *D. melanogaster* and *D. simulans*. *Chromosoma* (*Berl.*) **27**, 64–85.

Ashburner, M. & Lemeunier, F. 1972 Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. VII. Homology of puffing patterns on chromosome arm 3L in *D. melanogaster* and *D. yakuba* with notes on puffing in *D. teissieri*. *Chromosoma* (*Berl.*) **38**, 283–295.

Ashburner, M. & Lemeunier, F. 1976 Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (*Sophophora*). I. Inversion polymorphisms in *Drosophila melanogaster* and *Drosophila simulans*. *Proc. R. Soc. Lond.* B **193**, 137–157.

Bauer, H., Demerec, M. & Kaufmann, B. P. 1938 X-ray induced chromosomal aberrations in *Drosophila melanogaster*. *Genetics* **23**, 610–630.

Bernstein, N. & Goldschmidt, E. 1961 Chromosome breakage in structural heterozygotes. *Am. Nat.* **95**, 53–56.

Bock, I. R. & Wheeler, M. R. 1972 The *Drosophila melanogaster* species group. *Univ. Texas Publ.* no. **7213**, 1–102.

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

Carson, H. L. 1973 Ancient chromosomal polymorphism in Hawaiian *Drosophila*. *Nature, Lond.* **241**, 200–202.

Carson, H. L. & Stalker, H. D. 1969 Polytene chromosome relationships in Hawaiian species of *Drosophila*. IV. The *D. primaeva* subgroup. *Univ. Texas Publ.* no. **6918**, 85–93.

Clayton, F. E., Carson, H. L. & Sato, J. E. 1972 Polytene chromosome relationships in Hawaiian species of *Drosophila*. VI. Supplementary data on metaphase and gene sequences. *Univ. Texas Publ.* no. **7213**, 163–177.

David, J., Lemeunier, F., Tsacas, L. & Bocquet, C. 1974 Hybridation d'une nouvelle espèce, *Drosophila mauritiana* avec *D. melanogaster* et *D. simulans*. *Annls Génét.* **17**, 235–241.

David, J. & Tsacas, L. 1975 Les Drosophilidae (Diptera) de l'Ile de la Réunion et de l'Ile Maurice. III. Biologie et origine des espèces. *Beitrage z. Entomol.* **24** (in press).

Dobzhansky, Th. & Pavan, C. 1950 Local and seasonal variation in relative frequencies of species of *Drosophila* in Brazil. *J. Animal Ecol.* **19**, 1–14.

Dobzhansky, Th. & Socolov, D. 1939 Structure and variation of the chromosomes of *Drosophila azteca*. *J. Hered.* **30**, 3–19.

Dubinin, N. P., Sokolov, N. N. & Tiniakov, G. G. 1937 Intraspecific chromosome variability. *Biol. Zh.* **6**, 1007–1054. (In Russian with English summary.)

Helfer, R. G. 1941 A comparison of X-ray induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*. *Genetics* **24**, 278–301.

Horton, I. H. 1939 A comparison of the salivary gland chromosomes of *Drosophila melanogaster* and *D. simulans*. *Genetics* **24**, 234–243.

Hsu, T. C. 1949 The external genital apparatus of male Drosophilidae in relation to systematics. *Univ. Texas Publ.* no. **4920**, 80–142.

Krivshenko, J. D. 1963 The chromosomal polymorphism of *Drosophila busckii* in natural populations. *Genetics* **48**, 1239–1258.

Koller, P. C. & Ahmed, I. A. R. S. 1942 X-ray induced structural changes in the chromosomes of *Drosophila pseudoobscura*. *J. Genet.* **44**, 53–72.

Kunze-Mühl, E. 1961 Untersuchungen über die Verteilung der Brachstellen naturlich und Strahleninduzierter Chromosomendislokationen bei *Drosophila subobscura* Coll. *Chromosoma* (*Berl.*) **12**, 286–309.

Kunze-Mühl, E. & Müller, E. 1958 Weitere Untersuchungen über Chromosomale Struktur und die naturlichen Strukturtypen von *Drosophila subobscura* Coll. *Chromosoma* (*Berl.*) **9**, 559–570.

Lachaise, D. 1971 Répartition des espèces des Drosophiles du sous-genre *Sophophora* groupe *melanogaster* dans une mosaique 'savane à palmiers Rôniers-galerie forestière' (Cote d'Ivoire). *C. r. hebd. Séanc. acad. Sci., Paris* D **273**, 1527–1530.

Lachaise, D. 1974 Les *Drosophilidae* des savanes préforestières de la region tropicale de Lamto (Cote d'Ivoire). I. Isolement écologique des espèces affines et sympatriques, rythmes d'activité saisonnière et circadienne; rôle des feux de brousse. *Ann. Univ. Abidjan* E **7** (1), 7–152.

Lachaise, D. & Tsacas, L. 1974 Les *Drosophilidae* des savanes préforestières de la région tropicale de Lamto (Cote d'Ivoire). II. Le peuplement des fruits de *Pandanus candelabrum* (*Pandanacées*). *Ann. Univ. Abidjan* E **7** (1), 153–192.

Lemeunier, F. 1971 Etude monographique d'une drosophile africaine: *Drosophila yakuba* Burla (Diptères: Drosophilidae). Thèse presentée à la Faculté des Sciences de Paris.

Narayanan, Y. 1973 The phylogenetic relationships of the members of the *Drosophila robusta* subgroups. *Genetics* **73**, 319–350.

Novitski, E. 1946 Chromosome variation in *Drosophila athabasca*. *Genetics* **31**, 508–524.

Novitski, E. 1961*a* The regular reinversion of the roughest^3 inversion. *Genetics* **46**, 711–717.

Novitski, E. 1961*b* Chromosome breakage in inversion heterozygotes. *Am. Nat.* **95**, 250–251.

Pätau, K. 1935 Chromosomenmorphologie bei *Drosophila melanogaster* und *Drosophila simulans* und ihre genetische Bedeutung. *Naturwissenschaften* **23**, 537–543.

Rothfels, K. H. & Fairlie, T. W. 1957 The nonrandom distribution of inversion breaks in the midge *Tendipes decorus*. *Can. J. Zool.* **35**, 221–263.

Seecof, R. L. 1957 Cytological analysis. In W. S. Stone, M. R. Wheeler, W. P. Spencer, F. D. Wilson, J. T. Neuenschwander, T. G. Gregg, R. L. Seecof & C. L. Ward: Genetic studies of irradiated natural populations of *Drosophila*. *Univ. Texas Publ.* no. **5721**, 260–316.

Sturtevant, A. H. 1929 The genetics of *Drosophila simulans*. *Carnegie Inst. Wash. Publ.* **399**, 1–62.

Tsacas, L. 1971 *Drosophila teissieri*, nouvelle espèce africaine du groupe *melanogaster* et note sur deux autres especès nouvelles pour l'Afrique. (Diptera: Drosophilidae). *Bull. Soc. Ent. France* **76**, 35–45.

Tsacas, L. & David, J. 1974 *Drosophila mauritiana* n.sp. du groupe *melanogaster* de l'Ile Maurice (Dipt.: Drosophilidae). *Bull. Soc. Ent. France* **79**, 42–46.

Tsacas, L. & Lachaise, D. 1974 Quatre nouvelles especès de la Cote d'Ivoire du genre *Drosophila*, groupe *melanogaster*, et discussion de l'origine du sous-groupe *melanogaster* (Diptera: Drosophilidae). *Ann. Univ. Abidjan* E **7** (1), 193–211.

White, M. J. D. 1973 *Animal cytology and evolution*. Cambridge University Press.