Hereditas 113: 157–168 (1990)

The phylogeny of nine species of the *Drosophila obscura* group inferred by the banding homologies of chromosomal regions

II. Element E

ANTONIO BREHM and COSTAS B. KRIMBAS

Department of Genetics, Agriculture University of Athens, Athens, Greece

BREHM, A. and KRIMBAS, C. B. 1990. The phylogeny of nine species of the *Drosophila obscura* group inferred by the banding homologies of chromosomal regions. II. Element E. — *Hereditas 113*: 157–168. Lund, Sweden. ISSN 0018-0661. Received June 19, 1990. Accepted August 21, 1990

The phylogenetic relationships among nine species of *Drosophila* belonging to the *obscura* group were investigated by establishing (according to their banding similarities) the homologous chromosome segments of element E (equivalent to chromosome O of *D. subobscura*). The phylogenetic relationships were based on the existence of segments in different triads of species, which could only be produced by overlapping inversions. This permitted the ordering of the species belonging to each triad. *Drosophila obscura*, *D. ambigua* and *D. tristis* were found to be very closely related and thus forming a cluster in which *D. ambigua* occupies an intermediate position between the other two species. *Drosophila obscura* seems to be the species more directly linked to three other separate lineages, that of *D. subsilvestris*, the two African species (*D. microlabis* and *D. kitumensis*), and the *subobscura* cluster. The species from this last cluster may be ordered as follows: *D. subobscura* — *D. madeirensis* — *D. guanche*. It is not clear which species of this triad is the direct link to *D. obscura*. These results completely agree with those produced in an independent study, where element B was considered for the same nine species. Furthermore, the present study clarifies some ambiguities concerning the phylogenetic relationships which remained obscure due to the conservative nature of chromosome B.

Costas B. Krimbas, Department of Genetics, Agriculture University of Athens, 75 Iera Odos, 11855 Athens, Greece

In the previous paper of this series (BREHM et al. 1990) we have reported the results of a study concerning the homologies of segments belonging to element B (according to the terminology of MULLER 1940) for nine species of the *obscura* group. From these data, phylogenetic relationships among these species were inferred. In the present study we pursue further the examination of the phylogenetic relations with data concerning element E for the same nine species. Thus, we are in a position to compare phylogenies derived from independent chromosomes and discuss their agreement.

The ultimate aim of these studies is to produce phylogenetic trees that will depict accurately the sequence of natural events that have occurred. Trees based on several studies of chromosomal data (there are five long chromosomal elements that can be studied independently) will be compared with DNA sequence data gathered from the same species. A consensus tree, eventually produced, could be considered as a good approximation to the "natural" tree. It can then be used to replace invented trees in simulation procedures in order to test the performance of different genetic distance estimators and/or algorithms for tree construction when electrophoretic and molecular data are utilized. The adoption of the most efficient distance estimators and algorithms is a valuable information for those using electrophoretic or, possibly, other kinds of data to detect phylogenetic relationships.

Until now, some studies have appeared in which banding similarities are used in order to ascertain homologies between sections or regions of salivary gland chromosomes of *Drosophila* (for the *D. melanogaster* subgroup, see LEMEUNIER and ASHBURNER 1976, 1984, for the Hawaiian picture wing *Drosophila*, see CARSON and KANESHIRO 1976, for the *repleta* group WASSERMAN 1982, and for the *virilis* group, see THROCKMORTON 1982), but these generally concern species that are evolutionary closely related. It is indeed difficult, but by no ways impossible (STALKER 1972), to deal with *Drosophila* species further apart. Regarding the *obscura* group of species, phylogenies based on overlapping inversions have been constructed for the *pseudoobscura* cluster (DOBZHANSKY 1970), the *bifasciata* cluster

(YAMAGUCHI 1973), and for the subgroup *affinis* (MILLER 1977). Finally, KRIMBAS and LOUKAS (1984) and BREHM and KRIMBAS (1990a), homologized the species from the *subobscura* cluster. However, a serious work trying to link the different clusters from the subgroup *obscura* using such a classical approach has not been conducted until now.

Attempts to establish a phylogeny for the *obscura* group of species have been made using diverse techniques. LAKOVAARA et al. (1976), LOUKAS et al. (1984), CABRERA et al. (1983), and CARIOU et al. (1988) constructed phylogenetic trees using electrophoretic data. Most of the times the patterns depicted by such trees are not in agreement with each other. The first two works appear to be the ones more consistent either with morphological characters or chromosome banding pattern data. In the tree depicted by Cariou et al., the *obscura* cluster seems to occupy a central position in the group, from which the *subobscura*, the African, and the *pseudoobscura* clusters may have departed, which is supported by the data we present here.

Mitochondrial DNA also has been used to track phylogenetic relationships. The technique, while having a wide range of applications between populations of the same species (AVISE et al. 1979a) or very closely related species (AVISE et al. 1979b), has severe limitations. LATORRE et al. (1986) could not find the European population of *D. subobscura* from which the colonizers of Chile (and the rest of the New World) may have originated and, attempting to do a phylogeny for the *obscura* group, clustered the species in evident opposition to data obtained from morphological characters, chromosomal homologies, and electrophoretic markers (LATORRE et al. 1988).

More recently GODDARD et al. (1990), using DNA-DNA hybridization, made a phylogeny for 5 species from the *obscura* group, but none in common to the nine studied by us.

The present study and the data in BREHM et al. (1990) prove that using an especially rich photographic material chromosomal homologies can be established for most part, if not for the entire length, of the chromosome even if the species involved are not so closely related.

Material and methods

D. subobscura strains used in this study, originated from Crete (monomorphic for the O_{3+4} gene arrangement) and from Switzerland (Kusnacht, monomorphic for the O_{st} gene arrangement). *D. madeirensis* and *D. guanche* strains originated, respectively, from the Madeira and Canary Islands. One strain of *D. kitumensis* and another of *D. microlabis* both originated from Kenya (CARIOU et al. 1988). A number of European strains of *D. obscura* were investigated but the gene arrangement depicted in the photographs is found in a strain from Switzerland. One strain of *D. tristis*, one from *D. ambigua*, and one from *D. subsilvestris*, all from Switzerland, were also used.

Details on culture of strains, salivary gland preparations and photography of slides are as the ones described in BREHM et al. (1990).

Except for the species belonging to the *subobscura* cluster (*D. subobscura*, *D. madeirensis* and *D. guanche*) the species studied do not hybridize.

Results

From the comparison of a number of homologous segments produced by overlapping inversions between triads of the nine species studied, it became apparent that the arrangement displayed by *D. obscura* occupies an intermediate position. Thus, we decided to use this species as a pivotal extant gene arrangement with which all others are directly or indirectly compared. Of course this species displays an extremely rich inversion polymorphism which is discussed elsewhere (BREHM and KRIMBAS 1990b). We chose one common gene arrangement, which we present here, in order to proceed to comparisons with the other species studied. This gene arrangement was divided in 13 sections in order to facilitate identification of segments in the other species.

Except for *D. obscura*, *D. subobscura* and *D. kitumensis* all the other species are considered to be monomorphic for this chromosome. *D. obscura* is polymorphic for 6 inversions but none of them is useful for determining phylogenetic relationships with any of the other 8 species studied. *D. kitumensis* is polymorphic for an inversion by which it differs from its closely related species, *D. microlabis*.

Segment of chromosomes from all species, displayed in Fig. 3 to 5, are compared with segments of *D. obscura* that can be exactly located in the entire sequence of this species, displayed in Fig. 1 and 2. In order to avoid doubtful inferences, the overlapping homologies used to construct the unrooted phylogenetic tree presented in the Discussion are made of segments for which the banding patterns are clearly identical in a given triad of species.

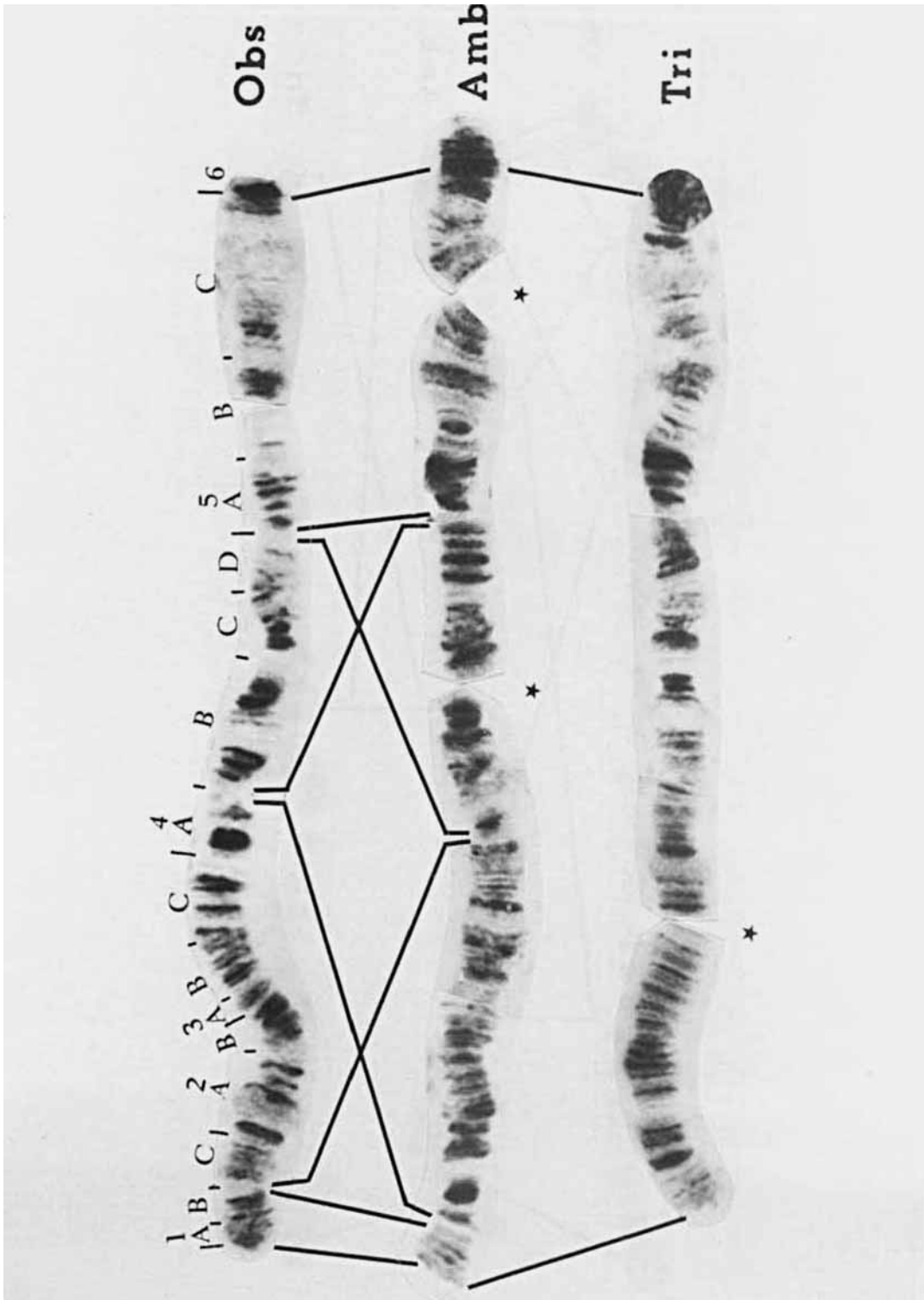


Fig. 1.

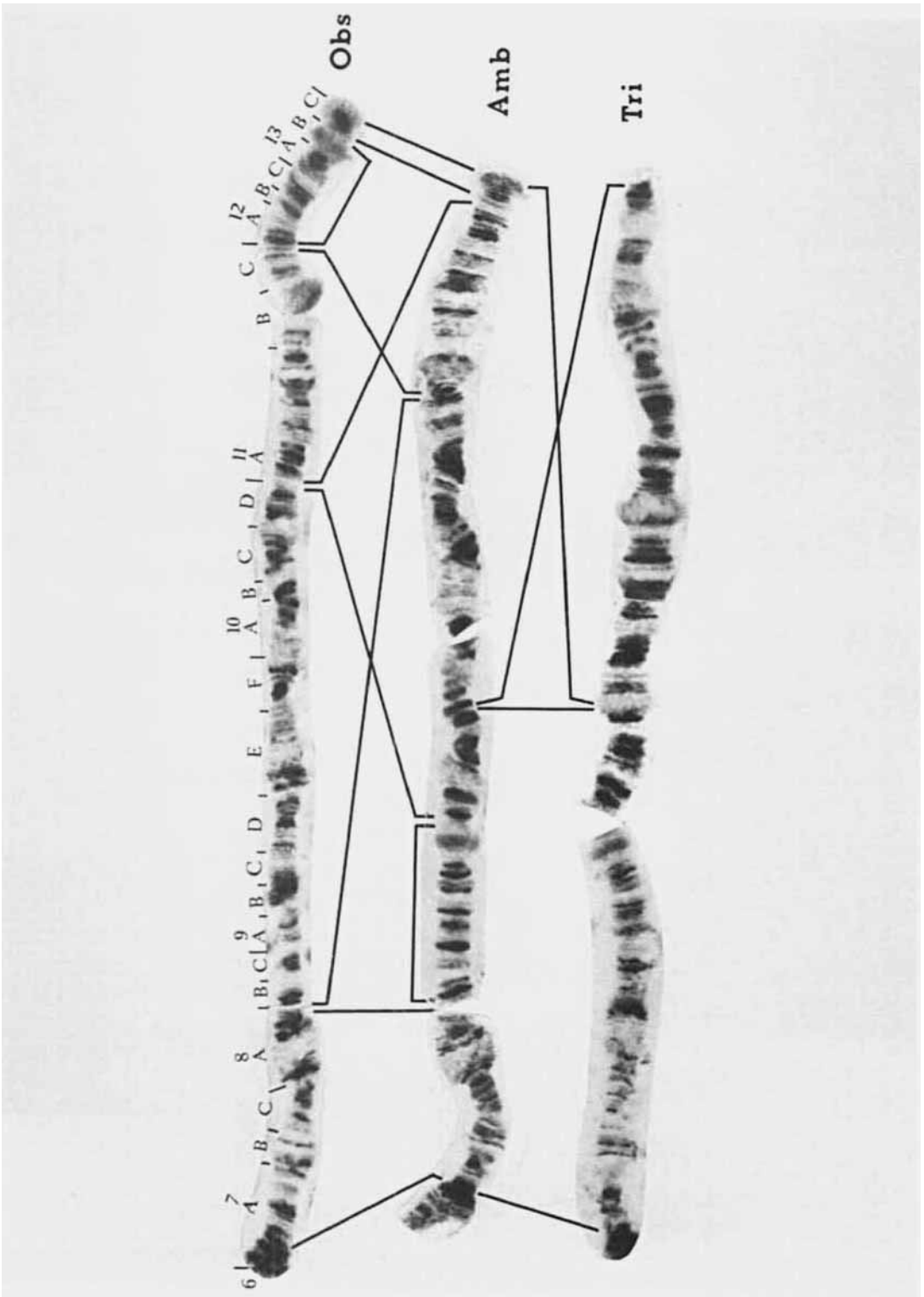


Fig. 2.

It seems relatively simple to derive *D. ambigua*'s gene arrangement from that of *D. obscura* (Fig. 1 and 2). Only four inversions are needed for that, two located in the left arm and two overlapping ones in the right arm; these last ones produce the displacement of a segment comprising sections 12A to 13B by inverting it twice subsequently. *D. tristis* differs only by one inversion from *D. ambigua*; however, this inversion is an overlapping one on the previously mentioned inversions in the right arm of *D. ambigua*. This observation permitted us to order the gene arrangements of the three species depicted in the following sequence, according to the principles stated by STURTEVANT and DOBZHANSKY (1936) and DOBZHANSKY (1937):

D. obscura — *D. ambigua* — *D. tristis*

The gene sequences of these two species in relation to the *D. obscura*'s standard gene arrangement are

ambigua 1AB/4A–1C/4DCB/5A–8A/12A–13B/10D–8B/11CBA/13C
tristis 1AB/4A–1C/4DCB/5A–8A/12A–13B/10DCB/13C/11ABC/8B–10A

The chromosomal element E of *D. guanche* compared with that of *D. obscura* indicates extensive rearrangements. *D. guanche* (as well as its closely related *D. subobscura* and *D. madeirensis*) have an acrocentric chromosome while in all other species examined it is metacentric. The centromere of *D. guanche*'s element E lies at the extreme left part of the chromosome, as depicted in Fig. 3. The homology of segments 3C/4A is not completely convincing, but after consulting a great amount of photographs for this region we are inclined to interpret them as homologous. For other segments we could not find any correspondence between the two species. The remaining segments show fairly good homologies and some could be easily identified with the help of landmarks as, for example, the puff regions of subsections 5BC and 11B.

The situation between the three species, *D. subobscura, D. madeirensis* and *D. guanche* is quite clear because of the presence of overlapping inversions. *D. madeirensis* has a gene arrangement characterized by inversion 0_3 (which is fixed in all chromosomes 0, equivalent to the E element), while in *D. guanche* over the inversion 0_3 another one is superimposed specific to it (KRIMBAS and LOUKAS 1984). *D. subobscura* has several arrangements deriving from two basic ones: one bears the combination 3+4 (that is an inversion 0_4 superimposed (overlapping) on inversion 0_3), and a second one is 0_{st}. The order of gene arrangements, according to the rule of Sturtevant and Dobzhansky, is

$$0_{st} \text{ —— } 0_3 \begin{cases} 0_{3+4} \\ 0_{3+g} \end{cases}$$

where g is an inversion specific to *D. guanche*.

D. subobscura natural populations contain both 0_{st} and 0_{3+4} gene arrangements but not 0_3. We may suppose that, originally, 0_3, the middle gene arrangement of the triad, was sometimes present also in *D. subobscura* but has been lost later on, probably according to WALLACE's rule (1953a, b). This rule predicts that the middle member of a triad tends to be selectively lost in order to preserve coadaptated gene blocks included in the two extreme members of the triad. Taking all this into consideration, the ordering of the three *subobscura* cluster species may be indicated as follows either:

D. guanche — *D. madeirensis* — *D. subobscura*

or, if we take into consideration that *D. subobscura* once contained also the 0_3 gene arrangement:

D. guanche — D. madeirensis / D. subobscura

From the data gathered it is not possible to know which one of the three species is directly related to *D. obscura*. This species could be more directly derived either by an 0_{3+g}, 0_3 or 0_{st} arrangement but not by 0_{3+4}. The arrangement 0_{3+4} could not be a link to *D. obscura*. As shown in Fig. 3, the segment 7AB and part of C is clearly homologized to *D. guanche*. The inversion 4 (from the arrangement 0_{3+4}), marked as 2/2 on the chromosome of this last species, would split the segment 7ABC into two. The breakage points for the other inversions (0_3, 0_{3+g} and 0_{st}) do not lie in any homologized segment

Fig. 1 and 2. Homologies of the small and long arms of element E from *D. obscura* (**OBS**), *D. ambigua* (**AMB**), and *D. tristis* (**TRI**). The Standard gene arrangement of *D. obscura* is depicted and divided in sections in order to facilitate the recognition of segments in the other species. In order to get a better visualization of the homologies, the photographs were cut in appropriate places to make them linear. Thus all intervals (nicks, *) between places of chromosome photographs do not correspond to genetic material.

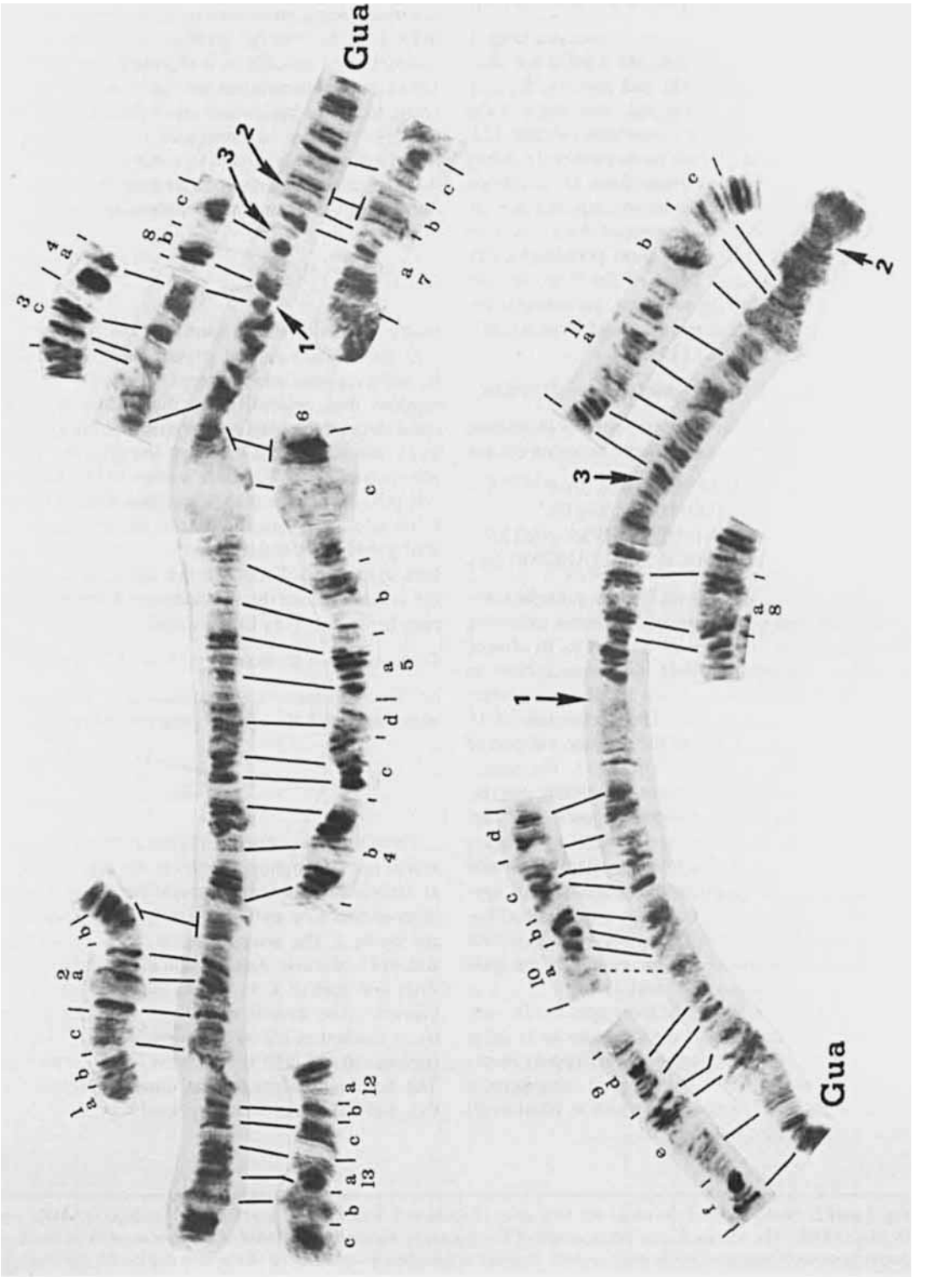


Fig. 3.

and thus can not provide a clue for inferring which of them is the link to the standard arrangement of *D. obscura*.

In Fig. 3 we indicate the breakage points of these inversions by arrows on the *D. guanche* chromosome: 1/1 refers to the inversion specific to *D. guanche*, 3/3 to that of the 0_3, and 2/2 to that of 0_4. These breakage points may not coincide with those of MOLTO and MARTINEZ-SEBASTIAN (1986) and MOLTO et al. (1987) but have been derived from the comparison of photographs of the gene arrangements mentioned above.

All three species from the *subobscura* cluster could have been derived only from *D. obscura* (and not from *D. ambigua* or *D. tristis*). The proof for this assertion is segment 4B–5C, which is intact only in *D. obscura* and every species of the *subobscura* cluster.

D. guanche could not be derived directly from *D. subsilvestris*. In *D. guanche*, segment 10ABCD is intact, as it is in *D. obscura*, but in *D. subsilvestris* it is split into two. Besides, in *D. guanche*, the segment 8BC is not followed by the bands of section 9A. Segment 8BC9A is a landmark for the remaining species (it appears unchanged in *D. subsilvestris*, the African, and the *obscura* cluster).

The gene sequence of *D. guanche* chromosome expressed in terms of the standard map of *D. obscura* is the following:

...13B-12B/.../1C-2B/4B-5C/.../3C4A/8BC/7A-C/.../9F-D/10A-D/.../8A/.../11A-11C/...

In Fig. 4, the relationships of segments from *D. subsilvestris*'s chromosome have been established with respect to those of *D. obscura*. In spite of having large homologous portions, *D. subsilvestris* comprises many displaced and/or inverted segments.

The picture on the small arm of both species is clear, with the exception, perhaps, of subsection 3B. The other arm, on the contrary, presents more difficulties. Homologies of subsections 12C/13A are doubtful and should be simply considered as our interpretation. The other photographs are convincing enough for the remaining segments. Subsection 13B, in spite of being just a small puff, is a well-known landmark. The gene sequence of *D. subsilvestris* in relation to that of *D. obscura* is the following:

3C-5B/1BA/3B-1C/5C-8A/10AB/9B-F/13B/10DC/12C/9A-8B/13A/12B-11A/13C.

D. subsilvestris could not be derived from *D. ambigua* or *D. tristis* but only from *D. obscura*. The segment 4B to 5C is carried by *D. obscura* and *D. subsilvestris* but not by the other two species. It contains one of the breakage points for an inversion in the transition from *D. obscura* to *D. ambigua* (and consequently to *D. tristis*). The same segment excludes all hypotheses in which any of the African species is an intermediate step between the two species *D. obscura* and *D. subsilvestris*.

In Fig. 5 the gene arrangement of another cluster of species is depicted, that of the two African, *D. kitumensis* and *D. microlabis*. The direct comparison is made between *D. kitumensis* and *D. obscura*. Some small segments in this case remain unidentified as far as their correspondence is concerned. *D. kitumensis* is polymorphic for an inversion by which it differs from the gene arrangement of *D. microlabis*, as indicated in Fig. 5.

Segments of the *D. obscura* element E were homologized either to *D. microlabis* or to *D. kitumensis*, depending of the quality of photographs. Some of the homologies were arrived at with the help of classical landmarks like the puffs of subsections 5C and 11B. If the small arm is reasonably recognized (with the exception of two small segments and subsection 1AB, which are not so clear), problems arise with the longer arm. We did not find any correspondence for the entire section 7 and subsections 13BC. However, an important segment, 8A–9A, was easily homologized, and in *D. subsilvestris* its resemblance with *D. kitumensis* is even more clear. This segment includes one breakage point of a big inversion, which makes the difference between *D. kitumensis* and *D. microlabis*, fixing a seriation of this cluster with *D. obscura* as

D. microlabis — *D. kitumensis* — *D. obscura*

Segment 10CD11A can be found in *D. kitumensis* and in *D. obscura*, but not in *D. subsilvestris*, where it is splitted in two separate segments.

A careful reading of subsections 10AB shows

Fig. 3. Homologies of element E between *D. guanche* (**GUA**) and *D. obscura*. *D. guanche* is shown in its complete sequence. The number 6 always represents the centromere. Arrows with numbers 1 to 3 are breakpoints of known inversions of the *subobscura* cluster (1 is of the g inversion, 2 of 0_3 and 3 of 0_4).

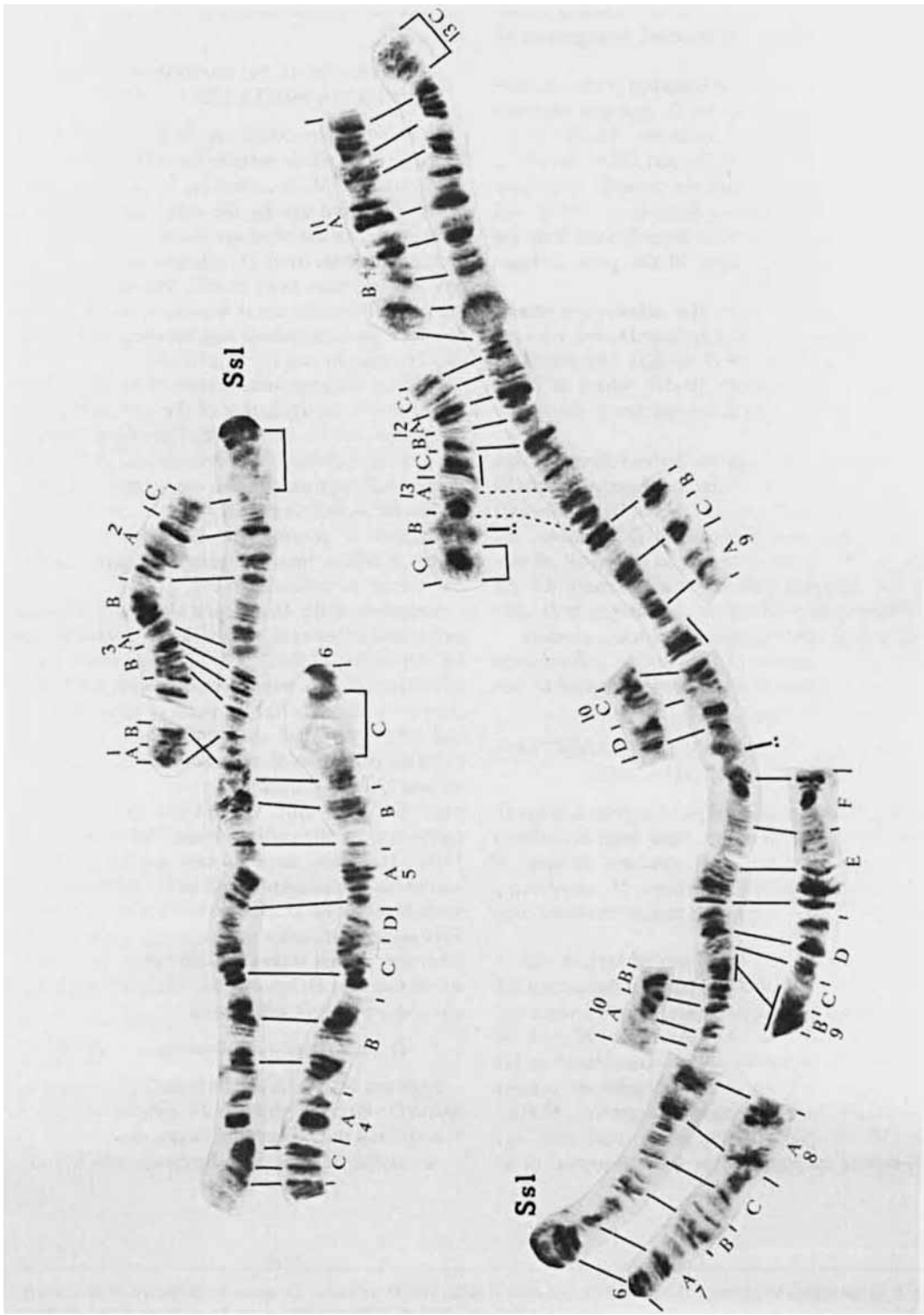


Fig. 4. Homologies of element E between *D. subsilvestris* (**SSL**) and segments of *D. obscura*. *D. subsilvestris* is shown in its complete gene sequence.

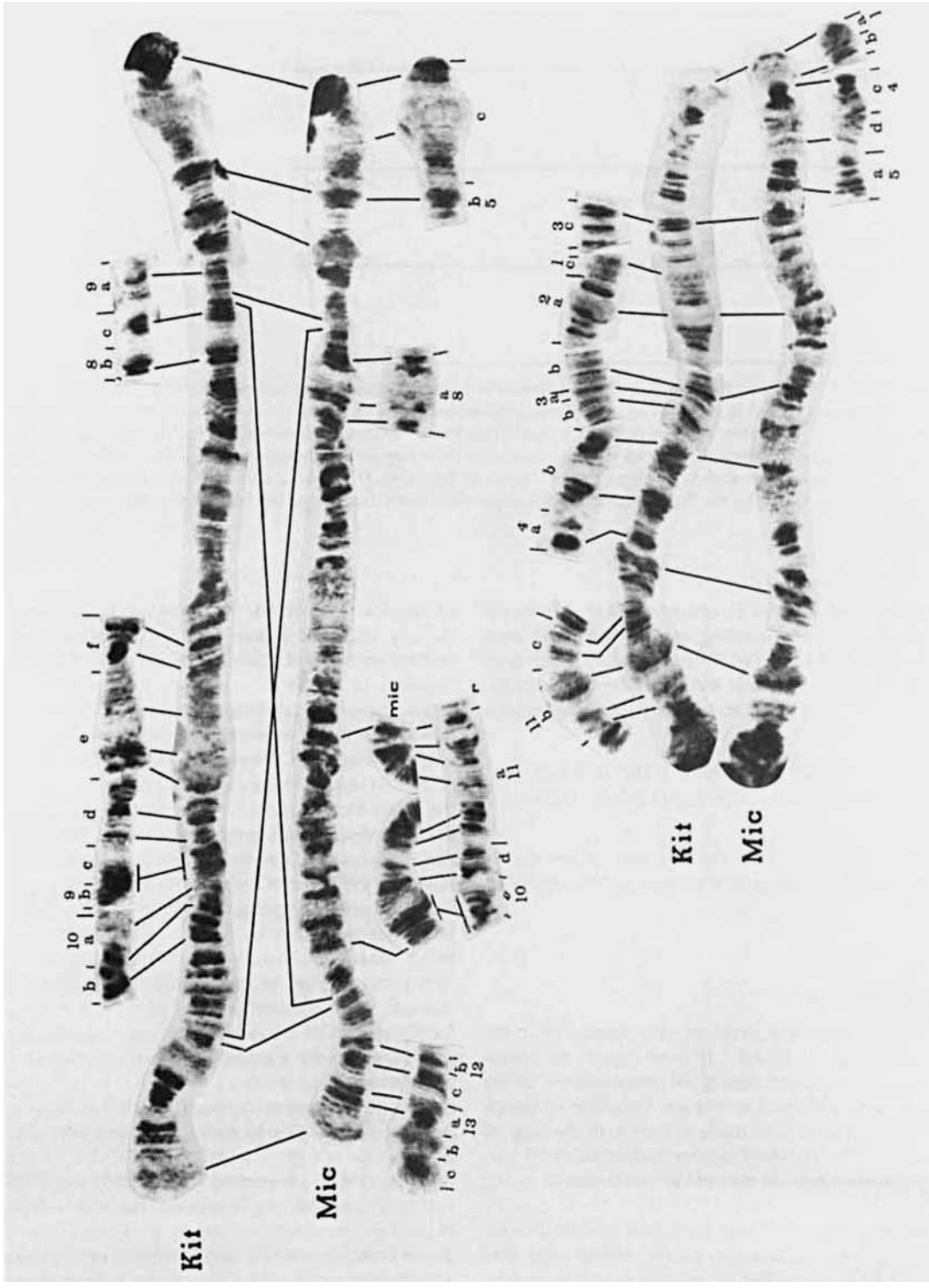


Fig. 5. Homologies of element E between the two species of the African cluster, *D. microlabis* (**MIC**) and *D. kitumensis* (**KIT**) with segments from *D. obscura*'s Standard sequence. The two African species are shown in their complete gene sequence.

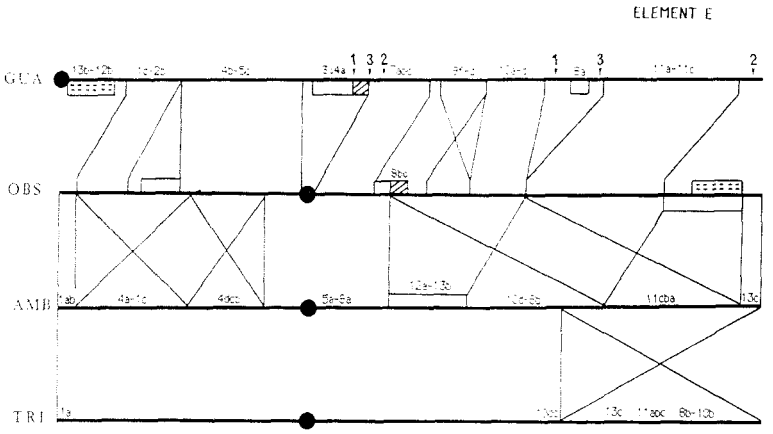


Fig. 6 and 7. Schematic representation of homologies between the nine species used in the present study. Segments that are found inverted in two species are indicated by crossed lines or boxes with arrows with inverted directions. When there are more, then one pair of boxes is used between two species, their correspondence is indicated by using different patterns. Sections of each segment are numbered according to the photographic map of *D. obscura*, and in accordance to the ones depicted in Fig. 1 to 5. Numbers 1 to 3 on *D. guanche* chromosome indicate the specific inversions differentiating the three species of the *subobscura* cluster (see text). The black circle represents the centromere.
Fig. 6.

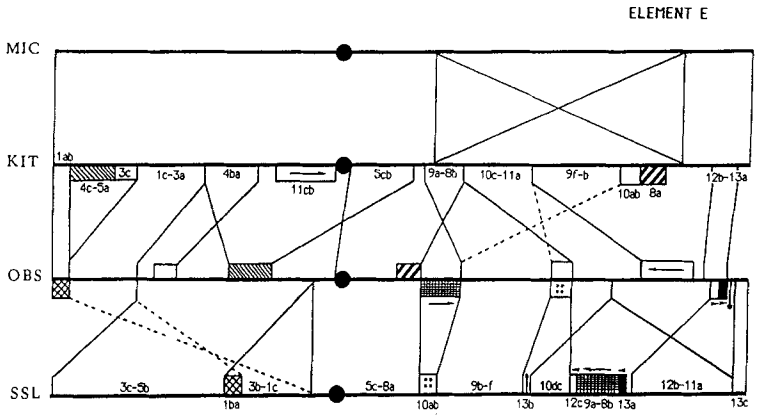
that it is the same in *D. obscura* and *D. kitumensis* but some other remaining segments are unknown or doubtful, because of their small size, which does not permit to establish with certainty the homologies. The complete gene sequence of *D. kitumensis* is

1AB/4C-5A/$\overline{3C}$/1C-3A/4BA/3B/11CB/6/5CB/.../9A-8B/10C-11A/9F-B/10AB/8A/.../12B-13A/...

where $\overline{3C}$ means that the segment is inverted in relation to the position it occupies in *D. obscura*.

Discussion

The data presented here can be summarized in the drawings of Fig. 6 and 7. In these Figures the homologies of segments among all chromosomes of the nine species studied are shown. Detection of homologous sections was made in part with the help of six puff regions which appear unchanged in all species studied and are situated in subsections 2A, 5C, 8A, 11A (part), 11B and 13B from the map of *D. obscura*. These puff regions behave like landmarks, having the same function as, for example, the sites of label of a number of repetitive genes used by Steinemann et al. (1984) in an attempt to investigate chromosomal homologies in the *obscura* group of species. Two of the recombinant DNA clones (λDmir 1023 and λDmir 1025) reported by these authors are situated in element E in the four species common to our work (*subobscura, obscura, ambigua, subsilvestris*). One other clone (12D8) was labeled on the E element in *subobscura* and *obscura*, but on the B element in *ambigua* and *subsilvestris*. In none of these cases do the authors present the exact localization of the label sites as well as the complete homologous segments of the same chromosome in all species studied. Also Felger and Pinsker (1987) made a phylogeny for 7 species, all from the *obscura* group, using the histone genes' label sites as landmarks, in spite of these genes not being located in homologous chromosomal elements and varying in their number in the species studied. They constructed two phylogenetic trees for the species of the *subobscura* and *obscura* clusters, based on the number of inversions needed to pass from one to another species. However, these authors do not present a comparison of chromosome banding homologies to make these numbers reliable. We do not agree with the data of Felger and Pinsker (1987) concerning the species from the *subobscura* cluster (see Brehm and Krimbas 1990a) as well as those concerning the E element, which do not coincide with the data presented here. Felger and Pinsker found a four inversions difference between *ambigua* and *obscura*, four between *ambigua* and *tristis*, and eight between *obscura* and *tristis*.



in opposition to our present results which are four, one and five, respectively. However, it is possible that the differences are due to the use of strains with more complicated gene arrangements.

The present work is the first that presents element E banding sequence homologies for the nine species studied. Based on these homologies and using *only* qualitative criteria, that is to say, the rule of triads of gene arrangements enunciated by Sturtevant and Dobzhansky, we may be able to arrive at a phylogenetic topology, an unrooted phylogenetic tree, in the following way. It has been noted already that the three species of the *subobscura* cluster, regarding the inversions 0g, 0_3 and 0_4, can be arranged in the linear way:

D. subobscura — D. madeirensis — D. guanche

Furthermore, based on the inversions of the right arm of *D. tristis* and the two other species of the *obscura* cluster we may arrive at the ordering:

D. obscura — D. ambigua — D. tristis

In case *D. kitumensis* would lose its polymorphism and get fixed for the alternative gene arrangement to that fixed in *D. microlabis*, we would also produce an ordering of a third triad.

D. microlabis — D. kitumensis — D. obscura

Three more orderings may be added to these, all of them deriving from the data summarized in Fig. 6 and 7:

D. subsilvestris — D. obscura — D. kitumensis,

based on the inversions of the small arm of element E;

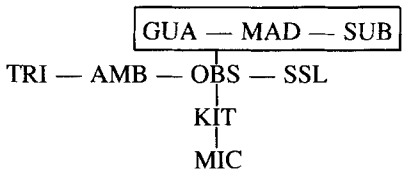
D. kitumensis — D. obscura — D. guanche,

based on the segments 4AB and 4B-5C; and finally

D. subsilvestris — D. obscura — D. guanche,

based on the segments 3C-5B and 4B-5C. To these data one should add that *D. subsilvestris. D. obscura,* and the two African species share a segment, 8BC9A, which is not found intact in the remaining species studied here.

There is only one way to arrange all these orderings, respecting also this constraint, in an unrooted phylogenetic tree:



This topological arrangement agrees completely with the one presented in the first paper of this series, dealing with element B, in which three separate lineages (that of the two African species, *D. subsilvestris,* and the *subobscura* cluster) derived independently from the members of the *obscura* cluster. Due to the conservative nature of the B element this tree contained a restricted amount of information. Element E is a much richer source of information, and we can furthermore clarify several features of this topology. Thus the species of the *subobscura* cluster are resolved, as well as those of

the *obscura* cluster; it is not clear which one of the species of the *subobscura* cluster is directly linked to *D. obscura*. This last species helds a pivotal place, from which four different lineages are derived. The excellent agreement between two trees, derived from independent sources of data (two independent chromosomes), is a strong indication for the validity of the qualitative method used.

References

AVISE, J., GIBLIN-DAVIDSON, C., LAERM, J., PATTON, J. and LANSMAN, R. 1979a. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher *Geomys pinetis*. — *Proc. Natl. Acad. Sci. USA 76*: 6694–6698

AVISE, J., LANSMAN, R. and SHADE, R. 1979b. The use of restriction endonuclease to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. — *Genetics 92*: 279–295

BREHM, A. and KRIMBAS, C. B. 1990a. Evolution of the *obscura* group *Drosophila* species. III. Phylogenetic relationships in the *subobscura* cluster based on homologies of chromosome A. — *Heredity* (in press)

BREHM, A. and KRIMBAS, C. B. 1990b. Inversion polymorphism in *Drosophila obscura*. — *J. Hered.* (in press)

BREHM, A., KRIMBAS, C. B., SOURDIS, J. and CARIOU, M. L. 1990. The phylogeny of nine species of the *Drosophila obscura* group inferred by the banding homologies of chromosomal regions. I. Element B. (submitted)

CABRERA, V., GONZALEZ, A., LARRUGA, J. and GUILLON, A. 1983. Genetic distance and evolutionary relationships in the *Drosophila obscura* group. — *Evolution 37*: 675–689

CARIOU, M. L., LACHAISE, D., TSACAS, L., SOURDIS, J., KRIMBAS, C. and ASHBURNER, M. 1988. New African species in the *Drosophila obscura* species group: genetic variation, differentiation and evolution. — *Heredity 61*: 73–84

CARSON, H. L. and KANESHIRO, K. Y. 1976. *Drosophila* of Hawaii: Systematics and ecological genetics. — *Annu. Rev. Ecol. Syst. 7*: 311–345

DOBZHANSKY, TH. 1937. *Genetics and the Origin of Species*. — *Colombia University Press. New York*

DOBZHANSKY, TH. 1970. *Genetics of the Evolutionary Process*. — *Columbia University Press. New York*

FELGER, I. and PINSKER, W. 1987. Histone gene transposition in the phylogeny of the *Drosophila obscura* group. — *Z. Zool. syst. Evol. Forsch. 25*: 127–140

GODDARD, K., CACCONE, A. and POWELL, J. R. 1990. Evolutionary implications of DNA divergence in the *Drosophila obscura* group. — *Evolution* (in press)

KRIMBAS, C. B. and LOUKAS, M. 1984. Evolution of the *obscura* group of *Drosophila* species. I. Salivary chromosomes and quantitative characters in *Drosophila subobscura* and two closely related species. — *Heredity 53*: 469–482

KRIMBAS, C. B. and SOURDIS, J. 1987. Recent improvements in handling allelic isozyme data for tree construction. — *Isozymes. Curr. Top. Biol. Med. Res. 15*: 49–62

LAKOVAARA, S., SAURA, A., LANKINEN, P. POHJOLA, L. and LOKKI, J. 1976. The use of isoenzymes in tracing evolution and in classifying Drosophilidae. — *Zool. Scripta* 5: 173–179

LATORRE, A., MOYA, A. and AYALA, F. 1986. Evolution of mitochondrial DNA in *Drosophila subobscura*. — *Proc. Natl. Acad. Sci. USA 83*: 8649–8653

LATORRE, A., BARRIO, E., MOYA, A. and AYALA, F. 1988. Mitochondrial DNA evolution in the *Drosophila obscura* group. — *Mol. Biol. Evol. 5*: 717–728

LEMEUNIER, F. and ASHBURNER, M. 1976. Relationships within the *melanogaster* species group of the genus *Drosophila (Sophophora)*. II. Phylogenetic relationships between six species based upon polytene chromosome banding sequences. — *Proc. R. Soc. London Ser. B 193*: 275–294

LEMEUNIER, F. and ASHBURNER, M. 1984. Relationships within the *melanogaster* species group of the genus *Drosophila (Sophophora)*. IV. The chromosomes of two new species. — *Chromosoma 89*. 343–351

LOUKAS, M., KRIMBAS, C. and VERGINI, Y. 1984. Evolution of the *obscura* group *Drosophila* species. II. Phylogeny of ten species based on electrophoretic data. — *Heredity 53*: 483–493

MILLER, D. 1977. Salivary gland chromosome variation in the *Drosophila affinis* sub-group. VI. Comparison of X, B and E chromosome patterns in *D. athabasca* and five related species. — *J. Hered. 68*: 105–113

MOLTO, M. D. and MARTINEZ-SEBASTIAN, M. J. 1986. Gene arrangements in polytene chromosomes of *Drosophila guanche* differing from standard arrangements in *D. subobscura*. — DIS *63*: 97–98

MOLTO, M. D., DE FRUTOS, R. and MARTINEZ-SEBASTIAN, M. J. 1987. The banding pattern of polytene chromosomes of *Drosophila guanche* compared with that of *D. subobscura*. — *Genetica 75*: 55–70

MULLER, H. J. 1940. Bearings of the *Drosophila* work on systematics. — In: *The New Systematics. (ed* J. HUXLEY), *Oxford University Press, London*, p. 185–268

KUNZE-MÜHL, E. and MÜLLER, E. 1958. Weitere Untersuchungen über die chromosomale Struktur und natürlichen Strukturtypen von *Drosophila subobscura*. — *Chromosoma 9*: 559–570

SOURDIS, J. and KRIMBAS, C. B. 1987. Accuracy of phylogenetic trees estimated from DNA sequence data. — *Mol. Biol. Evol. 4*: 159–166

SOURDIS, J. and KRIMBAS, C. B. 1990. Inferring chromosome trees from inversion data. — In: *Drosophila Inversion Polymorphism (eds* C. B. KRIMBAS *and* J. R. POWELL), *CRC Press, Boca Raton, Florida* (in press)

STALKER, H. D. 1972. Intergroup phylogenies in *Drosophila* as determined by comparison of salivary banding patterns. — *Genetics 70*: 457–474

STEINEMANN, M., PINSKER, W. and SPERLICH, D. 1984. Chromosome homologies within the *Drosophila obscura* group probed by in situ hybridization. — *Chromosoma 91*: 46–53

STURTEVANT, A. H. and DOBZHANSKY, TH. 1936. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura* and their use in the study of the history of the species. — *Proc. Natl. Acad. Sci. USA 22*: 448–450

THROCKMORTON, L. H. 1982. The *virilis* species group. — In: *The Genetics and Biology of Drosophila, vol 3 b (eds* M. ASHBURNER, H. L. CARSON *and* J. N. THOMPSON JR), *Academic Press, London*, p. 227–296

WALLACE, B. 1953a. Coadaptation and the gene arrangements of *Drosophila pseudoobscura*. — In: *International Union of Biological Sciences Symposium on Genetics of Population Structure. Fusi, Pavia*. p. 67–94

WALLACE, B. 1953b. On coadaptation in *Drosophila*. — *Am. Nat. 87*: 343–358

WASSERMAN, M. 1982. Evolution of the *repleta* group. — In: *The Genetics and Biology of Drosophila (eds* M. ASHBURNER, H. L. CARSON, *and* J. N. THOMPSON JR), *Academic Press, London*, p. 61–139

YAMAGUCHI, D. 1973. Salivary gland chromosome relationship between *Drosophila bifasciata* and *D. imaii*. — *Cytologia 38*: 603–614