Molecular Organization of the *X* Chromosome in Different Species of the Obscura Group of Drosophila

Carmen Segarra and Montserrat Aguadé

Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, 08071 Barcelona, Spain

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ABSTRACT

Nine single copy regions located on the *X* chromosome have been mapped by *in situ* hybridization in six species of the obscura group of Drosophila. Three Palearctic species, *D. subobscura*, *D. madeirensis* and *D. guanche*, and three Nearctic species, *D. pseudoobscura*, *D. persimilis* and *D. miranda*, have been studied. Eight of the regions include known genes from *D. melanogaster* (*Pgd*, *zeste*, *white*, *cut*, *vermilion*, *RNA polymerase II 215*, *forked* and *suppressor of forked*) and the ninth region (λ*DsubF6*) has not yet been characterized. In all six species, as in *D. melanogaster*, all probes hybridize to a single site. Established chromosomal arm homologies of Muller's element *A* are only partly supported by present results since two of the probes (*Pgd* and *zeste*) hybridize at the proximal end of the *XR* chromosomal arm in the three Nearctic species. In addition to the centric fusion of Muller's *A* (=*XL*) and *D* (=*XR*) elements, the metacentric *X* chromosome of the Nearctic species requires a pericentric inversion to account for this result. Previously proposed homologies of particular chromosomal regions of the *A* (=*X*) chromosome in the three species of the *D. subobscura* cluster and of the *XL* chromosomal arm in the three species of the *D. pseudoobscura* cluster are discussed in light of the present results. Location of the studied markers has changed drastically not only since the divergence between the melanogaster and obscura groups but also since the Palearctic and Nearctic species of the obscura group diverged.

In situ hybridization to polytene chromosomes of identified markers is a powerful technique to establish unambigously chromosomal homologies among *Drosophila* species. Before development of this technique, arm homologies between distant species were established by comparing linkage groups of morphological and electrophoretic markers. Such studies determined chromosomal homologies of MULLER's (1940) elements with chromosomes of *D. subobscura* (LOUKAS *et al.* 1979), *D. pseudoobscura* (PRAKASH 1977) and *D. melanogaster* (CAVENER 1977), among others. As summarized by LAKOVAARA and SAURA (1983), MULLER's element *A* is considered homologous to the *A* (=*X*) acrocentric chromosome of *D. subobscura*, to the *XL* arm of the sexual metacentric chromosome of *D. pseudoobscura* and to the *X* acrocentric chromosome of *D. melanogaster*.

Study of polytene chromosomes in interspecific hybrids also gives information about the homology of particular chromosomal regions between closely related species, although it can only be applied to species that can produce viable hybrids. This methodology has been used to study chromosomal homologies between *D. subobscura* and *D. madeirensis* (KRIMBAS and LOUKAS 1984; PAPACEIT and PREVOSTI 1989, 1991; BREHM and KRIMBAS 1990a), *D. madeirensis* and *D. guanche* (KRIMBAS and LOUKAS 1984), *D. pseudoobscura*, *D. persimilis* and *D. miranda* (DOBZHANSKY and TAN 1936), and *D. pseudoobscura* and *D. persimilis* (MOORE and TAYLOR 1986).

Direct analysis of banding patterns using particular landmarks, such as developmental and heat-shock puffs or points with a tendency to spontaneously break, can also be used to identify homologies of particular chromosomal regions between species with slight differences in chromosomal morphologies. This kind of study allowed MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN (1987) to build a map of salivary gland chromosomes of *D. guanche* by comparison with that of *D. subobscura* (KÜNZE-MÜHL and MULLER 1958) and it allowed BREHM and KRIMBAS (1990a,b) to build a putative phylogeny based on chromosomal arrangements fixed during the divergence of different species of the obscura group.

Mapping by *in situ* hybridization of single copy regions located along chromosomal arms offers a new approach to the study of chromosomal homologies that gives more accurate and unambiguous information about the bands and interbands that are identical in different species. A few such studies have been carried out in different species of the obscura group of Drosophila (STEINEMANN 1982; STEINEMANN, PINSKER and SPERLICH 1984; FELGER and PINSKER 1987; LOUKAS and KAFATOS 1988; STEINEMANN and STEINEMANN 1990). These studies confirmed previously established arm homologies, but no attempt was made

to establish homologies of particular chromosomal regions in a single chromosome.

In the present study, nine single copy regions presumably located in a single chromosome have been mapped by *in situ* hybridization in six species of the obscura group of Drosophila. Eight of these regions correspond to known genes isolated in *D. melanogaster* that map on the *X* chromosome of this species. These genes are: *phosphogluconate dehydrogenase* (*Pgd*), *zeste* (*z*), *white* (*w*), *cut* (*ct*), *vermilion* (*v*), *RNA polymerase II 215* (*RpII215*), *forked* (*f*) and *suppressor of forked* (*su-f*), ordered according to their location from the tip to the proximal end of the *X* chromosome. The ninth region, λ*DsubF6*, is an additional marker that has not yet been characterized.

Regions on the *X* chromosome of *D. melanogaster* have been chosen in order to cover the whole length of this chromosome, but also to be able to test whether tightly and more loosely linked genes were maintained in the obscura group. In this sense two groups of tightly linked markers (*Pgd-z-w* and *v-RpII215*) were chosen.

The six species analyzed can be grouped into two different clusters. One cluster includes three originally Palearctic species, *D. subobscura* with a broad geographic distribution, and the two closely related species *D. madeirensis* and *D. guanche*, endemic to Madeira and to the Canary Islands, respectively. The second cluster includes three Nearctic species, *D. pseudoobscura*, *D. persimilis* and *D. miranda*.

The results test previously established chromosomal homologies of the *A* (=*X*) chromosome among the three species of the *D. subobscura* cluster and of the *XL* chromosomal arm of the Nearctic species that form the *D. pseudoobscura* cluster. At the same time, the data should allow identification of homologous regions between the *A* (=*X*) and *XL* chromosomes of the Nearctic and Palearctic species. Moreover, accurate cytological location of particular genes in these species of the obscura group will contribute to a better genetic characterization of these species, and to their possible use as genetic markers of particular gene arrangements.

MATERIALS AND METHODS

D. pseudoobscura, *D. persimilis* and *D. miranda* stocks were kindly provided by R. C. LEWONTIN. *D. subobscura*, *D. madeirensis* and *D. guanche* strains were available in our laboratory. The *chcu* strain of *D. subobscura* with the standard *A* (=*X*) arrangement was used.

Fly stocks were grown in uncrowded culture bottles at 17° on standard cornmeal medium. After dissecting salivary glands from third-instar larvae, polytene chromosome preparations suitable for *in situ* hybridization were performed according to MONTGOMERY, CHARLESWORTH and LANGLEY (1987).

Recombinant DNA plasmids including totally or partially *z* (MARIANI, PIRROTTA and MANET 1985), *w* (BINGHAM, LEVIS and RUBIN 1981), *ct* (JACK 1985), *v* (SEARLES and VOELKER 1986), *RpII215* (JOKERST *et al.* 1989), *f* (PARKHURST and CORCÉS 1985) and *su-f* (A. MITCHELSON, M. SIMONELIG and K. O'HARE, personal communication) genes from *D. melanogaster* were used as probes. Screening of a *D. subobscura* genomic library (AGUADÉ 1988) with a 4.7-kb *Eco*RI fragment that includes the first exon of the *Pgd* gene of *D. melanogaster* (J. C. LUCCHESSI, personal communication), allowed the isolation of a positive phage (J. M. MARTÍN-CAMPOS, personal communication) (λ*DsubPgd*) that was later used as probe to identify the location of this gene in the obscura group species. The same *D. melanogaster* fragment used for the screening of the genomic library was biotinylated and hybridized as a control to polytene chromosomes of *D. pseudoobscura* and *D. persimilis*. λ*DsubF6* probe (S. CIRERA, personal communication) is a recombinant phage of the *D. subobscura* genomic library that has not yet been characterized.

Probes were labeled with biotin-11-dUTP by nick translation. Prehybridization, hybridization and detection were as described by MONTGOMERY, CHARLESWORTH and LANGLEY (1987) using the more sensitive ABC-Elite Vector Laboratories kit. Micrographs were obtained by phase contrast with a Zeiss Ortoplan Photomicroscope at approximately 800× using EKTAR-25 Kodak film and a blue filter.

The following polytene chromosome maps, including some photomaps, have been used to identify the location of hybridization sites: *D. subobscura* (KÜNZE-MÜHL and MULLER 1958), *D. madeirensis* (PAPACEIT and PREVOSTI 1991), *D. guanche* (MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN 1987), *D. pseudoobscura*, *D. persimilis* and *D. miranda* (DOBZHANSKY and TAN 1936; STOCKER and KASTRITSIS 1972; ANDERSON, AYALA and MICHOD 1977; MOORE and TAYLOR 1986).

RESULTS

D. melanogaster probes always hybridized at the expected sites on *D. melanogaster* polytene chromosomes. These probes gave a weaker signal at a single position when hybridized to the obscura group species polytene chromosomes.

Figure 1 shows the hybridization sites on the polytene chromosomes for the different probes and species. Figure 2 is a linear representation of the cytological location of the different markers on Muller's element *A* in the seven species studied.

Table 1 summarizes the location of the nine probes on the polytene chromosomes of the six species of the obscura group and in *D. melanogaster*. *D. pseudoobscura*, *D. persimilis* and *D. miranda* sections are indicated according to DOBZHANSKY and TAN (1936). The proximal sections of the *XL* chromosomal arm are difficult to identify on this map and slight differences in the position of these sections are detected when comparing with STOCKER and KASTRITSIS (1972), ANDERSON, AYALA and MICHOD (1977), and MOORE and TAYLOR (1986).

All probes hybridize on the *A* (=*X*) chromosome of the *D. subobscura* cluster. However, only seven probes (*w*, *ct*, *v*, *RpII215*, *f*, *su-f* and λ*DsubF6*) are located on the homologous *XL* chromosomal arm of the *D. pseudoobscura* cluster. The remaining two probes (*z* and

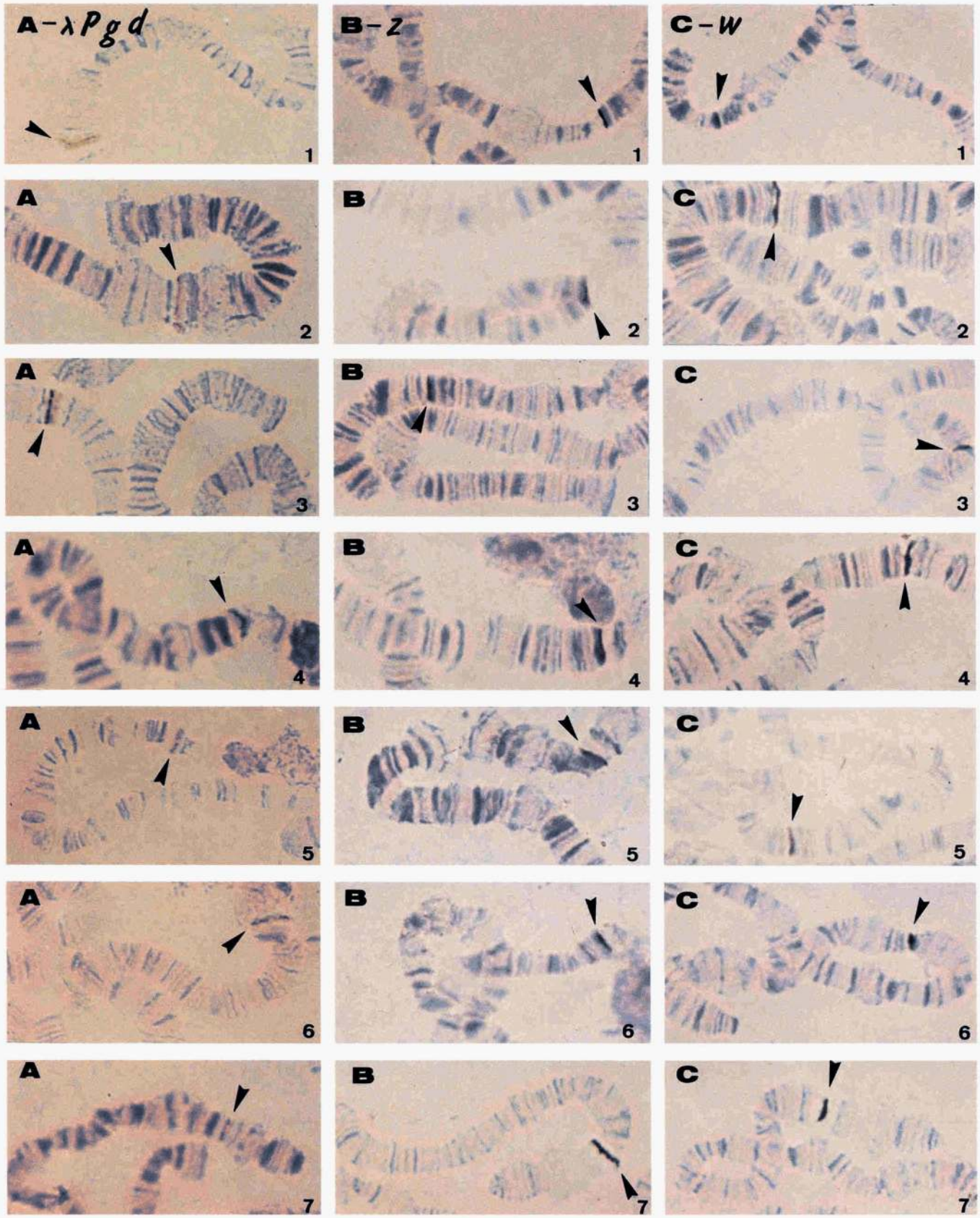

FIGURE 1.—Hybridization of nine probes to the polytene chromosomes of six species of the obscura group of Drosophila and to *D. melanogaster*. Each column, A to I, corresponds to a single probe, indicated on the top. Probes are indicated as follows: λ*Pgd* (λ*DsubPgd*), *z* (*zeste*), *w* (*white*), *ct* (*cut*), *v* (*vermilion*), *RpII215* (*RNA polymerase II 215*), *f* (*forked*), *su-f* (*suppressor of forked*) and λ*F6* (λ*DsubF6*). Species are indicated by numbers: 1, *D. subobscura*; 2, *D. madeirensis*; 3, *D. guanche*; 4, *D. pseudoobscura*; 5, *D. persimilis*; 6, *D. miranda* and 7, *D. melanogaster*. In all photographs hybridization signal is arrowheaded.

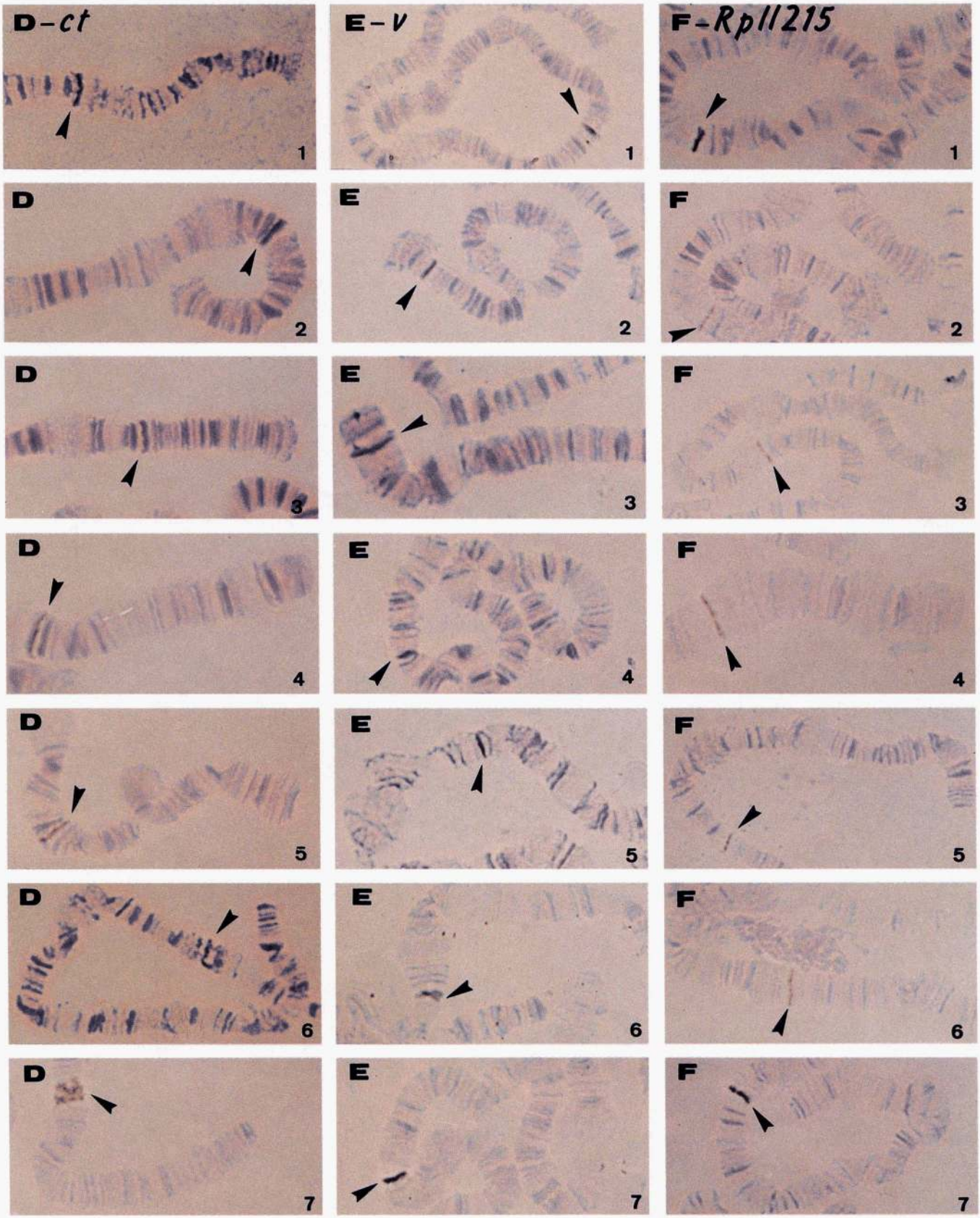

FIGURE 1.—Part 2

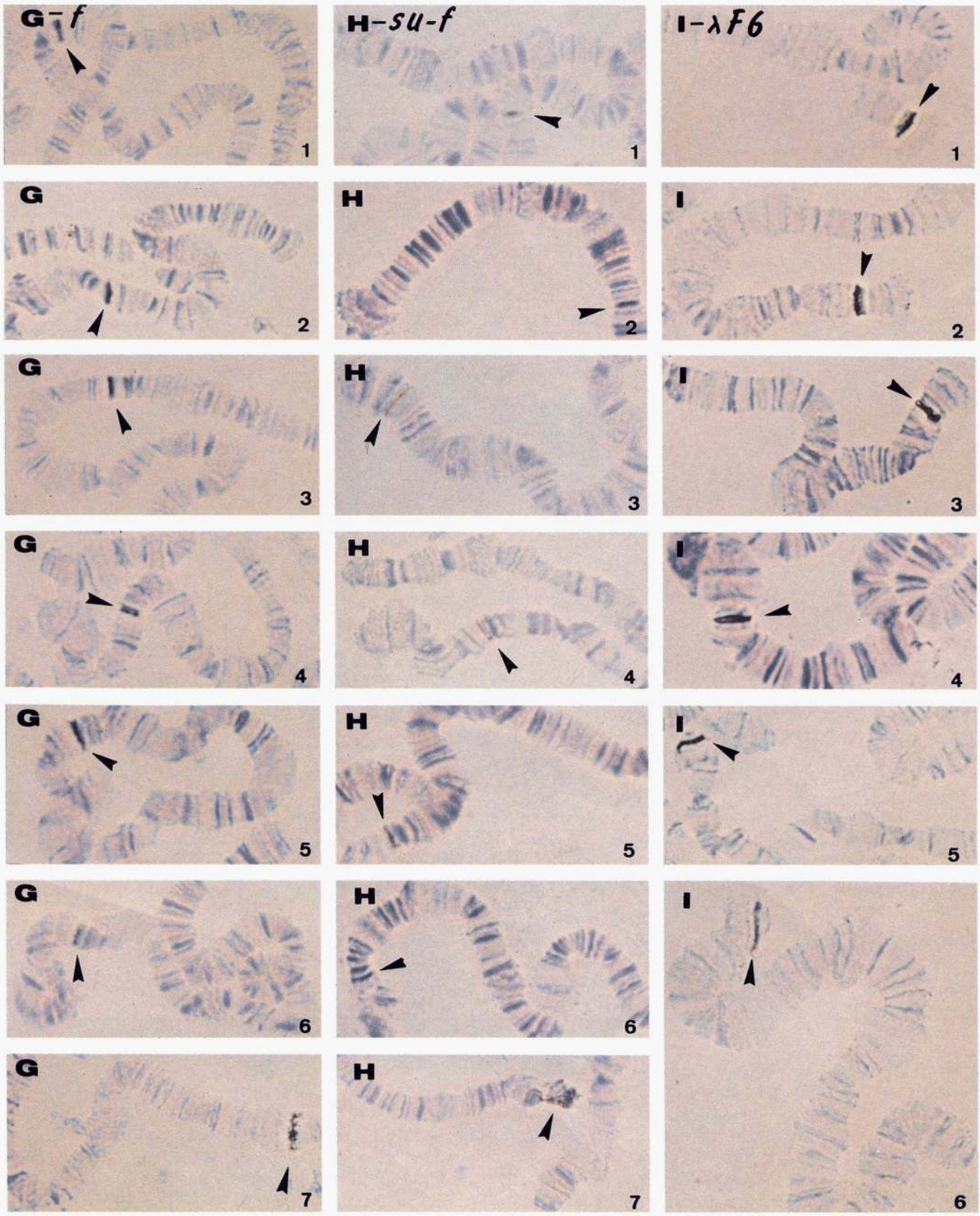

FIGURE 1.—Part 3

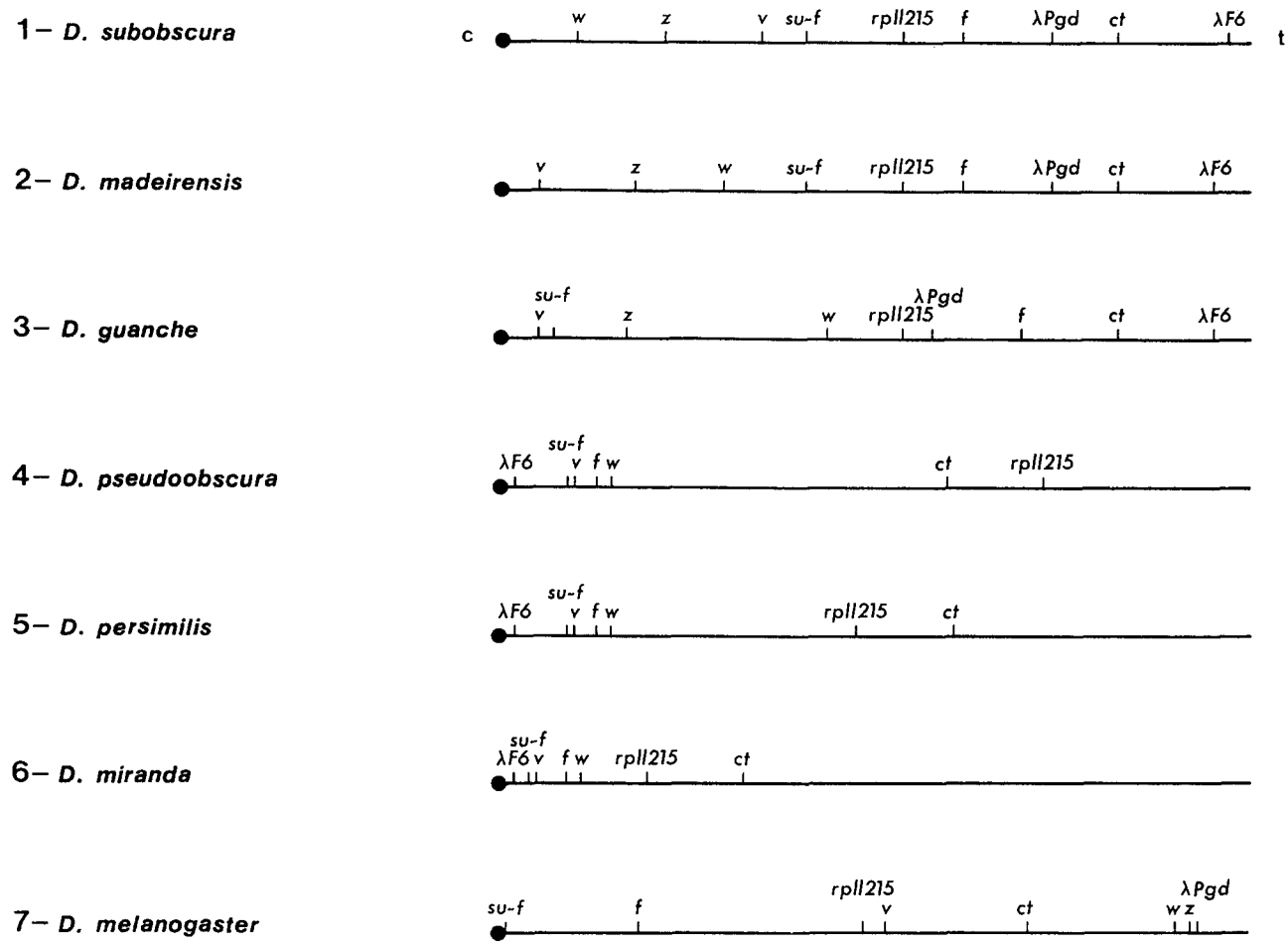

FIGURE 2.—Linear representation of the cytological location of the different probes on MULLER's element *A* of the studied species. The different elements have been given the same length to show the relative position of markers among them. In the Nearctic species location of *Pgd* and *z* are not indicated since these probes hybridize on MULLER's element *D* in these species. Location of the centromere is indicated by a dot. c = centromere. t = telomere. See legend to Figure 1 for notation of markers.

TABLE 1

Cytological location of the nine probes on the sexual chromosome of the different species studied

Drosophila species	*λPgd*	*z*	*w*	*ct*	*v*	*RpII215*	*f*	*su-f*	*λF6*
1. *D. subobscura*	*A-13A*	*A-5A*	*A-3A*	*A-14B*	*A-7A*	*A-10A*	*A-11B*	*A-8A*	*A-16C*
2. *D. madeirensis*	*A-13A*	*A-5A*	*A-3A*	*A-14B*	*A-7A*	*A-10A*	*A-11B*	*A-8A*	*A-16C*
3. *D. guanche*	*A-13A*	*A-5A*	*A-3A*	*A-14C*	*A-7C*	*A-10A*	*A-11B*	*A-7B*	*A-16D*
4. *D. pseudoobscura*	*XR-18*	*XR-18*	*XL-3*	*XL-11*	*XL-2*	*XL-13*	*XL-3*	*XL-2*	*XL-1*
5. *D. persimilis*	*XR-18*	*XR-18*	*XL-3*	*XL-11*	*XL-2*	*XL-13*	*XL-3*	*XL-2*	*XL-1*
6. *D. miranda*	*XR-18*	*XR-18*	*XL-3*	*XL-11*	*XL-2*	*XL-12*	*XL-3*	*XL-2*	*XL-1*
7. *D. melanogaster*	*X-2D*	*X-3A*	*X-3C*	*X-7B*	*X-10A*	*X-10C*	*X-15F*	*X-20E*	

See legend to Figure 1 for notation of markers.

Pgd) hybridize on the *XR* chromosomal arm of the Nearctic species. The *Pgd* hybridization site has been corroborated in *D. pseudoobscura* and *D. persimilis* using as probe a DNA fragment that includes part of the *Pgd* gene of *D. melanogaster* (result not shown).

The *D. melanogaster Pgd-z-w* linkage group is almost completely lost in the obscura species. Only *z* and *Pgd* remain linked in the *D. pseudoobscura* cluster. Both genes are located on section *18* that is the more proximal of the *XR* chromosomal arm, with *Pgd* closer to the centromere. The linkage between *RpII215* and *v* found in *D. melanogaster* has disappeared in the six species of the obscura group. The *su-f* gene is located in β-heterochromatin at the proximal end of *X* chromosome section *20* of *D. melanogaster* (YAMAMOTO *et al.* 1990), and it hybridizes at an intersticial position in the other studied species.

The discrepancy shown in Table 1 between the location of *ct* in *D. guanche* (*14C*) and *D. subobscura* (*14B*), might be attributable to a slightly different

interpretation of the location of the subsections in the map of *D. subobscura* (KÜNZE-MÜHL and MULLER 1958). The same argument would hold for the location in different subsections of λ*DsubF6* in *D. guanche* (*16D*) as compared to *D. subobscura* (*16C*).

Five of the seven probes that map on the *XL* chromosomal arm of the Nearctic species are located within three adjacent sections. λ*DsubF6* hybridizes at section *1*; *su-f* and *v* at section *2*, being *su-f* closer to the centromere; *f* at section *3* though at the band just on the edge of section *2*; and *w* at section *3*.

DISCUSSION

The available linkage map of *D. pseudoobscura* (ANDERSON and NORMAN 1977) includes the location on chromosomal arm *XL* of three morphological markers analyzed in the present study: *ct* (21.9), *w* (65.3) and *v* (69.1). Cytological location of these genes, as identified in the present study, is in agreement with their location in the recombination map.

Chromosomal arm homologies previously established for the species studied are only partly supported by present results. MULLER's (1940) element *A* is considered homologous to chromosome *A* (=*X*) from *D. subobscura* and related species and to chromosomal arm *XL* from *D. pseudoobscura* and relatives. However, two of the probes used, *Pgd* and *z*, hybridize near the proximal end of the *XR* chromosomal arm in the three Nearctic species studied. The location of *z* and *Pgd* on element *D* (MULLER 1940) indicates that, in addition to the centric fusion between acrocentric elements *A* and *D* needed to produce the metacentric sexual chromosome of the American species, a pericentric inversion has occurred in the chromosomal evolution of these species.

Chromosomal homologies between *D. subobscura* and *D. madeirensis* have been established by studying polytene chromosomes in interspecihichybrids and by interspecific comparison of banding patterns (KRIMBAS and LOUKAS 1984; PAPACEIT and PREVOSTI 1989, 1991; BREHM and KRIMBAS 1990a). The *A* (=*X*) chromosome of both species differs by two nonoverlapping paracentric inversions *Am1* and *Am2* (PAPACEIT and PREVOSTI 1991). *Am1* was first described as the polymorphic gene arrangement A_1 of *D. subobscura* (KRIMBAS and LOUKAS 1984). Later, PAPACEIT and PREVOSTI (1989) located the inversion breakpoints in subsections *7C/7D* and probably *1A/1B*, therefore neither corresponds to inversion A_1 nor to A_6, another polymorphic inversion in *D. subobscura*. The inverted orientation of genes *w*, *z* and *v* in both species (Figure 2) not only confirms the existence of inversion *Am1* in *D. madeirensis* as described by PAPACEIT and PREVOSTI (1989) but also delimits its distal breakpoint. The present results therefore invalidate the distal breakpoint proposed by BREHM and KRIMBAS (1990a), section *6E/7A*, since this would not explain the inverted position of *v*. *Am2* is a small inversion located on the distal end of the chromosome involving only section *16*. The slightly different position of the λ*DsubF6* probe in both species confirms the existence of this inversion.

Putative chromosomal homologies between *D. subobscura* and *D. guanche* have been established by KRIMBAS and LOUKAS (1984), MOLTÓ and MARTÍNEZ-SEBASTIÁN (1986), MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN (1987), and BREHM and KRIMBAS (1990a). The medial and distal regions of the *A* (=*X*) chromosome of *D. subobscura* and *D. guanche* differ by two nonoverlapping paracentric inversions. One of these, only involves section *16* and coincides with inversion *Am2* that differentiates *D. subobscura* and *D. madeirensis*. The larger inversion has been finally located between sections *10C* and *13A/B* (MOLTÓ and MARTÍNEZ-SEBASTIÁN 1986; MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN 1987; BREHM and KRIMBAS 1990a). The inverted orientation of markers *f* and *Pgd* between both species confirms both the existence of this inversion and its breakpoints, discarding previously described breakpoints: sections *9* and *14* as proposed by KRIMBAS and LOUKAS (1984), since in this case gene *RpII215* should also be inverted, and sections *10/11* and *12C* as proposed by FELGER (1985, in MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN 1987) because these latter could not account for the inverted position of *Pgd*.

The proximal region of the *A* (=*X*) chromosome differs significantly between *D. subobscura* and *D. guanche*. BREHM and KRIMBAS (1990a), and MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN (1987) proposed that at least four paracentric inversions became fixed in this region during the divergence of these species, although these authors have located the inversions breakpoints in different positions. The close linkage between *v* and *su-f* in *D. guanche* cannot be explained by any of the putative sequences of inversions proposed by these authors. The different location of both genes (*v* and *su-f*) in *D. guanche* (*7C* and *7B*) compared to *D. subobscura* (*7A* and *8A*) (Table 1) is therefore spurious and is accounted for by the wrong sequence of inversions assumed by MOLTÓ, DE FRUTOS and MARTÍNEZ-SEBASTIÁN (1987) when building the *D. guanche* map. The order and relative distances among *v*, *z*, *w* and *su-f* when comparing *D. subobscura* and *D. guanche* (Figure 2) also indicate that at least four overlapping inversions are needed to explain the differences in gene arrangements. The similar location of *v* in *D. guanche* and *D. madeirensis*, near the centromere, might implicate inversion *Am1* as one of the inversions differing between *D. guanche* and *D. subobscura*.

DOBZHANSKY and TAN (1936) proposed chromo-

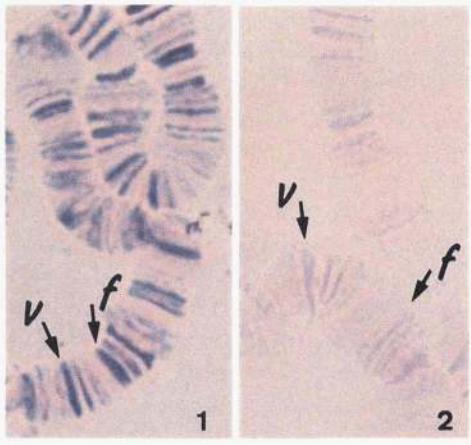

FIGURE 3.—Photographic composition showing the differential chromosomal region between *vermilion* and *forked* markers when comparing *D. pseudoobscura* and *D. miranda*. 1, *D. pseudoobscura* and 2, *D. miranda*. *v* (=*vermilion*) and *f* (=*forked*).

somal homologies among *D. pseudoobscura*, *D. persimilis* and *D. miranda* based both on banding patterns and on analysis of polytene chromosomes in interspecific hybrids. They suggested that chromosomal arm *XL* of *D. pseudoobscura* and *D. persimilis* differs by a single paracentric inversion with breakpoints in the middle of sections *7* and *12*. Later, MOORE and TAYLOR (1986), also studying interspecific hybrids, proposed that the distal breakpoint should be located on section *14* or between sections *13* and *14*. The inverted orientation of *RpII215* and *ct* in both species confirms this last interpretation, since the *RpII215* gene should not be affected by the inversion if the distal breakpoint was that proposed by DOBZHANSKY and TAN (1936).

D. pseudoobscura and *D. miranda XL* chromosomal arms differ in several aspects. The major difference is a paracentric inversion with breakpoints *6/5* and *11/12* (DOBZHANSKY and TAN 1936). The inverted position of the *RpII215* region cannot be explained by an inversion with the proposed distal breakpoint. Therefore, this breakpoint should be located at a more distal position, possibly in section *13* or *13/14*. The different location of *RpII215* in *D. pseudoobscura*, section *13*, and *D. miranda*, section *12*, (Table 1) is again spurious and due to the wrong location of this distal breakpoint in DOBZHANSKY and TAN'S (1936) map. The present results indicate that in their proposed junction of sections *12* and *86* in *D. miranda* there should be correspondence to section *13* of *D. pseudoobscura*.

DOBZHANSKY and TAN (1936) also pointed out that *D. pseudoobscura* and *D. miranda XL* chromosomal arms differ in the centromeric sections *1–3*. The order of the five markers located in these sections (λ*DsubF6*, *su-f*, *v*, *f* and *w*) is identical in both species and therefore does not indicate any change in these sections. However, comparison of the banding pattern in the region between *v* and *f* (Figure 3) confirms the existence of some differences between them as indicated by DOBZHANSKY and TAN (1936). They observed hybridization of this region with section *90* of *D. pseudoobscura* fourth chromosome. Other differences between the *XL* chromosomes of these species pointed out by these authors cannot be contrasted with the present results.

A priori, we expected that a comparison of nine genes unambiguously located on the *X* chromosome of *D. subobscura* (as representative of the Palearctic species), *D. pseudoobscura* (as representative of the Nearctic species) and *D. melanogaster* would have allowed us to identify homologous segments of this chromosome in the different species. The number of markers has however proved insufficient to do that given the high number of chromosomal rearrangements (mainly inversions) that have been fixed not only since the divergence of the melanogaster and obscura groups but also since the Nearctic and Palearctic species of the obscura group diverged from its common ancestor. None of the 10 approximately equidistant segments that the nine probes delineate in the *A* (=*X*) chromosome of *D. subobscura* is conserved in the *XL* chromosomal arm of *D. pseudoobscura* or in the *X* chromosome of *D. melanogaster*. Only the linkage between *Pgd* and *z* found in *D. melanogaster* has been kept in the three Nearctic species. Otherwise, a new group of closely linked genes (λ*DsubF6*, *su-f*, *v*, *f* and *w*) has been formed in the *D. pseudoobscura* cluster. These five genes are located together in a region that represents approximately 13% or less of the *XL* chromosomal arm length of the Nearctic species. The only similarity when comparing the *A* (=*X*) and *XL* chromosomes seems to be the close linkage between *v* and *su-f* markers found in *D. guanche* and in the three Nearctic species. The significance of this linkage for chromosome phylogeny is unclear.

The present study supports the idea that MULLER'S element *A* has conserved its integrity except for the interchange of genetic material between the *XL* and *XR* chromosomal arms of the Nearctic species of the obscura group. However, it also demonstrates that this element has evolved and changed drastically during species divergence, which shows extensive reorganization within the sex chromosome through the course of Drosophila speciation.

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