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In Situ Hybridization Analysis of Chromosomal Homologies in *Drosophila melanogaster* and *Drosophila virilis*

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ABSTRACT

Twenty-four biotin-labeled recombinant-DNA probes which contained putative unique-sequence *Drosophila melanogaster* DNA were hybridized to larval salivary-gland chromosomes of *D. melanogaster* and *Drosophila virilis*. All probes hybridized to *D. melanogaster* chromosomes at the expected sites. However, one probe hybridized to at least 16 additional sites, and one hybridized to one additional site. Thirteen probes hybridized strongly to *D. virilis* chromosomes, four hybridized weakly and infrequently, and seven did not hybridize. Probes representing two multigene families (β-tubulin and yolk-protein) hybridized as would be expected if all sites had been conserved in the two species on the same chromosomal elements. The multiple hybridization sites of a third probe which may represent a multigene family were also conserved. The results were consistent with H. J. Muller's proposal that chromosomal elements have been conserved during evolution of this genus.

MULLER (1940) proposed that the ancestral haploid karyotype of the genus *Drosophila* originally consisted of five large chromosomes (elements *A–E*) and a very small "dot" chromosome (element *F*) which have remained largely intact, as chromosomes or chromosomal arms, throughout the subsequent evolutionary history of the genus. Paracentric inversions, which occur relatively frequently in Drosophila (CLAYTON and GUEST 1986), are an obvious mechanism for rearranging the gene order within each chromosomal element. Centric fusions have occurred in certain groups (CLAYTON and GUEST 1986) but pericentric inversions and translocations are thought to be rare. MULLER's proposal thus suggests that paracentric inversions and fusions have produced the karyotypic diversity found in this genus. Accordingly, it should be possible to identify chromosomal homologies and establish a single chromosomal numbering system for the genus.

MULLER's proposal has been tested by several types of comparative studies: linkage analysis of genes which are thought to be homologous in different species (STURTEVANT and NOVITSKI 1941; PATTERSON and STONE 1952; ALEXANDER 1976); pairing of apparently homologous regions of polytene chromosomes in interspecific hybrids (HUGHES 1939; HSU 1952; THROCKMORTON 1982; KRIMBAS and LOUKAS 1984); comparison of banding patterns of salivary-gland chromosomes (STALKER 1972; YOON, RESCH and WHEELER 1972); and similarity of chromosomal puffing patterns in developing larvae (ASHBURNER and BERENDES 1978).

Results of these studies have generally supported MULLER's proposal, encouraging researchers such as FOSTER *et al.* (1981) to expand the proposal to include other families. However, as useful as most of these studies have been, they all share a similar problem. They are compromised in their ability to directly demonstrate sequence similarity between putatively homologous loci. For example, a gene which controls a particular phenotype in one species is not always homologous to a gene which controls a similar phenotype in another species (ALEXANDER 1976). Comparison of puffing patterns is subject to similar uncertainties. Banding pattern similarities can be used to establish chromosomal homologies but analysis is difficult and often uncertain when comparing distantly related species (STALKER 1972; FOSTER *et al.* 1980). Analysis of polytene chromosome pairing in interspecific hybrids is limited to closely related species (STONE, GUEST and WILSON 1960).

In situ hybridization studies provide the direct test of homology that is critical to evaluating MULLER's proposal. Such studies using probes containing unique-sequence DNA have generally supported the proposal, while those using probes containing repeated sequences have not (COHEN 1976a, b; WIMBER and WIMBER 1977; EVGEN'EV *et al.* 1978; COHEN, RAE and TSAI 1980; STEINEMANN 1982; BROCK and ROBERTS 1983; STEINEMANN, PINSKER and SPERLICH 1984; LOUKAS and KAFATOS 1986; LOUKAS and KAFATOS 1988; JEFFERY, FARMER and PLILEY 1988).

We have hybridized recombinant DNA probes containing putative unique-sequence *D. melanogaster* DNA to polytene chromosomes of *D. melanogaster* and *D. virilis*. These two species were chosen for several

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TABLE 1

Source and description of probes

Probe	Description	Reported hybridization site	Source
116H2	Hybridizes to abundant poly-A RNA	*102CD*	R. LEVIS
506	Hybridize to head-specific poly-A RNA	*82F*	J. MANNING
512		*92CD*	
514		*51B*	
521		*73DEF*	
527		*34F*	
538		*28C*	
547		*66D*	
548		*15AB*	
555		*43AB*	
3103	*Bithorax* sequences	*89E*	D. HOGNESS
3104			
adm63BC.1	*hsp83* sequence	*63BC*	R. LIS
DTB1	β-Tubulin 1 sequence	*97EF*[a]	J. NATZLE
DTB3	β-Tubulin 3 sequence	*60C*[b]	
lambdaA57	Hybridizes to maternal-specific poly-A RNA	*18CD*	E. STEPHENSON
p7R6	Vitellin-membrane sequence	*26A*	M. HIGGINS and R. MACINTYRE
pDCg2	Collagen-like sequence	*19EF-20AB*	J. NATZLE
pDm131	Metallothienein sequence	*85E*	G. MARONI
pkdm2G6	cDNA to intermolt puff-stage 1 poly-A RNA	*68C*	D. HOGNESS
pm11.5	*white* sequences	*3C*	G. RUBIN and R. LEVIS
pm12.3			
pm12.8			
pYP3	Yolk-protein sequence	*12BC*	T. BARNETT

[a] Minor hybridization sites at *56C*, *60C* and *85D*.
[b] Minor hybridization sites at *56C*, *85D* and *97EF*.

reasons. They are in different subgenera (*Sophophora* and *Drosophila*, respectively, WHEELER 1981); a large number of probes containing *D. melanogaster* DNA is available (MERRIAM *et al.* 1986); the karyotype of *D. virilis* is thought to be similar to the ancestral karyotype of the genus (CLAYTON and GUEST 1986); and their chromosomal elements have been extensively compared by linkage analysis of putatively homologous loci (STURTEVANT and NOVITSKI 1941; PATTERSON and STONE 1952; ALEXANDER 1976; GUBENKO and EVGEN'EV 1984).

MATERIALS AND METHODS

Polytene chromosome preparation: *Drosophila melanogaster gt* w^a and *D. virilis* wild-type stocks were grown in well-yeasted, uncrowded culture bottles at 17° on instant Drosophila medium (Carolina Biological). Polytene chromosomes were prepared by the method of ATHERTON and GALL (1972) as modified by PLILEY, FARMER and JEFFERY (1986).

Labeling, hybridization and detection of probes: Twenty-four recombinant probes containing *D. melanogaster* DNA, representing all six chromosomal elements, were obtained from several sources (Table 1). Probes were nick-translated with biotin-11-dUTP, hybridized to polytene chromosomes, and detected as described in WHITING, FARMER and JEFFERY (1987). Probes were hybridized to both *D. melanogaster* and *D. virilis* chromosomes at the same stringency (identical salt concentration, probe concentration, and temperature). Hybridization was done at 58.5° (generally for 6 hr) and posthybridization washing was done at 53.5°. We were able to identify hybridization sites unambiguously by photographing chromosomes both before (black-and-white) and after (Kodacolor VRG) hybridization. Color photography with phase contrast optics highlighted the blue color of the stained hybridization site (Figure 1).

Identification of sites: We used the *D. melanogaster* cytological map of LEFEVRE (1976) and the *D. virilis* cytological map of GUBENKO and EVGEN'EV (1984).

RESULTS

Hybridization to *D. melanogaster* chromosomes: Twenty-two of the probes hybridized to *D. melanogaster* polytene chromosomes at the expected sites (Tables 2 and 3). Two probes, 506 and 547, hybridized to additional sites, as discussed below.

Probes that hybridized strongly to *D. virilis* chromosomes: Seventeen of the 24 probes hybridized to *D. virilis*. Thirteen of them hybridized strongly: pYP3, p7R6, DTB1, DTB3, pDm131, aDm63BC.1, 506, 514, 521, 538, 547, 548 and 555 (Figures 1 and 2, Tables 2 and 3).

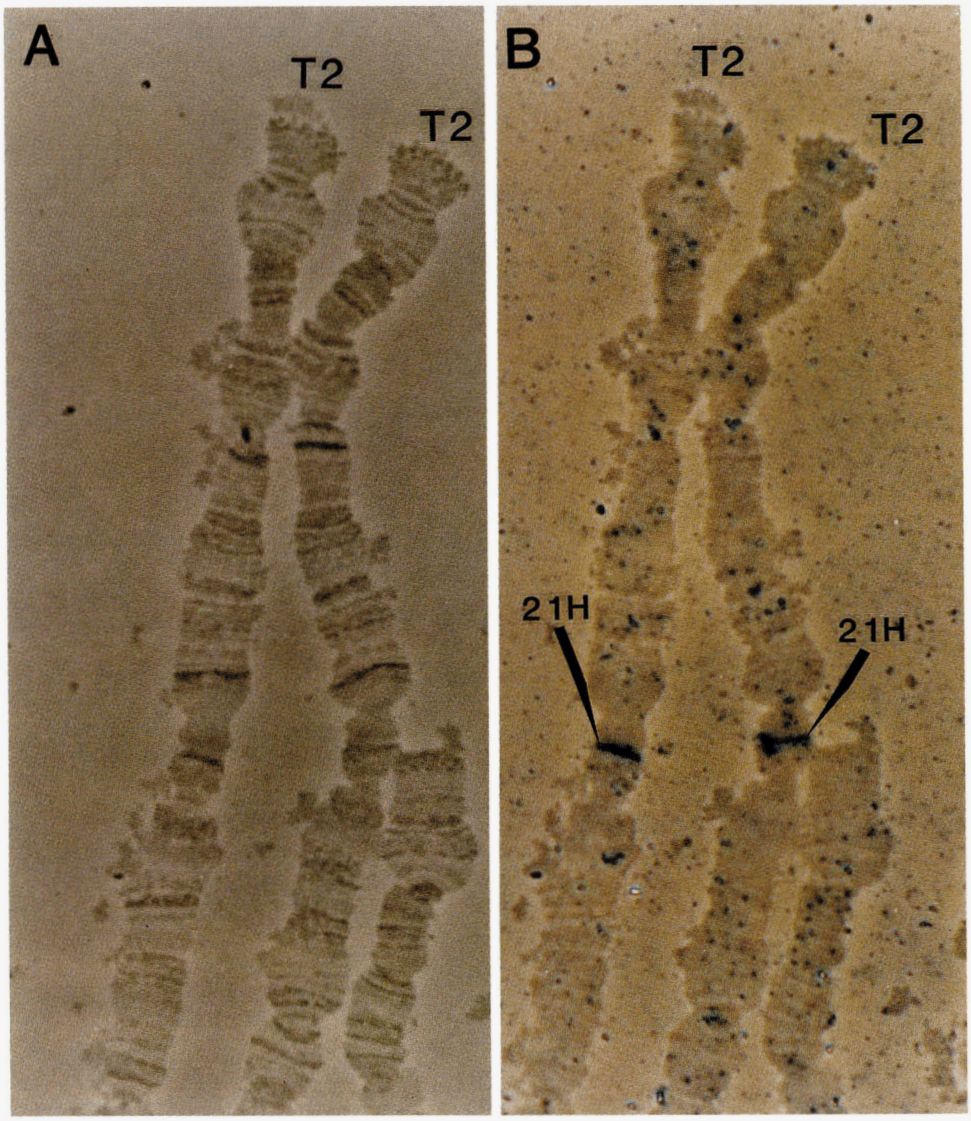

FIGURE 1.—DTB1 hybridized to *21H* on *D. virilis* chromosome *2*. Chromosomes are shown before hybridization (A) and after hybridization (B). The photographs show two *D. virilis* chromosome 2s lying side by side. The tips (T2) of each chromosome are labeled.

Probes that hybridized weakly to *D. virilis* chromosomes: Four of the 17 probes hybridized weakly and infrequently to *D. virilis* chromosomes: 512, 527, 3103 and pm12.8. Although precise hybridization sites have not been determined, preliminary information is given in Table 3.

Probes that did not hybridize to *D. virilis* chromosomes: Despite numerous attempts, we could not detect hybridization with seven of the twenty-four probes: pm11.5, pm12.3, lambdaA57, pDCg2, pkdm2G6, 3104 and 116H2 (Table 3).

Hybridization of DTB1 and DTB3 to *D. melanogaster* chromosomes: NATZLE and MCCARTHY (1984) reported that DTB1 and DTB3 represent two members of a small multigene family. We observed that DTB3 hybridized strongly to *60C* on *2R* (in agreement with NATZLE and MCCARTHY). DTB3 also hybridized weakly to *56C* on *2R* and *85D* and *97EF* on *3R*. DTB1 hybridized strongly to *97EF* on *3R* (in agreement with NATZLE and MCCARTHY). This site is indistinguishable from one minor site of DTB3. DTB1 also hybridized weakly to *60C* on *2R*, a site indistinguishable from the major site of DTB3.

Hybridization of DTB1 and DTB3 to *D. virilis* chromosomes: DTB3 hybridized strongly to two or three closely spaced bands at *57D* on *5* (Figure 3). It also hybridized weakly to three other sites: *21H* and *26A* on *2* (Figure 2) and *55EF* on *5* (a single band located at the intersection of *55E* and *55F*, a region that is not clearly resolved on the map of GUBENKO and EVGEN'EV; Figure 2). DTB1 hybridized strongly to *21H* on *2* (Figure 1) and weakly to *57D* on *5* (Figure 2).

Hybridization of pYP3 to *D. melanogaster* chromosomes: BARNETT *et al.* (1980) reported that there were three *X*-linked yolk-protein genes in *D. melanogaster*. One (from which pYP3 was derived) was located at *12BC* and the other two were located very close together at *8F-9A*. We also observed that pYP3 hybridized strongly to *12BC* and weakly to *8F-9A*.

TABLE 2

Homologous chromosomal elements in *D. melanogaster* and *D. virilis* and sites to which probes hybridized in each species

Probe	MULLER element	*D. melanogaster* site	*D. virilis* site
pYP3	A	*X (12BC)*[a]	*X (17B)*[a]
	A	*X (8F-9A)*[b]	*X (15A)*[b]
548	A	*X (15AB)*	*X (13D)*
p7R6	B	*2L (26A)*	*4 (47B)*
538	B	2L *(28C)*	*4 (41B)*
DTB3	C	*2R (60C)*[a]	*5 (57D)*[a]
	C	*2R (56C)*[b]	*5 (55EF)*[b,c]
	E	*3R (97EF)*[b]	*2 (21H)*[b]
	E	*3R (85D)*[b]	*2 (26A)*[b]
555	C	*2R (43AB)*	*5 (51A)*
514	C	*2R (51B)*	*5 (56C)*
adm63BC.1	D	*3L (63BC)*	*3 (33E)*
547	D	*3L (66D)*[a]	*3 (32E)*[a]
	B	*2L (28A1-2)*[b]	*4 (42D)*[b]
521	D	*3L (73DEF)*	*3 (32D)*
DTB1	E	*3R (97EF)*[a]	*2 (21H)*[a]
	C	*2R (60C)*[b]	*5 (57D)*[b]
506	E	*3R (82F)*[a,d]	*2 (29B)*[a]
			4 (42D)[b]
pDm131	E	*3R (85E)*	*2 (21G)*

[a] Major site (strong hybridization).
[b] Minor site (weak hybridization).
[c] Hybridized to a single *D. virilis* band at a site which is not clearly defined on GUBENKO and EVGEN'EV's (1984) maps.
[d] Reported hybridization site in *D. melanogaster* (LEVY *et al.* 1982; LEVY and MANNING 1982). In our experiments, the probe hybridized to at least 16 additional sites and to the chromocenter.

Hybridization of pYP3 to *D. virilis* chromosomes: Probe pYP3 hybridized strongly to *17B* on *X* and weakly to *15A* on *X* (Figure 2).

Hybridization of 547 to *D. melanogaster* chromosomes: Probe 547 hybridized strongly to *66D* on *3L* (LEVY *et al.* 1982) and weakly to *28A1-2* on *2L*, a previously unreported site (Figure 4).

Hybridization of 547 to *D. virilis* chromosomes: Probe 547 hybridized strongly to *32E* on *3* and weakly to *42D* on *4* (Figure 2).

Hybridization of probe 506 to *D. melanogaster* chromosomes: Probe 506 hybridized strongly to the expected site at *82F* on *3R*. It also hybridized to the chromocenter as well as to at least 16 additional sites. These sites have not been identified although it is clear that they are found on all six chromosomal elements (Figure 5).

TABLE 3

Probes which hybridized weakly or not at all to *D. virilis* chromosomes

Probe	MULLER element	*D. melanogaster* site	*D. virilis* site
512	E	*3R (92CD)*	*2 (?)*[a]
527	B	*2L (34F)*	*? (?)*[b]
3103	E	*3R (89E)*	*2 (?)*[c]
pm12.8	A	*X (3C)*	*X (?)*[d]
116H2	F	*4 (102CD)*	
3104	E	*3R (89E)*	
lambdaA57	A	*X (18CD)*	
pDCg2	A	*X (19EF-20AB)*	
pkdm2G6	D	*3L (68C)*	
pm11.5	A	*X (3C)*	
pm12.3	A	*X (3C)*	

[a] Weakly hybridized to chromosome *2*.
[b] Hybridized close to the chromocenter on an unidentified chromosome.
[c] Weakly hybridized to the proximal half of chromosome *2*.
[d] Weakly hybridized to chromosome *X*.

Hybridization of probe 506 to *D. virilis* chromosomes: Probe 506 hybridized to only two sites in *D. virilis*: strongly to *29B* on *2* and weakly to *42D* on *4*. We did not detect hybridization to the chromocenter (Figure 2).

Hybridization to bands and interbands: Hybridization sites were observed in each of the three possible chromosomal regions: dark bands, diffuse bands, and interbands (Figures 1 and 2).

Summary of results: Figure 6 shows the sites on *D. virilis* chromosomes to which probes hybridized. Figure 7 shows a schematic comparison of hybridization sites on chromosomes of both species.

DISCUSSION

MULLER-elements A-E: The homologies between the chromosomal elements of *D. melanogaster* and *D.*

FIGURE 2.—*D. melanogaster* probes hybridized to *D. virilis* polytene chromosomes. Photographic composites A–G, and J–S, show chromosomes as they appear before hybridization (1) and after hybridization (2). Photographs H and I show hybridized chromosomes only. Arrows indicate stained bands of hybridization. (A1 and A2) pYP3 hybridized to *17B* on chromosome *X* (major site). (B1 and B2) pYP3 hybridized to *15A* on chromosome *X* (minor site). (C1 and C2) 548 hybridized to *13D* on chromosome *X*. (D1 and D2) p7R6 hybridized to *47B* on chromosome *4*. (E1 and E2) 538 hybridized to *41B* on chromosome *4*. (F1 and F2) DTB3 hybridized to *57D* on chromosome *5* (major site). (G1 and G2) DTB3 hybridized to *55EF* on chromosome *5* (minor site). (H) DTB3 hybridized to *21H* on chromosome *2* (minor site). (I) DTB3 hybridized to *26A* on chromosome *2* (minor site). (J1 and J2) 555 hybridized to *51A* on chromosome *5*. (K1 and K2) 514 hybridized to *56C* on chromosome *5*. (L1 and L2) adm63BC.1 hybridized to *33E* on chromosome *3*. (M1 and M2) 547 hybridized to *32E* on chromosome *3* (major site). (N1 and N2) 547 hybridized to *42D* on chromosome *4* (minor site). (O1 and O2) 521 hybridized to *32D* on chromosome *3*. (P1 and P2) DTB1 hybridized to *57D* on chromosome *5* (minor site). It also hybridized to *21H* on chromosome *2* (major site; see Figure 1). (Q1 and Q2) 506 hybridized to *29B* on chromosome *2* (major site). (R1 and R2) 506 hybridized to *42D* on chromosome *4* (minor site). (S1 and S2) pDM131 hybridized to *21G* on chromosome *2*. An overall summary of probe locations on *D. virilis* chromosomes is shown in Figure 6 and corresponding *D. melanogaster* sites are indicated in Table 2.


A
1
2
B
1
2
C
1
2
D
1
2
E
1
2
F
1
2
G
1
2
H
I
J
1
2
K
1
2
L
1
2
M
1
2
N
1
2
O
1
2
P
1
2
Q
1
2
R
1
2
S
1
2

FIG. 2

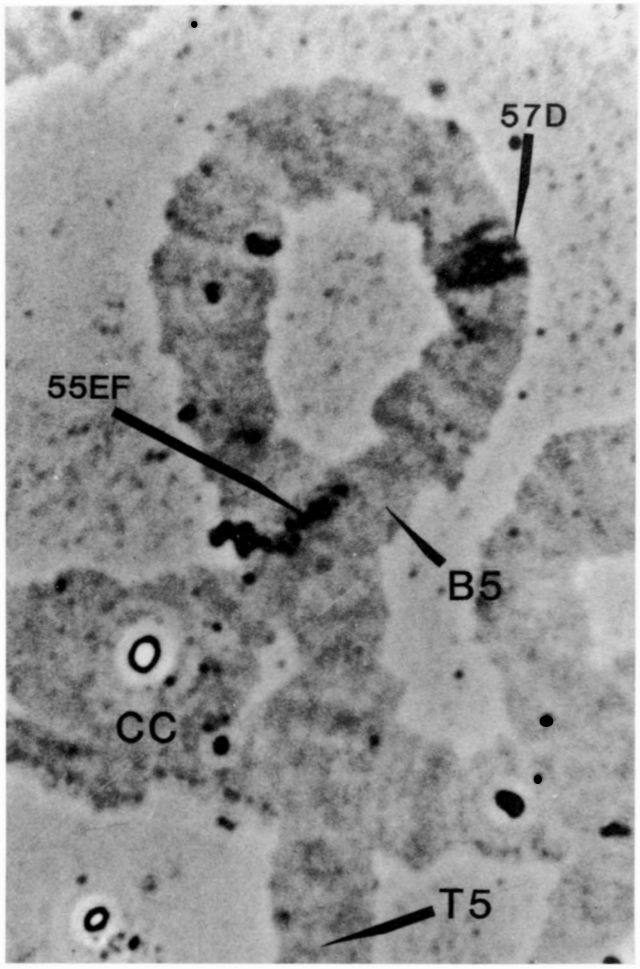

FIGURE 3.—DTB3 hybridized to *57D* on *D. virilis* chromosome *5*. The site consists of two or three closely spaced but clearly separated bands of stain. One additional DTB3 hybridization site at *55EF* is also shown. The tip (T5) and base (B5) areas of chromosome *5*, and the chromocenter (CC), are labeled.

virilis which have been proposed (MULLER 1940; STURTEVANT and NOVITSKI 1941; PATTERSON and STONE 1952) are as follows:

	MULLER elements					
	A	*B*	*C*	*D*	*E*	*F*
D. melanogaster	*X*	*2L*	*2R*	*3L*	*3R*	*4*
D. virilis	*X*	*4*	*5*	*3*	*2*	*6*

Our results (Figure 7 and Table 2) are in complete agreement.

MULLER-element *F*: Probe 116H2 hybridized strongly to *D. melanogaster* chromosome *4* but failed to hybridize to *D. virilis*. However, given the widespread occurrence of this "dot" chromosome throughout the genus (CLAYTON and GUEST 1986) and the similarity of mutants associated with the chromosome (STURTEVANT and NOVITSKI 1941), we see no reason to question the proposed chromosomal homology.

The β-tubulin multigene family: β-Tubulins in *D. melanogaster* are coded by a multigene family, with at least four members, which hybridize to four sites: DTB1, *97EF* on *3R*; DTB2, *56C* on *2R*; DTB3, *60C* on *2R*; and DTB4, *85D* on *3R* (NATZLE and MCCARTHY 1984). DTB2 also hybridized weakly to *60C*. Using Southern blots and low-stringency filter-hybridization, NATZLE and MCCARTHY observed that each of the four probes hybridized to all of the restriction fragments representing the four members of the family. Our *in situ* results confirm the cross hybridization between DTB3 and the other three β-tubulin sites, since DTB3 hybridized strongly to *D. melanogaster* site *60C* and weakly to the other three.

LOUKAS and KAFATOS (1986) presented evidence for the existence of two β-tubulin loci in *D. virilis* using *in situ* hybridization of a probe (clone 3.12) derived from the *97F* site of *D. melanogaster*. Clone 3.12 hybridized strongly to *D. virilis* site *21H* on *2* and weakly to *23C* on *2*. They suggested that these two sites in *D. virilis* corresponded to *97F* and *85D* in *D. melanogaster*, respectively. However, they were unable to detect hybridization to any sites that might be homologous to the *D. melanogaster* sites *56C* and *60C*. They concluded that these latter two β-tubulin loci were probably not present in *D. virilis*.

We observed that DTB3 hybridized strongly to *D. virilis* site *57D* on *5* and weakly to *21H, 26A* on *2*, and *55EF* on *5*. DTB1 hybridized strongly to site *21H*, confirming the sequence similarity to *D. melanogaster* site *97EF* which was proposed by LOUKAS and KAFATOS. DTB1 also hybridized weakly to *57D*, the major binding site for DTB3. We did not observe hybridization of DTB1 or DTB3 to *D. virilis* site *23C*, the minor site reported by LOUKAS and KAFATOS. Hence we have confirmed one of LOUKAS and KAFATOS' sites and identified three more. DTB1 and DTB3 thus showed a "cross-hybridization" pattern. Each hybridized strongly to a single major site and weakly to the major site of the other. These results suggest that both species have four β-tubulin loci which have diverged in sequence from a common ancestral gene. If so, the four loci must have been present early in the evolution of the genus, before the two species diverged. Assuming that the MULLER proposal is correct, we have tentatively identified *D. virilis* site *55EF* as corresponding to *D. melanogaster* site *56C*. This assignment conserves the synteny seen in *D. melanogaster* where DTB2 and DTB3 are located on the same element (NATZLE and MCCARTHY 1984). Using the same reasoning, we have tentatively identified *D. virilis* site *26A* as corresponding to *D. melanogaster* site *85D*.

DTB3 major hybridization site is a doublet or triplet: In *D. melanogaster*, staining of the DTB3-hybridized chromosomes consistently produced a single band of stain at the *60C* site. In *D. virilis*, staining frequently produced two or three closely spaced but clearly separated bands of stain at *57D* (Figure 3). It is likely that two or three β-tubulin genes or pseudo-

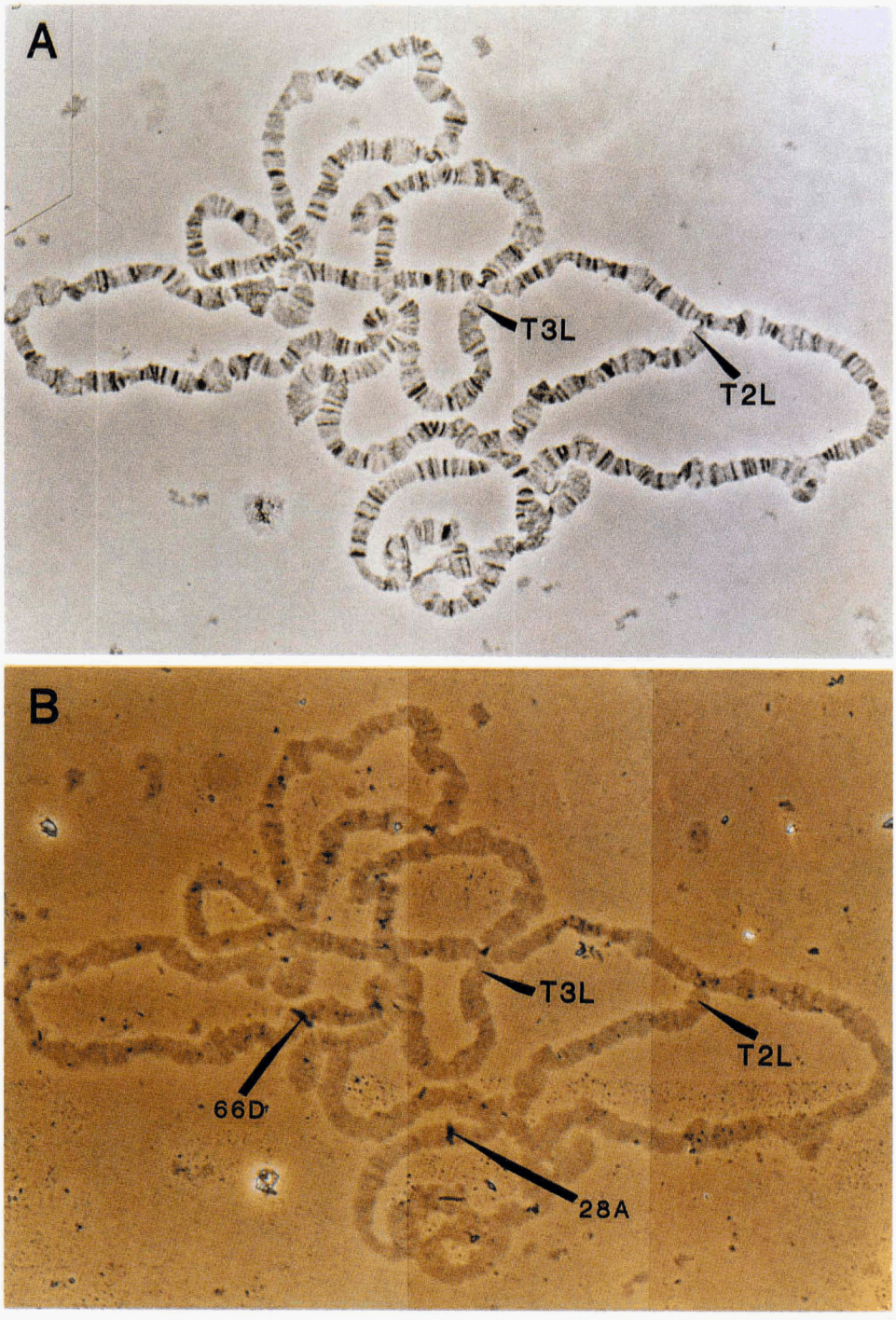

FIGURE 4.—547 hybridized to *66D* on *D. melanogaster* chromosome *3L*, and *28A1-2* on chromosome *2L*, a previously unreported site. Chromosomes are shown before hybridization (A) and after hybridization (B). The tips of *2L* (T2L) and *3L* (T3L) are indicated.

genes are located within the major *D. virilis* hybridization area. It is possible that the putative duplicate genes are also present at the homologous *D. melanogaster* site but too narrowly separated to be resolved. This possibility is strengthened by the observation that the DTB3 stained band in *D. melanogaster* is consistently wider (in the axial direction) than that of other probes.

Yolk protein-multigene family: The pYP3 probe hybridized most strongly to *12BC* on *X*, one of three yolk-protein sites located in the *D. melanogaster* genome (BARNETT *et al.* 1980). The other two sites are at *8F-9A* on *X* and are so closely linked that they appeared as a single hybridization band. Hybridization of pYP3 to *D. virilis* chromosomes identified a major site at *17B* and a minor site at *15A*, both on *X*. These probably correspond to *D. melanogaster* sites *12BC* and *8F-9A*, respectively. Like the β-tubulin multigene family, the yolk-protein multigene family seems to have been conserved during evolution.

Probe 547: 547 hybridized to one major site and one minor site in each species. If these sites are ho-

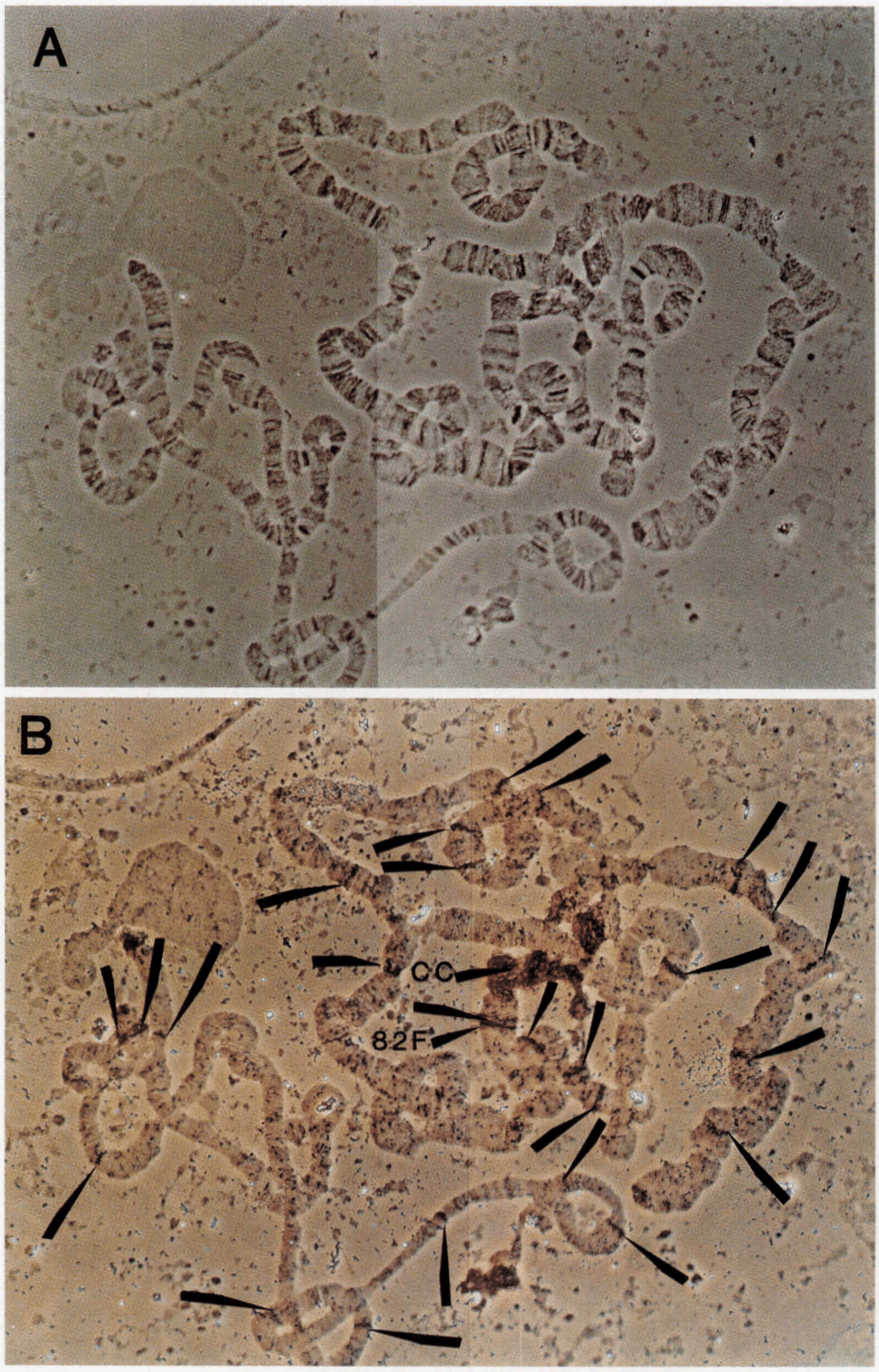

FIGURE 5.—506 hybridized to multiple sites in *D. melanogaster* including 82F (the previously reported site; Table 1), at least 16 additional sites, and the chromocenter (CC). Chromosomes are shown before hybridization (A) and after hybridization (B). Arrows indicate the locations of the hybridization sites.

mologous, they are on the elements which would be predicted from MULLER's proposal. We propose that *D. virilis* positions *32E* and *42D* correspond to *D. melanogaster* positions *66D* and *28A*, respectively.

Probe 506: The hybridization of probe 506 to multiple sites in *D. melanogaster*, including the chromocenter, had not previously been reported (LEVY and MANNING 1982; LEVY *et al.* 1982). The major sites are *82F* on *3R* in *D. melanogaster* and *29B* on *2* in *D. virilis*. If these sites are homologous, they are on the elements which would be predicted from MULLER's proposal. The minor site in *D. virilis* could correspond to any one of several minor sites in *D. melanogaster*. If most of the multiple minor sites in *D. melanogaster* were caused by the inclusion of transposable element DNA in 506, their absence from *D. virilis* would be understandable. However, we have no data to support this possibility.

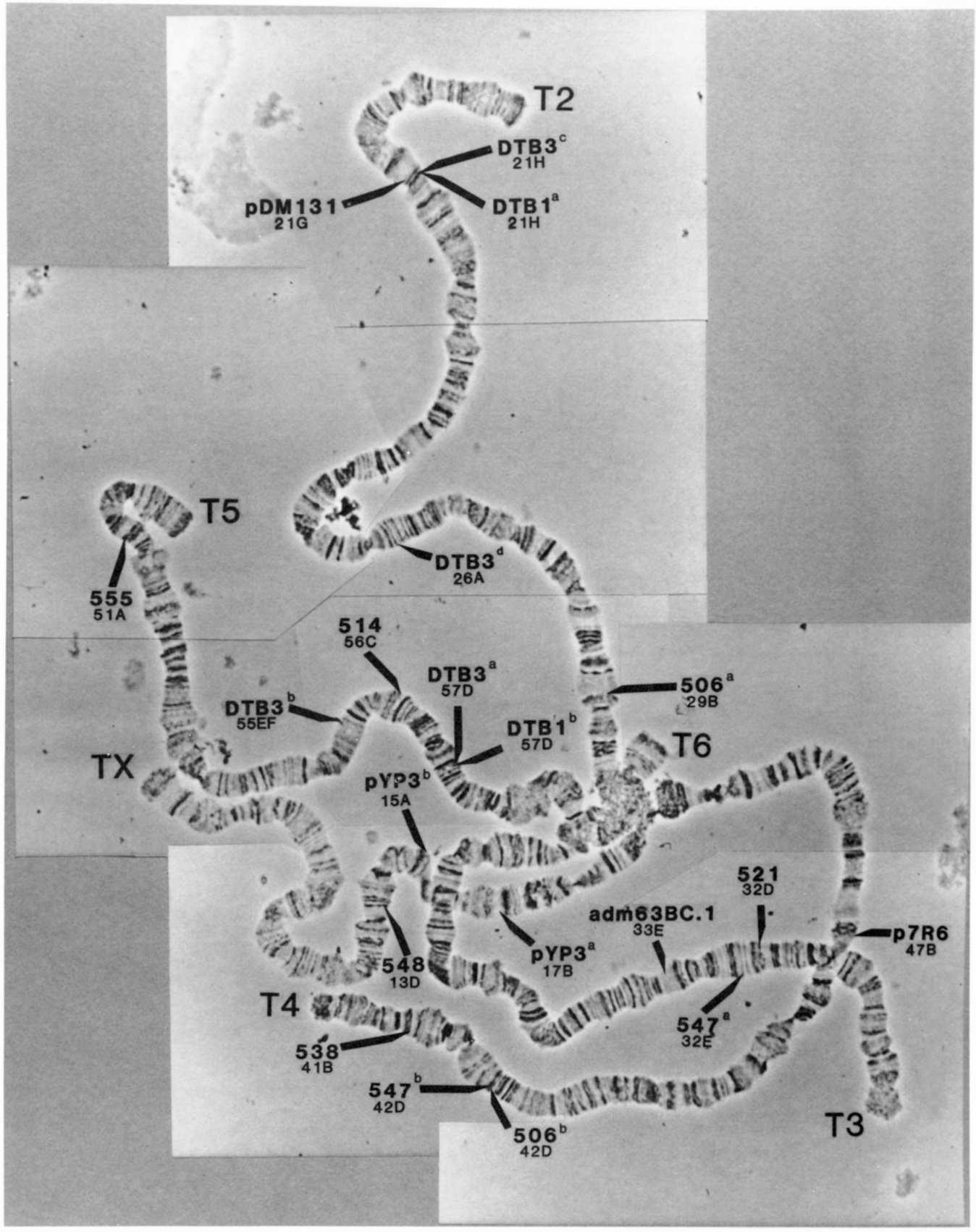

FIGURE 6.—Summary of *D. virilis* hybridization sites. The chromosomes in this photographic montage are unstained and are representative of how *D. virilis* chromosomes appear before denaturation and hybridization. The locations of where *D. melanogaster* probes hybridized to *D. virilis* chromosomes are indicated by the arrows.

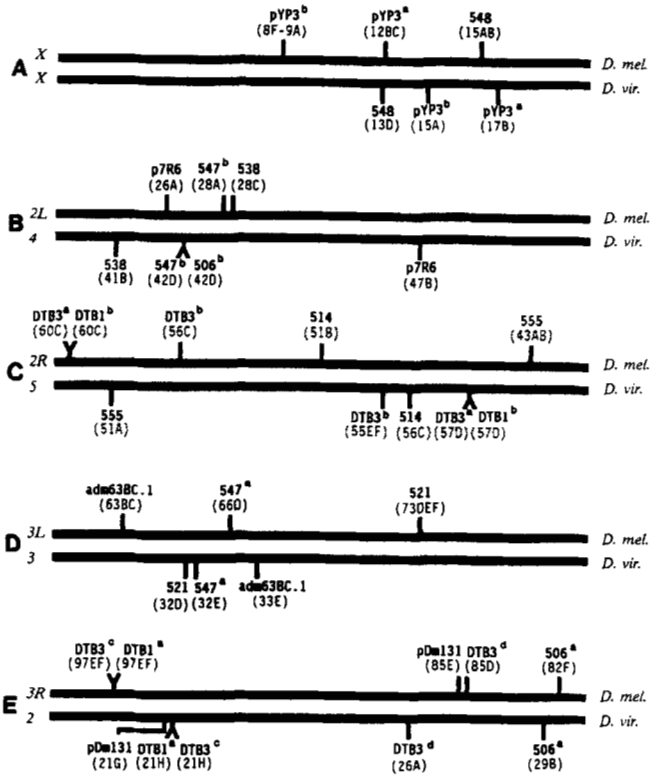

FIGURE 7.—Schematic comparison of homologous *D. melanogaster* and *D. virilis* chromosomes showing sites to which probes hybridized. Only probes that hybridized to both species are shown. The elements have been drawn the same length in order to show the relative positions of the sites. The tips of the chromosomes are located to the left.

Probes that did not hybridize: Since *in situ* hybridization is not foolproof, we cannot say that the probes which did not hybridize to *D. virilis* are not homologous to any sequences in that species.

Probes hybridized to bands and interbands: It was possible to very accurately locate a hybridization position when the chromosomes were photographed both before and after hybridization. We applied the technique primarily to *D. virilis* chromosomes. The hybridization site of a probe could accurately be located to a dark band, diffuse band, or interband region. This observation supports HILL and RUDKIN's (1987) proposal that genes are located in both bands and interbands.

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