LOCATION OF THE *LSP-1* GENES IN DROSOPHILA SPECIES BY *IN SITU* HYBRIDIZATION

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Manuscript received May 25, 1982
Revised copy accepted August 13, 1982

ABSTRACT

The locations of the larval serum protein one (*LSP-1*) α, β and γ genes were determined in *Drosophila melanogaster* and in 14 other species of Drosophila by *in situ* hybridization to polytene chromosomes. The *LSP-1* α gene mapped to bands 11B on the X chromosome, the *LSP-1* β gene mapped to bands 21D-E on chromosome 2*L*, and the *LSP-1* γ gene mapped to band 61A in all the melanogaster subgroup species. In eight other species, both the *LSP-1* α and β genes mapped to one site on Muller's element E which corresponds to chromosome 3*R* of *D. melanogaster*. No hybridization of *LSP-1* γ was detected in these eight species. Restriction enzyme digestion and analysis of genomic DNA by filter transfer hybridization confirmed the presence of *LSP-1* α-like and β-like genes in seven of these species. These results are discussed with respect to conservation of the chromosomal elements in the genus Drosophila.

CHROMOSOME fusions and paracentric inversions that change gene order are common in Drosophila, but translocations and pericentric inversions that change the constitution of the linkage groups are very rare because such individuals are under severe selective disadvantage in large populations (WRIGHT 1941; STURTEVANT and NOVITSKI 1941; PATTERSON and STONE 1952). Nonetheless, there is evidence for establishment of both translocations and pericentric inversions in natural populations (PATTERSON and STONE 1952). Despite the occasional observation of changes in linkage groups, studies on mutants with similar phenotypes in different Drosophila species led MULLER (1940) and STURTEVANT and NOVITSKI (1941) to propose that the five major chromosome arms found in primitive Drosophila species have been conserved largely intact during the evolution of the genus. This type of study has recently been extended by FOSTER *et al.* (1981), who have established homologies between mutants found on each of the five chromosomes of *Lucilia cuprina* with those of *Musca domestica* and *D. melanogaster*.

Establishment of chromosome homologies by comparison of mutants is of low resolution because the number of suitable mutants is small and because mutants of different genes may have similar phenotypes. These difficulties have been overcome by mapping specific enzymes (LAKOVAARA and SAURA 1971), by

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mapping genes that affect pteridines (GREGG and SMUCKER 1965), and by using *in situ* hybridization. Genes mapped in this way include 5S RNA (ALONSO and BERENDES 1975; COHEN 1976; WIMBER and WIMBER 1977), 18/28S RNA (STUART *et al.* 1981) and heat-shock proteins (EVGENEV *et al.* 1978; PIERCE and LUCCHESI 1980). These studies have generally confirmed assignments of chromosome homology made by earlier authors. However, the location of some pteridine loci appear to reflect the occurrence of a pericentric inversion (GREGG and SMUCKER 1965), the principal 5S RNA cluster in *D. hydei* is not found on the homologous chromosomal element in *D. melanogaster* (ALONSO and BERENDES 1975), and STUART *et al.* (1981) have recently shown that the 18/23S RNA genes of some Hawaiian Drosophila species are autosomal rather than X linked.

In *D. melanogaster* the genes coding for the α, β and γ subunits of *LSP-1* map to the X chromosome, chromosome *2L* and chromosome *3L*, respectively (ROBERTS and EVANS-ROBERTS 1979). These genes have been shown to be homologous by peptide mapping (BROCK and ROBERTS 1980) and by heteroduplex mapping (SMITH *et al.* 1981). To investigate the arrangement and dispersal of these genes in the genus Drosophila, and to investigate homology of the chromosome arms, cloned *LSP-1* α, β and γ DNA was hybridized *in situ* to polytene chromosomes of 15 Drosophila species. Interpretation of these results required analysis of genomic DNA by restriction enzyme analysis and filter transfer hybridization experiments.

MATERIALS AND METHODS

Drosophila stocks: The *melanogaster* subgroup species were obtained from DR. M. ASHBURNER at the University of Cambridge. All other species were obtained from the Drosophila stock center at the University of Texas, Austin, TX. Species were routinely maintained on wheatmeal medium, but larvae were grown on yeast-glucose medium (ROBERTS and EVANS-ROBERTS 1979) at 18° for preparation of polytene chromosomes.

Preparation of probes: PAT 153 plasmids containing *LSP-1* α, β and γ genes were the generous gift of DR. D. GLOVER. The plasmids were grown up and purified according to the method of SMITH *et al.* (1981).

The whole plasmid was nick translated for *in situ* hybridization. For analysis of genomic DNA the appropriate restriction fragments were eluted from 1.2% agarose gels (Seaplaque, Marine Colloids Ltd.) using the method of LANGRIDGE, LANGRIDGE and BERGQUIST (1980).

Plasmid DNA was nick translated with (5-^{3}H)-dCTP and (8-^{3}H)-dGTP to a specific activity of 3–6 $\times$ 10^6 dpm/μg for *in situ* hybridization and labeled with (α-^{32}P)-dATP to a specific activity of 1–4 $\times$ 10^7 dpm/μg for filter transfer hybridization following the method of RIGBY *et al.* (1977).

In situ hybridization: Chromosomes were prepared for *in situ* hybridization following the method of STROBEL, DUNSMUIR and RUBIN (1979), except that all preparations were acetylated to reduce background (HAYASHI *et al.* 1978). Hybridization occurred overnight in 15 μl of a solution containing 2 $\times$ 10^5 dpm of probe, 0.4 M NaCl, 50% formamide, 10 mM 1,4-piperazinediethanesulfonic acid (PIPES), pH 6.4, at 37°. The slides were washed for 15 min at 37° in the hybridization buffer, followed by two 15-min washes in 2 $\times$ NaCl/Cit (1$\times$ is 0.15 M NaCl, 15 mm sodium citrate). The slides were dipped in Ilford L4 emulsion diluted 1:1 with distilled water and exposed and developed according to standard procedures (PARDUE and GALL 1975).

Analysis of genomic data: DNA was purified from adult flies according to the method of ISH-HOROWICZ *et al.* (1979), except that after the first phenol extraction the sample was treated with 100 μg/ml boiled pancreatic RNase for 2 hr at 37°.

Restriction enzymes were purchased from New England Biolabs and used in the buffers recommended by the suppliers. Genomic DNA was digested overnight with 1 enzyme unit/μg of DNA at 37°.

After electrophoresis in 1% agarose gels, the DNA was depurinated (WAHL, STERN and STARK 1979) and transferred to nitrocellulose filters (SOUTHERN 1975). The filters were hybridized using the protocol of WAHL, STERN and STARK (1979), for 16–20 hr, and then washed three times for 5 min each in 2 × NaCl/Cit, 0.1% sodium dodecyl sulfate (SDS) at room temperature, and then washed in 0.1 × NaCl/Cit, 0.1% SDS twice for 30 min each at either 50° or at 60° as appropriate. The filters were exposed to X-ray film at −70° with intensifying screens.

RESULTS

Figure 1 shows a phylogeny adapted from THROCKMORTON (1975) of the species used in this study. THROCKMORTON (1975), PATTERSON and STONE (1952) and BOCK and WHEELER (1972) should be consulted for further details. *D. melanogaster, D. simulans, D. mauritiana, D. erecta, D. orena, D. yakuba* and *D. teissieri* form the seven-member *melanogaster* sibling species subgroup. *D. willistoni, D. saltans* and *D. pseudoobscura* were chosen as representatives of other major species groups in the subgenus Sophophora. *D. hydei, D. virilis, D. funebris* and *D. immigrans* represent major species groups in the subgenus Drosophila, and *D. busckii* is the sole member of the subgenus Drosophila.

Plasmids containing the *LSP-1* α, β and γ genes were hybridized *in situ* to polytene chromosomes from all of the aforementioned species. The probes contained flanking as well as transcribed sequences, but only *LSP* coding sequences hybridized to genomic DNA of species outside the *melanogaster* sibling species in filter transfer hybridization experiments (H. W. BROCK, unpublished observations). Interpretation of the *in situ* results was straightfor-

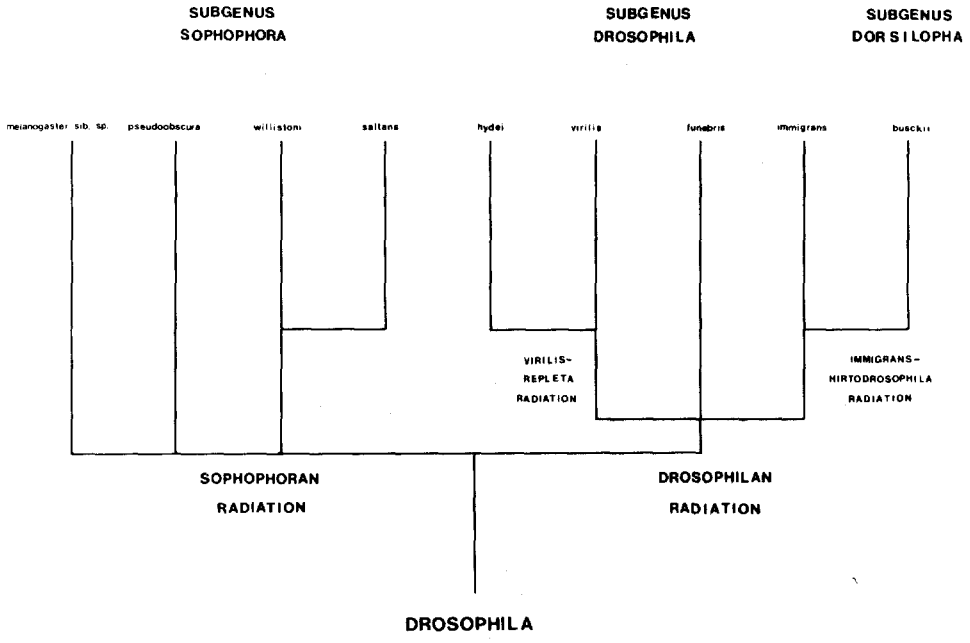

FIGURE 1.—Phylogeny of the Drosophila species used in this study adapted from THROCKMORTON (1975). The vertical scale indicates postulated order of species divergence but is not proportional to the absolute time of divergence.

ward in the *melanogaster* sibling species because the ratio of specific to nonspecific binding was high, but in more distantly related species this ratio was low. A site was scored as positive only if the grains were deposited over the full width of the chromosome arm parallel to the bands and interbands, if no grains in the vicinity of the site had a similar orientation to the chromosomal grains, and if the site was found several times on one slide, and also on at least two different slides. Using these criteria we are unlikely to have identified false positives, but we may well have missed sites of genuine hybridization that showed up infrequently.

As shown in Figure 2, the *LSP-1* α probe hybridizes to a site near the prominent constriction of bands 11A-B in all of the *melanogaster* sibling species despite the presence of inversions that have changed the position of bands 11A-B relative to their position in *D. melanogaster* (LEMEUNIER and ASHBURNER 1976). Similarly, the *LSP-1* β probe maps to bands 21D-E, and the *LSP-1* γ probe to band 61A in all of the *melanogaster* sibling species.

By comparison with the *LSP-1* α and β probes, the number of grains deposited with the *LSP-1* γ probe in the *melanogaster* sibling species was low and often patchy. This may be because the chromosomal DNA near the telomere reanneals more rapidly than chromosomal DNA at other *LSP-1* sites, thus lowering the amount of hybridization possible. An alternative hypothesis is that the *LSP-1* γ gene is under-replicated relative to the other *LSP-1* genes in these species. These results show that the *LSP-1* subunit genes have occupied these positions since before the divergence of the *melanogaster* subgroup, and that they have not changed their relative positions in the genome since this divergence.

The salivary chromosome maps of the following authors were used to identify the location of the *LSP-1* genes in the Drosophila species: *D. willistoni*, DOBZHANSKY (1950); *D. saltans*, CAVALCANTI (1948) and BICUDA (1973); *D. pseudoobscura*, STOCKER and KASTRITSIS (1972) and TAN (1937); *D. hydei*, BERENDES (1963); *D. virilis*, HSU (1952); *D. funebris*, PERJE (1954); *D. immigrans*, CALVEZ (1953); *D. busckii*, KRIVSHENKO (1963).

Figure 3 shows that the *LSP-1* β probe hybridizes to region 93-94 on chromosome 3 of *D. willistoni*. We found the chromosome maps of *D. saltans* difficult to interpret, but the *LSP-1* β probe hybridized to chromosome 3, which was identified because it was the longest arm and because of the location of the proximal weak points (CAVALCANTI 1948). The *LSP-1* α probe hybridized to the same location as the *LSP-1* β probe in these species on several nuclei of the 500 examined (data not shown). The *LSP-1* γ probe did not hybridize consistently to any one site in these species.

As shown in Figure 4, the *LSP-1* β probe hybridizes to region 54 on chromosome 2 of *D. pseudoobscura*, which is the same region to which the *LSP-1* α probe hybridizes. The *LSP-1* β probe also hybridizes to region 90-91 on chromosome 4.

The *LSP-1* α and β probes hybridized to the same site on the polytene chromosomes of *D. hydei*, *D. virilis*, *D. funebris*, *D. immigrans* and *D. busckii* as shown in Figures 4 and 5. These joint sites are 33A on chromosome 2 of *D. hydei*, 2C-D on chromosome 2 of *D. virilis*, A3-4 on chromosome 2 of *D. funebris*,

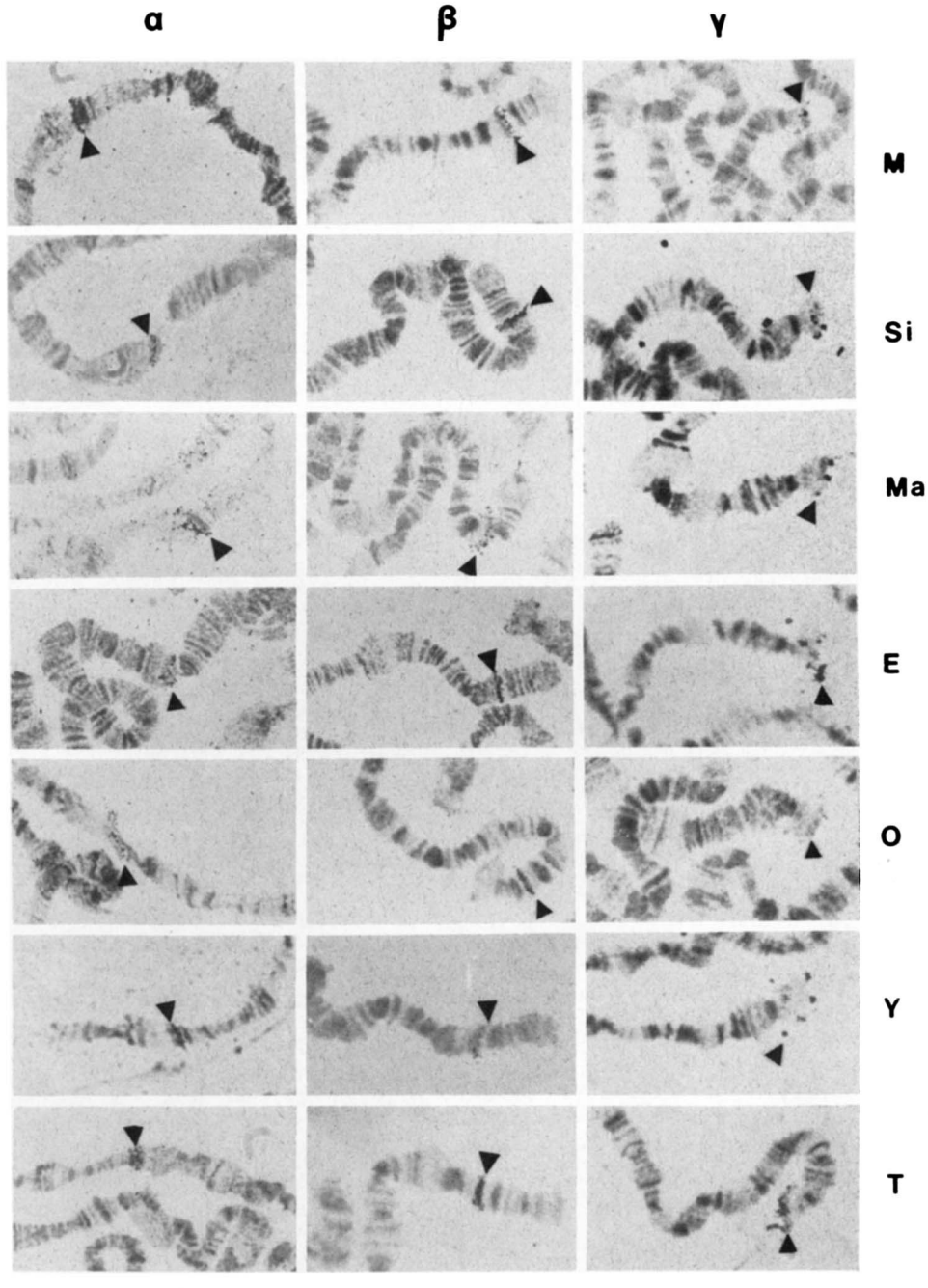

FIGURE 2.—*In situ* hybridization of the *LSP-1* α, β and γ probes to the polytene chromosomes of the *melanogaster* subgroup species. Grains are indicated with arrows. The species abbreviations are as follows: M, *D. melanogaster*; Si, *D. simulans*; Ma, *D. mauritiana*; E, *D. erecta*; O, *D. orena*; Y, *D. yakuba*; T, *D. teissieri*.

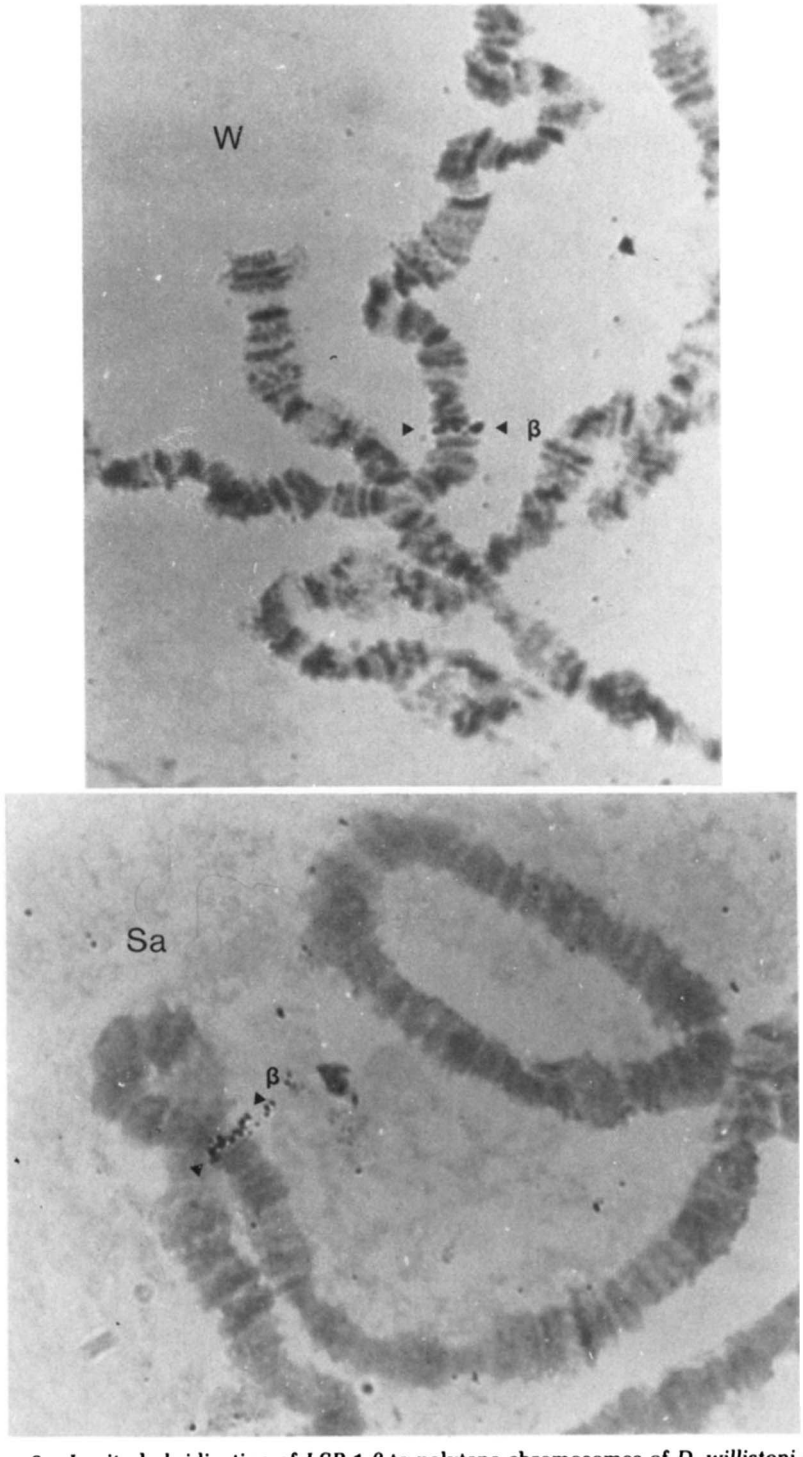

FIGURE 3.—*In situ* hybridization of *LSP-1 β* to polytene chromosomes of *D. willistoni* (W) and the hybridization of *LSP-1 β* to *D. saltans* (Sa). The *LSP-1 α* and *β* probes hybridize to the same site in region 93-94 of *D. willistoni* and to the same site on chromosome 3 of *D. saltans* (only *β* shown).

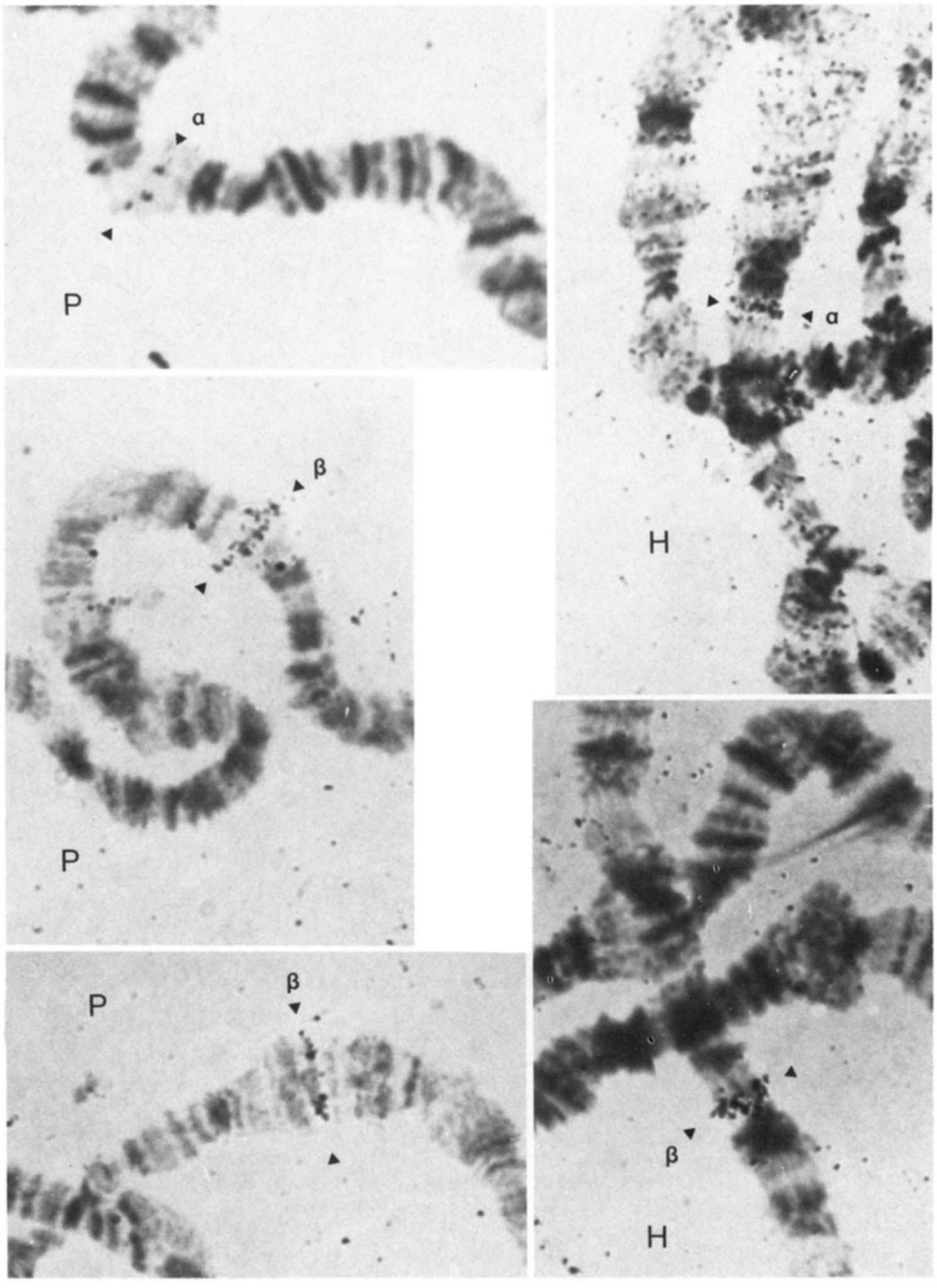

FIGURE 4.—*In situ* hybridization of *LSP-1* α and β to polytene chromosomes of *D. pseudoobscura* (P) and *D. hydei* (H). The *LSP-1* α and β probes hybridize to the same puff in region 54 of chromosome 2 of *D. pseudoobscura*, and the *LSP-1* β probe hybridizes in addition to region 90-91 on chromosome 4. The *LSP-1* α and β probes both hybridize to region 33A on chromosome 2 of *D. hydei*.

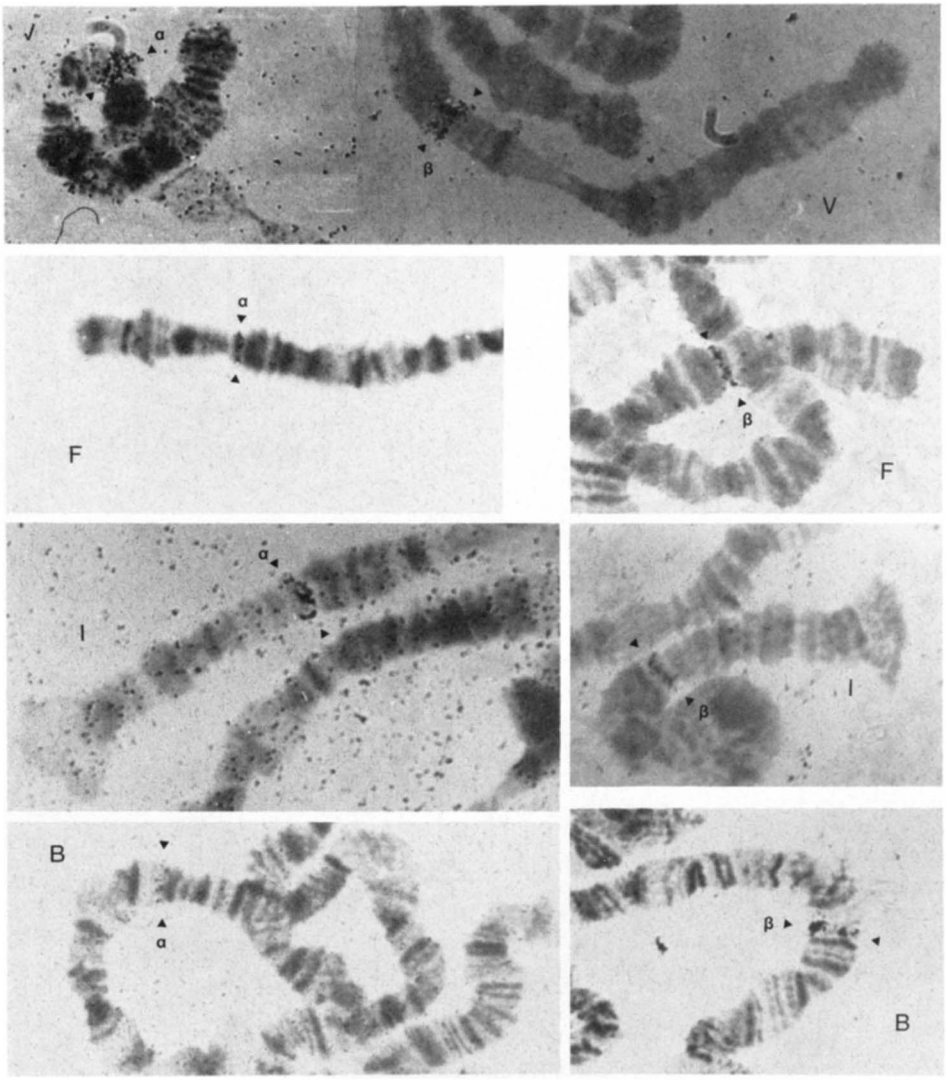

FIGURE 5.—*In situ* hybridization of the *LSP-1* α and β probes to polytene chromosomes of *D. virilis* (V), *D. funebris* (F), *D. immigrans* (I) and *D. busckii* (B). In each species both *LSP-1* probes hybridize to one site. This site is region 2C-D on chromosome *2* of *D. virilis*, A3-4 on chromosome *2* of *D. funebris*, 3A3 on chromosome *3* of *D. immigrans* and region 33-34 on chromosome *2L* of *D. busckii*.

3A3 on chromosome *3* of *D. immigrans* and region 33-34 on chromosome *2L* of *D. busckii*.

In general, more grains are deposited at one site with the *LSP-1* β probe than with the *LSP-1* α probe, despite both probes having similar specific activities and both sets of slides being exposed for the same length of time. The most likely explanation for these results is that the *LSP-1* genes in these species are more conserved relative to the *LSP-1* β probe than they are to the *LSP-1* α probe.

The detection of the *LSP-1* α site in *D. funebris*, *D. immigrans* and *D. busckii* is only just possible owing to the low number of grains deposited at these sites relative to the background. We are confident for the reasons mentioned before that these sites are genuine. However, it is possible in these species that additional sites of hybridization with the *LSP-1* α probe were missed. This problem is discussed in light of evidence from analysis of genomic DNA.

The *LSP-1* γ probe did not hybridize detectably to the polytene chromosomes of any of these species, even when the probe was made twice the specific activity of the *LSP-1* α and β probes and slides were exposed twice as long. As will be reported, the *LSP-1* γ probe did not bind specifically to genomic DNA from these species in Southern transfer experiments. These results together suggest that, if these species possess an *LSP-1* γ-like gene, this gene does not preserve sufficient DNA sequence homology to hybridize to the *D. melanogaster* probe.

The results of the *in situ* hybridization experiments are summarized in Table 1. Also shown in the table are the postulated homologies between the chromosome arm to which the *LSP-1* probes hybridize and the chromosome arms of *D. melanogaster* based on studies of mutants. STURTEVANT and NOVITSKI (1941) claim that element E is always the longest arm. Therefore, despite the lack of homology studies using mutants, chromosome 3 of *D. saltans*, chromosome 2 of *D. funebris* and chromosome 3 of *D. immigrans* are homologous to element E in Table 1 because they are the longest chromosome arms in each species. It is striking that, in every species except the *melanogaster* sibling species and one of the *LSP-1* β sites in *D. pseudoobscura*, the *LSP-1* α and β probes hybridize to element E, which is homologous to chromosome 3R of *D. melanogaster*.

Interpretation of these data is complicated because in every species two probes hybridize to the same location. This could be because there are two

TABLE 1

Location of LSP-1 *genes on the polytene chromosomes of Drosophila species*[a]

Species	Probe	Chromosome element	Homologous chromosome in *D. melanogaster*	Reference
melanogaster species subgroup	α	A	X	MULLER 1940
	β	B	2L	
	γ	D	3L	
D. willistoni	α, β	E	3R	STURTEVANT and NOVITSKI 1941
D. saltans	α, β	E	3R	Longest arm
D. pseudoobscura	α, β	E	3R	STURTEVANT and TAN 1936
	β	B	2L	
D. hydei	α, β	E	3R	SPENCER 1949
D. virilis	α, β	E	3R	CHINO 1936
D. funebris	α, β	E	3R	Longest arm
D. immigrans	α, β	E	3R	Longest arm
D. busckii	α, β	E	3R	STURTEVANT and NOVITSKI 1941

closely linked *LSP-1* genes at this site, or because the *LSP-1* α and β probes from *D. melanogaster* retain sufficient homology to a single *LSP-1* gene to cross-hybridize, or that a mixture of the two possibilities obtains in different species. To determine *LSP-1* gene number, DNA was purified from each species, digested with restriction enzymes, electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized to nick-translated *LSP-1* DNA.

Preliminary experiments showed that, when the complete *LSP-1* α and β coding sequences were used as probes, they cross-hybridized to genomic DNA fragments from *D. melanogaster*. This cross-hybridization could be eliminated by increasing the stringency of the wash conditions. However, in the distantly related Drosophila species in order to detect the *LSP-1* DNA sequences that have diverged from the *D. melanogaster LSP-1* genes, stringent wash conditions cannot be used. This fact introduces a complication into the analysis of the data. If transfers are washed at low stringency, the probability of cross-hybridization of the *LSP-1* α and β probes to the same fragment increases. Therefore, the filters were washed under conditions of increasing stringency and were interpreted with the assumption that loss of hybridization signal should be least in the fragment that preserves the most homology to the probe.

Analysis of the data is further complicated because the relative conservation of different domains of *LSP-1* genes in distantly related species may be different for each probe. For example, if two different probes hybridize to a single fragment, this may mean that there is one gene to which both probes cross-hybridize, or that there is conservation of restriction sites in two separate genes. Conversely, if two probes hybridize to different bands, this may mean that there are two different genes, or that there is one gene with a restriction site in the middle, and that one probe hybridizes to the 5′ fragment, and the other probe hybridizes to the 3′ end. Therefore, we used probes that are subsets of the complete coding sequence to increase the information available to us.

Figure 6 shows restriction maps of the *LSP-1* genes after SMITH *et al.* (1981). The *LSP-1* α 5′-probe is the 1.6-kb *Bam*HI fragment, and the *LSP-1* α 3′-probe is the 0.7-kb *Bam*HI fragment. The *LSP-1* β 5′-probe is the 1.3-kb *Bam*HI/*Pst*I fragment, and the *LSP-1* β 3′-probe is the 1.0-kb *Bam*HI fragment. The *LSP-1* γ 5′- and 3′-probes are the entire plasmids that contain 2.2 and 0.6 kb of coding sequence, respectively. After hybridization the filters were washed at 50° or 60°C. Comparison of the two filters allowed us in most cases to classify genomic DNA fragments as being more similar to *LSP-1* α or *LSP-1* β of *D. melanogaster*.

Figure 7 shows complete results of a genomic Southern analysis for *D. hydei*. As will be shown, results from the other species may be interpreted similarly. The DNA was digested with a mixture of *Eco*RI and *Hind*III. The *LSP-1* α 5′- and 3′-probes both hybridize to 4.6 and 3.4-kb fragments but more strongly to the 4.6-kb fragment. When the filters are washed at 60° hybridization to the 3.4-kb fragment by the *LSP-1* α 3′-probe almost disappears. Similar results are obtained with the α 5′-probe (results not shown). This suggests that the 4.6-kb fragment contains sequences that are more homologous to the *LSP-1* α probe than does the 3.4-kb fragment. The *LSP-1* β 5′-probe hybridizes only to the 3.4-kb fragment, whereas the *LSP-1* β 3′-probe hybridizes to the same fragment

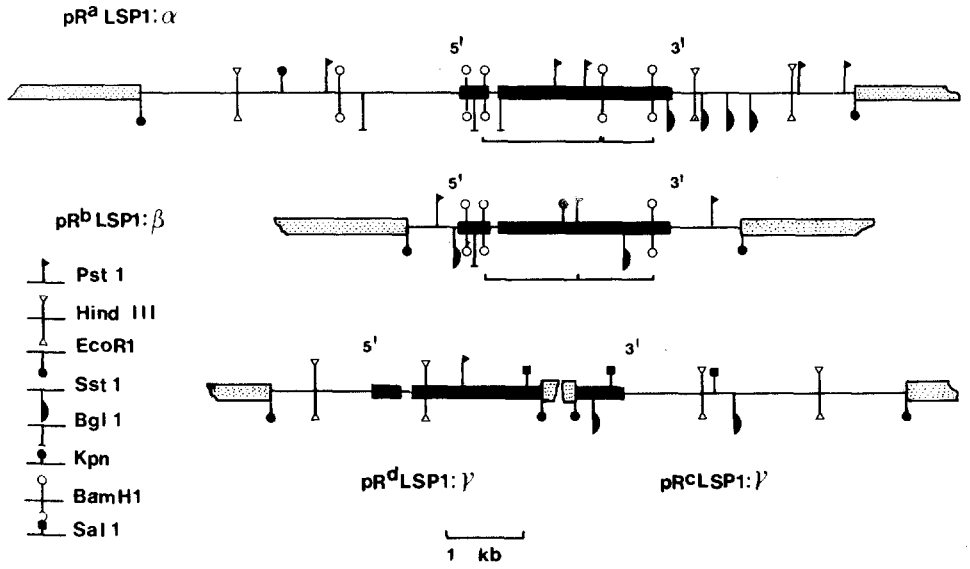

FIGURE 6.—Restriction maps of the *LSP-1* plasmids. These maps are adapted from SMITH *et al.* (1981). The restriction fragments corresponding to the *LSP-1* α and β 5′- and 3′-probes are indicated underneath each plasmid.

and a 3.0-kb fragment in addition when the filters are washed at 50°. The same pattern of hybridization is seen when the filters are washed at 60°. Since the *LSP-1* β probes hybridize weakly or not at all to the 4.6-kb fragment, the washes at different stringencies show that the 4.6-kb fragment contains sequences that are more similar to the *LSP-1* α probe, and that the 3.4- and 3.0-kb fragments contain sequences that are more similar to the *LSP-1* β probe. Single digests with *Eco*RI and *Hind*III alone gave results completely consistent with the results shown here, and the results with double digests were obtained independently at least three times with each species.

The simplest interpretation of these data is that there are two *LSP-1* genes in *D. hydei*, one α-like and one β-like. These results are consistent with *LSP-1* α-like 5′ and 3′ sequences being included in the 4.6-kb fragment, and the *LSP-1* β-like gene's 5′ sequences being included in the 3.4-kb fragment, along with some 3′ sequences. The 3.0-kb fragment contains sequences homologous to the *LSP-1* β 3′ sequences. However, the 3.4-kb fragment must also retain sufficient homology to the *LSP-1* α probe to permit cross-hybridization. Presumably, the *LSP-1* α-like gene of *D. hydei* has diverged too far from the *D. melanogaster* *LSP-1* β probe to allow cross-hybridization to be detectable.

Figure 8 shows the results of similar analyses with six other species: *D. willistoni*, *D. pseudoobscura*, *D. virilis*, *D. funebris*, *D. immigrans* and *D. busckii* using the 3′ α and β probes washed at 50° and 60°. The results from each species may be interpreted similarly to those of *D. hydei*. In each case the 5′ and 3′ *LSP-1* α probes hybridize strongly to a 4.6-kb fragment and less strongly to a 3.4-kb fragment at 50°. When the filter is washed at 60°, hybridization to the 3.4-kb fragment markedly diminishes relative to the 4.6-kb fragment. Results are

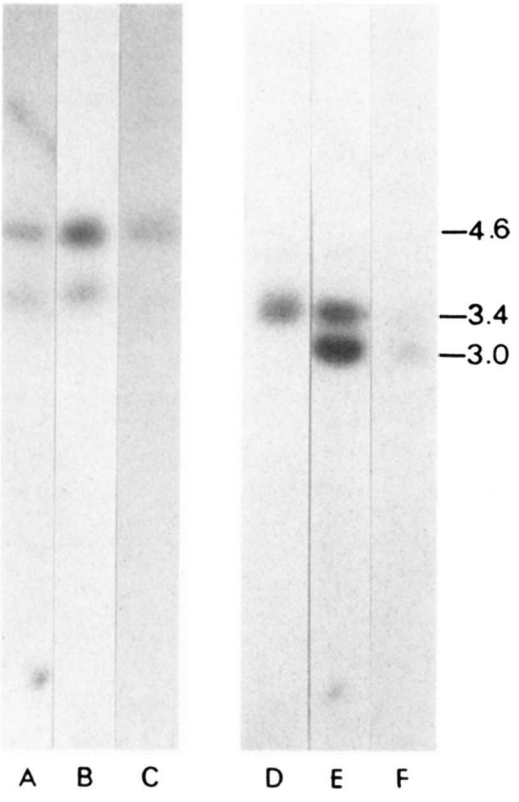

FIGURE 7.—Filter transfer hybridization analysis of *D. hydei* DNA digested with *Eco*RI and *Hind*III. The DNA was electrophoresed after digestion in 1% agarose gels and transferred to nitrocellulose filters. The filters were hybridized in turn with the *LSP-1* α and β 5′- and 3′-probes and washed at either 50° or 60° in 0.1 × NaCl/Cit, 0.1% SDS, and exposed to X-ray film with intensifying screens at −70° for 1 to 2 wk. A, *LSP-1* α 5′-probe, 50° wash; B, *LSP-1* α 3′-probe, 50° wash; C, *LSP-1* α 3′-probe, 60° wash; D, *LSP-1* β 5′-probe, 60° wash; E, *LSP-1* β 3′-probe, 50° wash; F, *LSP-1* β 3′-probe, 60° wash. The size of restriction fragments in kilobases was determined by reference to standards co-electrophoresed on the same gel and is indicated at the side of the tracks. These data show that there are separate α- and β-like genes in *D. hydei*. The *LSP-1* α-like gene is included in a 4.6-kb fragment containing both 5′ and 3′ sequences. Hybridization of the *LSP-1* α probes to the 3.4-kb fragment diminishes as the stringency of wash increases suggesting that this is cross-hybridization. The *LSP-1* β-like gene spans a 3.4-kb fragment containing 5′ and 3′ sequences and a 3.2-kb 3′ fragment.

shown here for only the 3′ probe. In *D. willistoni*, *D. virilis* and *D. funebris* the 3′ α probe hybridizes in addition to smaller fragments of varying sizes. Hybridization to these smaller fragments also diminishes when the filters are washed at 60°. The *LSP-1* β 5′-probe hybridizes strongly to the 3.4-kb fragment at both washing stringencies (not shown), and the *LSP-1* β 3′-probe hybridized strongly to both the 3.4- and 3.0-kb fragments at both 50° and 60°C. In *D. willistoni* the results were more complex because the *LSP-1* α and β 5′-probes hybridized in addition to 1.4- and 1.2-kb fragments (data not shown).

The parsimonious explanation of these data is that there are at least two *LSP-1* genes in these species, one that is α-like and one that is β-like, for reasons that

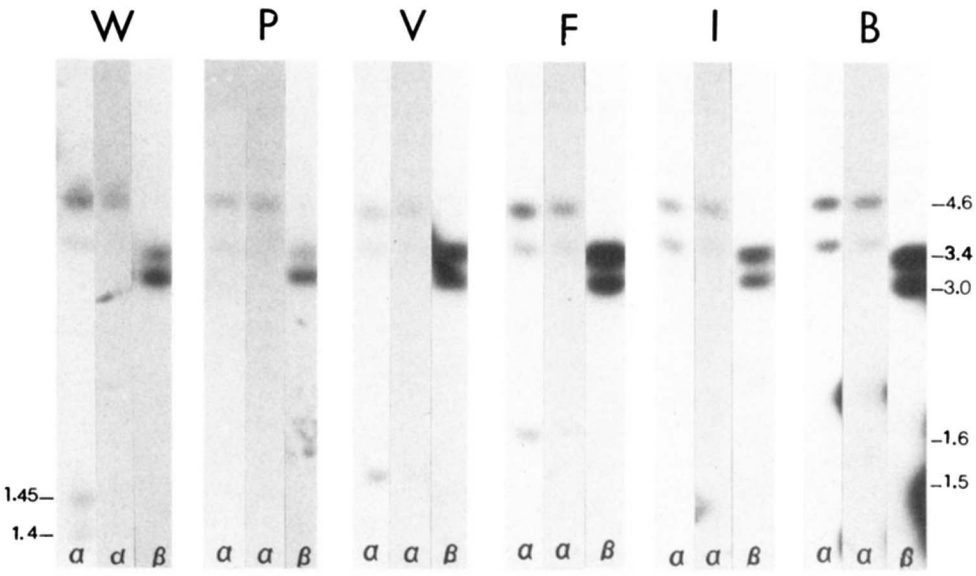

FIGURE 8.—Genomic Southern analysis of DNA from six Drosophila species. Conditions and abbreviations are the same as described for Figure 7 and previous figures. For each species are shown three filters that are in order from left to right: *LSP-1* α 3′-probe, 50° wash; *LSP-1* α 3′-probe 60° wash; *LSP-1* β 3′-probe, 50° wash. The results show that the *LSP-1* α probe binds most strongly to the 4.6-kb fragment and that hybridization to the 3.4 kb and smaller fragments diminishes at 60°, suggesting that the 4.6-kb fragment contains sequences most homologous to the *LSP-1* α probe. The *LSP-1* β 3′-probe hybridizes to a 3.4-kb fragment and to a 3.0-kb fragment. The *LSP-1* β 5′ probe binds only the 3.4-kb fragment (not shown). These results are consistent with an *LSP-1* β-like gene spanning a 3.4-kb 5′ fragment that also contains some 3′ sequences and a 3.0-kb fragment that contains only 3′ sequences.

are analogous to those advanced above for *D. hydei*. The 4.6-kb fragment contains 5′ and 3′ sequences which are more homologous to *LSP-1* α. It may be that there is a restriction site just inside the 3′ boundary of the coding sequence, and that in *D. willistoni*, *D. virilis* and *D. funebris* sufficient homology is retained at the 3′ end to detect smaller DNA fragments at 50° washing temperatures. The additional argument can be made in these species that the 0.7-kb *LSP-1* α 3′-probe could not hybridize to 4.6 and 3.4 kb and a smaller fragment unless there were at least two *LSP-1* genes. The *LSP-1* β-like gene spans a 3.4-kb fragment containing 5′ and 3′ sequences and a 3.0-kb fragment containing only 3′ sequences. The 3.4-kb fragment must contain sequences that are homologous to both probes. In *D. willistoni* it was not possible to assign all fragments as being α- or β-like, so it is possible that there are more than two *LSP-1* genes in this species. It should be noted that there may be more than two *LSP-1* genes in the other species, but the data show that there are at least two.

In these species the *LSP-1* β probe gave more intense hybridization to filter-bound genomic DNA than did the *LSP-1* α probes. This is consistent with the *in situ* data and argues that the β-like gene of these species retains more homology to the *LSP-1* β gene of *D. melanogaster* than do the α-like genes to the *LSP-1* α gene of *D. melanogaster*. In *D. hydei* and *D. virilis* in which large numbers of

grains are deposited with both probes after *in situ* hybridization we are unlikely to have missed a second site of hybridization. Therefore, these data show that for these species the *LSP-1* α-like and β-like genes are closely linked on chromosome *3R*. With the other species the number of grains deposited by the *LPS-1* probe is low relative to the background, so it is possible that the grains observed at the *3R* site with this probe represent cross-hybridization to the β-like site, and that the true α-like site was missed, perhaps because of under-replication or chromosomal location. However, it is highly likely that the *LSP-1* α and β-like species are closely linked in all species. It is not clear why two sites were detected with the *LSP-1* β probe by *in situ* hybridization in *D. pseudoobscura*, yet only one β-like gene appears to be present in the genomic Southern analysis, even though six different restriction enzymes were used in an attempt to find evidence of gene duplication in the β-like gene of this species. This observation suggests that estimates of gene number obtained from the filter hybridization analysis are more likely to be underestimates.

One striking feature of these data is the similarity of restriction sites between species in both the *LSP-1* α and β-like genes. Since the organization of the *LSP-1* genes, in addition to the size, number and position of the coding and intervening sequences in these species relative to the restriction sites, is unknown, and because few restriction sites have been sampled, it is not clear what significance this observation has. Accurate determination of *LSP-1* gene number and organization will require analysis of cloned DNA. The *LSP-1* γ probes were also hybridized to filters containing genomic DNA digested with *Eco*RI and *Hind*III. In each of these species faint hybridization to the 3.4- and 3.0-kb fragments was seen after long exposure times. If these species have a separate *LSP-1* γ gene it has either conserved restriction sites relative to the *LSP-1* β-like genes of these species or its DNA sequence has diverged sufficiently to prevent detection by hybridization. The latter implies that the β-like gene of these species retains some homology to the *LSP-1* γ probe. This result shows that under our conditions filter hybridization is more sensitive than is *in situ* hybridization since no grains were detected at the *LSP-1* β-like site with the *LSP-1* γ probe. H. W. BROCK and D. B. ROBERTS (unpublished data) have shown that γ-like proteins are detectable with an *LSP-1* γ-specific antibody in these species. It may be that DNA sequence divergence has been great enough to prevent detection of a γ-like gene by hybridization, but the product of this gene still retains some antigenic sites recognized by the antibody.

It has not been shown that the α-like and β-like sequences detected in these species by filter hybridization analysis are active. *LSP-1* is synthesized in the fat body, and when fat body mRNA is analyzed by filter transfer hybridization with *LSP-1* α and β 3′ probes strong hybridization to an mRNA which comigrates with *LSP-1* message is detected with both probes in all species when the filters are washed at 60° (H. W. BROCK and D. B. ROBERTS, unpublished results). Because at 60° there is no cross-hybridization of the *LSP-1* β probe to the α-like genes in these species, this result shows that the β-like gene in these species is active. Because the *LSP-1* α probe gives a strong hybridization signal at 60°, it is likely that the α-like gene is also active, although this remains to be demonstrated conclusively.

DISCUSSION

The data presented here support the hypothesis that chromosome elements are largely conserved in Drosophila evolution because the *LSP-1* genes are found on element E in eight widely diverged Drosophila species. These data suggest that the ancestor of the genus Drosophila probably had its *LSP-1* genes on element E. It would be of interest to determine the location of the *LSP-2* gene in these species. FOSTER *et al.* (1981) suggested recently that the chromosome 2 of *L. cuprina* is homologous to element B of *D. melanogaster* on the basis of homology of lucilin to *LSP-1* β and of two enzymes involved in tyrosine metabolism. Our study suggests that the *LSP-1* β gene of *D. melanogaster* is not a good gene to use for determining chromosome homologies in distant species.

Because the *LSP-1* genes of the *melanogaster* subgroup are found on elements A, B and D, the conservation of linkage groups is not absolute. As mentioned previously, there have been other examples of genes that have apparently been exchanged between elements. The mechanism by which this occurs is not clear. STUART *et al.* (1981) propose a model based on translocations and inversions to explain movement of the *18/28S RNA* genes from the X chromosome to an autosome, but they point out that conservation of elements is maintained because the translocation and pericentric inversion required occur in heterochromatin. GREGG and SMUCKER (1965) suggest that pteridine genes may have been exchanged between arms by means of a pericentric inversion. The metaphase configurations of the *melanogaster* group do show wide variation (CLAYTON and WHEELER 1975), but as discussed before, establishment of translocation and pericentric inversions is extremely rare in Drosophila. Three translocations or three fusions and pericentric inversions are required to account for the dispersal of *LSP-1* genes in the *melanogaster* subgroup species, so it is very doubtful if *LSP-1* dispersal can be explained by these means.

An alternative explanation is that the *LSP-1* genes have been dispersed by transposable elements. These elements have been shown to be able to move genes in the genome (GREEN 1969; ISING and RAMEL 1976). An organism in which genes have been dispersed by this mechanism may not face such severe selection pressure against establishment of the change as there would be if the genes were dispersed by translocation or pericentric inversion. The observation that the *LSP-1* genes are in the same location in all of the *melanogaster* sibling species suggests that transposition is not a frequent event. It would be of interest to determine the location of the *LSP-1* genes in other species of the *melanogaster* species group to determine whether dispersal of each *LSP-1* gene occurred independently and to get an idea of the frequency of *LSP-1* gene dispersal.

The analysis of genomic DNA shows that in seven widely diverged Drosophila species there are separate *LSP-1* α and β genes, and the *in situ* data show that these genes are closely linked. These observations suggest that the *LSP-1* genes arose by tandem duplication and that dispersal of the *LSP-1* genes occurred subsequent to their duplication. It is interesting that the genes for the homologous serum proteins in *L. cuprina* and *C. stygia* are clustered in these species (THOMSON *et al.* 1976; KEMP *et al.* 1978). Further molecular analysis is required to determine the arrangement of *LSP-1* α and β genes in these species. The *LSP-1* genes in different Drosophila species provide an example of a small gene

family that can be either clustered or dispersed and as such offers a system to test hypotheses about clustering and dispersal with regard to gene expression and evolution.

We thank DR. M. ASHBURNER for helpful discussions. This work was supported by a Science Research Council grant to DR. D. B. ROBERTS. H. W. BROCK acknowledges the assistance of a Commonwealth Scholarship.

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Corresponding editor: A. CHOVNICK