J Mol Evol (1990) 30:182–188

Journal of Molecular Evolution

Conservatism of Sites of tRNA Loci among the Linkage Groups of Several *Drosophila* Species

John Tonzetich,[1] Shizu Hayashi,[2] and T.A. Grigliatti[3]

[1] Department of Biology, Bucknell University, Lewisburg, Pennsylvania 17837, USA
[2] Department of Biochemistry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada
[3] Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada

Summary. The sites of seven tRNA genes (Arg-2, Lys-2, Ser-2b, Ser-7, Thr-3, Thr-4, Val-3b) were studied by in situ hybridization. ^{125}I-labeled tRNA probes from *Drosophila melanogaster* were hybridized to spreads of polytene chromosomes prepared from four *Drosophila* species representing different evolutionary lineages (*D. melanogaster, Drosophila hydei, Drosophila pseudoobscura,* and *Drosophila virilis*). Most tRNA loci occurred on homologous chromosomal elements of all four species. In some cases the number of hybridization sites within an element varied and sites on nonhomologous elements were found. It was observed that both $tRNA_2^{Arg}$ and $tRNA_2^{Lys}$ hybridized to the same site on homologous elements in several species. These data suggest a limited amount of exchange among different linkage groups during the evolution of *Drosophila* species.

Key words: *Drosophila* species — tRNA — In situ hybridization — Polytene chromosomes — Chromosomal evolution

Introduction

The study of chromosome evolution in the speciation process is dependent upon determining the homology of chromosomal elements among different karyotypes. Among the *Drosophila* species, chromosomal lineages are based upon a basic karyotype of six of these elements designated A–F (Muller 1940; Sturtevant and Novitski 1941). Variations in these lineages arise primarily by chromosomal fusions and paracentric inversions (Patterson and Stone 1952). Homologies among chromosomes have been established by sites of similar mutations, comparison of banding patterns, and the extent of pairing in hybrids (Table 1). The shortcomings of these analyses in demonstrating homologous loci have been discussed extensively by Whiting et al. (1989). Recently, the technique of in situ hybridization has provided a very effective means of directly determining sites of homologous genes on polytene chromosomes, as hybridization is based on nucleotide sequence similarities (Steinemann 1982; Brock and Roberts 1983; Loukas and Kafatos 1986; Whiting et al. 1989).

Genes having highly conserved sequences among species facilitate hybridization comparisons. The tRNAs demonstrate a strong sequence conservation among eukaryotes and could potentially function as effective interspecific probes. In *Drosophila melanogaster* there are an estimated 59 different tRNAs encoded by approximately 590 genes (Weber and Berger 1976). These genes are arranged in dispersed clusters found at several chromosomal sites, each cluster having a number of identical genes for a specific isoacceptor tRNA (Hayashi et al. 1980). Exceptional clusters having tRNA genes with differences limited to one to three nucleotides outside the anticodon region have been observed (Hosbach et al. 1980; Sharp et al. 1981). Other exceptional clusters contain genes of more than one species of tRNA (Yen and Davidson 1980).

We have compared the hybridization sites of seven tRNAs isolated from *D. melanogaster* (Hayashi et al. 1982) on polytene chromosomes from four

Offprint requests to: S. Hayashi

Table 1. Chromosomal homologies in *Drosophila* species

Drosophila species	Elements						References
	A	B	C	D	E	F	
virilis	X	4	5	3	2	6	Alexander (1976)
hydei	X	3	5	4	2	6	Hess (1976)
pseudoobscura	XL	4	3	XR	2	5	Lancefield (1922)
melanogaster	X	2L	2R	3L	3R	4	Sturtevant and Novitski (1941)

Table 2. Sites of hybridization of ^{125}I-labeled $tRNA^{Val}_{3b}$ to the polytene chromosomes of four species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	B	4	48F	7.1 ± 4.1	21
	E	2	21C4	9.8 ± 3.8	21
	E	2	28G2	6.2 ± 2.9	21
	E	2	29A1	26.2 ± 9.3	21
D. hydei	E	2	25A	8.0 ± 4.0	53
	E	2	33BC	20.3 ± 5.5	53
	E	2	35B	8.6 ± 3.7	53
D. pseudo-obscura	E	2	43	21.5 ± 4.9	20
	E	2	45	4.5 ± 2.4	20
	E	2	47	6.1 ± 3.2	20
D. melano-gaster	E	3R	84D	30.1 ± 8.3	38
	E	3R	90BC	8.4 ± 5.5	38
	E	3R	92B	18.6 ± 6.3	38

[a] Average number of grains at a site ± SD

[b] Total number of chromosomal regions analyzed

selected drosophilids. The four species studied are *Drosophila virilis* and *Drosophila hydei* (subgenus *Drosophila*) and *Drosophila pseudoobscura* and *D. melanogaster* (subgenus *Sophophora*). The tRNAs include $tRNA^{Arg}_{2}$, $^{Lys}_{2}$, $^{Ser}_{2b}$, $^{Ser}_{7}$, $^{Thr}_{3}$, $^{Thr}_{4}$, $^{Val}_{3b}$. Hybridizations of these tRNAs to *D. melanogaster* chromosomes have been previously described (Hayashi et al. 1980, 1981, 1982). The in situ map of the tRNA loci is further evidence that linkage groups are essentially conserved in the evolution of drosophilids.

Materials and Methods

In Vitro Labeling of tRNAs with ^{125}I. Transfer RNAs were isolated and purified as described in Hayashi et al. (1982), then labeled with ^{125}I by one of two methods. Transfer$^{Ser}_{2b}$ and $^{Thr}_{4}$ were labeled according to Commerford (1971) with modifications as described by Dunn et al. (1979). This method randomly labels cytosine residues. Transfer RNA^{Arg}_{2}, $^{Ser}_{7}$, $^{Thr}_{3}$, and $^{Val}_{3b}$ were labeled at the 3'-ends with ^{125}I-CTP (Hayashi et al. 1981). Both methods were used to label $tRNA^{Lys}_{2}$. The subsequent purification of the iodinated tRNAs was according to Hayashi et al. (1982).

In Situ Hybridizations. Polytene chromosomes from the salivary glands of late third instar larvae of *D. melanogaster* Oregon R strain, *gt^{1}w^{a}/y sc In(l)gt^{X11}*; *D. pseudoobscura* (from Dr. A. Beckenbach, Simon Fraser University); *D. virilis* Texmilucan and *D. hydei* (both from the species stock center in Austin, Texas) were prepared for in situ hybridization as described in Hayashi et al. (1980). The hybridizations were carried out in 0.16 M NaH_2PO_4, pH 7.0, 10 mM EDTA at 65°C or in 70% formamide/phosphate buffer at 45°C, according to the procedures described in Hayashi et al. (1980, 1981).

Analysis of Autoradiograms. The labeling of chromosomes was detected using Ilford K2 or K5 emulsion, followed by staining with 0.04% toluidine blue O in 2× SSC (0.3 M NaCl, 0.03 M Na citrate, pH 7.0). The frequency of grains at specific sites of hybridization was determined. Sites were identified from published maps of polytene chromosomes of *D. melanogaster* (Lefevre 1976), *D. virilis* (Gubenko and Evgen'ev 1984), *D. pseudoobscura* (Kastritsis and Crumpacker 1966; Stocker and Kastritsis 1972), and *D. hydei* (Berendes 1963; Ananiev and Barsky 1982).

Results

The sites of tRNA hybridization to polytene chromosomes from the four species of *Drosophila* and the homologous chromosomal element (see Table 1) on which the site resides are recorded in Tables 2–8. The mean number of grains per hybridization site provides an approximation of the relative number of genes of the tRNA at the site. The tRNAs are presented in order from the tRNAs showing the greatest conservation of sites on homologous elements to those with increasing variations.

Complete chromosomal homology of the sites of hybridization among the four species was not found for any of the tRNAs studied. Except for a minor site on element B in *D. virilis*, $tRNA^{Val}_{3b}$ consistently hybridized at three sites on the same chromosomal element, namely element E, in all four species (Table 2 and Fig. 1). Moreover, the intensity of label at these three sites varied. In *D. virilis*, *D. hydei*, and *D. pseudoobscura* there was one major site of high density labeling and two minor sites; in *D. melanogaster* there were two strong sites.

Transfer RNA^{Ser}_{2b} was studied in *D. virilis*, *D. hydei*, and *D. melanogaster* (Table 3). Data were not available for *D. pseudoobscura*. As with $tRNA^{Val}_{3b}$, three sites were found on chromosomal element E. In addition to these three sites, a fourth very weakly labeled locus on element D was detected in both *D. virilis* and *D. hydei*. The distribution of grains over

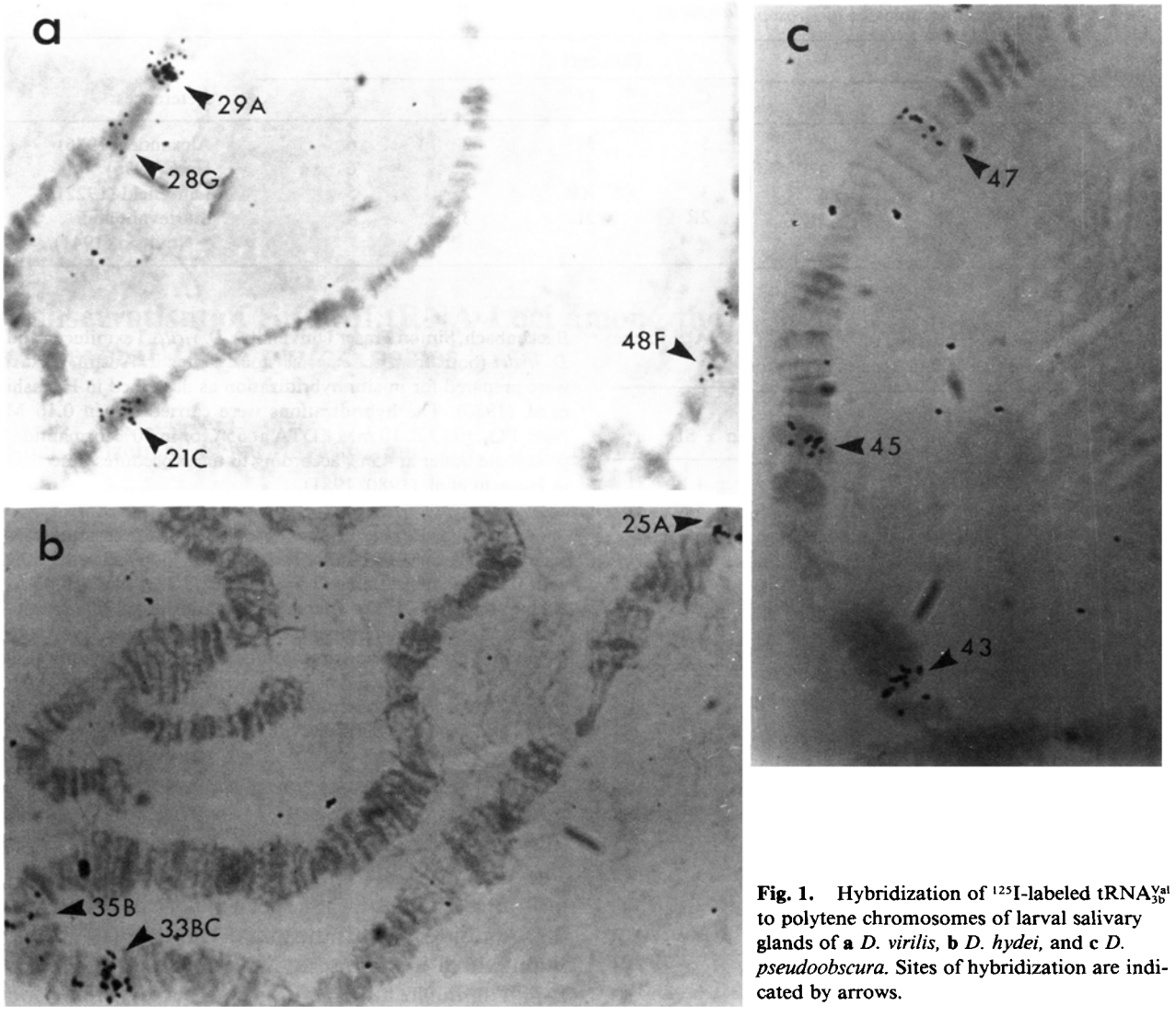

Fig. 1. Hybridization of ^{125}I-labeled $tRNA_{3b}^{Val}$ to polytene chromosomes of larval salivary glands of **a** *D. virilis*, **b** *D. hydei*, and **c** *D. pseudoobscura*. Sites of hybridization are indicated by arrows.

the three main sites varied between species. *Drosophila virilis* had one definite strong site, *D. melanogaster* had one site that was stronger than the other two, and *D. hydei* had all three sites equally labeled.

The pattern of hybridization of $tRNA_2^{Arg}$ (Table 4) was equally varied as that found for $tRNA_{2b}^{Ser}$. In the two species studied from the subgenus *Drosophila*, a single site was found on element C. In the subgenus *Sophophora* two sites were labeled in each of the species studied. In *D. pseudoobscura*, in addition to a site on element C, a minor one was found on element B. In *D. melanogaster*, again element C had a strongly labeled site, but, in this case, the second one was on element E.

As with $tRNA_2^{Arg}$ and $^{Ser}_{2b}$, the hybridization of $tRNA_3^{Thr}$ (Table 5) was consistent between elements in the subgenus *Drosophila* but differed in *Sophophora*. In *D. virilis* and *D. hydei* strongly labeled sites of hybridization were found on elements C and E,

Table 3. Sites of hybridization of ^{125}I-labeled $tRNA_{2b}^{Ser}$ to the polytene chromosomes of three species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	D	3	35B	1.6 ± 2.0	21
	E	2	20A5–6	3.0 ± 2.0	21
	E	2	22C4	6.0 ± 4.3	21
	E	2	25H1–2	2.9 ± 2.2	21
D. hydei	D	4	78D	3.3 ± 2.2	16
	E	2	23D	5.7 ± 3.4	16
	E	2	37D	6.9 ± 3.6	16
	E	2	47C	6.7 ± 3.8	16
D. melanogaster	E	3R	86A	9.8 ± 4.6	37
	E	3R	88A	10.4 ± 4.6	37
	E	3R	94A	15.8 ± 7.7	37

[a] Average number of grains at a site ± SD
[b] Total number of chromosomal regions analyzed

Table 4. Sites of hybridization of ^{125}I-labeled $tRNA_2^{Arg}$ to the polytene chromosomes of four species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	C	5	58E	17.0 ± 5.7	20
D. hydei	C	5	95D	18.5 ± 7.9	17
D. pseudo-obscura	B	4	83	3.0 ± 1.5	11
	C	3	80	14.4 ± 3.6	21
D. melano-gaster	C	2R	42A	19.9 ± 4.5	17
	E	3R	84F	10.4 ± 3.1	17

[a] Average number of grains at a site ± SD
[b] Total number of chromosomal regions analyzed

Table 5. Sites of hybridization of ^{125}I-labeled $tRNA_3^{Thr}$ to the polytene chromosomes of four species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	A	X	12B	3.6 ± 2.6	23
	C	5	55B2	18.3 ± 5.8	23
	E	2	29E1	15.1 ± 5.8	23
D. hydei	A	X	9A	4.0 ± 2.7	11
	C	5	101B	20.1 ± 4.7	22
	E	2	32C	20.0 ± 5.5	23
D. pseudo-obscura	A	XL	17	5.1 ± 2.7	48
	C	3	73	10.1 ± 4.4	48
D. melano-gaster	C	2R	47F	41.3 ± 11.7	30
	E	3R	87B	22.6 ± 6.8	30
	E	3R	93AB	3.7 ± 2.6	30

[a] Average number of grains at a site ± SD
[b] Total number of chromosomal regions analyzed

with a weak site on element A. *Drosophila pseudoobscura* no longer showed hybridization to element E, whereas *D. melanogaster* lost the site on element A and gained a second, albeit very minor one, on element E. The single common site among the four species on element C was also the one most heavily labeled in all four.

The results of the hybridization of $tRNA_2^{Lys}$ (Table 6) maintain the distinction between the subgenera *Drosophila* and *Sophophora* made by the three previous tRNAs. *Drosophila virilis* and *D. hydei* consistently had one site of hybridization on element E and three on element C. In contrast, *D. melanogaster* had one site on element D and four on element C. For *D. pseudoobscura,* the data are incomplete, as the specific activity of the labeled tRNA in this case was 10 times lower than that normally obtained [10^{17} disintegrations/min (dpm)/mole compared with 10^{18} dpm/mole]. Only two sites of labeling were found, both on element C. It is interesting to note that in all four species the strongest labeling site of $tRNA_2^{Lys}$ is also the strongest labeling site of $tRNA_2^{Arg}$.

Table 6. Sites of hybridization of ^{125}I-labeled $tRNA_2^{Lys}$ to the polytene chromosomes of four species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	C	5	58BC	9.2 ± 4.4	12
	C	5	58E	21.7 ± 6.0	12
	C	5	59A	8.4 ± 5.0	12
	E	2	26BC	5.7 ± 3.2	12
D. hydei	C	5	95D	11.1 ± 4.7	7
	C	5	96D	6.1 ± 3.5	6
	C	5	98D	5.4 ± 2.0	8
	E	2	23B	5.8 ± 1.8	8
D. pseudo-obscura[c]	C	3	63	2.1 ± 1.2	7
	C	3	80	6.2 ± 2.2	13
D. melano-gaster	C	2R	42A	31.0 ± 13.7	45
	C	2R	42E	19.7 ± 7.5	45
	C	2R	50B	4.6 ± 2.8	45
	C	2R	56EF	7.3 ± 4.2	45
	D	3L	63B	8.7 ± 4.7	45

[a] Average number of grains at a site ± SD
[b] Total number of chromosomal regions analyzed
[c] Data incomplete, see Results

Table 7. Sites of hybridization of ^{125}I-labeled $tRNA_4^{Thr}$ to the polytene chromosomes of three species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	E	2	20A1	12.9 ± 5.0	15
	E	2	27A1	7.3 ± 4.9	15
D. hydei	E	2	23B	9.3 ± 3.5	14
	E	2	47B	21.1 ± 5.7	14
D. melano-gaster	E	3R	93E	13.9 ± 4.8	32

[a] Average number of grains at a site ± SD
[b] Total number of chromosomal regions analyzed

The hybridization of $tRNA_4^{Thr}$ (Table 7) follows the pattern set by $tRNA_2^{Arg}$, $tRNA_3^{Thr}$, and $tRNA_2^{Lys}$. *Drosophila virilis* and *D. hydei* both had two sites of labeling on element E, whereas *D. melanogaster* had only one site on this element. The data for the hybridization to chromosomes of *D. pseudoobscura* were not available.

The greatest diversity in the pattern of hybridization to the chromosomal elements in the four species was found with $tRNA_7^{Ser}$ (Table 8). *Drosophila virilis* had one site on each of elements A and C and two on element D, whereas *D. hydei* had the same with the addition of a site on element B. Closer inspection of *D. virilis* element B indicated a very weak signal at 46B2 but this labeling was not consistent. *Drosophila melanogaster* was the same as *D. hydei,* except it had only one site on element D. *Drosophila pseudoobscura* differed from *D. melanogaster* in that it had a site on element E instead of on D.

Discussion

The four species studied occur in two widely separated taxa, the subgenus *Drosophila* and the subgenus *Sophophora*. According to Throckmorton (1975), *D. hydei* diverged from the *D. virilis* line in the subgenus *Drosophila*, whereas *D. melanogaster* and *D. pseudoobscura* diverged at approximately the same time from an earlier common line in the subgenus *Sophophora*. Results of the present study indicate that among these distantly related species there is a strong conservation of tRNA loci on homologous linkage groups or elements. All four species share one to several sites on common linkage groups, but variation does occur with some species having increased numbers of sites on additional elements.

The hypothesis that homologous elements have remained essentially intact is best supported by the results of the hybridization of $tRNA_{3b}^{Val}$ and $tRNA_{2b}^{Ser}$. Each tRNA hybridized to three sites on chromosomal element E in all the species tested. These results are comparable to the conservatism found in non-tRNA genes by other investigators: the actin gene family whose six genes are distributed on homologous linkage groups in several drosophilid subgenera (Loukas and Kafatos 1986); the alcohol dehydrogenase gene on element B both in *D. pseudoobscura* (Schaeffer and Aquadro 1987) and in *D. melanogaster* (O'Donnell et al. 1977); and the heat shock puffs at 48D and 93D in *D. hydei* and *D. melanogaster*, respectively, both on element E, which were demonstrated to be equivalent by Ryseck et al. (1987).

The linkage homology of the remaining five tRNAs studied was also conservative in pattern, but greater variations in number of loci and linkage group associations did occur. In the subgenus *Drosophila*, that is, in *D. virilis* and *D. hydei*, four of these tRNAs, $tRNA_2^{Arg}$, $tRNA_3^{Thr}$, $tRNA_2^{Lys}$, and $tRNA_4^{Thr}$, maintained sites of hybridization on the same chromosomal elements. In contrast, in the subgenus *Sophophora*, $tRNA_2^{Arg}$, $tRNA_3^{Thr}$, and possibly $tRNA_2^{Lys}$ showed variations. But even here, whenever a tRNA hybridized to element C, hybridization to this element was conserved in *D. melanogaster* and *D. pseudoobscura*. The distinction in the conservation of linkage groups between the subgenera has been noted by others. Brock and Roberts (1983) who studied chromosomal locations of larval serum protein 1 genes reported greater variations in the subgenus *Sophophora* than in the subgenus *Drosophila*, especially between *D. melanogaster* and *D. pseudoobscura*. Steinemann (1982) reported two tandemly repeated genes, 5S RNA and histone, also on different elements in *D. melanogaster* and *D. pseudoobscura*.

The hybridization of $tRNA_7^{Ser}$ showed the greatest variation, although linkage homology of elements A and C could still be demonstrated in all four species. Part of this apparent variation might be attributable to the fact that $tRNA_7^{Ser}$ also hybridizes to the genes of another serine isoacceptor, $tRNA_4^{Ser}$, as the genes of the two tRNAs from *D. melanogaster* differ by only three nucleotides (Cribbs et al. 1987). Sequence analysis showed that the sites on elements B and D contain genes of $tRNA_7^{Ser}$, the site on element C contains a gene of $tRNA_4^{Ser}$, and the site on element A, genes of both tRNAs (Leung et al., unpublished). Thus, the variation expected for a single isoacceptor tRNA would potentially be doubled. In addition, it has been demonstrated in *D. melanogaster* and its sibling species, *Drosophila erecta* and *Drosophila yakuba*, that hybrid genes resulting from permutations of the three differences between the two serine isoacceptors also reside at the site of hybridization found on the X chromosome (Leung et al., unpublished).

Although a detailed molecular analysis of tRNA genes was not made, evidence for the conservation of genes within a cluster was found. For example, a site on element C with the genes for $tRNA_2^{Arg}$ and $tRNA_2^{Lys}$ has been retained in all four species. That four genes for $tRNA_2^{Arg}$ and five for $tRNA_2^{Lys}$ are present in this cluster in *D. melanogaster* has been verified by sequence analysis of DNA from region 42A (Yen and Davidson 1980). Another example is the association of other $tRNA_2^{Lys}$ genes with the 5S RNA cluster. In *D. virilis* one of the sites of hybridization of $tRNA_2^{Lys}$, 58E, is also one of the

Table 8. Sites of hybridization of ^{125}I-labeled $tRNA_7^{Ser}$ to the polytene chromosomes of four species of *Drosophila*

Species	Element	Chromosome	Site	Mean ± SD[a]	n[b]
D. virilis	A	X	19B2	16.6 ± 4.1	10
	C	5	51C2	7.3 ± 2.6	9
	D	3	35E3	9.4 ± 2.0	11
	D	3	32C3	7.0 ± 1.9	10
D. hydei	A	X	14B	7.7 ± 4.2	7
	B	3	64B	14.6 ± 7.9	5
	C	5	119D	12.4 ± 4.2	5
	D	4	80B	14.5 ± 3.7	6
	D	4	89C	7.2 ± 3.1	5
D. pseudo-obscura	A	XL	4	33.5 ± 7.0	12
	B	4	87–88	12.6 ± 5.2	12
	C	3	65	6.7 ± 4.1	12
	E	2	54	8.1 ± 4.9	12
D. melano-gaster	A	X	12DE	28.2 ± 10.4	12
	B	2L	23E	13.9 ± 3.8	12
	C	2R	56D	5.0 ± 3.2	12
	D	3L	64D	6.2 ± 5.6	12

[a] Average number of grains at a site ± SD

[b] Total number of chromosomal regions analyzed

two sites of 5S RNA genes (Cohen 1976); in *D. melanogaster* 56EF is also the single locus for 5S RNA genes (Wimber and Steffensen 1970). Note that in these species the associated sites are on element C. In *D. hydei* $tRNA_2^{Lys}$ and the 5S RNA genes (Alonso and Berendes 1975) share chromosomal site 23B. In this case the common site is on element E. This analogy between the $tRNA_2^{Lys}$ and 5S RNA genes could not be extended to include *D. pseudoobscura,* as adequate data were not available. Finally, in both *D. melanogaster* and *D. pseudoobscura,* Suter and Kubli (1988) report a $tRNA^{Tyr}$ gene associated with decapentaplegic (*dpp*), one of the genes required for the development of the fly (Gelbart et al. 1985). They suggest a possible regulatory interaction between the two genes. Thus the conservation of sites of tRNA genes appears to include not only associations with linkage groups but also with specific tightly linked genes.

The results of the hybridization of tRNA genes generally support the hypothesis that chromosomal elements remain largely conserved among the *Drosophila,* although differences in the number and location of certain tRNA loci have no immediate explanation. Translocations and pericentric inversions do not appear to be a satisfactory mechanism by which these changes arose. Dispersal by transposable elements has been proposed by Brock and Roberts (1983) as a means of varying the chromosomal residence of larval serum protein 1 genes. There is also evidence that sequences from tRNA genes are an integral part of a few *Drosophila* transposable elements. The 3′ terminal 18 nucleotides of the $tRNA_7^{Ser}$ and $tRNA_{CGA}^{Arg}$ are complementary to the putative primer binding sites of *Drosophila* retrotransposons, 297 and 412, respectively (Inouye et al. 1986; Yuki et al. 1986). Therefore, transposition may be one means by which variation in the location of tRNA genes occurs during evolution. If this were true, our results would suggest that transposition frequencies are much lower in *D. virilis* and *D. hydei* than in *D. pseudoobscura* and *D. melanogaster.*

Acknowledgments. We thank Dr. Andy Beckenbach for his critical reading of the manuscript. This research was supported by grant MT1279 from the Medical Research Council of Canada and the N.S.E.R.C. grant 3005 to T.G.

References

Alexander ML (1976) The genetics of *Drosophila virilis.* In: Ashburner M, Novitski E (eds) The genetics and biology of *Drosophila,* vol 1c. Academic Press, London, pp 1365–1427

Alonso C, Berendes HD (1975) The location of 5S (ribosomal) RNA genes in *Drosophila hydei.* Chromosoma 51:347–356

Ananiev EV, Barsky VE (1982) A photographic map of *Drosophila hydei* polytene chromosomes. Chromosoma 87:239–245

Berendes HD (1963) The salivary gland chromosomes of *Drosophila hydei* Sturtevant. Chromosoma 14:195–206

Brock HW, Roberts DB (1983) Location of the LSP-1 genes in *Drosophila* species by in situ hybridization. Genetics 103:75–92

Cohen M Jr (1976) Evolution of 5S ribosomal RNA genes in the chromosomes of the *virilis* group of *Drosophila.* Chromosoma 55:359–371

Commerford SL (1971) Iodination of nucleic acids in vitro. Biochemistry 10:1993–1999

Cribbs DL, Leung J, Hunter Newton C, Hayashi S, Miller RC Jr, Tener GM (1987) Extensive microheterogeneity of serine tRNA genes from *Drosophila melanogaster.* J Mol Biol 197: 397–404

Dunn R, Hayashi S, Gillam IC, Delaney AD, Tener GM, Grigliatti TA, Kaufman TC, Suzuki DT (1979) Genes coding for valine transfer ribonucleic acid-3b in *Drosophila melanogaster.* J Mol Biol 128:277–287

Gelbart WM, Irish VF, St Johnston RD, Hoffmann FM, Blackman RK, Segal D, Posakony LM, Grimaila R (1985) The decapentaplegic gene complex in *Drosophila melanogaster.* Cold Spring Harbor Symp Quant Biol 50:119–125

Gubenko IS, Evgen'ev MB (1984) Cytological and linkage maps of *Drosophila virilis* chromosomes. Genetica 65:127–139

Hayashi S, Gillam IC, Delaney AD, Dunn R, Tener GM, Grigliatti TA, Suzuki DT (1980) Hybridization of tRNAs of *Drosophila melanogaster* to polytene chromosomes. Chromosoma 76:65–84

Hayashi S, Addison WR, Gillam IC, Grigliatti TA, Tener GM (1981) Hybridization of tRNAs of *Drosophila melanogaster* to the region of the 5S RNA genes of the polytene chromosomes. Chromosoma 82:385–397

Hayashi S, Gillam IC, Grigliatti TA, Tener GM (1982) Localization of tRNA genes of *Drosophila melanogaster* by in situ hybridization. Chromosoma 86:279–292

Hess O (1976) Genetics of *Drosophila hydei* Sturtevant. In: Ashburner M, Novitski E (eds) The genetics and biology of *Drosophila,* vol 1c. Academic Press, London, pp 1343–1363

Hosbach HA, Silberklang M, McCarthy BJ (1980) Evolution of a *D. melanogaster* glutamate tRNA gene cluster. Cell 21: 169–179

Inouye S, Saigo K, Yamada K, Kuchino Y (1986) Identification and nucleotide sequence determination of a potential primer tRNA for reverse transcription of a *Drosophila* retrotransposon, 297. Nucleic Acids Res 14:3031–3043

Kastritsis CD, Crumpacker DW (1966) Gene arrangements in the third chromosome of *Drosophila pseudoobscura.* J. Hered 57:150–158

Lancefield DE (1922) Linkage relations of the sex-linked characters in *Drosophila obscura.* Genetics 7:335–384

Lefevre G Jr (1976) A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In: Ashburner M, Novitski E (eds) The genetics and biology of *Drosophila,* vol 1a. Academic Press, London, pp 31–66

Loukas M, Kafatos FC (1986) The actin loci in the genus *Drosophila*: establishment of chromosomal homologies among distantly related species by in situ hybridization. Chromosoma 94:297–308

Muller HJ (1940) Bearings of *Drosophila* work on systematics. In: Huxley J (ed) The new systematics. Oxford University Press, Oxford, pp 185–268

O'Donnell J, Mandel HC, Krauss M, Sofer W (1977) Genetic and cytogenetic analysis of the *ADH* region in *Drosophila melanogaster.* Genetics 86:553–566

Patterson JT, Stone WS (1952) Evolution in the genus *Drosophila.* Macmillan Company, New York

Ryseck R-P, Walldorf U, Hoffmann T, Hovemann B (1987) Heat shock loci 93D of *Drosophila melanogaster* and 48B of

Drosophila hydei exhibit a common structural and transcriptional pattern. Nucleic Acids Res 15:3317–3333

Schaeffer SW, Aquadro CF (1987) Nucleotide sequence of the *Adh* gene region of *Drosophila pseudoobscura*: evolutionary change and evidence for an ancient gene duplication. Genetics 117:61–73

Sharp S, DeFranco D, Silberklang M, Hosbach HA, Schmidt T, Kubli E, Gergen JP, Wensink PC, Soll D (1981) The initiator tRNA genes of *Drosophila melanogaster*: evidence for a tRNA pseudogene. Nucleic Acids Res 9:5867–5882

Steinemann M (1982) Analysis of chromosomal homologies between two species of the subgenus *Sophophora*: *D. miranda* and *D. melanogaster* using cloned DNA segments. Chromosoma 87:77–88

Stocker AJ, Kastritsis CD (1972) Developmental studies in *Drosophila*. III. The puffing patterns of the salivary gland chromosomes of *D. pseudoobscura*. Chromosoma 37:139–176

Sturtevant AH, Novitski E (1941) The homologies of the chromosome elements in the genus *Drosophila*. Genetics 26:517–541

Suter B, Kubli E (1988) $tRNA^{Tyr}$ genes of *Drosophila melanogaster*: expression of single-copy genes studied by S1 mapping. Mol Cell Biol 8:3322–3331

Throckmorton LH (1975) The phylogeny, ecology and geography of *Drosophila*. In: King RC (ed) Handbook of genetics, vol 3. Plenum, New York, pp 421–469

Weber L, Berger E (1976) Base sequence complexity of the stable RNA species of *Drosophila melanogaster*. Biochemistry 15:5511–5519

Whiting JH Jr, Pliley MD, Farmer JL, Jeffery DE (1989) In situ hybridization analysis of chromosomal homologies in *Drosophila melanogaster* and *Drosophila virilis*. Genetics 122: 99–109

Wimber DE, Steffensen DM (1970) Localization of 5S RNA genes on *Drosophila* chromosomes by RNA–DNA hybridization. Science 170:639–641

Yen PH, Davidson N (1980) The gross anatomy of a tRNA gene cluster at region 42A of the *D. melanogaster* chromosome. Cell 22:137–148

Yuki S, Inouye S, Ishimaru S, Saigo K (1986) Nucleotide sequence characterization of a *Drosophila* retrotransposon, 412. Eur J Biochem 158:403–410

Received May 30, 1989/Revised August 2, 1989