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Widely differing degrees of sequence conservation of the two types of rDNA insertion within the *melanogaster* species sub-group of *Drosophila*

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We have examined the distribution of sequences homologous to the type I and type II rDNA insertions of *Drosophila melanogaster* in its sibling species. Each of the six species we have examined has sequences homologous to the type I insertion, which have undergone extensive divergence by the criterion of their *Eco*RI, *Bst*I and *Hind*III restriction patterns. We have isolated cosmid clones containing type I sequences from *D. simulans* and *D. mauritiana*, the two species most closely related to *D. melanogaster*. Southern hybridisation analysis of these clones indicates that, as in *D. melanogaster*, the type I sequences can exist independently of rDNA and can also dissociate to give sub-components homologous to the right hand segment of the *D. melanogaster* type I insertion. The type II sequences, on the other hand are present in five out of the six species, but their restriction endonuclease cleavage profile is highly conserved. The differences in the degree of conservation of the two types of insertion sequence are discussed.

Key words: rDNA insertions/*Drosophila melanogaster*/sibling species

Introduction

The rDNA of *Drosophila melanogaster* can contain two types of insertion in its 28S rRNA genes. They occur at positions in the 28S rRNA gene which are 60 nucleotides apart (Roiha *et al.*, 1981; Dawid and Rebbert, 1981; Roiha and Glover, 1981). Both types of insertion are inefficiently transcribed, but whereas the major transcripts of the type I insertions are 1-kb cytoplasmic RNA molecules (Long and Dawid, 1979), the major type II transcript corresponds to the full length of the insertion and does not leave the nucleus (Kidd and Glover, 1981). The two insertion types differ in their chromosomal distribution: whereas the type II sequences are present in the rDNA of both the X and Y chromosomes, the type I insertion is only found in X rDNA (Tartof and Dawid, 1976; Wellauer *et al.*, 1978). Unlike the type II sequences, however, type I elements occur at other chromosomal sites, principally as tandem arrays in the chromocenter (Kidd and Glover, 1980; Dawid *et al.*, 1981; Peacock *et al.*, 1981). This, together with the nucleotide sequence analysis of the junctions of type I elements suggests that they are capable of site-specific transposition (Roiha and Glover, 1981).

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Two previous studies have examined the restriction endonuclease cleavage profiles of rDNA within the sibling species of *D. melanogaster*, but both have given only scant attention to the insertion sequences (Tartof, 1979; Coen *et al.*, 1982b). Since the conservation of nucleic acid sequence in the course of evolution is an indication of the selective constraints upon the generation of diversity, we decided to examine the extent of sequence divergence of the two insertion types in the *melanogaster* species subgroup. We report our surprising findings that whereas there is extensive divergence of the restriction endonuclease cleavage profile of type I sequences between the six species we examined, the type II pattern is strongly conserved. Paradoxically, however the type II species are not present in one of the species, *D. erecta*.

Results

The restriction profile of rDNA and type I insertion sequences

We have examined the conservation of the two types of rDNA insertion in the *melanogaster* species sub-group, by looking at the length variation of restriction fragments characteristic of these sequences as they occur in *D. melanogaster* itself. In *D. melanogaster* there are essentially three types of rDNA unit: those which do not have sequence interruptions in the transcription unit and those in which the 28S gene contains one of two types of insertion. The most common arrangements of these three types of unit are shown in Figure 1. This is an over simplification, since both types of in-

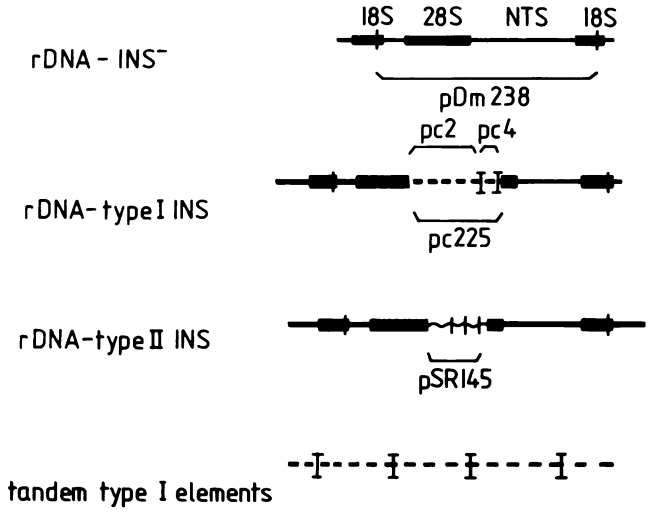

Fig. 1. The major patterns of organisation of the rDNA and its insertion sequences in *D. melanogaster*. This diagram is an over-simplification since both types of insertion as well as the NTS show differing degrees of length heterogeneity. The type I insertion is indicated by a dashed line, the type II insertion by a wavy line and the rRNA genes (28S; 18S) by shaded blocks. The restricton sites are: I, *Eco*RI; I, *Bst*I. pDm238 is a 12-kb *Eco*RI fragment which represents one complete repeating unit of uninterrupted rDNA (Roiha *et al.*, 1981); pC2 is a *Hind*III/*Bst*I fragment, pC4 the *Bst*I fragment from the 5.6-kb type I insertion of the clone cDm103 (Kidd and Glover, 1980); pc225 contains both of the above fragments from cDm103; pSR145 contains the 0.67- and 0.63-kb *Eco*RI fragments of the type II insertion together with the adjacent *Sal*I/*Eco*RI fragment (Kidd and Glover, 1981).

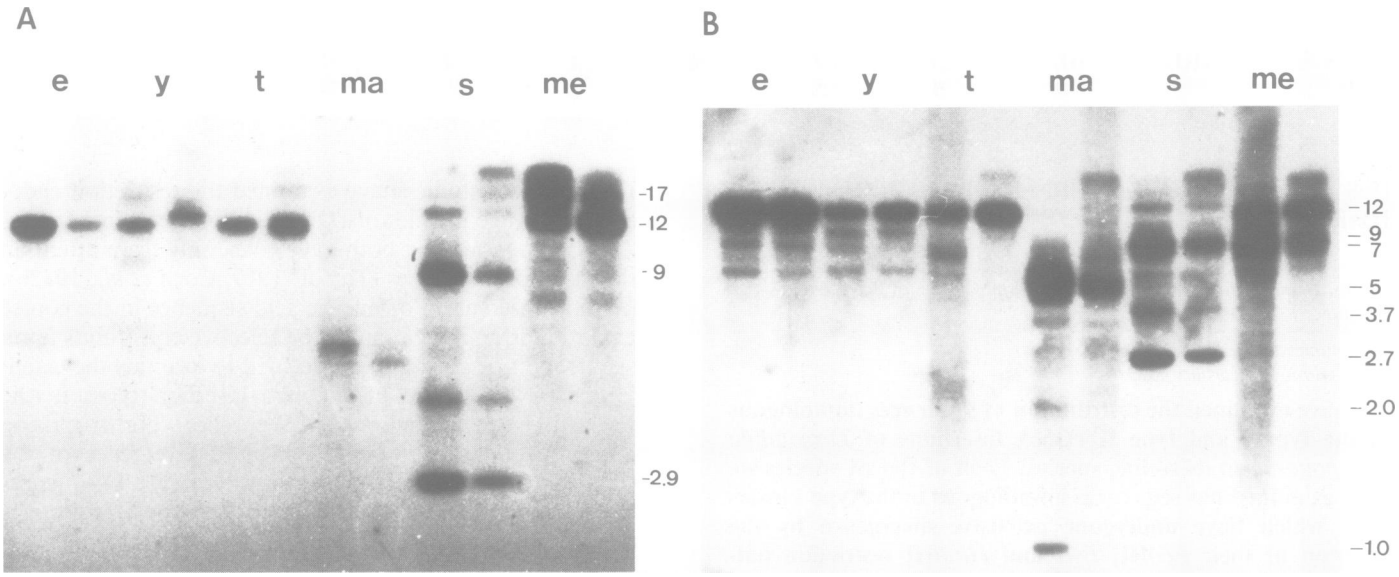

Fig. 2. Restriction patterns of rDNA of the *D. melanogaster* sibling species. DNA was prepared from a female and male fly from each species, as described in Materials and methods. DNAs on **gel A** were digested with *Eco*RI, and on **gel B** with *Eco*RI and *Bst*I before electrophoresis on a 0.8% agarose gel. Following electrophoresis, the DNA was transferred onto nitrocellulose for hybridisation with pDm238 DNA. The species are as follows: e, *D. erecta*; y, *D. yakuba*; t, *D. teissieri*; ma, *D. mauritiana*; s, *D. simulans*; me, *D. melanogaster*. For each pair of samples, DNA from the female fly is in the left track and from the male is in the right. Fragment lengths, indicated in kbs alongside each gel, were calculated following rehybridisation of the filter to a probe for the internal markers.

sertion show length variations. These are most extensive for the type I insertions for which there are a sub-set of elements consisting of sequences from the right hand side of the most common 5-kb insertion (Dawid and Rebbert, 1981; Roiha and Glover, 1981). The type I sequences also occur outside the nucleolus organiser, principally as tandem arrays in the heterochromatin of the X-chromosome (Kidd and Glover, 1980; Dawid *et al.*, 1981). This type of sequence arrangement is also shown in Figure 1.

As a probe for uninterrupted rDNA, we have used the cloned 12-kb *Eco*RI fragment pDm238 (Figure 1), A 'Southern' transfer hybridisation in which this probe has been hybridised to DNA extracted from single adult males and females from six species in the sub-group is shown in Figure 2. In the *Eco*RI digest of *D. melanogaster* DNA, the strongest hybridisation is to 17-kb rDNA fragments which contain the 5-kb type I insertion and 12-kb fragments of uninterrupted rDNA. There are fewer rDNA units which contain type II insertion sequences and these can barely be seen on this autoradiogram as bands of ~7.5 and 5.5 kb. The rDNA patterns of the two most closely related species, *D. simulans* and *D. mauritiana* appear very different from that of *D. melanogaster*, largely as a consequence of sequence divergence within the non-transcribed spacer (NTS) region. This has been examined by Coen *et al.* (1982b) who showed that in both of these species the NTS is cleaved by *Eco*RI. Consequently the repeating uninterrupted rDNA units in *D. simulans* are primarily comprised of 9-kb and 2.9-kb *Eco*RI fragments and in *D. mauritiana* of 4.8-kb and 5.8-kb *Eco*RI fragments. The prominent band of hybridisation in *D. teissieri, D. yakuba* and *D. erecta* DNA is at 12 kb, although there are additional minor bands at higher mol. wt. which are not easily visible in this exposure.

The majority of type I insertion sequences in *D. melanogaster* contain *Bst*I cleavage sites (*Bst*I is an isoshizomer of *Bam*HI). Consequently, in a double digest with *Eco*RI and *Bst*I, the 17-kb fragment of *D. melanogaster* rDNA breaks down into bands of 9 kb and 7 kb together with an internal insertion fragment which is not picked out by this probe (Figure 2B). The rDNA pattern of *D. teissieri, D. yakuba* and *D. erecta* is broadly similar, whereas the patterns with *D. simulans* and *D. mauritiana* are more complex. The distributions of the major insertion sequences are more clearly seen in Figure 3, where comparable digests have been probed with the type I insertion probe, pcDm225 (see Figure 1). In *D. melanogaster* the type I insertions do not have *Eco*RI sites and consequently the tandemly arranged type I elements give a high mol. wt. smear when cleaved with this enzyme. *Eco*RI fragments containing type I insertions, principally of 17 kb, are superimposed over this smear. The insertion element contains one or two closely spaced *Bst*I sites, and so in the double digest with *Eco*RI and *Bst*I (Figure 3B), the tandem arrays are cleaved into a compact set of fragments of 4 kb to 5.6 kb. The 17-kb rDNA on the other hand gives 9-kb, 0.8-kb and 7-kb fragments of which the latter does not hybridise with this probe. The patterns given by the homologous sequences in the other species differ radically. The strongest hybridisation signals are with *D. mauritiana* and *D. erecta* DNA. These two species both have discrete *Eco*RI or *Eco*RI/*Bst*I fragments which hybridise strongly to the type I probe and yet were not detected by the rDNA probe. It seems, therefore, that in these species the type I insertions contain internal *Eco*RI sites. Such fragments could originate from insertion in the rDNA or from elements existing independently of rDNA. The hybridisation of the type I probe to *D. teissieri* and *D. yakuba* DNA is much weaker, but it is possible to detect fragments which hybridise to both rDNA and type I sequences and others which only hybridise to the latter.

Cosmids containing type I sequences from D. simulans and D. mauritiana

In order to get a clearer idea of the arrangement of type I

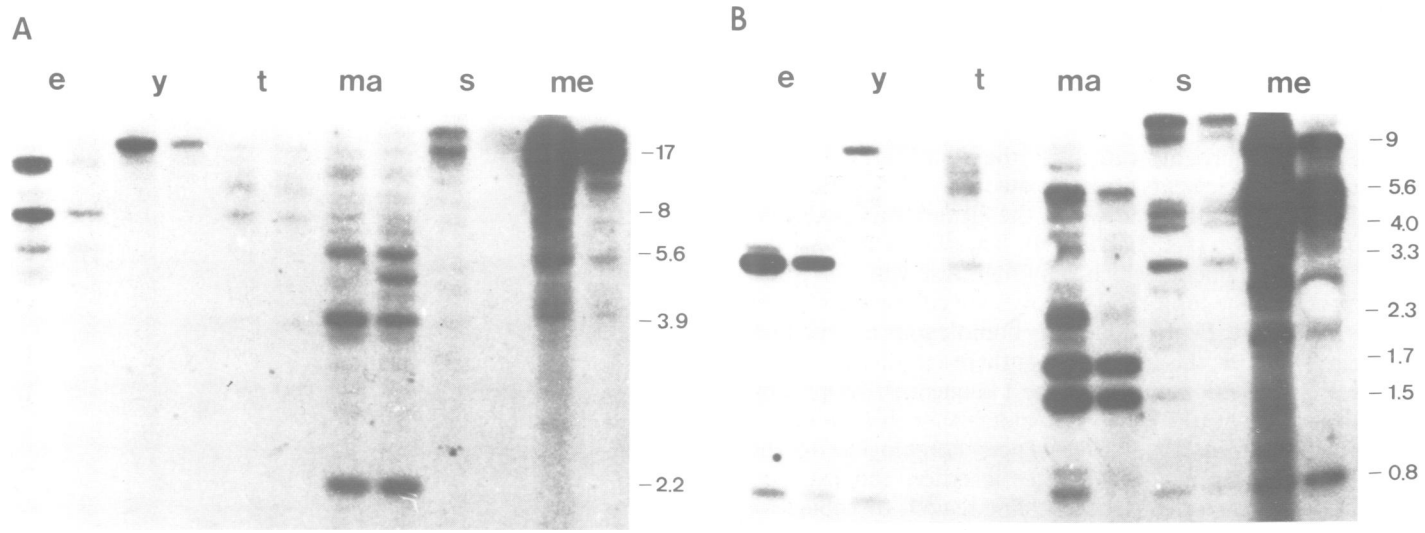

Fig. 3. Restriction patterns of type I sequences in the *D. melanogaster* sibling species. Autoradiograms of *Eco*RI digests **(panel A)** and *Eco*RI/*Bst*I digests **(panel B)** of DNA from single flies of the sibling species as described in the legend to Figure 2, but probed with ^{32}P-labelled pc225 DNA.

Table I. *Eco*RI cleavage pattern of cosmids containing type I sequences from *D. simulans* and *D. mauritiana*

	Lengths of *Eco*RI fragments (kb)										Total length of cloned *Drosophila* DNA (kb)
D. simulans											
A36 (*rDNA*$^+$, *INS-L*$^-$, *INS-R*$^+$)	5.4^v	12.5^{r,R}	8.1^{r,R}	4.9^{r,R}	4.1^r	2.9^r					32.5
B6 (*rDNA*$^+$, *INS-L*$^+$, *INS-R*$^+$)	5.4^v	13.9^{r,L,R}	7.83^r	6.33^r	2.9^r						31.0
B12 (*rDNA*$^-$, *INS-L*$^+$, *INS-R*$^+$)	5.4^v	21^{L,R}	4.9	4.3							30.2
D. mauritiana											
A89 (*rDNA*$^+$, *INS-L*$^+$, *INS-R*$^+$)	5.4^v	5.67^{R,r}	4.46^r	3.63^{L,R,r}	2.72	2.44	1.53^L	1.27			21.7
B60 (*rDNA*$^+$, *INS-L*$^+$, *INS-R*$^+$)	5.4^v	5.02^r	4.78^r	4.23^R	4.02^{L,R,r}	2.81^L	2.22	1.94^r	1.84^r	1.58^L	28.4
A84 (*rDNA*$^-$, *INS-L*$^-$, *INS-R*$^+$)	5.4^v	12.42	10.45^R	8.42^R	6.08^R	4.97	1.28	1.11			44.7
A123 (*rDNA*$^-$, *INS-L*$^+$, *INS-R*$^+$)	5.4^v	7.56^{L,R}	6.62^L	5.46^L	4.41^{L,R}						24.1

DNA from *D. simulans* and *D. mauritiana* was partially digested with *Eco*RI* and cloned in the cosmid vector, as described in Materials and methods. Cosmids were selected which contained sequences homologous to pcDm225. This Table shows the size of the *Eco*RI fragments in kb from seven of these cosmids. The gels were blotted onto nitrocellulose following the procedure of Southern (1975), for hybridisation with cloned segments of rDNA or type I insertion sequences from *D. melanogaster*. The probes are all indicated in Figure 1. The fragments showing hybridisation with one or more of the probes are indicated by superscripts, as follows: r (rDNA), pDm238; L (left hand part of type I insertion), pc2; R (right hand part of insertion), pc4; v (vector). The complementary of the cloned *Drosophila* DNA to these probes is also summarised in parenthesis next to alphanumeric designation of the clone.

sequences in *D. simulans* and *D. mauritiana*, we decided to isolate cosmid clones containing these sequences. Cosmids permit the cloning of large segments of DNA and therefore provide a convenient way to look at the distribution of type I sequences between rDNA and 'non-rDNA' chromosomal segments. Table I shows the *Eco*RI restriction pattern of representative cosmids isolated from *D. simulans* and *D. mauritiana* libraries by screening with the type I sequence probe, pcDm225 (see Materials and methods). These fragments were electrophoretically fractionated and transferred onto nitrocellulose for hybridisation with probes for rDNA and sequences from the left and right hand segments of the *D. melanogaster* type I insertion. The superscripts in the Table indicate which fragments show hybridisation to these probes. The clones B6 and A36 from *D. simulans* contain type I sequences associated with rDNA. In these and all other such clones from *D. simulans* we find that the type I probe always hybridises to *Eco*RI fragments which also contain rDNA. This suggests that the type I elements of *D. simulans*, like that of *D. melanogaster*, do not contain internal *Eco*RI fragments. This is also borne out by clones like B12 which has type I sequences but no rDNA. The clone B12 exemplifies a set of clones which have type I sequences contained in *Eco*RI fragments equal to or greater than 20 kb in length, sometimes associated with fragments homologous to neither rDNA nor type I elements. This strongly resembles the pattern which would be obtained in *D. melanogaster* from the type I elements which are not associated with rDNA units (Kidd and Glover, 1980; Dawid *et al.*, 1981). The clone A36 is an example from *D. simulans* of rDNA associated only with sequences homologous to the right end of the *D. melanogaster* insertion. This would be analogus to the rDNA units which

contain short type I insertions in *D. melanogaster* (Dawid and Rebbert, 1981; Roiha and Glover, 1981). In both species, therefore, sequences from the right hand of the type I element can exist independently of those on the left.

The *Eco*RI patterns of *D. mauritiana* clones are very different. We can broadly categorise the clones into those in which the type I elements are associated with rDNA (B60 and A89, Table I) and those in which the elements are independent of the rDNA (A123 and A84). The rDNA clones have some *Eco*RI fragments which only hybridise with the insertion probe and the 'non-rDNA' clones are comprised of a set of lower mol. wt. *Eco*RI fragments homologous to type I sequences. Together, this indicates that there are internal *Eco*RI sites within the *D. mauritiana* type I sequences, supporting our interpretations of the genomic blots. As with *D. melanogaster* and *D. simulans*, the sequences homologous to the right hand of the *D. melanogaster* insertion can exist independently of those from the left hand region. In Table I we show an example of a cloned segment, A84, in which such sequences are independent not only of the left hand region of the insertion, but also of rDNA.

The restriction profile of the type II insertion sequences

In order to look at the distribution of the type II insertion sequences in the sibling species, we have probed *Eco*RI digests of their DNA with pSR145 (see Figure 1). This hybridises to three *Eco*RI fragments of *D. melanogaster* rDNA: a 5.5-kb fragment consisting of the left part of the insertion linked to the 5′ end of the 28S rRNA gene and 0.67-kb and 0.63-kb fragments of the insertion. This pattern is strikingly conserved between *D. melanogaster*, *D. simulans* and *D. mauritiana* (Figure 4A). Of these three species only *D. simulans* shows a slight difference in pattern and has a faint fourth band at 2.6 kb. The pattern is also strongly conserved in *D. teissieri* and *D. yakuba*, except that *Eco*RI does not cleave at the position corresponding to the right-most *Eco*RI site and there is only one internal fragment of type II sequence of 0.63 kb. The left and right hand segments of the insertions in these two species are linked to fragments of 5.5 kb and 8 kb, repectively. These patterns suggest that the length of the major type II insertion is conserved between these species. We confirmed this by digesting with *Hind*III, which does not cleave within the insertion, but only in the highly conserved flanking rDNA sequences (Roiha and Glover, 1980). In each of these five species we saw a major band of hybridisation corresponding to such 8.3-kb fragments (data not shown).

Curiously, the type II sequences seem to be absent from *D. erecta* DNA. To convince ourselves that this DNA had been loaded onto the gel shown in Figure 4A, we hybridised the filter with an rDNA probe. The result of this second hybridisation is shown in Figure 4B where it can be seen that the rDNA is giving a comparable hybridisation in all the tracks. In repeated experiments with larger amounts of *D. erecta* DNA, we have been completely unable to detect sequences homologous to the type II insertion in *D. erecta* under conditions where we can easily detect single copy sequences.

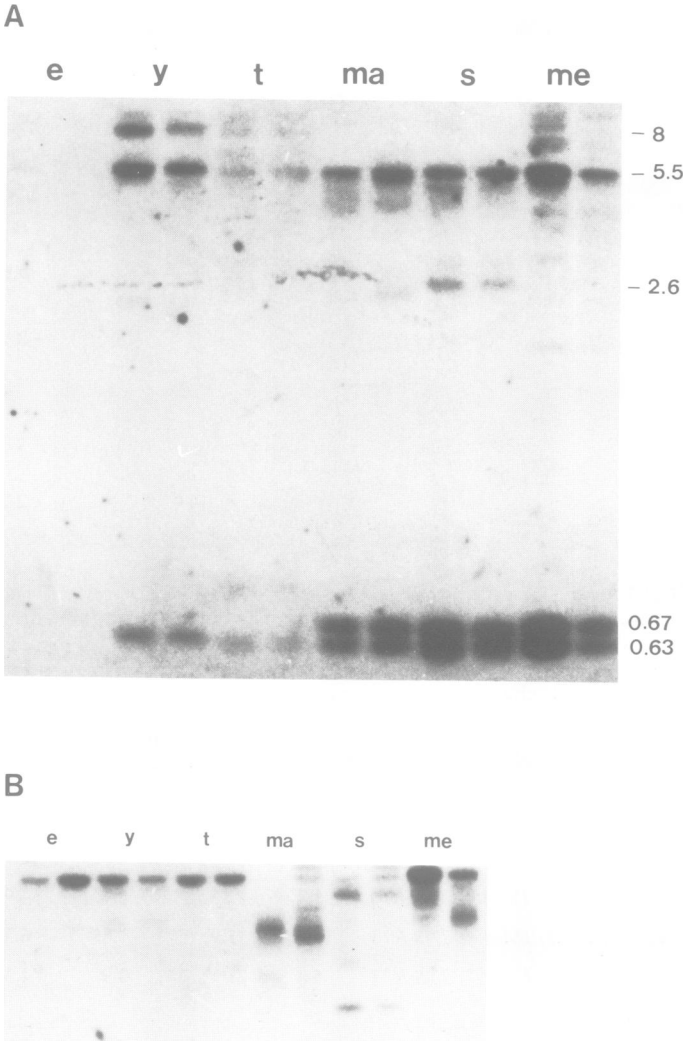

Fig. 4. Restriction patterns of type II sequences in the *D. melanogaster* sibling species. DNA was prepared from the indicated species (key as in Figure 2) and digested with *Eco*RI. Following electrophoresis on 1.2% agarose gel, it was blotted onto nitrocellulose for hybridisation with ^{32}P-labelled pSR145. A 2-week autoradiographic exposure of the filter is shown in **A**. The hybridised counts were then removed by washing in 0.3 M NaOH for 10 min and then neutralising the filter with 1 M Tris pH 8.0. The filter was then reprobed with ^{32}P-labelled pDm238. A 1 day exposure following this second round of hybridisation is shown in **B**.

Discussion

The main finding reported here is that whereas the type I rDNA insertion sequences show considerable sequence divergence in the *melanogaster* species sub-group, the type II insertion sequences are highly conserved. The cleavage pattern with *Eco*RI and *Hind*III indicates that the length of the insertion element has remained constant in these species. The only cleavage site difference which we detect with this admittedly limited range of enzymes is that whereas *D. melanogaster*, *D. simulans* and *D. mauritiana* have three internal *Eco*RI sites in the type II insertion, only two of these sites are present in *D. yakuba* and *D. teissieri*. The distribution and cleavage patterns of the type II sequences between the six species are completely consistent with the established phylogeny of the species sub-group which is shown in Figure 5. This phylogeny was originally proposed from morphological and chromosomal evidence. The group *D. melanogaster*, *D. simulans* and *D. mauritiana* have similar banding patterns and differ from *D. teissieri*, *D. yakuba* and *D. erecta* by at least seven fixed autosomal inversions (Lemeunier and Ashburner, 1976). The phylogeny is also supported by the distribution of allozyme alleles (Eisses *et al.*, 1979), satellite DNA sequences (Barnes *et al.*, 1978; Cseko *et al.*, 1979) and their courtship

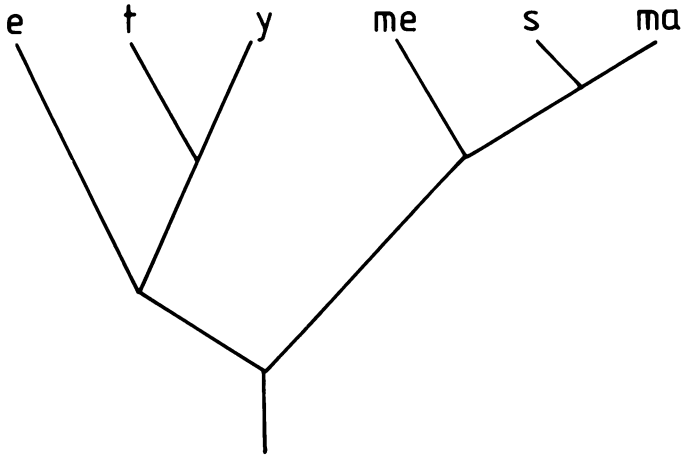

Fig. 5. Phylogenetic relationships between species examined in this study.

songs (Cowling and Burnet, 1981). The type II sequences are completely absent from *D. erecta* and could therefore have been acquired by the progenitor of *D. yakuba* and *D. teissieri* or lost at the time these species diverged.

The type I sequences show a completely different restriction pattern in the six species we have examined. This was expected since there is considerable heterogeneity of these sequences even within the genome of *D. melanogaster*. This very complexity makes it very difficult to interpret genomic blots of the sibling species probed with type I sequences. Our analysis of cloned chromosomal segments containing type I sequences from *D. simulans* and *D. mauritiana* shows that, as in *D. melanogaster*, the type I sequences can be associated with rDNA but can also occur independently. Furthermore, the *D. simulans* and *D. mauritiana* sequences corresponding to the right hand region of the *D. melanogaster* type I insertion can exist independently of the full unit. This is known to occur in *D. melanogaster*, where there are a set of rDNA clones containing shorter type I insertions which together form a co-terminal sub-set of sequences from the right hand side of the 5.6-kb insertion (Dawid and Rebbert, 1981; Roiha and Glover, 1981). These shorter insertions are flanked by the duplication, variable in length, of a sequence present as a single copy in uninterrupted rDNA units. We have previously discussed this and other evidence which indicates that the type I elements are capable of site-specific transposition (Roiha and Glover, 1981). We suggest that it is this ability of the element to undergo transposition and to exist as independent components which leads to polymorphism within a species and consequently the extensive variation between the sibling species. Previous work has shown that homology can be detected between the insertions corresponding to type I sequences in a wide range of Dipteran species (Barnett and Rae, 1979). There may, therefore, be a functional requirement to retain an internal sequence within the element. The extent of divergence of the type I sequence is comparable with that of the NTS region (Coen *et al.* 1982b). In *D. melanogaster* the NTS contains multiple copies of sequences found at the initiation site for transcription (Kohorn and Rae, 1982a; Coen and Dover, 1982; Miller *et al.*, 1983). The divergence of the NTS presumably accounts for the inability of *D. melanogaster* RNA polymerase I to initiate transcription correctly on *D. virilis* rDNA *in vitro* (Kohorn and Rae, 1982b).

Dover has suggested that unequal exchange and genetic drift together are insufficient to account for the fixation of variants in the NTS. This suggestion arises from considerations of the dynamics of NTS sequence divergence in the *melanogaster* species sub-group (Coen *et al.*, 1982b) and within *D. melanogaster* itself (Coen *et al.*, 1982a). The latter task is difficult, but one measurement was attempted by looking at the spacer pattern in some sub-lines of *D. melanogaster* (strain OK) established from single females and maintained apart for 230 generations. It is difficult to know how to interpret this experiment since one has no idea of the number of X chromosomes contributed by the original mating pairs and, furthermore, the variations in cleavage patterns observed could in some cases be a consequence of incomplete digestion. This is one line of work which has led Dover to propose a mechanism of molecular drive which he defines as 'the fixation of variants in a population as a consequence of stochastic and directional processes of family turnover' (Dover, 1982). This is a conceptual generality in which it is imagined that fixation is accelerated by events such as transposition or biased gene conversion. Within the concept one could imagine that transposable elements within the rDNA would be one means by which molecular drive could be engaged to rapidly shift variants around chromosomes. It is, however, difficult to ascertain the influence that transposition of type I sequences might have had on the evolution of the rDNA gene cluster as a whole, since it is not clear whether these elements can also mobilise rDNA.

In their analysis of the species sub-group, Coen *et al.* (1982b) noted the presence of type I and type II sequences in seven sibling species. They suggest that the data on the distribution of both types of insertion implies a process of continual re-insertion into rDNA units. They failed, however, to notice the extraordinary difference in the degree of sequence conservation between the two insertion types. We can see nothing to suggest that the type II sequences might be mobile. The conservation of the type II sequences suggests that either they are functional in their own right or that they are passively carried with the rDNA and conserved by the correction mechanisms which maintain the homogeneity of rDNA. The type II sequences show a very different pattern of transcription to type I sequences (Kidd and Glover, 1981). The main type II transcript is a 3.4-kb RNA molecule corresponding to the length of the insertion. In addition there are a set of RNA molecules in which the insertion transcript is linked to rRNA upstream in the transcription unit. This pattern could be explained by termination of the transcription of the insertion sequence at a poly(A) tract at the right hand junction with rDNA. Alternatively, the insertion transcript could be spliced from the primary rRNA transcript in a reaction that occurs more rapidly at the right hand junction than at the left. This has led to the suggestion that the type II insertions may be analogous to introns in other genes. The only situation which has been described for which one might expect conservation of intron sequences is in *Tetrahymena* rDNA in which splicing is an autocatalytic property of the intron (Kruger *et al.*, 1982). Much remains to be done in the study of the processing of the type II insertion in *D. melanogaster*, however, before we could even begin to speculate that this could be a reason why the type II sequence is conserved. At the moment, therefore, the high degree of sequence conservation of the type II insertion remains an enigma.

Materials and methods

DNA extraction, restriction and fractionation

Individual flies homogenised in 100 μl 10 mM Tris:Cl pH 7.5, 60 mM NaCl, 10 mM EDTA, 0.15 mM spermine, 0.15 M spermidine, 5% sucrose with the Eppendorf tip used to dispense the solution. 100 μl 1.25% SDS, 0.3 M Tris:Cl pH 9.0, 0.1 M EDTA, 5% sucrose was added and the tubes were incubated at 65°C for 30–40 min. 30 μl 8 M potassium acetate were added to each extract. The tubes were left on ice for 45 min and spun in a microfuge for 10 min in the cold. The supernatant was collected, care being taken to avoid the lipid layer, and the DNA precipitated with two volumes of ethanol. The pellet was washed twice with 70% ethanol, dried briefly and resuspended in 20 μl Tris:Cl pH 8.0, 1 mM EDTA for 10 min at 37°C. 5 μl single fly DNA was digested together with 0.2 μg lambda phage DNA or adenovirus 2 DNA with 2 units of the appropriate restriction endonuclease in 10 μl final volume 100 mM Tris:Cl pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl at 37°C for 2 h. The viral DNAs serve to monitor the completeness of the digestion. 10 μl per track of the fly DNA digests were loaded containing 1 μg/ml ethidium bromide and electrophoresis was carried out in 40 mM Tris:acetate pH 7.8, 5 mM sodium acetate, 1 mM EDTA. The fractionated DNA was depurinated, denatured and blotted overnight onto nitrocellulose. Hybridisation and autoradiography was carried out as previously described (Kidd and Glover, 1980). Plasmids were prepared as previously described (Roha *et al.*, 1981) and labelled by nick-translation (Rigby *et al.*, 1977).

Cosmid libraries

Cosmid libraries were constructed as described by Chia *et al.* (1982). Cosmids containing type I sequences were selected by colony hybridisation (Grunstein and Hogness, 1975) using gel purified fragments from the type I insertion corresponding to those in the clone pc225 (Figure 1).

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References

Barnes,S.R., Webb,D.A. and Dover,G. (1978) *Chromosoma,* **67**, 341-363.
Barnett,T. and Rae,P.M.M. (1979) *Cell,* **16**, 763-775.
Chia,W., Scott,M.R.D. and Rigby,P.W.J. (1982) *Nucleic Acids Res.,* **10**, 2503-2520.
Coen,E. and Dover,G. (1982) *Nucleic Acids Res.,* **10**, 7016-7026.
Coen,E., Thoday,J.M. and Dover,G. (1982a) *Nature,* **295**, 564-568.
Coen,E., Strachan,T. and Dover,G. (1982b) *J. Mol. Biol.,* **158**, 17-35.
Cowling,D.E. and Burnet,B. (1981) *Anim. Behav.,* **29**, 924-935.
Cseko,Y.M.T., Dower,N.A., Minoo,P., Lowenstein,L., Smith,G.R., Stone,J. and Sederoff,R. (1979) *Genetics,* **92**, 459-484.
Dawid,I.B., Long,E.O., Di Nocera,P.P. and Pardue,M.L. (1981) *Cell,* **25**, 399-408.
Dawid,I. and Rebbert,M.L. (1981) *Nucleic Acids Res.,* **9**,5011-5020.
Dover,G. (1982) *Nature,* **299**, 111-117.
Eisses,K.T., Van Dijk,H. and Van Delden,W. (1979) *Evolution,* **33**, 1063-1068.
Grunstein,M. and Hogness,D.S. (1975) *Proc. Natl. Acad. Sci. USA,* **72**, 3961-3965.
Kidd,S.J. and Glover,D.M. (1980) *Cell,* **19**, 103-119.
Kidd,S.J. and Glover,D.M. (1981) *J. Mol. Biol.,* **151**, 645-662.
Kohorn,B.D. and Rae,P.M.M. (1982a) *Nucleic Acids Res.,* **10**, 6879-6886.
Kohorn,B.D. and Rae,P.M.M. (1982b) *Proc. Natl. Acad. Sci. USA,* **79**, 1501-1505.
Kruger,K., Grabowski,P.J., Zang,A.J., Sands,J., Gottschling,D.E. and Cech,T.R. (1982) *Cell,* **31**, 147.
Lemeunier,F. and Ashburner,M. (1976) *Proc. R. Soc. Lond., Ser. B,* **193**, 275-294.
Long,E.O. and Dawid,I.B. (1979) *Cell,* **18**, 1185-1196.
Miller,J.R., Hayward,D.C. and Glover,D.M. (1983) *Nucleic Acids Res.,* **11**, 11-20.
Peacock,W.J., Appels,R., Endow,S. and Glover D.M. (1981) *Genet. Res.,* **37**, 209-214.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.,* **113**, 237-251.
Roiha,H. and Glover,D.M. (1980) *J. Mol. Biol.,* **140**, 341-355.
Roiha,H. and Glover,D.M. (1981) *Nucleic Acids Res.,* **9**, 5521-5532.
Roiha,H., Miller,J.R., Woods,L.C. and Glover D.M. (1981) *Nature,* **290**, 749-753.
Southern,E.M. (1975) *J. Mol. Biol.,* **98**, 503-517.
Tartof,K. (1979) *Cell,* **17**, 607-614.
Tartof,K.D. and Dawid,I.B. (1976) *Nature,* **263**, 27-30.
Wellauer,P.K., Dawid,I.B. and Tartof,K.D. (1978) *Cell,* **14**, 269-278.