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Dispersed repeats in *Drosophila virilis*: Elements mobilized by interspecific hybridization

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Abstract. A new class of moderately repetitive elements, designated the pDv sequences of *Drosophila virilis*, was cloned and molecularly analysed. The pDv elements occupy about 200 sites in the *D. virilis* genome. Their number decreases in related species with increasing phylogenetic distance. The pDv elements are characterized by a rather stable localization within the *D. virilis* genome, by intensive transcription and by partial polytenization in salivary gland chromosomes. Furthermore, they are capable of transposition in interspecific hybrids. DNA sequence analysis revealed that the pDv element contains tandemly arranged 36 bp repeat units which are flanked by imperfect direct terminal repeats of about 80 bp and inverted terminal repeats of 4 bp.

Introduction

Mobile dispersed genetic elements (mdg) of *Drosophila melanogaster* (Finnegan et al. 1978; Ilyin et al. 1978) are characterized by high species specificity. Dispersed elements of *D. virilis* are similarly restricted but they have been found to be present, in much smaller quantities, in the genomes of other species of the "*virilis*" group (Evgen'ev et al. 1982). These observations argue against a specific biological function of mdg elements in the cell, and the respective elements might represent "egoistic" components within the *Drosophila* genome (Orgel and Crick 1980).

First indications of the possible role of mdg elements in spontaneous mutagenesis are accumulating, in particular in genetically unstable strains (Rubin et al. 1982; McGinnis et al. 1983). The evident ability of P factors to induce multiple chromosome aberrations and the transposon character of mdg elements may account for rapid sympatric speciation. From this point of view, it would be interesting to study the evolutionary significance of mdg elements and their effects in a model system such as a group of closely related species with well known genealogical relationships. The *Drosophila* "*virilis*" group (Patterson and Stone 1952; Evgen'ev and Polyanskaya 1976; Throckmorton 1982) represents such a model system; *D. virilis* exhibits a supposedly primitive chromosome complement and is characterized by the absence of chromosomal rearrangements (Patterson and Stone 1952; Throckmorton 1982). Furthermore, the "*virilis*" group consists of two phylades, "*virilis*" and "*montana*", each represented by several species.

In the present work we used *D. lummei* and *D. texana* from the "*virilis*" phylad and *D. littoralis* from the "*montana*" phylad. The species of this group can be crossed under experimental conditions and give partially fertile progeny. We have recently cloned copies of a repeated element of *D. virilis* (designated the pDv sequence) which transposed into the chromosomes of related species in hybrids of different species of the same phylad (Evgen'ev et al. 1982). In the present paper we describe the molecular features of a novel repeated DNA element of *D. virilis* which is different, at the sequence level, from all other mdg elements known in *Drosophila*.

Materials and Methods

Drosophila strains. Strain 160 of *D. virilis*, carrying recessive markers in all autosomes, was constructed by us in 1975, using different mutant strains obtained from D. Stone (Texas Univ. USA).

The wild type *D. virilis* strain 9 was collected in Batumi, Georgia, USSR. Different wild-type strains of *D. texana*, *D. lummei*, *D. littoralis*, and *D. pinicola* from the *Drosophila* collection of the Institute of Developmental Biology, Moscow, USSR, were used.

DNA isolation and labelling. DNA was prepared from cultured *D. virilis* cells, from adult flies of different species and from salivary glands by the phenol-detergent method. DNA was labelled with ^{3}H, ^{32}P, or ^{125}I by nick translation (Rigby et al. 1977) and purified on a Sephadex G-50 column. The in situ hybridization of ^{3}H-labelled DNA was performed as described by Bonner and Pardue (1976).

Digestion of DNA and hybridization procedure. For digestion of DNA, endonucleases Hind III, Eco RI, Pst I, and Bgl II were used. The restricted fragments were electrophoresed in agarose gels and then transferred to nitrocellulose filters according to Southern (1975).

Hybridization was carried out for 24 h in 4 × SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate), 50% formamide, 2 × Denhardt's solution, 0.05 M phosphate buffer, pH 7.0, 0.2% SDS at 42° C. After hybridization, the filters were extensively washed with 2 × SSC, 0.2% SDS at 60° C.

RNA isolation and hybridization. RNA of *D. virilis* and related species was prepared either from larvae or from narcotized flies by homogenization in a solution containing 4 M

guanidinthiocyanate, 50 mM Tris-HCl, pH 7.6, 10 mM EDTA, 2% Sarcosyl; an equal volume of phenol was added before homogenization, and afterwards deproteinization was carried out with a phenol/chloroform mixture (1/1). Polyadenylated RNA was obtained by repeated chromatography on an oligo(dT)-cellulose column as described by Maniatis et al. (1982). Polyribosomes were prepared as described by Evgen'ev et al. (1978). RNA was extracted from polyribosomes with phenol/chloroform.

RNA samples containing 50% formamide, 6% formaldehyde and 20 mM sodium phosphate, pH 7.0, were subjected to electrophoresis in 1% agarose containing 6% formaldehyde and transferred to a nitrocellulose sheet. The blotting buffer was 10 × SSC. The intactness of RNA on blots as well as the amount loaded was tested by hybridization with nick-translated rDNA.

In vitro deletions and DNA sequencing of clone HR2. A set of in vitro deletion variants was constructed from clone HR2 using a modification of the kilodeletion procedure described by Barnes and Bevan (1983). The Eco RI site of pBR322 was chosen as a starting point for deletions which protruded into the insert. The resulting deletion subclones were accurately sized on an agarose gel to ensure that a set with a decrement of 200–400 bp between individual clones was obtained. Seven clones from this screening were sequenced by a modification of the Maxam-Gilbert procedure (1977). Sequencing gels were 6%, with a Tris-borate buffer, pH 8.8, gradient of 70–400 mM, run under controlled temperature at 50° C in the Macrophore apparatus (LKB, Sweden). Further processing of the gels was according to the instructions for the apparatus.

Sequence data handling was performed with a Wicat 150 WS computer using the programs of Staden (1982).

All other procedures were performed essentially as described by Maniatis et al. (1982).

Results and discussion

Isolation of repeated elements and construction of restriction maps

The plasmid pDv111 contains a *D. virilis* DNA segment of 14 kb (Evgen'ev et al. 1982). In situ analysis (Evgen'ev et al. 1982) has demonstrated that part of this DNA segment hybridizes to about 200 sites in *D. virilis* polytene chromosomes. Using this clone as a probe, we isolated, in a colony filter hybridization screen, five additional clones with strong homology to pDv111 DNA from a plasmid library constructed from Hind III fragments of *D. virilis* genomic DNA.

In situ hybridization analysis revealed for each clone a localization pattern identical to that of the original pDv111 in *D. virilis* chromosomes. In situ hybridization of these clones to *D. lummei* chromosomes showed that pDv111 DNA is preferentially localised at the chromocentre, and only faint hybridization is found over the chromosome arms. In contrast, one of the newly isolated clones, designated pDv14, strongly hybridized to one site of the fifth chromosome of *D. lummei* (Fig. 1a). This indicates that the cloned DNA originates from the corresponding 56F region of chromosome *5* (Gubenko and Evgen'ev 1984).

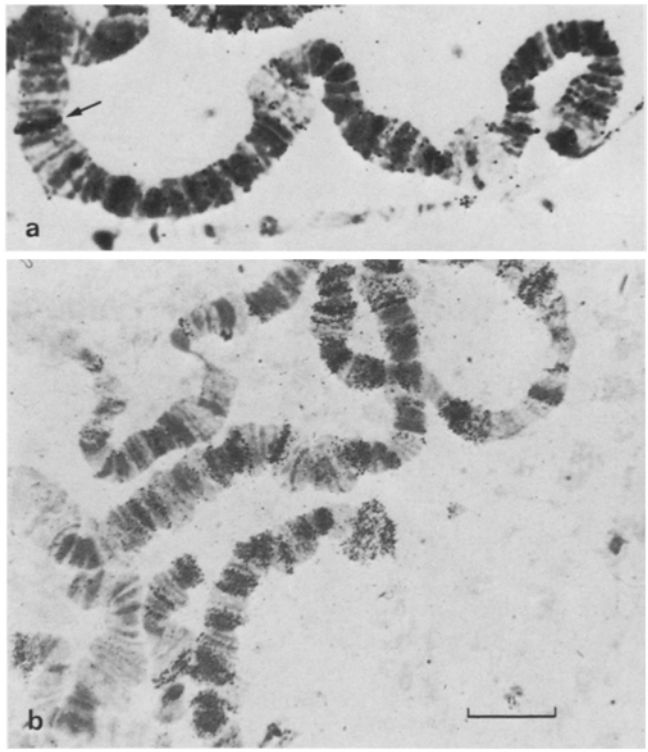

Fig. 1a, b. In situ hybridization of ^{3}H-labelled pDv elements with *Drosophila lummei* and *D. virilis* polytene chromosomes. **a** *D. lummei* chromosome *5* after in situ hybridization with pDv14 DNA. The heavily labelled 56F region from where this clone originated is indicated by an *arrow*. **b** Multiple regions of the *D. virilis* genome contain sequences homologous to H15 DNA. Numerous examples of light and heavy hybridization are seen in the chromosome arms. Exposure time 14 days. Giemsa stained. Bar represents 10 μm

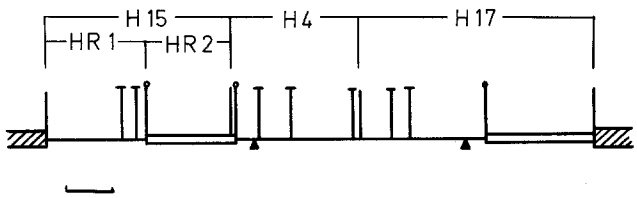

Fig. 2. Restriction endonuclease cleavage map of *Drosophila virilis* cloned pDv14 DNA. The map shows the inserted pDv14 DNA and its orientation in the pBR322 plasmid (*hatched bar*). The *letters* above the line designate restriction fragments cloned in pBR322 and used in further analysis. Repetitive DNA belonging to the pDv family is represented by an *open bar*. Restriction sites ⌈ Eco RI, | Hind III, ⌉ Pst I, ▲ Bgl II. Bar represents 1 kb

The DNA of pDv14 is composed of three Hind III fragments. Since the plasmid library had been constructed from Hind III-digested genomic DNA we had to eliminate the possibility of artifactual ligation of random Hind III fragments during the cloning. We therefore subcloned each Hind III fragment (see Fig. 2; H4, 2.9 kb; H15, 4.1 kb; H17, 4.8 kb) and analysed their chromosomal origin by in situ hybridization. Each of the repeat fragments hybridized at the expected sites and single copy DNA exclusively to the 56F region. This argues against a cloning artefact.

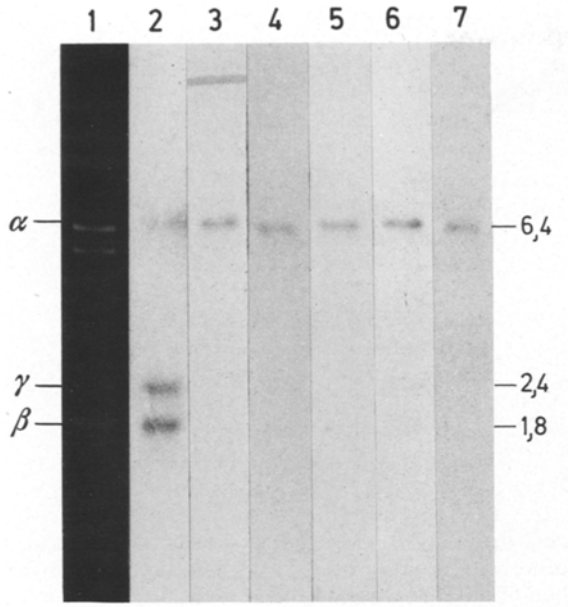

Fig. 3. Examination of total genomic and salivary gland DNA of *Drosophila virilis*, *D. lummei* and *D. littoralis* for the presence of different repeats within pDv14 DNA. pDv14 DNA was digested to completion with Eco RI, the fragments were separated by electrophoresis in agarose, blotted on to nitrocellulose and hybridized to genomic (adult flies) and salivary gland DNA of *D. virilis* and the two other species. *Lane 1* represents such a gel stained with ethidium bromide; *lanes 2* and *3*, hybridization with ^{32}P-labelled *D. virilis* adult flies and salivary gland DNA, respectively; *lanes 4* and *5*, hybridization with ^{32}P-labelled *D. lummei* adult flies and salivary gland DNA, respectively and *lanes 6* and *7*, hybridization with ^{32}P-labelled *D. littoralis* adult flies and salivary gland DNA, respectively. *Greek letters* denote restriction fragments containing genomic repeats. Sizes are given in kb. The exposure time was identical for all filters

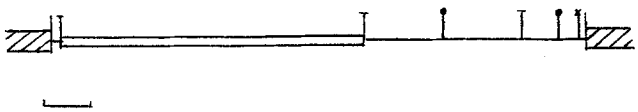

Fig. 4. Restriction endonuclease cleavage map of *Drosophila virilis* cloned pDv8 DNA. The map shows the inserted pDv8 DNA and its orientation in the pBR322 plasmid (*hatched bar*). Repetitive DNA belonging to the pDv family is represented by an *open bar*. Restriction sites: ↑ Eco RI, ↑ Pst I; ↑ Sal I, | Hind III. Bar represents 1 kb

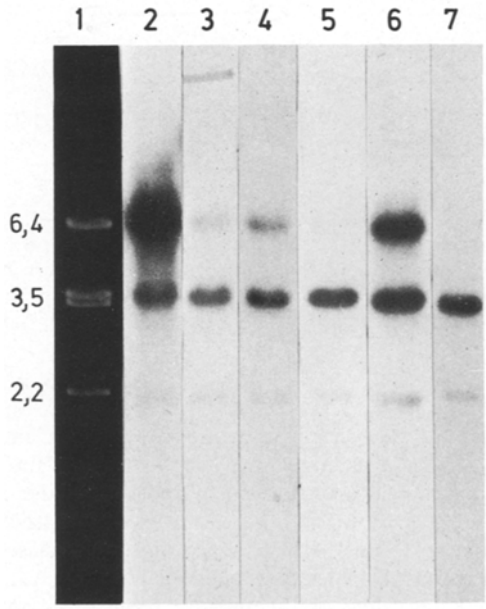

Fig. 5. Examination of total genomic and salivary gland DNA of *Drosophila virilis*, *D. lummei* and *D. littoralis* for the presence of different repeats within pDv8 DNA; pDv8 DNA was digested to completion with Pst I. Restricted DNA fragments were separated by electrophoresis in agarose, blotted on to nitrocellulose and hybridized to genomic and salivary gland DNA of *D. virilis* and the two other species. *Lane 1* represents such a gel stained with ethidium bromide. Sizes of fragments are given in kb. *Lane 2* and *3*, hybridization with ^{32}P-labelled *D. virilis* adult flies and salivary gland DNA; *lanes 4* and *5*, hybridization with ^{32}P-labelled *D. lummei* adult flies and salivary gland DNA, and *lanes 6* and *7*, hybridization with ^{32}P-labelled *D. littoralis* adult flies and salivary gland DNA, respectively. The exposure time was identical for all filters

Certain classes of repeats within pDv14 are underreplicated during polytenization

DNA sequences homologous to pDv111 DNA have been found in smaller quantities in the genomes of related species but are differently arranged there, however (Evgen'ev and Zelentsova 1984). In analogous experiments we hybridized Southern filters of Eco RI-digested pDv14 DNA using ^{32}P-DNA of *D. virilis*, *D. lummei* and *D. littoralis* as genomic DNA probes. Figure 3 demonstrates that the signal intensity over the 6.4 kb fragment is independent of the intensity over the 1.8 kb and 2.4 kb fragments, suggesting that different repeated elements were contained within pDv14 DNA. The 6.4 kb fragment includes subclone HR1 DNA (Fig. 2) which contains repeats ("α-sequences") with diffuse hybridization in *D. virilis* chromosomes (Fig. 3). Fragments of 1.8 kb (subclone H15) and 2.4 kb (subclone H17) contain homologous sequences which are represented by many copies of similar if not identical localization in the *D. virilis* genome. In pDv14 DNA, they are separated by a unique sequence (see Fig. 2); we denoted these repeats (Fig. 3) as β and γ sequences.

In *Drosophila*, genomic DNA replicates unevenly during polytenization; satellite DNA (sDNA), for example, is not replicated and rDNA is underreplicated in salivary gland nuclei (Gall and Atherton 1974; Hennig and Meer 1971; Endow and Glover 1979). The pDv sequences may fall into either of the latter categories. We tested this assumption using nick-translated ^{32}P-DNA from either salivary glands or flies of *D. virilis*, *D. lummei* and *D. littoralis*, as a hybridisation probe for Southern filters containing Eco RI-restricted and size-fractionated pDv14 DNA. The hybridization showed that the α-sequences of the pDv14 clone are equally represented in the salivary gland DNA of the three species. In contrast, β and γ sequences are present in lower quantities in the salivary gland DNA of *D. virilis*. This effect was even more pronounced in *D. littoralis* and *D. lummei* (Fig. 3, lanes 3, 5, 7). These experiments suggest underreplication of pDv14 DNA during salivary gland chromosome polytenization.

To test whether underreplication is a common feature of pDv111 homologous sequences, we used a second clone, pDv8, which contains a fragment of about 6.4 kb (Fig. 4), homologous to β and γ sequences of pDv14, for similar studies. As shown in Figure 5 the pDv8 cloned DNA and,

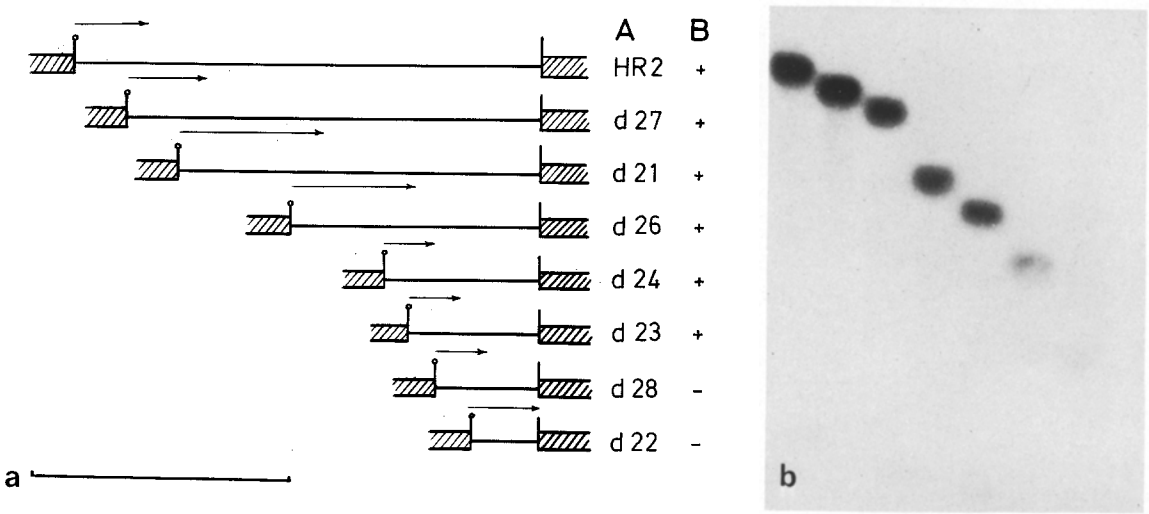

Fig. 6a, b. Deletions used in sequence analysis. **a** Schematic diagram showing the sizes of deletions used and strategy of sequencing. Restriction sites: | Hind III, † Eco RI, *single-hatched bars*, pBR flanking sequence. *A* The designations of the deletions used in the analysis. *B* The presence or absence of 36 bp units. **b** Southern blot hybridization of different cloned deletions with ^{32}P-labelled total DNA isolated from *Drosophila virilis* flies. Each lane contained an equal quantity of deleted insert DNA. Note very faint hybridization in the case of the d28 deletion and lack of hybridization with the d22 fragment apparently containing unique sequence. Bar represents 1 kb

therefore, the homologous repetitive sequences included in both clones, are characterized by underreplication in the course of polytenization. It is important to note that underreplication of tandemly repeated DNA such as sDNA (see above) has been reported for different *Drosophila* species, while underreplication of dispersed genetic elements is not necessarily expected in view of the normal replication of two *D. melanogaster* mdg families, Dm297 and Dm412, during polytenization (Lifshitz 1983). The underreplication of pDv14 DNA may relate to partial underreplication at each position in the polytene chromosomes similar to that observed for sequences during macronucleus formation in *Tetrahymena* (Yao et al. 1983). Alternatively, most of the pDv sequences may be associated with heterochromatin and, therefore, may be subject to underreplication. The latter possibility does not require a specific mechanism, and thus, seems more likely to account for the underreplication observed for pDv sequences.

Sequence data and general organization of HR2 in D. virilis and related species

A series of deletion clones (Fig. 6) was constructed from clone HR2 (see Fig. 2), which possibly contains a full-length repeat unit. The subclones were used to hybridise genomic DNA on Southern blots and chromosomes in situ. Part of the analysis is shown in Figures 6 and 7, and it indicates that sequence analyses should focus on an entire repeat unit (Fig. 8).

The first notable feature of this sequence is a tandem array of 15 nearly identical units of 36 bp (underlined in Fig. 8), having GTCGTTTTGGAACGTCATATCTCC-GCGCGGAGTTAT as a consensus sequence. Changes in nucleotides rarely take place at the left end (position 6, 8, 9) of this unit but they frequently occur at the right end (position 25, 31, 33–35 and, in particular, at 28). The most prominent characteristics of the flanking sequences are imperfect direct repeats of about 80 bp found on either side

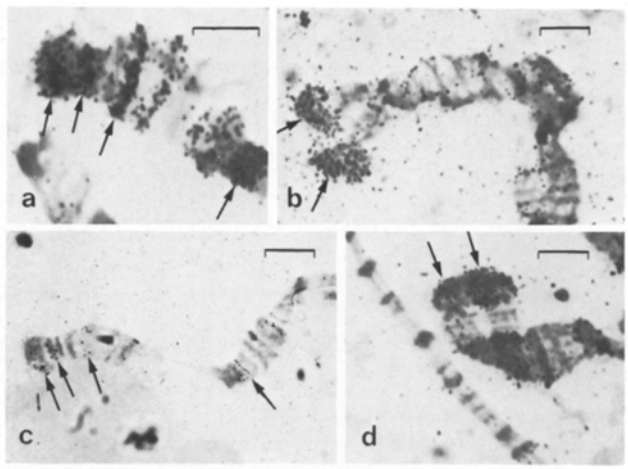

Fig. 7a–d. In situ hybridization of different subclones containing deletions with polytene chromosomes of *Drosophila virilis* and an interspecific hybrid. Salivary gland chromosomes of *D. virilis* were hybridized with ^{3}H-labelled d23 DNA (**a**) and d28 DNA (**c**). It is evident that these sequences hybridized with *D. virilis* chromosomes with different intensities. Subclone d23 containing several 36 bp tandem units gives much more prominent hybridization. The sites of hybridization in common at the distal end of chromosome *2* are indicated by *arrows*. In situ hybridization of ^{125}I-d28 DNA (**b**) and ^{3}H-d23 DNA (**d**) with the distal end of the *D. virilis* × *D. lummei* hybrid *X* chromosome. *Arrows* indicate comparatively even distribution of grains in homeologous chromosomes in the first case and the quantitatively different level of hybridization in the latter case. Exposure 21 days. Giemsa stained. **a** 1300×, **b** 950×, **c** 950×, **d** 950×

of the tandem repeats. In addition, there are short inverted repeats of 4 bp at the very end of the direct repeats. The general structure of the repeated element is outlined in Figure 9. It should be noted that we did not find short duplications near the junctions of pDv elements and the flanking

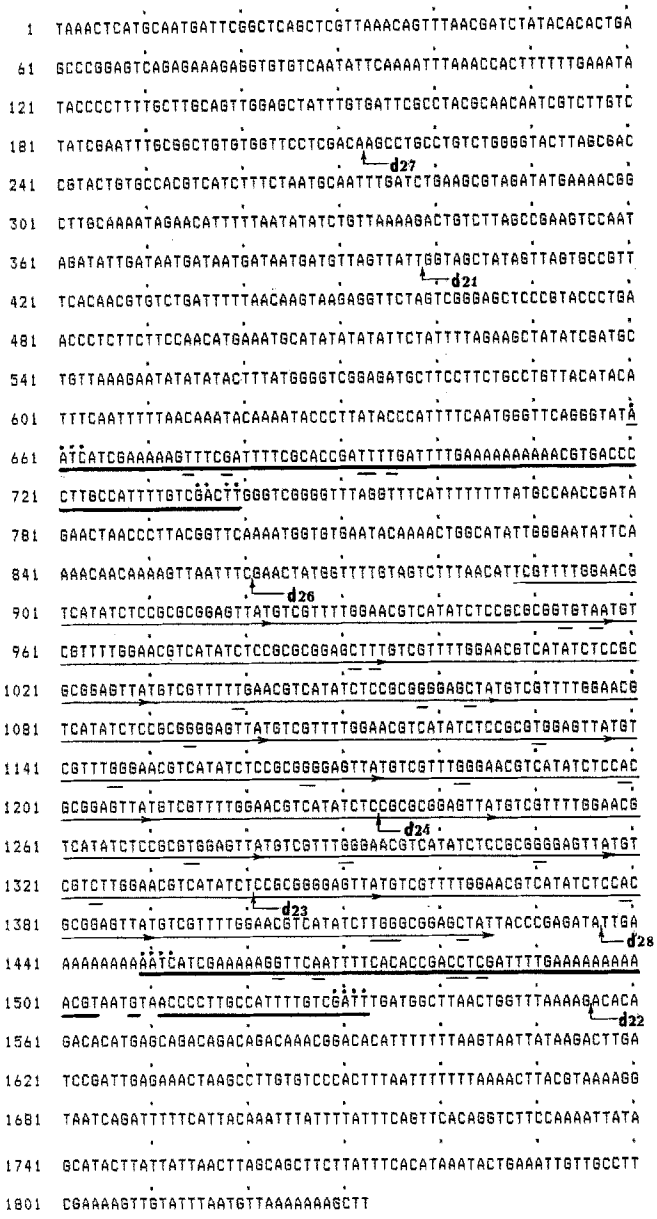

Fig. 8. Nucleotide sequence of the HR2 cloned fragment. Terminal direct repeats are marked by *solid lines* and inverted repeats are marked by *filled circles*. Tandem repeats are marked by *thin arrows*. *Underlined* bases deviate from the consensus sequence. The end points of deletions used in sequencing are indicated by *arrows*

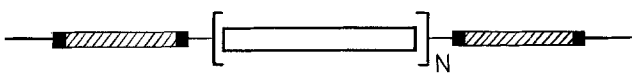

Fig. 9. The organization of pDv sequences. *Open bar* represents 36 bp tandem units, *single-hatched bars* represent direct terminal repeats, *solid bars* represent short inverted repeats flanking direct terminal repeats at both sides

sequences normally representing the "target site duplication" characteristic for transposition events.

In situ hybridisation experiments with pDv sequences on *D. virilis* chromosomes showed a high degree of variability in the number of grains. This is probably due to the presence of tandemly repetitive clusters with varying numbers of the 36 bp units. The same interpretation may

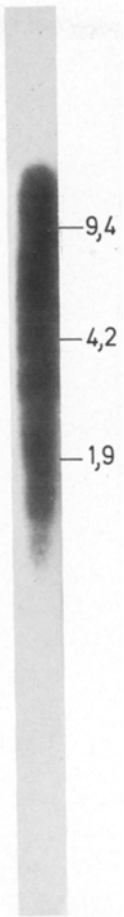

Fig. 10. Hybridization of ^{32}P-labelled HR2 fragment with total *Drosophila virilis* DNA digested by Hind III, electrophoresed and transferred to a nitrocellulose filter. Molecular weights of markers (in kb) are indicated

hold for the different levels of hybridization at homologous loci of *D. virilis* and the related species. Figure 7c, d illustrates the in situ hybridization pattern of ^{125}I-labelled d28 DNA and ^{3}H-labelled clone d23 containing tandem repeats with chromosome *1* of a *D. virilis* × *D. lummei* hybrid. While d28 DNA hybridized with both homeologues in the hybrid to the same extent (Fig. 7c) in the second case with d23 DNA (Fig. 7d) quantitatively quite different levels of hybridization were observed – the *D. virilis* homeologue exhibiting stronger labelling.

Further information on the possibility of a different repeat number per fragment was obtained from whole genomic Southern blots using endonucleases that do not cleave within the HR2 sequence (Fig. 10). These results show that there are sequences within the *D. virilis* genome, ranging from 0.2 kb to 20 kb, homologous to HR2.

Expression of pDv elements and flanking sequences

Northern blots of poly(A)$^+$-RNA and poly(A)$^-$-RNA from *D. virilis* adult flies, and nuclear and cytoplasmic RNA from *D. virilis* cell cultures, were hybridized with labelled HR2 clone DNA (Fig. 11). Strong hybridization was observed with a heterogeneous set of poly(A)$^-$-RNAs from adults, ranging in size from 15 kb to 1 kb. Most of the transcripts encoded by HR2 DNA were found in the nuclei.

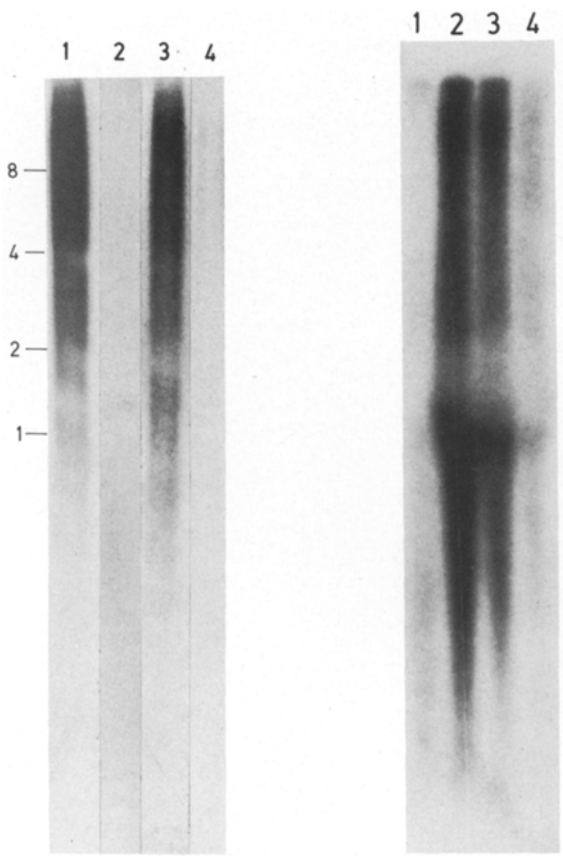

Fig. 11

Fig. 12

Fig. 11. RNA molecules homologous to HR2 sequences. RNA was separated by electrophoresis in denaturing gels, transferred to nitrocellulose paper and hybridized with 2×10^6 cpm/ml of labelled HR2 DNA; 10 µg RNA were hybridized in each case. Sizes are given in kb. The white band of about 4 kb is due to competition for binding to nitrocellulose paper at this position by nuclear 28S rRNA. *Lane 1* poly(A)$^-$-RNA; *lane 2* poly(A)$^+$-RNA. RNA was isolated from adult flies. Poly(A)$^+$-RNA was purified by oligo(dT)-cellulose fractionation. *Lanes 3* and *4* contain RNA isolated from the nuclei and cytoplasm of *Drosophila virilis* cell culture, respectively

Fig. 12. Transcripts complementary to HR2 DNA in polysomal RNA from *Drosophila virilis* cell culture. Equal amounts (15 µg) of nonpolysomal RNA (*1*) and RNA extracted from monosomes (*2*), light (*3*) and heavy (*4*) polysomes were fractionated on a 1% agarose slab gel and transferred to a nitrocellulose filter. Southern hybridization was performed using ^{32}P-labelled HR2 clone as a probe

However, after prolonged exposure RNA homologous to pDv elements was also found in the cytoplasm. To test whether this poly(A)$^-$-RNA is associated with polyribosomes and if so, it most likely represent poly(A)$^-$-mRNA, we investigated the presence of RNA complementary to HR2 DNA in polysomal fractions.

Fractions of heavy and light polysomes, monosomes and free nonpolysomal RNA were separated by velocity centrifugation in a sucrose gradient. RNA was extracted from each fraction and, after gel separation, loaded onto Northern blots. RNA homologous to the HR2 probe was found in light and heavy polysomes and in monosomes and thus may represent mRNA being translated (Fig. 12).

Having demonstrated the presence of pDv sequences in the genomes of several species of the "*virilis*" group,

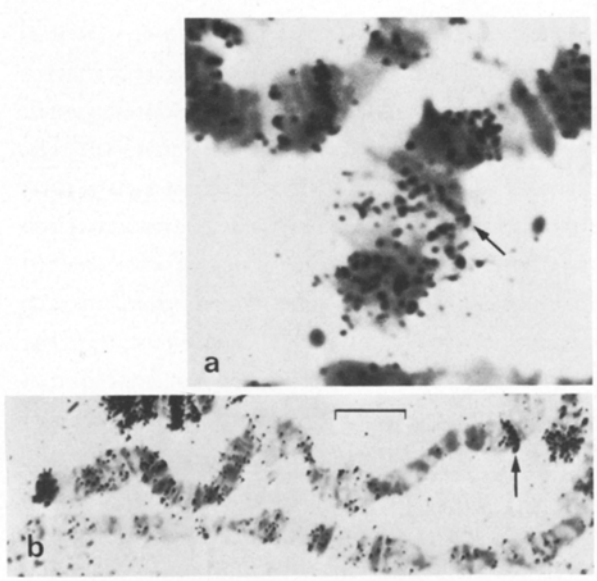

Fig. 13a, b. Demonstration that pDv sequences are mobile elements. **a** Salivary gland chromosomes from progeny of *Drosophila virilis* interstock hybrid (160 × 9) were hybridized with ^{3}H-labelled HR2 DNA. Heterozygosity in labelling at the 20CD heat-shock locus is indicated by *arrow*. **b** Autoradiograph of giant chromosomes from the hybrid stock where transposition in *D. lummei* chromosome *5* took place. The chromosomes were hybridized with the HR2 probe. The distinct hybridization at region 53C of *D. lummei* chromosome *5* marks the site of transposition (*arrow*). Slides stained with Giemsa. Exposure 20 days. Bars represent 10 µm

we were now interested in whether they are expressed as well. To test this in different species, we used a semi-quantitative dot-hybridization procedure (Kafatos et al. 1979) with total RNA preparations from flies of the following species: *D. virilis*, *D. texana*, *D. lummei*, *D. littoralis*, and, in addition, *D. pinicola* which does not belong to the "*virilis*" group. RNA sequences complementary to HR2 DNA were present in RNA from *D. virilis*, *D. lummei*, and *D. texana*, but not in RNA from *D. littoralis* or *D. penicola* (data not shown). These data are corroborated by in situ hybridization data with polytene chromosomes indicating that HR2 DNA is localized predominantly in the chromocentre of *D. littoralis*, which contains heterochromatin, and by the apparent lack of HR2 sequences in *D. pinicola*. The transcripts seen in the other species are therefore species specific; their function remain to be established.

pDv sequences are mobile elements

The transposable nature of pDv sequences is indicated by several independent lines of evidence. The cytological analysis was performed with two *D. virilis* strains of different origin, 160 and 9 (see Materials and methods). Individuals of both strains can be crossed to yield fertile progeny. The salivary gland chromosomes of these stocks have homosequential banding patterns and usually pairs perfectly in F_1 progeny. In situ hybridization of HR2 sequences of polytene chromosomes of the parental stocks and their F_1 hybrids showed an almost identical pattern of chromosomal sites in both stocks, with a major difference in the hybridization pattern in region 20CD of chromosome *2*, the site of a heat-induced puff in *D. virilis* (Gubenko and Baritcheva 1979). In stock 160, two sites of hybridization with pDv

sequences are evident in region 20CD, while in stock 9 only one site is seen. The F_1 hybrid chromosomes are shown in Figure 13a. Apart from this characterized difference, other differences between stocks and individuals to exist. This indicates that HR2 sequences can transpose, at a low frequency, in the DNA of *D. virilis*, and HR2 sequences, therefore, have characteristics of transposable elements.

We have recently noted that transpositions of pDv sequences occur in hybrid males after the introduction of chromosomes of the related species *D. littoralis* into the *D. virilis* genotype (Evgen'ev et al. 1982). Since crossing over in males has been demonstrated in *D. littoralis* (Lummei and Lankinen 1981), the transposition may be a result of double recombination in F_1 males from the cross *D. virilis* × *D. littoralis*. To test this assumption, we performed substitutional crosses using *D. lummei* as the sequence-recipient species and introduced different chromosomes of this species into the *D. virilis* karyotype. Using in situ hybridization to identify the chromosomal sites of the HR2 sequences, we detected transposition of pDv sequences into the fifth chromosome of *D. lummei*. This transposition (Fig. 13b) occurred in region 53C, a site void of HR2 sequences in both *D. lummei* and *D. virilis* chromosome *5*. To test whether the signal observed relates to transposition or whether amplification of a single preexisting 36 bp unit had occurred at this *D. lummei* chromosome *5* site (which would be left undetected by our probe), we hybridized the hybrid chromosomes with clone d28, which contains only the terminal direct repeat. This clone hybridized with locus 53C only in the stock with transposition (data not shown). Since we failed to notice any male recombination in *D. lummei*, we conclude that transposition of pDv sequences is a common phenomenon in hybrid stocks carrying different combinations of *D. virilis* chromosomes and those of related species. On mechanistic grounds, the transposition may occur via circular DNA, as suggested for copia, a transposable element of *D. melanogaster* (Flavell and Ish-Horowicz 1983, Ilyin et al. 1984). In fact, we observed circular DNA molecules of two sizes hybridizing with pDv DNA in the salivary gland cells of the hybrids, and these molecules contained the direct repeats flanking pDv sequences at both sides (Evgen'ev et al., in preparation).

The pDv sequences described here share some features with the well-studied copia class of transposable elements: fold-back sequences described by Potter (1982), scrambled elements discovered by Wensink (1979) in *D. melanogaster* and Cla-sequences described in detail by Schmidt (1984), in closely related species of *Chironomus*. We, therefore, conclude that the pDv elements characterize a new class of transposable sequences specific for the *virilis* group.

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