The evolutionary history of *Drosophila buzzatii*. XXIII. High content of nonsatellite repetitive DNA in *D. buzzatii* and in its sibling *D. koepferae*

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Corresponding Editor: B. Golding

Received February 4, 1992

Accepted July 6, 1992

MARIN, I., LABRADOR, M., and FONTDEVILA, A. 1992. The evolutionary history of *Drosophila buzzatii*. XXIII. High content of nonsatellite repetitive DNA in *D. buzzatii* and in its sibling *D. koepferae*. Genome, **35**: 967–974.

The frequency and types of repetitive nonsatellite DNA of two sibling species of the *repleta* group of *Drosophila*, *D. buzzatii*, and *D. koepferae* have been determined. For each species, the analysis is based on a sample of more than 100 clones (400 kb) obtained from genomic DNA. A theoretical model has been developed to correct for the presence of a mixture of repetitive and unique DNA in these clones. After correction, a high content of repetitive DNA has been demonstrated for both species (*D. buzzatii*, 19–26%; *D. koepferae*, 27–32%). The repetitive sequences have been classified according to their hybridization pattern when used as probes against genomic DNA and by their *in situ* hybridization signals on polytene chromosomes. Data suggest that the main nonsatellite component of these species is simpler and more repetitive than that of *D. melanogaster*, pointing to a wide variability in content and class size distribution of repetitive DNA among *Drosophila* species.

Key words: repetitive DNA, DNA evolution, *Drosophila*, *repleta* group, sibling species.

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La fréquence et les types d'ADN répétitif non-satellite chez deux espèces jumelles de *Drosophila* du groupe *repleta*, les *D. buzzatii* et *D. koepferae*, ont été déterminées. Pour chaque espèce l'analyse s'est appuyée sur un échantillon de plus de 100 clones (400 kb) obtenus de l'ADN génomique. Un modèle théorique a été développé pour corriger la présence dans ces clones d'un mélange d'ADN répétitif et d'ADN unique. Après correction, une teneur élevée en ADN répétitif a été démontrée chez les deux espèces : de 19–26% chez le *D. buzzatii* et de 27–32% chez le *D. koepferae*. Les séquences répétitives ont été classées d'après leur pattern d'hybridation en tant que sondes dirigées contre l'ADN génomique et leurs signaux d'hybridation *in situ* sur les chromosomes polytènes. Les données obtenues suggèrent que le principal composant répétitif non-satellite de ces espèces est plus simple et plus répétitif que celui de *D. melanogaster* et font ressortir la grande variabilité dans la teneur et la distribution de la dimension des classes d'ADN répétitif chez les espèces de *Drosophila*.

Mots clés : ADN répétitif, évolution de l'ADN, *Drosophila*, groupe *repleta*, espèces jumelles.

[Traduit par la rédaction]

Introduction

In *Drosophila melanogaster*, DNA reassociation analysis provided an estimation of the percentage of the repetitive genomic DNA and led to the discovery of its characteristic long interspersion pattern, i.e., blocks of repetitive DNA 0.5–13 kb long separated by even longer blocks of unique DNA (Manning *et al.* 1975). With the advent of recombinant DNA techniques, another approach has been used to estimate the repetitive DNA content, consisting in obtaining a large random sample of genomic DNA clones representative of the whole genome. These studies have shown that approximately 28% of *D. melanogaster* clones contain repetitive DNA (Young 1979; Dowsett and Young 1982). In *D. simulans*, a sibling species of *D. melanogaster*, a very different result has been obtained, that is, only 10% of its clones carry repetitive DNA (Dowsett and Young 1982). Another study with comparable results is that of Riede *et al.* (1983) for *D. virilis* showing that 31% of its clones contain repetitive DNA. The percentage of repetitive clones in *D. melanogaster* (28%) is considerably higher than the percentage of nonsatellite repetitive DNA estimated from reassociation experiments (12%) (Spradling and Rubin 1981). Because *D. melanogaster* satellites are not cloned when using conventional procedures (Lohe and Brutlag 1987) this discrepancy has to be explained by the fact that most clones carry both repetitive and unique DNA. These results raise two important points. First, one may ask whether these large differences for repetitive DNA content among closely related species of *Drosophila* represent the rule or just a peculiar trait of the *melanogaster* species group. Second, the quantification of repetitive DNA from a sample of cloned DNA has to be corrected for the presence of single copy DNA.

To address these points, we studied the repetitive DNA component of two sibling species, *D. buzzatii* and *D. koepferae* (formerly *D. serido*; Fontdevila *et al.* 1988), that belong to the *repleta* group (Wasserman 1982), a group phylogenetically very distant from the *melanogaster* group (Throckmorton 1982). Southern blot analysis and *in situ* hybridizations on polytene chromosomes have allowed us to detect and classify the repetitive component of our species. A simple theoretical model that considers an interspersion pattern similar to that found in *Drosophila melanogaster* has been used to cope with the simultaneous presence of both repetitive and unique DNA in most clones, allowing the comparison of our results with those of previous experiments with other species. Our results show that the repetitive DNA content in our species is larger than in the *melanogaster* group and the main repetitive component

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Printed in Canada / Imprimé au Canada

TABLE 1. Distribution of repetitive DNA by clone size

Class (kb)	Average size, $\bar{s}$ (kb)	Total no. of clones	Repetitive clones	
			No.	r
		D. buzzatii		
<1	0.83	8	1	12.5
1–2	1.82	26	9	34.6
2–3	2.24	13	2	15.4
3–4	3.41	19	4	21.1
4–5	4.23	16	7	43.8
5–6	5.33	8	6	75.0
6–7	6.14	7	5	71.4
>7	8.32	11	6	54.5
	3.64	108	40	37.0
		D. koepferae		
<1	0.72	5	2	40.0
1–2	1.45	30	7	23.3
2–3	2.49	14	7	50.0
3–4	3.24	22	7	31.8
4–5	4.12	14	7	50.0
5–6	5.11	9	5	55.6
6–7	6.14	7	5	71.4
>7	10.37	14	9	64.3
	3.87	115	49	42.6

NOTE: $\bar{s}$, weighted mean size of clones; r, percentage of repetitive clones.

is qualitatively different from that found in the species of the *melanogaster* group.

Material and methods

Drosophila *stocks*

Three *D. buzzatii* and five *D. koepferae* stocks have been used. Their geographical origins are as follows: *D. buzzatii*—BSL, San Luis, Argentina; BU2, Evora, Portugal; BU13, Adeje, Gran Canaria, Spain; *D. koepferae*—KSL, San Luis; KO2, San Luis; KO4, Vipos; KO6, Mazan; all of them from Argentina.

Nucleic acid preparations

DNA extractions from *Drosophila* stocks were made using a procedure described elsewhere (Piñol *et al.* 1988). Plasmid DNA was obtained either by the alkaline lysis procedure of Dowsett and Young (1982) or by the boiling method as described in Maniatis *et al.* (1982). High-quality supercoiled plasmid DNA for sequencing was extracted using Magic Miniprep columns (Promega, Madison, Wis.).

Genomic libraries

Two genomic libraries were obtained from stocks BSL and KO4 following the protocol described in Dowsett and Young (1982) modified for the use of a different cloning vector (pUC18, Norrander *et al.* 1983) and of a different restriction enzyme (*Hin*dIII) for cutting both plasmid and genomic DNA. Only those clones larger than 0.6 kb have been included in this study.

Reverse genomic hybridization (Pirrotta 1986)

We have chosen this routine method used in walking studies to determine if a clone carries repetitive DNA. This method utilizes total genomic DNA as a probe against the DNA of recombinant clones. Under appropriate conditions only those clones that carry repetitive DNA will show a hybridization signal (see for example Pirrotta *et al.* 1983; Bauman *et al.* 1987; Healy *et al.* 1988).

Around 100 ng of each recombinant clone were cut with *Hin*dIII, plasmid and insert were separated by electrophoresis in 0.6% agarose, and transferred to Hybond-C extra nitrocellulose filters (Amersham International, Buckinghamshire, England) by Southern procedure as described in Maniatis *et al.* (1982). Approximately 1 μg of total genomic DNA from adults of each species was labelled by nick translation with biotin-11-dUTP (Sigma Chemical Co., St. Louis, Mo.) and used as the probe. Hybridization and development procedures are detailed in the protocol provided with the BluGENE Kit (Bethesda Research Labs, Bethesda, Md.). The highest stringency of posthybridization washes was performed at 50°C and 0.16× SSC (medium according to Stacey *et al.* 1986).

Hybridizations against genomic DNA

About 1 μg of cloned DNA was labelled as indicated above. Total genomic DNA (3–5 μg) from adult BSL and KO4 strains was cut with *Hin*dIII, separated by electrophoresis, and transferred to Hybond-C extra filters. Hybridization and development procedures were as cited previously.

In situ *hybridizations on polytene chromosomes*

Probes were obtained by random primed labelling 300 ng of plasmid DNA with digoxigenin-11-dUTP (Boehringer Mannheim, Mannheim, Germany). Slides were obtained and hybridized as described in Labrador *et al.* (1990). Posthybridization washes and detection procedures have been developed according to DeFrutos *et al.* (1989).

DNA sequencing of the cDk27 clone

It was performed from double-stranded DNA by the dideoxy-chain termination method (Sanger *et al.* 1977) using T7 DNA polymerase (Pharmacia Labs, Uppsala, Sweden) by extending it from the universal and reverse primers of the pUC18 plasmid.

Results

Estimation of the percentage of repetitive DNA in D. buzzatii *and* D. koepferae

Forty of 108 clones of *D. buzzatii* (37%) and 49 of 115 clones of *D. koepferae* (43%) showed hybridization signal in reverse genomic hybridization (Table 1). An example of hybridization results is shown in Fig. 1 for 18 of these *D. buzzatii* clones. Since some mixed clones containing repetitive and single-copy DNA would show a positive signal, a correction was applied that considered the average size of the clone (the probability for a clone to include repetitive DNA increases with its size) and the sensitivity of the detection procedure. Assuming that repetitive DNA is interspersed in blocks separated by extensive zones of unique DNA, a relationship between the frequency of repetitive DNA and the frequency of clones detected by reverse genomic hybridization as repetitive DNA carriers can be formulated as

$$[1] \quad r = R + (N(s - 2d))/T$$

where r is the frequency of detected clones, R is the real frequency of repetitive DNA, N is the total number of repetitive blocks in the considered genome, s is the size of the clones, d is the minimum size of repetitive DNA that can be detected by the procedure used, and T is the fraction of the genome that has been considered (see Appendix). This formula establishes a linear relationship between r and s

$$[2] \quad r = A\,s + B$$

where $A = N/T$ and $B = R - (2Nd)/T$, so

$$[3] \quad R = B + 2\,A\,d$$

R can be estimated for a given species knowing the linear equation that expresses r in front of s when a fixed value is assigned to d. Equations for each species have been

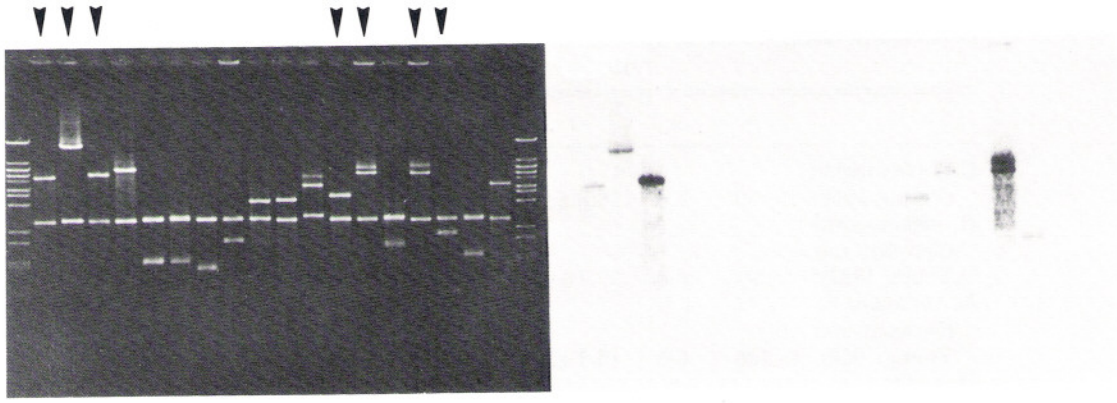

FIG. 1. Reverse genomic hybridization. Photograph on the left side shows an ethidium bromide stained gel with 18 DNA clones of *D. buzzatii* after separating plasmids from their inserts (at the extremes, lambda DNA cut with *Bst*EII serving as the molecular weight marker). Arrowheads point to those clones showing positive signals after reverse hybridization against total genomic DNA (right side).

obtained assigning values of 0 for nondetected clones and 1 for detected ones. Results are $r = 0.121 + 0.070s$ for *D. buzzatii* and $r = 0.216 + 0.054s$ for *D. koepferae*. To estimate d, 54 negative clones from *D. buzzatii* have been hybridized. Forty-five behaved as unique DNA (either one band or one principal band and a secondary one, probably a restriction polymorphism) and nine clones showed a greater number of bands or a tenuous smear (data not shown). If we consider the latter as repetitive cryptic clones, that is, those that carry repetitive DNA but are not detected, the percentage of repetitive DNA in our species would be even greater so our main conclusion remains unaffected. An estimation of the average sensitivity of our procedure of selection can be made from the percentage of cryptic clones, that is related with d (see Appendix): $r_c = 2\,Nd/T$. In *D. buzzatii*, taking $N/T = 0.070$ from the regression equation and $r_c = 9/94$ (9 of 94 clones tested were not detected by reverse genomic hybridization even though they contained repetitive DNA), we obtain $d = 0.68$ kb. Table 2 shows the values of R estimated from the regression analysis and from values of d between 0.5 and 1.0. Variance of R cannot be estimated by using standard statistical procedures from [3] because d covaries with A and B in a way impossible to asses from our experimental data. To circumvent this difficulty, we have used Tukey's jackknife method (Sokal and Rohlf 1981) to obtain R variance estimates.

The clones obtained by other authors (Young 1979; Dowsett and Young 1982; Riede *et al.* 1983) had a greater average size than ours, so the percentage of repetitive DNA must be even much smaller than the percentage of carrier clones. Unfortunately, their results do not specify the size distribution of those clones without repetitive DNA, so it is impossible either to fit a regression line to their results or to estimate d values. A rough approximation can be obtained by assuming that all the blocks of repetitive DNA have the same size (x), so $R = Nx/T$ (see Appendix), or, transforming algebraically by means of formula [1], $R = rx/(x + s - 2d)$. Table 3 shows different estimations for blocks of 5.5 kb, the average size of the block of repetitive DNA as calculated by Manning *et al.* (1975), taking into account the medium size of the clones of Young (1979), Dowsett and Young (1982), and Riede *et al.* (1983). The ranges of the estimated percentages are 12–15% for *D. melanogaster*, 4–6% for *D. simulans*, and 10–11% for *D. virilis*. The values for *D. melanogaster* are perfectly compatible with the results

TABLE 2. Estimated percentage of repetitive DNA ($R \pm$ SE) for *D. buzzatii* and *D. koepferae* for different d values

	$d = 0.5$	$d = 0.68$	$d = 1$
D. buzzatii	19.2 ± 6.1	21.7 ± 5.7	26.2 ± 5.0
D. koepferae	27.0 ± 5.7	28.9 ± 5.5	32.3 ± 5.0

found in the reassociation experiments previously cited. Interspecies comparisons can be established by estimating from regression lines the expected percentages of repetitive DNA bearing clones in each species for a determined clone size (for example, the expected percentage of *D. buzzatii* repetitive clones for a size of 7.6 kb, the *D. melanogaster* average clone size in Young 1979, is 65.3%) and comparing values by means of a t-test of equality of percentages (Sokal and Rohlf 1981). All t comparisons of *D. buzzatii* and *D. koepferae* values with those of *D. melanogaster*, *D. simulans*, and *D. virilis* are highly significant ($p < 0.001$). However, differences between *D. koepferae* and *D. buzzatii* are not significant. This procedure assumes that the sensitivity of detection of repetitive clones is similar in all the experiments. In fact, the sensitivity of the direct hybridization of each clone against genomic DNA, the procedure used by previous authors, is higher than that of reverse genomic hybridization (see results above). It means that true differences are even more significant than those estimated here.

Southern and in situ *hybridization patterns of clones detected by reverse genomic hybridization*

All those clones detected by reverse genomic hybridization have been taken as probes for hybridizations against genomic DNA of our two species. All but one, from *D. koepferae*, show consistent hybridization patterns in both species and characteristic of moderately to highly repetitive DNA. Figure 2 shows a sample of each of the five patterns in both species: continuous hybridization patterns with (Fig. 2*b*) or without bands (Fig. 2*a*), numerous bands (Fig. 2*c*), a main smear of hybridization (Fig. 2*d*; as the "repetitious DNA" described in Pirrotta *et al.* 1983) or a very intense main band, most probably an internal fragment of a repetitive sequence (Fig. 2*e*). Roughly 80% of the

TABLE 3. Estimated percentages of repetitive DNA ($R \pm$ SE) for *D. melanogaster*, *D. simulans*, and *D. virilis* for different d values assuming a uniform repetitive block size (x = 5.5 kb)

	N	s	r	$d = 0$	$d = 0.5$	$d = 1$
D. melanogaster (Young 1979)	80	7.6	28.8 ± 5.1	12.1 ± 2.1	13.1 ± 2.3	14.3 ± 2.5
D. melanogaster (Dowsett and Young 1982)	94	6.4	27.7 ± 4.6	12.8 ± 2.1	14.0 ± 2.3	15.4 ± 2.6
D. simulans (Dowsett and Young 1982)	126	6.6	10.3 ± 2.7	4.7 ± 1.2	5.1 ± 1.3	5.6 ± 1.5
D. virilis (Riede *et al.* 1983)	26	11.5	30.8 ± 9.2	10.0 ± 3.0	10.6 ± 3.2	11.3 ± 3.4

NOTE: N, total number of clones examined; s, average size of the clones (in kb); r, percentage of repetitive clones (± SE).

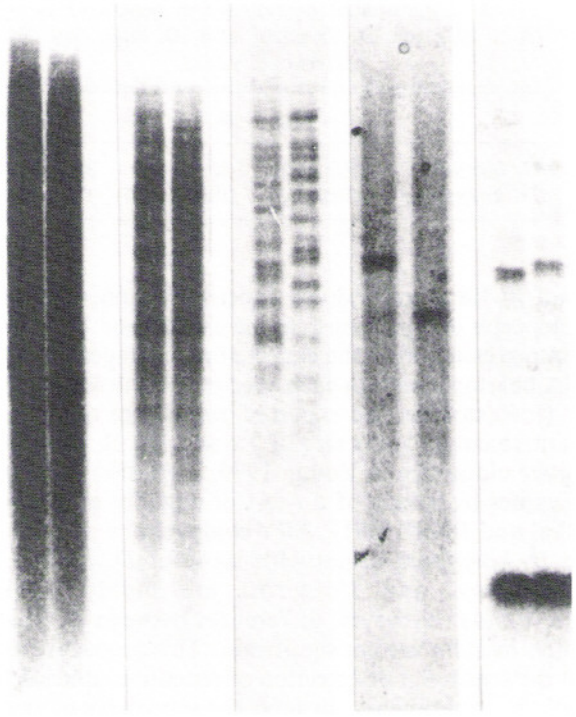

FIG. 2. The five characteristic patterns of the repetitive DNA bearing clones when hybridized against genomic DNA: continuous hybridization without (*a*) or with bands (*b*), numerous bands (*c*), main band with a smear of hybridization (*d*), and internal fragment (*e*). Left lane, *D. buzzatii*; right lane, *D. koepferae*.

repetitive clones found in our species present continuous patterns similar to those shown in Figs. 2*a*, 2*b*, and 2*d*.

Eight *D. buzzatii* and 11 *D. koepferae* clones have been hybridized against polytene chromosomes of strains of the species from which they were obtained. Their name, size, Southern and *in situ* hybridization patterns, and strains employed are detailed in Table 4. Characteristic patterns of hybridization are shown in Fig. 3. Four clones hybridized on single euchromatic bands (Fig. 3*a*). Three clones showed a gradient of hybridization, higher in centromeric zones and decreasing along the chromosomes (Fig. 3*b*). Six clones hybridized on pericentromeric regions, with occasional euchromatic bands (Figs. 3*c* and 3*d*). Three clones showed a disperse pattern of discrete euchromatic signals plus centromeric regions (Fig. 3*e*). The three remnant clones showed peculiar patterns: cDb65 hybridized on the nucleolus (Fig. 3*f*); cDb67, along euchromatin, without centromeric signal (Fig. 3*g*); finally, cDk254 hybridized on telomeres (Fig. 3*h*), and sometimes on the centromeric regions, with a variable pattern among strains.

Sequence of the clone cDk27

The sequence of the short cDk27 clone has been fully determined (Fig. 4). It contains two stretches of low-complexity DNA, an alternating purine–pyrimidine zone (nucleotides 436–493) and an AT-rich zone (nucleotides 576–639). Searching in EMBL databases has failed to uncover significant homologies of cDk27 with other known sequences.

Discussion

We describe a simple procedure to detect repetitive DNA bearing clones, as well as to quantify the percentage of non-satellite repetitive DNA from the number of detected clones. Moreover, we present an analysis of a number of repetitive DNA clones using Southern and *in situ* hybridization techniques. The species analyzed here show the highest content of this repetitive DNA among all *Drosophila* species studied so far (Tables 2 and 3). As Table 2 shows, variances associated to this type of quantification are large for the number of analyzed clones (around 100). A higher number of clones should be analyzed to produce better estimates of R values. However, in spite of this limitation, our percentages of repetitive DNA clones are high enough to render very significant statistical differences in all comparisons with other *Drosophila* species. Therefore, our method has demonstrated to be a good tool for establishing interspecific comparisons.

With such clear quantitative differences, the question arises of whether they are produced by underlying qualitative changes or not. To investigate this interesting question, we have examined the molecular characteristics of our repetitive clones. Southern hybridization patterns of all analyzed clones can be classified in just five classes (Fig. 2). These types are not equally represented. Roughly 80% of the clones

TABLE 4. Patterns of *in situ* hybridization of selected *D. buzzatii* (cDb) and *D. koepferae* (cDk) clones

Clone No.	Size (kb)	Southern pattern	*In situ* pattern	Strains tested
cDb7	1.3	*a*	Hybridization along all chromosomes, higher intensity near centromeres	BU2, BU13
cDb65	1.0	*e*	Nucleolus (Fig. 3*f*)	BU2, BU13
cDb67	0.9	*b*	Hybridization along chromosomes, except centromeres (Fig. 3*g*)	BU2, BU13
cDb96	1.5	*e*	1 euchromatic band (Fig. 3*a*)	BU2, BU13
cDb99	1.9	*a*	Hybridization along all chromosomes, higher intensity near centromeres	BU2, BU13
cDb116	4.3	*b*	15–20 euchromatic bands plus centromeres of all the chromosomes	BU2, BU13
cDb120	3.6	*b*	1 euchromatic band	BU2, BU13
cDb197	3.5	*c*	1 euchromatic band plus centromeres of all the chromosomes	BU2, BU13
cDk2	4.5	*c*	Pericentromeric zones (Fig. 3*d*)	KSL, KO2
			Pericentromeric zones and 1 euchromatic band	KO5
cDk27	0.7	*b*	Pericentromeric zones (Fig. 3*c*)	KSL, KO2, KO5
cDk72	7.3	*d*	1 euchromatic band	KO2, KO6
cDk210	1.8	*c*	6–14 euchromatic bands plus centromeres of all the chromosomes	KO2, KO5, KO6
cDk254	2.6	*c*	Telomeres of chromosomes 3 and 4 (Fig. 3*h*)	KO2
			Telomere and centromere of chromosome 5	KO4
			Telomeres of chromosomes X and 5, centromeres of the same chromosomes	KO5
			Telomere chromosome X	KO6
cDk265	5.0	*a*	Hybridization along all chromosomes, higher intensity near centromeres (Fig. 3*b*)	KO2, KO6
cDk270	4.9	*b*	5–10 euchromatic bands plus centromeres of all the chromosomes (Fig. 3*e*)	KSL, KO2, KO4, KO5, KO6
cDk271	2.3	*c*	Centromere of chromosome 3 plus 1 conservative euchromatic band	KSL, KO2, KO4
cDk323	5.1	*c*	Centromere of chromosomes X and 3	KO6
			Centromere of chromosomes X and 3 plus 1–2 euchromatic bands	KSL, KO2
cDk596	9.3	*d*	1 euchromatic band	KO2, KO6
cDk665	3.9	*b*	Centromeres of all the chromosomes	KSL, KO6
			Centromeres plus 2 euchromatic bands	KO4

NOTE: Letters refer to the classes of Southern hybridization shown in Fig. 2: *a*, continuous hybridization; *b*, continuous hybridization and bands; *c*, discrete bands; *d*, main band plus a tenuous smear; *e*, internal segment.

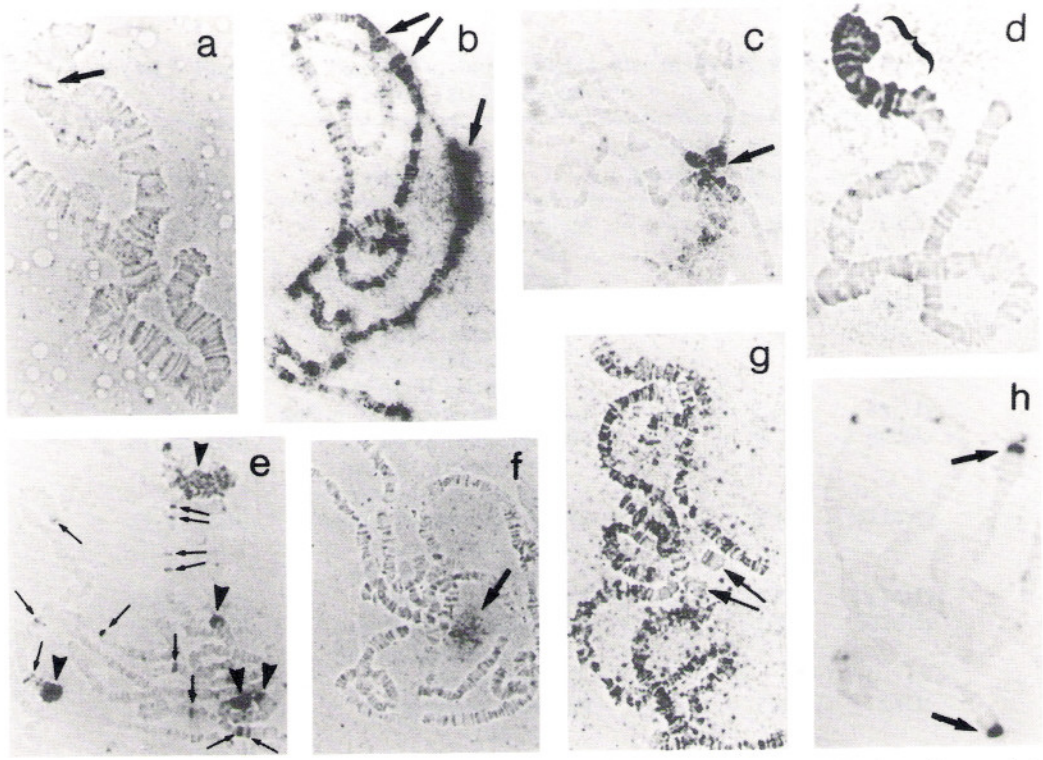

FIG. 3. Characteristic patterns of *in situ* hybridization on polytene chromosomes. (*a*) cDb96, single euchromatic band (arrow); (*b*) cDk265, hybridization along all chromosomes, with a higher signal near centromeres (arrows); (*c*) cDk27, pericentromeric hybridization. Arrow points to chromocenter; (*d*) cDk2, a higher magnification of the same pattern as in (*c*). Note the extensive pericentromeric hybridization (key); (*e*) cDk270, a clone with disperse euchromatic bands (arrows) as well as pericentromeric hybridization (arrowheads); (*f*) cDb65, hybridization on the nucleolus (arrow). (*g*) cDb67, hybridization along chromosomes, except on their centromeric termination. Dot chromosome is not hybridized either (arrows). (*h*) cDk254, hybridization on telomeres of chromosomes 3 and 4 of the KO2 stock (arrows).

show either continuous patterns or a band plus a smear hybridization signal (Figs. 2*a*, 2*b*, and 2*d*). This kind of repetitive sequence is scarce in the genome of *D. melanogaster* (Young 1979; Dowsett and Young 1982). Yet, sequences of similar characteristics have been described in specific genomic regions of *D. melanogaster*, usually in heterochromatic–euchromatic transitional zones or close to intercalar heterochromatin (Pirrotta *et al.* 1983; Miklos *et al.* 1988; Healy *et al.* 1988). On the other hand, sequences similar to those described here are found very frequently in the repetitive DNA fraction of *D. subobscura* (Felger and Sperlich 1989), suggesting important differences among species.

The molecular characterization of the different classes of repetitive DNA is being carried out in our laboratory by combining *in situ* hybridization on polytene chromosomes with sequencing. Although sequence analysis is far from being complete, the results reported here allow us to interpret some of the hybridization patterns found. (*i*) A pattern of hybridization with a number of disperse euchromatic bands is typical of mobile DNA. Sequencing of some of our clones has shown that they indeed carry fragments of mobile elements. For example, cDk210 bears around 0.5 kb of a new element of the *Ac* family (Calvi *et al.* 1991) of DNA-transposing elements (I. Marín and A. Fontdevila, in preparation). Characteristically, this type of clone shows definite bands in Southern hybridization. (*ii*) Clones with pericentromeric localizations probably carry a heterogeneous mixture of repetitive DNA types, as has been demonstrated in *D. melanogaster* (Vaury *et al.* 1989). One of these clones, cDk27, has been fully sequenced and was shown to contain short stretches of low-complexity DNA, which probably explains their smeared pattern of hybridization. It is important to note that the pericentromeric *in situ* hybridizations found are very different from those detected when satellites are used as probes (Miklos and Cotsell 1990). For example, *D. hydei* (*repleta* group) satellites hybridize almost exclusively in the tightly compacted α-heterochromatin (Renkawitz 1978*b*), but our hybridizations (Figs. 3*c* and 3*d*) are similar to *D. hydei* middle repetitive DNA signals (Renkawitz 1978*a*). (*iii*) Three clones have shown a disperse "continuous" pattern of *in situ* hybridization, with a higher number of hybridized bands and a stronger signal near centromeres. These clones typically show a strong smear when hybridized against genomic DNA. Similar sequences have been described in *D. algonquin* (Hey 1989) and *D. subobscura* (Felger and Sperlich 1989). The nature of these sequences remain to be elucidated. They could just be "sequence motifs," i.e., clusters of partially homologous sequences without a defined structure, as those found in cDk27. Alternatively, they could be short repetitive sequences capable of autonomously transposing, as the *Cla* family of *Chironomus* (Schmidt *et al.* 1980; Schmidt 1984; Hankeln and Schmidt 1987). Finally, their pattern of *in situ* hybridization could be produced by the presence of different repetitive sequences with a clustered–scrambled arrangement inside the clones (Wensink *et al.* 1979). (*iv*) Clones with a single euchromatic position probably belong to two different

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AAGCTTGAAAATTGGATGGTTCAGTTACTAATGTCCACCTCTATC
GATGACCCAAAAATGATTTTTCAGCCTCTTTGGGGGCGTTGGCAT
ATCGAAAAATACCCTGTAATTTTCTCATTTTTGGAATGTAAGCAA
CCAAATGCAGTCATGTTAGCCTCGGCTCTTTCTAAGAACTGGCCA
AAAATTATGCGTCTACGCCCACTGGGGGGCTGGCAAATCGGCAAA
GAAGTTTGTTTTTTATTTTTTNNCCAACTGCCATAAATTCATGCA
TACAATTAGCTTATTAGTAATGCTAAGTCGAATTGTCAATGAATC
AATCAAAAAAAATTTCAGTGGGATTCGCCCAGGTCTTTTGAAAAA
ATAACAATGTTAAATCGTTTAGCTAAAACCTAAAATTAAAGTTTT
CACTGCTTATTATTCACTTTGGATTGTTGTTATACATGTAGAAAC
ATATGTATACACATACATACATATATACATGTATGCATGTATAGA
AAAATTGCAACAATTTCTAGTGATTAAATGCACAATTCATATGCG
TATGTTTATAAATTATTATCAATTGGCAGCAAGCATTTTATAATA
AATATACAAATATAAAAAAAATTAAAAAATTATATTTAAAAAAAA
AAAAAAAAG
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FIG. 4. Sequence of the cDk27 clone. Low-complexity DNA zones have been underlined.

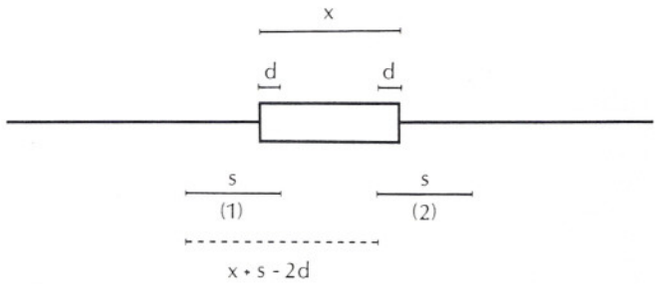

FIG. 5. Schematic drawing that shows the apparent size of a block of repetitive DNA when a sample of clones spanning its length is selected by reverse genomic hybridization.

classes. First, cDb96 can be an example of a tandem repeat, and we have found an internal repetitive structure inside it (unpublished data). This could be the single clone where a satellite or a minisatellite has been cloned in our screening, but the evidence is still too preliminary to be conclusive. The other clones could also be explained as bearing localized repeats, but we believe that their *in situ* hybridization pattern is an artifact owing to the low sensitivity of nonradioactive probes (Pardue 1986). The percentage of repetitive DNA included in these clones is probably so small that it cannot be detected on polytene chromosomes by our usual procedures. (*v*) Finally, the other three patterns found have already been described in other *Drosophila* species. Sequences that hybridize on telomeric ends, as cDk254, have been found in *D. melanogaster* (Rubin 1978; Young *et al.* 1983) and a sequence with different hybridizations among telomeres have also been described (Renkawitz-Pohl and Bialojan 1984). Nucleolus hybridizations, as in cDb65, have been found not only in *D. melanogaster* but also in *repleta* group species when rDNA is used as probe (Hennig *et al.* 1982). Finally, hybridization along all chromosomes excluding centromeric zones (cDb67) has been described in *D. melanogaster* when simple sequences are tested (Lowenhaupt *et al.* 1989).

Summarizing, the genomes of our sibling species show particular and interesting features. The number of repetitive-bearing clones is higher than that found in other *Drosophila* species. When data are corrected according to a model that takes into account that clones can carry both unique and repetitive DNA, it is concluded that *D. buzzatii* and *D. koepferae* repetitive DNA content is very high. Most of these repetitive sequences show a smeared pattern of Southern hybridization. *In situ* hybridization data suggest that those patterns could be produced by highly repetitive sequences found dispersed along all chromosomes or concentrated at pericentromeric zones and that cloned satellites, if present, are very scarce. Only a few sequences, in spite of extensive searching, have revealed patterns compatible with active mobile DNA, although inactive mobile elements could be found in centromeres. All these data suggest that the repetitive component of our species is different from that found in *D. melanogaster*. In this species most of the DNA cloned by similar procedures is middle repetitive and disperse (Young 1979; Dowsett and Young 1982). Lacking definitive sequence analysis, our data suggest that the main repetitive component of our species is simpler and more repetitive than that of *D. melanogaster*. We cannot fail to note the interesting parallelism between our results and those found when the repetitive component of *D. subobscura* is studied (Felger and Sperlich 1989). Because *D. subobscura* is closer to *D. melanogaster* than to our species, it raises the interesting question that the *melanogaster* model, considered the paradigm of *Drosophila* genome organization (Spradling and Rubin 1981), may in fact be a particular, even infrequent, outcome of the genome evolution of repetitive DNA in the genus *Drosophila*.

Acknowledgements

We thank Dr. J. Modolell for critically reading an early version of this paper and Dr. M. Santos for his advice in the statistical analysis of the data. We also thank two anonymous reviewers for their constructive criticisms, which have improved this paper. This work was made possible by grant PB86/0064 from the Ministerio de Educación y Ciencia, Spain (DGICYT) awarded to A.F. as principal investigator and from the Generalitat de Catalunya (CIRIT) to M.L. I.M. is a recipient of a fellowship from the Programa de Formación d'Investigadors (Generalitat de Catalunya).

Appendix

A block of repetitive DNA of size x is included in a unique DNA zone (Fig. 5). Because d is the minimum length of repetitive DNA in a clone of size s that can be experimentally detected, the dashed line defines the range of all positions where a clone detected as the repetitive DNA carrier can start. Thus, (1) and (2) are the first and last carrier clones of a series progressing from left to right, and the apparent size of the block is greater than x, being in fact $x + s - 2d$. The correction $s - 2d$ is independent of the block size if $s - 2d > 0$. If there are N_x blocks in fraction T of the genome, the real frequency (R) of repetitive DNA $\left(R = \sum_{x=0}^{x_{\max}} N_x \frac{X}{T}\right)$ will increase $N(s-2d)/T$, so the frequency of clones detected as carriers of repetitive DNA (r) will be

$$r = \sum_{x=0}^{x_{\max}} \frac{N_x\,(x + s - 2d)}{T} = R + \frac{N(s - 2d)}{T}$$

Moreover $r_c = 2Nd/T$ will be the frequency of cryptic clones, i.e., those not detected by the procedure used even if they contain repetitive DNA.

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