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Distribution of the copia transposable element in the *repleta* group of *Drosophila*

O Francino, O Cabre*, A Fontdevila

Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra, Barcelona, Spain

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Summary – The occurrence of the copia transposable element in 18 species of the *repleta* group of *Drosophila* has been studied using the Southern technique. The homologous sequence of copia was detected, either with radioactive or non-radioactive nucleic acid detection systems, as a pattern of multiple bands in species of the *mercatorum* and *mulleri* subgroups. Nevertheless, this sequence was not detected in the *hydei* subgroup. The intraspecific polymorphism in the pattern of bands indicates that this sequence is likely to be mobile. Some of the results could suggest the existence of restriction polymorphism of the copia homologous sequence in *D koepferae* populations. The partial sequencing of 2 independent clones isolated from *D buzzatii* clearly establishes that these elements are related and are likely to be the same.

copia transposable element / *Drosophila* / *repleta* group

Résumé – Distribution de l'élément transposable *copia* dans le groupe *repleta* de la drosophile. *La présence de l'élément* copia *a été recherchée dans 18 espèces de drosophiles du groupe* repleta *par la technique de Southern. Plusieurs bandes ont été détectées dans les sous-groupes* mercatorum *et* mulleri *à l'aide de sondes radioactives et non radioactives. En revanche, aucune séquence n'a été décelée dans le sous-groupe* hydei. *Le polymorphisme intraspécifique de la position des bandes indique que ces séquences sont vraisemblablement mobiles. Chez* D koepferae *il existe un polymorphisme des sites de restriction de la séquence homologue* copia. *Enfin, la séquence partielle obtenue pour 2 clones indépendants de* D buzzatti *indique que les 2 éléments sont apparentés et probablement les mêmes.*

élément transposable *copia* / *drosophile* / groupe *repleta*

* Correspondence and reprints

INTRODUCTION

Copia retrotransposon from *D melanogaster* is 1 of the best known retroviral type elements in the genus *Drosophila* (Mount and Rubin, 1985; Emori *et al*, 1985). Retrotransposons are recognized by structural and functional similarities to integrated retroviruses. They are bound by long terminal repeats (LTRs) at their termini and contain open reading frames resembling *gag* and *pol* genes from retroviruses (Finnegan 1989; see Bingham and Zachar, 1989 for review). There are 2 distinct lineages of retrotransposons based on the order of the gene complement and reverse transcriptase (RT) amino-acid sequence relationships (Xiong and Eickbush, 1988, 1990; McClure, 1992). More closely related to retroviruses and sharing a common ancestor with caulimoviruses, is a group including several retrotransposons of *D melanogaster* (gypsy, 17.6, 412, 297, micropia), *S cerevisae* (*Ty3*) and *B mori* (*Mag*). On the other hand, copia-like elements have a gene order which is different from all other retroid family members in that the integrase domains are located at the amino terminal of the RT domain. Retrotransposons from distantly related taxonomic groups such as *D melanogaster* (copia and 1731), *S cerevisae* (*Ty*1 and *Ty*2), *A thaliana* (*Ta*1) and *N tabacum* (*Tnt*1) are clustered in this latter group (Xiong and Eickbush 1990, McClure 1992).

The presence of the copia element has been reported in the major *Drosophila* radiations, suggesting an ancient origin of this component in the genome (Martin *et al*, 1983, Stacey *et al*, 1986). Nevertheless, the distribution of copia is discontinuous within the different radiations analysed. In the *virilis-repleta* radiation, hybridizing sequences have been found in the *mulleri* and *mercatorum* subgroups (*repleta* group), but no detectable hybridization was observed in the *hydei* subgroup (*repleta* group) or in any of the representatives of the *virilis* group. However, even in closely related species, the relative abundance of the copia element can be highly variable. In the *melanogaster* subgroup the number of dispersed copies of copia ranges from 60 in *D melanogaster* (Finnegan *et al*, 1978) to 0 in *D yakuba* and *D erecta* (Dowsett, 1983). Similar differences were observed in the *obscura* group, with more than 30 copies of the homologous sequence in *D pseudoobscura* and no detectable copies in *D subobscura* (Martin *et al*, 1983).

A preliminary approach to the molecular evolution of the transposable elements is to investigate their presence (or absence) in a species group in which the biogeographic and phylogenetic relationships are known. The *repleta* group of *Drosophila* has been thoroughly studied and its phylogeny and biogeography have been deduced (Wasserman 1982; Fontdevila 1982; Ruiz *et al*, 1982). It is distantly related to the *melanogaster* group (Throckmorton, 1982), but copia homologous sequences have been detected in some of its species (Martin *et al*, 1983; Stacey *et al*, 1986).

Here we expand the survey to 18 species of this group comprising 3 different subgroups (*mulleri, mercatorum* and *hydei*). The 2 sibling species *D buzzatii* and *D koepferae* have been studied in more detail by analysing strains from different geographic origins. Moreover, partial sequencing of 2 independent clones isolated from *D buzzatii* demonstrates the presence of copia itself in this species.

Characterization of copia in different species is a tool to solve some questions, such as which molecular features act as functional determinants and the nature of the evolutionary dynamics of the element in the genus *Drosophila*.

MATERIAL AND METHODS

Drosophila stocks

The strains used were originated from collections made by 1 of us (AF) and coworkers; there are some exceptions: *D mulleri* and *D wheeleri* were provided by W Heed; *D buzzatii* populations from Tunis and Chile were provided by J David and D Brncic, respectively; and *D borborema* and *D serido* were purchased from Bowling Green.

Probe

The pDmcopia was kindly provided by J Modolell. It is a full-length sequence of copia obtained from cDm5002 (Dunsmuir *et al*, 1980), cloned in pUC8.

Restriction enzymes

The enzymes were purchased from Boehringer Mannheim and used according to the supplier's instructions.

Genomic DNA extraction, agarose gel electrophoresis and Southern blotting

Genomic DNA extraction was performed as described previously (Pinol *et al*, 1988). Digested genomic DNA was loaded on a 0.6% agarose gel (0.5 × 14 × 20 cm). Electrophoresis was carried out at 20–25 V overnight. When using non-radioactive DNA detection methods, the amount of DNA loaded in each lane was adjusted by a correction factor obtained from the densitometric analysis of an electrophoresis previously carried out. Blotting on a nitrocellulose filter (Hybond C and Hybond C-EXTRA) was as described in Maniatis *et al* (1982).

Hybridization

The pDmcopia probe was labelled with either ^{32}P-ATP, biotin-11-dUTP (using nick-translation) or digoxigenin-11-dUTP (using a random primed reaction). When using ^{32}P-ATP-labelled probes the hybridization conditions were the same as those described in Maniatis *et al* (1982). The post-hybridization washes were always carried out at 65°C, twice in 2 × SSC for 15 min, and once in 2 × SSC 0.1% SDS for 30 min, which represents medium stringency wash conditions (Stacey *et al*, 1986). The autoradiography was exposed 24–36 h at −70°C with an intensifying screen. When using biotin- or digoxigenin-labelled probes, the hybridization was performed at 42°C in 50% formamide and washes at rt twice in 2 × SSC, 0.1% SDS for 5 min, and then at 50°C twice in 0.1 × SSC, 0.1% SDS for 15 min (described

in the non-radioactive nucleic acid detection systems from BRL and Boehringer Mannheim).

Cloning and sequencing

The genomic library from *D buzzatii* DNA was prepared as described by Piñol *et al* (1988) and screened with pDmcopia probe. DNA from positive lambda clones was prepared, BamHi, EcoRI, HinDIII and SalI digested and hybridized with the same probe. Restriction fragments containing copia from independent lambda clones were subcloned into pTZ–18U (US, Biochemical) and partially sequenced by the dideoxy chain termination method using Sequenase (US Biochemical) or T7 DNA polymerase (Pharmacia). For sequence comparisons the FASTA program from the EMBL data bank was used.

RESULTS

Distribution of copia in the repleta *group*

In order to test the presence of copia in different species of the *repleta* group, an initial qualitative screening was carried out with species belonging to clusters *buzzatii*, *martensis* and *mulleri* (*mulleri* subgroug, Wasserman, 1982). These clusters were chosen because the presence of copia in *D mulleri* has previously been described (Stacey *et al*, 1986). Southern blots of EcoRI-digested DNAs were hybridized with ^{32}P-labelled pDmcopia probe. Under medium stringency wash conditions, autoradiography shows patterns of multiple and discrete bands (fig 1–3). The time required to obtain a visible signal in the *repleta* group species clearly overexposes the band corresponding to *D melanogaster*. The patterns were different for each species tested, and indicate the presence of a repetitive sequence homologous to copia in the *repleta* group. Some of the bands detected are shorter than the copia element which is 5 kb long, suggesting that the homologous sequence has at least 1 internal EcoRI restriction site or some defective representatives in the species tested.

Twelve strains of *D buzzatii* populations from different geographic localities were analysed for the genomic distribution of the copia element (fig 2). Some differences are detected in the relative intensity and in the presence or absence of a given band, but the different strains share most of their bands, suggesting a similar distribution of copia in the genome of this species.

Major differences are observed in patterns obtained for populations of *D koepferae* (Fontdevila *et al*, 1988) and its symmorphic species *D serido* (fig 3). In the Argentinian populations of *D koepferae*, all of the signal is virtually reduced to an intense 3.4 kb band, while the rest of the bands are extremely faint. This pattern could be due to either an internal EcoRI fragment or a tandem organization of the element in these populations. In order to test the origin of this prominent band, EcoRI- and HindIII-digested genomic DNA from Bolivian and Argentinian populations were hybridized with digoxigenin-labelled pDmcopia probe. A pattern of multiple bands was observed in HindIII digestions (fig 4b), which favours the idea of the presence of an EcoRI internal fragment instead of a tandem array of

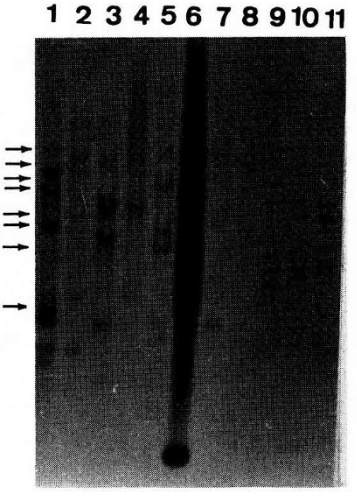


Fig 1. Southern blot patterns of copia transposable element from species of the *repleta* group of *Drosophila*. DNA samples ware EcoRI-digested, electrophoresed, transferred to a nitrocellulose filter and probed with a full-length copia labelled with ^{32}P by nick-translation. The species tested are: (1) *D uniseta*, (2) *D venezolana*, (3) *D starmeri* and (4) *D martensis* from cluster martensis; (5) *D aldrichi*, (7) *D huaylasi* and (8) *D nigrodumosa* from cluster *mulleri*; (9) *D borborema*, (10) *D buzzatii* and (11) *D buzzatii* (laboratory strain originated from 10) from cluster *buzzatii*; and (6) *D melanogaster* (as control). The arrows indicate positions of the BstEII λ DNA fragments of 8.4, 7.2, 6.3, 5.8, 4.8, 4.3, 3.6, and 2.3 kb, respectively.

the element in the genome of *D koepferae*. The intensity of bands is greater for the lanes corresponding to Argentinian populations when the same amount of DNA is loaded (fig 4, bands 2–4).

We have also used a biotin-labelled pDmcopia probe to extend the survey of the presence of copia in the *mulleri* subgroup species. We included DNA from *hydei* and *mercatorum* subgroups as additional reference points, for it is known that copia is detected in *D mercatorum* but not in *D hydei* DNA (Martin *et al*, 1983; Stacey *et al*, 1986). The DNA loaded in each band was adjusted beforehand (see *Material and methods*) in order to obtain both qualitative and quantitative results. As it can be seen in figure 5, a sequence homologous to the copia element was detected with the biotin-labelled pDmcopia probe in all the *mulleri* subgroup species tested, but no detectable hybridization was observed in the *hydei* subgroup (represented here by *D hydei* and *D hydeoides*). The relative intensity of the bands was greater for the lanes corresponding to *D mercatorum, D mulleri* and *D buzzatii.*

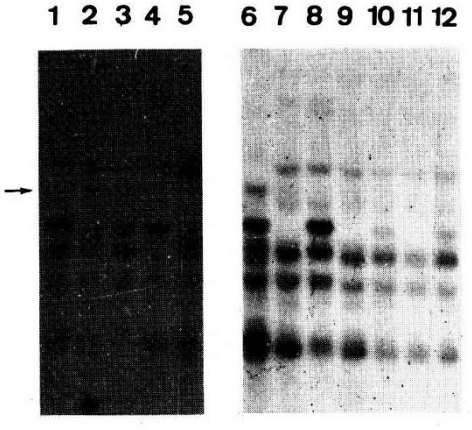


Fig 2. Genomic distribution of copia in different strains of *D buzzatii*. Samples from different geographic localities were analysed as in figure 1. The different samples showed here are from: (1) Los Negros, Bolivia; (2) Quilmes, Argentina; (3) Dean Funes, Argentina; (4) Arroyo Escobar, Argentina; (5) C-9 laboratory strain from San Luis, Argentina; (6) Melocotón, Chile; (7) Adeje, Canary Islands; (8) Oasis Nasrallah, Tunis; (9) Bahía Azul, Balearic Islands; (10) Carboneras, Spain; (11) Caldetes, Spain; and (12) Plasencia, Spain. The arrow indicates position of a 5 Kb-long DNA fragment.

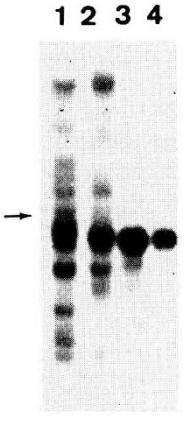


Fig 3. Genomic distribution of copia in the symmorphic species *D serido* and *D koepferae*. Southern blot analysis of EcoRI-digested DNA from: (1) *D serido* from Cafarnaum (Brazil); (2) *D kopferae* from Los Negros I (Bolivia); (3) *D koepferae* from San Luis (Argentina); and (4) *D koepferae* from Quilmes (Argentina). The sample of band 2 is overloaded. The arrow indicates position of a 5 kb-long DNA fragment.

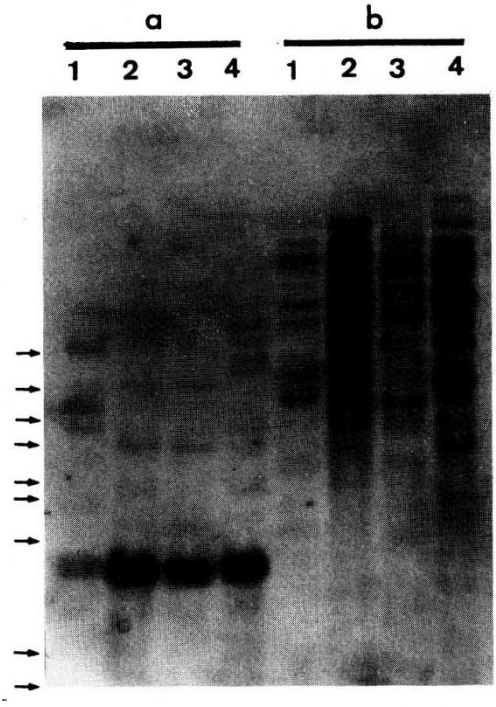


Fig 4. Analysis of genomic distribution of copia in Bolivian and Argentinian populations of *D koepferae* by a non-radioactive method. (a): EcoRI-digested genomic DNA. (b): HindIII-digested genomic DNA. The populations tested were: (1) Los Negros II, Bolivia; (2) Yacochuya, Argentina; (3) Quilmes, Argentina; and (4) San Luis, Argentina. The arrows indicate the mobilities of the BstEII λ DNA fragments of 8.4, 7.2, 6.3, 5.8, 4.8, 4.3, 3.6, 2.3 and 1.9 kb, respectively.

Isolation of copia from D buzzatii

As a preliminary step for the molecular characterization of the copia element in *D buzzatii*, a genomic library was screened with digoxigenin-labelled pDmcopia probe. Two independent clones were isolated and restriction fragments hybridizing with pDmcopia were subcloned and partially sequenced.

The alignment of the sequences with copia from *D melanogaster* (Dm copia) is shown in figure 6. The sequenced region of each of the clones aligns with Dm copia in different positions: Db 07X (λ 5) aligns in the 3′ region of integrase while Db 05TqE (λ 12) corresponds to reverse transcriptase. The identity between *D buzzatii* subclones and DM copia is higher than 75% at the nucleotide level (77.4% for integrase and 76.5% for reverse transcriptase) and about 70% at the amino-acid level (74.2 and 68.9%, respectively). When considering similarities at the amino-acid level, the percentage increases to 95.2% for integrase region and to 88.8% for reverse transcriptase. It is noteworthy that copia from *D melanogaster* is the only *Drosophila*-transposable element sequence that aligns with our subclones at the

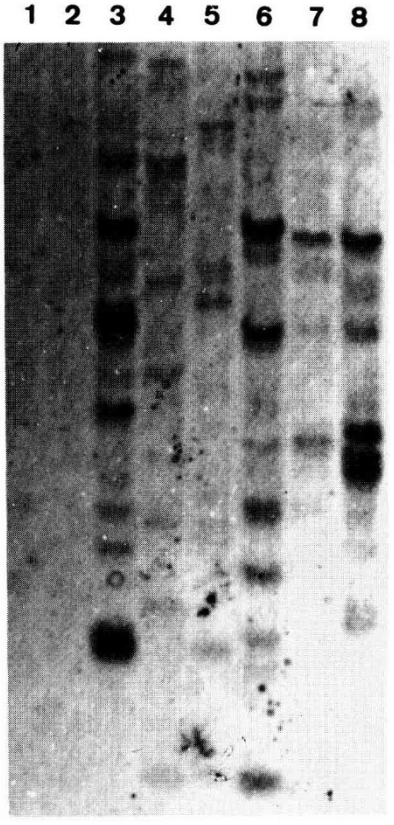


Fig 5. Non-radioactive detection of copia in several species of the *repleta* group. Southern blot analysis of EcoRI-digested genomic DNA hybridized with biotin-11-dUTP-labelled Dmcopia probe: (1) *D hydei*; (2) *D hydeoides*; (3) *D mercatorum*; (4) *D majovensis*; (5) *D arizonae*; (6) *D mulleri*; (7) *D wheeleri*; (8) *D buzzatii*.

nucleotide level when using the FASTA program. The sequence identity with other elements is not enough to allow their alignment with *D buzzatii* subclones.

On the other hand, amino-acid sequences obtained for putative ORFs of both the integrase and reverse transcriptase regions align with elements from distantly related taxonomic species, such as *Nicotiana tabacum* or *Arabidopsis thaliana*, but with no other *Drosophila*-transposable element. Only 1731 from *D melanogaster* is aligned with Db 05TqE at the amino-acid sequence level (reverse transcriptase), but the percentage of identity changes from 68.9% between *D buzzatii* subclone and Dm copia to 32.2% between the same subclone and 1731.

In order to test the reliability of pDmcopia hybridization signals in the *repleta* group species, the Db 05TqE subclone from *D buzzatii* was used as a probe for *D buzzatii* and *D koepferae* EcoRI-digested DNA (fig 7). The hybridization patterns obtained for *D koepferae* were compared with those obtained with the pDmcopia

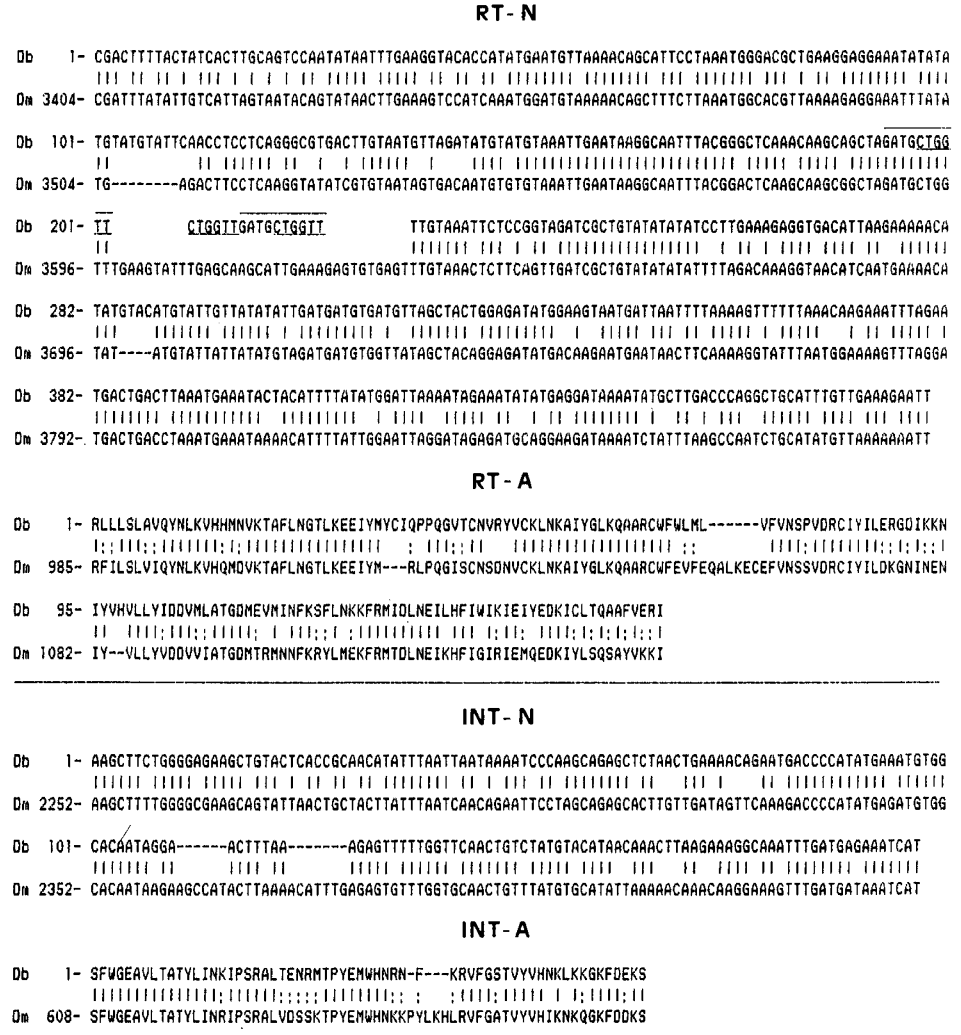


Fig 6. Sequence alignment of 2 independent clones from *D buzzatii* with copia from *D melanogaster*. Db = *D buzzatii*. Dm = *D melanogaster*. RT = reverse transcriptase. INT = integrase. N = nucleotide sequence. A = amino-acid sequence. Matches are indicated as: | = coincident, : = conservative. Direct repetitions in *D buzzatii* RT subclone are marked with a line above or below. When deletion is considered as a single event, the identity between *D buzzatii* sequences and the copia element from *D melanogaster* is 76.5% for RT and 77.4% for INT at the nucleotide level. The identity at amino-acid level is 68.9% for RT and 74.2% for INT, whereas the similarity rises to 88.8% and 95.2%, respectively.

probe when the same strains were used (see fig 4, bands 1,4; fig 7, bands 1, 2). The 3.4 kb EcoRI internal fragment is observed with both probes in the bands corresponding to Argentinian populations (fig 4, band 4; fig 7, band 2). The hybridization signal is greater for the Db 05TqE probe, since it contains a fragment of the element from a closely related species and a higher sequence conservation is expected. However, the relative intensity of the faint bands in relation to the internal fragment in each band is equivalent with both probes. Moreover, the signal is always more intense for the Argentinian than the Bolivian populations when the same amount of DNA is loaded. The coincidence of these results demonstrates the specificity of pDmcopia hybridization in the *repleta* group.

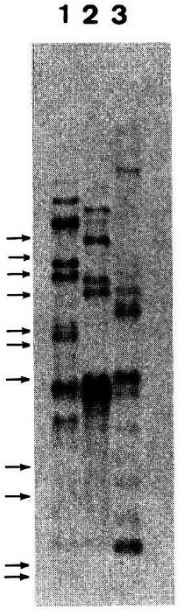
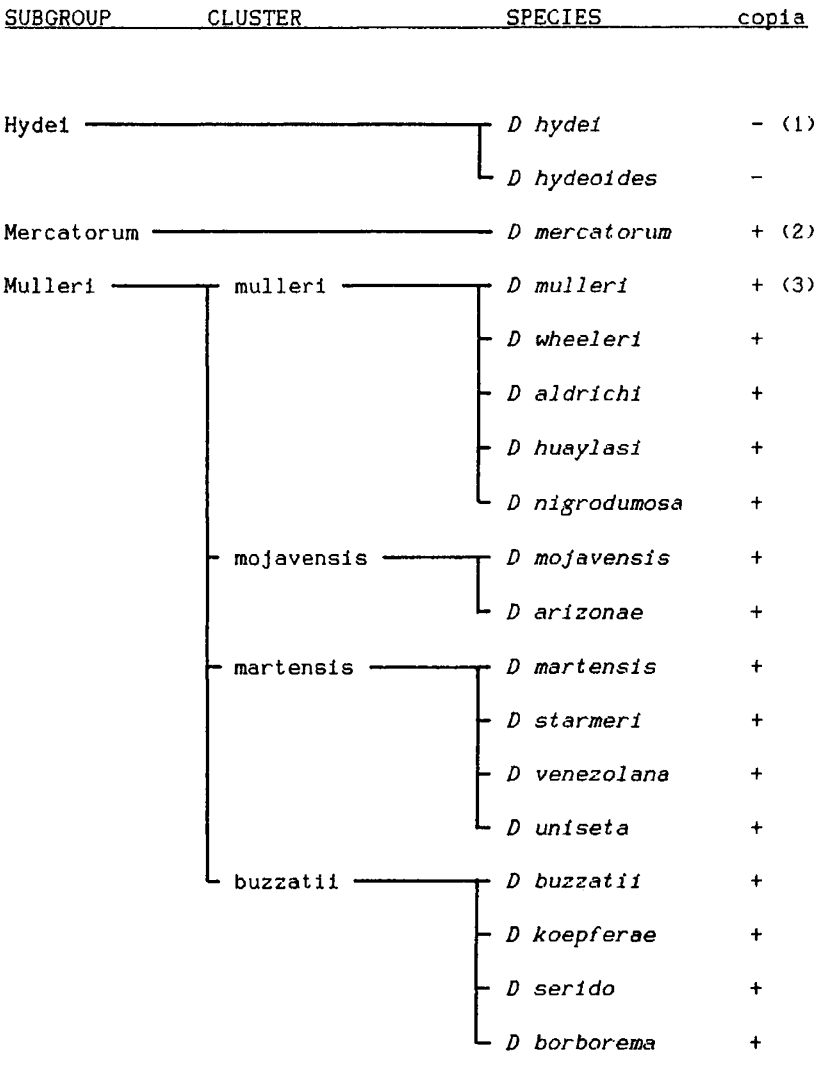


Fig 7. Hybridization of *D buzzatii* and *D koepferae* EcoRI-digested DNA with *D buzzatii* 05TqE probe. (1) *D koepferae* from Los Negros II, Bolivia. (2) *D koepferae* from San Luis, Argentina. (3) *D buzzatii* from San Luis, Argentina. Probe was labelled with digoxigenin-11-dUTP.

DISCUSSION

We have analysed the occurrence of copia in the *repleta* group. The results obtained are summarized in table I. It can be seen that a sequence homologous to copia from *D melanogaster* (Dm copia) is detected in all the tested species from the *mulleri* and *mercatorum* subgroups. Therefore, using both radioactive and non-radioactive detection methods, our results are in good agreement with those reported by Martin *et al* (1983) and Stacey *et al* (1986), where a sequence homologous to Dm copia was detected in the *repleta* group species *D mulleri* and *D mercatorum*.

Table I. Distribution of the copia transposable element in the *repleta* group of *Drosophila*. The results marked with numbers have also been reported by Martin *et al* (1983) (1, 2) and Stacey *et al* (1986) (2, 3).



The negative result obtained here for *D hydei* is also in agreement with the work of Martin *et al* (1983), where no complementary sequences were detected in this species. We have also shown that copia is not detected in *D hydeoides*. The negative result in both species of the *hydei* subgroup could be explained by either the absence of copia in this subgroup or a greater divergence rate of the element in these species, which would avoid detection by hybridization with the pDmcopia

probe. Both alternatives suggest particular evolutionary events of the copia element in the *hydei* subgroup in relation to other *repleta* subgroups.

In the *mulleri* subgroup species tested, the similarity with the pDmcopia probe is enough to detect the homologous sequence in the *repleta* group species under medium stringency wash conditions (Stacey *et al*, 1986). The hybridization signal is heterogeneous between species, suggesting different degrees of similarity between the copia element from the *repleta* group and *D melanogaster*. However, similar patterns of hybridization are obtained with both the pDmcopia and *D buzzatii* probes for *D buzzatii* and *D koepferae* DNA, although the degree of divergence between them is nearly 30% at the DNA level. We therefore deduce that weak signals obtained with pDmcopia probe in Southern blots are due to sequence divergence or the low number of copies of the copia element rather than cross hybridization of the probe with other transposable elements present in these species.

Differences are also observed between closely related species such as *D koepferae* and *D buzzatii*. Populations from different geographic localities from both species were analysed for the genomic distribution of copia. Polymorphism in the genomic location of the elements is detested as heterogeneity in the patterns of the bands obtained for strains of the same species, according to the great variability in the reported chromosomal distribution of copia (Strobel *et al*, 1979; Montgomery and Langley, 1983; Biémont *et al*, 1985; Pasyukova *et al*, 1986; Ronsseray and Anxolabéhère, 1986; Leigh-Brown and Moss, 1987).

The most striking differences in the pattern of bands are observed between Argentinian and Bolivian populations of *D koepferae*. The prominent band observed in the former could be due to the presence of an internal EcoRI fragment or a cluster where the copia element and its flanking regions would be regularly interspersed (Rubin 1983; Yamaguchi *et al*, 1987; Belyaeva *et al*, 1984, Crozatier *et al*, 1988; Di Franco *et al*, 1989).

Using a second restriction enzyme, HindIII, a pattern of multiple bands is obtained with a pDmcopia probe in both Argentinian and Bolivian populations of *D koepferae* (fig 4b). It is noteworthy that hybridizing fragments are longer than 5 kb, suggesting the lack of a HindIII restriction target site in the *copia* sequence. The pattern of multiple bands obtained removes the possibility of a tandem arrangement of the element and suggests that the prominent band in the Argentinian populations is due to the presence of a 3.4 kb-long EcoRI internal restriction fragment.

It is interesting to note that a single change in an internal EcoRI site could explain the pattern observed. In the populations where only 1 EcoRI internal site is present, a pattern of multiple bands is expected, with the fragment lengths determined by the external flanking EcoRI sites. The presence of a second EcoRI internal site generates a pattern with a prominent band corresponding to the internal restriction fragment. Therefore, if copies of the element with either 1 or 2 internal EcoRI sites coexist in the same genome, pattern of bands obtained in EcoRI-digested DNA will depend on the relative frequency of each class of element in the population tested.

The relative intensity of the EcoRI internal fragment in relation to the other bands is lower in Bolivian than in Argentinian populations. Moreover, a clear difference in both number and intensity of bands is observed between *D koepferae* populations of different geographic origins. These results suggest the existence of

polymorphism in the copia element between Argentinian and Bolivian populations of *D koepferae*, in which a certain degree of genetic divergence has previously been described (Fontdevila *et al*, 1988).

On the other hand, patterns of bands obtained for South American and European populations of *D buzzatii* are rather similar, which means that polymorphism in the genomic distribution of copia in this species is very low. Such a regular distribution of the element could be due to the absence of recent transposition events or to genetic drift of a common ancestral set of inactive copies of the element.

Knowing the degree of divergence, we can expect that the homologous elements will remain unsolved until both active and inactive copies of the same element are characterized in closely and distantly related species. We have analysed 2 closely related species, *D koepferae* and *D buzzatii* in more detail, and different situations are observed. In one, an EcoRI restriction polymorphism is observed in the element. In the other a set of ancestral inactive copies is likely to be responsible for the observed patterns of hybridization.

The partial sequencing of 2 independent clones isolated from *D buzzatii* reveals a 70–75% identity in both nucleotide and amino-acid sequences between *D buzzatii* and Dm copia (the similarity raises to 89–95% at the amino-acid level). It is interesting to note that no other transposable element from *D melanogaster* is similar enough to be aligned with *D buzzatii* sequences in the EMBL data bank at the nucleotide level with the FASTA program, and only the amino-acid sequence of the 1731 element from *D melanogaster* is aligned with the *D buzzatii* RT subclone. In this case the amino-acid identity percentage goes from 68.9 to 32.2% in relation to Dm copia.

It is well known that divergence rate between homologous retroviral proteins is faster than for structural genes and the high mutation rate is attributed to the low fidelity of RT (for a review, see Doolittle *et al*, 1989). Although RT is the slowest changing of the retroviral gene products, the amino-acid sequence divergence among different retrotransposons is greater than 60%. Moreover, retrotransposons are clustered in 2 different branches according to RT phylogenies. The copia element from *D melanogaster* is clustered with retrotransposons from very different organisms, such as yeasts (Ty1, *S cerevisiae*) or plants (Tnt1, *N tabacum*; Ta1, *A. thaliana*), and only with one other from *Drosophila* (1731, *D melanogaster*).

The nucleotide identity percentage between *D Buzzatii* isolated sequences and Dm copia is similar to that obtained for structural genes, such as Adh (72.3% at the nucleotide level). If we consider a higher rate of divergence for retrotransposons than for structural genes, we could postulate any mechanism accounting for the conservation of the element sequence between *D melanogaster* and *D buzzatii*, such as horizontal transmission of the element between these species. Evidence for the horizontal transmission of other *Drosophila* elements between phylogenetically distant species has previously been described (Maruyama and Hartl, 1991, Daniels *et al*, 1990). However, the copia element is detected in all the tested *mercatorum* and *mulleri* subgroup species, and the absence of any homologous sequence is confirmed in the *hydei* subgroup. In this case, we postulate transmission of the copia element into the *mulleri* subgroup after the separation of *hydei* subgroup and before the irradiation of the *mulleri* and *mercatorum* subgroups. From that moment, the copia element in these species would have changed in relation to *D melanogaster* according

to the predicted rate of divergence for retrotransposons. On the other hand, if the copia element was present in ancestral species before the irradiation of the *repleta* group, we can postulate the loss of the element in the *hydei* subgroup genomes.

Other retrotransposons have been isolated and sequenced from the *virilis-repleta* radiation species such as micropia from *D hydei* (Lankenau *et al*, 1988, 1990) and gypsy from *D virilis* (Mizrohki *et al*, 1991). Amino-acid identity percentage ranges from 70 to 90% between homologous retrotransposons from these species and *D melanogaster*, which agrees with our results.

Therefore, the high levels of nucleotide and amino-acid sequences identity between the *D buzzatii* element and the copia from *D melanogaster* clearly establishes that the elements are related and are likely to be the same.

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