Homologous sequences to the P transposable element in Drosophila subobscura

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Summary - The distribution of P homologous sequences among different strains of D subobscura is described. Southern analysis of genomic DNAs from the strains restricted with different enzymes showed rather homogeneous hybridization patterns. P homologous sequences are ubiquitous in D subobscura although they are present in a very low copy number. They are strongly divergent from that of D melanogaster and are probably not functional. These results are consistent with the idea that P elements are very old components of the D subobscura genome.

D subobscura / molecular evolution / P transposable element

Résumé - Séquences homologues à l'élément transposable P chez Drosophila subobscura. La distribution des séquences homologues à l'élément transposable P a été étudiée chez D subobscura. L'analyse de l'ADN génomique de différentes souches, réalisée par la technique de Southern-blot, a montré l'existence de profils de bandes très homogènes. Ces résultats suggèrent une présence généralisée, mais en faible nombre de copies, de ces séquences chez D subobscura. Ces séquences sont probablement non-fonctionnelles, faiblement homologues à l'élément P trouvé chez D melanogaster et très anciennes dans le génome de D subobscura.

D subobscura / élément transposable P / évolution moléculaire

INTRODUCTION

Transposable elements are widely distributed among the genomes of living organisms. The role of mobile elements as a cause of spontaneous mutagenesis is well established. In spite of their evolutionary significance as a source of genetic variability, the screening and identification of the different element families from the different species of eukaryotes is just starting. In this respect, D melanogaster is one of the species whose transposable elements are best understood. Up to now, approximately 30 different families of transposable elements in this species have
been described (Finnegan and Fawcett, 1986). Among them are the hybrid dysgenesis inducing families, P, I and hobo, which are especially interesting. The phenomenon of hybrid dysgenesis occurs when males bearing complete copies of these elements (P, I or hobo) are crossed with females lacking these elements (M, reactive and empty strains respectively). P, I and hobo families share a common feature. They show a temporally and spatially discontinuous distribution among *D. melanogaster* populations (Anxolabèhère et al, 1985b, 1988; Bucheton et al, 1986; Boussy et al, 1988; Périquet et al, 1989). An even more patchy distribution among *Drosophila* species has been described (Stacey et al, 1986). This pattern of distribution of these sequences strongly supports the rapid-invasion hypothesis proposed by Kidwell (1979, 1983), based on the analysis of dysgenic potential of P strains.

The P element family is composed of a group of sequences heterogeneous in size: a complete 2.9 kb functional element (O’Hare and Rubin, 1983) and various smaller defective nonfunctional elements (Spradling and Rubin, 1982; O’Hare and Rubin, 1983; Voelker et al, 1987). There have been analyses of the distribution of these sequences among *Drosophila* species other than *D. melanogaster*. Although in early studies no evidence for complete or deleted sequences in other species of the *melanogaster* group was reported (Brookfield et al, 1984; Lansman et al, 1985), recent studies demonstrate the presence of these sequences in some of the species of the *montium* subgroup (Daniels et al, 1990). Among the other 3 *Drosophila* species groups: willistoni, saltans and obscura, P homologous sequences are widely distributed. Extensive Southern analyses of the distribution of P sequences among the willistoni and saltans groups (Daniels and Strausbaugh, 1986) show that complete element sequences are present in most species of these groups. Recent reports on sequences from *D. nebulosa* (Lansman et al, 1987) and *D. willistoni* (Daniel et al, 1990) indicate that a complete highly homologous element is carried by both species. While the P sequence from *D. willistoni* is completely functional, no transposition ability is shown by *D. nebulosa* P sequences. Because of this distribution, the *willistoni* species group has been proposed as the source of the sequences invading the *D. melanogaster* genome (Daniels and Strausbaugh, 1986; Daniels et al, 1990).

Bearing in mind the phylogenetic relationships among the 4 groups of *Drosophila* species, it seems especially interesting to analyse the distribution of sequences amongst the obscura group. As in the willistoni and saltan groups, P homologous sequences were found to be widely distributed among obscura group species as detected by blotting analysis but only in low stringency conditions (Anxolabèhère et al, 1985a; Stacey et al, 1986; de Frutos et al, in preparation). While recent horizontal transfer seems to have occurred from some willistoni group species to the *D. melanogaster* genome, P homologous sequences among the obscura species group seem to be old components of the genome. The studies reported here were designed to analyse the distribution of P homologous sequences among natural populations of a species of the obscura group: *D. subobscura*. This species, which has been extensively analysed from cytogenetic and evolutionary points of view (Krimbas and Loukas, 1980), was originally Palearctic but has recently invaded North and South America (Brncic et al, 1981; Prevosti et al, 1988). Thus, it would be especially interesting to analyse the transposable elements in an expanding species, which may be subject to rapid changes in its genome.
MATERIALS AND METHODS

D subobscura strains

From Europe: H271 from Finland; Up, Su, Ga and Sn from Sweden; Eie, Ef, Ro, Bas, Die, Ká and Bu from Switzerland; Fp from Italy; PM, Sub, Sag, Col, LC, Ald, Che and R from Spain. From North-Africa: Bi from Tunisia; Ch and Mar from Morocco. From North-Atlantic Islands: Az from Azores, Ma from Madeira and Ra from the Canary Islands. From North-America: PC from British Columbia; Al from Washington; CJ from Oregon; Da, Er, Eu, Gr and Wi from California. From South-America: Ba from Argentina.

D melanogaster strains

Harwich is a strong P strain that contains many P element copies, and it was used as a positive control. Gruta and Canton-S are true M strains devoid of P elements, and they were used as negative controls.

Probes

The p25.7 BWC plasmid was used as a probe. This plasmid was constructed by K O’Hare and is a derivative of p25.7 (Spradling and Rubin, 1982). This clone contains a nearly complete P element from D melanogaster which lacks 39 pb from its 5’end and 23 pb from its 3’end.

Experimental procedures

Plasmid DNA was extracted following standard methods (Maniatis et al, 1982). Drosophila genomic DNAs were extracted following the method described by Junakovic et al (1984). Nick translated probes labeled with $^{32}$P were hybridized to the genomic DNAs from D subobscura by squash blotting (an especial dot blot designed by Tchen et al, 1985) and Southern blotting (Southern, 1975). Genomic DNAs were restricted with enzymes which cut twice inside the P element: AccI, PvuII and PstI. Complete P elements of D melanogaster digested with these enzymes yield 2.4 kb, 0.9 kb and 0.7 kb fragments respectively (fig 1). In addition, genomic DNAs were digested with AvaII which generates specific discrete 0.63 kb and 0.48 kb fragments from deleted KP elements (Black et al, 1987).

Fig 1. Restriction-site map of D melanogaster P element.
Hybridization conditions for squash blot
The hybridization was performed in 2X SSC, 1X FPG (0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% glycine), 22 mmol/l KH2PO4 pH 7.2, 0.1% SDS, 2 mmol/l EDTA, 10% dextran sulphate, at 65°C overnight. Washes were performed at 65°C in 2X SSC, 0.1% SDS twice for 15 min and in 1X SSC, 0.1% SDS twice for 30 min.

Hybridization conditions for Southerns
The hybridization was performed overnight at 65°C in 5X SSC, 5X FPG, 0.5% SDS, 150 µg/ml sheared salmon sperm DNA. Washes were performed at 45°C in 3X SSC, 0.1% SDS twice for 30 min, and at 45°C in 2X SSC, 0.1% SDS twice for 1 h.

RESULTS
Filters with DNA from flies from the 38 strains of D subobscura used were probed with the P element of D melanogaster. Weak positive signals were detected in all strains analysed. No differences in the intensity of the signals were detected depending on geographic origin of the samples. As an example we show in figure 2 an autoradiogram from North-European samples.

Fig 2. Squash blot analyses of D subobscura individuals from North-European samples. Four individuals were taken from each strain. 1 to 4: Sn, Su, Gå and UP strains from Sweden; 5: H271 strain from Finland. 6: Tü strain from Germany. 7: individuals from Harwich (positive control) and Gruta (negative control) are shown in alternate positions.

More information about the molecular structure of homologous P sequences of D subobscura can be obtained from Southern analysis. Genomic DNAs from different D subobscura strains restricted with AccI, PvuII, PstI and AvaII were probed with complete p25.7 BWC plasmid. AccI, PvuII and PstI produce 2.4 kb, 0.9 kb and 0.7 kb specific internal fragments from the complete P element of D melanogaster. The 2.4 kb fragment produced by AccI digestion encompasses most of the P element sequence which has been used to determine whether or not complete P elements are present in the D subobscura genome. Figure 3 shows an autoradiogram of some samples of D subobscura genomic DNAs restricted with AccI. Some features of
the $P$ homologous sequences in $D$ subobscura can be seen in this picture. First, sequences homologous to $P$ element of $D$ melanogaster are present in the genome of $D$ subobscura. Positive hybridization signals were found in all the 38 analysed strains (data not shown). Secondly, the strongly hybridizing 2.4 kb fragment, indicative of the presence of a complete $P$ element (lane 1 fig 3), is missing in $D$ subobscura. A very weak band of similar size is detected in lanes 3, 4, 7 and 8, but these signals can be due to the low stringency washes. We have no information about the size of $P$ sequences in $D$ subobscura. It is possible that no complete $P$ elements are present in this species. Alternatively, it is possible that an element of approximately 2.9 kb is present in $D$ subobscura but 1 or both target sites for AccI are missing in this sequence. Instead of the 2.4 kb fragment, 2 bands of 4 kb and 2 kb tend to be present in all the analysed strains. However, some variability in the banding pattern among strains was detected. This variability is not correlated with the geographical distribution of the strains. Thus, identical patterns are shown by BA and MA strains (lanes 3 and 6, fig 3) which come from Argentina and from the Madeira Islands respectively, LC and PC strains (lanes 8 and 4, fig 3) which come from Spain and British Columbia respectively and so on.

![Figure 3](image)

**Fig 3.** *Southern blot* analyses of some $D$ subobscura strains. Genomic DNAs were digested with AccI and probed with the $p$B25.7 Bwc plasmid. Samples are as follows: 1, $D$ melanogaster $P$ strain, Harwich. 2, $D$ melanogaster $M$ strain, Canton-S. 3, Ba (Argentina). 4, PC (British Columbia). 5, Mar (Morocco). 6, Ma (Madeira Islands). 7, LC (Spain). 8, Kå (Switzerland). 9, H271 (Finland).

Similar results were obtained from samples digested with PvuII and PstI (figs 4 and 5). The presence of a complete $P$ element of $D$ melanogaster produces a 0.9 kb PvuII fragment. As can be observed in figure 4, the 0.9 kb fragment (lane 10) is not present in the $D$ subobscura genome. Genomic DNAs of $D$ subobscura digested with PvuII and probed with the $P$ element exhibit bands of 3.1 kb and 2.7 kb (lanes 1 to 8, fig 4, and lanes 1, 3 and 5, fig 5). A highly homogeneous banding pattern is generated by this enzyme in all the strains analysed. Similarly, the 0.7 kb PstI fragment does not appear in the $D$ subobscura genome. Instead, a strong 1.8 kb band and some other weak signals were found in all the strains analysed (examples of this pattern are shown in lanes 2, 4 and 6, fig 5). From these patterns
we can deduce that variation exists between $P$ sequences of $D$ melanogaster and $D$ subobscura. $Pvu$II, $Pst$I and probably $Accl$ target sites were missing. It can also be concluded that only one or very few $P$ elements are present in $D$ subobscura genome.

Figure 6 shows the results of digesting genomic DNAs from some samples with $Ava$II. The digestion of the $D$ melanogaster DNA with this enzyme produces a 1.8 kb fragment and 2 smaller 0.54 kb and 0.48 kb fragments from complete $P$ elements as can be seen in lane 9. $Ava$II also produces 2 specific 0.64 kb and 0.48 kb small fragments from the deleted $KP$ element. A homogeneous hybridization pattern is shown by $D$ subobscura strains, but fragments of a similar size of those of the $D$ melanogaster complete $P$ element and not of the $KP$ element were found in this species (lanes 1 to 7, fig 6).

**DISCUSSION**

Sequences homologous to $D$ melanogaster $P$ elements were found in $D$ subobscura. The distribution pattern of $P$ sequences amongst the strains analysed shows strong differences from that of $D$ melanogaster. All the strains bear $P$ sequences, independently of their geographic origin. This differs from the temporally and geographi-
cally patchy distribution found among populations of *D. melanogaster*. We have no information about temporal changes in the presence or absence of *P* elements in *D. subobscura*. Most of the strains used in this analysis came from wild individuals captured recently. However, currently, *D. subobscura* is undergoing an interesting new evolutionary process. Until 1978 *D. subobscura* was a Palearctic species. In that year it was detected in Puerto Montt, Chile (Brncic et al., 1981; Prevosti et al., 1988). Since then this species has invaded extensive areas in South and North America. This interesting evolutionary process has been accompanied by a rapid genomic reconstruction, e.g. new clines of inversions have been found (Prevosti et al., 1988). Similarly, changes in transposable elements could be expected. However, no differences have been found between European and American populations.

Not only are *P* sequences ubiquitous in *D. subobscura* but there are also strong similarities between the elements. The intensity of the hybridization signals is very similar in all strains and no strong homologies with the *D. melanogaster* *P* sequences were found. Positive hybridization signals appeared exclusively at low stringency washes. This agrees with other results of the distribution of *P* homologous sequences among the *obscura* group species reported by Anxolabéhère et al. (1985a) and de Frutos et al. (in preparation).

We can draw some conclusions about the structure of *P* homologous sequences in *D. subobscura* from the Southern analysis. Firstly, samples from the 38 *D. subobscura* strains, coming from different geographic locations, show a rather homogeneous hybridization pattern, which indicates that no active elements are present in the *D. subobscura* populations. Secondly, a very low number of bands are generated by the restriction enzymes used in this study. We can deduce that very few homologous *P* sequences occur in the genome of *D. subobscura*. Thirdly, depending on the restriction enzyme used we can obtain some additional information about the structure of these sequences. *PvuII* and *PstI* produce 0.9 kb and 0.7 kb internal fragments, respectively, from the complete *P* sequence of *D. melanogaster*. Neither the 0.9 kb nor the 0.7 kb fragment has been found in any strain of *D. subobscura*. **Fig 6. Southern blot analyses of some *D. subobscura* strains.** Genomic DNAs were digested with *AvaiI*. Samples are as follows: 1, H271 (Finland). 2, Kä (Switzerland). 3, LC (Spain). 4, Ma (Madeira Islands). 5, Mar (Morrocco). 6, PC (British Columbia). 7, Ba (Argentina). 8, *D. melanogaster M* strain, Canton-S. 9, *D. melanogaster P* strain, Harwich.
In addition from AccI and AvaII digestions we can deduce that neither complete
P nor deleted KP elements exist in the D subobscura genome. Alternatively, it is
possible that both complete and deleted elements are kept in the D subobscura
genome but target sequences from AccI and AvaII enzymes have been lost in this
species. To summarize, no targets for any of the cited enzymes are conserved in the
P homologous sequences of D subobscura.

Thus, P homologous sequences are present in the D subobscura genome, but
they are probably not functional and they are strongly divergent from those of D
melanogaster. A very low number of sequences exist in the genome of this species.
Although we are using the P element of D melanogaster as a reference, it is highly
probable that this species has recently been invaded by P (Kidwell, 1979, 1983) and
that this sequence comes from D willistoni or any other species of the willistoni
group (Daniels and Strausbaugh, 1986; Daniels et al, 1990). In this context we can
conclude that P sequences from D subobscura are strongly diverged from those of
D willistoni.

Simple models based on vertical transmission do not account for the distribution
of P sequences among Sophophora subgenus species. In a strictly vertical model,
P elements must have been present among Drosophilidae family members before
Sophophora radiation. In this model we would have to postulate that after their
radiation, the willistoni-saltans lineages kept active elements. Meanwhile, the P
sequence among the obscura group probably lost their function quickly and diverged
strongly. Most of the species of the melanogaster group including D melanogaster
would have had to lose these elements. If the Sophophora radiation occurred about
50 My ago (Throckmorton, 1985) we would have to postulate that, during this
time P elements disappeared completely in most of the species. Nevertheless, P
sequences reappeared recently in D melanogaster.

Obviously, horizontal transference of genetic elements between reproductive iso-
lated species is not the usual method of transposable element dispersion. However,
some evidence supports the hypothesis of some kind of transport of P elements from
some species of the willistoni group to the D melanogaster genome (Daniels and
Strausbaugh, 1986; Daniels et al, 1990). It is not known whether this is a recurrent
phenomenon. Other apparent transposable element homologies could be explained
by a mechanism of horizontal transmission (eg the close relationship between the
copia element of D melanogaster and Ty1 of yeast, or gypsy group also from D
melanogaster and plant viruses, Doolittle et al, 1989). Assuming that horizontal
transfer can occur, the distribution of P sequences in the 4 lineages of Sophophora
can be explained in the following way. P elements are widely distributed among
the willistoni-saltans lineages, but not to such an extent among the melanogaster-
obscura lineages. Thus, there were probably P sequences before Sophophora radi-
ation. Since P related sequences have also been detected in other Diptera outside
the Drosophilidae family (Anxolabéhere and Périer, 1987), they should have been
present from more ancient times. P sequences seem to have evolved differently in the
4 Sophophora lineages. While in willistoni-saltans lineages these P sequences have
remained from these times (and moreover they are active in D willistoni), in most
of the species of the melanogaster group they have been lost and D melanogaster
should have been recently invaded by these sequences. In the obscura group strong
changes seem to have occurred. Few elements remain in the D subobscura genome,
and some of them seem to be located in the heterochromatin. Preliminary in situ hybridization results, using as probe a D subobscura clone (homologous to the P element of D melanogaster), indicate the presence of P related sequences at the 68D locus on E chromosome and at centromeric region (Paricio et al, in preparation). If P sequences were transposed early from the euchromatic to the heterochromatic region it is possible to expect that they were submitted to an early inactivation, and that they diverged quickly.

Although horizontal transmission could be invoked to explain the distribution of P elements among Sophophora species, our results suggest that no such evolutionary event has recently occurred in D subobscura.

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