Genet Sel Evol (1990) 22, 307-316

Original article

Homologous sequences to the *P* transposable element in *Drosophila subobscura*

N Paricio, L Pascual, MJ Martínez-Sebastián, M Pérez-Alonso, R de Frutos

Universidad de Valencia, Facultad de Ciencias Biológicas
Departamento de Genética
Dr Moliner 50. 46100 Burjassot. Valencia, Spain

(Received 29 January 1990; accepted 13 June 1990)

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 (Anxolabéhère *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 (Anxolabéhère *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, Gä and Sn from Sweden; Eie, Ef, Ro, Bas, Die, Kä 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 *pπ25.7 BWC* plasmid was used as a probe. This plasmid was constructed by K O'Hare and is a derivative of *pπ25.7* (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: *Acc*I, *Pvu*II and *Pst*I. 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 *Ava*II which generates specific discrete 0.63 kb and 0.48 kb fragments from deleted *KP* elements (Black *et al*, 1987).

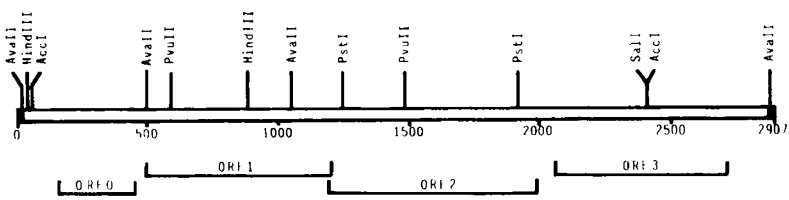

Fig 1. Restriction-site map of *D melanogaster P* element.

***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 KH_2PO_4 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.

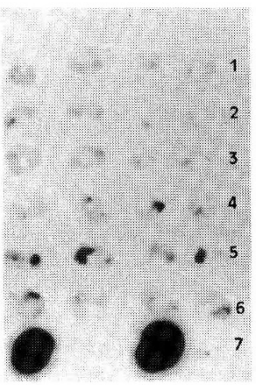

Fig 2. *Squash blot* analyses of *D subobscura* individuals from North-European samples. Four individuals were taken from each strain. 1 to 4: Sn, Su, Gä and UP strains from Sweden; 5: H271 strain from Finland. 6: Tü strain from Germany. 7: individuals from Harwich (positive control) and Gruta (negative 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 *Acc*I, *Pvu*II, *Pst*I and *Ava*II were probed with complete *pπ25.7 BWC* plasmid. *Acc*I, *Pvu*II and *Pst*I 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 *Acc*I 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 *Acc*I. 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 *Acc*I 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.

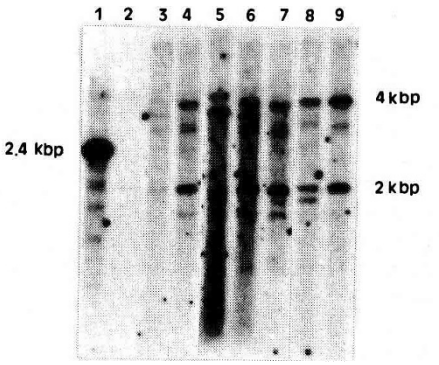

Fig 3. *Southern blot* analyses of some *D subobscura* strains. Genomic DNAs were digested with *Acc*I and probed with the pπ25.7 Bwc plasmid. Samples are as follows: 1, *D melanogaster P* strain, Harwich. 2, *D melanogaster M* strain, Canton-S. 3, Ba (Argentina). 4, PC (British Columbia). 5, Mar (Morocco). 6, Ma (Madeira Islands). 7, LC (Spain). 8, Kä (Switzerland). 9, H271 (Finland).

Similar results were obtained from samples digested with *Pvu*II and *Pst*I (figs 4 and 5). The presence of a complete *P* element of *D melanogaster* produces a 0.9 kb *Pvu*II 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 *Pvu*II 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 *Pst*I 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

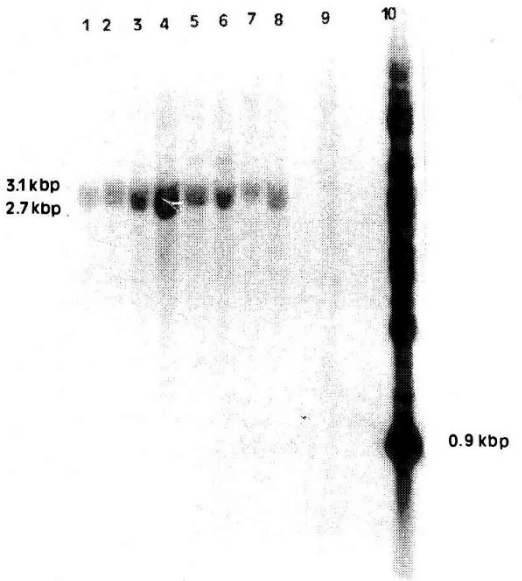

Fig 4. *Southern blot* analyses of some *D subobscura* strains. Genomic DNAs were digested with *Pvu*II. Samples are as follows: 1 to 3 Sn, Gä and Su from Sweden. 4, H271 (Finland). Lanes 5 to 8 Bu, Die, Eie and Bas from Switzerland. 9, *D melanogaster M* strain, Gruta 10, *D melanogaster P* strain, Harwich.

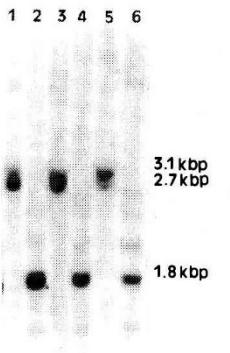

Fig 5. *Southern blot* analyses of some *D subobscura* strains. Genomic DNAs were digested with *Pvu*II (lanes 1, 3 and 5), or *Pst*I (lanes 2, 4 and 6). Samples are as follows: lanes 1 and 2, R strain (Spain). Lanes 3 and 4, UP strain (Sweden). Lanes 5 and 6, Tü strain (Germany).

we can deduce that variation exists between *P* sequences of *D melanogaster* and*D subobscura*. *Pvu*II, *Pst*I and probably *Acc*I 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-

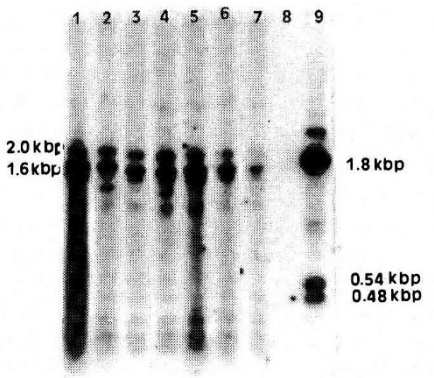

Fig 6. *Southern blot* analyses of some *D subobscura* strains. Genomic DNAs were digested with *Ava*II. Samples are as follows: 1, H271 (Finland). 2, Kä (Switzerland). 3, LC (Spain). 4, Ma (Madeira Islands). 5, Mar (Morrocco). 6, PC (British Columbia). 7, Ba (Argentina). 8, *D melanogaster M* strain, Canton-S. 9, *D melanogaster P* strain, Harwich.

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, *eg* 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. *Pvu*II and *Pst*I 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*.

In addition from *Acc*I and *Ava*II 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 *Acc*I and *Ava*II 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 isolated 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* radiation. Since *P* related sequences have also been detected in other *Diptera* outside the *Drosophilidae* family (Anxolabéhère and Périquet, 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 heterocromatic 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*.

ACKNOWLEDGMENTS

This work was supported by Grant PB87-0952 from the Comisión de Investigación Científica y Técnica.

REFERENCES

Anxolabéhère D, Nouaud D, Périquet G (1985a) Séquences homologues à l'élément *P* chez des espèces de *Drosophila* du groupe *obscura* et chez *Schaptomiza pallida (Drosophilidae). Génét Sél Evol* 17, 579-584

Anxolabéhère D, Nouaud D, Périquet G, Tchen P (1985b) *P*-element distribution in Eurasian populations of *D melanogaster*: A genetic and molecular analysis. *Proc Natl Acad Sci USA* 82, 5418-5422

Anxolabéhère D, Périquet G (1987) *P* homologous sequences in *Diptera* are not restricted to the *Drosophilidae* family. *Genet Iber* 39, 211-222

Anxolabéhère D, Kidwell MG, Périquet G (1988) Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of *D melanogaster* by mobile *P* elements. *Mol Biol Evol* 5, 252-269

Black DM, Jackson MS, Kidwell MG, Dover GA (1987) *KP* elements repress *P*-induced hybrid dysgenesis in *D melanogaster*. *EMBO J* 6, 4125-4135

Boussy IA, Healy MJ, Oakeshott JG, Kidwell MG (1988) Molecular analysis of the *P-M* gonadal dysgenesis cline in Eastern Australian *D melanogaster*. *Genetics* 119, 889-902

Brncic D, Prevosti A, Budnik M, Monclús M, Ocaña J (1981) Colonization of *D subobscura* in Chile. I. First population and cytogenetic studies. *Genetica* 56, 3-9

Brookfield JFY, Montgomery E, Langley CH (1984) Apparent absence of transposable elements related to the *P* elements of *D melanogaster* in other species of *Drosophila*. *Nature* 310, 330-332

Bucheton A, Simonelig M, Vaury Ch, Crozatier M (1986) Sequences similar to the *I* transposable element involved in *I-R* hybrid dysgenesis in *D melanogaster* occur in other *Drosophila* species. *Nature* 322, 650-652

Daniels SB, Strausbaugh D (1986) The Distribution of *P*-element sequences in *Drosophila*: the *willistoni* and *saltans* species groups. *J Mol Evol* 23, 138-148

Daniels SB, Peterson KR, Strausbaugh LD, Kidwell MG, Chovnick A (1990) Evidence for horizontal transmission of the *P* transposable element between *Drosophila* species. *Genetics* 124, 339-355

Doolittle RF, Feng DF, Johnson MS, McClure MA (1989) Origins and evolutionary relationships of retroviruses. *In: The Quarterly Review of Biology* Vol 64(1), 1-30

Finnegan DJ, Fawcett DM (1986) Transposable elements in *D melanogaster*. *In: Oxford Surveys on Eukayotic Genes.* Oxford University Press, vol 3, 1-62

Junakovic N, Caneva R, Ballario P (1984) Genomic distribution of copia-like elements in laboratory stocks of *D melanogaster*. *Chromosoma (Berl)* 90, 378-382

Kidwell MG (1979) Hybrid dysgenesis in *D melanogaster*: the relationship between the *P-M* and *I-R* interaction systems. *Genet Res* 33, 205-217

Kidwell MG (1983) Evolution of hybrid dysgenesis determinants in *D melanogaster*. *Proc Natl Acad Sci USA* 80, 1655-1659

Krimbas CB, Loukas M (1980) The inversion polymorphism of *D subobscura*. *In: Evolutionary Biology.* (Hecht MK, Steere WC, Wallace B, eds) Vol 12, 163-234

Lansman RA, Stacey SN, Grigliatti TA, Brock HW (1985) Sequences homologous to the *P* mobile element of *D melanogaster* are widely distributed in the *subgenus Sophophora*. *Nature* 318, 561-563

Lansman RA, Shade RO, Grigliatti TA, Brock HW (1987) Evolution of *P* transposable elements: Sequences of *D nebulosa P* elements. *Proc Natl Acad Sci USA* 84, 6491-6495

Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular cloning: A Laboratory Manual.* Cold Spring Harbor, NY

O'Hare K, Rubin GM (1983) Structures of *P* transposable elements and their sites of insertion and excision in the *D melanogaster* genome. *Cell* 34, 25-35

Périquet G, Hamelin MH, Bigot Y, Lepissier A (1989) Geographical and historical patterns of distribution of *hobo* elements in *D melanogaster* populations. *J Evol Biol* 2, 223-229

Prevosti A, Ribó G, Serra L, Aguadé M, Balaña J, Monclús M, Mestres F (1988) Colonization of America by *D subobscura*: Experiment in natural populations that supports the adaptive role of chromosomal-inversion polymorphism. *Proc Natl Acad Sci USA* 85, 5597-5600

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98, 503-517

Spradling AC, Rubin GM (1982) Transposition of cloned *P* elements into *Drosophila* germline chromosomes. *Science* 218, 341-347

Stacey SN, Lansman RA, Brock HW, Grigliatti TA (1986) Distribution and conservation of mobile elements in the genus *Drosophila*. *Mol Biol Evol* 3, 522-534

Tchen P, Anxolabéhère D, Nouaud D, Périquet G (1985) Hybridization on squashed flies: a method to detect gene sequences in single *Drosophila* individuals. *Anal Biochem* 150, 415-420

Throckmorton LH (1985) The phylogeny, ecology and geography of *Drosophila*. *In: Handbook of Genetics.* (King RC, ed) Plenum Press, New York, 421-469

Voelker RA, Stamatis N, Monastirioti M, Hatzopoulos P, Louis C (1987) *Hobo* is responsible for the induction of hybrid dysgenesis by strains of *D melanogaster* bearing the male recombination factor 23.5 MRF. *Cell* 49, 487-495