Heredity **73** (1994) 377–385
Received 10 February 1994
©The Genetical Society of Great Britain

The *mariner* transposable element in the Drosophilidae family

FREDERIC BRUNET, FABIENNE GODIN, JEAN R. DAVID & PIERRE CAPY*
Laboratoire Populations, Génétique et Evolution, CNRS, 91198 Gif/Yvette Cedex, France

The distribution of the *mariner* transposable element among Drosophilidae species was investigated using three different techniques, i.e. squash blots, Southern blots and PCR amplification, using two sets of primers (one corresponding to the Inverted Terminal Repeats and the other to two conserved regions of the putative transposase). Our results and those of others show that the distribution of *mariner* is not uniform and does not follow the phylogeny of the host species. An analysis of geographical distribution, based on endemic species, shows that *mariner* is mainly present in Asia and Africa. At least two hypotheses may be proposed to explain the specific and geographical distributions of this element. Firstly, they may be the results of several horizontal transmissions between *Drosophila* species and/or between *Drosophila* species and one or several donor species outside the Drosophilidae family. Secondly, these particular distributions may correspond to the evolution of the *mariner* element from an ancestral copy which was present in the ancestor of the Drosophilidae family.

Keywords: *Drosophila*, *mariner*, transposon, phylogeny.

Introduction

The distribution of the *mariner* transposable elements in *Drosophila* was previously investigated by Maruyama & Hartl (1991a). In their analysis, species belonging to different groups, subgenera and genera were screened, showing a distribution with a major gap between the *melanogaster* group and the *Zaprionus* subgenus. On the basis of this specific distribution and of the high similarities of DNA sequences of copies extracted from different species, it was suggested that horizontal transfers had occurred between *D. mauritiana* and *Z. tuberculatus* or between species related to these.

The horizontal transfer hypothesis is commonly suggested from the analysis of transposable element distributions and from the comparison of their sequences (Daniels *et al.*, 1990; Mizrokhi & Mazo, 1990; Lawrence *et al.*, 1992; Robertson, 1993). On the other hand, several alternative hypotheses, among which genetic drift, existence of ancestral elements on which high selective constraint may act in a small part of the element, ancestral polymorphism, different rates of evolution according to the activity level of the copies and according to the host species, could also explain the phylogenetic oddities of several elements (Capy *et al.*, 1994). In all cases, the evolutionary history of these elements is not simple and more information is necessary about the variability within and between more or less closely related species.

In this respect, we have investigated the Drosophilidae family to extend the previous work of Maruyama & Hartl (1991a), which included 78 species. The number of species analysed in this family is now 127. For several groups, we have screened almost all the available species. Moreover, three more or less sensitive techniques, i.e. squash blots, Southern blots and PCR (polymerase chain reaction) have been used here while Maruyama & Hartl (1991a) only used a Southern blot detection.

The aim of this analysis was to obtain a better idea of the specific and geographical (based on endemic species) distributions of the *mariner* element. This is a first step before a detailed analysis, at the nucleotidic level, of the polymorphism within and between species. Our results show that some *mariner*-related sequences can be detected in many *Drosophila* species. Moreover, in many species, a particular class of deleted elements seems to represent the major part of the copies. However, in different species, different classes of deleted elements exist. The geographical and specific distributions among species will be discussed with reference to the different hypotheses reviewed by Capy *et al.* (1994).

*Correspondence.

Materials and methods

Species

Eighty-three species belonging to the Drosophilidae family were screened. Most of them belong to the huge *Drosophila* genus. This genus is subdivided into subgenera among which two, the *Sophophora* and *Drosophila* subgenera, contain a large number of species. These subgenera are themselves classically divided into groups (e.g. the *melanogaster* or the *obscura* groups) and groups containing too many species are subdivided into subgroups (e.g. the *melanogaster* or the *ananassae* subgroups).

Particular attention was paid to the *ananassae* and *montium* subgroups belonging to the *melanogaster* group, to the *obscura* group and to the *Zaprionus* genus. Moreover, three other genera, *Chymomyza*, *Scaptodrosophila* and *Scaptomyza*, were also considered. Among the 83 species here analysed, 34 were in common with Maruyama & Hartl (1991a).

Squash blots and Southern blots

Squash blots were performed as described by Tchen *et al.* (1985). For Southern blots, DNA was extracted from about 20–30 flies using the technique of Maruyama & Hartl (1991a). Total DNA was then digested by *Hin*dIII and *Bam*HI, two restriction enzymes that do not cut in the *peach* sequence, an inactive element described in *D. mauritiana* (Jacobson *et al.*, 1986). Gels were transferred according to the Southern technique (Southern, 1975) and probed with a mixture of *pchIV* and *pchV* probes described in Maruyama & Hartl (1991a).

PCR amplifications

PCR amplifications were performed using two sets of primers (Fig. 1). Firstly, the inverted repeats of the *D. mauritiana peach* element and, secondly, primers corresponding to the WVPHEL and YSPDLAP conserved regions in the putative transposase (see Robertson, 1993; Langin *et al.*, 1994). These particular primers deduced from the comparison of *mos*-1 (an active *mariner* element of *D. mauritiana*, Bryan *et al.*, 1987) and *MLE* (an inactive *mariner*-like element of the lepidopteran *Hyalophora cecropia*, Lidholm *et al.*, 1991), will be called the 'internal primers'. The PCR amplification was performed as follows: less than 10 ng of DNA from regular DNA extraction or 1 μL of a rough extraction following the protocol of Gloor & Engels (1991) were used in 50 μL of a total reaction volume containing 0.5 units of Taq polymerase (Promega). Amplifications were performed on the Trio-Thermoblock of Biometra. Prior to the first cycle, a denaturation at 95 °C during 5 min was performed, then the basic cycle corresponded to a denaturation at 95 °C for 30 s, an annealing phase at 52 °C for 1 min and an elongation phase at 72 °C for 2 min. After the last cycle, the temperature was decreased to 4 °C. The total number of cycles varied between 35 and 40. To be sure that the amplified fragments were related to *mariner*, the gels with the PCR products were transferred on a nylon filter and hybridized with *mariner* probes of *D. mauritiana* as for squash blots and Southern blots.

Results

Investigations were mostly carried out on species of the *Sophophora* subgenus of *Drosophila*, i.e. the *ananassae* and the *montium* subgroups within the *melanogaster* group, the *obscura* group and on species of *Zaprionus* with its two subgenera (*Zaprionus* and *Anaprionus*). Several other species randomly sampled from the Drosphilidae family were also tested.

The *ananassae* subgroup

Twelve of the 20 species known in this subgroup have been screened. Detailed results are summarized in

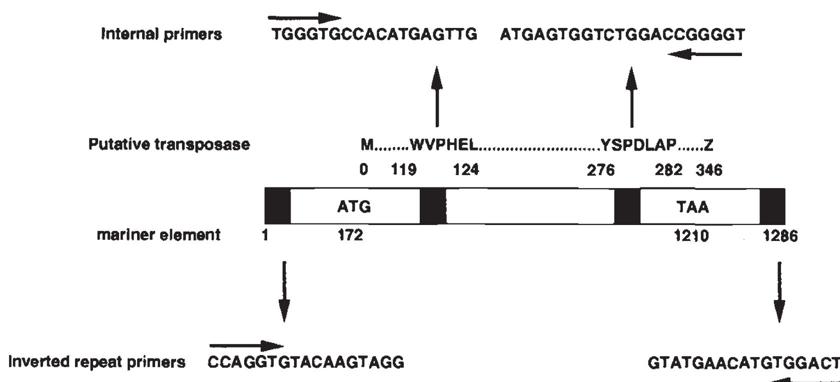

Fig. 1 PCR primers used for the amplification of the *mariner* element from total genomic DNA. The WVPHEL and YSPDLAP regions were determined from the comparison between the *mariner*-like element (MLE) of *Hyalophora cecropia* (Lidholm *et al.*, 1991) and the *peach* element of *D. mauritiana* (Jacobson *et al.*, 1986).

Table 1 Distribution of the *mariner* element detected by three different techniques in the species of the *ananassae* subgroup in the *melanogaster* group

Species	Squash blot	Southern blot (no. of bands)	PCR ITR	PCR NT
bipectinata complex				
D. bipectinata	+ +	1–2	+	+
D. malerkotliana	+ + +	9	+	+
D. parabipectinata	+ +	10–15	+	+
D. pseudoananassae	+ +	0	+	+
ananassae complex				
D. ananassae	+ +	3–5	+	+
D. atripex	+ + +	8	+	+
D. monieri	+/−	7	+	+
D. ochrogaster			+	+
ercepeae complex				
D. ercepeae	+ + + +	10–20	+	+
D. vallismaia		15–20	+	+
D. n.sp. Madagascar	+	5		
Ungrouped species				
D. varians	+	2	+	−

A blank means that the technique was not used.

Table 1. The species are distributed among the four complexes of this subgroup, i.e. the *bipectinata* (four species), the *ananassae* (four species), the *ercepeae* (three species) and the nonclassified species (one species) complexes. In this subgroup, only two species were previously screened by Maruyama & Hartl (1991a).

The three techniques of detection were used for all species. For most of them, some positive signals were observed (Fig. 2). The only exception is *D. varians* (nonclassified species) for which no amplification was obtained with internal primers. The hybridization signals and the numbers of bands observed by Southern blots vary greatly from one species to another. Assuming that, in this subgroup, the two restriction endonucleases used do not cut into the *mariner* copies, our results suggest that the number of copies is highly variable. Indeed, estimations of the number of copies, based on the number of bands observed by Southern blots, show that this number could vary from 1 to 2 in *D. bipectinata* and *D. varians* to 10 to 20 in the species of the *ercepeae* complex. For *D. monieri* no signal was detected by squash blot while faint hybridizing bands were observed by Southern blot. The main difference between these two tech-

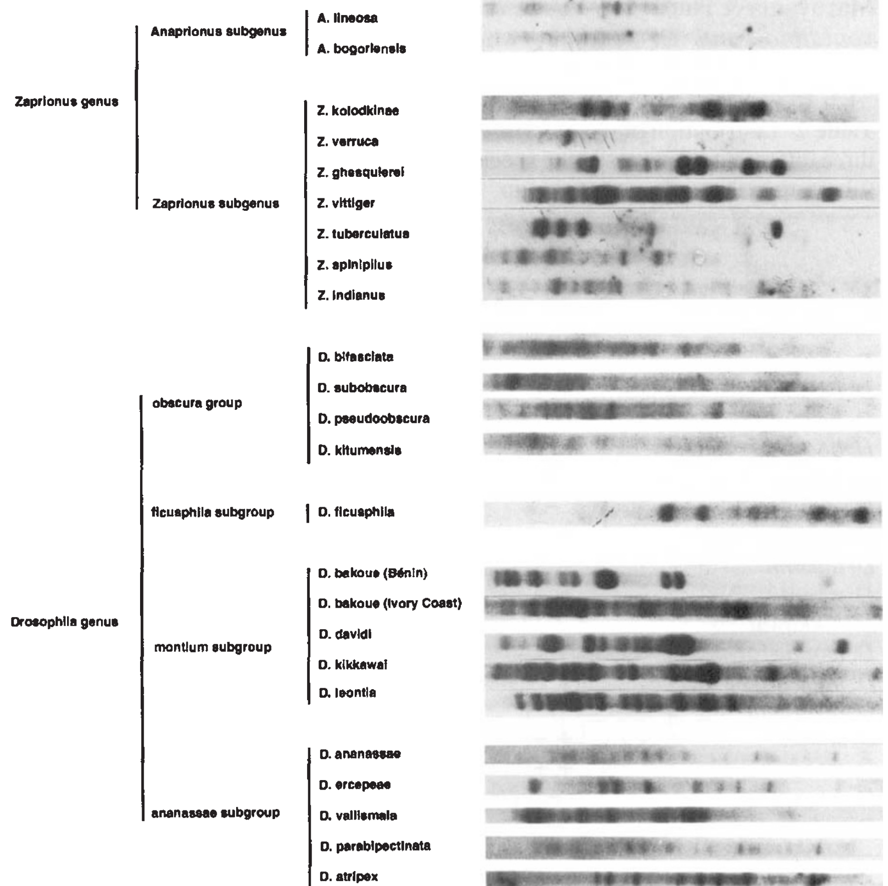

Fig. 2 Southern blot hybridizations. In each case, the total genomic DNA was extracted from about 20–30 individuals using the technique described by Maruyama & Hartl (1991a). In the *Anaprionus* subgenus very faint bands are detected.

niques was that the washing stringency was generally higher for the former compared with the latter. Therefore, it is possible that the similarity between the *mariner* copies of this species and the *D. mauritiana* probe is lower than for the copies detected in the other species of this subgroup.

The montium *subgroup*

In this subgroup, 14 species were previously screened by Maruyama & Hartl (1991a). In the present work, 18 species were tested corresponding to 13 newly investigated species and five species already analysed. The results are given in Table 2 and some hybridizations are shown in Fig. 2.

Considering the species in common, the results of the two analyses are in agreement with the exception of *D. serrata*. Here, Maruyama & Hartl (1991a) found a positive signal by Southern blot whereas we failed to find anything with the three techniques used. However, because two different strains were used, it is possible that this species is polymorphic for the presence or absence of the element.

With regard to the new species, the *montium* subgroup is quite heterogeneous. Several species seem to be free of the *mariner* element (*D. auraria* from Maruyama & Hartl (1991a), *D. burlai*, *D. chauvacae*, *D vouidibioi and D. vulcana*) whereas several others contain a large number of copies (assuming that no restriction sites for *Hin*dIII and *Bam*HI exist in these copies). Such is the case for *D. bakoue*, *D. davidi*, *D. kikkawai* and *D. leontia* in which the average number of copies is about 14. All these copies were detected by Southern blots. By PCR amplification, no product was obtained for the eight species tested, using either set of primers. Therefore, the elements detected may correspond to deleted copies (in particular in the regions of the PCR primers). On the other hand, while these copies are easily detectable by Southern hybridization, it is possible that the divergence with the *D. mauritiana* elements is strong enough to prevent the hybridization of the PCR primers.

Table 2 Distribution of the *mariner* element detected by three different techniques in the species of the *montium* subgroup in the *melanogaster* group

Drosophila species	Squash blot	Southern blot (no. of bands)	PCR ITR	PCR INT
D. bakoue (Bénin)	+ +	14	–	–
D. bakoue (Congo)	+ +	9	–	–
D. bakoue (Ivory Coast)	+	12	–	–
D. bocqueti	+ / –	0	–	–
D. burlai	–			
D. chauvacae	–	0	–	–
D. davidi	+ +	16	–	–
D. dossoui	+ +	4	–	–
D. greeni	+ +			
D. kikkawai	+ + / –	14		
D. leontia		16		
D. malagassya	+ +			
D. nikananu	+ + +			
D. serrata	+ –	0	–	–
D. tsacasi	+ +			
D. vouidibioi	–			
D. vulcana	–			

A blank means that the technique was not used.

Other subgroups in the melanogaster *group*

Within the *melanogaster* group, some *mariner*-like elements were detected in seven out of the eight subgroups analysed (Fig. 3). Only the *elegans* and *eugracilis* subgroups appear to be free of such elements. In the *takahashii* subgroup, some elements seem to be present in *D. lutescens* and *D. pseudotakahashii* but not in *D. takahashii* itself. Therefore, the *mariner* elements seem to be randomly distributed within and between the different subgroups which compose this group. Moreover, the hybridization intensities vary from one species to another. Such a result could be explained by different degrees of similarities with the *D. mauritiana* probe. In this case, it will be interesting to sequence several of these elements to determine their phylogenetic relationships.

The obscura *group*

This group, which also belongs to the same *Sophophora* subgenus, was previously investigated by Maruyama & Hartl (1991a). However, none of the six species analysed contained any *mariner* copies. In the present work, 10 species were tested, three of them being in common with the previous investigation. In contrast with the previous results, all the species tested seem to harbour some *mariner* elements (Table 3). The main difference between the two investigations is probably the washing stringency of the hybridized filters. Therefore, it is quite possible that the elements detected in this group are more divergent from those of *D. mauritiana*. It must be stressed, however, that some amplifications were obtained using the PCR primers corresponding to the ITR.

The Drosophila, Zaprionus *and other genera*

For the remaining species tested belonging to the *Drosophila* genus, the results are summarized in Table

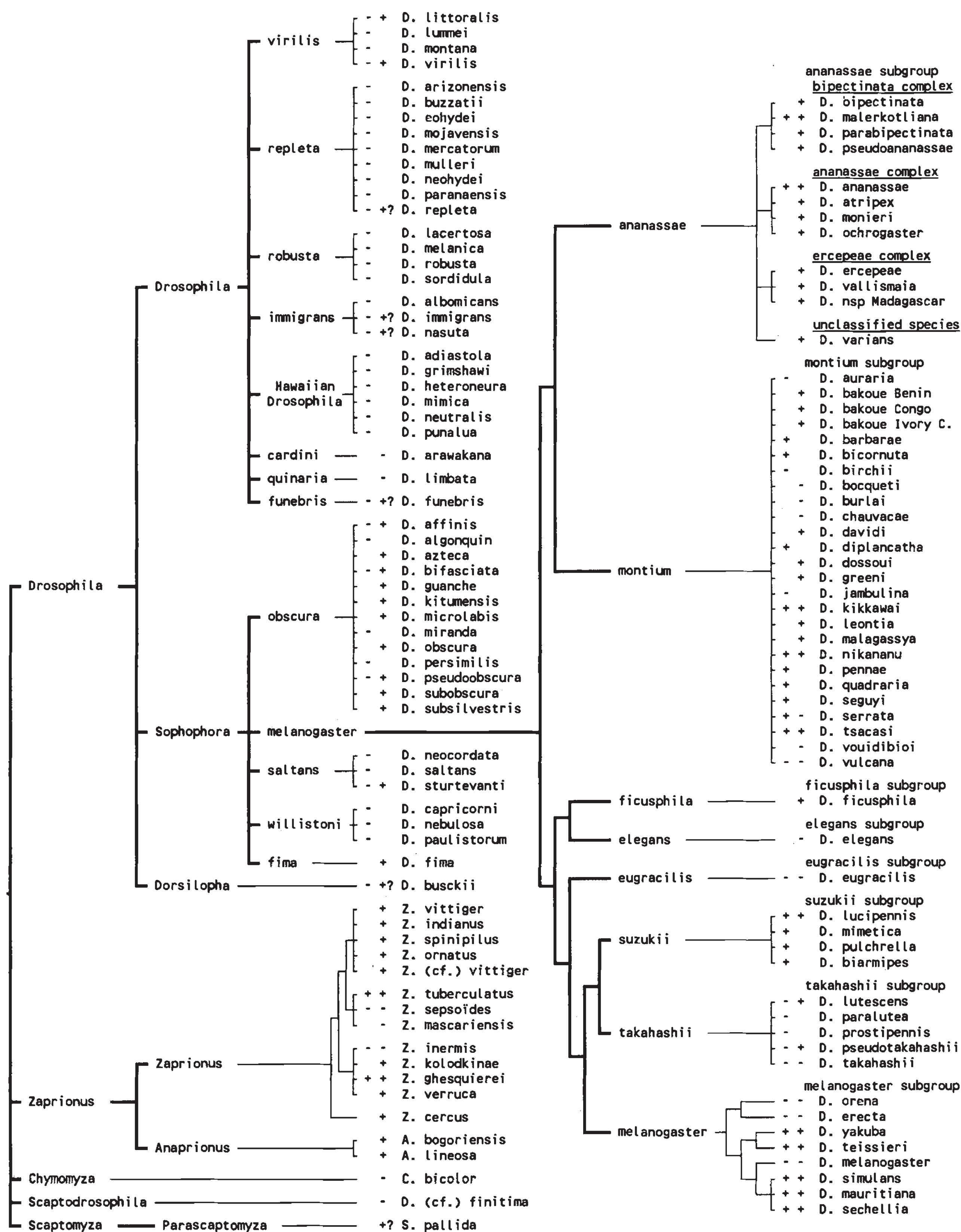

Fig. 3 Summary of the different results obtained by Maruyama & Hartl (1991a), in the left column, and in the present work, right column. +, existence of hybridizing signal(s); −, no hybridizing signal.

Table 3 Distribution of the *mariner* element detected by three different techniques in the species of the *obscura* group

Drosophila species	Squash blot	Southern blot (no. of bands)	PCR ITR	PCR INT
Palaeartic species				
D. bifasciata	+	12	+ +	
D. guanche	+			
D. obscura	+		+	
D. subobscura	+	5	+	
D. subsilvestris	+			
American species				
D. affinis	+		+ +	
D. azteca	+			
D. pseudoobscura	+	8		
African species				
D. kitumensis	+	5	−	+
D. microlabis	+			

A blank means that the technique was not used.

Table 4 Distribution of the *mariner* element detected by three different techniques in some species of the *Drosophila* genus

Drosophila genus	M & H Southern blot	Squash blot	PCR ITR	PCR INT
Sophophora subgenus				
saltans group				
D. sturtevanti	−	+/−		
fima group				
D. fima		+ + +		
Drosophila subgenus				
virilis group				
D. littoralis	−	+ + +		
D. virilis	−	+ +		+ ?
repleta group				
D. repleta	−			+ ?
immigrans group				
D. immigrans	−	+/−		+ ?
D. nasuta	−			+ ?
funebris group				
D. funebris	−		+ ?	
cardini group				
D. arawakana		−		
quinaria group				
D. limbata		−		
Dorsilopha subgenus				
D. busckii	−	−		+ ?

M & H Southern blot refers to the work of Maruyama & Hartl (1991a).
A blank means that the technique was not used.

4. Among these data we can observe the putative absence of *mariner* in *D. arawakana* (*cardini* group) and in *D. limbata* (*quinaria* group) whereas some hybridization was obtained on *D. fima* genomic DNA (*fima* group). Moreover, a few species seem to be positive while they appeared negative from the Maruyama & Hartl (1991a) analysis, e.g. *D. virilis* and *D. littoralis* (*virilis* group).

Concerning the *Zaprionus* genus, two species belonging to the *Anaprionus* subgenus and 13 species of the *Zaprionus* subgenus were tested (Table 5). With the exception of *Z. sepsoïdes*, *Z. mascariensis* and *Z. inermis*, all the species showed some hybridising bands with *D. mauritiana* probes. The signals are generally weak in the *Anaprionus* subgenus but can be strong in the *Zaprionus* subgenus for almost all the positive species. This result suggests a better identity between *D. mauritiana* and the species of the *Zaprionus* subgenus than with the two species of the *Anaprionus* subgenus.

Concerning the species belonging to the three other genera (*Chymomyza*, *Scaptodrosophila* and *Scaptomyza*) no signal was detectable by Southern hybridization. However, some amplification products were obtained from total genomic DNA of *Scaptomyza pallida* using the two sets of primers.

Discussion

Detection of the elements

Figure 3 summarizes all the data concerning the distribution of the *mariner* element among the species of the Drosophilidae family. The data of Maruyama & Hartl (1991a) and of the present work were pooled. It appears that some gaps exist and that the element can be present in a given species but absent in a closely related one. Such is the case between the species of the *montium* subgroup. Moreover, in the *melanogaster* subgroup, our results confirm those of Maruyama & Hartl (1991a), i.e. the absence of the *mariner* element in *D. melanogaster, D. erecta* and *D. orena*.

In this respect, it must be stressed that it is not possible to state that a species is free of the *mariner* element. For instance, in the present study the genomic DNAs of the species tested were probed using the *D. mauritiana* sequences as a reference. In other words, if a species contains some homologous sequences which are homologous but diverged strongly from our reference sequence, it will be difficult to detect them. That is the reason why, complementing the classical analysis by squash and Southern blots, some investigations by PCR amplification were performed using two sets of primers taken from regions suspected to evolve at a slower rate than the other parts of the element.

Table 5 Distribution of the *mariner* element detected by three different techniques in the species of the *Zaprionus* genus and other related genera

Species	M & H Southern blot	Present study		PCR	
		Squash blot	Southern blot (no. of bands)	ITR	INT
Zaprionus genus					
Zaprionus subgenus					
Z. indianus (= *Z. collarti*)		+ + +	10–20	+(700 bp)	–
Z. ornatus		+ +			
Z. spinipilus		+ +	7–12	+	+
Z. vittiger		+ +	15–20	+	
Z. (cf.) vittiger (Cape Town)		+ +			
Z. mascariensis		–	0		
Z. sepsoïdes	–	–	0		
Z. tuberculatus	+	+ +	2–7	+	+
Z. ghesquieri	+	+ +	6–13	+	+
Z. inermis	–	–	0	+	+
Z. kolodkinae			3–6		
Z. verruca		+ +	2–3	+(1800 bp)	+
Z. cercus			0	+	+
Anaprionus subgenus					
A. bogoriensis		+ +	5		
A. lineosa			11		
Chymomyza genus					
C. bicolor		–		–	–
Scaptodrosophila genus					
D. (cf.) finitima		–			
Scaptomyza genus					
Parascaptomyza subgenus					
S. pallida				+	+

M & H Southern blot refers to the work of Maruyama & Harth (1991a).
A blank means that the technique was not used.

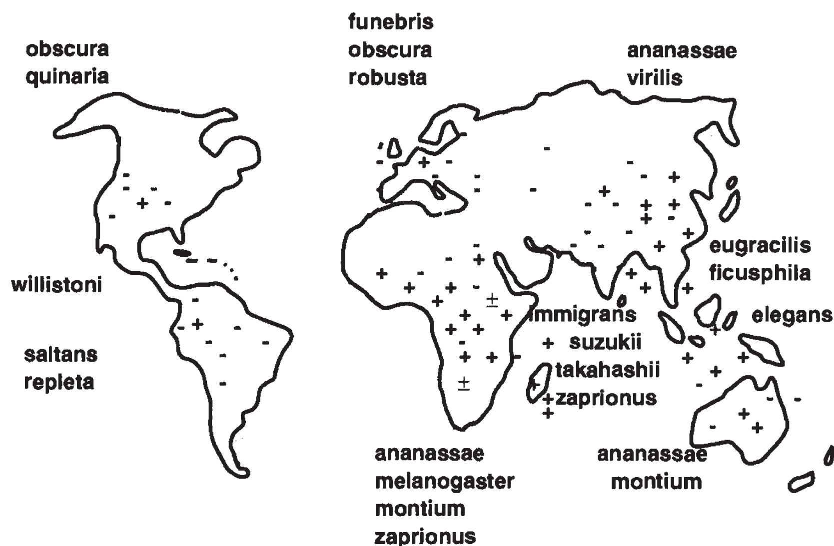

Fig. 4 Geographical distribution of the *mariner*-like elements based on the distribution of endemic species of the Drosophilidae family.

Geographical distribution

To try to understand the origin of the *mariner* element in the Drosophilidae family, its geographical distribution was analysed considering only endemic species. This compilation, summarized in Fig. 4, shows that *mariner* is mainly present on the Asiatic and the African continents. In other words, with the exception of species belonging to the *obscura* group, none of the species endemic to the Palaearctic region and to the American continent contains any *mariner* element hybridizing with the *D. mauritiana* probes.

Evolutionary hypotheses

At least two hypotheses may be proposed to interpret the results. Firstly, the apparent random distribution between species of this element and its restricted geographical distribution suggest the existence of horizontal transmission. Such a phenomenon has already been proposed by Maruyama & Hartl (1991b) to explain the high similarity observed between the sequences of *Zaprionus* and some species of the *melanogaster* subgroup. However, to explain the complete *mariner* distribution among the Drosophilidae, it must be assumed that this phenomenon was relatively frequent. On the other hand, the geographical distribution restricted to the Asiatic and to the African continents also suggests a repeated introduction of this element by horizontal transmission in species endemic in these regions.

The second hypothesis which can be proposed is that the *mariner* element is an ancient element, as suggest by Kidwell (1993) and by Robertson (1993). In this respect, assuming that a *mariner* element was present in the ancestor of the family Drosophilidae, the random distribution of this element among the species of this family can be interpreted as the result of loss in some lineages. For instance, Kaplan *et al.* (1985) showed that it is possible to lose a transposable element by genetic drift when the average number of copies is relatively low. We also may consider that if, in a species, all the elements become inactive they will be easily lost by genetic drift.

Throckmorton (1975) and Wheeler (1981) suggested that the origin of the Drosophilidae family was probably in south-east Asia. Therefore, the hypothesis of an ancestral origin of the *mariner* element in this family is reinforced by its geographical distribution. Indeed, it is quite possible that *mariner* was lost in several lineages during speciation, as stated above, but also during the colonization of the new area. Thus, this could explain the absence of *mariner* in the Palaearctic region and on the American continent.

These two hypotheses are not mutually exclusive and it is quite possible that a *mariner* element was present in the ancestor of the Drosophilidae family, that it was lost in some lineages and was reacquired by horizontal transmission. In this respect, analysing *mariner* in other insects, Robertson (1993) showed the presence of different types of *mariner* in several species suggesting the existence of several *mariner* subfamilies. Therefore, even if *mariner* were an ancient element, it could be reacquired several times from several donors by horizontal transmission. Thus, we need more information, especially on sequence polymorphism within and between more or less related species.

Note added in proof

The presence of the *mariner* element in *D. erecta* (*melanogaster* subgroup) has been recently mentioned by A. R. Lohe, E. N. Moriyamo, D. A. Lidholm and D. L. Hartl (manuscript submitted). The copies sequenced are 97 per cent identical to those of the cat flea *Ctenocephalides felis*, and 50 per cent divergent from the *Mos-1* element of *D. mauritiana*. This result strongly suggests the existence of horizontal transmission. This could explain why these copies were not detected with the *mauritiana* probes used in this work.

Acknowledgements

We thank the anonymous referees from their helpful comments. This work has benefitted from CNRS/NSF (no. 693) and GREG (no. 48) grants.

References

BRYAN, G. J., JACOBSON, J. W. AND HARTL, D. L. 1987. Heritable somatic excision of a *Drosophila* transposon. *Science*, **235**, 1636–1638.

CAPY, P., ANXOLABÉHÈRE, D. AND LANGIN, T. 1994. The strange phylogenies of transposable elements: are horizontal transfers the only explanation? *Trends Genet.*, **10**, 7–12.

DANIELS, S. B., PETERSON, K. R., STRAUSBAUGH, L. D., KIDWELL, M. G. AND CHOVNICK, A. 1990. Evidence for horizontal transmission of the *P* element between *Drosophila* species. *Genetics*, **124**, 339–355.

GLOOR, G. AND ENGELS, W. 1991. Single-fly DNA preparations for PCR. *Drosph. Inf. Serv.*, 17–18.

JACOBSON, J. W., MEDHORA, M. M. AND HARTL, D. L. 1986. Molecular structure of a somatically unstable element in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8684–8688.

KAPLAN, N., DARDEN T. AND LANGLEY, C. H. 1985. Evolution and extinction of transposable elements in Mendelian populations. *Genetics*, **109**, 459–480.

KIDWELL. M. G. 1993. Voyage of an ancient *mariner*. *Nature*, **362**, 202–202.

LANGIN, T., CAPY, P. AND DABOUSSI, M. J. 1994. The transposable element, *Impala*, a fungal member of the *Tc1-mariner* super family. *Mol. Gen. Genet.* (in press).

LAWRENCE, J. G., OCHMAN, H. AND HARTL, D. L. 1992. The evolution of insertion sequences within enteric bacteria. *Genetics,* **131**, 9–20.

LIDHOLM, D. A., GUDMUNDSSON, G. H. AND BOMAN, H. G. 1991. A highly repetitive *mariner*-like element in the genome of *Hyalophora cecropia*. *J. Biol. Chem.*, **266**, 11518–11521.

MARUYAMA, K. AND HARTL, D. L. 1991a. Evolution of the transposable element *mariner* in *Drosophila* species. *Genetics,* **128**, 319–329.

MARUYAMA, K. AND HARTL, D. L. 1991b. Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zaprionus*. *J. Mol. Evol.*, **33**, 514–524.

MIZROKHI, L. J. AND MAZO, A. M. 1990. Evidence for horizontal transmission of the mobile element *jockey* between distant *Drosophila* species. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 9216–9220.

ROBERTSON, H. M. 1993. The *mariner* transposable element is widespread in insects. *Nature,* **362**, 241–245.

SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, **98**, 503–517.

TCHEN, P., ANXOLABÉHÈRE, D., NOUAUD, D. AND PERIQUET, G. 1985. Hybridization on squashed flies: a method to detect genes sequences in single *Drosophila* individuals. *Analyt. Biochem.*, **150**, 415–420.

THROCKMORTON, L. H. 1975. The phylogeny, ecology and geography of *Drosophila*. In: King, R. C. (ed.) *Handbook of Genetics*, vol. 3, pp. 421–469. Plenum Press, New York.

WHEELER, M. R. 1981. The Drosophilidae: a taxonomic overview. In: Ashburner, M., Carson H. L. and Thompson J. N., Jr. *The Genetics and Biology of Drosophila*, vol. 3a, pp. 1–97. Academic Press, New York.