Distribution of the Retrotransposable Element 412 in *Drosophila* Species

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Copy numbers of sequences homologous to the *Drosophila melanogaster* retrotransposable element 412, their distribution between the chromosome arms and the chromocenter, and whether they contain full-size copies were analyzed for 55 species of the *Drosophila* genus. Element 412 insertion sites were detected on the chromosome arms of *D. melanogaster, Drosophila simulans,* and a few species of the *obscura* group, but the chromocenter was labeled in almost all species. The presence of element 412 sequences in the majority of species shows that this element has a long evolutionary history in Drosophilidae, although it may have recently invaded the chromosomes in some species, such as *D. simulans*. Differences in copy number between species may be due to population size or specific endogenous or environmental factors and may follow the worldwide invasion of the species. Putative full-length copies were detected in the chromocenters of some species with no copies on the chromosome arms, suggesting that the chromocenter may be a shelter for such copies and not only for deleted ones.

Introduction

Transposable elements (TEs), which were thought for a long time to be only parasitic elements (Doolittle and Sapienza 1980; Orgel and Crick 1980), are increasingly seen as essential components of the genomes of many organisms (Britten 1996; Löwer, Löwer, and Kurth 1996; Pardue et al. 1996), and even as a way of causing DNA sequence variation (Charlesworth 1996). Knowledge of their structure and of mechanisms controlling transposition and regulating copy number has increased greatly in recent years, but we still have no clear understanding of the population biology of these elements (Biémont 1992; Biémont et al. 1997*a,* 1997*b*). Population data have come mostly from the fruit fly, *Drosophila melanogaster,* which has a large number of TEs, highly scattered over the chromosomes. Extensive surveys have been carried out for various species of *Drosophila* to detect elements, with the aim of studying their distribution according to *Drosophila* radiation. Most of the studied elements, generally homologous to those of *D. melanogaster,* are distributed according to phylogenetic classification (Dowsett and Young 1982; Martin, Wiernasz, and Schedl 1983; Brookfield, Montgomery, and Langley 1984; Stacey et al. 1986; Daniels et al. 1990; de Frutos, Peterson, and Kidwell 1992; Alberola and de Frutos 1993; Montchamp-Moreau et al. 1993; Sezutsu, Nitasaka, and Yamazaki 1995). Some elements, however, present an erratic distribution with large differences in copy number between species (Martin, Wiernasz, and Schedl 1983; Silber et al. 1989; Capy, David, and Hartl 1992; Simmons 1992; Francino, Cabre, and Fontdevila 1993; Brunet et al. 1994; Csink and McDonald 1995; Regner et al. 1996; Vieira and Biémont 1996*a*). Such a patchy distribution may suggest horizontal transmission events. Horizontal transfers are indeed well documented for the transposons P (Clark, Maddison, and Kidwell 1994; Hagemann, Haring, and Pinsker 1996; Clark and Kidwell 1997) and mariner (Maruyama and Hartl 1991*a*), and are also proposed for hobo (Daniels, Chovnick, and Boussy 1990; Simmons 1992), the retroposons I (Bucheton et al. 1986, 1992) and jockey (Mizrokhi and Mazo 1990), and the retrovirus gypsy (Stacey et al. 1986; Alberola and de Frutos 1993) (see Clark, Maddison, and Kidwell [1994] and Capy, Anxolabéhère, and Langin [1994] for discussion).

Overall, these results suggest that most of the elements were present in ancestral species and had long evolutionary histories. The numbers of element copies rapidly increased in some species (Maruyama and Hartl 1991*b*), whereas elements were lost in others, possibly due to genetic drift (Engels 1981) associated with small effective population sizes (Kaplan, Darden, and Langley 1985; Cariou 1987; Hey and Kliman 1993; Sezutsu, Nitasaka, and Yamazaki 1995). However, the distribution of the elements, their copy numbers in euchromatin and heterochromatin, and the extent to which potentially active copies are present in most *Drosophila* species are not well understood. Such information is required to understand the behavior of TEs in the various species, and to select the most interesting species for further detailed analysis of the mechanisms restricting or increasing TE copy number. In this study, we investigated the presence and total genomic copy numbers of sequences homologous to the *D. melanogaster* retrotransposable element 412 (Finnegan et al. 1978). We studied their distribution over the chromosome arms and in the chromocentric regions, and whether putative full-length copies were present, in 55 species of Drosophila.

Materials and Methods
Fly Stocks

All the species of the Drosophilidae family used in this study, except *D. lebanonensis* (a gift from R. Allemand), *D. madeirensis* (collected by A. Brehm in Madeira), *D. melanogaster* (collected by us in Valence, France), and *D. simulans* (from Amieu, New Caledonia;

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a gift from C. Montchamp-Moreau), were laboratory-maintained strains from the collection of the Populations, Génétique et Evolution laboratory in Gif-sur-Yvette.

DNA Extraction and Southern Blotting

DNA was extracted from about five adult females (more if the flies were very small) of each species by a standard phenol-chloroform-salt method with proteinase K digestion. The DNA was digested with restriction enzymes according to the manufacturer's recommendations. Agarose gel electrophoresis, transfer of DNA to membranes, prehybridization, and hybridization were carried out as described in Junakovic, Caneva, and Ballario (1984) and Di Franco et al. (1989). Membranes were washed at 65°C in $2 \times$ SSC (twice for 15 min) and then in $0.1 \times$ SSC, 0.1% SDS (10 min). We estimated that under these high-stringency conditions, the sequences and the probe might possess at least 70%–80% identity for bands to be detected on the filters (Dove and Davidson 1962; Bonner et al. 1973). For the species not belonging to the *melanogaster, ananassae,* or *montium* subgroup, the $0.1 \times$ SSC wash was too stringent, implying more time for faint bands seen with only $2 \times$ SSC to be visualized: therefore, the filters corresponding to these species were washed only with $2 \times$ SSC.

To estimate the overall amount of 412 elements, genomic DNA was cut with the *Hin*dIII restriction enzyme, subjected to electrophoresis, and probed with the internal 1.4-kb *Eco*RI fragment of the 412 element from *D. melanogaster.* Putative complete copies homologous to the 412 element of *D. melanogaster* were detected by cutting DNA with *Bsr*BI and probing it with the internal 6.9-kb *Bsr*BI fragment. Probes were labeled with a Megaprime kit (Amersham). X-ray film exposure time was overnight to 4 days, with intensifying screens at −80°C.

For each blot, DNA samples of both *D. melanogaster* and *D. simulans* were added. The autoradiographs were scanned, and the patterns were assessed with a DNA molecular weight ladder and a comparison with the *D. melanogaster* and *D. simulans* profiles.

In Situ Hybridization

Polytene chromosome squashes from salivary glands of third-instar female larvae were prepared and probed with pAT153 containing the 412 element from *D. melanogaster,* nick-translated, and biotinylated (Biémont 1994). The probes were prepared with 50% formamide, as used for homologous DNA hybridizations (formamide: 50%; dextran: 8%; $20 \times$ SSC: 8%; water). Less stringent conditions with 35% formamide were used for species in which no insertion site was initially detected under 50% formamide conditions, to confirm that there were no labeled sites. Insertion sites were visualized as brown bands produced by a dye-coupled reaction with peroxidase substrate and diaminobenzidine. Genomic DNA sequences associated with the 412 probe hybridized to the 21D region of the 2L chromosome.

The signal at this region was therefore used as a positive control, indicating hybridization.

Results

Distribution of the 412 Element as Detected by Southern Blotting

The restriction patterns of genomic DNA digested with *Hin*dIII and probed with the 1.4-kb *Eco*RI fragment (fig. 1) show that sequences homologous to the 412 element of *D. melanogaster* were detected in almost all of the species analyzed, but the number of bands detected differed greatly between species. For example, all three species of the *melanogaster* subgroup, *D. melanogaster, D. simulans,* and *D. sechellia,* generated large numbers of bands, whereas *D. mauritiana, D. yakuba, D. teissieri, D. orena,* and *D. erecta* generated fewer bands, which differed in intensity. The *montium* species subgroup, particularly *D. greeni, D. dossoui,* and *D. nikananu* species, and the *takahashii, eugracilis, elegans,* and *ficusphila* species subgroups also presented patterns involving many bands. The *ananassae* and *suzukii* species subgroups had fewer bands than did the subgroups of the melanogaster group. *Drosophila pseudoobscura, D. obscura,* and *D. microlabis,* of the obscura group, and *D. funebris* generated many bands, but the intensity of hybridization was weaker than that for *D. melanogaster* and *D. simulans.* There were also many bands detected for *Zaprionus tuberculatus,* although this species is only very distantly related to *D. melanogaster.*

Based on the structure of the 412 element from *D. melanogaster,* we expected to detect bands longer than 4.0 kb when *Hin*dIII-digested DNA was probed with the 1.4-kb *Eco*RI fragment. The bands detected for *D. melanogaster* and *D. simulans* were longer than 4.0 kb, but those for the other species were smaller. These patterns of bands produced by copies of elements with sequences differing from that of the 412 canonical element of *D. melanogaster* suggest that there has been divergence of the 412 sequence in the genomes of the Drosophilidae. A large number of small high-intensity bands were found in many species of the *montium* subgroup, in almost all species of the *obscura* group, and in *D. immigrans, D. funebris,* and *Z. tuberculatus.* Some of these small bands migrated to the same position in the gel for different species, suggesting that they may be 412 copies with the same type of deletion or polymorphism, rather than having comigrated only by chance.

We are aware that the use of internal DNA probes in the Southern blots may have missed some short deleted elements or elements with a small internal sequence amplified which could exist in the heterochromatin of some species. However, the comparison below between in situ hybridization and Southern blots gives compatible results in the sense that a low labeling or an absence of labeling of the chromocenter of the polytene chromosomes was always associated with few bands in Southern blots.

Chromosome Distribution of the 412 Element

In situ hybridization to polytene chromosomes (table 1) showed that a few species with large numbers of

bands on Southern blots had numerous insertion sites scattered over the chromosomes, as seen for *D. melanogaster* and *D. simulans* (see table 1 and fig. 2). In the *melanogaster* subgroup, only the *melanogaster* complex species, including *D. melanogaster, D. simulans, D. mauritiana,* and *D. sechellia,* had 412 element insertion sites on the chromosomal arms, with variable copy numbers, whereas the *yakuba-teisseiri* complex and *orena-erecta* complex species (Lachaise et al. 1988) had no such euchromatic copies of the 412 element. The centromeric regions were, however, labeled for all species of the *melanogaster* subgroup. In the *melanogaster* complex, *D. sechellia* had only 2–3 euchromatic copies, whereas it had as many bands on Southern blots as *D. melanogaster* and *D. simulans,* which had, on average, 30 and 14 sites, respectively (Vieira and Biémont 1996*b*), as detected by in situ hybridization. This suggests that the bands detected in *D. sechellia* were mostly heterochromatic copies. *Drosophila mauritiana* and *D. sechellia,* which both had only 2–3 copies in their euchromatin, had completely different Southern blot profiles, with many more bands for *D. sechellia* than for *D. mauritiana.* In situ hybridization also showed that the labeling of the chromocenter was stronger and more scattered for *D. sechellia* than for *D. mauritiana.* This shows that the 412 element behaves differently in the heterochromatic regions of these species.

In the other species of the *melanogaster* group tested, only *D. greeni, D. dossoui,* and *D. kikkawai* of the *montium* subgroup and *D. ananassae* of the *ananassae* subgroup had any insertion sites on the chromosome arms. In Southern blots, all of these species except *D. ananassae* generated many bands, as did *D. sechellia.* The chromocenter was strongly stained in species such as *D. greeni* and *D. dossoui* (fig. 2), suggesting that the majority of bands detected by Southern blotting were due to various 412 insertions into heterochromatin. The labeling of the chromocenter differed greatly between species, from a large homogeneous mess-like mass in *D. greeni* and *D. dossoui* to a strong brown spot in *D. vallismaïa* (fig. 2). Such differences raise questions about the precise influence of the 412 sequences in shaping the heterochromatic regions. The generation of high-intensity bands by these species shows that the heterochromatin may contain many 412 copies with a specific deletion or polymorphism.

In the obscura group, *D. pseudoobscura, D. subobscura, D. madeirensis, D. obscura,* and, to a lesser extent, *D. bifasciatia* had few copies on their chromosomal arms and weak labeling in their chromocentric regions. The small number of bands detected by Southern blotting, as for *D. subobscura* and *D. bifasciata,* confirms that there were only a few heterochromatic copies. For *D. madeirensis,* different larvae showed different euchromatic 412 labeled sites on their chromosome arms, suggesting either the presence of putative active 412 copies in this species or a residual polymorphism of actually inactive copies.

Presence of Putative Complete Copies

We looked for complete copies of the 412 element by digesting genomic DNA with *Bsr*BI. This enzyme cuts the DNA at the extreme ends of the 412 sequence of *D. melanogaster,* resulting in a 6.9-kb fragment. The restriction pattern for each species tested is given in figure 3. We expected complete copies to become more difficult to detect as the phylogenetic distance of the species from *D. melanogaster* increased, if no horizontal transfer was involved. The 6.9-kb fragment was detected in various populations of *D. melanogaster* and its sibling species *D. simulans* (data not shown). For these two species, the band was very strong, suggesting that most of the 412 element copies were full-size. However, a few other faint bands longer or shorter than 6.9 kb were observed in *D. simulans,* suggesting that the 412 element had undergone various deletions and had become polymorphic in *D. simulans.* This 6.9-kb fragment was not detected in most of the other species, indicating that the 412 element has indeed diverged in Drosophilidae. This fragment was nevertheless detected in some species of the *melanogaster* subgroup (*D. mauritiana, D. yakuba, D. teissieri,* and *D. erecta*), but the band was fainter than those for *D. melanogaster* and *D. simulans. Drosophila sechellia* had euchromatic copies of the 412 element, but it did not generate the 6.9-kb fragment, unlike *D. mauritiana.* This suggests that the euchromatin of species such as *D. sechellia* had no full-length copies of the 412 element, or that the copies had diverged from the 412 canonical *D. melanogaster* sequence such that they were not detectable by *Bsr*BI digestion. *Drosophila yakuba* and *D. erecta* had no insertion sites on the chromosome arms, but full-size copies were detected by Southern blotting. The 6.9-kb *Bsr*BI fragment was also detected in *D. cauveri, D. vulcana,* and *D. buzzatii,* but the intensity of the labeling was low. This band may thus be generated by a small number of complete copies in the chromocenter. Such results support the idea that the heterochromatic regions may bear potentially full-length copies of the 412 element. We cannot eliminate the hypothesis that a band at 6.9 kb corresponds to fortuitous comigrating sequences generated by divergent 412 elements. The presence of this 6.9-kb band in many species, sometimes with strong labeling, suggests, however, that this band really corresponds to a specific sequence.

Although a few copies of the 412 element were detected on the chromosome arms of the obscura group species, none of these species generated the 6.9-kb fragment. The 2.3-kb fragment, detected as a high-intensity band in almost all the obscura group species, may be a major copy of the 412 element in this group. *Drosophila microlabis* did not generate this band. It did, however, generate a major 6.8-kb band of high intensity which may result from 412 copies with internal deletions. *Bsr*BI bands of various sizes were detected for most of the species analyzed, reflecting the presence of various kinds of deleted fragments or the existence of a restriction site polymorphism due to the long evolutionary history of the 412 retrotransposable element in the Drosophila genus.

Discussion

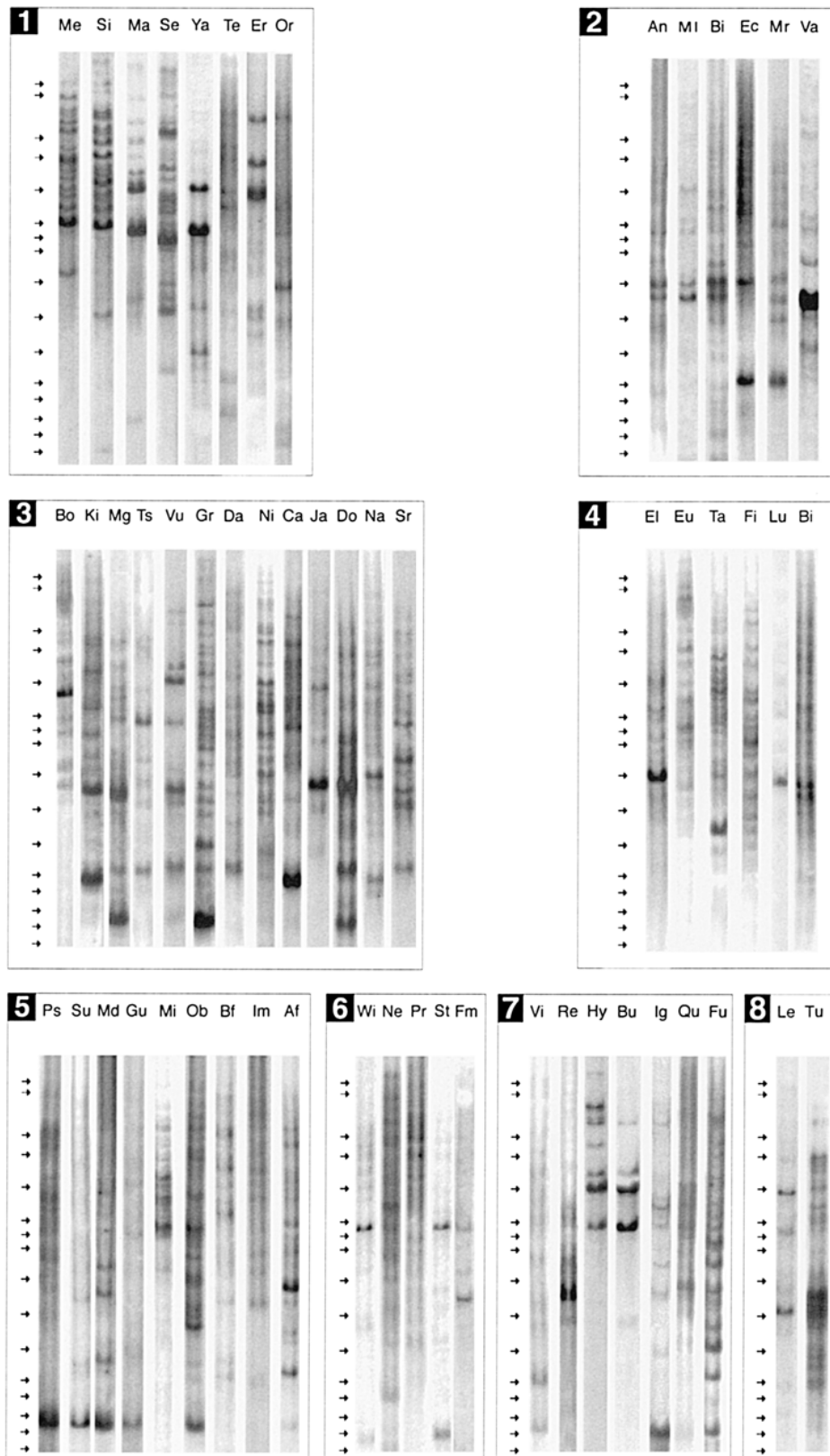The 412 element was found in almost all of the 55 species of Drosophila, with the exception of a few spe-

Fig. 1.—Southern blot hybridization of genomic DNA from species of the Drosophila genus. DNA was digested with *Hin*dIII and transferred to filters, and the filters were probed with the internal 1.4-kb *Eco*RI fragment of the 412 element from *D. melanogaster*. The species analyzed were: (1) *melanogaster* subgroup—*D. melanogaster* (Me), *D. simulans* (Si), *D. mauritiana* (Ma), *D. sechellia* (Se), *D. yakuba* (Ya), *D. teissieri* (Te), *D. erecta* (Er), *D. orena* (Or); (2) *ananassae* subgroup—*D. ananassae* (An), *D. malerkotliana* (Ml), *D. bipectinata* (Bi), *D. ercepeae* (Mr) *D. merina* (Er), *D. vallismaïa* (Va); (3) *montium* subgroup—*D. bocqueti* (Bo), *D. kikkawai* (Ki), *D. malagassya* (Mg), *D. tsacasi* (Ts), *D. vulcana* (Vu), *D. greeni* (Gr), *D. davidi* (Da), *D. nikananu* (Ni), *D. cauveri* (Ca), *D. jambulina* (Ja), *D. dossoui* (Do), *D. nagarholensis* (Na), *D. serrata*

cies, such as *D. lucipennis,* and a few lineages, such as the fima group. There were considerable differences in copy numbers, even between closely related species. Sequences homologous to the 412 element were also detected in species more distantly related to Drosophila, such as *Z. tuberculatus.* Consistent with previous analyses (Martin, Wiernasz, and Schedl 1983; Stacey et al. 1986; de Frutos, Peterson, and Kidwell 1992; Francino, Cabre, and Fontdevila 1993), these data suggest that the 412 retrotransposable element appeared early in the evolution of the Drosophilidae family and was subsequently lost in some lineages and species, or that it was only recently acquired by some species during diversification of the Drosophila genus. Although we cannot rule out the possibility that this element was acquired recently, it is generally thought that it had been lost from certain species, as this could account for its discontinuous distribution in Drosophilidae. Population bottlenecks, chance excision, and specific recombination events are often involved in lineages that lack copies of the TE analyzed. Thus, species with no euchromatic copies that have a signal in the centromeric and pericentromeric regions are thought to be at a stage just short of stochastic loss, as suggested by Hartl et al. (1997) for the mariner transposon. Many of the species analyzed in this study are presumably at such a stage for the 412 retrotransposable element. The species devoid of 412 element copies may have totally lost this element, although they may have a few ancient 412 copies sufficiently divergent to not be detected.

The distribution of the 412 element in the Drosophila genus contrasts with that of the copia retrotransposable element (Dowsett and Young 1982; Stacey et al. 1986; Csink and McDonald 1995; Francino, Cabre, and Fontdevila 1993; unpublished data). The 412 element is present in most species, at least in the chromocenter, whereas copia is absent from many species, being detected only in *D. yakuba, D. erecta, D. orena, D. kikkawai,* and *D. jambulina,* by labeling of the centromeric region only. This suggests that 412 is an older element than copia, and that the 412 element was present in ancestors of the Drosophila species, whereas copia appeared more recently. It is also possible that copia was present before 412 and was either lost in almost all of the species or has sufficiently diverged from the canonical sequence of *D. melanogaster* to not be detected. The copia and 412 elements thus have different evolutionary histories and may still behave independently among different species. The distribution of the element and the regulation of its copy number thus depend on the TE considered, although some of the overall genomic char-

acteristics of the species may also be involved in this regulation. This is illustrated by *D. simulans,* which has mainly three fixed sites for copia (Leibovitch et al. 1992; Vieira and Biémont 1996*b*), whereas 412 is invading the genome of this species (Vieira and Biémont 1996*a*).

All the species of the *melanogaster* subgroup, which comprises the eight species most closely related to *D. melanogaster,* contain 412 elements in the chromocenter. Only the four species of the *melanogaster* complex (*melanogaster, simulans, mauritiana, sechellia*) have copies on the chromosome arms, the number of which depends on the host species. The 412 element may have been present in the common ancestor of the *melanogaster* subgroup and invaded euchromatin in the *melanogaster* complex species, but was limited to only the centromeric heterochomatin in *D. erecta, D. orena, D. yakuba,* and *D. teissieri* species.

The differences in the restriction profiles of the *melanogaster* complex species show that the 412 retrotransposable elements had different histories or behaved differently in closely related species, as also reported for the mariner (Capy et al. 1991) and hobo transposons (Periquet et al. 1994). The large difference in the distribution of 412 in *D. simulans, D. sechellia,* and *D. mauritiana,* three species of the *D. simulans* clade, is particularly important given the debate about the phylogenetic relationships between these three species. Recent data suggest that *D. sechellia* and *D. mauritiana* diverged from *D. simulans* (Hey and Kliman 1993; Caccone et al. 1996; but see Coyne and Charlesworth 1997), consistent with the observation that the three species have homosequential polytene chromosomes and slight differences in the heterochromatin distribution of their mitotic chromosomes. The C banding pattern of the mitotic chromosomes of *D. sechellia* is more similar to that of *D. mauritiana* than to that of *D. simulans* or *D. melanogaster* (Lemeunier, Dutrillaux, and Ashburner 1978). This study, however, shows that 412 is distributed very differently in *D. sechellia* and *D. mauritiana. Drosophila sechellia* had a large number of heterochromatic 412 element copies but did not generate the fragment that would be expected if full-size copies were present, whereas *D. mauritiana* was only weakly labeled in the chromocenter but generated this fragment. These differences observed among the *melanogaster* complex species may be due to the geographical distribution and breeding sites specific to each species (reviewed in Lemeunier, David, and Tsacas 1986; Lachaise et al. 1988). Indeed, *D. melanogaster* and *D. simulans* are cosmopolitan generalist species with similarly wide ecological niches, whereas *D. mauritiana* and *D. sechellia* are en-

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(Sr); (4) other subgroups of the melanogaster group—*D. elegans* (El), *D. eugracilis* (Eu), *D. takahashii* (Ta), *D. ficusphila* (Fi), *D. lucipennis* (Lu), *D. biarmipes* (Ba); (5) obscura group—*D. pseudoobscura* (Ps), *D. subobscura* (Su), *D. madeirensis* (Md), *D. guanche* (Gu), *D. microlabis* (Mi), *D. obscura* (Ob), *D. bifasciata* (Bf), *D. imaii* (Im), *D. affinis* (Af); (6) other groups of the *Sophophora* subgenus—*D. willistoni* (Wi), *D. nebulosa* (Ne), *D. prosaltans* (Pr), *D. sturtevanti* (St), *D. fima* (Fm); (7) *Drosophila* subgenus—*D. virilis* (Vi), *D. repleta* (Re), *D. hydei* (Hy), *D. buzzatii* (Bu), *D. immigrans* (Ig), *D. quadrilineata* (Qu), *D. funebris* (Fu); (8) *Scaptodrosophila* subgenus—*D. lebanonensis* (Le) and Zaprionus genus: *Z. tuberculatus* (Tu). The species are ordered as in table 1. A DNA molecular weight ladder is shown, with fragments from 18 to 754 bp. The washing conditions were 2 × SSC followed by 0.1 × SSC for subgroups 1, 2, and 3 and only 2 × SSC for subgroups 4, 5, and 6.

Table 1
Distribution of Sequences Homologous to the Retrotransposable Element 412 in Species of the Drosophila Genus

| | | | | In Situ Hybridization | | | |
| | | | | Insertion Site Number on Chromosome Arms[a] | Presence in Chromocenter[b] | Southern Blots[c] | Literature Data[d] |
Subgenus	Species Group	Subgroup	Species				
Sophophora	melanogaster	melanogaster	melanogaster*	26–35	+	+++	+
			simulans*	1–73	+	+++	+[e,f]
			mauritiana	3	+	++	+[e,f]
			sechellia*	2–3	+	+++	
			yakuba	0	++	+	+[f]
			teissieri	0	w	+	w[f]
			erecta	0	++	+	
			orena	0	+	+	
		ananassae	ananassae	1–3	+	+	+[f]
			malerkotliana	0	++	+	
			bipectinata	0	+	+	+[f]
			ercepeae	0	+	+	
			merina	0	+	+	
			vallismaïa*	0	++	++	
		montium	bocqueti	0	+	+	
			kikkawai	1	+	++	
			malagassya	0	w	++	
			tsacasi	0	w	+	
			vulcana	0	++	+	
			greeni*	2–3	++	+++	
			davidi	0	++	++	
			nikananu	0	w	+++	
			cauveri	0	++	++	
			jambulina	0	++	+	
			dossoui*	1–3	++	+++	
			nagarholensis	0	+	++	
			serrata	0	++	++	
		elegans	elegans	0	+	++	
		eugracilis	eugracilis	0	+	+	
		takahashii	takahashii	0	+	++	
		ficusphila	ficusphila	0	+	++	
		suzukii	lucipennis	0	+	w	
			biarmipes	0	+	+	
	obscura	obscura	pseudoobscura	0–1	+	++	+[f,g]
			subobscura	1–4	+	+	+[f,g]
			madeirensis	3–8	+	++	+[f]
			guanche	0	+	w	+[g]
			microlabis	0	+	++	
			obscura	2–10	++	++	+[h]
			bifasciata	1–2	++	+	+[g]
			imaii	0	++	+	
		affinis	affinis	0	+	++	?[f,g,i]
	willistoni		willistoni	0	++	+	
			nebulosa	0	++	++	
			paulistorum	ND	ND	ND	+[f]
	saltans	saltans	prosaltans	0	++	+	
		cordata	neocordata	ND	ND	ND	+[f]
		sturtevanti	sturtevanti	0	++	ε	
	fima	fima	fima	ND	ND	+	
Drosophila.	virilis	virilis	virilis	0	+	+	+[f]
			montana	ND	ND	ND	+[f]
			littoralis	ND	ND	ND	+[f]
	repleta	melanopalpa	repleta	0	w	++	
		hydei	hydei	0	w	+	0[f]
		mulleri	buzzatti	0	0	+	
		mercatorum	mercatorum	ND	ND	ND	+[f,i]
	immigrans	immigrans	immigrans	0	+	++	
			quadrilineata	1	++	+	
	funebris	funebris	funebris	0	w	+++	
Scaptodrosophila. . . .			lebanonensis	0	0	ε	
Genus Zaprionus. . . .			tuberculatus	1–2	+	+++	
			vittiger	ND	ND	ND	+[f]

demic to Mauritius and the Seychelles, respectively. *Drosophila mauritiana* is a generalist species (David et al. 1989), whereas *D. sechellia* is a specialist, feeding on the fruit of *Morinda citrifolia* (R'Kha, Capy, and David 1991). Differences in effective population size may thus be responsible for the differences in 412 element copy number between these two species. Small effective population size or severe bottlenecks may account for the loss of element copies by genetic drift, as suggested for *D. sechellia* (Cariou et al. 1990; Capy et al. 1991; Capy, David, and Hartl 1992) and for *D. erecta,* which feeds on the fruits of *Pandanus* (Lachaise et al. 1988). This confirms that the distribution of the TE depends on the overall characteristics of the genome of the species. *Drosophila simulans* has a large population size and is still segregating variation (Hey and Kliman 1993), so why is 412, but not copia, invading this species? The idea that a higher effective species population size for *D. simulans* than for *D. melanogaster* accounts for there being fewer transposable elements overall in *D. simulans* is based on the notion that selection against TE insertions is similar in the two species (Aquadro, Lado, and Noon 1988; Aquadro 1992). From an analysis of 412 copy numbers in many samples from natural populations of *D. melanogaster* and *D. simulans,* Vieira and Biémont (1996*b*) concluded that this selection is stronger in *D. simulans* than in *D. melanogaster,* suggesting specific characteristics of each species in their way of dealing with their TEs.

For many species in which the 412 element was detected only in the chromocenter, full-size, potentially functional copies were not detected, suggesting that heterochromatic copies are old defective elements that were already present in the ancestors of *Drosophila* before radiation of the genus. Heterochromatic copies may thus be the dead-end hallmark of an ancient invasion, suggesting that heterochromatin is a trap of ancient, mostly defective copies, mostly in fixed positions (Bucheton et al. 1992; Dimitri 1997). It may be that elements inserted into heterochromatin are not as easily eliminated by recombination and natural selection as euchromatic insertions. It is not clear, however, that insertion into heterochromatic regions means that the TEs concerned are doomed to extinction (Hartl et al. 1997). Indeed, during evolution, heterochromatic TEs may have lost their transposition capacity and acquired new functions (von Stenberg et al. 1992; McDonald 1993; Dimitri 1997).

Transposable elements in fact appear more and more to be major structural components of *Drosophila* heterochromatin (Pimpinelli et al. 1995). However, the presence of complete, putatively active 412 element copies in the chromocenters of some species may demonstrate the potential of this element to invade genomes from heterochromatin, as suggested for the I retroposon in *D. melanogaster* (Bucheton et al. 1992). Transposable elements may thus have long periods of stasis interrupted by several waves of genomic invasion by copies from the centromeric regions. Heterochromatic copies could occasionally be rearranged to produce new functional elements. The conditions and mechanisms responsible for such rearrangement and activation are not known, but it is proposed that the number of TE copies in the heterochromatic portion of the genome may change much more extensively than would be expected if only recombinations were involved (Terrinoni et al. 1997). This, of course, does not rule out the possibility of horizontal transfer (Capy, Anxolabéhère, and Langin 1994).

Our data raise the question of the precise fate of heterochromatic copies. Do such copies participate in the regulation of total copy number? Are they potential genomic invaders, which would make them of real importance in evolutionary terms (McDonald 1990)? All of the species with large numbers of insertions into the chromocenter are thus of potential importance for increasing our understanding of TE dynamics and the possible effect of TEs on evolution. The behavior of TEs through evolution is far from being well known, but it should be investigated. The extent to which TEs are involved in species differentiation is, however, a matter of debate.

←

NOTE.—* indicates the species for which pictures of polytene chromosomes are presented in figure 2. ND = not determined. The species are classified following Throckmorton (1975), Wheeler (1981), Beverley and Wilson (1984), Lemeunier, David, and Tsacas (1986), Cariou (1987), deSalle and Grimaldi (1991), and Russo, Takezaki, and Nei (1995).

[a] The number of insertion sites over the chromosomes was estimated on three or more larvae per species. For *D. melanogaster* and *D. simulans,* the values are from Vieira and Biémont (1996*a,* personal communication).

[b] ++, +, and w denote strong-, normal-, and weak-labeling chromocenter, respectively.

[c] +, ++, and +++ indicate the number of bands, increasing from + to +++, and ε indicates that very few weak bands were detected on the Southern blots.

[d] References for *D. melanogaster* are numerous and are omitted from the table. +: presence; 0: absence; w: weak labeling.

[e] Data from Brookfield, Montgomery, and Langley (1984).

[f] Data from Martin, Wiernasz, and Schedl (1983).

[g] Data from de Frutos, Peterson, and Kidwell (1992).

[h] Data from Francino, Cabre, and Fontdevila (1993).

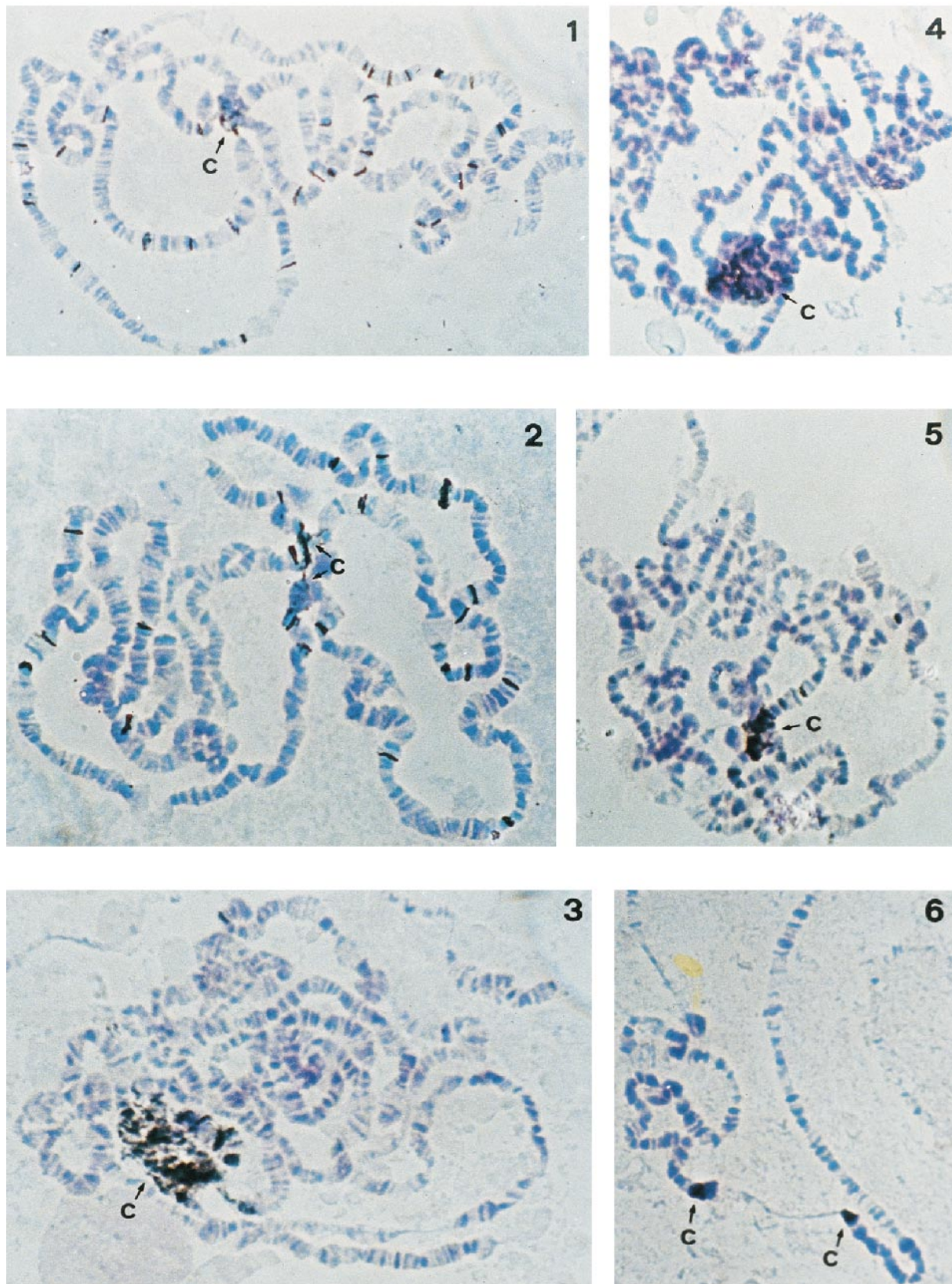[i] Data from Stacey *et al.* (1986).

FIG. 2.—Photographs of salivary gland polytene chromosomes of *D. melanogaster* (1), *D. simulans* (2), *D. greeni* (3), *D. dossoui* (4), *D. sechellia* (5), and *D. vallismaïa* (6), hybridized in situ with the 412 element DNA probe. The brown bands indicate 412 element insertion sites. C = chromocenter
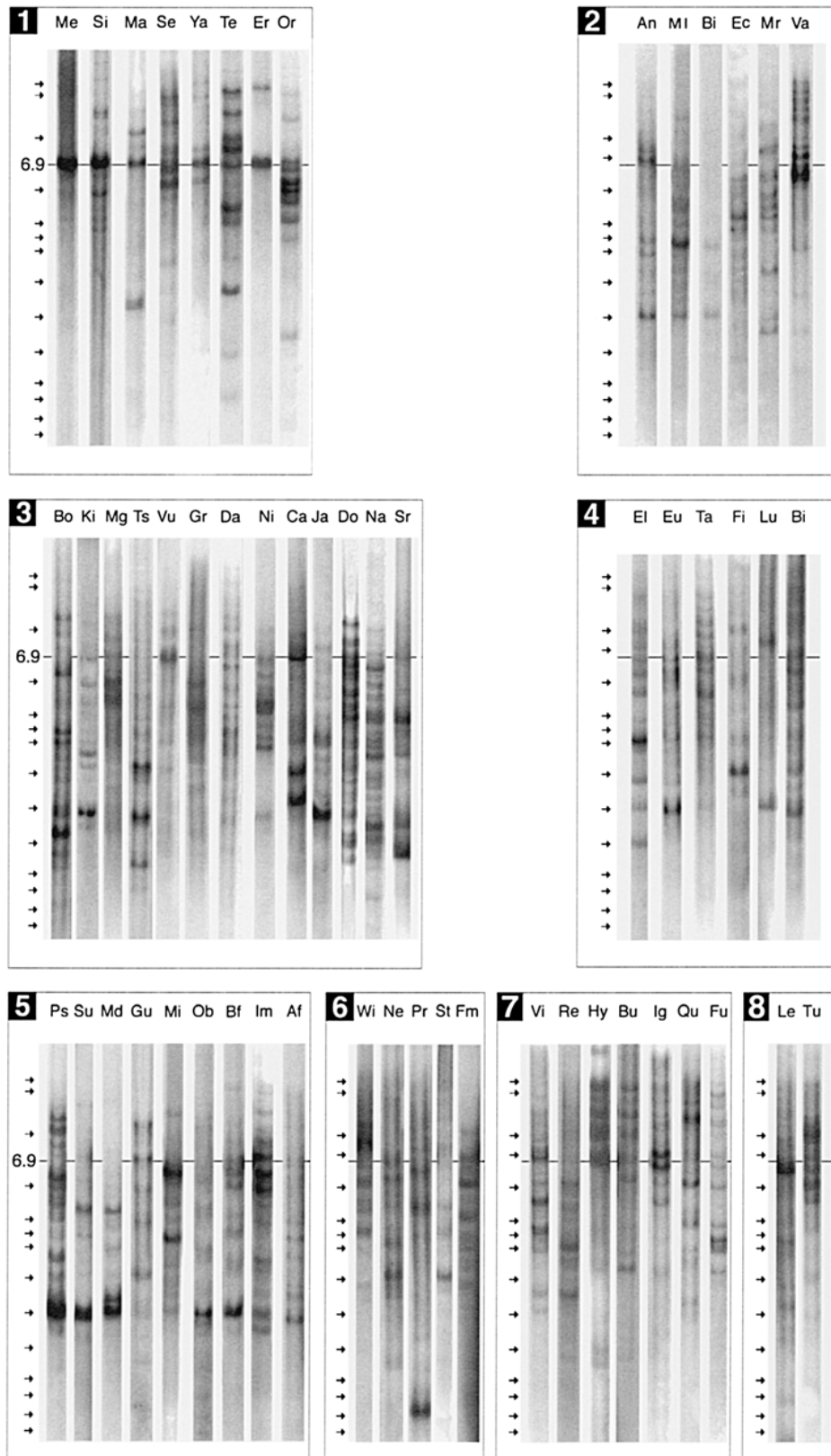
FIG. 3.—Southern blot hybridization of genomic DNA from species of the Drosophila genus. DNA was digested with *Bsr*BI, transferred to filters, and probed with the internal 6.9-kb *Bsr*BI fragment of the 412 element from *D. melanogaster*. A band at 6.9 kb indicates the presence of a putative full-size 412 element in the species concerned. The species and washing conditions are as for figure 1.

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