Distribution and Conservation of Mobile Elements in the Genus *Drosophila*[1]

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Essentially nothing is known of the origin, mode of transmission, and evolution of mobile elements within the genus *Drosophila.* To better understand the evolutionary history of these mobile elements, we examined the distribution and conservation of homologues to the P, I, gypsy, copia, and F elements in 34 *Drosophila* species from three subgenera. Probes specific for each element were prepared from *D. melanogaster* and hybridized to genomic DNA. Filters were washed under conditions of increasing stringency to estimate the similarity between *D. melanogaster* sequences and their homologues in other species. The I element homologues show the most limited distribution of all elements tested, being restricted to the *melanogaster* species group. The P elements are found in many members of the subgenus *Sophophora* but, with the notable exception of *D. nasuta,* are not found in the other two subgenera. Copia-, gypsy-, and F-element homologues are widespread in the genus, but their similarity to the *D. melanogaster* probe differs markedly between species. The distribution of copia and P elements and the conservation of the gypsy and P elements is inconsistent with a model that postulates a single ancient origin for each type of element followed by mating-dependent transmission. The data can be explained by horizontal transmission of mobile elements between reproductively isolated species.

Introduction

Mobile elements are widespread throughout the genus *Drosophila* (Martin et al. 1983). Studies on the distribution of mobile elements suggest that a number of different repetitive dispersed DNAs have appeared or disappeared during the radiation of the genus *Drosophila* (Dowsett and Young 1982; Dowsett 1983; Martin et al. 1983). The evolutionary instability of mobile elements suggests that these sequences do not play an essential role in the *Drosophila* genome. However, mobile elements may play an important role in speciation and evolution in the Drosophilidae and hence in other organisms. All transposable elements used in this study can cause mutations when they insert into or near a normal gene (Bingham and Judd 1981; Modolell et al. 1983; Bucheton et al. 1984; Levis et al. 1984). Furthermore, given the appropriate mating, P and I elements can produce a syndrome known as hybrid dysgenesis, which results in high frequencies of sterility and genetic damage (reviewed in Bregliano and Kidwell 1983; Engels 1983). Mobile elements persist in wild populations despite the fact that they appear to be detrimental to their hosts.

1. Key words: Mobile element, P element, I element, F element, gypsy, copia, *Drosophila* species.

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We know very little about the origin of mobile elements. Mobile elements might arise de novo within a species by modification of the host genome and be amplified (Orgel and Crick 1980; Martin et al. 1983). Alternatively, mobile elements could arise outside a species and infect a species that formerly lacked the element. Some transposable elements resemble the genomes of retroviruses in their structure and hence may be derived from an infectious virus. Copia and gypsy elements resemble retroviruses (Modolell et al. 1983; Flavell 1984; Baltimore 1985; Emori et al. 1985; Marlor et al. 1986), yet no available data suggest horizontal transmission of copia or gypsy elements. However, since transposition of copia-like elements proceeds through an RNA intermediate and reverse transcription (Flavell 1984) and because transcripts of copia are found in virus-like particles, infectious transmission of copia-like elements remains a real possibility.

The P transposable element of *Drosophila* may provide a glimpse of the initial invasion and subsequent dispersal of mobile elements in a population and perhaps a genus. Kidwell (1979, 1983) has proposed that the P elements have recently invaded wild populations of *D. melanogaster.* The recent-invasion hypothesis generally assumes that initial entry of the mobile element was by horizontal transmission, as a result of some infectious process. The recent-invasion hypothesis is supported for P elements because the sibling species of *D. melanogaster* lack P elements altogether (Brookfield et al. 1984) and because populations of *D. melanogaster* maintained in the laboratory for more than 50 years lack the element (Bingham et al. 1982).

An important question yet to be satisfactorily answered is how and when these hypothetical invasions originated. This problem cannot be addressed without knowledge of the distribution and conservation of different mobile elements within the genus *Drosophila.* The Drosophilidae have been subjected to extensive analyses to determine both their geographic distribution and phylogenetic relationships (Throckmorton 1975). Therefore, they provide an excellent system in which to examine the evolutionary history of transposable elements. We have undertaken an extensive survey of the distribution of the P (Bingham et al. 1982; Rubin et al. 1982), I (Bucheton et al. 1984), and copia elements (Finnegan et al. 1978), the copia-like element gypsy (Modolell et al. 1983), and a novel element, the F element (Di Nocera et al. 1983), in the genus *Drosophila* to determine when these mobile elements entered the genus. Our results show that copia, gypsy, F, and P elements are widespread within the genus and that the I element has a much more restricted distribution. These results are discussed with respect to both time of entry into the genus and mode of transmission of these mobile elements within the genome.

Material and Methods

Stocks

All species stocks were obtained from the National Drosophila Species Resource Center, Bowling Green State University, Bowling Green, Ohio. The species are listed in table 1. In each case the strain used was the first listed of that species in the 1984 Drosophila Species Stock List prepared by the Bowling Green Stock Center.

DNA Isolation

DNA was prepared from adult flies using either slight modifications of a published procedure (Kidd et al. 1983) or as follows: Approximately 100 adult flies were anesthetized and examined under a dissecting microscope to confirm their identity. Flies

Table 1

Classification of the *melanogaster* Species,[a] Their Geographic Origins, and the Distribution of Transposable-Element Homologues

Subgenus	Species Group	Subgroup	Species	Geographic Origin	Element			
					P	I	Copia	Gypsy
Sophophora . . .	*melanogaster*	*ananassae*	*ananassae*	Mexico	0	H	M	M
		suzukii	*lucipennis*	Taiwan	L	N/A	H	L
		montium	*kikkawai*	Colombia	L	H	H	M
		elegans	*elegans*	Philippines	0	H	M	M
		eugracilis	*eugracilis*	New Guinea	0	H	0	M
		takahashii	*takahashii*	Nepal	0	0	H	L
		ficusphila	*ficusphila*	Taiwan	L	H	H	M
	obscura	*affinis*	*affinis*	Nebraska	M	0	H	H
		obscura	*pseudoobscura*	Arizona	M	0	M	H
	willistoni	*willistoni*	*willistoni*	Nicaragua	H	0	M	L
			equinoxialis	Honduras	H	0	N/A	L
			tropicalis	El Salvador	H	0	M	L
			paulistorum	Mesitas	H	0	M	M
			paulistorum-like	Mexico	H	0	M	M
			nebulosa	Colombia	H	0	M	L
			succinea	Colombia	H	0	M	L
			capricorni	Colombia	H	0	M	L
			fumipennis	Colombia	H	N/A	M	M
	saltans	*saltans*	*saltans*	Costa Rica	H	0	M	M
			australosaltans	Brazil	H	0	M	L
			prosaltans	Costa Rica	H	N/A	M	L
		cordata	*neocordata*	Brazil	0	0	M	0
		sturtevantii	*sturtevantii*	Costa Rica	H	0	M	L
		emarginata	*emarginata*	Costa Rica	0	0	M	L
Drosophila	*virilis*	*virilis*	*virilis*	California	0	0	0	H
	melanica	*melanica*	*melanica*	Florida	0	0	0	H
	robusta	*robusta*	*robusta*	Alabama	0	0	0	H
	repleta	*mulleri*	*mulleri*	Texas	0	0	M	L
		melanopalpa	*repleta*	Barbados	0	0	0	H
		mercatorum	*mercatorum*	Brazil	0	0	M	M
	mesophragmatica	*pavani*	*pavani*	Argentina	0	0	M	H
	pinicola	*pinicola*	*pinicola*	California	0	0	M	H
	immigrans	*nasuta*	*nasuta*	India	M	0	M	0
Dorsilopha	*busckii*		*busckii*	Costa Rica	0	0	0	H

NOTE.—All stocks were obtained from the National Drosophila Species Resource Center, Bowling Green State University, Bowling Green, Ohio. Origin refers to the original collection site of the strains used, as quoted in the center's catalogue. The columns at the right show the highest stringency wash at which hybridization to *D. melanogaster* probes could be detected. Distribution of homologues is noted as follows: 0 = No hybridization detected; L = hybridization readily detected at low-stringency wash; M = hybridization readily detected at low- and medium-stringency washes; H = hybridization readily detected at low-, medium-, and high-stringency washes; N/A = data not available. Wash conditions for the various stringencies are described in Material and Methods. The distribution within the Sophophoran subgenus of P homologues has been reported previously (Lansman et al. 1985).

[a] After Patterson and Stone (1952).

were homogenized in 100 μl of 50 mM Tris (hydroxymethyl) methylamine (Tris), pH 8.0, 100 mM ethylenediaminetetraacetic acid (EDTA) containing 200 μg/ml of proteinase K. The volume was increased to 500 μl by the addition of 50 mM Tris (pH 8.0), 100 mM EDTA, and the preparations were left on ice for 10 min. Sodium dodecyl sulfate (SDS) was added to a final concentration of 1%, and the preparations were incubated at 65 C for 30 min. The preparations were cooled to 37 C, and proteinase K was added for the second time to a concentration of 200 μg/ml, followed by incubation for 3 h at 37 C. An ethidium bromide/cesium chloride density gradient was made by adding the contents of the preparation to 7.0 g of cesium chloride and bringing

this to a final volume of 7.0 ml with TE (TE = 10 mM Tris, pH 8.0, 1 mM EDTA), followed by addition of ethidium bromide to 1 mg/ml. The preparations were kept on ice for 10 min to precipitate RNA and then cleared by centrifugation for 10 min at 10,000 rpm in a Sorvall SS34 rotor. The supernatant was transferred to ultracentrifuge tubes and centrifuged for 36 h at 45,000 rpm. The chromosomal DNA was collected by side puncture, and the ethidium bromide was extracted into isopropanol. Samples were dialyzed overnight against several changes of TE, precipitated with ethanol, and resuspended in TE.

Genomic Hybridization

Approximately 1 μg of genomic DNA was digested with *Pvu*II, separated by electrophoresis on 0.6% agarose, and transferred to nitrocellulose (Southern 1975). Whole plasmids or gel-purified fragments from cloned *D. melanogaster* transposable elements were labeled with ^{32}P-dCTP by nick translation to a specific activity of 0.5–1 × 10^8 dpm/μg and used as probes (Rigby et al. 1977). The probes used were as follows: (1) P element—an 840-bp internal *Hin*dIII fragment of p25.1 (O'Hare and Rubin 1983), (2) I element—a 1-kb 5′ internal *Hin*dIII fragment of pI407 (Bucheton et al. 1984), (3) copia element—the 3.4-kb *Xba*I fragment from Dm5002, containing the 3′ portion of the copia element and a very short segment of flanking DNA (Levis et al. 1980), (4) gypsy element—the entire plasmid gypsy *Xho*I, which consists of most of the gypsy element but lacks the extreme 5′ and 3′ ends of the long terminal repeats (W. Bender, personal communication), and (5) F element—the 1.5-kb *Eco*RI-*Hin*dIII 3′ internal fragment of plasmid pSL25 (Di Nocera et al. 1983).

Hybridization was carried out in 6 × SSPE (1 × SSPE = 0.18 M NaCl, 10 mM NaH_2PO_4, pH 7.4, and 1 mM EDTA), 50% formamide, 5 × Denhardt's solution (5 × Denhardt's solution = 0.1% bovine serum albumen, 0.1% polyvinylpyrrolidone, and 0.1% ficoll), 10 mM EDTA, 0.5% SDS for 12–16 h at 37 C. The filters were washed three times for 30 min each under the following conditions: low stringency (2 × SSPE, 0.1% SDS, room temperature [25 C]); medium stringency (0.2 × SSPE, 0.1% SDS, 53 C); high stringency (0.2 × SSPE, 0.1% SDS, 63 C). Individual filters were washed first at low stringency, exposed to film, and then rewashed at higher stringency and reexposed to film. Control experiments in which filters were washed at medium or high stringency immediately following hybridization provided comparable results (data not shown).

It is possible to estimate the sequence conservation detectable at each wash stringency, since the T_m depends on the temperature, ionic strength, and number of mismatched base pairs. The T_m of a 50% G/C DNA in 0.2 × SSPE is 80 C (Marmur and Doty 1962), and the T_m decreases 1 degree C for every 1% increase in the number of mismatched base pairs (Bonner et al. 1973). Therefore, under our high- and medium-stringency wash conditions, the sequences must possess ≥83% and ≥73% sequence identity, respectively. The T_m of a DNA is increased by 18.5 times the log of the ratio of the ionic strengths of each buffer (Dove and Davidson 1962), and so, under our low-stringency wash, sequences possess ≥30% sequence identity. We assume that fragments that hybridize to the probe are anastrally related to the probe, even at low stringency. We will henceforth call them homologues.

Results

It has been estimated that the Drosophilidae arose ~50–60 Myr ago (Throckmorton 1975; Beverly and Wilson 1984). The evolution of the genus *Drosophila* is

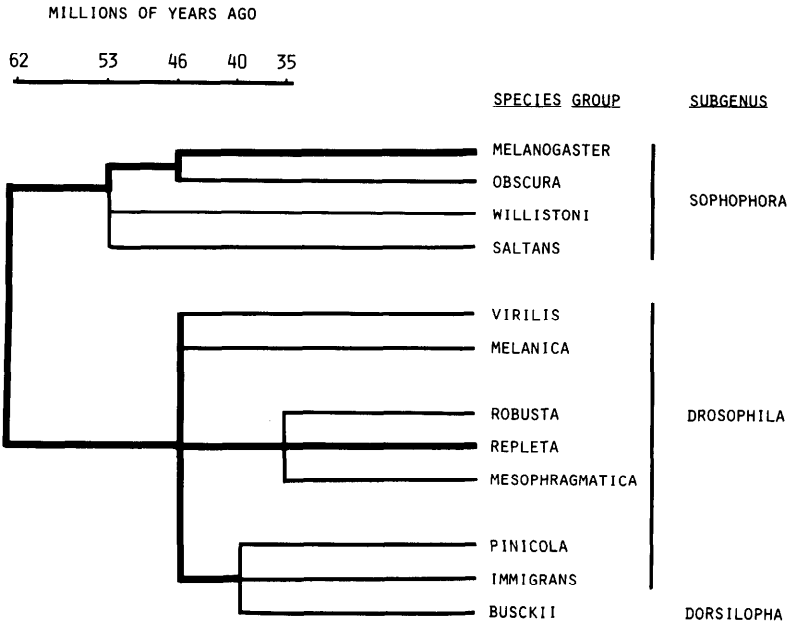

FIG. 1.—Phylogeny and estimated divergence times of *Drosophila* species groups. The figure shows the phylogenetic relationships of the species groups used in this study, together with their subgenus. The estimated divergence times of the species groups are shown above the figure, which has been modified from Beverly and Wilson (1984). The thick black lines represent species groups whose divergence times have been calculated by immunological methods (Beverly and Wilson 1984). The divergence times of the species groups drawn with thin lines are less certain and have been estimated from geographical and morphological data. We have added the *busckii, melanica,* and *mesophragmatica* species groups to the figure presented in Beverly and Wilson (1984), according to the phylogeny of Throckmorton (1975).

complex. *Drosophila* subgenera are thought to have arisen in the Old World tropics and then spread to the New World tropics. Meanwhile, some species that arose in the Old World tropics now live in temperate forests. This pattern can be found in four of the five subgenera of *Drosophila.* The *Scaptodrosophila* arose first and gave rise to the Sophophoran radiation, which in turn gave rise to the *Drosophila* and Hirtodrosophilan radiations. These four subgenera are further divided into species groups that live in different habitats. The fifth subgenus, *Dorsilopha,* is represented by only one species, *D. busckii.* Figure 1 shows the phylogenetic relationships of the subgenera and species groups of the species used in this study, together with an estimate of the divergence time from each other of the subgenera, as taken from Beverly and Wilson (1984) and Throckmorton (1975). Thirty-four species from three subgenera were chosen to represent as wide a range as possible of habitat and phylogenetic relatedness. Table 1 shows the geographic origin of the strain of each species used in this study, together with a classification following Patterson and Stone (1952).

DNA samples were prepared from the species listed in table 1, digested with *Pvu*II, separated electrophoretically, and transferred to nitrocellulose. The nitrocellulose filters were then probed with nick-translated *D. melanogaster* transposable-element probes. The filters were washed subsequently at low, medium, and high stringencies to obtain an estimate of the conservation between the *D. melanogaster* elements and any homologues that might be present. The results are summarized in table 1, which

shows whether the homologues of the P, I, gypsy, and copia element are present or absent. If a transposable-element homologue is present, the table indicates the highest conditions of stringency under which the probe was retained on the filter. Representative autoradiograms are shown in figures 2 and 3.

Homologues of the I element were detected only in members of the *melanogaster* species group. Therefore, of the five elements tested, the I-element homologue appears to have the most limited distribution. The I-element homologues are well conserved, since the *D. melanogaster* probe is retained under high-stringency wash conditions, but only *D. eugracilis* shows an intensity of hybridization similar to the *D. melanogaster* control. The copy number of these homologues is reduced relative to that of *D. melanogaster,* as judged by the number of bands present on Southern (1975) blots. This appears to be a general property of transposable-element homologues in other *Drosophila* species (Dowsett and Young 1982; and see below). The most highly conserved and highest-copy-number I-element homologue was found in *D. eugracilis,* originally collected in New Guinea. Preliminary examination of a *Hin*dIII-*Pst*I genomic digest showed that this sample contains a 2.3-kb fragment that comigrates with the 2.3-kb internal fragment from the *D. melanogaster* I element. The presence of this fragment in genomic digests has been used to distinguish inducer (I) and reactive (R) strains in *D. melanogaster* (Bucheton et al. 1984) and therefore suggests that the I-element homologue in *D. eugracilis* may retain its function. It is also interesting to note the presence of I-element homologues in *D. ananassae.* Hinton (1984, and references therein) has described a number of systems in this species that resemble hybrid dysgenesis. Only *D. takahashii* of the *melanogaster* species group did not possess I-element homologues.

The P-element homologues are more widespread than the I-element homologues. We have shown previously that P-element homologues are found in all species groups within the subgenus *Sophophora* but that not every species has P-element homologues (Lansman et al. 1985). In this study, P-element homologues were not detected in any species in the subgenera *Dorsilopha* or *Drosophila,* with the exception of *D. nasuta* (fig. 3A). To our knowledge, this is the first demonstration of the presence of a P-element homologue in a non-Sophophoran species. Surprisingly, the most highly conserved P-element homologues relative to *D. melanogaster* were found in members of the South American *willistoni* and *saltans* species groups, even though these species are much more distantly related to *D. melanogaster* than are other members of the Sophophoran subgenus.

It has been reported that a strain of *D. pseudoobscura* from Bogata exhibits hybrid sterility when outcrossed to other *D. pseudoobscura* strains (Prakash 1972; Dobzhansky 1974). Figure 3A shows that both the Bogata and an Arizona strain of *D. pseudoobscura* have P-element homologues. Since both strains contain P-element homologues, it is unlikely that the hybrid dysgenesis observed in crosses between these strains results from presence or absence of P-element homologues, unless one strain possesses only nonfunctional P-element homologues. On Southern (1975) blots, both the number and position of restriction fragments detected differ between these two strains. This could either reflect recent movement of this P-element homologue or, less interestingly, result from restriction-site polymorphisms between the two strains. Brookfield et al. (1984) did not find P-element homologues in *Drosophila,* perhaps because of strain differences in this species or because of differences in hybridization and wash conditions.

The copia and gypsy elements are widely distributed among the *Drosophila* species. Copia-element homologues are present in 23 of 24 Sophophoran species, 5 of 9 Dro-

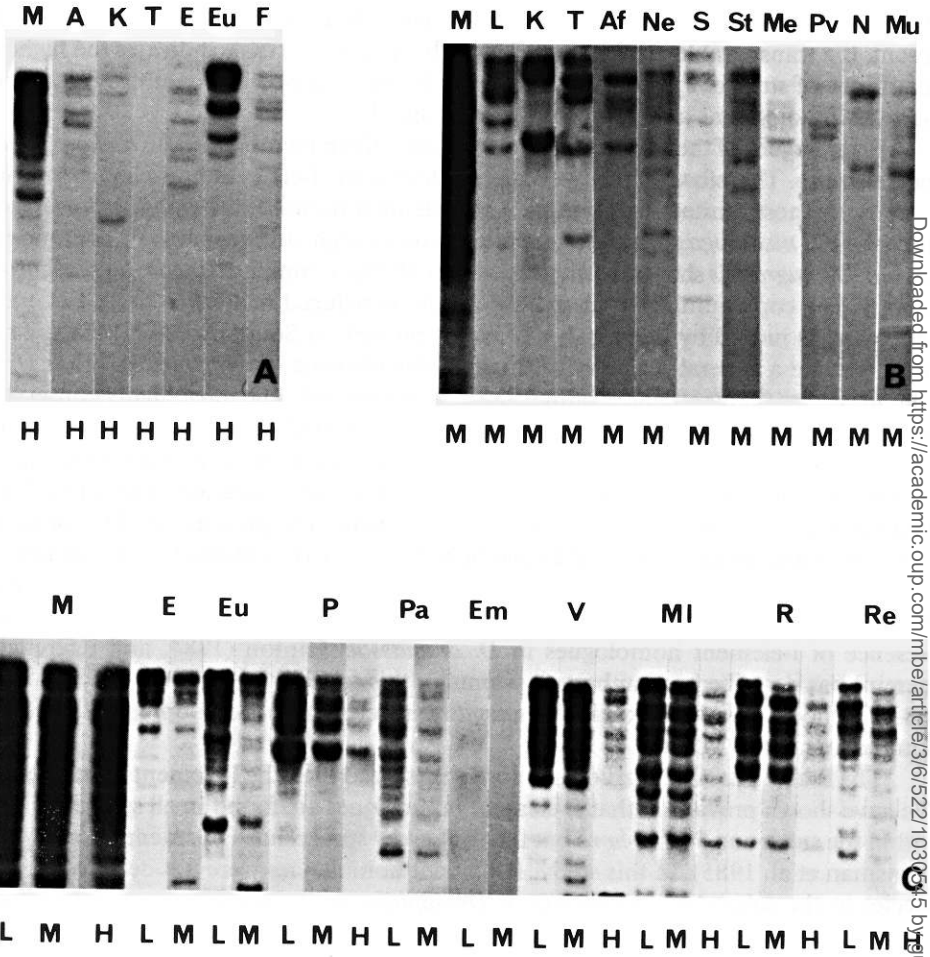

FIG. 2.—Hybridization of transposable elements to genomic DNA of *Drosophila* species. The I, copia, and gypsy mobile elements of *D. melanogaster* were hybridized to genomic DNA of *Drosophila* species. After restriction-enzyme digestion and transfer to nitrocellulose, the filters were probed, washed at low stringency, exposed to film, and then rewashed at higher stringency and reexposed to film. The wash stringency is indicated below each sample lane, using the following abbreviations: L, low stringency (2 × SSPE at 25 C); M, medium stringency (0.2 × SSPE at 53 C); H, high stringency (0.2 × SSC at 63 C). The following abbreviations were used for the species illustrated in the figure: A, *D. ananassae;* Af, *D. affinis;* E, *D. elegans;* Em, *D. emarginata;* Eu, *D. eugracilis;* F, *D. ficusphila;* K, *D. kikkawai;* L, *D. lucipennis;* M, *D. melanogaster;* Me, *D. mercatorum;* Ml, *D. melanica;* Mu, *D. mulleri;* N, *D. nasuta;* Ne, *D. nebulosa;* P, *D. pseudoobscura;* Pa, *D. paulistorum;* Pv, *D. pavani;* R, *D. robusta;* Re, *D. repleta;* S, *D. saltans;* St, *D. sturtevanti;* T, *D. takahashii;* and V, *D. virilis.* 2A, Hybridization of the I mobile element to genomic DNA from *melanogaster* subgroups species, subgenus *Sophophora.* Only *D. takahashii* lacks homologues to the I element. 2B, Hybridization of the copia mobile element to genomic DNA of *Drosophila* species. In general, the number of hybridizing fragments and the intensity of the hybridization signal decreases as phylogenetic distance increases. 2C, Hybridization of the gypsy mobile element to genomic DNA of *Drosophila* species. Sequences homologous to the probe are detectable after high-stringency washes in the *Drosophila* subgenus species *D. virilis, D. melanica, D. robusta,* and *D. repleta.*

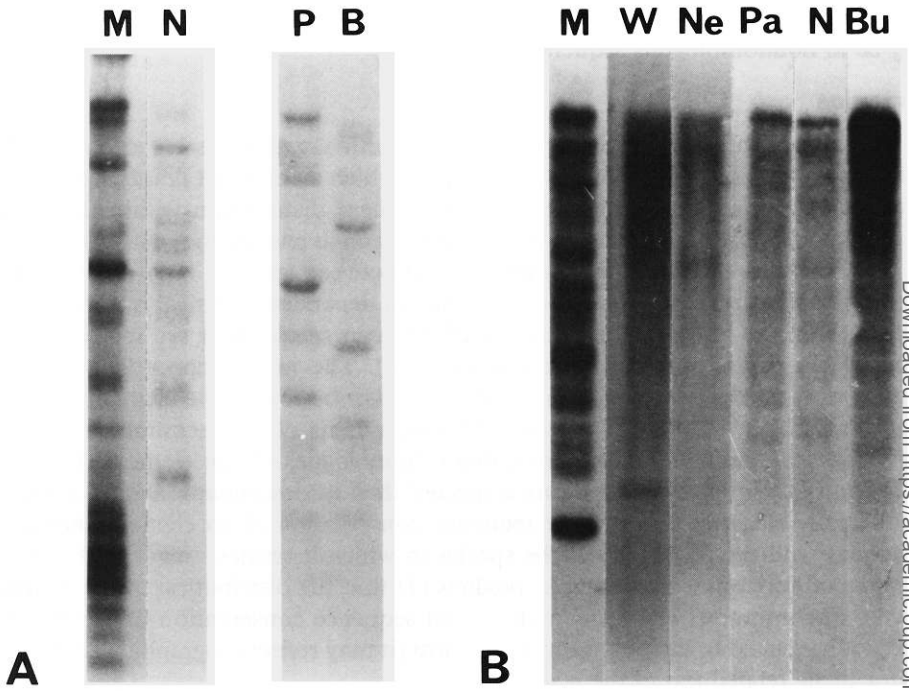

FIG. 3.—Hybridization of P and F elements to genomic DNA of *Drosophila* species. 3A, Hybridization of the *D. melanogaster* P element to genomic DNA of *D. melanogaster* (M) and *D. nasuta* (N) and the *D. pseudoobscura* strains from Tucson (P) and Bogata (B). All filters shown here were washed at medium stringency (2 × SSPE at 53 C). This is the first demonstration of sequences homologous to the P element in a subgenus *Drosophila* species. 3B, Hybridization of the *D. melanogaster* F element to genomic DNA of *Drosophila* species: M, *D. melanogaster;* W, *D. willistoni;* Ne, *D. nebulosa;* Pa, *D. paulistorum;* N, *D. nasuta;* and Bu, *D. busckii.* The filters shown here were washed at medium stringency. The hybridization of the F element was generally detectable as a smear, although bands can be clearly detected in *D. busckii.*

sophilan species, and absent in *D. busckii.* Gypsy-element homologues are present in 23 of 24 Sophophoran species, 7 of 9 Drosophilan species, and are also present in *D. busckii.* Copia-element homologues showed a decrease in both the number of bands observed and the degree of conservation as the phylogenetic distance from *D. melanogaster* increased (see fig. 2B). In contrast to the results observed with copia, the correlation between phylogenetic distance and conservation of gypsy-element homologues relative to *D. melanogaster* was very poor. As shown in figure 2C the most-conserved gypsy-element homologues relative to *D. melanogaster* were observed in species from the subgenus *Drosophila.* The gypsy-element homologues detected in Sophophoran species were generally less conserved relative to the gypsy element of *D. melanogaster.*

Sequences homologous to the F-element probe were detected at high stringency in all species examined. In *D. melanogaster,* separate bands are clearly seen, but in all other species sequences hybridizing to the F-element probe are visible only as a smear (fig. 2B). This element is known to be highly heterogeneous within *D. melanogaster* (Di Nocera et al. 1983; Levis et al. 1984). We suspect that each sample examined here contained a highly heterogeneous group of elements at differing chromosomal positions. Such variation between individual flies in the sample could produce

smears like those observed. However, we cannot rule out that the observed hybridization may be to nonhomologous sequences.

Discussion

To understand the evolutionary history of a particular transposable element within a genus, it is necessary to determine the time at which an element first became established in the genus and the mode of its subsequent distribution within the genus. However, before estimating when a transposable element entered the genus *Drosophila,* we must show how that element is transmitted between species. Transmission of an element between species could be strictly mating dependent (vertical or orthologous transmission), or transmission could occur between species that are reproductively isolated (horizontal or xenologous transmission). The mating-dependent mode of transmission makes two predictions. First, the distribution of the element within a genus should be virtually continuous. All descendants of an ancestral species that contained that element should also contain homologues of the element. If during evolution the element were lost from a species, then its descendants should not have acquired the element. Second, the sequence conservation of an element should be congruent with the phylogeny of the species in which it resides. In contrast, the occurrence of horizontal transmission predicts (1) that the distribution of the element may be discontinuous or patchy and (2) that sequence conservation of the element need not be related to the phylogeny of the host (it may reflect geographic distribution and/or niche relatedness of the hosts).

The predictions about continuity of distribution and congruence of sequence conservation with phylogeny that are derived from each mode-of-transmission hypothesis depend on the assumption that sequence divergence or loss of homologous elements in different species occurs at the same rate. Little is known about divergence rates of mobile elements in the genus *Drosophila* or how divergence rates depend on mobility of the transposable element. In addition, vertical transmission and horizontal transmission are not mutually exclusive. The existence of horizontal transmission, even at a low level, would allow an element to enter a genus several times. These events, which could be widely separated in time, would provide multiple foci for the subsequent vertical transmission of the element. Ignorance of divergence rates for a given element in different species, together with the possibility that horizontal transmission could have occurred several times during evolutionary history, make it difficult to distinguish between strict vertical transmission and vertical transmission accompanied by occasional horizontal transmission. To accept horizontal transmission as a possibility, it must be shown that the conservation of the element between closely and distantly related species is so variable that the observed differences in conservation cannot be explained simply on the basis of species-specific differences in mobile-element sequence-divergence rates. Similarily, horizontal transmission might be considered plausible only if the distribution of an element within a species group or subgenus is so patchy that the pattern is not easily explained by species-specific differences in the rates at which the element is eliminated from a species.

To examine the distribution and conservation of the transposable elements, Southern (1975) blots of genomic DNA were hybridized with radiolabeled, cloned, transposable elements. Both intact elements and internal fragments were used as probes in separate experiments. The results obtained in each experiment were identical. This eliminates the possibility that the observed hybridization reflects binding of conserved DNA adjacent to the elements. The filters were initially washed under low-stringency conditions to maximize the likelihood of detecting homologues. The conditions used

should allow us to detect elements whose sequences are only 30% conserved relative to the *D. melanogaster* probe. At this stringency, it is possible that the signals detected do not always represent hybridization of the probe to homologous sequences. However, many species show no signal at low stringency, suggesting that nonhomologous hybridization is not frequent. Furthermore, any element detected after washes at higher stringency was previously observed after the low-stringency hybridization, indicating that the observed hybridization at low stringency is specific. Nonetheless, this low stringency was chosen purposefully, since, even if some nonhomologous hybridization does occur, this should only bias the results in favor of the vertical transmission hypothesis, since nonspecific hybridization would increase the apparent number of species possessing a mobile element, leading to an apparent continuity in the distribution of the element.

The I element has the most restricted distribution of any element tested; it is highly conserved within the *melanogaster* species group, and it is not found in any other species. These observations are consistent with the idea that the I element arose after the separation of the Sophophoran species groups but before the radiation of species within the *melanogaster* species group. There are two ways in which an element might arise in a genus: either the element was assembled de novo from preexisting sequences, or it invaded the founder species. We believe that invasion of the *melanogaster* species group (horizontal transmission) by an outside source of the I element is more likely than de novo assembly of the I element. Once established in the species group by horizontal transmission, the I element could have spread afterward by vertical transmission. In this case, *D. takahashii* must have either lost the element or never possessed it. We believe that horizontal entry of the I element into the *melanogaster* species group is more probable than the alternative—that is, ancient origination of the I element followed by loss in all *Drosophila* species except the *melanogaster* species group—although the latter possibility cannot be ruled out.

We and others have shown that the P element has a very patchy distribution within the subgenus *Sophophora* (Brookfield et al. 1984; Daniels et al. 1984; Lansman et al. 1985). We have extended these observations to show that only *D. nasuta* in the subgenus *Drosophila* has sequences homologous to the P element. The large number of discontinuities in its distribution is consistent with horizontal transmission of the P element within the genus. This suggestion is strongly supported by the observation that the sequences homologous to the *D. melanogaster* P-element probe are much less divergent from the phylogenetically distantly related *willistoni* and *saltans* species groups and *D. nasuta* than they are from the phylogenetically closely related *melanogaster* species group. We have cloned P-element homologues from *D. nebulosa* and *D. saltans* whose DNA sequences differ by <4% from the P element of *D. melanogaster* (R. A. Lansman, H. W. Brock, and T. A. Grigliatti, unpublished data). These findings support the conclusions about homology that were derived from the blot-hybridization data presented above.

The patchy distribution and lack of correlation between the divergence of P elements and the phylogenetic distance of the species is not consistent with strict vertical transmission of the P element unless the rate at which the homologues diverged (or were eliminated) is much lower in the *willistoni, saltans,* and *nasuta* species than it is in other *Drosophila* species. If it is assumed that the P element entered the subgenus *Sophophora* before the radiation of the species groups, then the divergence rate of P-element homologues within the *saltans* and *willistoni* groups is the product of the difference in sequence (4%) divided by the divergence time in millions of years (53 Myr) and is equal to 0.075% nucleotide substitutions/Myr. Since sequences homologous

to the P element within the *melanogaster* species group are detectable only at low stringency, their divergence rate is bounded by the maximum and minimum conservation that would be detected under our low-stringency conditions (72%–30%). Therefore, we calculate that the divergence rate of P elements in the *melanogaster* species is between 0.51% and 1.32% nucleotide change/Myr. The divergence rate of noncoding DNA in *Drosophila* has been estimated at 2.5%/Myr (Langley et al. 1982). Therefore, if P elements were spread by strict vertical transmission, the divergence rates in some species must be ≥7–19 times higher than it is in the *willistoni, saltans,* or *nasuta* species—and close to the divergence rate of unconserved DNA. We favor the hypothesis that horizontal transmission, rather than vertical transmission accompanied by large differences in divergence rate, most easily accounts for the data.

The majority of the examined species that contain P elements have geographic ranges that either include or overlap with South America. This geographic overlap is consistent with horizontal transmission and suggests that the source for P elements may originate in South America. However, this hypothesis does not account for the presence of P elements in *D. nasuta,* an old-world species.

The data for the F, copia, and gypsy elements are more equivocal. These elements are more widely distributed than either P or I elements, which is consistent with ancient origin followed by vertical transmission. Under this hypothesis, species that lack copia or gypsy elements either never possessed or have lost the element. The latter possibility is the stochastic-loss hypothesis (Engels 1981) and cannot be disproved. However, Kaplan et al. (1985) have proposed, on theoretical grounds, that all mobile elements should be eliminated slowly from the genome, and it is more probable that mobile elements would persist in the genome in the same way that pseudogenes of structural genes persist. The distribution of F, copia, and gypsy elements could also be explained by horizontal transmission agents with different infectivities.

The conservation of sequences homologous to the F and copia elements is consistent with the *Drosophila* phylogenies proposed by Throckmorton (1975) and Beverly and Wilson (1984) and supporting vertical transmission of these elements. On the other hand, conservation of sequences homologous to the gypsy element are inconsistent with *Drosophila* phylogeny, since sequences found in members of the phylogenetically more remote *Drosophila* subgenus are much less divergent from the *D. melanogaster* probe than are sequences found within the *melanogaster* species group. This result could be explained by vertical transmission accompanied by different divergence rates in different species, or it could be explained by horizontal transmission. The latter explanation must be considered a valid possibility since horizontal transmission has probably occurred in the case of P and I elements.

It is clear from this and previous studies that a simple examination of the distribution and conservation of transposable elements can give limited information about their history. In the future, it will be necessary to compare the DNA sequences of mobile-element homologues from several species and to determine whether these elements are functional. It remains to be determined whether horizontal transmission occurs as a consequence of infectious properties of the mobile element itself or whether mobile elements are spread by a vector that is itself parasitic on *Drosophila.*

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