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Invasion of *Drosophila virilis* by the *Penelope* transposable element

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Abstract The *Penelope* family of transposable elements (TEs) is broadly distributed in most species of the *virilis* species group of *Drosophila*. This element plays a pivotal role in hybrid dysgenesis in *Drosophila virilis*, in which at least four additional TE families are also activated. Here we present evidence that the *Penelope* family of elements has recently invaded *D. virilis.* This evidence includes: (1) a patchy geographical distribution, (2) genomic locations mainly restricted to euchromatic chromosome arms in various geographical strains, and (3) a high level of nucleotide similarity among members of the family. Two samples from a Tashkent (Middle Asia) population of *D. virilis* provide further support for the invasion hypothesis. The 1968 Tashkent strain is free of *Penelope* sequences, but all individuals collected from a 1997 population carry at least five *Penelope* copies. Furthermore, a second TE, *Ulysses*, has amplified and spread in this population. These results provide evidence for the *Penelope* invasion of a *D. virilis* natural population and the mobilization of unrelated resident transposons following the invasion.

Introduction

The *virilis* species group is one of several major species groups in the subgenus *Drosophila*. It has been the subject of many earlier, cytogenetic and evolutionary studies (e.g., Sturtevant and Novitski 1941; Patterson and Stone 1952; Throckmorton 1982). Classical chromosomal studies of this group have described the karyotypic evolution of species of the group. Polymorphic and fixed inversions are present, and often abundant, in all species of the group, with one exception (Stone et al. 1960; Throckmorton 1982; Spicer 1992): *Drosophila virilis* is completely free from inversions and is therefore of special interest because of its relatively primitive karyotype.

Studies of transposable elements (TEs) in the genus *Drosophila* have provided much new information about the evolution of transposable sequences and their possible role in the evolution of their host genomes. In particular, several families of TEs have been shown to be activated in hybrid dysgenesis. Hybrid dysgenesis in *Drosophila melanogaster* results in high sterility and mutation rates, male recombination, segregation distortion and chromosomal aberrations resulting from the activation of *P* (Kidwell et al. 1977), *I* (Bucheton et al. 1984), and *hobo* (Blackman et al. 1987) elements. There is strong evidence that the *P, I* and *hobo* element families have only entered the *D. melanogaster* genome in the last century following horizontal transfer from other species (Kidwell 1983; Bucheton et al. 1992; Simmons 1992).

A hybrid dysgenesis syndrome has also been described in *D. virilis* (Lozovskaya et al. 1990; Scheinker et al. 1990). This was first observed (Lozovskaya et al. 1990) when males from an old Japanese laboratory strain (160) were crossed to females of a wild-type strain, collected in 1970, in Batumi (Georgia, former USSR). In contrast to the *D. melanogaster* hybrid dysgenesis systems in which individual families of repeats are activated independently of one another, the *D. virilis* system involves at least five different unrelated families of repeats that are mobilized simultaneously (Petrov et al. 1995; Evgen'ev et al. 1998; Vieira et al. 1998). The elements mobilized include *Penelope*, *Ulysses*, *Paris* and *Helena* and *Telemac* (Petrov et al. 1995; Vieira et al. 1998). Evgen'ev et al. (1997) concluded that the *Penelope* family is primarily responsible for the hybrid dysgenesis syndrome of *D. virilis*. This conclusion was based on element localization in parental strains, the determination of

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transcription patterns and the results of injection experiments. Additional evidence for this conclusion has been independently obtained in genetic experiments (Vieira et al. 1998).

The distributions of *Penelope* and *Ulysses* appear to be restricted to the *virilis* species group of *Drosophila* (Zelentsova et al. 1999). Both elements appear to have had a long presence in most species of this group. Except for *Drosophila kanekoi*, *Drosophila lummei*, and some strains of *D. virilis*, species of the group carry full-sized and at least potentially functional copies of both element families. It was of interest to carry out a more detailed analysis to compare the distribution of *Penelope* with that of other element families in this species. Here we describe the distributions of *Penelope*, *Ulysses* and other TEs in *D. virilis* strains that were collected from different geographical locations at different times in the past. Of particular interest is the comparison of two samples from a population in Tashkent, Middle Asia, collected 30 years apart, and examined for the presence of *Penelope* and other elements. The data provide strong evidence for the recent invasion and spread of the *Penelope* family of TEs in the *D. virilis* genome following a postulated horizontal transfer.

Materials and methods

Drosophila virilis strains

A list of the geographical strains examined is given in Table 1. The year of collection is indicated when known. The progeny of seven individual females caught in the same winery, in Tashkent in 1997, were included in the analysis. We also examined a number of old laboratory strains carrying visible markers that have been kept in the Moscow Stock Center for more than 40 years. Marker strains are only of limited use, however, because of the absence of information on their origin and date of establishment. Stocks were maintained on standard medium at 25°C.

Measurement of gonadal dysgenesis

Female and male gonadal dysgenesis were measured in the same way as described previously for *D. virilis* (Lozovskaya et al. 1990). Consistent with previous usage, *Penelope*-free strains are designated as "M-like" and *Penelope*-bearing strains as "P-like" (Evgen'ev et al. 1997) The M-like standard reference was strain 9, a wild-type strain collected in 1970 in Batumi (Georgia Republic, former USSR). The P-like standard reference was strain 160, an old laboratory strain from Japan carrying recessive markers on all autosomes (Lozovskaya et al. 1990).

DNA isolation and Southern analysis

DNA from different *D. virilis* strains was prepared as described previously (Zelentsova et al. 1986). Twenty micrograms of genomic DNA was used for a typical restriction enzyme digestion. Southern blots of genomic DNA were prepared following electrophoresis on 1% agarose gels, denaturation, capillary-blotting onto nylon membranes, according to the manufacture's protocol, and fixation by UV cross linking, using a UV Stratalinker 2400 (Stratagene). Standard high-stringency hybridization and wash conditions were used.

Probes for Southern hybridization

The *Penelope* probe used was 8 kb in length and represents the largest copy of this element so far isolated (Evgen'ev et al. 1997). It has an organization suggestive of a tandem array of two copies of *Penelope*, one of them containing the complete open reading frame of the element (Evgen'ev et al. 1997). This sequence, along with flanking sequences of about 1 kb, was cloned into a Puc18-SacI site. Sometimes we isolated the 2.6 kb XhoI-XhoI fragment of this clone, representing a full-sized copy of the element and used it in our in situ and Southern studies. In most cases, we used the whole clone that does not contain other repetitive sequences. Therefore a good internal control for intensity of hybridization and efficiency of blotting is provided by a unique flanking sequence present in the clone. We also used a *Ulysses* probe containing the 1.9 kb NdeI-ClaI fragment of this element corresponding to the long terminal repeat (LTR) and isolated from agarose after digestion with corresponding restriction endonucleases, a *Helena* probe containing a 691 bp fragment of the *Helena* element cloned into an EcoRI site of the BlueScript vector (Petrov et al. 1995) and a

Table 1 List of *D. virilis* strains tested and their *Penelope* element copy numbers. (BG, Bowling Green; Mos, Moscow)

Collection location	Collection date	Stock center	Stock no.	Copy no.	Collection location	Collection date	Stock center	Stock no.	Copy no.
Europe & Middle Asia					Americas & Far East				
Berlin	Old strain	Mos	102	0	Pasadena, NY	1913	BG	1051.0	0
Uman, Ukraine[a]	1965	Mos	2003	26	Japan	"Old"	Mos	101	0
Tashkent, Uzbekistan	1968	Mos	40	0	Mexico[a]	1947	BG	1051.48	23
Magarach, Crimea[a]	1970	Mos	2005	1	China[a]	1948	BG	1051.47	20
Batumi, Georgia	1972	Mos	9	0	Argentina[a]	1950	BG	1051.49	15
Krasnodar, Russia	1975	Mos	13	0	TK, Tokyo, Japan	1951			10
Mtskheta, Georgia	1975	Mos	25	0	Chile[a]	1956	BG	1051.51	32
Yalta, Crimea	1975	Mos	42	0	Takuman, Chile	1976			>20
Yerevan, Armenia	1975	Mos	1	0	Seychelle Islands	1985	Mos	59	0
Baku, Azerbajan	1980	Mos	10	0	Hunan, China	1988			15–20
Leeds, UK	1982			15–20	Nanjing, China	1988			15–20
Leeds, UK	1995			15–20	Sapporo, Japan	1996			16
Tashkent (7 isofemale lines)	1997			5–20	Truckee, Calif[a]		BG	1051.8	66
Jerez, Spain	1998			20+	Sendai, Japan[a]		BG	1051.9	28
					New Mexico		Mos	117	>20

[a] Strains subjected to in situ hybridization with *Penelope*- and *Ulysses*-containing probes

probe consisting of the 2.9 kb HindIII-HindIII fragment of the *gypsy* element isolated from a *D. virilis* strain 160 genomic library (M.B. Evgen'ev, unpublished results).

Cytological analysis and in situ hybridization to polytene chromosomes

For in situ hybridization studies, larvae were grown at 18°C and live yeast solution was added to the culture 2 days before the larvae were examined. Salivary glands from males and females of different *D. virilis* strains were dissected from third instar larvae in 45% acetic acid and squashed according to procedures developed by Lim (1993). DNA probes were biotinylated by nick translation using biotin 14-dATP essentially as described by Lim (1993). All localizations were made using a photographic map of *D. virilis* chromosomes (Gubenko and Evgen'ev 1984).

Results

Distribution of *Penelope* in *D. virilis*

To investigate the distribution of *Penelope* in *D. virilis*, we carried out Southern blot analysis of the strains listed in Table 1. Genomic DNA from adult flies was cleaved with XhoI and hybridized with the labeled *Penelope* probe (see Materials and methods). Figure 1A provides examples of typical Southern blots showing the distribution of *Penelope* in 12 different geographical strains. As expected, each lane shows hybridization to the flanking sequence included in the probe. In addition, a strong hybridization signal, corresponding to the 2.6 kb XhoI fragment present in functional *Penelope* elements, is evident in lanes 2, 8 and 10 (for strains 160, New Mexico and Sendai, respectively). The intensity of this band, indicated by an arrow in Fig. 1A, gives an indication of the relative numbers of potentially functional *Penelope* copies in individual strains (Evgen'ev et al. 1997). It is evident from Fig. 1A and Table 1 that some strains appear to lack *Penelope* elements altogether, while others are highly heterogeneous with respect to copy number and restriction fragment size. Multiple signals corresponding to single-copy equivalents observed in all *Penelope*-containing strains (lanes 2, 8 and 10) indicate a high level of structural heterogeneity of the *Penelope* copies present.

In situ hybridization experiments carried out to localize *Penelope* insertion sites also provided an independent, more accurate, estimate of copy number. From Table 1 it is seen that when *Penelope* is present, copy number usually varies between 15 and 40. However, there are exceptions: at one extreme, the Magarach strain carries only one *Penelope* copy and at the other extreme, the Truckee strain carries as many as 66 copies.

With respect to the cosmopolitan distribution, it is seen that, with two exceptions, Uman with 26 copies, and Magarach with only one copy, *Penelope* was completely absent from all strains originating in Middle Asia and Europe before 1982. Of particular interest is a comparison of strains collected in the same winery in Tashkent, Uzbekistan, in 1968 and 1997. Although the earlier collection does not carry *Penelope* elements, the recent collection has more than 20 copies with variable insertion locations among individual flies. (A more detailed analysis of the 1997 Tashkent population is given below.) On the other hand, with three exceptions, strains collected in the Americas and Far East all carry multiple *Penelope* elements. Two exceptions are very old strains from Japan and New York. The third exception was collected in the geographically isolated Seychelles Islands. Although the geographical origin of the mutant strains is not known, these strains are known to have been maintained in various laboratories for more than 40 years. Eight of these strains were positive, and three negative, for *Penelope*. These data are consistent with the idea that *D. virilis* has been in the process of invasion by *Penelope* since the earlier years of this century. Whether or not the putative invasion is still continuing, or whether it is now complete, remains to be determined from more extensive new collections from natural populations.

Distribution and copy number of other families of mobile elements in *D. virilis*

We carried out parallel Southern analyses using as probes four additional mobile elements isolated from the *D. virilis* genome. Figure 1B shows that, unlike *Penelope*, *Ulysses* is present in approximately the same genomic copy number in all strains examined. A comparatively low frequency of restriction fragment polymorphism is also seen. A similar pattern of relatively low copy number variation among strains was also observed when *Helena* (Fig. 1C), *gypsy* (Fig. 1D) and *Paris* (data not shown) were used as probes. Unlike the other elements examined here, preliminary experiments did not reveal any induction of transposition of the *gypsy* element in the course of hybrid dysgenesis (data not shown). We also failed to locate *gypsy* within any mutation obtained in the progeny of dysgenic crosses (Evgen'ev et al. 1997). Although, like *Ulysses*, *Helena* and *Paris*, all strains analyzed contain *gypsy* at approximately the same copy number, unlike these three elements, restriction analysis of *gypsy* revealed a relatively high level of variation in the structure of individual copies.

Southern analysis of lines derived from the 1997 Tashkent population

In order to test the invasion hypothesis directly, in November 1997 we resampled the *D. virilis* population from Tashkent City (Uzbekistan, Middle Asia), which is associated with a small, local winery. Flies collected at this location in 1968 had been used to establish the Tashkent laboratory strain (strain 40, see Table 1). This old laboratory strain was shown by in situ hybridization, Southern blot analysis and polymerase chain reaction studies to be free of *Penelope*-related sequences (data not shown). From the sample of *D. virilis* flies collected in 1997 the progeny of seven fertile females

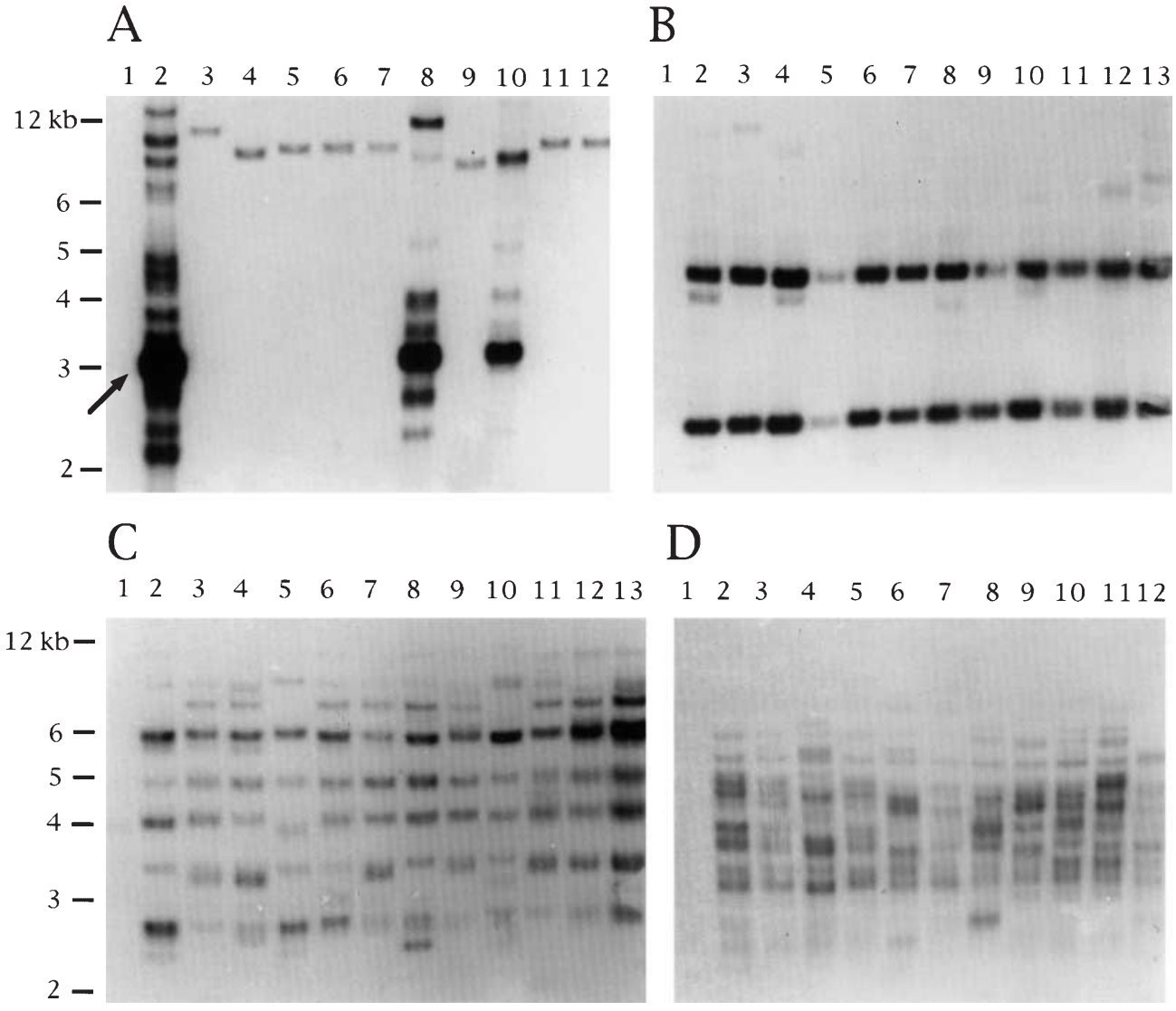

Fig. 1A–D Hybridization spectra of 12 *Drosophila virilis* strains collected from different geographical locations. **A** *Penelope*. DNA was digested with XhoI, electrophoresed on a 1% agarose gel, and blotted onto a nitrocellulose filter. The filter was then hybridized with the *Penelope*-containing probe, labeled with ^{32}P-dNTP. *Lanes*: *1 Drosophila melanogaster* Oregon, control; *2 D. virilis* 160; *3* Yerevan; *4* Seychelle Islands; *5* Old Japanese; *6* Baku City; *7* Krasnodar; *8* New Mexico; *9* Tashkent; *10* Sendai; *11* Mzheta; *12* Yalta. The *arrow* indicates the position of the 2.6 kb band corresponding to the size of complete *Penelope* elements. **B** *Ulysses*. DNA was digested with ClaI, electrophoresed on a 1% agarose gel, and blotted onto a nitrocellulose filter. The filter was then hybridized with the *Ulysses*-containing probe, labeled with ^{32}P-dNTP. *Lanes: 1 D. melanogaster* Oregon, control; *2 D. virilis* 160; *3* Yerevan; *4* Seychelle Islands; *5* Old Japanese; *6* Baku City; *7* Krasnodar; *8* New Mexico; *9* Tashkent; *10* Sendai; *11* Mzheta; *12* Yalta; *13* Batumi, Georgia. **C** *Helena*. The blot described in **B** was stripped of label by boiling in 0.1×SSC 0.15 M NaCl, 0.015 M sodium citrate, for 5 min and hybridized with the *Helena* probe labeled with ^{32}P-dNTP. **D** *Gypsy*. DNA was digested with HindIII and hybridized with a *gypsy*-containing probe labeled with ^{32}P-dNTP. *Lanes: 1 D. melanogaster* Oregon, control; *2 D. virilis* 160; *3* Chile; *4* Argentina; *5* Mexico; *6* China; *7* Truckee, Calif.; *8* Berlin; *9* Pasadena; *10* Uman; *11* Magarach; *12* strain 110

were used for Southern blot analysis and in situ hybridization.

Figure 2 depicts the results of Southern blot analysis of genomic DNA isolated from the progeny of five individual *D. virilis* females caught in 1997 in the Tashkent winery and from the old Tashkent strain 40. The DNA was digested with different restriction endonucleases and hybridized with labeled *Penelope*, *Ulysses* and *gypsy* probes (Fig. 2A, B and C, respectively). A significant level of polymorphism in the hybridization patterns in the progeny of individual females is evident with both the *Penelope* and *Ulysses* probes. It is clear from Fig. 2A, however, that in contrast to the 1968 Tashkent strain, flies from the 1997 Tashkent population carry multiple copies of *Penelope*. Also, because the majority of the hybridization is represented by the 2.6 kb band, most *Penelope* elements in these lines are full-sized, potentially active, copies. On the other hand, a less uniform picture in terms of restriction fragment polymorphisms is evident in the “old” and “new” Tashkent strains when *Ulysses* and *gypsy*-containing clones were used as probes (Fig. 2B, C).

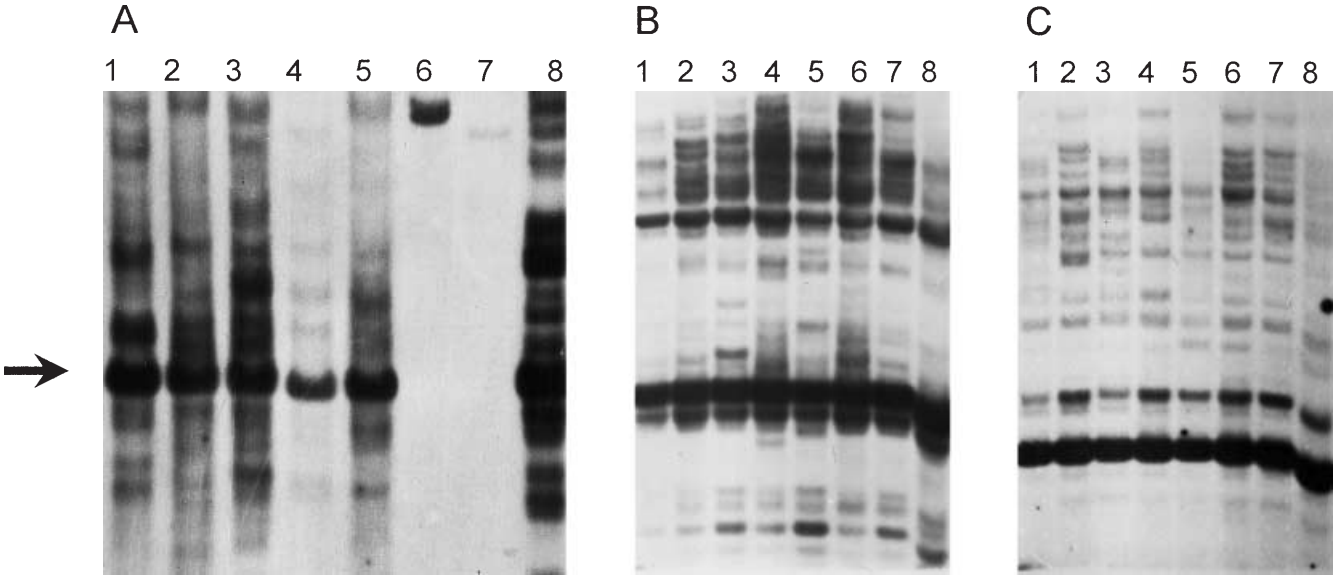

Fig. 2A–C Southern blot analysis of genomic DNA from Tashkent strains. **A** Ten micrograms of total DNA was digested with XhoI and hybridized with the *Penelope* probe (see Materials and methods). The 2.6 kb hybridization band is indicated by an *arrow*. *Lanes 1–5* 1997 Tashkent isofemale lines 1, 2, 5, 6, and 7; *lane 6* strain 9 (M-like strain control); *lane 7* strain 40 (Tashkent 1968); *lane 8* strain 160 (P-like strain control). The high molecular weight single band hybridization seen in *lane 6* is due to the presence of a 1 kb genomic flanking sequence in the *Penelope*-containing probe. **B** The same DNAs as in **A** (above) were digested with EcoRI and hybridized with a *Ulysses* probe containing a 2.5 kb internal fragment of the element cloned into Puc19, EcoRI and BamHI restriction sites. **C** After the membrane described in **B** (above) was stripped of the label it was hybridized with a *D. virilis gypsy* clone containing the 2.9 HindIII-ApaI internal fragment of the element

Table 2 Percentage of F1 female and male progeny with normal gonads from 15 crosses involving either the *D. virilis* 1968 Tashkent strain (40), or one of the 1997 Tashkent strains (T5 or T6)

Maternal strain	Sex	Paternal strain				
		9	160	40	T5	T6
9 (M-like strain)	F			100	99	100
	M			100	98	99
160 (P-like strain)	F			98		95
	M			95		99
40	F	98	64		100	94
	M	99	63		99	89
T5	F	99	98	97		
	M	100	100	100		
T6	F	99	99	90		
	M	100	99	100		

Changes in the hybrid dysgenesis properties of the Tashkent population

Previously we proposed that, by analogy with the P-M dysgenesis system in *D. melanogaster*, *D. virilis* strains can be to classified as P-like, M-like, or Q-like, based on their behavior in dysgenic crosses (Lozovskaya et al. 1990). All strains described so far that lack *Penelope* belong to the M-like category (Lozovskaya et al. 1990; Evgen'ev et al. 1997, 1998). The 1968 Tashkent strain that lacks *Penelope* was previously classified as a typical M-like strain having a significant frequency of male and female gonadal sterility in the F1 progeny of females from this strain with males from the P-like strain 160 (M.B. Evgen'ev, unpublished results).

We carried out new gonadal dysgenesis assays of the 1968 Tashkent line (strain 40) along with two of the 1997 Tashkent isofemales lines T5 and T6. The results are presented in Table 2. They indicate that in contrast to strain 40, which is still classified as M-like, the 1997 Tashkent lines can be classified either as Q-like (neutral), or very weak P-like strains. Therefore the hybrid dysgenesis potential of lines from the contemporary Tashkent population has changed, consistent with the changed *Penelope* characteristics determined at the molecular level.

Comparison of TE locations in the 1968 and 1997 Tashkent populations

Samples of larvae from the 1968 and 1997 Tashkent populations were examined by in situ hybridization for the chromosomal locations of *Penelope*, *Ulysses* and *gypsy* insertion sites. Because *Ulysses* and *gypsy* have a low frequency of transposition, we expected that, if the 1997 Tashkent population had descended from the old one, the two populations would have a significant proportion of *Ulysses* and *gypsy* sites in common. Data presented in Table 3 indicate that this is apparently the case. A significant proportion of sites present in the old strain, (45% for *Ulysses* and 100% for *gypsy*) were retained in the recently caught flies. On the other hand, an extremely high level of variability in *Ulysses* and *Penelope* locations is

Table 3 Hybridization of *Penelope*, *Ulysses* and *gypsy* elements with the 1997 Tashkent population of *D. virilis*

Element	Total no. of sites	No. (%) repeats[a]	No. (%) sites occupied	% hot spots	% old sites retained	Mean	Variance
Penelope	103	30 (29.8)	0 (0)	78.5	0	11.7	24.1
Ulysses	128	56 (43.7)	1 (0.7)	88.0	45	22.2	64.2
Gypsy	12	8 (66.6)	3 (25.0)	0	100	4.8	1.7

[a] The data in this column represent the hybridization sites found in the progeny of more than one fertile female caught in the contemporary Tashkent population

evident among lines established from the contemporary Tashkent population (Table 3). Thus, of 103 sites of *Penelope* hybridization detected in the progeny of seven fertile females, 73 were unique (i.e., present in the progeny of only single females), while others were occupied by *Penelope* in the progeny of two or more females. We failed to find a single *Penelope* hybridization site that was present in the progeny of all fertile females studied from the same population. In the case of *Ulysses*, there was only one such site (60C in chromosome 6).

The asymmetrical hybridization that is frequently observed in unpaired regions of polytene chromosomes in the progeny of freshly caught females with both *Penelope* and *Ulysses* probes (Fig. 3A, B) indicates that an exceptionally high level of heterozygosity is present in the contemporary Tashkent population. The high level of polymorphism observed for *Penelope* and *Ulysses* (Table 3) may have resulted from the mating of local flies with ones that had migrated from a neighboring population. These immigrants might have differed in the chromosomal distribution of both *Penelope* and *Ulysses*. Another possibility is that the new *Penelope* and *Ulysses* insertion sites in the modern Tashkent population may have resulted from transposition following hybrid dysgenesis. In order to check the latter possibility, we examined the occupancy of *Penelope* and *Ulysses* "hotspots" in the progeny of recently caught females (Evgen'ev et al. 1998).

Previously, when studying 16 different geographical strains of *D. virilis*, we revealed 70 hotspots for *Penelope* (of 200 total sites of hybridization scored for all strains) and 33 hotspots for *Ulysses* (of 151 total sites of hybridization scored for all strains). A site was considered to belong to a hotspot category if it was occupied by a particular transposon in three or more strains studied (Evgen'ev et al. 1998). Surprisingly, more than 78% of *Penelope* hotspots and 88% of *Ulysses* hotspots identified in the earlier study were occupied by corresponding elements in one or more individuals checked in the contemporary Tashkent population (Table 3). This was surprisingly high given that in all *D. virilis* laboratory strains previously studied (Evgen'ev et al. 1998) the percentage of hotspots occupied by *Ulysses* and *Penelope* never exceeded 30%. These results suggest that, following the *Penelope* invasion, multiple transpositions of *Ulysses* and *Penelope* probably took place with preference for insertion into hotspots and these contributed to the high level of polymorphism in the chromosomal distribution of these elements.

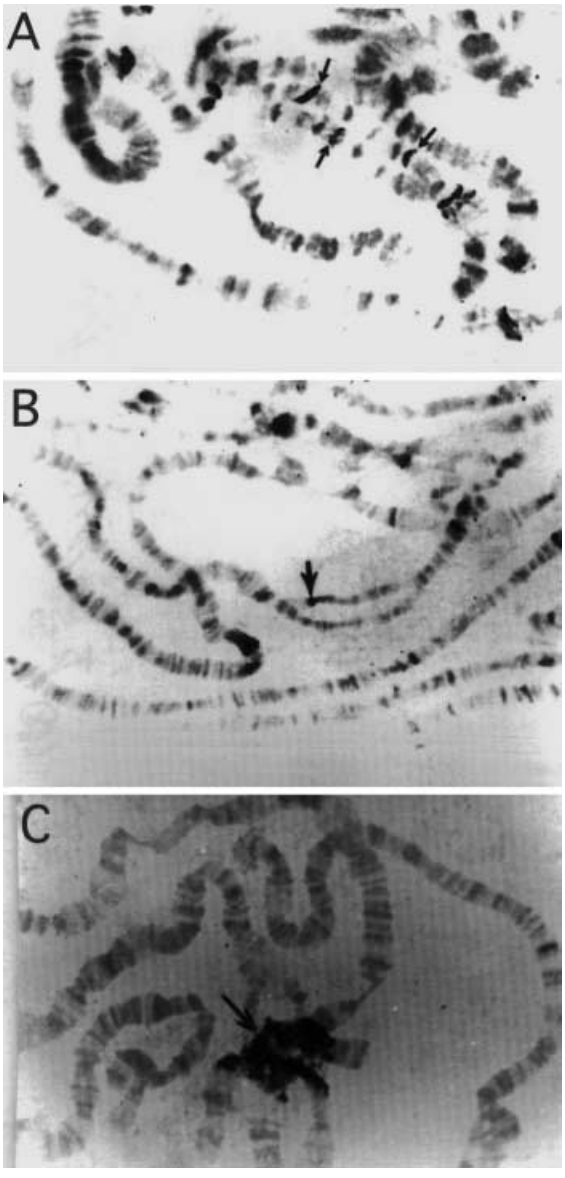

Fig. 3A–C In situ hybridization of polytene chromosomes of a 1997 Tashkent line with three element probes: **A** *Penelope*, **B** *Ulysses*, and **C** *gypsy*. The photographs in **A** and **B** represent typical patterns of asynapsis and asynchronous hybridization with *Penelope* and *Ulysses* that are often observed in the progeny of freshly caught females. Asymmetric hybridization sites present in only one of the homologs in **A** and **B** are indicated by *arrows*. In **C**, an *arrow* indicates the concentration of label in the chromocenter and bases of all the chromosomes

Finally, we investigated the chromosomal localization of *gypsy*, an element that can behave either as an endogenous infectious retrovirus in *D. melanogaster* (Song et al. 1994), or as an LTR retrotransposon. Although *gypsy* is not normally mobilized in hybrid dysgenesis, either in *D. melanogaster,* or in *D. virilis*, this element may undergo massive mobilization in some genetic environments (Prud'homme et al. 1995). In all strains of *D. virilis* examined in the present study, *gypsy* hybridized intensely to the chromocenter (Fig. 3C) and was found in high abundance in the basal euchromatin of most chromosome arms. These results, coupled with the comparatively similar patterns of hybridization revealed in the Southern hybridization experiment (Fig. 2C), suggest that *gypsy* is not active in these *D. virilis* strains.

Discussion

Here we have demonstrated that the *Penelope* element is not uniformly present in *D. virilis* populations, collected worldwide, but has a patchy geographical and temporal distribution. With a few exceptions, *Penelope* was completely absent from all strains originating in Middle Asia and Europe more than 17 years ago, but was present at that time in the American and Far Eastern populations studied. This distribution of *Penelope* is consistent with an hypothesis of a recent invasion of *D. virilis* that proceeded slowly from the Far East and Americas to Europe and Middle Asia. In contrast, other *D. virilis* mobile elements (*Ulysses*, *Helena*, *Paris* and *gypsy*) exhibit comparatively low variability in their copy numbers and distributions; we failed to find a single *D. virilis* strain that was devoid of any of these families of elements.

Of particular interest was the finding of *Penelope* in a 1997 collection of *D. virilis* from Tashkent, a location that was *Penelope*-free in 1968. All seven lines of flies from the 1997 Tashkent population were classified as neutral in the *Penelope* system of hybrid dysgenesis, even though they carry multiple copies of *Penelope.* RNA hybridization analysis failed to detect significant induction of *Penelope* transcription when males from the contemporary Tashkent population were crossed to females from a typical M-like strain (data not shown). Although the *Penelope* elements present in the modern Tashkent population do not produce a strong manifestation of hybrid dysgenesis, the level of *Penelope* transcription does appear to have been sufficient for recent activation of other transposons and for transposition of *Penelope* itself.

A second line of evidence supporting the recent invasion hypothesis comes from the results of in situ hybridization experiments that clearly indicate that *Penelope* insertion sites are located almost exclusively on chromosome arms; these sites are rarely observed in the centric heterochromatin, a location associated with long-established elements. This distribution pattern is similar to that of *P* elements (O'Hare et al. 1992) that have recently invaded *D. melanogaster* (Kidwell 1983). However, the pattern differs from that of diverged, nonautonomous *I* elements that have been present in *D. melanogaster* for some time and are usually located in the pericentromeric heterochromatin (Crozatier et al. 1988; Vaury et al. 1989). Similar to the distribution pattern described for *I* elements, we do observe some quantitative interstrain differences in the distribution of *Ulysses*, *Helena* and *gypsy* in euchromatic chromosome arms, but all these elements also hybridized strongly with the chromocenter.

A third piece of evidence for recent invasion comes from the high nucleotide similarities among individual *Penelope* elements. *Penelope* copies from the same and different strains exhibited >97% similarity at the nucleotide level when analogous regions were aligned (M.B. Evgen'ev, unpublished results). A similar high level of similarity was observed among *P* elements from the same and different geographical strains (Sakoyama et al. 1985; O'Hare et al. 1992) following the recent invasion of *D. melanogaster* by these elements.

Our data indicate that *Penelope*-bearing and *Penelope*-free strains have coexisted in natural populations of *D. virilis* for a considerably longer period than was the case for *P* and *hobo* following their invasion of *D. melanogaster* (Anxolabéhère et al. 1988; Kidwell 1994). The slower rate of *Penelope* invasion might be attributable to the difference in population structure between the two species. Unlike *D. melanogaster, D. virilis* appears to be limited to semidiscrete local populations that are probably currently experiencing reduction of size, and even extinction, due to habitat destruction.

The *gypsy* element appears not to be active in the 1997 Tashkent strains. This conclusion follows from the results of in situ hybridization using the *gypsy* probe (Fig. 3C) coupled with the comparatively similar patterns of Southern hybridization (Fig. 2C). It seems likely that *gypsy* has lost its transposition activity by mutation and stochastic loss (for a review, see Arkhipova et al. 1995). Therefore, if the Tashkent population was recently invaded by *Penelope*, it would be expected to exhibit a *gypsy* hybridization pattern that is identical, or very similar to, that of the ancestral population. Indeed, it was shown that although all seven hybridization sites that are characteristic of the 1968 Tashkent population are retained in the contemporary one (Table 3), five new sites appeared in the progeny of newly caught flies. These data indicate that, contrary to the *Penelope* and *Ulysses* cases, no massive transposition and spread of *gypsy* is evident in the 1997 Tashkent population.

We cannot be certain at present whether *D. virilis* was previously invaded by *Penelope* or whether the invasion we have recently witnessed is the first encounter of the species with this family of TEs following horizontal transfer from another species. One possibility is that there was a much earlier *Penelope* invasion of this species and that these old elements were either mostly lost or became diverged beyond recognition. A recent amplification of an endogenous copy could have initiated a new invasion of the species. Alternatively, active *Penelope* elements may have been introduced or reintroduced

by horizontal transfer. Under the recent horizontal transfer scenario, the identity of the donor species is unknown. One possibility is that *Penelope* may have recently entered the *D. virilis* genome by a rare outcross with a species from the same phylad, such as *Drosophila texana*, in which *Penelope* is an old component of the genome (Evgen'ev et al. 1997; Zelentsova et al. 1999). Species of the same phylad have been successfully crossed under experimental conditions and have produced partially fertile progeny (Patterson and Stone 1952). There is also evidence that *D. virilis* may cross in the wild with other species of the *virilis* phylad (Tsuno and Yamaguchi 1991).

In conclusion, an observation by Throckmorton (1982) is pertinent. He noted that it was unfortunate that most, if not all, our knowledge of *D. virilis* derives from the domestic, cosmopolitan form. Cytogenetic and molecular investigation of putative ancient wild populations that may still exist in Eastern Asia may help to find the intermediate forms necessary for understanding both the karyotypic and molecular evolution of the group. Parallel investigations of the distribution and evolution of *Penelope* and other families of repeats in other species of the *virilis* group show considerable promise for understanding the possible role of the *D. virilis* hybrid dysgenesis syndrome in chromosome reorganization of the whole group.

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