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Evolution

P-element distribution in Eurasian populations of *Drosophila melanogaster*: A genetic and molecular analysis

(transposable elements/hybrid dysgenesis/DNA hybridization/geographical variation)

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ABSTRACT **Genetic and molecular investigations were carried out with Eurasian *Drosophila melanogaster* populations on the *P–M* system of hybrid dysgenesis. In 27 strains sampled from France to Middle Asia, a clear gradient exists between Western Europe, in which most modern strains are of the *Q* type, and eastern areas, in which *M*-cytotype strains predominate. Molecular analysis on individual flies was performed with two complementary probes of the cloned 2.9-kilobase *P* element. The results provide evidence for a gradually decreasing frequency of *P* elements from west to east, but the presence of *P*-homologous sequences has been ascertained in all of the wild *M*-cytotype populations analyzed. Moreover, some active *P* elements with *GD* sterility potential were revealed in the majority of *M*-cytotype populations when tested with a highly sensitive reference line. The gradual change in distribution of the polymorphic *P* family in Eurasia is discussed in relation to the structure of the elements together with the theories of *P–M* evolution and is interpreted as the present invasion of Eurasian populations by these elements.**

About 10% of the genome of *Drosophila melanogaster* exists as dispersed moderately repetitive sequences belonging to different families (1). The *P* family is composed of mobile dispersed genetic elements implicated in the *P–M* system of hybrid dysgenesis. This phenomenon, which is manifested in certain interstrain hybrids, results in a number of correlated aberrant genetic traits—e.g., high frequencies of gonadal sterility (*GD* sterility), mutation, and male recombination (2). Three types of individuals, *P*, *Q*, and *M*, have been described on the basis of their cross-effect properties. Hybrids between *P* males and *M* females show dysgenic traits that are reduced or absent in the reciprocal hybrids. *Q* individuals do not exhibit *GD* sterility in any cross-combinations but produce mutations and male recombinations in crosses with *M* females (3, 4).

In the *P–M* system, hybrid dysgenesis results from interactions between chromosomally linked factors (*P* factors) and a particular type of cellular state referred to as the *M* cytotype (5). The *P* factors are active genetic elements of the *P* family, whose members are heterogeneous in size [0.5–2.9 kilobases (kb)], but which share substantial sequence homology (6–8). All *P* and *Q* strains thus far examined bear 30–50 copies of the *P* family (7). *Q* individuals are thought to carry a subset of the *P*-element family that apparently lacks sterility potential while retaining mutator activity and other *P*-element functions (7, 9, 10). Conversely, all long-established laboratory *M* strains that have been examined completely lack homology with the *P*-element family (7). Some strains showing the *M* cytotype but with some homology to *P* sequences have also been found in laboratory collections (7). In this paper, such strains will be called *M'*, the term *M* strain being reserved for strains of the *M* cytotype with no *P* homology at all.

The *M*-cellular state component of the *P–M* interaction may be considered as a "susceptibility" to the action of active *P* elements that tend to be destabilized in the *M* cytotype. Inversely, individuals with the *P* cytotype are seemingly resistant to the action of *P* elements that remain stable within this cellular state (5, 11). These properties might be determined either by an episome with a limited potential for self-replication (11) or by active *P* elements themselves encoding a regulator (8).

At the population level, temporal trends in the distribution of strains have been observed with a frequency of *M*-cytotype strains positively correlated with laboratory age. *P* strains have only been revealed in samples taken since 1950 and are increasingly more frequent in subsequent collections (12). Pronounced geographical differences have been shown, however, between the Americas, Africa, and Eurasia in the actual worldwide distribution of population tested for their *GD* sterility potential (13–15). We report here the results of a survey of Eurasian strains of *D. melanogaster* in terms of the presence of *P* elements at the molecular level and their influence on the dysgenic potential of the population. There is a gradual change in the distribution of the *M* cytotype, rare in the west of Europe and predominating in central and eastern areas. However, all flies from these samples of natural populations contain at least some *P*-element homology. This is interpreted as the result of the present spread of *P* elements in Eurasian populations.

MATERIAL AND METHODS

Strains Employed. Twenty-seven wild strains derived from diverse areas between France and Middle Asia were investigated in this study. Whenever possible, each strain was derived from a large number (>30) of individuals collected in 1982–1983. They were kept under standard laboratory conditions by mass culture of about 500 individuals and normally were analyzed during the first to fifth generation following capture.

Three reference strains were used to test the dysgenic potential of the wild strains: a strong *P* strain, Harwich (H), the strong *M* strain, Canton S (C), and the *M'* strain: *y, sn^w; bw; st (sn^{w}M')*. The latter strain is stable (16) and reacts as a more sensitive *M* cytotype than Canton S (see *Results*).

Estimation of the Frequency of *GD* Sterility. Three crosses were routinely made for each population. Thirty individuals of the population tested were mass-mated at 28.5°C as follows:

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Abbreviation: kb, kilobase(s).

Cross A_1: Canton S ♀♀ × ♂♂ tested
Cross A_2: *sn*^{w}*M'* ♀♀ × ♂♂ tested
Cross A*: ♀♀ tested × ♂♂ Harwich

All crosses were duplicates or triplicates. Dissection of 50 F_1 females was used to estimate the frequency of dysgenic ovaries and to classify the strains (17, 18).

Detection of *P* Elements by Direct Hybridization on *Drosophila* Prints. The presence of *P*-element sequences in single individuals (squashed flies) was investigated by hybridization (19, 20). Flies squashed on a nylon filter (Pall Posidyne) were lysed by layering successively the filter on Whatman 3MM papers soaked with 10% $NaDodSO_4$ (2 min), 0.5 M NaOH/2.5 M NaCl (two times, 5 min each), and 3 M sodium acetate (pH 5) (three times, 2 min each). Chitinous parts were removed, and the filter was dried at room temperature (30 min) and then baked (80°C, 1 hr). Two ^{32}P-labeled probes were used: the 0.84-kb *Hin*dIII fragment (P1 probe) and the 1.5-kb *Hin*dIII–*Sal* I fragment (P2 probe) from the Pπ 25.1 plasmid (Fig. 2*A*). Prehybridization and hybridization were carried out at 65°C in 2× concentrated NaCl/citrate/FPG solution/25 mM KH_2PO_4, pH 7/2 mM EDTA/0.5% $NaDodSO_4$/10% dextran sulfate (FPG solution = 0.02% Ficoll type 400/0.02% polyvinylpyrrolidone type 350/0.02% glycine). Washings were done at 65°C: 2× concentrated NaCl/citrate/0.1% $NaDodSO_4$ (two times, 30 min each); 0.2× concentrated NaCl/citrate (30 min). Control individual flies were taken from the strong *P* Harwich strain (positive control) and from the strong *M* Gruta strain (negative control).

RESULTS

Geographical Distribution of Potential for *GD* Sterility. Fig. 1 and Table 1 contain the results of wild-type strains tested for their *GD* sterility potential when crossed with Harwich and Canton S. Fig. 1 is the updated version of the current distribution, including the present observations and available data obtained previously for populations sampled in these areas between 1980 and 1983 (15).

A marked difference is evident in the present survey between western Europe (France, Federal Republic of Germany, Switzerland) and the rest of Europe, including Spain, the Mediterranean Basin, and Middle Asia. Most of the current strains in France are *Q*, and the few observed *M*-cytotype strains are primarily Mediterranean. The Pyrenees appear to be a natural barrier for the *P–M* system when we use the *GD* criterion in crosses with Canton S, but a gradual variation is observed over the Eurasian area. As shown in Table 1, column 1, western populations reveal *P* cytotypes (0–2% of *GD* sterility), whereas eastern populations vary in *M* cytotypes (80–100% of *GD* sterility in the USSR). Nevertheless, the observation of intermediate levels of *GD* sterility (30–60%) in Central Europe suggests the existence of cytotype polymorphism in this area, as has been shown in North Africa (21) and Japan (22). As seen in Table 1, column 2, the *GD* sterility levels observed in F_1 daughters from ♀♀ Canton S × ♂♂ wild type are practically null for eastern populations but can reach levels ranking from 2% to 9% in French populations. This suggests the presence of some *GD* potential *P* elements among the set of elements carried by those *P* cytotype populations. To determine if *P* elements are present in western populations and absent in eastern populations, as in old laboratory *M* strains, we carried out molecular level experiments.

Geographical Distribution of the Presence of *P* Elements. In 15 populations, randomly taken among the 27 listed in Table 1, samples of 50 females were tested for the presence of *P* elements by direct hybridization on *Drosophila* prints: 25 with the P1 probe and 25 with the P2 probe (Fig. 2*A*). As these two probes are free of the chromosomal sequences surrounding the *P* element in Pπ 25.1, all hybridization signal must be attributed to the presence of *P*-homologous sequences in the genomic DNA analyzed.

The results are illustrated in Fig. 2 *B* and *C*. The two probes give a very strong signal with Harwich flies (positive control) and no detectable hybridization with the old collected Gruta strain (negative control). They were found to hybridize with

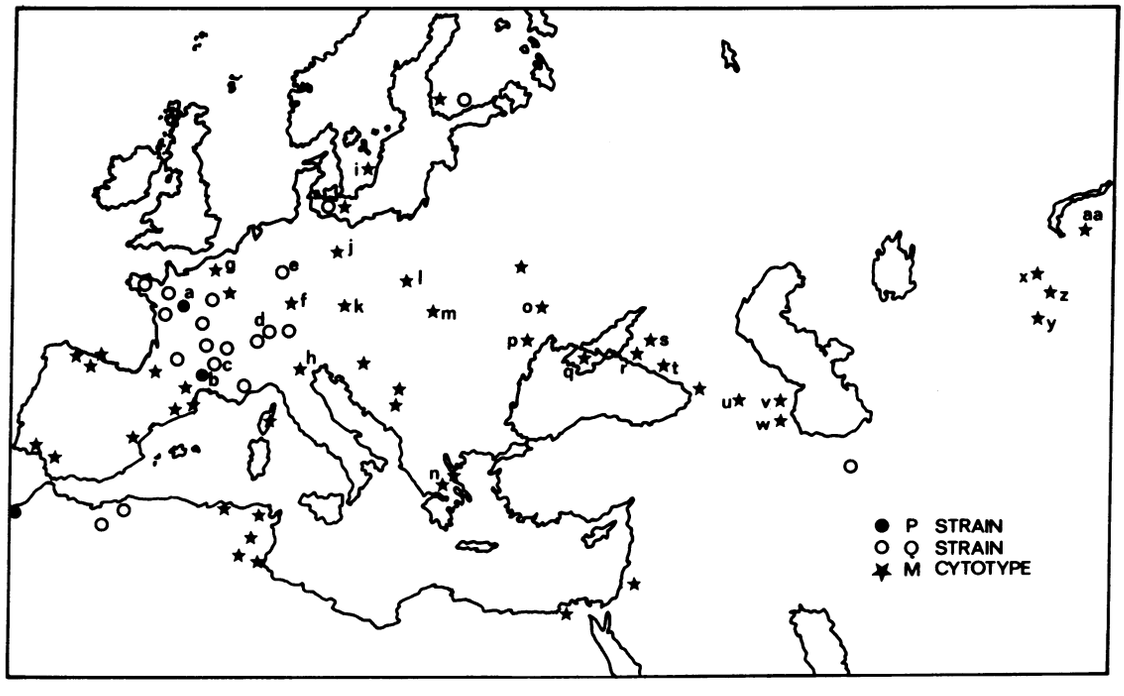

FIG. 1. Geographical distribution of Eurasian population of *D. melanogaster* according to the *GD* sterility potential in the *P–M* system. Letters indicate the populations sampled in this study and listed in Table 1.

Table 1. Description of *P–M* status of western to eastern Eurasian populations

Strain tested*	Cytotype cross A* ♀ T × ♂ H	*P* potential Cross A_1 ♀ C × ♂ T	*P* potential Cross A_2 ♀ *sn*^{w}*M'* × ♂ T	Intrastrain sterility	Signal level P1	Signal level P2
Angers (a)	2	9	18	0	5	4
St. Christol (b)	0	5	8	0	5	4
Pinols (c)	0	1	5	0	5	4
Oron (d)	2	0	5	0	5	4
Giessen (e)	0	3	18	0		
Tubingen (f)	37	0	2	0.5	5	3
Lille (g)	53.5	2	0	0		
Padova (h)	61	0	4	0.5		
Djursvick (i) (iso-female line)	62.5	0	3	0	5	2
Berlin (j)	78	0	0	0	5	3
Ceske Budejovice (k)	86	0	2	0		
Krakow (l)	90	0	1	1	4	3
Uzhgorod (m)	72.5	1	3	0		
Athens (n)	77.5	0.5	1	2	4	3
Uman (o)	100	0	1	0		
Kischinev (p)	87.5	0	1	0	4	1
Magarach (q)	71	0	2	1		
Gelendzich (r)	99.5	0	2	0		
Krasnodar (s)	92	0	9	1	5	2
Pitsunda (t)	78	0	2	1.5		
Ubinskaya (u)	86	0	3	1		
Alexejevka (v)	92	0	7	0		
Lerik (w)	83	0	5	0	4	1
Dusanbe 82 (x)	95.5	0	1	3	4	1
Tachkent 81 (y)	94.5	0.5	1	1		
Chimkent (z)	99	0	4	0	3	1
Alma Ata 81 (aa)	97	0	3	0	2	ε

Percentages of F_1 females with gonadal dysgenesis in tests for the *M* cytotype (cross A*), *P*-factor activity (crosses A_1 and A_2), and interstrain sterility are given in parallel with the signal intensities obtained in hybridization experiments with the two complementary P1 and P2 probes of the cloned 2.9-kb *P* element.

*Letters in parentheses refer to populations indicated in Fig. 1.

all individuals tested from natural populations. Each fly of every population gave a similar signal intensity (Fig. 2*B*), but significant differences were obtained between strains (Fig. 2*C*). On a scale from 5 to 0, the visual signal intensity ranged from very dark spots similar to Harwich spots (noted 5) to very light spots (noted 1) (or ε for the weakest) but was always greater than the null Gruta spots (Table 1, columns 5 and 6).

The most western strains gave the strongest signals and an intensity gradient clearly appeared from east to west, in parallel with the results obtained by genetic analysis. Within natural populations, the signal intensity was generally weaker with the P2 probe than with the P1 probe, a difference not shown with the Harwich control. The most unexpected result was that all Eurasian flies tested gave a positive signal with each probe, showing the presence and the abundance of homologous *P* sequences in all of the populations analyzed in that area. This finding raised the question of the nature of such elements.

Investigation for Low Levels of *GD* Potential. To screen for the presence of some *P* elements with *GD* potential among the set of *P* sequences shown previously, tests with the *sn*^{w}*M'* strain were performed. Table 2 shows the higher susceptibility of this strain to detect *P* elements with *GD* potential. The *GD* sterility induced in crosses between *P–Q* reference males with *sn*^{w}*M'* females was up to 10 times greater than that observed in crosses with Canton S females. According to these tests, the so-called *Q* strains might in fact be classified as weak *P* strains (10, 23). Conversely, when *sn*^{w}*M'* females were crossed with Canton S males, known to be devoid of *P* elements (7), the resulting frequency of atrophic ovaries was very low, at 3.5×10^{-3} ($n = 1000$ F_1 females in 20 independent replicates). Similarly, the level of *sn*^{w}*M'* intrasterility was only 7.5×10^{-3} ($n = 1000$). Thus, the use of the *sn*^{w}*M'* strain leads to the more efficient demonstration of the presence of rare *P* factors in the Eurasian population analyzed here.

The results of these crosses are presented in Table 1 (column 3). The levels of induced *GD* sterility were significantly higher (25 times over 27 samples; $P < 0.001$ by the sign test) than those obtained with Canton S and thus revealed the presence of some *P* factors in the majority of Eurasian populations. A correlative difference exists between *P*-cytotype western populations with relatively high levels of induced *GD* sterility and *M*-cytotype (central and eastern) populations with low levels of induced *GD* sterility (Table 1, Fig. 2*C*). This result corroborates the pattern of a gradual distribution of the polymorphic *P*-element family between France and Middle Asia.

DISCUSSION

The genetic and molecular investigations of the present survey provide evidence that the variable distribution of *P* elements explains the geographical distribution of the *P–M* system. Differences observed between natural populations are the reflection of the relative frequencies of *P*-, *Q*-, and *M*-type individuals in those populations. In this sense, the geographical pattern observed from France to Middle Asia can be described as a gradually decreasing frequency of *P* elements in *D. melanogaster* populations.

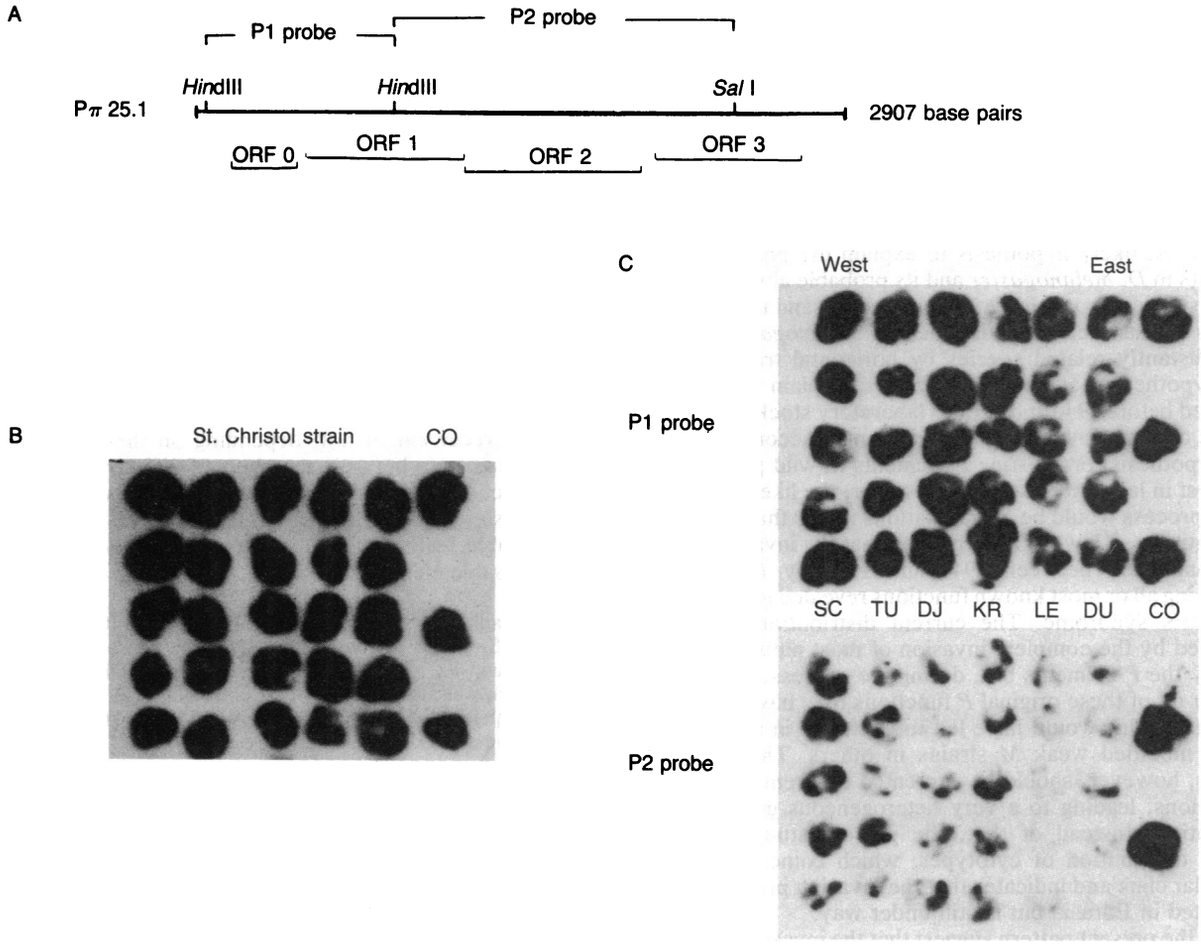

FIG. 2. (*A*) Structure of the 2.9-kb P 25.1 element showing the two restriction fragments *Hin*dIII (P1) and *Hin*dIII–*Sal* I (P2) used to probe individual flies from natural populations by direct hybridization on prints. ORF, open reading frame. (*B*) Direct hybridization with the P1 probe on individuals from the St. Christol population. Each fly presents a positive signal with about the same intensity. CO, control. (*C*) Example of response gradient obtained by hybridization with P1 and P2 probes on individual flies of six populations randomly taken from west to east. SC, St. Christol; TU, Tubingen; DJ, Djursvick; KR, Krasnodar; LE, Lerik; DU, Dusanbe. In the control (CO) column, individuals from Harwich (positive control) and Gruta (negative control) are shown in alternate vertical positions.

At the phenotype level, this variation explains the presence of western *P*-cytotype and eastern *M*-cytotype populations, assuming the switch from an *M* to a *P* cytotype results from the accumulation of *P* elements in the genome (either in their total number or, more likely, in the number of active *P* elements encoding the required functions). At the molecular level, the gradually decreasing frequency of *P* elements results in the observed gradient of hybridization signals. Their level is known to be correlated with the number of *P* sequences in the tested fly (19). As a first approximation, a western population giving a response of the same intensity as that obtained with Harwich might harbor 30–50 copies of *P*

Table 2. Use of the *sn*^{w}*M* strain to reveal low levels of *GD* potential in males of different *P–Q* and *M* reference strains

Strain tested	Cytotype T H	*P* potential T C	*P* potential T *sn*^{w}*M*	Intrastrain sterility
2	2	98	100	0
Harwich	0.15	98	100	0.15
TOO7Cy				
[]	0	75	94.5	0
[cy]	0	0.5	1	
Loua 83	0	15.5	40	1
Raleigh 82	0	13	39	0
MRh12Cy				
[]	0	6	64	0
[Cy]	0	2.5	26	
Mont Carmel	4	0	1	1
MR GB 39	24	0.5	1.5	1
Canton S	98	0	0.35	0
sn^{w}*M*	100	0	0.75	0.75

elements, as in this reference strain (7). In the Middle Asian populations, none of the flies tested failed to give a positive signal with the two probes, but some individuals showed very weak signals. These were of the same order of magnitude as those of the $sn^{w}M$ strain, and the harboring individuals might possess a very limited number of *P* sequences (about three), as in this reference strain (4). In fact, all natural *M*-cytotype populations from France to Middle Asia today appear to be of the *M* type.

The most likely hypothesis to explain the presence of *P* elements in *D. melanogaster* and its probable absence in the sibling species *Drosophila simulans* (ref. 24 and unpublished results) is that *P* elements invaded *D. melanogaster* from a more distantly related species by horizontal transfer (25). Two hypotheses have been advanced to explain why strains collected before 1940 and kept as laboratory stocks are of the *M* type and are devoid of any *P* elements. According to the first hypothesis, preexisting *P* elements in wild populations were lost in laboratory stocks. However, the likely outcome of this process would be *M* populations rather than *M* ones. Alternatively, Kidwell (12) proposed that the invasion might have happened between 1940 and 1960 by *P* elements possessing all or most known functions revealed in the hybrid dysgenesis syndrome. The current distribution might be explained by the complete invasion of most natural populations by the *P* elements but, during the process, the loss of some or all of these original *P* functions may have occurred frequently. This would have led to *Q* strains in some areas and to modified weak *M* strains in others. This type of process, however, should have created different patches of populations, leading to a very heterogeneous geographical distribution. Instead of that, the present study shows a gradual distribution of cytotypes, which coincides with a molecular cline and indicates that the invasion process is not completed in Eurasia but is still under way.

Does the present pattern suggest that the invasion began in Middle Asia and spread westward, ultimately reaching the American continent and leaving behind *M* populations? Or did the invasion occur the other way around? This latter hypothesis is favored by the findings that the oldest laboratory strains with *P* activity are North American samples collected between 1950 and 1960 (12), whereas the oldest French ones date back to the middle of the 1960s (13). Moreover, only one such *P* strain has been found in Central European and USSR samples since 1936 (unpublished data). We propose the invasion might have started in North America and spread to South America, Australia, and Central Africa, where *P–Q* populations are found today. In Europe and North Africa, the invasion presently would be spreading to eastern localities, where the *M* strains represent the advancing wave front. During this process the frequency of *P* elements increases by transposition, inducing mutations (26, 27) and progressively switching the *M* cytotype. A similar situation would happen the other way around, with the *P* elements presently spreading in Chinese populations.

Moreover, both the number and structure of elements are of importance. Our observations indicate that left and right parts of the 2.9-kb *P* element do not appear equally frequent in Eurasian populations. This may correspond to parts having different roles and different dynamics. The right part might be involved in the determination of the *P* cytotype (as the eastern females with the strongest *M* cytotype only poorly hybridize with the P2 probe) and might be preferentially deleted during transposition.

The occurrence of a high frequency of *P* elements inducing *GD* sterility, restricted to the Americas, Central Africa, and Australia, may be due either to a different timing of the invasion process, to foundation effects on the relative number of active elements, to the genomic response of the recipient population, or to environmental factors not found in Eurasia.

The invasion hypothesis also has been applied to the *I*-element family, which was thought to have totally invaded the global *D. melanogaster* between 1930 and 1970. However, recent evidence has shown that *I*-homologous sequences are also present in old laboratory strains (28). By using available data (12), a similar invasion scenario might be proposed, assuming not necessarily a second horizontal transfer but the activation of some previously silent elements and their propagation.

The occurrence of two such takeover events in this century would imply an unreasonably high rate when considered on an evolutionary time scale. The population genetics of transposable elements may in fact deal with very rapid phases, recurrent or not, depending on the compatibilities and interactions between members of different families (14). The outcome of studies of the geographical distribution of the *P–M* system could help in answering some of those questions and enable testing of the theory of speciation induced by transposable elements (29).

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