

Molecular Characteristics of Diverse Populations Are Consistent with the Hypothesis of a Recent Invasion of *Drosophila melanogaster* by Mobile *P* Elements¹

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Approximately 100 strains derived from natural populations of *Drosophila melanogaster* were tested for the presence or absence of *P*-element sequences by using two molecular probes derived from internal regions of a full-sized *P* element. Strains that had been collected from several continents at varying times during the past 60 years were examined. The oldest available strains, representing most major geographical regions of the world, exhibited no detectable hybridization to the *P*-element probes. In contrast, all recently collected natural populations that were tested carried *P*-element sequences. The earliest appearance of *P* elements occurred in collections made during the 1950s and early 1960s in the Americas and during the late 1960s on other continents. The youngest strains that were completely devoid of *P* elements originated in populations sampled during the mid-1960s in America, but as late as 1974 in populations from the USSR. There are differences in the patterns of hybridization to the two *P*-element probes between populations from different geographical regions. These differences are consistent with the varying P-M phenotypic properties of these populations. Taken together with the results of phenotypic tests reported in earlier studies, the available evidence is consistent with the hypothesis of a worldwide *P*-element invasion of *D. melanogaster* during the past 30 years and suggests that the putative invasion of the Americas possibly preceded by approximately a decade that in Europe, Africa, and the rest of the world.

Introduction

Among those eukaryotic mobile elements that have been adequately studied, the *P*-element family of *Drosophila melanogaster* is unusual in its genomic distribution. *P* elements are present in multiple copies per genome in individuals of some populations yet are completely absent from others (see, e.g., Bingham et al. 1982; Todo et al. 1984; Sakoyama et al. 1985). Previous results from phenotypic tests had suggested that presence or absence of *P* elements is closely related to the length of time that has elapsed since collection of a population sample from the wild (Kidwell 1983). Further, major differences in both the phenotypic and molecular properties of the *P*-element family have been observed in populations having different geographical origins (Anxolabéhère

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et al. 1982, 1984, 1985*b*; Kidwell and Novy 1985; Boussy 1987). The study of these distributional differences is providing interesting and important clues to the population dynamics and evolutionary history of this transposable-element family.

P elements constitute one of several structural classes of mobile elements that are found in *D. melanogaster* (Rubin 1983). Although *P* elements are not present in species that are closely related to *melanogaster* (Brookfield et al. 1984), these elements are common in a number of more distantly related *Drosophila* species, such as those of the *willistoni-saltans* and *obscura* groups (Anxolabéhère et al. 1985*a*; Lansman et al. 1985; Daniels and Strausbaugh 1986; Stacey et al. 1986). In *D. melanogaster* the degree of sequence similarity is high among different members of the *P*-element family, but there is considerable variability in sequence length. On the basis of structure, *D. melanogaster* *P* elements can be divided into two types, complete (autonomous) elements and those that are nonautonomous or defective. The complete element (sometimes called the *P* factor) is 2.9 kb in length and has 31-bp terminal repeats. It has four open reading frames, all of which are used to encode a transposase enzyme (O'Hare and Rubin 1983). Nonautonomous elements are smaller and variable in size and are derived from autonomous elements by internal deletions. The frequency of such deletions is high under conditions when transposition is actively taking place (Daniels et al. 1985). Autonomous elements are able to catalyze their own transposition as well as that of some nonautonomous elements that lack this ability on their own (Spradling and Rubin 1982).

Populations and strains of *Drosophila* may be characterized on the basis of two properties related to the phenotypic effects of their *P* elements. Different strains may vary in their potential for mobilizing *P* elements when they are in an unregulated or susceptible state. We refer to this property as "P activity potential." Strains may also vary in their ability to regulate or suppress the activity of the *P* elements present in their genomes. We refer to this property as "P susceptibility"; it embraces the joint action of all mechanisms affecting *P*-element regulation, including that of cytotype (Engels 1979).

On the basis of these two properties, strains of *D. melanogaster* may be divided into two broad types, P strains and M strains, according to their phenotypic characteristics in diagnostic test crosses. P strains have the potential for P activity, but the level of this activity may vary from high to low. A subset of P strains that produce <10% gonadal sterility (Schaefer et al. 1979) on the basis of standard phenotypic tests are called Q strains (Kidwell 1979). In addition to the potential for P activity, P strains also have low levels of P susceptibility, a regulatory capacity that is often referred to as P cytotype (Engels 1979). However, recent work suggests that, in addition to P cytotype, other mechanisms may also be involved in *P*-element regulation (Kidwell 1985).

M strains rarely have any significant level of P activity potential. There are two main subtypes of M strains, true M and M' (pseudo-M). True M strains have extremely high *P*-element susceptibility, but that of M' strains varies from high to moderately low.

Major continental differences in the genomic distribution of *P* elements are observed when present-day American and European populations are compared. Almost all American populations are of the P/Q type (Kidwell and Novy 1985), but in European populations P strains are rare and M' strains are common. The level of *P*-element susceptibility varies widely across a gradient from west to east (Anxolabéhère et al.

1985b). Further, American populations differ in the level of their potential for P activity, but that of European populations is low and relatively invariable.

There is also evidence for distributional gradients in *P*-element properties in other continental areas. For example, a latitudinal gradient has been observed along the east coast of Australia (Boussy 1987; Boussy and Kidwell 1987). Populations vary from strong P in the north, to Q in the southeast, to M' in the south and Tasmania.

Kidwell (1979, 1983) attempted to integrate the spatial and temporal distribution patterns of *P*-element properties and discussed possible explanations for their existence. She proposed a sequence of events in which *P* elements invaded the species during the previous 30-year period and suggested that such a recent invasion might account for the observed distributional patterns.

A deficiency of many of the previous *P*-element population surveys is that they were carried out before the availability of *P*-element molecular probes; they were based on the phenotypic properties of P strains as determined by genetic crosses. The *P*-element family is notable in being associated with germ-line anomalies that produce a syndrome of phenotypic traits known as hybrid dysgenesis (Kidwell et al. 1977). Hybrid dysgenesis occurs when *P* elements are destabilized in germ-line cells in the presence of the maternally derived cytoplasm from a susceptible M strain. Dysgenic traits include temperature-dependent sterility, transmission-ratio distortion, and increased frequencies of mutation, recombination, and chromosomal aberrations. These traits are most dramatically produced in the F₁ hybrids of crosses between strong M females and strong P males. Phenotypic tests allow an accurate distinction between the two main types of P and M strains but cannot discriminate between M strains that are completely lacking in *P* elements (true M strains) and those that carry *P* elements with little or no phenotypic manifestations (M' strains). However, the squash-blot method has recently been developed (Tchen et al. 1985), which, together with different *P*-element probes, allows a determination of *P*-element properties at the molecular level and is suitable for use in population surveys.

The purpose of the present paper is to report the analysis of strains from diverse sources by using the squash-blot method in conjunction with tests of the strains' P-M phenotypic properties. The results of the present analysis, together with those of previous reports, are consistent with the hypothesis of a recent invasion of *D. melanogaster* by the *P* element during the past half-century. They also provide some information on the timing and direction of the invasion but raise a number of questions about the population dynamics and evolution of this process.

Material and Methods

Strains

The *Drosophila melanogaster* strains used in the present study had varied origins. Thirty-five strains that had originally been tested by Kidwell et al. (1983) were reanalyzed in one phase of the study. In addition, 71 European strains obtained from many different sources were also subjected to molecular and phenotypic tests.

Hybridization Techniques

The presence of *P*-element sequences were detected by the squash-blot technique using two sequences (P₁ and P₂) from the p π 25.1 *P* element as probes (Anxolabéhère et al. 1985a; Tchen et al. 1985). Figure 1 provides a restriction-enzyme map of the intact *P* element, illustrating the structures of the two probes. P₁ contains the 0.85-kb *Hind*III fragment of the *P* element that does not include the left-end inverted terminal

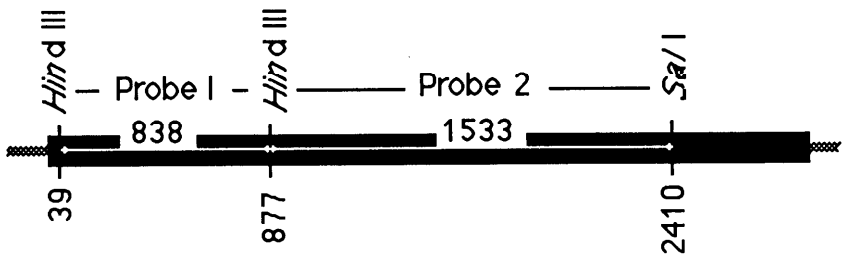


FIG. 1.—Restriction map of the complete *P* element, showing the structure of the P_1 and P_2 probes.

repeats. Because of P_1 's location at the left end, most *P* elements, including defectives, are expected to include at least some of the P_1 sequences. P_2 contains the internal 1.5-kb *Hind*III/*Sal*I *P*-element fragment. Because of P_2 's location, many defective *P* elements are expected to be lacking at least some—and sometimes all—of the P_2 sequence. The two probes were gel purified, and reassurance that they were not contaminated with genomic flanking DNA was obtained for each filter by observation of no detectable hybridization to the same probes when the M strain Gruta was used.

Twenty-five female *Drosophila* were squashed for each of the P_1 and P_2 tests. Each fly was crushed on a nylon filter membrane (Pall Biodyne), and the DNA fixed on the filter was denatured in 0.5 NaOH–2.5 M NaCl, neutralized in 3 M NaCH₃CO₂, prehybridized in $2 \times (0.15 \text{ M NaCl}, 0.015 \text{ M sodium citrate; SSC}) - 5 \times \text{FPG solution}$ (0.02% Ficoll 400/0.2% polyvinylpyrrolidone 350/0.02% glycine) for 2 h, and hybridized overnight at 65 C in $2 \times \text{SSC} - 1 \times \text{FPG}$ and 10% dextran sulfate solution with ³²P-labeled 0.85-kb *Hind*III restriction fragment (P_1) or the 1.5-kb *Hind*III/*Sal*I fragment (P_2). After hybridization the filter was washed for 1 h at 65 C in $2 \times \text{SSC}$, 0.1% SSC, 0.1% sodium dodecyl sulfate (SDS) and then rapidly at room temperature in $0.1 \times \text{SSC}$, 0.1% SDS.

Since both probes contain only *P*-element DNA, all hybridization signals must be attributed to the presence of the *P* homologous sequences in the genomic DNA analyzed. The strong *P* strain, Harwich, was used as a positive control, and the true M strain, Gruta, was used as a negative control. With this method, the signal intensity is roughly proportional to the amount of genomic DNA homologous to the probe (Tchen et al. 1985). In the present experiments we recognize five levels of signal intensity: S = strong, equivalent to the signal intensity of the positive control strain Harwich; I = intermediate; W = weak; E = extremely weak, equivalent to the signal intensity of the M' strain, singed weak (Tchen et al. 1985); and 0 = null, equivalent to complete absence of hybridization as seen in the negative control, the true M strain, Gruta. Examples of these five levels are illustrated in figure 2.

Phenotypic Tests

The standard tests for measuring gonadal (GD) sterility potential (Kidwell et al. 1983) were used. Two reference crosses were made for each tested strain: cross A = Canton S ♀♀ × ♂♂ of tested strain, and Cross A* = ♀♀ of tested strain × Harwich ♂♂. Cross A provides a measure of the *P* activity potential of a tested strain. Cross A* provides a measure of *P* susceptibility.

For each cross, 20–30 pairs of flies were mated en masse in half-pint milk bottles and immediately placed at 29 C. Approximately 2 days following the onset of eclosion, F₁ progeny were collected and allowed to mature for 2–3 days at room temperature

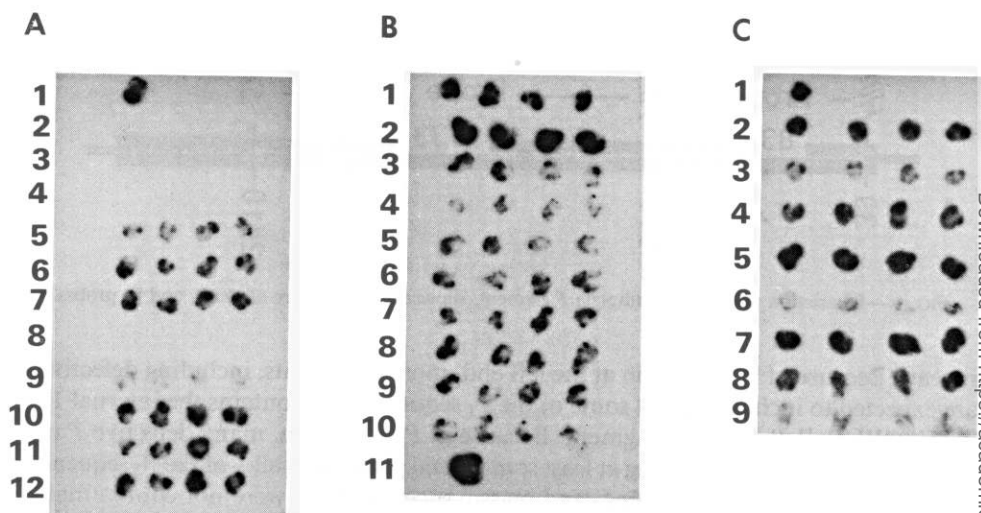


FIG. 2.—Examples of the responses to direct hybridization with the P_1 and P_2 probes. Five levels of signal intensity are recognized: S = strong; I = intermediate; W = weak; E = extremely weak; 0 = null. Four individuals were taken from each strain collected in three geographical areas: A, the Americas (P_2 probe): row 1, controls (S 0 0 0); row 2, Oregon R 1925 (0); row 3, Lausanne S 1938 (0); row 4, Cockaponsett Forest 1938 (0); row 5, Idaho Falls 1938 (I); row 6, Bermuda 1954 (I); row 7, Blacksburg 1954 (I); row 8, Painesville 1966 (0); row 9, Mount Sterling 1966 (E); row 10, Margarita 1970 (I); row 11, Marion 1970 (I); row 12, New Jersey 1971 (I). B, France (P_1 probe): row 1, Tautavel 1967 (S); row 2, Meze 1971 (S); row 3, Tostes 1973 (I); row 4, Chevreuse 1976 (I); row 5, Brouilly 1976 (I); row 6, Tostes 1976 (I); row 7, Meze 1978 (I); row 8, Menerbes 1979 (I); row 9, La Sirole 1981 (I); row 10, Tautavel 1982 (I); row 11, controls (S 0 0 0). C, the USSR (P_1 probe): row 1, controls (S 0 0 0); row 2, Krasnodar 1974 (S); row 3, Tashkent 1977 (I); row 4, Vitebsk 1978 (S); row 5, Gelendzhik 1978 (S); row 6, Alma Ata 1978 (W); row 7, Uman 1979 (S); row 8, Sini Gay 1979 (S); row 9, Alma Ata 1981 (W). In each part of the figure the four control individuals are Harwich (S) and Gruta (0 0 0).

(22 C). Fifty females were then taken at random for dissection. Dissected ovaries were scored as either normal or dysgenic (Schaefer et al. 1979), and the frequency of GD sterility was calculated by dividing the number of dysgenic ovaries by the total number of ovaries scored.

Results

To elucidate certain aspects of the P -element distribution that were previously ambiguous or poorly understood, the squash-blot method was used, together with standard phenotypic tests (see Material and Methods), to compare the properties of strains sampled from different geographical regions. A direct comparison of samples from different continents derived from the same time period was often not possible owing to limited availability. The strategy was therefore to attempt to identify, in each of the regions for which some material was available, the oldest strain carrying P -element sequences and the youngest strain not carrying these sequences.

The results of phenotypic and molecular tests on 35 strains collected from the Americas at varying time periods between 1925 and 1971 are presented in table 1. A large number of strains from North America have been collected and tested since 1971 (see, e.g., Engels and Preston 1980; Kidwell and Novy 1985; Simmons 1986). The results have provided strong indications that P elements were present in most, if not all, American populations after 1971. Because of limitations in the number of

Table 1

Phenotypic and Molecular Characteristics of Strains Collected in North and South America between 1925 and 1986

YEAR(s); STRAIN	P POTENTIAL ^a		P SUSCEPTIBILITY ^b		PROBE ^c	
	DIS ^d	Retest ^e	DIS ^d	Retest ^e	P ₁	P ₂
1925; Oregon R, USA	0		100		0	0
1935; Canton-S, USA	0		98		0	0
1936; Inbred, USA	0		92		0	0
1938; Lausanne S, USA	4		100		0	0
1938; NB1, Connecticut, USA	0		76		0	0
1938; Cockaponsett Forest, USA	0		61		0	0
1938; IF-38, Idaho Falls, USA	8	3	38	16	S	I
1950-55; Boa Esperanca, Brazil	0		96		0	0
1950-55; Gruta, Argentina	4		100		0	0
1950-55; San Miguel, Argentina	0		73		0	0
1952; 731C St. Paul, USA	4		97		0	0
1952; 91C St. Paul, USA	0	1	64	99	W	0
1954; NO1, New Orleans, USA	25	0	100	100	0	0
1954; NO2, New Orleans, USA	0		96		0	0
1954; RC1, Riverside, USA	12	1	95	100	0	0
1954; BER2, Bermuda	54	76	0	0	I	I
1954; BV1, Blacksburg, USA	13	9	5	0	I	I
1957-58; SC1, Santiago, Chile	2		91		0	0
1957-58; Ica, Peru	0	0	96	82	I	0
1961; CO3, Commack, USA	1		100		0	0
1961; MO1, Monroe, USA	3		100		0	0
1962; BOG1, Bogota, Columbia	0		94		S	I
1962; BOG3, Bogota, Columbia	25		95		S	S
1962; Ottawa, Canada	0		100		0	0
1963; RVC2, Riverside, USA	8	4	100	100	0	0
1963; Madison, USA	0		96		W	W
1966; 2A, Painesville, USA	0		100		0	0
1966; 3C, Mt. Sterling, USA	5		100		S	E
1966; 4B, Mammoth Cave, USA	4	0	92	100	0	0
1966; 5C, Red Top Mountain, USA	0		100		0	0
1966; 10E, S. Carolina, USA	0		100		0	0
1966; 11A, Oxford, USA	12	2	96	100	0	0
1970; Margarita, Venezuela	0		43		S	I
1970; Marion, USA	0	3	84	20	S	I
1971; SH-71G, New Jersey, USA	0	2	41	4	S	I
1971-86; 338 N. American iso♀lines	See text		See text			

^a Measured by % GD sterility in diagnostic cross A.

^b Measured by % GD sterility in diagnostic cross A*.

^c Strength of hybridization to the probes: S = strong; I = intermediate; W = weak; E = extremely weak; 0 = hybridization completely undetectable.

^d Results originally reported by Kidwell et al. (1983).

^e Results of retests carried out in France during April 1985.

strains that could be handled, the strains in table 1 collected after 1960 are not a representative sample of those actually available from this region and period; they were restricted to the available strains that were previously classified as phenotypically M, in order to make a distinction between true M and pseudo-M strains. All strains that were classified phenotypically as P are assumed to carry *P* elements.

Table 2
Phenotypic and Molecular Characteristics of Strains Collected in France
between 1938 and 1986

COLLECTION YEAR, STRAIN	P POTENTIAL ^a	P SUSCEPTIBILITY ^b	PROBE ^c	
			P ₁	P ₂
1938, Banyuls	0	99	0	0
1942, Champetieres	0	100	0	0
1945, Paris	7	100	0	0
1946, Charolles	0	87	0	0
1952, Banyuls	0	94	0	0
1957, Ajaccio	0	100	0	0
1965, Marseillan	0	90	0	0
1966, Chateaneuf	0	98	0	0
1967, Tautavel	28	2	S	S
1968, Lapeyrouze	0	100	0	0
1969, Saillé	0	96	0	0
1969, Kerbinou	100	3	S	S
1969, Menetreol	7	0	ND	ND
1969, Luminy	10	1	S	S
1970, Le Chatelet	61	0	S	S
1971, Meze	36	0	S	S
1972, Guerande (B2')	7	3	ND	ND
1972, Beaune	2	45	ND	ND
1973, Tostes	0	27	I	I
1973, Le Montet	0	5	ND	ND
1973, Tulle	0	53	ND	ND
1973, Meze	0	20	I	I
1976, Chevreuse	0	38	I	I
1976, Brouilly	0	30	I	I
1976, Tostes	0	0	I	I
1978, Meze	1	0	I	I
1978, Marseillan	2	0	I	I
1979, Menerbes	3	0	I	I
1981, La Sirole	0	0	I	I
1983, Tautavel	0	32	I	I
1984, Meze	4	1	I	I
1985, Tautavel	2	11	I	I
1986, Meze	0	2	I	I

^a Measured by % GD sterility in diagnostic cross A.

^b Measured by % GD sterility in diagnostic cross A*.

^c Strength of hybridization to the probes: S = strong; I = intermediate; W = weak; E = extremely weak; 0 = hybridization completely undetectable; ND = not determined.

The results of the molecular tests presented in the last two columns of table 1 indicate that the majority of strains collected in the Americas before 1960 had no detectable similarity to the *P*-element probes. However, there were several exceptional strains from different time periods that appeared to carry *P*-element sequences. The oldest tested strain from the American continent with detectable *P*-element sequences was collected in 1938. The youngest strains that showed no detectable hybridization to the *P*-element probe were collected from nature in 1966.

In table 2 are presented the results of phenotypic and molecular tests of strains collected in France between 1938 and 1985. In this instance, almost all the strains

available as late as 1981 have been included in the analysis. Unfortunately the number of available strains with collection dates prior to 1970 is meager for this and many European countries. No tested strain collected after 1969 was completely lacking in *P*-element sequences. However, the oldest French strain with detectable hybridization to the *P*-element probe was collected as late as 1967 (Tautavel). The youngest strain lacking *P*-element sequences was collected in 1969.

Table 3 presents comparable data from strains collected in Czechoslovakia and the USSR. Again, all available strains have been included in the analysis and collections prior to 1970 are small in number. There is good agreement with the data from France (table 2) in that the oldest strain carrying *P* elements was collected in 1967. Several strains collected from the USSR as late as 1974 are completely lacking in *P*-element similarity.

When the results of tables 1–3 are considered together, there is a good correlation between low *P* activity potential, high *P* susceptibility, and absence of any hybridization signal. Moreover, previously observed differences between North America, France, and the USSR are confirmed. In particular, recently collected strains from North America are either *P* or *Q*, those from France are predominantly *Q*, and those from the USSR and Czechoslovakia are *M'*.

A broad view of the temporal and geographical trends in the distribution of *M* and *P* strain types for four major geographical regions may be obtained from the summary presented in table 4. All the available cross *A** data on GD sterility, taken from the present study and several published and unpublished sources (Kidwell 1983; Kidwell et al. 1983; Anxolabéhère et al. 1984, 1985*b*; Kidwell and Novy 1985; M. G. Kidwell, unpublished results), have been included in this table. The cross *A** test (Material and Methods) provides a measure of *P*-element susceptibility. This test provides an unambiguous indication of the presence of *P*-elements because *P* susceptibility occurs only in the absence of *P* factors (Sved 1987).

The summary data of table 4 are illustrated in the world maps, presented in temporal sequence, in figure 3. The maps illustrate the changes in distribution of strain phenotypic properties during five consecutive time periods between 1920 and 1986. It is seen that, according to the cross *A** criteria, no *P/Q* strains appear anywhere before 1950. *P/Q* strains subsequently appear, first in the Americas and later on other continents. This observation, together with the consistent patterns of increasing frequencies of these types of strains, indicates that their early geographical distribution is nonrandom. In all time periods after 1950, the proportion of tested strains that are *P/Q* is consistently higher in the Americas than in any other continent.

Discussion

The results of the molecular analysis indicate that the majority of laboratory strains derived from natural populations in several geographical regions before the mid-1960s are completely lacking in *P*-element sequences. It was also determined that strains collected as late as 1966 in North America and as late as 1974 in the USSR were completely devoid of *P* elements. In contrast, all recently derived and present-day populations that have been tested carry *P* elements. These results are concordant with and parallel to the results of the phenotypic analyses and show a progressive increase in the presence of *P* elements over a very short evolutionary time period.

On the basis of the combined results of the molecular and phenotypic analyses, the patterns of *P*-element distribution over time do not appear to be identical for all continents. In figure 4 the available strains are shown schematically below the horizontal

Table 3

Phenotypic and Molecular Characteristics of Strains Collected in Czechoslovakia and the USSR between 1936 and 1984

COUNTRY AND COLLECTION YEAR, STRAIN	P POTENTIAL ^a	P SUSCEPTIBILITY ^b	PROBE ^c	
			P ₁	P ₂
Czechoslovakia:				
1969, Crkwenica	0	100	0	0
1969, Hodejice	0	7	I	W
1972, Moravski Pisek	0	100	W	W
1973, Krizanovica	0	0	S	I
1977, Zabcice	2	96	S	W
1978, Kravi Hora	0	19	S	I
USSR:				
1936, Crimea	0	100	0	0
1936, Samarkand	0	100	0	0
1960-65, Magarach (LM)	8	100	0	0
1961, Gurzuf	1	100	0	0
1964, Dilizhan	0	96	0	0
1966, Uman	1	100	0	0
1966, Dilizhan	6	100	I	W
1967, Magarach	93	1	I	I
1967, Dilizhan	7	89	0	0
1968, Zolotonosha	3	88	0	0
1970, Uman	10	100	0	0
1970, Frunze	5	100	0	0
1974, Borsk	4	100	0	0
1974, Gorky	0	100	W	0
1974, Krasnodar	3	14	S	0
1977, Kurdamir	1	67	S	W
1977, Tashkent	0	99	I	E
1978, Gelendzhik	10	0	S	W
1978, Vitebsk	1	98	S	W
1978, Anapa	1	97	I	E
1978, Alma Ata	5	100	W	W
1978, Shahrinai	11	97	S	W
1979, Uman	3	98	S	W
1979, Sini Gay	0	74	S	W
1981, Alma Ata	1	97	W	E
1982, Dushanbe	1	96	S	W
1983, Uman	1	99	S	W
1983, Kischinev	1	88	S	W
1983, Krasnodar	5	92	S	I
1983, Lerik	3	83	S	W
1983, Chimkent	2	97	I	W
1984, Magarach	3	95	S	W

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^a Measured by % GD sterility in diagnostic cross A.

^b Measured by % GD sterility in diagnostic cross A*.

^c Strength of hybridization to the probes are: S = strong; I = intermediate; W = weak; E = extremely weak; 0 = hybridization completely undetectable.

lines, which represent the three geographical regions that were studied. Above the lines are shown the strains that were actually tested, with the first two P and M' strains and the last two true M strains specifically indicated. With one exception (IF-38), it

Table 4
Numbers (Percentages) of Tested Strains Collected in Four Major Geographical Regions during Five Time Periods according to Their Ability to Suppress P Activity

PERIOD	REGION							
	Americas		Europe and Asia		Africa		Orient and Australia	
	M ^a	P ^b	M ^a	P ^b	M ^a	P ^b	M ^a	P ^b
1920-49	11 (100)	0 (0)	10 (100)	0	3 (100)	0
1950-59	11 (84.6)	2 (15.4)	11 (100)	0	4 (100)	0	11 (91.7)	1 (8.3)
1960-69	6 (31.6)	13 (68.4)	24 (85.7)	4 (14.3)	4 (80)	1 (20)	9 (75.0)	3 (25.0)
1970-79	4 (7.5)	49 (92.5)	35 (51.5)	33 (48.5)	4 (67.7)	2 (33.3)	9 (39.1)	14 (60.9)
1980-86	1 (3.6)	27 (96.4)	50 (55.5)	40 (44.5)	11 (36.7)	19 (63.3)	16 (43.2)	21 (56.8)

^a Strains that have high susceptibility to P factor activity.

^b Strains that have low susceptibility to P factor activity (i.e., strains that are classified as P or Q).

is seen that the first P and M' strains cluster together between 1952 and 1954 in the American samples. This is in contrast to the clustering of the first P and M' strains in the late 1960s and early 1970s in France and the USSR. The latest appearance of true M strains was in 1966 in North America, in 1969 in France, and in 1974 in the USSR.

Because of the difficulties posed by the unequal availability of samples from different geographical regions and periods, it seems important that not too much weight be attached to precise dates of collection in certain instances. However, the date of the last appearance of true M strains in a geographical region provides an indication that such strain types existed in nature at least that recently. Accurate identification of the time when the first P and M' strains appeared is difficult because of the possibility of contamination of strains during their subsequent culture in the laboratory. It seems likely that P-element contamination of a few strains may have occurred in some instances, subsequent to their establishment in the laboratory. Contamination is likely to have been more frequent in areas in which P strains appeared first in the wild than in those in which they appeared later. Thus, there is a possibility that some of the earliest-collected American P strains may actually have been contaminated M strains. In particular, the origin of P elements in the strain IF-36 is questionable because its properties are somewhat inconsistent with those of other strains collected before 1950.

It is difficult to explain the overall distribution pattern in terms of the hypothesis of recent loss of P elements (Engels 1981b, 1986). There are two properties of Eurasian populations that suggest that they may lose their P elements faster than do North American strains: (1) they tend to have fewer P elements, and (2) they are more likely to have M cytotype. However, that period of <30 years during which, under this hypothesis, Eurasian-derived laboratory stocks would have to lose all their P elements is extremely short. Neither empirical studies on the effect of laboratory culture on genomic P-element copy number (M. G. Kidwell, unpublished results) nor theoretical studies (Charlesworth 1985; Kaplan et al. 1985) provide any support for the idea that a genome can rid itself of all its elements in such a short period of time.

On the other hand, the data reported here are fully consistent with the hypothesis of a worldwide recent invasion of *Drosophila melanogaster* (Kidwell 1979, 1983); further, they provide some evidence concerning the location of the origin of P elements in this species. The following invasion scenario is consistent with the available data:

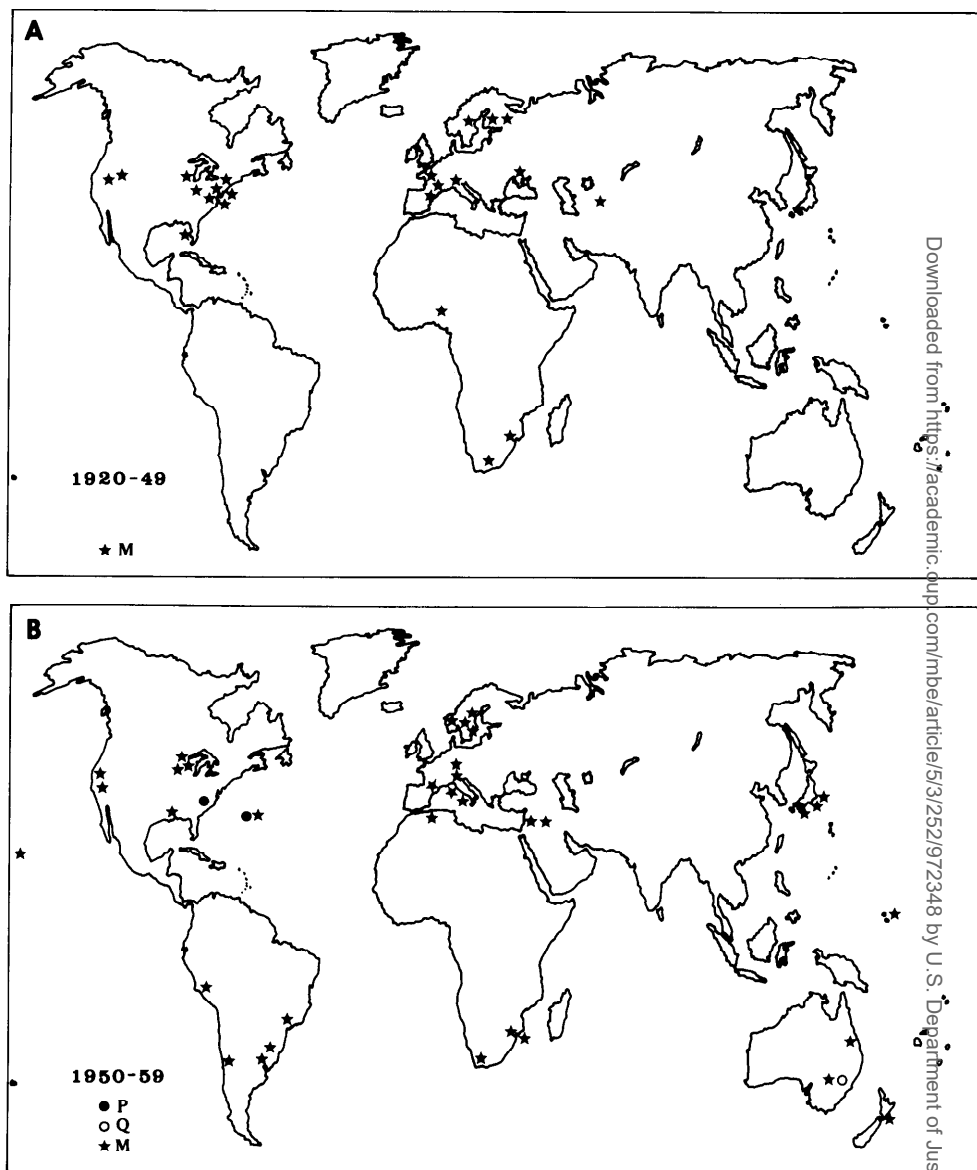


FIG. 3.—The geographical distribution of strains collected from the wild during five time periods and classified according to their cross A* phenotypic characteristics in the P-M system of hybrid dysgenesis. A, 1920–49; B, 1950–59; C, 1960–69; D, 1970–79; E, 1980–86. Strains and isofemale lines collected at the same location and having the same characteristics are represented by a single symbol.

1. The *P*-element family entered the *D. melanogaster* species some time prior to 1950. The time of origin of *P* elements in the species cannot even be approximately determined on the basis of our limited data because *P* elements may have been present in this species for a long period of time but restricted to a small local population prior to their rapid spread (Uyenoyama 1985). The present data are consistent with the idea that the *P* element may have originated in *D. melanogaster* in an American population; but, because of poor sampling, other possibilities cannot be excluded.

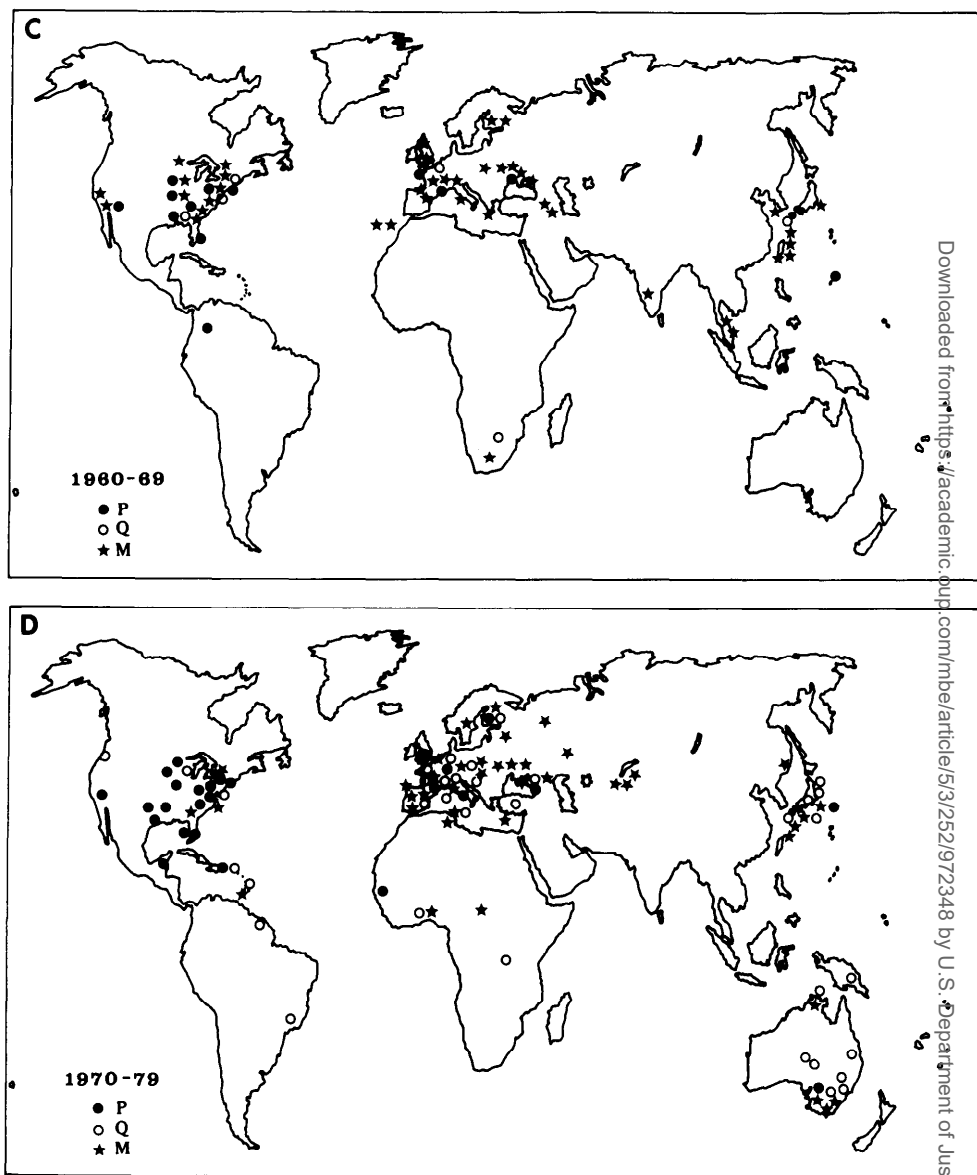


FIG. 3.—Continued.

2. The *P* element started to invade North- and South-American populations during the 1950s and spread to most regions of these continents during the 1960s and 1970s. It is not clear, however, whether all American populations are now at equilibrium with respect to their *P*-element properties; but there is evidence that some of them may be. Single-location samples taken over a 10-year period (Kidwell and Novy 1983), although showing seasonal annual fluctuations in mean *P* activity potential and *P* susceptibility, did not show any consistent changes over the whole period. Also, Simmons (1985) found little phenotypic evidence for *P* activity in a recently sampled natural population in California.

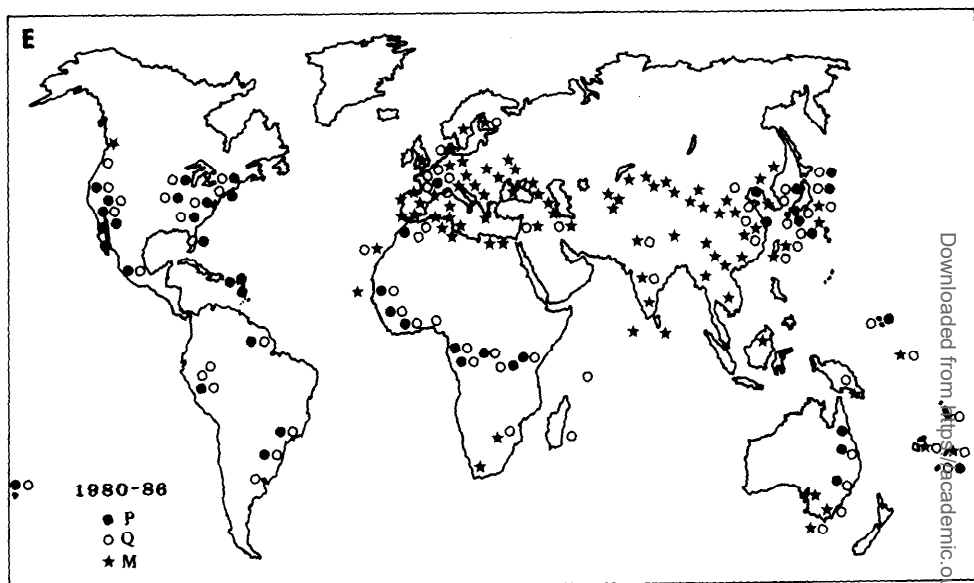


FIG. 3.—Continued.

3. The postulated *P*-element invasion of Europe and other continental regions was largely initiated during the 1960s and early 1970s, and the process may not yet be complete. The existence of isolated island reservoirs of true *M* populations cannot be excluded. The current distribution of *P*-element properties in other continental regions differs in several important respects from that in the Americas. Only in the

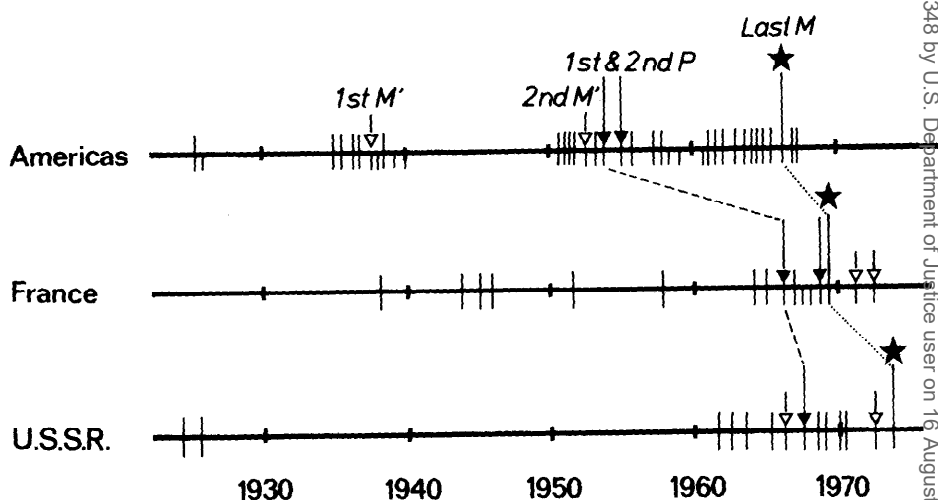


FIG. 4.—Graphic representation of the extensiveness of sampling of available strains up to the date of collection of the last true *M* and the first and second *P* and *M'* strains in the three main geographical regions surveyed. Each vertical line that extends both above and below the horizontal lines represents a strain that was included in both the phenotypic tests and the molecular survey. Strains that were subjected to phenotypic tests only are shown as vertical lines below the horizontal lines. Dotted lines are used to join dates of appearance of the first *P* and the last true *M* strains that were identified in each of the geographical areas.

Americas is the widespread distribution of the P/Q type found throughout the continent; this type is currently present in several local areas such as northeastern Australia and central Africa, but by no means throughout the whole of these continental areas. M' strains are currently abundant in southeastern Australia, North and South Africa, Europe, Asia, and the Far East, and the existence of geographical gradients in *P*-element properties from P to M' appears to be a common feature in several areas.

There are several independent observations that support this scenario. In 1973, in two geographically separated populations from the Caucasus and middle Asia an outburst of mutability of the singed-bristle locus was reported (Berg 1974; Ivanov and Golubovsky 1977; Golubovsky and Belyaeva 1985). The X-linked singed-bristle locus is known to be a hot spot for P-M insertional mutagenesis (Golubovsky and Belyaeva 1985). Some *P* insertions at this locus are also known to be hypermutable and revert at inordinately high rates (Engels 1981a). Thus, the timing and other properties associated with the mutability wave are consistent with the *P* element-invasion scenario.

Watanabe et al. (1976) reported a sudden doubling of the frequency of lethal second chromosomes in a natural population from Katsunuma, Japan, between 1968 and 1970. The frequency of sterility also increased at that time. Mukai et al. (1985) found that by 1983 the lethal load in the Katsunuma population had returned to the original level. They accounted for this rise and fall by citing the invasion of a "*P*-type element." Although the history of *P* elements in Japan has not been directly addressed in the present report, the temporal distribution of these elements appears to be similar to that of elements in European populations (Kidwell et al. 1983).

There is some evidence that potential P-factor activity is higher in older than in younger populations, in both American and European populations. Kidwell (1983) provided evidence that P/Q populations collected during the 1960s in North America had, on average, a higher level of P activity than did those collected during the following decade. Also, perusal of tables 2 and 3 of the present report shows that strong P strains also tend to be restricted to the late 1960s and early 1970s in France and the USSR, at approximately the time when the postulated mutation "wave" (Berg 1974) was at its peak.

One of the most difficult unanswered questions is how, under the invasion hypothesis, the striking qualitative differences in *P*-element distribution between geographical regions could have arisen. The difficulty appears to be due to our current ignorance about the detailed molecular mechanisms that regulate *P*-element transposition. The recent work of H. Robertson and W. R. Engels (personal communication) and Black et al. (1987) may provide a clue to the resolution of this issue. It was originally proposed that *P*-element regulation is always closely associated with the development of a cellular state called P cytotype (Engels 1979, 1981a), which is itself dependent on the presence of *P* factors in the genome (Sved 1987). The molecular basis of P cytotype is not clearly understood; the two-component model (O'Hare and Rubin 1983) proposes that a *P* element-encoded regulator molecule is involved. H. Robertson and W. R. Engels (personal communication) have found preliminary evidence for such a molecule. The alternative, one-component model (Simmons and Bucholz 1985) suggests that P cytotype may be determined by a *P*-element titration mechanism involving the terminal repeats of both autonomous and nonautonomous elements. Mixed-population experiments (Kidwell et al. 1981; Kiyasu and Kidwell 1984) have shown that when strong P and true M strains were mixed, *P* elements rapidly took over the population. The P cytotype and P activity of the resulting mixed population achieved, in a few generations, the same levels as those of the original P

strain. It appears that the pattern of regulation of *P* elements, at least in North America, is consistent with the development of *P* cytotype.

In other experiments (Anxolabéhère et al. 1986), it has been shown that the outcome of evolution in mixed populations depends on the type of strains involved. When strong *P* and different *M'* or *Q* populations were combined, *P* susceptibility rapidly disappeared, but *P* activity potential reached different equilibrium levels, which were positively correlated with the level of *P* susceptibility of the initial *M'* or *Q* strains used. When weak *P* and true *M* or *M'* strains were mixed, *P* susceptibility decreased slowly over 30 generations, with sometimes some residue remaining, and *P* activity potential decreased slowly to values at or near zero. These results lead to the hypothesis of innovative stepping-stone invasions (Anxolabéhère et al. 1986, 1987). This hypothesis proposes that, during the initial invasion of American populations, *P* elements underwent internal deletions leading to moderate *P* strains that harbored a mixture of autonomous and nonautonomous elements. The subsequent invasion of Eurasia resulted from the introduction of individuals of such strains into that region and led to weak *P/Q* populations in France (the first region postulated to have been invaded). New deletions occurred during this process, and these subsequently radiated into the rest of Europe and Asia and now are found in the *M'* strains recently sampled from these areas.

This hypothesis is consistent with the results of the present study and with the work of Black et al. (1987), who found a 1.15-kb nonautonomous *P* element, called the *KP* element, which is present in many (up to approximately 30) copies in many European strains. The single internal deletion sustained by the *KP* element removes all sequences homologous to the *P*₂ probe used in the present study (see fig. 1). Indeed, perusal of table 3 indicates that, almost without exception, all *P* element-bearing strains from Czechoslovakia and the USSR show significantly weaker hybridization to the *P*₂ probe than to the *P*₁ probe. This is not true, however, for the recently collected French strains, many of which are classified as *Q* strains (table 2) and in which the strength of hybridization to the two probes is almost always equal. Evidence from genetic experiments has been obtained (Black et al. 1987; M. Jackson, personal communication) suggesting that *KP* elements have a regulatory role in suppressing both *P* activity and the production of dysgenic traits in those individuals in which they are present in multiple copies. Thus, it is possible that two different types of regulatory mechanisms may be operating to determine the qualitatively different patterns of *P*-element distributions in different geographical regions, such as North America and central Europe. Moreover, the distribution of *P* elements in Eurasia also shows quantitative variation. The gradual decrease in strength of hybridization to the *P*-element probes from west to east (Anxolabéhère et al. 1985b) has been confirmed by *in situ* hybridization, revealing a correlated decrease in the number of *P* sequences (S. Ronsse- ray, personal communication). Many outstanding questions about how this distribution evolved will have to be answered if we are to fully understand the dynamics of *P* elements in natural populations.

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