Phenotypic stability of the P-M system in wild populations of Drosophila melanogaster

Masanobu Itoh*,1 Tomokazu Fukui,1 Miyako Kitamura,1 Takahisa Uenoyma,2 Masayoshi Watada3 and Masamitsu Yamaguchi1

1Department of Applied Biology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606-8585, Japan
2Kobe Gakuin Women’s College, Kobe 653-0861, Japan
3Department of Biology & Earth Sciences, Ehime University, Matsuyama 790-8577, Japan

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The P element appears to be one of the most recently invaded transposons of D. melanogaster. To study the dynamics and long-term fate of P elements in natural populations of D. melanogaster, 472 isofemale lines newly collected from 27 localities of Japan were examined for the P element-associated characteristics (abilities to induce and repress of P element transposition) and genomic P element composition (size classes and their numbers). There was variation in the P element-related phenotypes among local populations, but genomic P composition did not correlate strongly with the phenotype of each line: full-size P and KP elements predominated in their genomes (FP+KP predominance). Comparison with previous results suggests a stability in the P-M system in local populations over about 15 years. In some populations, phenotypic stability for particularly long times was found: for 30 years or more Q strains predominated in Hikone and Tanushimaru, P or Q strains around Inakadate, and M or Q strains around Tozukawa. There was no clear evidence of structural destruction underlying functional variation of P elements during this period. These results suggest that the current evolutionary status of P elements in the gene pool of D. melanogaster is not an intermediary stage predicted by the original recent invasion hypothesis, and that several other factors such as the position effect play important roles.

Key words: Drosophila melanogaster, P element, KP element, P-M system, transposon

INTRODUCTION

Transposable genetic elements (TEs) have been shown to represent a considerable fraction of eukaryotic genomes (Berg and Howe 1989; Kidwell and Lisch 2002). In Drosophila, recent analyses revealed that TEs account for 3.9% of the euchromatic and 52% of the heterochromatic sequences in the genome (Kaminker et al. 2002; Hoskins et al. 2002). Molecular studies have shown that TEs can influence gene expression and chromosome structure. On the other hand, the nature of their evolutionary processes is still controversial (Charlesworth and Langley 1989; Labrador and Corces 1997). P transposable elements are the causative agent of P-M hybrid dysgenesis in Drosophila melanogaster (for reviews see Engels 1996; Rio 2002). Their transposition depends on the 87 kDa transposase (O’Hare and Rubin 1983; Rio et al. 1986), the 66 kDa repressor protein associated with P cytotype (Karess and Rubin 1984; Laski et al. 1986; Misra and Rio 1990), and some host factors (Rio and Rubin 1988; Kaufman and Rio 1992; Mul and Rio 1997). Complete 2.9 kb P elements encode both transposase and repressor, production of which is regulated by alternative splicing. On the other hand, many smaller P elements with internal deletions coexist in the genome. Among them, derivatives with specific deletions involving intron 3 are called type I repressor elements (Gloor et al. 1993) because of encoding the 66 kDa repressor or its equivalent. Smaller elements with larger deletions, such as the KP element, are called type II repressor elements, based on laboratory demonstrations of their repressor ability (Black et al. 1987; Jackson et al. 1988; Andrews and Gloor 1995; Simmons et al. 2002). In the P-M system, fly lines can be classified into three types by their phenotypes (P transposition-inducing
and -repressing abilities): $P$ strains with both; $Q$ strains with repressing ability; and $M$ strains with neither (Kidwell 1977; Kidwell et al. 1977; Engels and Preston 1980). An $M$ strain carrying some $P$ sequences in the genome is called $M'$ (pseudo $M$) strain to distinguish it from “true $M$” strains which are completely devoid of $P$ elements (Bingham et al. 1982).

The $P$ element is one of the most recently invaded transposons in $D. melanogaster$ populations. It is thought to have intruded into the $D. melanogaster$ genome from $D. willistoni$ by a horizontal transmission, perhaps about 1950 in the Americas, and to have spread rapidly world-wide (recent-invasion hypothesis: Kidwell 1983). The first fly strains carrying $P$ elements were found in the 1950s in the USA, the 1960s in Eurasia, and later in Australia and East Asia, suggesting the timing of the invasion (Kidwell et al. 1983; Anxolabéhère et al. 1988). The last published collection of true $M$ strain from wild was made in 1974 in the former USSR (Anxolabéhère et al. 1990; Ronsseray et al. 1991; Bonnivard and Higuet 1998). Empirical evidence suggests that $P$ elements became virtually ubiquitous in wild $D. melanogaster$ in the 1970s. The recent-invasion hypothesis (Kidwell 1983) proposed a possible evolutionary scenario of $P$ elements in $D. melanogaster$. An updated version of the hypothesis includes different size $P$ elements with different properties (Black et al. 1987; Jackson et al. 1988; Brookfield 1996; Quesneville and Anxolabéhère 1998). In this model, although the originally invading $P$ elements were complete, having the abilities to transpose and to achieve $P$ cytotype, smaller elements heterogeneous in size and function were produced by various internal deletions during transposition. The internally deleted elements explain the polymorphism in P-M system phenotypes in populations as due to a process of succession of $M$, $P$, $Q$, and $M'$ phenotypes in that order. This model presumes a simple relationship between P-M system phenotypes and the genomic elements, with many complete and some repressor $P$ elements in $P$ strain, many repressor elements but no complete elements in $Q$ strains, and no or few functional $P$ elements in $M'$ strain genomes. Computer simulations showed that $M'$ strains could be an equilibrium state for populations with migration among each other (Quesneville and Anxolabéhère 1998).

In contrast to the succession predicted by the hypothesis, molecular surveys of isofemale lines demonstrated that full-size $P$ and $KP$ elements were currently predominant in Australia, Africa, and Asia (Itoh et al. 1999, 2001; Itoh and Boussy 2002). And also only weak correlations were found between genomic $P$ element compositions and the phenotypes of many wild populations (Todo et al. 1984; Anxolabéhère et al. 1985, 1988, 1990; Boussy and Kidwell 1987; Boussy et al. 1988, 1998; Biemont et al. 1990; Ronsseray et al. 1991; Bonnivard and Higuet 1999; Itoh et al. 1999, 2001; Itoh and Boussy 2002).

These intriguing facts raise questions: What determines the $P$ element-related phenotypes in wild populations? What is the current status of $P$ elements in $D. melanogaster$? What is the long-term fate of $P$ elements in the species? In an attempt to address these questions, we analyzed 472 isofemale lines collected from 27 localities of Japan. Here we report the results, and emphasize the persistence (up to 30 years) of particular P-M phenotypes in local populations. Our present results do not appear consistent with the simple expectation from the recent-invasion hypothesis of $P$ element dynamics.

**MATERIALS AND METHODS**

**Flies** Wild flies were collected using banana traps or by net-sweeping over *Drosophila*-attractive sites (e.g., piles of fermenting fruits waste in orchards, or pomace heaps near wineries) in 20 localities of Japan (Table 1). After collection, inseminated females were kept individually in vials to establish isofemale lines. In total, 373 isofemale lines were established from 1997 to 2001. Together with some already established populations (Itoh et al. 2001; Itoh and Boussy 2002), 472 isofemale lines from 27 localities in 1989) (Gamo et al. 1990; Nishino et al. 1993) were kindly gifted by S. Gamo. Flies were maintained on standard food medium at 25°C except for the cross for the GD test (see below).

**Gonadal dysgenesis (GD) tests and definition of lines** With Harwich and Canton S as $P$ and $M$ standards, respectively, two kinds of crosses, A (Canton S females x tested males) and A* (tested females x Harwich males), were performed at 29°C (Kidwell et al. 1977; Engels and Preston 1980). All F1 females were individually dissected and the GD score for each line was calculated as the percentage of undeveloped ovaries. More than 50 F1 females were dissected for each cross.

P-M characteristics were defined according to Kidwell et al. (1983) and Quesneville and Anxolabéhère (1998); $P$ strains (>10% GD in cross A and <10% GD in cross A*), $Q$ strains (<10% GD in both crosses), $M$ strains (<10% GD in cross A and >10% GD in cross A*), and exceptional $P$ strains (>10% GD in both crosses). Populations were classified into four types, which are characterized by the strains they comprise; type 1 by $P$ and $Q$, type 2 by $Q$ and $M'$, type 3 by all of $P$, $Q$, and $M'$, and type 4 by $Q$ alone (Matsuura et al. 1993).

**Genomic DNA and Southern blot hybridization** Fly genomic DNA was extracted from ten adults using standard methods. For Southern blot hybridization, genomic DNA was digested with *DdeI* and probed by an internal $P$ element probe, the *DdeI-BsiWI* 189 bp frag-
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ment of a canonical P element (positions 586–775) (Figure 1A). This hybridization system can detect full-size P elements as a 2.2 kb band and defective elements as bands of specific length, for example, KP elements as a 0.4 kb band. This short probe gives equal density bands from full-size and KP elements (Itoh et al. 1999). The relative numbers of full-size P and KP elements in the genomic DNA (KP/FP ratio) of a particular line were evaluated by calculating the ratio of the observed densities of the bands corresponding to fragment sizes expected from KP and full-size P elements in an autoradiograph. Scanning densitometry was performed by analyzing the autoradiograph with public domain image analysis software (NIH Image v. 1.62). Although the pDBs189 probe cannot detect many defective P elements that lack the nucleotide sequence homologous to the probe, re-probing experiments with a 5' sequence probe (canonical positions 50–727) demonstrated that the pDBs189 probe missed very few P elements in wild populations (Itoh and Boussy 2002).

RESULTS

P element-related phenotypes of Japanese wild populations of D. melanogaster To know the current status of the P-M system characteristics of wild pop-
Fig. 1. Analysis of the genomic $P$ elements. (A) Structure of full-size $P$ and $KP$ elements. Restriction fragments by $DdeI$ and the hybridization probe are designated by white bars and a solid bar, respectively. ORFs are indicated by light shaded boxes. B: $BstWI$, D: $DdeI$, X: $XhoI$. (B) Examples of the result of Southern blot hybridization. The name of each isofemale line is shown above the lane with the symbol for the individual population (see Table 1). (C) $P$ elements in the isofemale lines from Toyotomi (TY) and the results of GD test. CS: Canton S, Hw: Harwich.
ulation of *D. melanogaster*, 472 isofemale lines were examined by GD test (Table 1). Q strains were found most abundantly in all local populations examined and they are predominant overall (71.6%: 338/472). However, there was significant variation in phenotypes (Figure 2). For example, AZ, AS and TN were actually composed

![Map of Japan with results of the P-M phenotypic GD test.](image)

Fig. 2. Results of the P-M phenotypic GD test for the populations in Japan. In the A-A* graph (Yamamoto et al. 1984), intensities of inducibility and repressability of P transposition of the individual isofemale line is shown as a dot, and distribution pattern of the dots, therefore, tells us the total P-M characteristics of each local population, hence their phenotypic types.
of only $Q$ individuals (type 4). IN, OS, HG, IS, IR, and HT had some $P$ strains accompanied with $Q$ strains (type 1). Some $M$ with $Q$ strains were found in FK, KN, TZ, OZ and OM populations (type 2). In addition, TY, SP, TK, and KM had a mixture of $P$, $Q$ and $M$ strains (type 3). The distribution of $P$ and $M$ strains seemed sporadic, with no obvious geographical pattern. There were a few exceptional $P'$ strains in some populations.

**Genomic $P$ element complement in Japanese wild populations of $D. melanogaster$**  Southern blot hybridization was carried out to analyze the $P$ element composition of each isofemale line. As shown in the example, all lines examined had many $P$ copies in their genomes (Figure 1B). All phenotypically $M$ lines were, therefore, $M'$ by definition (Bingham et al. 1982). The 2.2 kb and 0.4 kb bands were two of the major signals in the blots, indicating that full-size $P$ and KP elements were the majority in these populations ($F'P+KP$ predominance). No other specific size class was found to be typical for $P$, $Q$ or $M$ strains. For example, there were no special bands specific for each phenotype in the TY population, which contained all kinds of P-M system phenotypes (Figure 1C). By a quantitative analysis with densitometry, no clear relationship was found between genomic $P$ element profiles and P-M phenotypic characteristics. The relative numbers of full-size $P$ and KP elements ($KP/FP$ ratio) were significantly higher in the type 2 ($M'$ and $Q$) populations, KN and OM, than in the type 1 ($P$ and $Q$; IN and IR), and the type 3 (mixed all; TY) populations. Those of the type 4 (HK(az) and TN) were intermediate (Figure 3). However, the ratios were significantly higher in $Q$ lines than in $M'$ lines in a population as seen in KN,

![Graph](image.png)

**Fig. 3.** Weak correlation between the $KP/FP$ ratio and the P-M system phenotypes. The bars of black depict results for $P$ strains, with dots for $Q$ strains, and blank for $M'$ strains.

but in OM. The ratios in $Q$ lines showed large variation among the populations, with a range from 0.38 in TY9 to 7.87 in OM3. There was only a weak correlation between the genomic $P$ element composition and the P-M phenotypes of the individual line.

The Hikone population is one of the wild populations most extensively studied for the P-M system in Japan. Hikone R established in 1952 had no $P$ elements in the genome, while Hikone H established in 1957 is a $Q$ strain with some genomic $P$ elements, suggesting the $P$ invasion occurred in the middle of 1950s in Japan (Gamo et al. 1990; Nishino et al. 1993). We analyzed the old Hikone lines as well as current HK(az) lines, which are entirely

![Image](image.png)

**Fig. 4.** Results of Southern hybridization of $P$ elements in the isofemale lines from Hikone region from 1952 to 2001.

<table>
<thead>
<tr>
<th>Hikone</th>
<th>HK(az)</th>
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<tbody>
<tr>
<td>R</td>
<td>1</td>
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<tr>
<td>H</td>
<td>4</td>
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<tr>
<td>N2</td>
<td>6</td>
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<td>N4</td>
<td>7</td>
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<td>N9</td>
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(kb)
Q strains. As for other recent Japanese lines, the 2.2 kb and 0.4 kb signals were found with only a few bands with other sizes in all of the Hikone lines except for Hikone R (Figure 4), indicating that P elements invaded into the population between 1952 and 1957 in Hikone. This survey also showed that KP elements and full-size P elements have coexisted from the early stage of their invasion to the present day in the population. By densitometry, the relative doses of KP and other defective elements to full-size elements were evaluated as 21.3 and 9.2 in 1957, and similarly but with standard deviation as 4.12 ± 3.84 and 3.04 ± 1.01 in 1989, and 3.11 ± 0.55 and 3.54 ± 1.14 in 2001, respectively. There was no clear evidence of structural destruction underlying a loss of function of P elements during this 40 years in Hikone.

**DISCUSSION**

**FP+KP predominance in the wild populations of D. melanogaster in Japan** Our present analysis demonstrates that full-size P and KP elements are the major genomic P elements (FP+KP predominance). The number of genomic P elements and the KP/FP ratios differ among the populations, and the profiles of genomic P elements cannot completely explain the difference of the P-M phenotypes. We isolated six P elements from a genomic library of a strong P-M strain, OM5, whose value of cross A* GD was 100%, and found that one was an intact P element and five were KP elements by nucleotide sequencing (data not shown). It is an emerging consensus that P element-related phenotypes of individual flies or lines are not determined only by the numbers of each type of element in the genome (Todo et al. 1984; Anxolabéhère et al. 1985, 1990; Ronsseray et al. 1991; Daniels et al. 1987; Bonnivard and Higuet 1999; Itoh et al. 2001; Itoh and Boussy 2002). Boussy and coworkers extensively studied genomic P element composition and its relationship to P-M phenotype in Eastern Australian wild populations and found a FP+KP predominance, weak correlations between genomic P element complements and P, Q, and M' phenotypes, and no correlation with maternally- or biparentally-transmitted repression (Boussy and Kidwell 1987; Boussy et al. 1988, 1998; Itoh et al. 1999). Although attention is now focused on position effect of individual elements (Robertson and Engels 1989; Biémont et al. 1990; Simmons et al. 1990; Misra et al. 1993; Rasmussen et al. 1993; Andrews and Gloor 1995), determination of P-M phenotype is still an open question.

**Stability in the P-M phenotypes** Many wild populations of D. melanogaster in Japan were previously surveyed for P-M system characteristics (Todo et al. 1984; Yamamoto et al. 1984, Sakoyama et al. 1985; Gamo et al. 1990; Nitasaka and Yamazaki 1994). In particular, Matsuura et al. (1993) analyzed 28 wild populations collected in the 1980s. They reported that type 1 populations were in the north part of the mainland and the southernmost islands, while type 2 populations were found near Fukui (FK), Nishinomiya (NM), Tozukawa (TZ), and Okumura (OM). This is very similar to the distribution of types that we found. Thus, the distribution of P and M’ strains does not seem to have changed much in this period, suggesting a stability in the P-M characteristics of each local population for about 15 years.

The Hikone populations presented even greater stability in phenotypes; it has been entirely Q from 1957 to 2001 (Figure 4). Since Hikone R, established in 1952, was true M strain, a rapid change from M to Q in a few years and a stability of the Q state for more than 40 years are likely in Hikone. Three other examples of long-term stabilities can be found (Figure 5). The Tanushimaru population (TN) was independently shown to be Q four times during the past 40 years (Yamamoto et al. 1984; Iwano et al. 1984; Matsuura et al. 1993; this study). The populations around Tozukawa (TZ) have always contained some M’ strain for the past 30 years (Gamo et al. 1990; Matsuura et al. 1993; this study). The populations around Inakadate (IN) have been characterized by some P strains since the 1970s (Kidwell et al. 1983; Matsuura et al. 1993; this study). Consequently, long-term stability of the P-M system is not rare at least in Japan. One simple interpretation is that after the initial invasion of P elements, each local population rapidly changed from M state to different state, P, Q, or M’, and was thereafter

![Fig. 5. Schematic representation of phenotypic changes in the wild populations. The boxes of black depict for P strain or P/Q type, with dots for Q strains, blank for M’ strain or M’/Q type, and blank with an asterisk for true M. HK: Hikone, TN: Tanushimaru, TZ: Tozukawa, IN: Inakadate. References: (1) Gamo et al. 1990, (2) Nishino et al. 1993, (3) Yamamoto et al. 1984, (4) Iwano et al. 1984, (5) Matsuura et al. 1993, (6) Kidwell et al. 1983.](image-url)
stable. This seems inconsistent with the assumption of sequential changes in P-M phenotype by Kidwell (1983) and others (see below).

A similar stability in P-M phenotypes was reported in Eurasia, Africa, and Oceania over about 15 years by Bonnivard and Higuet (1999), who attributed this stasis to “Q strain buffer zones” that prevent P element spreading. They proposed that the Q strain buffer zone in France is involved in the stability in Eurasia populations, and posited that such zones should also exist in China and Africa. Our present result may complement their hypothesis, although there is no direct evidence that Q buffering would work similarly in Japan, where small local populations having different phenotypes dwell close each other.

**Current status of the P element in the evolutionary history in D. melanogaster** The long-term fate of transposable genetic elements in a gene pool is thought to be molecular extinction following immobilization. Pinsker et al. (2001) proposed an evolutionary history of P elements in Drosophila as follows. After invading a naive gene pool, the P element enjoys vigorous propagation. However, transposition activity is gradually suppressed by various mechanisms, including repression by the P elements themselves, production of defective copies with repressive properties, and host-directed silencing, which contains heterochromatization and cosuppression. Immobilized elements will then become extinct by accumulation of mutations, although horizontal emigration and acquisition of a novel function as a host gene are possible escape routes from the extinction.

The current polymorphism in the P-M system is thought to represent different stages in the evolutionary process under the recent invasion hypothesis (Kidwell 1983; Anxolabéhère et al. 1988). Our present results are, however, inconsistent with this. First, our analysis of some series of temporal populations from single-location did not indicate any succession of phenotypes. Rather, stabilities of the P-M system were seen in local populations for 15 years and in some cases for 30 years or more, despite their phenotypic variety (P/Q, Q, or M'/Q). Second, structural destruction that might underlie phenotypic divergence was not found in their genomic P elements. Entire loss of full-length intact P elements was not the case even in the M’ strains in the populations. In addition, our result, together with those of others, suggests an important role of position effect in determining the phenotype. Heterochromatization (Roche and Rio 1998; Ronsseray et al. 1998) and post-transcriptional cosuppression (Dorer and Henikoff 1997) have been demonstrated to be associated with such effects. The FP+KP predominance is evidence for temporary silencing of the elements, because the observed phenotypic differences can be attained by partial inactivation of the elements without irreversible structural decay. Consequently, a simple succession in P-M phenotypes with loss of functional elements does not seem to be the case, at least in the populations examined. This may suggest that P elements are still in a very early stage of the evolutionary history in D. melanogaster and they are far before from entire immobilization followed by extinction. It is conceivable that the timescale for the sequential changes from P to Q and/or M’ are substantially longer than for the swift conversion of M to P.

Brookfield (1991; 1996) proposed possible evolutionary stable states of the P-M system by computational simulation studies. His result suggested that autonomous elements can persist in Q strains, with nonautonomous but powerful zygotic repressor elements. This model is consistent with our observation of phenotypic stability in this area where all isofemale lines contained autonomous P and KP elements and more than 70% of them were Q strains. It is noteworthy that the Hikone population has been Q since 1957, and has the FP+KP condition. Brookfield also suggested that the FP+KP condition can be stable in P strains when powerful P cytotype or zygotic repression is build up, and in M’ strains when the population size is small. Most P strains examined had strong P cytotype. As for the M’ strains, there is no information about their effective population sizes. According to Brookfield’s expectation, it is probable that the long-term stability of the phenotypes observed here could be an evolutionary stable state in the P-M system. Interestingly, a recent molecular survey of P elements demonstrated wide distribution of the FP+KP predominance in Asia, Australia, and Africa (Itoh et al. 1999; Itoh and Boussy 2002). More detailed molecular analysis and careful surveys of natural population are necessary.

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