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Genetics

Transposition rates of movable genetic elements in *Drosophila melanogaster*

(spontaneous mutations/*in situ* hybridization/adaptive evolution)

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ABSTRACT **A considerable proportion of visible mutations is reported to be caused by the insertion of mobile genetic elements in *Drosophila* and other organisms. We estimated transposition rates of some *Drosophila* mobile elements by using the lines AW and JH in which spontaneous mutations have been accumulated independently for about 400 generations. Occupied sites of the mobile elements were detected by *in situ* hybridization on the salivary gland chromosomes sampled from 40 AW and 30 JH lines. The rates of insertion and excision of the *copia* and two *copia*-like elements, *412* and *17.6*, are very low: Insertions occurred at up to 10^{-3} per second chromosome per generation (*17.6*) and excision occurred at about 10^{-5} per site per generation (*copia* and *412*). Insertions of the *I* and *hobo* elements occurred much more frequently. These estimates are not only important for assessing the actual rate of various types of mutations but also for developing an evolutionary theory of mobile elements themselves.**

A large amount of genetic variation commonly exists in natural populations of *Drosophila* and other organisms. Clearly, genetic variation is the source of adaptive microevolution and understanding the maintenance mechanism of such variation has been a core problem of evolutionary theory (1). Reliable estimate of spontaneous mutation rate is essential for quantitative analysis of genetic variation maintained in natural populations. Mutation rate is, however, known to be influenced by both environmental stresses and genomic components such as insertion sequences. It has been suggested that many of the spontaneous visible mutations are insertional ones (2). Recent molecular analysis of the white locus in *Drosophila melanogaster* suggests that the majority of spontaneous mutations may be due to insertion of moderately repetitive DNA or mobile elements (3). These elements are also shown to have induced lethal mutations (4), viability mutations (5, 6), and other mutations of quantitative traits such as bristle number (7) in *Drosophila*. Therefore, the determination of a reliable estimate of the transposition rate of such elements is necessary for assessing the actual rates of spontaneous mutations and estimating the evolutionary significance of mobile elements in natural populations. Several attempts have been made to estimate the transposition rate of mobile elements (e.g., refs. 8 and 9); however, there are very few cases in which experiments were designed strictly for direct estimation.

As a primary purpose of this report, we examined the transposition rates, insertion and excision rates, of several mobile elements by using long-term mutation accumulation lines.

MATERIALS AND METHODS

Accumulation of Mutation. Spontaneous mutations have been accumulated under minimum pressure of selection in many second chromosome lines derived from four stem chromosomes: two lethal chromosomes, l_{AW} and l_{JH}, and two *Cy* chromosomes (Fig. 1). Because of the lethality of original chromosomes, any contamination is easily checked by the appearance of normal phenotype. Forty AW and 30 JH lines were used in this experiment. The temperature employed for maintaining the *Drosophila* stocks and the experimental crosses was 25°C.

Plasmids. A 2.6-kilobase (kb) *Xho* I fragment from plasmid pH108 (11) was subcloned into pUC13 and used as a probe for the *hobo* element. A 2.4-kb *Eco*RI fragment from plasmid cDm2055 (12) was subcloned into pUC13 and used as a probe for the *copia* element. A 5.1-kb *Hin*dIII fragment of cDm412 (13) was subcloned into pBR322 and used as a probe for the *412* element. A 7.0-kb *Cla* I fragment from recombinant phage λhist17.6 (12) was subcloned into pBR322 and used as a probe for the *17.6* element. Whole plasmids pI407 (14) and pπ25.1 (3) were used as probes for the *I* and *P* elements, respectively. The plasmids cDm2055 and subcloned plasmids cDm412 and λhist17.6 were gifts from K. Saigo (University of Tokyo). The plasmids pI407 and pπ25.1 were gifts from T. Yamazaki (Kyushu University). The plasmid pH108 was a gift from N. Miyashita (Kyoto University).

***In Situ* Hybridization.** Experimental crosses were made between single male AW (JH) files and isogenic Canton S females. Salivary gland preparations were made using F_1 larvae with l_{AW} (l_{JH}) chromosomes. The plasmid DNAs were labeled with biotin-11-dUTP (BRL) by nick-translation (15). The hybridization solution contained 20 μl of 20× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7), 40 μl of 50% (wt/vol) dextran sulfate, 100 μl of formamide, 8 μl of carrier DNA (salmon sperm DNA at 10 mg/ml), and 4 μg of biotin-labeled probe DNA in a total of 200 μl. This solution was heated in boiling water for 5 min and quickly cooled on ice just before use. Salivary gland preparations were denatured in an alkaline solution (0.07 M NaOH) for 2 min. Hybridization solution (20 μl) was applied to slides and sealed with a cover slip. Slides were incubated for 12 hr at 37°C in a moist chamber. The detection of the probes was done using detection kit Ditek-If (Enzo Biochem). Detailed methods of hybridization followed Engels *et al.* (16).

RESULTS

The occupied sites for the retrovirus-like elements, *copia*, *412*, and *17.6*, are listed in Table 1. The original insertions of the parental AW and JH chromosomes are evidently inferred in the sites that are occupied by the elements in all or most of the lines, whereas the sites occupied in at most one line are judged as new insertions. There are five and nine original insertions for the *copia* element in the AW and JH lines, respectively. Both the AW and JH lines possess five original insertions for the *412* element. There are three and two original insertions for the *17.6* element in the AW and JH lines, respectively. No new insertions were observed for the *copia* and *412* elements. Four and 18 new insertions of the

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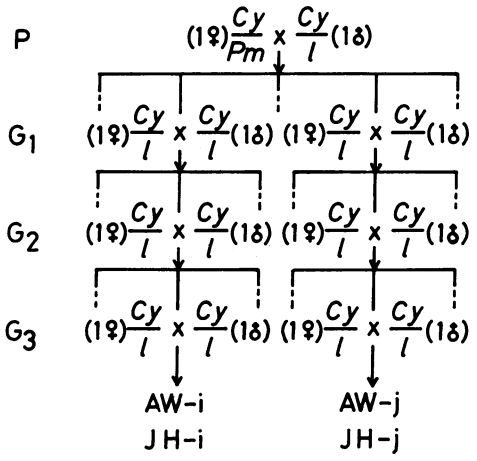

FIG. 1. Mating scheme for the accumulation of mutations modified after Yamaguchi and Mukai (10). Two lethal-carrying second chromosomes (l_{AW} and l_{JH}) were derived from cage population W-1, which was initiated from the stock collected in Erie, Pennsylvania, in 1954 by A. B. Burdick. They were extracted with the marked inversion technique using a balanced lethal stock, C160 [*In(2LR)SM1/In(2LR)bw^{V1}*, abbreviated as *Cy/Pm*] whose genetic background was isogenic and also originated in the Erie population. One heterozygous male (*Cy/l*) was crossed with one C160 female and expanded to 500 sublines by generation 3. The experiment started in 1967 and spontaneous mutations accumulated by successive pair matings of *Cy/l* × *Cy/l* until 1975. The letters i and j indicate the line numbers. After 1975 each line has been maintained in mass culture in single vials. The number of parents in the mass culture is about 20, and mutations whose selection coefficient is much less than 0.05 have been accumulated as effectively neutral. Under these conditions, the frequency of possible segregating mutations changes mainly by random genetic drift. Chromosomal location of mobile elements was determined using single males randomly sampled from each line. The total number of generations that mutations accumulated was about 400 when this experiment was conducted.

17.6 element were observed in AW and JH lines, respectively. One excision for the *copia* in the JH lines and two excisions for the *412* in the AW lines were observed. No excisions were observed for the *17.6* element.

A large variation in copy numbers on the second chromosomes was observed for both *I* and *hobo* elements. These elements as well as the *P* element are known to induce hybrid dysgenesis under certain mating systems in *Drosophila melanogaster* (18, 19). The chromosomal distribution of the *I* and *hobo* element insertions is shown in Fig. 2. The average copy number for the *I* element on the second chromosomes is 5.95 ± 0.37 (mean ± SEM) for the AW lines and 2.97 ± 0.30 for the JH lines. Three sites (26A, 55B, and 60E) are occupied by the *I* elements in all of the AW lines, indicating the original insertions. No excision occurred at these sites. The extremely low rate of excision for *I* elements as well as for the *copia* and two other *copia*-like elements, *412* and *17.6*, supports the view that the transposition of these elements is replicative and does not require excision (14). Furthermore, one site (22A) in the AW lines is occupied by the element in 12 of the 40 lines. This is most probably the site of an early insertion that occurred within the first three generations in the process of the original expansion (see Fig. 1) and not a hot spot of insertion since the corresponding site in the JH lines is not occupied by the element so frequently. Alternatively, this site may be very unstable and the original element may be excised at an unusually high rate (1.75×10^{-3} per site per generation in this case). No conserved sites for the *I* element were observed in the JH lines.

Much larger variation in copy number among lines was observed in the insertion of *hobo* elements. The average copy number for the *hobo* element on the second chromosome is 9.05 ± 1.47 (mean ± SEM) for the AW and 23.47 ± 2.51 for the JH lines. No conserved sites were found for the *hobo* element in either the AW or JH lines and original insertion sites are not clearly evident for the *hobo* element. However, there are several sites in which a *hobo* element insertion occurred in many of the lines. We call these polymorphic sites. Two apparently polymorphic sites (39D and 43A) were found for the *hobo* element in the AW lines. One is occupied by the element in 24 of the 40 AW lines and the other is occupied in 30 of the 40 AW lines. These sites may not be hot spots of insertion because the corresponding sites in JH lines are not polymorphic. However, it is not clear whether these sites were occupied by the element during the first three generations of expansion or were present in the original parental chromosomes. Twelve apparently polymorphic sites, 10 on the left arm (25E, F, 26A, 30F, 34A, B, F, 35B, C, and 38B) and 2 on the right arm (56D and 57B), were observed in the JH lines. They were occupied by the element in more than 10 JH lines. Because we have several reasons to believe that the original lethal second chromosomes carried several *hobo* elements, as discussed later, some of these polymorphic sites are likely to be the original insertion sites. This may indicate that frequent excision as well as insertion of the *hobo* element occurred in these lines.

The overall insertion and excision rates of the mobile elements except for the *hobo* element are summarized in Table 2. The exceptional polymorphic site (22A) for the *I* element of the AW chromosome was ignored in calculating the insertion and excision rates.

DISCUSSION

The transposition rates of *copia* and two other *copia*-like elements are relatively low in these lines. The highest insertion rate among *copia* and *copia*-like elements is observed for

Table 1. Occupied sites of *copia*, *412*, and *17.6* elements in the lethal second chromosomes of mutation accumulation lines

Element	AW							JH								
copia																
Site	35A	35C	35F	39C	41C			31A	36B	38C	41C	47E	54C	55B	56F	58A
Number	40	40	40	40	40			29	30	30	30	30	30	30	30	30
412																
Site	25A	51C	54E	58A	58E			35A	36D	37D	38A	58D				
Number	40	38	40	40	40			30	30	30	30	30				
17.6																
Site	32E	36B	46E	52F	53F	57A	58E	21D	23E	25E	25F	27E	28A	28F	29C	34A	35B
Number	1	40	40	40	1	1	1	1	1	1	1	1	1	1	1	1	30
Site								35D	35F	36C	37C	43F	52A	52E	52F	55B	58F
Number								1	1	1	1	1	1	1	1	30	1

Second chromosome occupied sites of the elements on the salivary gland polytene chromosome map (17) and the number of lines possessing the insertion at the sites are shown. Four new insertions of the *17.6* element occurred in 4 AW lines. Thirteen lines shared 18 new insertions of the *17.6* element in the JH lines. Nine lines had 1 new insertion. Three lines had 2 new insertions, and 1 line had 3 new insertions.

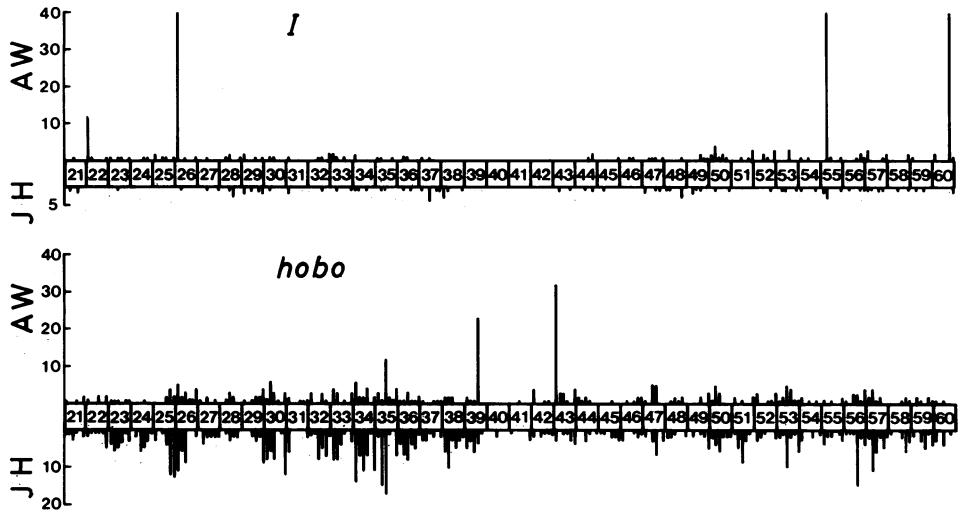

FIG. 2. Distribution of occupied sites of the *I* and *hobo* elements on the second chromosome of AW and JH lines. The bars above (AW) or below (JH) the salivary gland chromosome map (17) of the second chromosome indicate the number of lines possessing the elements at each position. The position of bars represents one of the six subdivisions (A–F) of each chromosomal region (21–60).

17.6. The insertion rate of the *17.6* element was significantly higher in the JH lines than in the AW lines. The differential insertion rate of the *17.6* element may reflect the different genetic backgrounds of the AW and JH lines. The total copy number of the *17.6* elements in individuals with diploid chromosome complements was determined by using five lines randomly sampled from each AW and JH line. The copy number except for the centromeric regions is 21.2 ± 2.07 (mean ± SEM) for the AW stock lines and 19.0 ± 1.23 for the JH stock lines. They are not significantly different. Therefore, the factor affecting the insertion rate in this case may not be copy number but rather the activity of the background elements. Transposition rate is sometimes defined as the probability that an element in a given individual produces a new copy that inserts elsewhere (9). By using this definition, the rate for the *17.6* element becomes 5.90×10^{-5} and 3.95×10^{-4} per element per generation for the AW and JH lines, respectively. The value obtained for the JH lines is very close to that of *copia*-like elements (4×10^{-4} per element per generation) estimated in a natural population (9). Note, however, that our estimate is the maximum value for *copia*-like elements examined, and the average value for these elements could be lower. Excision rates are also low for these elements with no significant differences among them. The reported spontaneous reverse mutation rate for the allele w^a in which a 5-kb *copia* element is inserted into a small intron (20–23) is 1/106,240 or 9.42×10^{-6} (24). This value is fairly close to the excision rate for the *copia* element obtained in the JH lines. The revertant of w^a was a partial one and was distinguishable from wild type phenotypically (24). This may indicate that the revertant occurred by incomplete excision of the *copia* element. Molecular analyses of the spontaneous (25) and x-ray-induced (26) partial revertants of w^a indicated that one long terminal repeat of the *copia* element was left at the previous insertion site. These observations suggest that excision of *copia* occurs by a mechanism similar to that of the *Ty* element of yeast in which reciprocal recombination between homologous long terminal repeats results in excision of the internal region of the element and leaves behind a single long terminal repeat (27–29).

Frequent chromosome rearrangements were reported for the AW and JH lines by Yamaguchi and Mukai (10) at about the 100th generation. Twenty and 92 chromosome rearrangements were observed in 500 of the AW and 500 of the JH lines, respectively. They included 108 inversions, 3 translocations, and 1 transposition. Eight unique inversions, 7 paracentric and 1 pericentric, in the JH lines and 3 paracentric unique inversions in the AW lines were also found in this experiment. All these inversions have insertions of the *hobo* element at the breakpoints. These findings strongly suggest that the claimed mutator factor in these mutation accumulation lines (10) is the *hobo* element. Nine of the 11 inversions found in this experiment were not found by Yamaguchi and Mukai (10). These observations suggest that the crosses used for establishing the mutation accumulation lines were dysgenic and induced a condition in which *hobo* elements are mobilized (30, 31). Such a condition may be comparable to that present in *P–M* hybrid dysgenesis (32) and may have continued for many generations. It is notable that some breakpoints reported by Yamaguchi and Mukai (10) recurred in many chromosome rearrangements. Among these breakpoints 26A and 34A in the JH lines are significant: they were

Table 2. Insertion and excision rates of four mobile elements

Element	Insertion rate		Excision rate	
	AW	JH	AW	JH
I	107/16,000 (6.7×10^{-3})	90/12,000 (7.5×10^{-3})	0/48,000 (0)	— (—)
copia	0/16,000 (0)	0/12,000 (0)	0/80,000 (0)	1/108,000 (9.3×10^{-6})
412	0/16,000 (0)	0/12,000 (0)	2/80,000 (2.5×10^{-5})	0/60,000 (0)
17.6	4/16,000 (2.5×10^{-4})	18/12,000 (15.0×10^{-4})	0/48,000 (0)	0/24,000 (0)

Numerators in the calculation of insertion or excision rates are the number of events. Denominator in the calculation of insertion rates is the number of chromosome generations (number of lines × number of generations). Denominator for the excision rates is the number of site generations (number of original sites × number of lines × number of generations). Numbers in parentheses are the rate of insertion (per second chromosome per generation) or the rate of excision (per site per generation). Signals of hybridization of these elements were also detected in the centromeric regions, but these were not counted.

involved in 28 chromosome rearrangements (27 inversions and 1 transposition) and 10 inversions, respectively. These hot spots of chromosomal breaks were also frequently occupied by the *hobo* element in the present investigation (see Fig. 2). It has been proposed that pairing between homologous *hobo* elements and subsequent recombination causes inversions when these elements are in opposite orientation on the chromosome and causes deletions when they are in the same orientation (33). Probably the two hot spots of chromosomal breaks mentioned above are the original sites of *hobo* insertion. The recombination between the original and the newly transposed *hobo* elements may have produced inversions. There are a total of 339 and 659 sites occupied by the *hobo* element in the AW and JH lines, respectively. Eleven JH lines had insertions at 26A and 14 lines had insertions at 34A. Assuming these two hot spots of chromosomal breaks are the only original insertion sites, the insertion rate is estimated to be 339/16,000 or 0.0212 per second chromosome per generation for the AW lines and 636/12,000 or 0.0530 per second chromosome per generation for the JH lines. Similarly, the excision rate is estimated to be 37/24,000 or 1.54×10^{-3} per site per generation for the JH lines. Although not detected cytologically, many small deletions may also have occurred in these lines.

All of the stock lines of AW and JH were examined for the existence of the *P* element by *in situ* hybridization. None of the lines carried the *P* element. Therefore, it is not involved in the frequently occurring chromosome aberrations observed in the present experiment. According to the rapid invasion hypothesis (34), the *P* element invasion in American natural populations began about 30 years ago, so it is likely that the sampling of the original chromosomes in the Erie population (in 1954) occurred before the invasion of the *P* element.

In early reports, cross-mobilization of the *gypsy* and other mobile elements has been claimed under conditions of *P–M* hybrid dysgenesis (35–38). As has been pointed out (8, 39), there may be a stimulus that causes mobilization of *copia*-like and other elements; however, *P–M* hybrid dysgenesis may not be the stimulus responsible for directly inducing such "bursts" (35) or "gene reshuffling" (40) in the genome. Similarly, our observations indicate that the *copia* and *copia*-like elements are relatively stable under constant laboratory conditions, even under the continuing pressure of hybrid dysgenesis induced by the *hobo* element. There is also no significant correlation in copy numbers between *hobo* and *I* elements. Therefore, transposition rates seem to be controlled independently for each transposable element.

Comparing the mutation rate estimated for mildly deleterious genes [0.12–0.17 per second chromosome per generation (41, 42)] to that of structural genes [0.008–0.040 per second chromosome per generation (1)], Mukai and Cockerham (1) proposed on the basis of the occurrence rates of various types of mutations that most viability and other fitness polygenes are located in controlling regions outside the structural genes. Mutation rates at visible recessive loci were not significantly increased in these lines (43, 44); however, a significant increase in the genetic variance of alcohol dehydrogenase activity was observed in these lines at about 300th generation (45). Frequent insertion of the *hobo* and *I* elements in noncoding regions or potential control regions may have induced such regulatory mutations. Regulatory mutations such as those altering enzyme activities may affect individual fitness and compose some fraction of genetic loads in natural populations. Molecular analyses of DNA regions including structural genes and their vicinities (46) showed that noncoding regions actually harbor extensive amounts of insertion sequences, mainly mobile elements. With the potential of rapid movement, mobile elements create a great amount of unique haplotypes in natural populations. Quantitative analysis of the evolutionary significance of mobile elements is thus possible based on the transposition rates as estimated in this experiment.

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