Comparative study of *P* element activity in two natural populations of *Drosophila melanogaster*

Ko HARADA¹, Shin-ichi KUSAKABE² and Terumi MUKAI¹

¹*Department of Biology, Faculty of Science, Kyushu University 33,
Fukuoka 812, Japan*

²*Division of Environmental Studies, Faculty of Integrated Arts and
Sciences, Hiroshima University, Hiroshima 730, Japan*

(Received 19 September 1991)

ABSTRACT

Population structure concerning *P* element activity was investigated in two natural *Drosophila* populations. These populations are very different as far as in the viability spectrum is concerned. In one population, the Raleigh, United States population, genetic loads related to viability have been kept at a fairly high level. In the other population, the Nagasaki, Japan, population, the genetic loads tend to be stable at very low levels. In the Raleigh population it is estimated that on the average 4 copies of intact *P* elements that possess transposase activity exist in the genome. On the other hand only 0.7 complete copies are estimated to exist in the genome of the Nagasaki population. Heterogeneity in the *P* element copy number and significant positive linkage disequilibrium among occupied sites were detected in the Raleigh population. Our results, with some evidences which indicate that high mutation rate was caused by the *P* element, suggests that the large genetic loads in the Raleigh population are caused by the rapid invasion of *P* element in this population.

1. INTRODUCTION

About seventeen percent of the *Drosophila* genome is composed of middle repetitive DNA, of which three fourths of them is estimated to be movable genetic elements or so called transposons (Young, 1979). More than thirty families of these elements are known in *Drosophila melanogaster* (Ajioka and Hartl, 1989). Some of them transpose at a considerably high rate in the genome. Thus they are suggested to be the major cause of spontaneous mutations (Green, 1980). Most of mutations caused by transposable elements are considered deleterious, however this may not always be so. Transposable elements can alter genes and genome composition in many ways. The evolutionary significance of those complex mutations caused by transposons are discussed by Finnegan (1989). Thus, it is important to study the population dynamics of transposable elements and evaluate the amount of mutations caused by them.

Some transposons, including *P*, *I* and *hobo* elements are known to be the agents of "hybrid dysgenesis" that is induced in the offsprings of certain unidirectional crosses and is represented by an array of abnormal phenotypes such as chromo-

somal rearrangements, male recombination, sterility and lethal mutations. The hybrid dysgenesis caused by the *P* element (P-M hybrid dysgenesis) also causes mutations in quantitative traits (Mackay, 1985) and other mutations related to fitness components such as viability (Yukuhiro et al., 1985; Mackay, 1986; Fitzpatrick and Sved, 1986). It is noticed that old laboratory strains are absent of the *P* elements, while almost all flies collected from natural populations within several decades contain *P* elements. From this observation, it is assumed that the *P* element was introduced in the *D. melanogaster* population about thirty to thirty five years ago and rapidly spread all over the world (Kidwell, 1983). This hypothetical process is called "rapid invasion". Proliferation of the *P* element in a population is most probably attained by high rate of transposition which offset the loss of the elements due to deleterious effect of insertions (Golding et al., 1986). During the process of rapid invasion, populations are assumed to have experienced a considerable amount of genetic loads because of increased mutation rate. The proportion of the loads created by hybrid dysgenesis is, however, expected to be lowered after the population acquired immunity to prevent transposition of the element. In this paper, we investigated the population structure as it relates to the *P* element activity in two populations showing very different viability spectra.

2. MATERIALS AND METHODS

(1) *Stocks*

Isofemale stocks derived from flies collected from two populations, Raleigh, North Carolina and Nagasaki, Japan, were used for this experiment. Collection of flies was done in 1984 in both of the populations. Most of the experiments were done in 1985.

(2) *Establishment of second chromosome lines and measurement of viability*

Second chromosome lines were established from the isofemale lines using the balanced lethal stocks of C-160 (*In(2LR)Cy/In(2LR)bw^{V1}*, abbreviated *Cy/Pm*). The genetic background of C-160 is the isogenic laboratory strain W-160. The viability of the second chromosome lines whose *P* element location was determined was measured by the *Cy* method (Wallace, 1955). The mating scheme for these methods is illustrated in Fig. 1.

(3) *G-D sterility test*

Gonadal sterility induced by P-M hybrid dysgenesis was tested following the method of Engels and Preston (1980). Sixty-five isofemale lines from the Raleigh population and 72 isofemale lines from the Nagasaki population were used for this experiment. The strains π_2 and Canton S were used as the typical P and M strain, respectively. For the test of *P* element activity, the following reciprocal

Fig. 1. Mating scheme for estimating the viability of second chromosomes. Males (+/+) were sampled from an isofemale line. Homozygote viability is expressed by the index, $2b/(a+1)$ (Haldane, 1956). Here, *a* and *b* represent the numbers of *Cy* and wild phenotype, respectively.

crosses, A and B, were done.

A: Canton S (♀) × U (♂)

B: U (♀) × Canton S (♂)

Here, U indicates a strain to be tested for *P* element activity. The cytotype of an unknown strain, U, was determined by the following reciprocal crosses, A* and B*.

A*: U (♀) × π_2 (♂)

B*: π_2 (♀) × U (♂)

In both of the experiments, flies were kept at 29°C and discarded on the fourth day of the cross. Vials were kept three more days at 29°C and then moved to 25°C. On the eleventh day, emerged flies were transferred to a new vial. Four days after transfer, female flies were placed in an egg laying chamber, which is a plastic 96-cell tissue culture plate prepared as described by Engels and Preston (1980). After three days, each female was scored for sterility. If no eggs were found, the female was dissected in saline. A female with two rudimentary ovary was scored as sterile.

(4) *Hypermutable of the sn^w allele*

To test whether the strain contains intact *P* elements, sn^w (singd-weak) hypermutability was examined (Engels, 1979; Engels and Preston, 1981). Fifty-six isofemale lines from the Raleigh and 47 isofemale lines from the Nagasaki

populations were randomly sampled and examined. A single male of each isofemale line was crossed with a single sn^w (M strain of $sn^w/y^+Y; bw; st$) female at 23°C. A male offspring was crossed with five females of $C(1)DX, y w f$ at 25°C. Male offspring with the phenotypes sn^w , sn^e and sn^+ (singled-weak, -extreme and -normal, respectively) were counted and the ratio $(sn^e + sn^+)/ (sn^w + sn^e + sn^+)$ was used as the index for mutability.

(5) *in situ hybridization*

The plasmid p π 25.1, which contains an intact *P* element cloned into pBR322 (Spradling and Rubin, 1981), was labelled with biotin-11-dUTP by nick translation (Rigby et al., 1977). Probe DNA was denatured by boiling for 5 minutes then hybridized with salivary gland chromosomes prepared from the second chromosome lines of the Raleigh and Nagasaki populations. The sites occupied by the *P* elements were detected using the Detek I-f (ENZO) detection kit.

3. RESULTS

Distribution of the occupied sites of P element

The numbers of sites occupied by *P* elements were determined by *in situ* hybridization for 46 Raleigh second chromosomes (29 standard gene arrangements and 17 *In(2L)t*) and 15 Nagasaki second chromosomes. All of the second chromosomes in the Nagasaki population had the standard gene arrangement. The results are summarized in Table 1. A significantly larger number of occupied sites are observed in the right arm (2R) than in the left arm (2L) except in the case of the *In(2L)t*-carrying chromosomes in the Raleigh population ($t=4.186$ with 28 degrees of freedom ($p<0.01$) for Raleigh standard chromosomes, $t=1.339$ with 16 degrees of freedom ($0.10<P<0.25$) for Raleigh *In(2L)t* chromosomes, $t=4.207$ with 45 degrees of freedom ($p<0.01$) for Raleigh pooled chromosomes and $t=3.108$ with 14 degrees of freedom ($p<0.01$) for Nagasaki chromosomes). Similar results were obtained by Yamaguchi et al. (1987) in a Canary Island cage population and by Eanes et al. (1988) in a Botswana population. This is probably due to the longer size of 2R and larger number of potential target sites for the *P*

Table 1. Mean copy numbers* of *P* element on the second chromosomes in the Raleigh and the Nagasaki populations

	n	2L	2R	Total
Raleigh (Standard)	29	6.52 $\pm$ 0.48	9.41 $\pm$ 0.63	15.93 $\pm$ 0.89
Raleigh (<i>In(2L)t</i>)	17	6.29 $\pm$ 0.70	6.93 $\pm$ 0.61	13.21 $\pm$ 1.04
Pooled	46	6.37 $\pm$ 0.37	8.54 $\pm$ 0.50	14.91 $\pm$ 0.71
Nagasaki	15	5.67 $\pm$ 0.58	7.53 $\pm$ 0.67	13.20 $\pm$ 1.10

* Copy numbers are shown as mean $\pm$ S.E.

element in 2R. It is reported that a total of 1136 salivary gland chromosome bands are observed in 2R, while 804 in 2L (Lefevre, 1976). χ^2 test was performed assuming the number of target sites is proportional to the number of bands. No significant difference from this hypothesis was observed in neither of Raleigh standard, *In(2L)t* and Nagasaki chromosomes.

Mean copy number on the second chromosome is significantly larger in Raleigh standard than in *In(2L)t* carrying chromosomes ($t=1.929$ with 44 degrees of freedom, $p<0.05$). This difference is caused by the difference in copy number in 2R ($t=2.617$ with 44 degrees of freedom, $p<0.01$). Total copy number is not significantly different either between Raleigh standard second chromosomes and Nagasaki second chromosomes or Raleigh *In(2L)t* chromosome and Nagasaki second chromosomes.

Variances in copy number are tend to be larger than the means; however, only the variance in the Raleigh second chromosomes (standard and *In(2L)t* pooled) is significantly larger than the mean ($\chi^2=70.40$ with 45 degree of freedom, $p<0.001$; see Sokal and Rohlf, 1981). This indicates the distribution of copy number in this population depart from that of Poisson.

The location of the occupied sites of the *P* elements on the second chromosomes was determined for 33 Raleigh and 11 Nagasaki second chromosome lines among the above mentioned lines (Fig. 2). Occupied sites were assigned to one of the 240 sections of the second chromosome (21A to 60F) of the Bridges's salivary gland map (Bridges, 1935; Lefevre, 1976). The goodness of fit to Poisson distribution was examined for these numbers of occupied sites in each region. Significant departure from Poisson distribution is observed in the Raleigh stan-

Fig. 2. Distribution of occupied sites of the *P* element on the second chromosome. Closed bars and open bars in the Raleigh population indicates the sites in the standard chromosomes and *In(2L)t* chromosomes, respectively. Second chromosome is divided into 20 major regions of 21 to 60 according to the salivary gland map of Bridges (Bridges, 1935; Lefevre, 1976). Each region is further divided into 6 minor regions of A to F in which occupied sites are located. The vertical measure indicates the frequency.

dard chromosomes ($\chi^2=31.67$ with 5 degrees of freedom, $p<0.001$) and in the pooled Raleigh chromosomes ($\chi^2=51.57$ with 6 degrees of freedom, $p<0.01$), but not in the Raleigh *In(2L)t*-carrying chromosomes ($\chi^2=5.80$ with 3 degrees of freedom) and Nagasaki ($\chi^2=3.02$ with 3 degrees of freedom) chromosomes. The distribution of the occupied sites for these regions in the Raleigh standard chromosomes is clumping. This may mean distribution of the potential target sites for the *P* element is not randomly located on the second chromosome. Departure from Poisson distribution in Raleigh chromosomes is due to the departure in standard chromosomes in the 2R ($\chi^2=35.3$ with 5 degrees of freedom, $p<0.001$). These findings indicate that there is significant heterogeneity in the distribution of the occupied sites of the *P* element in the Raleigh standard chromosomes especially in 2R region.

To test the independence of the distribution of the *P* elements in the Raleigh and Nagasaki populations, χ^2 test was performed for 40 chromosomal sections (21 to 60) on the Bridges's map. Nonsignificant result ($\chi^2=15.67$ with 19 degree of freedom, $0.9>p>0.5$) shows that the distribution of the occupied sites in two populations are similar.

Regression of homozygote viability as a function of the P element copy number

Homozygote viability was measured for these second chromosome lines. Excluding the lethal chromosomes, regression of the viability as a function of the *P* element copy number was analyzed for 42 second chromosomes in the Raleigh and 15 chromosomes in the Nagasaki population. The regression coefficient is -0.0049 for the Raleigh population and -0.0067 for the Nagasaki population. Both of the coefficients are not significantly different from zero; however, it is noticeable that the values are similar and both of them are negative. The 95% confidence interval is -0.0151 to 0.0015 for the former and -0.0304 to 0.0204 for the latter. Large confidence interval is shown in the Nagasaki population because of small sample size; however, the result obtained in the Raleigh population indicate that individual effect of the *P* element insertion is small and decrease less than 1% of the viability.

P element activity assay

The population structure concerning the *P* element activity (A cross) and the repressor activity (A* cross) is shown in Fig. 3 (the results of B and B* cross are not shown). According to the Kidwell's criteria (Kidwell, 1986), strains are classified into M, M', Q, weak or moderate P and strong P and summarized in Table 2. The most frequent class of the Raleigh population was moderate P strain and no M strains are observed. About half of the lines (47.7%) was P strain in the Raleigh population. By contrast, most of the lines belong to Q or M' strains in the Nagasaki population and no P strain was found.

Comparable result was obtained in the *sn^w* hypermutability test. The result is

Fig. 3. Population structure concerning P-M hybrid dysgenesis. A total of 65 isofemale lines in the Raleigh and 72 isofemale lines in the Nagasaki populations were tested for the sterility. The vertical measure indicates the frequency.

shown in Fig. 4. The mean mutability with standard error is 21.68 ± 2.00 (%) ($n=56$) for the Raleigh population and 3.02 ± 0.77 (%) ($n=47$) for the Nagasaki population. The number of lines showing no mutability is one out of 56 lines or 1.8% in the Raleigh population and 23 out of 47 lines or 48.9% in the Nagasaki

Table 2. Population structure concerning to the P-M hybrid dysgenesis in the Raleigh and the Nagasaki populations

Type*	Raleigh		Nagasaki	
	Frequency	%	Frequency	%
M	0	0.0	2	2.8
M'	7	10.8	28	38.9
Q	18	27.7	40	55.6
P (weak)	30	46.2	0	0.0
P (strong)	1	1.5	0	0.0
Others	9	13.8	2	2.8

* Type of the strains is conventionally determined following the Kidwell's criteria (1986). Strain shows 0% sterility in A cross and shows 100% sterility in A* cross is M type. M' is the type showing 0 to 10% sterility in A cross and 10 to 100% sterility in A* cross. Q type shows 0 to 10% sterility in both of A and A* crosses. P type shows 0 to 10% sterility in A* cross, and this class is subdivided into two classes of weak or moderate P and strong P type. Strain shows 10 to 80% sterility in A cross is weak or moderate P, and strain shows more than 80% sterility in A cross is strong P type.

Fig. 4. Distribution of *sn^w* mutability in the cross of males from each population and female of *sn^w* (M) strain. A total of 56 Raleigh and 47 Nagasaki lines were used. Closed bars indicate Raleigh lines and open bars indicate Nagasaki lines.

population. These results indicate that most of the lines in the Raleigh population contains at least one intact *P* element and about half of the lines in the Nagasaki population lack intact *P* element which can produce transposase. Assuming at least one intact *P* element is sufficient for inducing hypermutability of *sn^w* locus and the elements distribute following Poisson, the average number of the intact *P* element is estimated to be 4.03 ± 0.99 (S.E.) in the Raleigh and 0.71 ± 0.15 (S.E.) in the Nagasaki population.

4. DISCUSSION

In the present study, It is shown that many of the lines in the Raleigh population possess active *P* elements. However, about 90% of the lines are P or Q strains and it is assumed that P-M hybrid dysgenesis does not frequently occur in this population. Heterogeneity in the copy number of occupied sites of the *P* element was observed between standard and *In(2L)t* carrying second chromosomes. Significantly larger variance than expected from Poisson was observed in the Raleigh second chromosomes. Larger variance suggests that there is positive linkage disequilibrium among occupied sites, that is, there is tendency for the frequency of occupation of one site to be higher if a neighboring site is occupied (Charlesworth and Charlesworth, 1983; Charlesworth and Langley, 1989). Disequilibrium in the *P* element insertion sites suggest that recurrent migration of chromosomes with different composition of this element might have been occurred in the Raleigh population in fairly recent times. Migration of chromosomes with active *P* element might caused hybrid dysgenesis at the beginning. Subsequent shift of cytotype from M to P may have occurred in this population and caused decreasing frequency of hybrid dysgenesis. The shift of cytotype from M to P was demonstrated in the laboratory populations originated from a mixture of P and M strains (Kidwell, 1981; see Engels, 1989). Probably the similar process might have occurred in this population by 1984.

The above speculation is parallel to the observation in the change of genetic loads in this population. Considerably large genetic load concerning to viability for the second chromosomes was observed in the Raleigh population in 1970 (Mukai and Yamaguchi, 1974). Total homozygous load was 0.8343 and made of lethal and detrimental components of 0.5007 and 0.3336, respectively. Total genetic load decreased to 0.5065 (0.2891 lethal and 0.2174 detrimental loads in the native genetic background) in 1984 (Baba et al., 1986; Kusakabe et al., unpublished data). Large genetic loads in the Raleigh population in 1970 might be caused by high mutation rate in both of lethals and detrimentals. Cardellino and Mukai (1975) accumulated spontaneous mutations for 40 generations on 140 inversion free second chromosomes. These lines were extracted from the Raleigh 1970 population. Unusually large increase in dominance variance for viability was observed in these lines. Lethal load increased from 0.436 at

generation 0 to 1.645 at generation 40, while no significant increase was observed in detrimental load. Further investigation revealed that 63 new chromosomal aberrations including 55 inversions were found among these lines (Yamaguchi et al., 1976). Although it is not known when the *P* element was introduced in the Raleigh population, there are several evidences which indicate the high mutation rate in the Raleigh 1970 population was caused by P-M hybrid dysgenesis. Several unique inversions were found in the isofemale lines of the Raleigh population in 1984. *P* element insertions are detected at the breakpoints of these inversions by *in situ* hybridization (Kusakabe et al., 1990). Similarity of these inversions with *In(2R)**j* reported by Stalker (1976) in the Oklahoma population in 1973 suggests that these inversions already existed in the early '70s or late '60s in the southern United States.

By contrast, lines possessing active *P* element is very rare in the Nagasaki population although copy number detected by *in situ* hybridization was not significantly different from that of the Raleigh. Nonsignificant departure from Poisson distribution in copy number of *P* element on the second chromosomes suggests that the population is at near equilibrium and the chromosomes are not the recent immigrants from surrounding areas. It is assumed that this population had also experienced hybrid dysgenesis at the beginning of the *P* element invasion and during the course of the shift of cytoplasm. Genetic loads in the Nagasaki population seem stable at very low level. The results obtained in periodical estimates from 1977 were summarized in Table 3. The values obtained in the Nagasaki population are one of the lowest in the populations ever estimated in Japan. Lethal mutations are estimated to be lost more rapidly than detrimental mutations as has been shown in the change of D/L ratio in the Raleigh population. Low level of genetic loads in both of lethal and detrimental loads in the Nagasaki population suggests that longer time has passed in this population than in the

Table 3. Genetic loads concerning to the viability in the Nagasaki population

Load	1977-1979 ¹⁾	1984 ²⁾	1986 ³⁾
T	0.4459	0.2906	0.4097
D	0.1691	0.1628	0.1881
L	0.2768	0.1278	0.2216

1) Data from Ayaki et al. (1980)

2) Data from Harada et al. (1985)

3) Unpublished data of K. Harada

Genetic loads in 1) are the averages of the values estimated in autumn in 1977, spring and autumn in 1978 and spring in 1979. Genetic loads were estimated in the "native genetic background" in 1984 and in 1986 in which second chromosomes are extracted using *Cy/Pm* balancer stock whose genetic and cytoplasmic background is same as that of one of the isofemale lines in this population. T, D and L indicate total, detrimental and lethal loads, respectively.

Raleigh population since the first introduction of the *P* element. It must be noticed, however, that change in population structure is largely dependent on the composition of starting population concerning to *P* element activity (Kidwell, 1981).

Several authors measured the effect of hybrid dysgenesis on the fitness components and other quantitative characters. Eanes et al. (1988) estimated that the decrease in viability due to the newly inserted *P* element is 1.4 ± 0.6 (standard error) % on hemizygous X chromosomes. The increase of homozygous load in viability estimated in this study is 0.49% in the Raleigh population or 0.67% in the Nagasaki population for each insertion at face value. Although no statistical difference was detected among them, smaller values estimated in natural populations may indicate that purifying selection is operating in eliminating the insertions of deleterious effect. It is expected that larger number of transposons are accumulated in the regions in which recombination is restricted because these regions may be free from natural selection against chromosome rearrangement caused by ectopic exchange between elements (Charlesworth and Langley, 1989). Our result shows no such excess in copy number in the *In(2L)t* region. The age of *In(2L)t* in the Raleigh population was estimated to be about 4000 generations (Mukai and Voelker, 1977) and average frequency of *In(2L)t* estimated from flies collected in 1968 to 1974 is 0.056 ± 0.012 (Mukai and Voelker, 1977). Thus the inversion exists in the population in most of the case in heterozygous condition and actually no crossing over occurs in this region. Our result may indicate that the time after the invasion of *P* element is too short comparing to the age of the inversion to accumulate the excess number of the element in this region. No significant increase in the frequency of *In(2L)t* in 1984 (Kusakabe et al., 1990) suggest that there is not detectable advantage in inversion heterozygotes expected from inhibition of the ectopic exchange between elements.

It is shown that the DNA sequence of the element found in Ishigakijima, Japan is completely match to π_2 element isolated from a natural population in Madison, United States (Nitasaka et al., 1990). Although the element in the Nagasaki population is not sequenced, it is plausible that the elements found in Japan and in the United States are the same origin. Absence of the *P* element in old laboratory stocks and the observation that *P* elements are absent in closely related species of *D. melanogaster* suggest that *P* elements may have entered the melanogaster genome by horizontal transmission (e.g., Bingham, Kidwell and Rubin, 1982). Extensive survey of the genus *Drosophila* showed that *P* homologous sequences are essentially confined to the subgenus *Sophophora* (Daniels et al., 1990). These observations basically support the rapid invasion hypothesis. Thus, the change in population structure concerning to *P* element activity presented in this study must have occurred in very short time that is at most in 50 years.

We are grateful to Dr. D. A. Johnson, Seinan Gakuin University for reading and improving the manuscript. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- Ajioka, J. W. and Hartl, D. L. (1989). Population dynamics of transposable elements. In: *Mobile DNA* (eds.: I. Berg, D. E. Howe and M. Martha). Amer. Soc. Microbiol., Washington, D.C..
- Ayaki, S., Yoshikawa, I. and Shiomi, T. (1980). Genetic features in a Nagasaki population of *Drosophila melanogaster*. II. *Jpn. J. Genet.* **55**, 449.
- Baba, H., Maki, M., Kusakabe, S., Harada, K. and Mukai, T. (1986). Hybrid dysgenesis and genetic load in natural populations of *Drosophila melanogaster*. I. Dynamics of homozygous load in Raleigh population. *Jpn. J. Genet.* **61**, 572.
- Bingham, P. M., Kidwell, M. G. and Rubin, G. M. (1982). The molecular basis of P-M hybrid dysgenesis: the role of the P element, a P-strain-specific transposon family. *Cell* **29**, 995–1004.
- Bridges, C. B. (1935). Salivary chromosome maps. *J. Hered.* **26**, 60–64.
- Cardellino, R. A. and Mukai, T. (1975). Mutator factors and genetic variance components of viability in *Drosophila melanogaster*. *Genetics* **80**, 567–583.
- Charlesworth, B. and Charlesworth, D. (1983). The population dynamics of transposable elements. *Genet. Res.* **42**, 1–27.
- Charlesworth, B. and Langley, C. H. (1989). The population genetics of *Drosophila* transposable elements. *Ann. Rev. Genet.* **23**, 251–287.
- Daniels, S. B., Peterson, K. R., Strausbaugh, L. D., Kidwell, M. G. and Chovnick, A. (1990). Evidence for horizontal transmission of the P transposable element between *Drosophila* species. *Genetics* **124**, 339–355.
- Eanes, W. F., Wesley, C., Hey, J., Houle, D. and Ajioka, J. W. (1988). The fitness consequences of P element insertion in *Drosophila melanogaster*. *Genet. Res.* **53**, 17–26.
- Engels, W. R. (1979). Extrachromosomal control of mutability in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **76**, 4011–4015.
- Engels, W. R. (1989). P elements in *Drosophila melanogaster*. In: *Mobile DNA* (eds.: I. Berg, D. E. Howe and M. Martha). Amer. Soc. Microbiol., Washington, D.C.
- Engels, W. R. and Preston, C. R. (1980). Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster*. *Genetics* **95**, 111–128.
- Engels, W. R. and Preston, C. R. (1981). Identifying P factors in *Drosophila* by means of chromosome breakage hotspots. *Cell* **26**, 421–428.
- Finnegan, D. J. (1989). Eukaryotic transposable elements and genome evolution. *Tr. Genet.* **5**, 103–107.
- Fitzpatrick, B. J. and Sved, J. A. (1986). High levels of fitness modifiers. *Genet. Res.* **48**, 89–98.
- Golding, G. B., Aquadro, C. F. and Langley, C. H. (1986). Sequence evolution within populations under multiple types of mutation. *Proc. Natl. Acad. Sci. USA* **83**, 427–431.
- Green, M. M. (1980). Transposable elements in *Drosophila* and other Diptera. *Ann. Rev. Genet.* **14**, 109–120.
- Haldane, J. B. S. (1956). Estimation of viabilities. *J. Genet.* **54**, 294–296.
- Harada, K., Tohda, K., Hirose, K., Hurutani, A. and Mukai, T. (1985). Detection of transposons in a natural population of *Drosophila melanogaster* in Japan—Hirabaru (Nagasaki pref.) population. *Jpn. J. Genet.* **60**, 589.
- Kidwell, M. G. (1981). Rapid unidirectional change of hybrid dysgenesis potential in *Drosophila*. *J. Hered.* **72**, 32–38.
- Kidwell, M. G. (1983). Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **80**, 1655–1659.

- Kidwell, M. G. (1986). P-M mutagenesis. In: *Drosophila: a practical approach* (ed.: D. B. Roberts). IRL Press, Oxford.
- Kusakabe, S., Harada, K. and Mukai, T. (1990). The rare inversion with a P element at the breakpoint maintained in a natural population of *Drosophila melanogaster*. *Genetica* **81**, 111–115.
- Lefevre, G., Jr. (1976). A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In: *The Genetics and Biology of Drosophila*, Vol. 1a. (eds.: M. Ashburner and E. Novitski). Academic Press, New York.
- Mackay, T. F. C. (1985). Transposable element-induced response to artificial selection in *Drosophila melanogaster*. *Genet. Res.* **44**, 231–237.
- Mackay, T. F. C. (1986). Transposable element-induced fitness mutations in *Drosophila melanogaster*. *Genet. Res.* **48**, 77–87.
- Mukai, T. and Yamaguchi, O. (1974). The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. *Genetics* **76**, 339–366.
- Mukai, T. and Voelker, R. A. (1977). The genetic structure of natural populations of *Drosophila melanogaster*. XIII. Further studies on linkage disequilibrium. *Genetics* **86**, 175–185.
- Nitasaka, E. and Yamazaki, T. (1991). The relationship between DNA structural variations and activities of *P* elements in typical P and Q strains of *Drosophila melanogaster*. *Genet. Res.* (submitted).
- Rigby, P. W. J., Dieckmann, M. Rhodes, C. and Berg, P. (1977). Labelling deoxyribonucleic acids to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**, 237–251.
- Sokal, R. R. and Rohlf, F. J. (1981). *Biometry*, 2nd ed., 177pp., W. H. Freeman, New York.
- Spradling, A. C. and Rubin, G. M. (1981). *Drosophila* genome organization: Conserved and dynamic aspects. *Ann. Rev. Genet.* **15**, 219–264.
- Stalker, H. D. (1976). Chromosome studies in wild populations of *D. melanogaster*. *Genetics* **82**, 323–347.
- Wallace, B. (1955). Studies on irradiated populations of *Drosophila melanogaster*. *J. Genet.* **54**, 280–293.
- Yamaguchi, O., Cardellino, R. A. and Mukai, T. (1976). High rates of occurrence of spontaneous chromosome aberrations in *Drosophila melanogaster*. *Genetics* **83**, 409–422.
- Yamaguchi, O., Yamazaki, T., Saigo, K., Mukai, T. and Robertson, A. (1987). Distribution of three transposable elements, *P*, *297*, and *copia* in natural populations of *Drosophila melanogaster*. *Jpn. J. Genet.* **62**, 205–216.
- Young, M.W. (1979). Middle repetitive DNA: A fluid component of the *Drosophila* genome. *Proc. Natl. Acad. Sci. USA* **76**, 6274–6278.
- Yukuhiro, K. Y., Harada, K. and Mukai, T. (1985). Viability mutations induced by the *P* elements in *Drosophila melanogaster*. *Jpn. J. Genet.* **60**: 531–537.