Further genetic studies on the Katsunuma population of *Drosophila melanogaster*

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Changes in the genetic structure of the Katsunuma natural population of *Drosophila melanogaster* have been examined during the past 35 years. The frequency of recessive lethal genes on the second chromosome once increased from 15% to 30% in the early 1970s, then decreased to about 24% in the late 1970s, and thereafter showed no significant changes. Sterility genes, the frequency of which is always less than the lethals, showed a similar tendency. The *SD* (segregation distorter) mutant gene disappeared but some others such as *rbl* (reduced bristle) and *bw* (brown) persisted in the population. The frequency of inversion-carrying chromosomes gradually decreased in the period, such that the standard chromosome frequency in the second and third chromosomes increased from about 40% to more than 80%. Coincident with these frequency changes is the invasion of a transposable element *P* into the Katsunuma population. The *P* element should have invaded into Katsunuma in the late 1960s. It spread over the population apparently inducing deleterious mutations, causing the decrease in the allelism rate, and hence increasing the effective population size. Soon, however, most flies became resistant to the *P* element-mediated transposition as they began to harbor defective *P* elements. During the course of spreading, the *P* element must also have induced deleterious mutations on the polymorphic inversions, breaking up the heterotic gene complexes along the chromosomes, which probably caused the reduction in the frequency of inversion chromosomes. Temporal invasion of *D. simulans*, a sibling species of *D. melanogaster*, into Katsunuma occurred several times after 1978, and the species seems to have been settled since 1990. This, however, did not have any effect on the genetic structure of *D. melanogaster* population.

INTRODUCTION

Genetic variations concealed in natural populations of *D. melanogaster* have been studied by many investigators since the 1930s, for example, in American populations (Ives, 1945, 1954, 1970; Ives and Band, 1986), Japanese populations (Minamori and Saito, 1964; Watanabe, 1969a; Watanabe et al., 1976a; Mukai et al., 1985), and Korean populations (Paik, 1960, 1966; Choo and Lee, 1986). These works all focused on the contents of lethals and other deleterious genes in populations from the view point of mutation rate and natural selection. Inversion poly-

morphism of this species have also been studied, for example, by Mettler et al. (1977) for American populations, by Inoue and Watanabe (1979) and Inoue et al. (1984) for Japanese populations, and by Knibb et al. (1980) for Australian populations. They observed the cosmopolitan polymorphic inversions persisted in every natural population, especially in the lower latitudes.

Previous workers considered various genetic as well as non-genetic (environmental and ecological) factors influencing the genetic structure in local populations. Here we consider two causes relevant to the present study. The *P* transposable element was detected in *D. melanogaster* populations (Kidwell et al., 1977), and was shown to induce mutations in the *M* cytoplasm (Engels,

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1979). The *P-M* system was initially studied as the syndrome of hybrid dysgenesis, and it was considered as the most probable cause of spontaneous mutations in natural populations. Molecular studies on the *P* element revealed that the complete *P* element was 2.9 kb in length, and that incomplete or defective *P* with various lengths also existed (Bingham et al., 1982; Kidwell et al., 1983; O'Hare and Rubin, 1983). Todo et al. (1984) discussed the origin of polymorphic *P* elements observed in Japanese and Korean natural populations. The *P-M* system of hybrid dysgenesis in natural populations of the mainland of Japan has been examined extensively (Yamamoto et al., 1984; Gamo et al., 1990; Matsuura et al., 1993; Nitasaka and Yamazaki, 1994; Itoh and Takegawa, 1998). Most Japanese populations, including the Katsunuma population, examined in the 1980s were *Q* strains carrying many copies of *P*, which rendered flies not susceptible to mutation induction by mating. Thus, the invasion of the *P* element must have affected on the population structure.

Invasion of *D. simulans* (a sibling species of *D. melanogaster*) into the mainland of Japan, including Katsunuma, has been reported (Watanabe and Kawanishi, 1976, 1978; Watanabe et al., 1984; Watada et al., 1986). If a newly introduced species comes to occupy the same ecological niche, it may cause changes in genetic structure in another species that is already existing.

The purpose of this paper is to describe the changes of genetic structure of *D. melanogaster* in Katsunuma during the past 35 years. Possible causes for the genetic changes particularly in the contents of lethals and polymorphic inversions are discussed based on the influence of *P* transposable element. The effect of the presence of the sibling species, *D. simulans*, on the genetic structure of *D. melanogaster* population is also considered.

MATERIALS AND METHODS

Collection of flies. In the early October, *D. melanogaster* wild flies were collected 14 times in the period of 1974–1997 in Katsunuma, Yamanashi prefecture, Japan. Sampling was made by using a sweeping net in vineyards. They were established as isofemale lines. *D. simulans* wild flies were also collected in vineyards, and in addition in the garden of Hikawa shrine, near the vineyards, by using banana traps.

Genetic analysis. *D. melanogaster* wild flies were subjected to genetic analyses. Male flies were crossed individually to females of the balancer stocks, *SM1*, *Cy/Pm* or *SM1*, *Cy/Pm*; *TM3*, *Sb/Pr*, to isolate the second chromosomes or sets of the second and third chromosomes, respectively (Watanabe et al., 1976a). The balancer stocks used were previously replaced with the genetic backgrounds (chromosomes and cytoplasm) from the Katsunuma natural population. Each of the second chro-

mosomes and sets of the second and third chromosomes were made homozygous in the F_3 generation. Lines producing wild type flies, that are homozygous for the chromosomes from the Katsunuma population, at most 1% were classified as lethal-carrying. To test the male sterility, five such homozygous males from each line were mated with five virgin females with full fertility, Oregon R or *y*; *C(4)RM*, *spa^{pol}*. Female sterility was also examined by the reciprocal crosses. Visible mutations were examined when the homozygotes were obtained in the F_3 generation. The second chromosomes isolated in 1978 and 1979 were also examined whether they had the *SD* (segregation distorter) genes by the following way. Flies carrying the second chromosomes extracted from the natural population were individually mated with a laboratory stock homozygous for the second chromosome carrying *cn bw*. Heterozygous males (red eyes) were backcrossed to the *cn bw* females (white eyes). If the segregation in the progeny deviated from the expected ratio, the chromosome examined was categorized as *SD*-carrying. All experiments except for the GD test described below were done at 25°C.

Cytological analysis. Salivary gland chromosomes were examined from larvae of isofemale lines or from the homozygous lines established as above. The preparation of chromosomes and the description of inversion chromosomes were the same as used by Inoue and Watanabe (1979). The frequency of each inversion was calculated per major autosome arm.

Gonadal dysgenesis (GD) tests. A set of crosses, A (Canton-S females x wild males) and A* (wild females x Harwich males), were performed at 29°C (Kidwell et al., 1977; Engels and Preston, 1980) for the isofemale lines. All of F_1 females were individually dissected and the GD score for each line was calculated as the percentage of undeveloped ovaries. Following the conventional definition by Kidwell et al. (1983), a line showing less than 10% GD scores in both crosses was judged as *Q* strain, a line showing less than 10% GD score in the cross A and more than 10% in the cross A* as *M* strain, and a line showing more than 10% GD score in the cross A and less than 10% in the cross A* as *P* strain.

RESULTS

Frequencies of lethals and steriles. The data from the analyses of the Katsunuma populations collected in the 1974–1997 period are presented in Table 1. The overall frequency of the second chromosomes carrying recessive lethal genes was about 24%, ranging from 20% in 1979 to 28% in 1974. The 1979 sample was unusual in that it contained the lowest frequency of the second chromosome lethals (19.6%) and the highest frequency of the

Table 1. Frequencies (%) of lethals, steriles and other mutants in Katsunuma

Year	No. of Chromosomes	Second chromosome								Third chromosome	
		Lethal	Allelism	Sterile		Mutant				Lethal	
				Male	Female	<i>SD</i>	<i>rbl</i>	<i>bw</i>	<i>wx-l</i>		
1974	458	28.1									
1975	409	24.5		10.5	5.2						
1976	713	23.6		10.9	8.0		2.2	2.2	0.2		
1977	251	23.9	1.5	11.5	9.9		4.7	3.1	0		
1978	245	23.7		11.8	13.4	0	3.7	2.1	0.5		
1979	232	19.6		14.5	10.2	0				40.9	
1980	238	25.6					1.1	1.7	0.6	31.1	
1981	244	26.2					1.9	0	0.6	31.1	
1984	144	22.2								32.6	
1985	192	21.1 (23.2)					2.2	1.7		26.3 (23.2)	
1990	231	22.5		7.2	5.6						
1997	196	25.0	0.9	10.6							

Numbers in parentheses were obtained by using the balancer stock with the original (laboratory) genetic background (see Discussion).

Table 2. Frequencies (%) of polymorphic inversion in Katsunuma

Year	N*	Common cosmopolitan				Rare cosmopolitan				Endemic		Standard chromosome	
		<i>2Lt</i>	<i>2RNS</i>	<i>3LP</i>	<i>3RP</i>	<i>2LA</i>	<i>3LM</i>	<i>3RC</i>	<i>3Rmo</i>	<i>2LW</i>	<i>3LY</i>	Second	Third
1982	122	11.5	12.3	0	27.0	0	0	3.3	0	3.3	0.8	75.0	69.1
1985	209	11.0	12.0	0	15.3	0	0	4.8	0	1.4	0	77.0	79.9
1990	200	14.0	5.0	2.0	19.5	0	0	2.0	0.5	1.0	0	81.0	76.9
1991	200	14.0	3.5	1.5	25.5	0	0	1.5	0	1.0	0	82.0	74.9
1997	204	10.8	1.5	0	14.2	0	0	0	0	0.5	0	87.0	85.8

* Number of autosomes examined.

third chromosome lethals (40.9%), highly deviating from the data of neighboring years. The frequency of the third chromosome lethals was examined five times during the 1979–1985 period. The frequencies (26.3–40.9%) were always much higher than those of the second chromosome lethals (19.6–26.2%) in the same period.

The recessive steriles in the Katsunuma population were monitored seven times during the period (Table 1). The average frequency of male steriles was 11.0% (7 samples, 7.2–14.5%), while that of female steriles was 8.7% (6 samples, 5.2–13.4%). These frequencies are significantly less than the lethal content in the same period.

The frequencies of allelism for the second chromosome lethals were examined twice (1.5% in 1977 and 0.9% in 1997) during the period. These frequencies are significantly lower than those in the 1960s (3.50%) and in the early 1970s (3.43%) (Watanabe et al., 1976a). This means that the variation of lethal loci had increased since the middle of 1970s in the population. Recessive visible mutations, *rbl* (*reduced bristles*), *bw* (*brown*), and *wx-l* (*waxy-like*), were found with the frequencies of about 2.6%, 1.8%, and 0.4% on the average, respectively. We did not find any *SD* gene in the present study, in contrast to its persistent presence during 1963–1966 in the Katsunuma population (Watanabe, 1967).

Frequencies of inversions. The frequencies of inversions were examined from 1982 to 1997 (Table 2). No polymorphic inversions (inversions appearing in frequencies greater than 1%) were detected on the X chromosome in the period, in accordance with the previous results (Inoue and Watanabe, 1979). Inversions on the second and third chromosomes were gradually decreasing in frequency in this period, especially *In(2R)NS*, *In(3R)C* and *In(2L)W*, except for *In(2L)t* and *In(3R)P* which were relatively constant. We found no or only a very few chromosomes with *In(2L)A*, *In(3L)M*, *In(3L)P*, *In(3R)Mo* or *In(3L)Y* inversion, each of which had been found frequently in the same population during the 1960s and 1970s (Inoue et al., 1984). As a result, the standard chromosomes in both the second and third chromosomes increased and overwhelmed inversion-carrying chromosomes.

P-M analysis by GD tests. The results of GD test show that the Katsunuma populations in 1996 and 1997 are made up of many *Q* strains with substantial number of *M* strains (Fig. 1). No unequivocal *P* strain was found. Several females were susceptible to *P* transposition like an *M* strain from the cross A*, whereas males from one line inducing transposition was detected in 1996 (12.1%

GD value in the cross A). Southern blot analysis revealed that these *M* strains carried *P* elements in their genomes with varying lengths (data to be presented elsewhere).

Fig. 1. Results of the GD test for the Katsunuma population of *D. melanogaster*. A set of crosses, A and A* were performed for the 22 and 19 isofemale lines established in 1996 and 1997, respectively. All of F₁ females, number of which varied from 22 to 188, were dissected individually.

Table 3. Frequencies (%) of *D. simulans* in Katsunuma

Year*	Vineyard		Shrine	
1975			0	(850)
1976			0	(1375)
1977	0	(1332)	1	(288)
1978	0.1	(2294)	7	(431)
1979	0.5	(1321)	15	(264)
1980	7	(899)	23	(47)
1981	0	(545)	2	(271)
1982	0.5	(569)	0	(20)
1983	0	(551)	10	(29)
1984	1	(557)	31	(45)
1985	0.4	(1020)	44	(383)
1988	0	(580)	0	(24)
1990	9	(920)		
1991	18	(459)		
1996	30	(490)		
1997	22	(1851)	50	(316)

Numbers in parentheses are the total number of *D. simulans* and *D. melanogaster* collected.

* Data for 1975, 1976, and 1977–1985 are from Watanabe and Kawanishi (1976), Watanabe and Kawanishi (1978), and Watada et al. (1986), respectively.

Frequency of *D. simulans*. Since the first collection in Katsunuma in 1977, *D. simulans* increased its number especially in the shrine (Table 3). On the other hand, *D. simulans* in vineyards was scarcely found until 1980 (7%), but it suddenly increased after 1990, reaching 30% in 1996. Now, *D. simulans* is a successful species in Katsunuma. It is noted that the frequencies in vineyards were always lower than those in the shrine.

DISCUSSION

Fig. 2 summarizes the genetic changes of the Katsunuma population in the past 35 years. The data by Watanabe et al. (1976a) for 1963–1973 and Inoue et al. (1984) for 1963–1980 are incorporated so as to cover the 1963–1997 period. The frequency of the second chromosome lethals was about 15% in the 1963–1968 period, increasing rapidly to more than 30% in the 1970–1973 period. Then, it fell down to about 24% in 1975, and remained constant thereafter. The frequency of lethal-carrying third chromosomes was always higher than the frequency of lethal-carrying second chromosomes in the years 1979–1985. Band and Ives (1963) and Watanabe et al. (1976b) reported similar observations in American populations. The present study confirms those observations in the Katsunuma population. This may possibly be due to the fact that the third chromosome is longer than the second chromosome.

The increase and decrease of lethals in this population were also paralleled by sterility genes throughout the 1968–1997 period. The average frequency of lethals (24%), male steriles (11%) and female steriles (8.7%) in the present study (1974–1997) also paralleled those of spontaneous mutation rates estimated as 0.72%, 0.32%, and 0.19% per generation per chromosome, respectively (Watanabe and Lee, 1977).

Using Nei's formula (1968), the effective size of population (*N_e*) is estimated for three different periods (1963–1968, 1977, and 1997) for Katsunuma population. The population size had increased about three times in 1977 (*N_e* = 5203) and six times in 1997 (*N_e* = 10349) compared to that estimated for the 1960s (*N_e* = 1763). Although the frequency of lethals seems to have reached a new equilibrium at around 24% in 1975, the size of population was increasing until 1997. This increase in the effective population size may have been caused by the invasion of *P* into Katsunuma (see below).

The segregation distorter (*SD*) gene was found in Katsunuma in 1963 (Watanabe, 1967), and it was detected every year until 1966. No *SD* genes, however, was found in the 1967, 1972, and 1973 samples (Watanabe et al., 1976a). The present results for the 1978 and 1979 samples again showed the absence of *SD* in the Katsunuma population. On the other hand, three visible mutations *rbl*, *bw*, and *wx-l* are shown to have persisted

Fig. 2. Frequency changes of autosomes with lethals, steriles, and standard chromosomes in the Katsunuma population of *D. melanogaster* from 1963 to 1997. Data for the lethals and steriles in 1963–1973 are from Watanabe et al. (1976a), and data for the standard chromosomes in 1963–1980 are from Inoue et al. (1984).

in the population. Thus the mutation and selection systems for these visibles should be different from those for the *SD* gene. It has been noted that the *SD* gene was often found in linkage with an inversion, *In(2R)NS*, while the *rbl* gene was with another inversion, *In(2L)t* (Watanabe, 1967, 1969b). The frequency of the former inversion has been decreasing, while that of the latter has remained constant in the Katsunuma population (Table 2).

Another change is observed in chromosomal polymorphism. Continuous decreases of inversions are observed both in the second and third chromosomes. Consequently, standard chromosomes have been and are still increasing (Fig. 2). This is in sharp contrast to the lethal and sterile mutations showing new equilibria. Inversion polymorphism has often been explained by heterosis, that is, inversion heterozygotes are advantageous in fitness than the corresponding homozygotes. Watanabe and Watanabe (1973) observed that inversion heterozygotes were superior in female productivity. Even the lethal heterozygotes, when in combination with a polymorphic inversion *In(2L)t*, did not show any reduction in female productivity. The maintenance of chromosomal polymorphism in nature has been extensively studied in *D. pseudoobscura* (Dobzhansky, 1970), and the superiority of heterozygotes in egg production was shown by Nickerson and Druger (1973). The drastic increase in the effective population size in Katsunuma as estimated above also may be a factor responsible for changes in the population structure and hence causing the disappearance of inversions.

The transposable element *P* may have acted as a causative factor for the genetic changes observed in the present study. Previous surveys of the *P-M* system in the Katsunuma population showed that *Q* strains were already predominant in 1981 (Yamamoto et al., 1984), and in 1983 and 1984 (Matsuura et al., 1993). In the present study, we found many *Q* strains as well as a substantial number of *M* strains but no apparent *P* strains in samples from the 1996 and 1997 collections. The *P-M* character in Katsunuma thus did not change significantly in this 17-year period. A laboratory stock established from the Katsunuma population collected in 1963 was shown to be *M* (Kidwell et al., 1983). This means that the *P* element invaded into the Katsunuma population between 1963 and 1981. Gamo et al. (1990) reported that the earliest *P* element in Japan was found in 1957 at Hikone (Shiga prefecture), supporting the above assumption.

A possible scenario for the Katsunuma population of *D. melanogaster* thus is: In the late 1960s, *P* elements were introduced into the Katsunuma population which until then was made up of *M* strains. After the invasion, *P* elements spread rapidly in the population. Vigorous transposition of *P* elements resulted in the increase of lethals and steriles. As the number and heterogeneity of *P* elements increased in the population, the transposition became repressed. Most flies in the population would have become to carry defective *P* by 1973, and the mutation rates decreased to an ordinary level. The lethals and steriles once increased in the population were subjected to constant selection and reached a new equilibrium. As a result, the allelism rate of these deleterious genes

decreased, and hence the effective population size increased.

The effect of *P* element invasion on the Katsunuma population was first suggested by Mukai et al. (1985), who examined the population of 1983. When the second chromosomes were isolated by a balancer stock, *SM1, Cy/Pm*, in which genetic background had been substituted to that of the Katsunuma natural population, the frequency of lethals was 17.6%. When the balancer stock with a foreign genetic background (*M* cytotype) was used, the lethal frequency was 27.8%. They suggested that the difference came from the mutations newly occurred in the second chromosome during chromosome isolation. This point was taken up again in the present study. All 192 lines isolated in 1985 were re-crossed with females carrying the balancer chromosome *SM1, Cy/Pm* whose genetic background was a laboratory origin (*M* cytotype), and they were made homozygous after additional three generations (Table 1, shown in the parentheses). The frequencies of lethals both on the second and third chromosomes were almost the same as those obtained employing the balancer chromosome whose genetic background had been replaced to that of the Katsunuma natural population. The discrepancy between the present and previous results remains to be explained.

The invasion of *P* element might also have had an effect on the decrease of inversion frequencies in the population. The *P* element must have induced mutations on the inversion-carrying chromosomes as well as on the standard chromosomes. Watanabe and Yamazaki (1976) examined the deleterious effects of induced mutations on the viability of flies heterozygous for a polymorphic inversion-carrying chromosome and a standard chromosome. The heterozygotes showed a significantly lower viability, when mutations were induced on the inversion-carrying chromosome, whereas the viability was not changed when mutations were induced on the standard chromosomes. Induced mutations by the *P* element, therefore, might have broken a heterotic gene complex, disrupting an intrinsic heterosis carried by inversions. Without heterosis, inversion chromosomes are forced to disappear from the population. In contrast genetic variations such as lethals, steriles, and some visibles are maintained in population by the balance of recurrent mutations and selection in heterozygous condition (Hiraizumi and Crow, 1960).

D. simulans, a most closely related species to *D. melanogaster*, was first found in Katsunuma in 1977 (Watada et al., 1986). It was a minor species until the 1990s, especially in the vineyards where we collected *D. melanogaster*. A temporal fluctuation of this newly colonized species was clearly observed in the shrine. Activity of this species was depending on the winter temperature where they lived (Watanabe et al., 1984). The frequency of *D. simulans*, however, did not show any correlation with the genetic changes observed in *D. melanogaster*.

In conclusion, the genetic structure of the Katsunuma population of *D. melanogaster* has changed in the past, and is still changing. Although the frequencies of lethals, male steriles, and female steriles seem to be in an equilibrium, the size of population is still increasing. On the other hand, the polymorphic inversions are constantly decreasing since 1966. An invaded transposon, *P*, appears to have played an important role in changing the population structure.

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