ENZYME AND CHROMOSOME POLYMORPHISMS IN JAPANESE NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*[1]

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Manuscript received October 28, 1975

ABSTRACT

Collections of *D. melanogaster* from Japanese populations were analyzed for enzyme and chromosomal polymorphisms. Allelic frequencies at the *Adh* and *αGpd* loci were compared with polymorphic inversion (*In(2L)B*, *In(2R)C*) frequencies in the second chromosome. There was a significant positive correlation between the frequencies of Adh^S and *In(2L)B*, caused by linkage. On the other hand, inversion-free cage populations maintained in the laboratory for a long time showed considerably larger variation in the frequencies of these enzyme alleles, which seem very likely to be a consequence of random drift. Two fitness components of these enzyme and chromosomal variants were measured in two different environmental conditions; neither of the two loci showed heterozygote superiority in viability or productivity, while the inversion heterozygotes showed a superior productivity compared to the corresponding homozygotes in the fluctuating environment. These findings are compatible with the hypothesis that polymorphic isozyme genes are maintained by random drift of neutral genes in natural populations, and that association with linked inversions is a historical accident.

THE controversy over whether a major fraction of protein polymorphisms in natural populations is due to random drift of neutral mutations (neutral hypothesis, KIMURA and OHTA 1971) or to some kind of balancing selection (PRAKASH, LEWONTIN and HUBBY 1969; AYALA, POWELL and DOBZHANSKY 1971) remains unsolved, and it deserves thorough examination from every aspect.

A large amount of data on allozyme variability for a wide range of organisms, especially Drosophila, is now available, and several methods of testing the hypotheses have been offered. However, only a few investigations have been undertaken to examine relationships between allozymes and polymorphic chromosome inversions. Associations between the two polymorphisms have been reported in *D. pseudoobscura* and *D. persimilis* (PRAKASH and LEWONTIN 1968, 1971; DOBZHANSKY and AYALA 1973), in *D. pavani* (NAIR and BRNCIC 1971), in *D. willistoni* (AYALA, POWELL and DOBZHANSKY 1971), in *D. robusta* (PRAKASH and LEVITAN 1974) and in *D. melanogaster* (KOJIMA, GILLESPIE and TOBARI 1970; MUKAI, METTLER and CHIGUSA 1971; MUKAI, WATANABE and YAMAGUCHI 1974; LANGLEY, TOBARI and KOJIMA 1974). The most important points in these studies are (1) what kind of natural selection is acting, (2) which

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genotypes responded to the selection, and (3) how strong is the association between the polymorphisms of allozymes and inversions.

The present paper is the result of a survey of the second chromosomes from Japanese natural populations and stable cage populations of *D. melanogaster*. We report here on the fitness of flies heterozygous for allozymes and inversions estimated simultaneously in two different environments, and discuss the biological significance of the relationship found between the two polymorphisms of entirely different nature.

MATERIALS AND METHODS

Three Japanese natural populations of *Drosophila melanogaster* were sampled: Katsunuma, Yamanashi in the year 1972, Akayu, Yamagata in 1974, and Ishigaki, Okinawa in 1973 and 1974. Three large laboratory cage populations (actual adult population size is about 5,000 individuals) were sampled: Cage Su-62, 12 years old, which had originated from Suyama, Shizuoka in 1962; Cage Ka-63, 11 years old, which originated from Katsunuma, Yamanashi in 1963; and Cage LF, 7 years old, which had been started with lethal-free second chromosomes. These cage populations are at equilibrium for lethal gene frequency.

Each wild male collected from Katsunuma in 1972, Ishigaki in 1973, Akayu in 1974 and Cage Ka-63 was mated to three *Pm/(SM1)Cy* females. A single F_1 male whose genotype is $+/(SM1)Cy$, was backcrossed to the balancer stock. Males and virgin females from this mating, genotype $+/(SM1)Cy$, were mated to initiate the lines which were maintained in mass cultures. Among these lines, Katsunuma 1972 and Ishigaki 1973 were assayed for karyotypes and allozymes. Akayu 1974 and Cage Ka-63 lines were assayed only for allozymes.

Each wild female collected from Akayu in 1974, Ishigaki in 1974, Cage Su-62, Cage Ka-63 and Cage LF was kept in a vial as an iso-female line. In the following generation, lines from Ishigaki 1974, Cage Su-62 and Cage LF were assayed for karyotypes and allozymes. Akayu 1974 and Cage Ka-63 population lines were assayed only for karyotypes. From the Cage LF population, collections were made twice at a month interval, and we denote them SI and SII respectively.

The procedures for the cytological analysis and the nomenclature of the inversions were described in detail by WATANABE (1967). One larva per vial was taken and salivary gland chromosomes were observed. Homozygous inversions were recognized by the characteristic pattern of inverted bandings. We used the same electrophoretic technique for allozymes (*Adh*: Alcohol dehydrogenase and *α-Gpd*: α-Glycerophosphate dehydrogenase) as LANGLEY, TOBARI and KOJIMA (1974).

The estimations of viability and productivity were described in detail by WATANABE and OHNISHI (1975). Homozygote and heterozygote viabilities were estimated as follows. Crosses were made among three $+_i/(SM1)Cy$ females and three $+_i/(SM1)Cy$ males with four simultaneous replications in each chromosome line from Katsunuma 1972, where the subscript i indicates the line. In the offspring, $+_i/(SM1)Cy$ and $+_i/+_i$ flies are expected to segregate in 2 : 1 ratio. Similar matings were made for random heterozygotes, except that two successively numbered lines were combined, *i.e.*, $+_i/(SM1)Cy \times +_{i+1}/(SM1)Cy$, in order to secure random combinations of different chromosome lines. Three days after the cross, all six flies were transferred to a second vial. Three days after the transfer, all flies were discarded. Flies emerging from both vials, the original and transferred, were counted three times, until the 16th day after the cross or transfer. The numbers of Curly and wild-type flies from the pair of vials were pooled and considered as a single observation. The viability was expressed as the percentage of wild-type flies among the total number emerged. Homozygote and heterozygote viabilities were examined at the same time. All viabilities were standardized to the average viability of the heterozygotes.

Productivity was examined in the generation immediately following measurement of viability. Two wild-type females ($+_i/+_i$ in homozygotes, $+_i/+_{i+1}$ in heterozygotes) aged 48 hrs after eclosion were crossed to four Oregon-R males with four simultaneous replication for each line. Three days after the cross, all six flies in a vial were transferred to a second vial. Three

days after the transfer, all flies were discarded. Flies from both vials were counted three times, until the 16th day after the cross or transfer, and pooled as a single observation. The crosses and standardizations in the estimation of productivity were done in the same way as for viability.

A total of 1,232,861 flies were counted in the viability experiment and 890,643 in the productivity experiment. These experiments were carried out in two environments. One was a constant environment at 25° under a fluorescent lamp and the other a fluctuating environment where the temperature was changed cyclically from 18° to 32° each day, with 12 hrs dark in the low temperature and 12 hrs light in the high temperature.

RESULTS

1. *Allozyme and inversion frequency:* Table 1 gives a comparison of the frequencies of the allozymes and the inversions in the three Japanese natural populations and in the three laboratory cage populations. The frequency of the α-*Gpd*^{S} gene varies from 7% to 22% in the natural populations. However, it exceeds 50% in the two cage populations. On the other hand, the frequency of *Adh*^{S} gene ranges from about 40% to 80% in both natural and cage populations. Another characteristic difference between the natural and cage populations is the degree of variation. In nature, the allelic frequencies of the *Adh* gene are more divergent than those of the α-*Gpd* gene, but under cage conditions, α-*Gpd* genes are more divergent than *Adh* genes. The initial frequencies of these allozymes in the cage populations are not known. However, it is not unreasonable to assume that Cage Ka-63 and Cage LF populations were initially similar to the natural Katsunuma population of 1969, since they had been derived from the same Katsunuma origin in 1963 and 1967, respectively.

In Japanese natural populations, polymorphisms for the paracentric inversions were found in each arm of the second chromosome; *In(2L)B* having the same breakpoints as *In(2L)t* at 22D and 34A, and *In(2R)C* having the same break-

TABLE 1

A comparison of frequencies of allozymes and inversions in Japanese natural and cage populations of Drosophila melanogaster

Population	Allozyme frequency		Inversion frequency per arm	
	α-*Gpd*^{S}	*Adh*^{S}	*In(2L)B*	*In(2R)C*
Katsunuma 1969†	.153 (396)	.466 (1050)	.236 (123)	.172 (166)
Katsunuma 1970‡	.167 (264)	.395 (263)	.180 (233)	.138 (261)
Katsunuma 1972	.223 (197)	.421 (197)	.125 (200)	.045 (200)
Akayu 1974	.126 (215)	.493 (215)	.263 (300)	.147 (300)
Ishigaki 1973	.141 (78)	.744 (78)	.531 (81)	.358 (81)
Ishigaki 1974	.072 (292)	.784 (292)	.579 (252)	.373 (252)
Cage Su-62	.279 (480)	.665 (480)	0 (200)	0 (200)
Cage Ka-63	.787 (708)	.592 (708)	0 (200)	0 (200)
Cage LF-SI	.569 (480)	.381 (480)	0 (54)	0 (54)
Cage LF-SII	.575 (480)	.373 (480)	–	–

Sample size given in parentheses.
† From KOJIMA, GILLESPIE and TOBARI (1970).
‡ From LANGLEY, TOBARI and KOJIMA (1974)

points as *In(2R)NS* at 52A and 56F (WATANABE 1967). Frequencies of these inversions in natural and cage populations are shown in Table 1. The Ishigaki population maintains the inversions in both arms at high frequencies, and Akayu and Katsunuma of 1969 follow it. Recently, the Katsunuma population appears to have had a sudden change in the frequencies of the inversions and of recessive lethal genes (WATANABE, WATANABE and OSHIMA 1976). Both *In(2L)B* and *In (2R)C* become less frequent after 1969. At any rate, these natural populations have a fair number of inversions, while all the cage populations are found to be free from any inversions. They must have lost these chromosomal variants while the cages were kept in the laboratory with a constant environment, since the initial frequencies of the inversions were known to be:

	Cage Su-62	Cage Ka-63	Cage LF
In(2L)B	.150	.325	.250
In(2R)C	0	.270	.050

These inversions in Cage Su-62 and Cage Ka-63 had already disappeared in 1970 (7 years for Su-62 and 6 years for Ka-63). *In(2L)B* in Cage LF had been maintained for two years unchanged (WATANABE and WATANABE 1973), but it has not been detected in the recent examination.

2. *Association between allozyme and inversion:* Table 2 shows the associations

TABLE 2

Associations between two linked enzymes, and between enzymes and inversions

Population	*Adh—α-Gpd*				n†	χ^2	Significance
	F-F	F-S	S-F	S-S			
Katsunuma 1972	83	31	70	13	197	3.76	NS
ST	77	30	39	12	158	0.34	NS
In	6	1	31	1	39	1.30	NS
Ishigaki 1973	16	4	51	7	78	0.77	NS
ST	10	3	8	4	25	0.32	NS
In	6	1	43	3	53	0.52	NS
Akayu 1974	91	18	97	9	215	3.14	NS
Cage Ka-63	21	63	29	115	228	0.73	NS

Population	Allozyme—Inversion				n†	χ^2	Significance
	F-F	F-S	S-F	S-S			
Katsunuma 1972							
α-Gpd—2L	121	31	44	1	197	8.46	<1%
Adh—2L	113	2	52	30	197	42.71	<1%
α-Gpd—2R	144	8	44	1	197	0.75	NS
Adh—2R	110	5	78	4	197	0.03	NS
Ishigaki 1973							
α-Gpd—2L	26	41	11	0	78	14.18	<1%
Adh—2L	18	2	19	39	78	19.57	<1%
α-Gpd—2R	42	25	7	4	78	0.00	NS
Adh—2R	15	5	34	24	78	1.69	NS

† *n*: No. of chromosomes.

between two enzyme loci, and between enzyme and inversions. *Adh* and *α-Gpd* are located in the left arm. *In (2L)B* includes the *α-Gpd* locus but *Adh* is located between *In (2L)B* and the centromere (Grell 1967; Grell, Jacobson and Murphy 1965). The alleles at *Adh* and *α-Gpd* loci are in linkage-equilibrium. On the other hand, both allozymes show significant nonrandom association with the left arm inversions. A complete linkage of *In(2L)B* with Adh^S and with $\alpha\text{-}Gpd^F$ was found in Katsunuma of 1972. Two Adh^F—*In 2L* combinations shown in Table 2 were due to a unique inversion (28D—32D) and one $\alpha\text{-}Gpd^S$—*In 2L* combination was due to another unique inversion (37B—39C). However, in the Ishigaki population two Adh^F—*In(2L)B* combinations were found. This association is very rare among all published data. No linkage disequilibrium between the right arm inversion and the left arm allozyme was detected in the Katsunuma and Ishigaki populations.

Figure 1 shows the correlations of Adh^S and $\alpha\text{-}Gpd^S$ frequencies with *In(2L)B* frequency, in which the observations of six natural populations are presented with large circles. It is apparent that Adh^S frequency increases with the *In(2L)B* frequency. The correlation is positive, and the regression coefficient (*b*) of Adh^S on the *In(2L)B* is 0.88, which is statistically different from 0. On the other hand, $\alpha\text{-}Gpd^S$ frequency decreases as the inversion frequency increases. The face value of the regression coefficient (*b*) of the $\alpha\text{-}Gpd^S$ on the *In(2L)B* is —0.21, but this is not significantly different from 0. Samples from three cage populations are

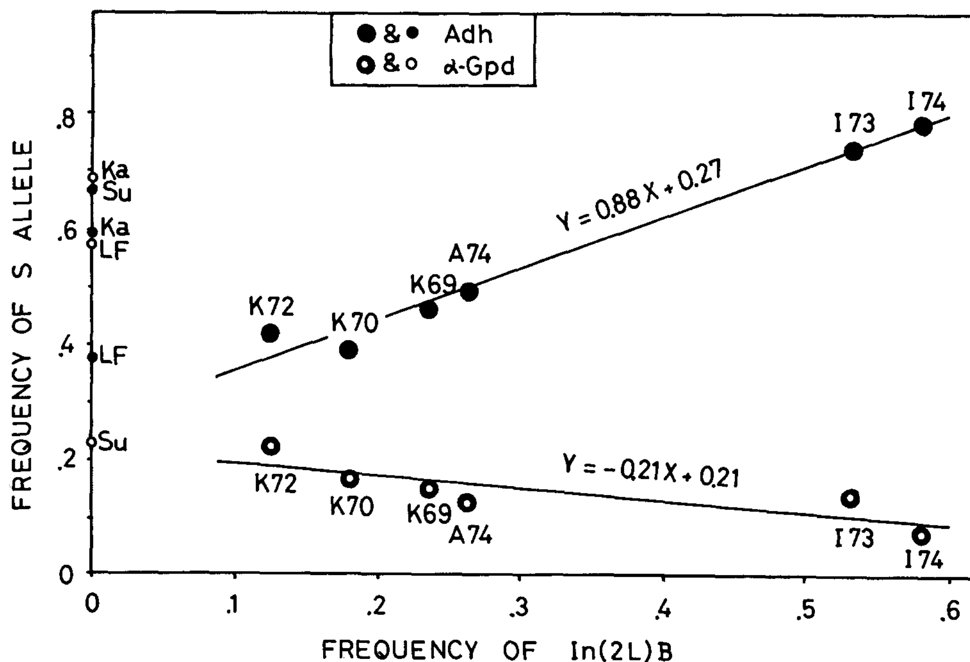

Figure 1.—Correlations between frequencies of allozyme and *In(2L)B*. Large circles represent natural populations while small circles represent cage populations.

presented by small circles along the vertical axis. This is because the cage populations have lost the inversion and presently have no chromosomal polymorphism.

3. *Zygotic frequencies of allozymes and inversions:* Analyses of zygotic combinations for allozymes from the iso-female lines are shown in Table 3. Most cases show numerically close agreement with the Hardy-Weinberg expectation for the two enzymes analyzed. Homozygote excess for *Adh* locus was observed in the first sample of Cage LF, but it was not confirmed in the second sample. The zygotic frequencies of inversions from Akayu 1974 are also given in Table 3. A significant homokaryotype excess was detected in *2L* chromosomes at Site 1, but the other results were close to the Hardy-Weinberg expectations.

4. *Viability and productivity of enzymes:* The viabilities and productivities of the flies from Katsunuma population of 1972 were examined with respect to the alleles at the enzyme loci. Table 4 shows the result which was obtained in the

TABLE 3

Zygotic frequencies for enzymes and inversions in natural and cage populations

Enzyme:

Population		S/S	S/F	F/F	n†	χ^2
Ishigaki 1974						
	Adh	98	33	15	146	16.09**
	α-Gpd	4	13	129	146	16.01**
Cage Su-62						
	Adh	105	109	26	240	0.08
	α-Gpd	15	104	121	240	1.42
Cage Ka-63						
	Adh	82	111	47	240	0.72
	α-Gpd	152	75	13	240	0.84
Cage LF-SI						
	Adh	24	135	81	240	8.88**
	α-Gpd	73	127	40	240	1.49
Cage LF-SII						
	Adh	33	113	94	240	0.01
	α-Gpd	76	124	40	240	0.80

Inversion:

Akayu 1974		ST/ST	ST/In	In/In	n†	χ^2
Site I	2L	100	38	11	149	6.28*
	2R	120	29	0	149	1.73
Site II	2L	82	57	11	150	0.06
	2R	108	34	5	147	1.25
Site III	2L	47	35	7	89	0.02
	2R	67	17	4	88	3.82

† n: No. of zygotes.
* 5% level of significance.
** 1% level of significance.

TABLE 4

Mean viabilities and productivities for isozyme genes in homozygous genetic backgrounds

Genotype	Viability			Productivity		
	n†	Constant	Fluctuating	n†	Constant	Fluctuating
Adh:						
F/F	114	.596 ± .042	.567 ± .029	76	.545 ± .034	.412 ± .029
S/S	83	.584 ± .049	.557 ± .048	52	.518 ± .034	.342 ± .029
α-Gpd:						
F/F	153	.591 ± .036	.561 ± .035	101	.522 ± .027	.367 ± .023
S/S	44	.590 ± .070	.567 ± .069	27	.578 ± .053	.446 ± .045

† n: No. of lines.

homozygous genetic backgrounds. For *Adh*- and *α-Gpd*-carrying chromosomes, no significant differences in mean viability or mean productivity were detected between F/F and S/S genotypes in constant or fluctuating environments.

To reveal the intrinsic effect of the enzyme genes, viabilities and productivities were then estimated in heterozygous genetic backgrounds. Second chromosome lines were combined heterozygously and the mean viability and productivity were measured. Results are presented in Table 5. Three genotypes (F/F, F/S and S/S) for the two loci were tested. Again, viabilities and productivities of these genotypes are very similar to each, with one exception. In the fluctuating environment, the viability of Adh^F/Adh^S was rather inferior to that of Adh^F/Adh^F. There was no evidence for heterozygote superiority, even if the mean of the two homozygotes was compared with the heterozygotes.

TABLE 5

Mean viabilities and productivities of genotypes for isozyme genes in heterozygous genetic backgrounds

Genotype	n†	Viability		Productivity	
		Constant	Fluctuating	Constant	Fluctuating
Adh:					
F/F	65	.995 ± .006	1.012 ± .006] **	.997 ± .019	.829 ± .020
F/S	94	1.000 ± .005	.986 ± .006] **	.996 ± .013	.793 ± .013
S/S	35	.999 ± .006	.993 ± .007	1.009 ± .020	.813 ± .024
Pooled hetero.	94	1.000 ± .005	1.000 ± .007] *	1.000 ± .013	1.000 ± .017
Pooled homo.	100	.997 ± .005	1.020 ± .005] *	1.005 ± .014	1.038 ± .019
α-Gpd:					
F/F	112	1.002 ± .005	.995 ± .005	1.000 ± .012	.813 ± .013
F/S	76	.999 ± .006	.998 ± .007	.997 ± .017	.801 ± .018
S/S	6	1.017 ± .017	.979 ± .012	1.014 ± .027	.818 ± .043
Pooled hetero.	76	1.000 ± .006	1.000 ± .007	1.000 ± .017	1.000 ± .022
Pooled homo.	118	1.012 ± .004	.996 ± .005	1.003 ± .012	1.016 ± .015

† n: No. of crosses.
* 5% level of significance.
** 1% level of significance.

TABLE 6

Homozygote viabilities and productivities for standard and inversion chromosomes

Chromosome	Viability n†	Viability Constant	Viability Fluctuating	Productivity n†	Productivity Constant	Productivity Fluctuating
Standard	160	.593 ± .035	.564 ± .034	105	.539 ± .027	.392 ± .023
Inversions	40	.602 ± .069	.576 ± .068	26	.504 ± .057	.318 ± .047
In(2L)B	25	.519 ± .089	.482 ± .087	14	.440 ± .073	.252 ± .054*
Others	15	.740 ± .103	.733 ± .099	12	.580 ± .086	.395 ± .077

† n: No. of line.
* 5% level of significance.

5. *Viability and productivity of inversions*: The viability and productivity of inversion-carrying individuals were examined in the same manner as the experiment for isozymes. Results are given in Table 6 for the homozygotes and in Table 7 for the heterozygotes. The homozygotes for inversion-carrying chromosomes, especially for *In(2L)B*. showed reduced productivity in the fluctuating environment, while the viabilities were not statistically different among the chromosome types. In the heterozygous genetic backgrounds, the heterokaryotypes were, on the average, superior to the homokaryotypes. A significant heterosis in productivity was detected in the fluctuating environment, although no such heterosis was found in viability. Thus, the polymorphic inversion, *In(2L)B*, is evidently heterotic.

DISCUSSION

AYALA, POWELL and DOBZHANSKY (1971) and PRAKASH and LEVITAN (1974) have noted from extensive Drosophila data that the most common allele is the same over a wide geographical area, and that is true for a number of loci. On the other hand, JOHNSON and SCHAFFER (1973) found distinct correlations between the patterns of gene frequency variations at enzyme loci and environmental factors. This sort of uniformity or correlation is what would be expected if there

TABLE 7

Heterozygote viabilities and productivities for standard and inversion chromosomes

Genotype	n†	Viability Constant	Viability Fluctuating	Productivity Constant	Productivity Fluctuating	
Homokaryotype	142	.999 ± .004	.998 ± .005	.997 ± .012	.796 ± .012	
Standard/Standard	134	.999 ± .004	.998 ± .005	.999 ± .012	.803 ± .012	
In(2L)B/In(2L)B	7	1.007 ± .022	1.009 ± .018	.974 ± .024	.676 ± .051	
Others/Others	1	.996	.990	1.069	.752	*
Heterokaryotype	58	1.001 ± .006	.991 ± .006	1.007 ± .016	.849 ± .019	*
Standard/*In(2L)B*	36	1.008 ± .007	.987 ± .007	1.025 ± .018	.855 ± .020	
Standard/Others	22	.990 ± .008	.997 ± .010	.977 ± .030	.839 ± .039	

† n: No. of crosses.
* 5% level of significance.

is selection, but one can also explain these results on the neutrality hypothesis by assuming the right amount of migration. The present study also revealed a considerable amount of variation at the enzyme loci, which is similar to the cases found by JOHNSON and SCHAFFER. The most extreme deviation was in the frequencies at the *α-Gpd* locus in the cage population that had been kept for more than 7 years in the laboratory.

Chromosomal polymorphisms involving inversion of a gene block have been known in natural populations of many Drosophila species. Heterotic balancing selection is thought to be responsible for maintenance of permanent polymorphisms (DOBZHANSKY 1970). In the case of the second chromosome of *D. melanogaster*, *In(2L)B* is the most common polymorphic inversion found throughout the world. Nearly all Japanese natural populations have *In(2L)B* inversion. WATANABE and WATANABE (1973) have reported that the heterokaryotypes for the inversion and the standard chromosome (*In(2L)B/ST*) show a clear superiority in female productivity. This heterotic balancing selection was confirmed here again in the fluctuating environment. However, the ecological factor(s) that determine the frequency in each population remains unsolved.

Testing the zygotic frequencies against the Hardy-Weinberg expectations enables us to infer the breeding structure and sampling bias. An excess of homozygotes is expected when a sample is taken from a population with some degree of inbreeding. The excess of heterozygotes is a consequence of sampling error rather than heterosis. Therefore direct estimations of fitness components are needed. MARINKOVIC and AYALA (1975a,b) have studied the fitness of allelic variation at five enzyme loci of *D. pseudoobscura*. Heterozygotes were sometimes best when all the fitness components (female fecundity, male mating capacity, egg-to-adult survival, rate of development) were considered. In our present experiment, the heterozygotes for the enzymes did not show any superiority, though the heterozygotes for inversions appeared to exhibit a clear heterosis in the simultaneously conducted experiment. MUKAI, WATANABE and YAMAGUCHI (1974) have found no evidence for differential viability or fecundity among different genotypes at each of three typically polymorphic loci. Moreover, they did not have cumulative effects of heterozygous loci on the fitness components at *α-Gpd*, *Mdh*, *Adh* and *Amy* loci.

POWELL (1973) periodically studied cage populations of *D. willistoni* for the frequency changes of allozymes. He observed apparent frequency changes in *Est-5* and *Lap-5* enzyme loci. However, these loci were nonrandomly associated with the polymorphic gene arrangements. In another experiment, the gene frequencies at *Est-7* loci did not change significantly, which is compatible with the result of *Est-5* loci in *D. pseudoobscura* (YAMAZAKI 1971). The latter two enzymes are sex-linked esterase loci with no associated inversion.

Associations of *Adh* and *α-Gpd* alleles with the second chromosome inversion, *In(2L)B*, are not random, though associations of *Adh—α-Gpd* and the allozymes —opposite arm inversion (*In(2R)C*) are random in Japanese natural populations of *D. melanogaster*. The same association has been reported from Texas populations (LANGLEY, TOBARI and KOJIMA 1974). A slightly different result was

reported from North Carolina populations (MUKAI, WATANABE and YAMAGUCHI 1974), in which *Adh—In(2R)NS* association was found to be nonrandom.

The allozyme—inverison linkage found here is quite rigid; that is, *In(2L)B* is linked always with Adh^S and α-Gpd^F alleles, with the exception of two chromosomes from the Ishigaki population. Although the history of the polymorphic inversion remains unknown, we can speculate that this adaptive chromosomal rearrangement has occurred in one standard sequence chromosome which had Adh^S and α-Gpd^F alleles and spread throughout the world populations. The *Adh* locus is located between the centromere and *In(2L)B*. The distance between the *Adh* locus and the proximal end of the inversion is so short that the linkage has been maintained by reduced recombination. The α-*Gpd* gene which is included in the inversion shares its fate with *In(2L)B*.

In natural populations, the frequency of allozymes, especially that of Adh^S, is correlated with the frequency of *In(2L)B*. Therefore the frequency of Adh^S seems to be determined by the frequency of *In(2L)B* in natural populations where the inversion appears to be maintained by selection, but not by the selection on the enzyme itself. If natural selection acts directly on the enzyme loci and the loci respond it, frequencies of Adh^S and α-Gpd^S would be more uniform throughout different inversion frequencies.

The elimination of the polymorphic inversions from cage populations can be explained at present in the following manner. In the cage environment, natural selection acting on the pre-adult stages of Drosophila might have dominated that acting on the post-adult stages. Therefore, the inversion heterozygotes which have superiority in productivity can not compensate for their lower viability. In addition, constant temperature environment might have taken the heterotic effect away from the inversion heterozygotes. At any rate, although the polymorphic inversions were eliminated from the cage populations, the enzyme polymorphisms have been maintained. Moreover, the frequencies of each allozyme have shown a large variation among populations. This is consistent with the random genetic drift of neutral genes, that is, genes with selection coefficients less than the reciprocal of the effective population size.

We thank DRS. TAKEO MARUYAMA and JAMES F. CROW for valuable discussions and for reading the manuscript. We are indebted to DR. YOSHIKO N. TOBARI for her kind analyses of enzymes. We also thank MESSRS. KENJI ITO, TADASHI OKAWA and MASAOKI KAWANISHI for their technical assistance. This work was supported in part by the grant from the Ministry of Education, Japan (954159).

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Corresponding editor: J. F. CROW