ASSOCIATION OF CHROMOSOME AND ENZYME POLYMORPHISMS IN NATURAL AND CAGE POPULATIONS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

The frequencies of a polymorphic inversion, *In(2L)t*, and of *Adh* and *αGpdh* alleles were analyzed in three natural populations of *Drosophila melanogaster* from Japan. Significant positive correlations between the frequencies of *In(2L)t* and Adh^S or $\alpha Gpdh^F$ were detected due to tight linkage. An analysis of correlation with latitude showed that the negative cline of Adh^S frequency could be explained entirely by its linkage with *In(2L)t*; the frequency of Adh^S on the standard chromosome did not show a latitudinal cline. To the contrary, the cline of $\alpha Gpdh^F$ frequency itself was positive, and its linkage with *In(2L)t* makes the positive cline unclear. These results suggest that the two allozymes themselves respond to latitudinal natural selection in different ways. When these populations were transferred to laboratory cages and maintained for a long time, they lost the chromosomal polymorphism but retained stable enzyme polymorphisms, although allele frequencies in the cage were not the same as in nature. The frequencies of *Adh* and *αGpdh* alleles were close to those in earlier cage populations of the same geographical origin.

DROSOPHILA populations have been surveyed in order to study the mechanisms maintaining genetic variability, such as enzyme and inversion polymorphisms. The second chromosome of *D. melanogaster* carries considerable genetic variation in nature. A polymorphic inversion on the left arm, *In(2L)t*, is frequently found in addition to the standard sequence chromosome, *ST*. Enzyme loci located on the same chromosome arm, *Adh* (alcohol dehydrogenase) and *αGpdh* (α-glycerophosphate dehydrogenase), carry *F* and *S* alleles which are fairly frequent. Polymorphic inversions are often subject to selection. WATANABE and WATANABE (1973, 1977) observed superior female productivity of *In(2L)t/ST* heterozygotes compared with the corresponding homozygotes, suggesting that heterotic balancing selection was working in nature (DOBZHANSKY 1970). In three widely separated regions, the eastern United States, Australia and Japan, latitudinal clines in the frequencies of the polymorphic inversions, higher toward the equator, have been reported (METTLER, VOELKER and

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MUKAI 1977; INOUE and WATANABE 1979; KNIBB, OAKESHOTT and GIBSON 1981). Moreover, nonrandom associations of inversions have occasionally been observed in some natural populations (STALKER 1976; KNIBB, OAKESHOTT and GIBSON 1981).

On the other hand, there is controversy about the maintenance of enzyme polymorphisms. Neutralists offer random processes, such as drift, as major factors (KIMURA and OHTA 1971). This hypothesis is supported by a number of experimental studies. There was no difference in viability or fecundity among genotypes of *Adh* or *αGpdh* (MUKAI, METTLER and CHIGUSA 1971; WATANABE and WATANABE 1977). Moreover, no association was observed between isozyme alleles if their loci were very close on the same chromosome arm (MUKAI, WATANABE and YAMAGUCHI 1974; MUKAI and VOELKER 1977; LANGLEY, ITO and VOELKER 1977). However, geographical clines in allele frequency have been observed for *Adh* (VIGUE and JOHNSON 1973; PIPKIN, RHODES and WILLIAMS 1973; OAKESHOTT *et al.* 1982), *αGpdh* (VOELKER *et al.* 1978; OAKESHOTT *et al.* 1982), *Est-C* and *Odh* (JOHNSON and SCHAFFER 1973). CAVENER and CLEGG (1978) reared *D. melanogaster* populations on medium supplemented with ethanol and determined allelic frequencies for eight polymorphic enzymes. Only *Adh* and *αGpdh* showed rapid gene frequency divergence between control and treated populations, with a decrease of the *S* allele for both enzymes.

Nonrandom associations between allozymes and inversions complicate the problem of both kinds of polymorphism. Nonrandom association was observed when enzyme locus and inversion were located on the same chromosome arm. Two alleles (*F*, *S*) of *Adh* and *αGpdh* showed significantly nonrandom associations with *In(2L)t*, although the two alleles of these enzyme loci were in linkage equilibrium (KOJIMA, GILLESPIE and TOBARI 1970; MUKAI, METTLER and CHIGUSA 1971; LANGLEY, TOBARI and KOJIMA 1974; VOELKER, MUKAI and JOHNSON 1977; WATANABE and WATANABE 1977).

WATANABE and WATANABE (1977) studied the frequencies of *In(2L)t*, Adh^S and $\alpha Gpdh^F$ in Japanese natural populations and in some cage populations. They found positive correlations between *In(2L)t* and Adh^S or $\alpha Gpdh^F$, although the correlation involving *In(2L)t* and $\alpha Gpdh^F$ was not statistically significant. The cage populations, which were more than 7 years old, lost the inversion polymorphism but maintained some types of isozyme polymorphisms. Allele frequencies in these cages differed from the original populations, and they varied among cages. The extinction of inversion polymorphism in cage populations was confirmed by INOUE (1979) with strains from other populations such as Ishigaki (Okinawa) and Katsunuma (Yamanashi) in 1976. This paper presents the results of a further survey of Japanese natural populations and of inversion-free cage populations. In addition, allozyme frequencies in the cage populations of INOUE (1979) were monitored during the course of inversion extinction.

MATERIALS AND METHODS

Japanese natural populations were sampled at Katsunuma, Yamanashi, in 1976, 1979, 1980 and 1981; at Akayu, Yamagata in 1977; and at Ishigaki, Okinawa, in 1976. The Katsunuma and Akayu

populations were at wineries, and that of Ishigaki was in a pineapple field. Cage populations were begun with 200 isofemale lines from samples of Katsunuma-1976, Akayu-1977 and Ishigaki-1976 and maintained under laboratory conditions at 25°. Older cage populations were also examined: Suyama-1962, Katsunuma-1963, Katsunuma-1967 LF, Ishigaki-1973 and Akayu-1974. The Katsunuma-1967 LF population was started with lethal-free second chromosomes. The four cage populations other than Akayu-1974 were the same as those used by WATANABE and WATANABE (1977). These older populations were already free from inversions.

Chromosome and allozyme analyses were carried out at the same time. Individual females from each sample were kept in vials as isofemale lines, and one F_1 larva was taken for salivary chromosome analysis (INOUE and WATANABE 1979). For the Katsunuma populations of 1979, 1980 and 1981 the allozyme and chromosome assays were done on the same chromosomes that were isolated from nature and kept in the laboratory using SM1 *Cy* and TM3 *Sb* balancers. The allozyme analyses (*Adh* and *αGpdh*) were carried out on equal numbers of male and female flies according to the electrophoretic techniques of LANGLEY, TOBARI and KOJIMA (1974).

RESULTS

Japanese natural populations: A total of six samples were analyzed from three populations. Katsunuma and Akayu are located on the mainland and Ishigaki, on the Southwest Islands. Table 1 shows the frequencies of *In(2L)t*, Adh^S and $\alpha Gpdh^F$, including data from earlier reports. Although inversion frequency was observed to decrease from the 1960s to the 1970s in the Katsunuma population (INOUE and WATANABE 1979), the frequencies of these three genes did not vary much in each locality but, rather, differed from locality to locality. *In(2L)t* was found in more than half of the *2L* chromosome arms observed from Ishigaki; in the Akayu and Katsunuma populations, frequencies were less

TABLE 1

Frequencies of In(2L)t, Adh^S, *and* $\alpha Gpdh^F$ *in three Japanese natural populations of* D. melanogaster[a]

Population		*In(2L)t*	Adh^S	$\alpha Gpdh^F$	Reference
Katsunuma	1969	0.236 (123)	0.466 (1050)	0.847 (396)	KOJIMA, GILLESPIE and TOBARI (1970)
	1970	0.180 (233)	0.395 (263)	0.833 (264)	LANGLEY, TOBARI and KOJIMA (1974)
	1972	0.125 (200)	0.421 (197)	0.777 (197)	WATANABE and WATANABE (1977)
	1976	0.115 (200)	0.374 (240)	0.749 (240)	This study
	1979	0.134 (232)	0.341 (232)	0.759 (232)	This study
	1980	0.147 (238)	0.382 (238)	0.761 (238)	This study
	1981	0.160 (244)	0.361 (244)	0.803 (244)	This study
Akayu	1974	0.263 (300)	0.493 (215)	0.874 (215)	WATANABE and WATANABE (1977)
	1977	0.225 (200)	0.407 (240)	0.916 (240)	This study
Ishigaki-Jima	1973	0.531 (81)	0.744 (78)	0.859 (78)	WATANABE and WATANABE (1977)
	1974	0.579 (252)	0.784 (292)	0.928 (292)	WATANABE and WATANABE (1977)
	1976	0.545 (200)	0.841 (240)	0.893 (240)	This study

[a] Sample size is given in parentheses.

than half of that at Ishigaki. The frequency of Adh^S was similar in the Katsunuma and Akayu populations, the range being 37–49%, whereas the range of frequencies at Ishigaki was 74–84%. On the other hand, the frequency of $\alpha Gpdh^F$ did not vary so much, ranging from 75 to 93% among the three localities. As WATANABE and WATANABE (1977) reported, allozyme frequencies in nature varied more at the *Adh* locus than *αGpdh*.

The left panels of Figure 1 and 2 show the correlations of Adh^S and $\alpha Gpdh^F$ frequencies with the frequency of *In(2L)t*, for 12 samples of three natural populations. The correlation was positive and significant in both cases, and the regression coefficients of Adh^S and $\alpha Gpdh^F$ on *In(2L)t* were 1.003 and 0.268, respectively, both statistically significantly different from zero.

Laboratory cage populations: Six cage populations maintained for 1–6 yr in the laboratory were analyzed for frequencies of Adh^S and $\alpha Gpdh^F$. The Akayu-1977 cage retained *In(2L)t* at 13%, but the other five cages lost the chromosomal polymorphism and contained only the standard chromosome. The results are shown in Table 2. Both allozyme frequencies seemed little changed during the several years after the polymorphic inversions were eliminated from the cage populations. The frequencies of Adh^S in the Katsunuma-1967 and Ishigaki-1973 cage populations were rather similar to those in the corresponding natural populations (Figure 1). The frequency was slightly higher in the Katsunuma-1963 cage than in nature, whereas the Akayu-1974 cage population showed a lower frequency than in nature. On the other hand, the $\alpha Gpdh^F$ frequencies in most cage populations were lower than in the natural ones (Figure 2); both Katsunuma-1963 and Katsunuma-1967 LF populations showed frequencies remarkably lower than the natural population.

In natural populations the variation of Adh^S frequency was higher than that

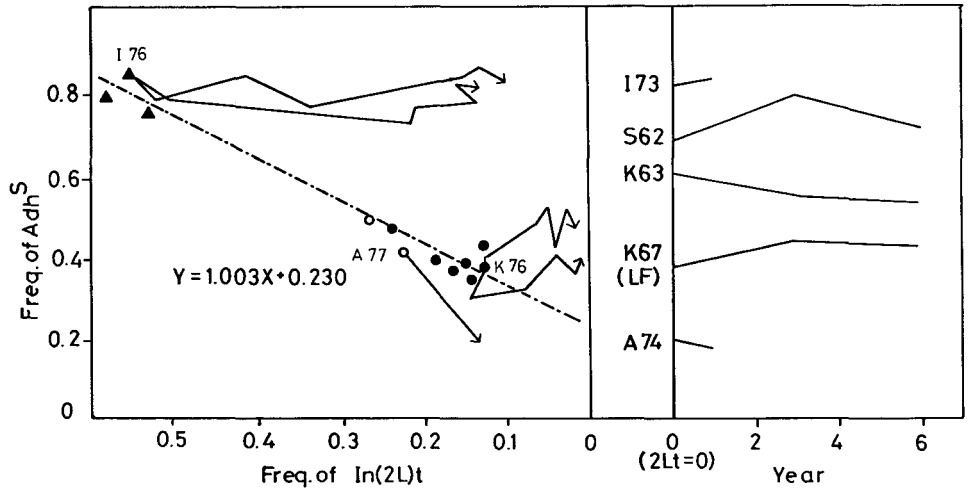

FIGURE 1.—Regression of Adh^S frequency on that of *In(2L)t* in natural populations from ▲, Ishigaki; O, Akayu; ●, Katsunuma. Arrows indicate their frequency changes in cages (left). The right panel shows Adh^S frequency changes after the inversions were eliminated from the cages. I, A, K and S denote original populations from Ishigaki, Akayu, Katsunuma and Suyama, and the numbers after them are the years of collection in nature.

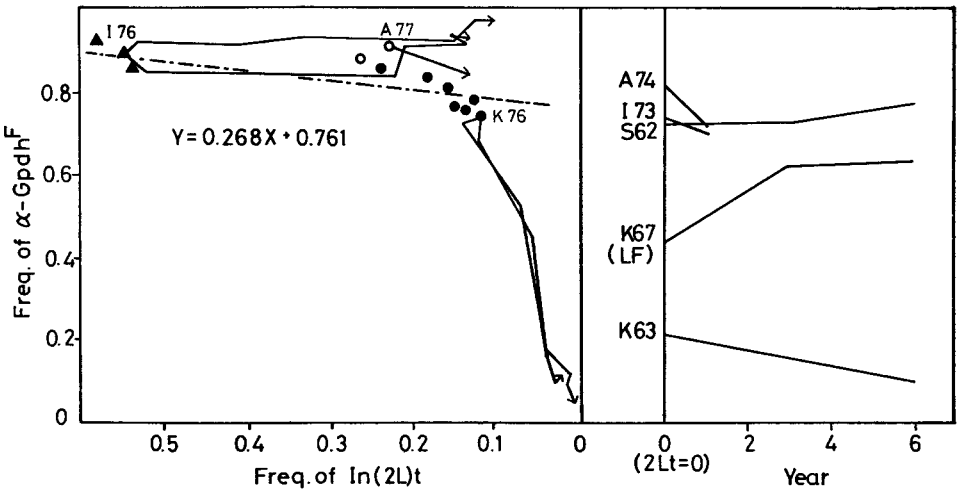

FIGURE 2.—Regression of $\alpha Gpdh^F$ frequency on that of $In(2L)t$ in natural populations from ▲, Ishigaki; O, Akayu; ●, Katsunuma. Arrows indicate their frequency changes in cages. The right panel shows $\alpha Gpdh^F$ frequency changes after the inversions were eliminated from the cages. Abbreviations are as in Figure 1.

TABLE 2

The frequencies of Adh^S *and* $\alpha Gpdh^F$ *in inversion-free cage populations*

Origin	No. of months in cage	No. of chromosomes tested	Adh^S	$\alpha Gpdh^F$
Katsunuma-1963	132[a]	708	0.592	0.213
	168	240	0.550	0.154
	204	240	0.533	0.100
Katsunuma-1967 (LF cage)	84[a]	480	0.381	0.431
	120	240	0.433	0.617
	156	240	0.433	0.625
Suyama-1962	144[a]	480	0.665	0.721
	180	240	0.787	0.725
	218	240	0.717	0.767
Akayu-1974	36	240	0.188	0.821
	48	240	0.175	0.717
Akayu-1977[b]	12	240	0.190	0.820
Ishigaki-1973	48	240	0.813	0.738
	60	240	0.833	0.696

[a] Data from WATANABE and WATANABE (1977).
[b] This cage still maintained the $In(2L)t$ with the frequency of 0.13.

of $\alpha Gpdh^F$, but in cage populations the situation reversed (Table 2, Figure 2, right panel). This was largely due to the remarkable change in the Katsunuma-1963 cage population. It is clear that the cage populations have lost the chromosomal polymorphism, as reported by WATANABE and WATANABE (1977) and

INOUE (1979), but they continued to maintain enzyme polymorphisms at relatively stable levels, although allele frequencies in the cages were not the same as in nature.

Periodic monitoring of the cage populations: INOUE (1979) reported that the polymorphic inversions were eliminated from cage populations with a constant selective intensity of 0.05 or 0.06. We used the same materials of his four cage populations; two each of which were established from the natural populations at Katsunuma (Katsunuma-1976) and Ishigaki (Ishigaki-1976). The frequencies of *In(2L)t*, Adh^S and $\alpha Gpdh^F$ were examined every 3 months for 1.5 yr. The results are shown in Table 3. Each set of two replicated cage populations showed similar tendencies. The frequency of *In(2L)t* decreased gradually in all cages: from 13 to 2–0.5% in the Katsunuma cages and from 55 to 13–10% in the Ishigaki cages. Adh^S frequencies were not changed as much from nature in all cages (see Figure 1, left panel). Similarly, $\alpha Gpdh^F$ frequencies in the two Ishigaki cages were as high as in nature. But $\alpha Gpdh^F$ frequency decreased drastically in the two Katsunuma cages, almost linearly from 75 to 5–13% (see Figure 2, left panel).

It is reasonable to expect that Adh^S and $\alpha Gpdh^F$ frequencies in the two Katsunuma-1976 cages would approach the equilibrium frequencies in the Katsunuma-1963 and Katsunuma-1967 LF cages, and that frequencies in the two Ishigaki-1976 cages would approach the frequency in the Ishigaki-1973 cage when *In(2L)t* was finally eliminated. The frequencies of Adh^S and $\alpha Gpdh^F$ decreased in the Akayu cage (Akayu-1977) over a year. Although *In(2L)t* was still present at a frequency of about 13%, both allozyme frequencies were expected

TABLE 3

Frequency changes of In(2L)t, Adh^S *and* $\alpha Gpdh^F$ *in the Katsunuma and Ishigaki cage populations*

No. of months	Replication	No. of chromosomes tested	Katsunuma-1976			Ishigaki-1976		
			In(2L)t	Adh^S	$\alpha Gpdh^F$	*In(2L)t*	Adh^S	$\alpha Gpdh^F$
3	A	200	0.140	0.292	0.725	0.530	0.792	0.921
	B	200	0.120	0.404	0.679	0.520	0.787	0.850
6	A	200	0.070	0.317	0.529	0.410	0.846	0.917
	B	200	0.055	0.487	0.450	0.220	0.729	0.842
9	A	200	0.040	0.411	0.183	0.335	0.783	0.925
	B	200	0.045	0.517	0.238	0.210	0.771	0.904
12	A	200	0.015	0.367	0.117	0.150	0.842	0.921
	B	200	0.040	0.425	0.162	0.130	0.771	0.917
15	A	200	0.015	0.371	0.096	0.125	0.862	0.975
	B	200	0.030	0.512	0.100	0.155	0.837	0.933
18	A	200	0.005	0.396	0.050	0.095	0.825	0.975
	B	200	0.020	0.475	0.131	0.130	0.818	0.925

to be like those of the Akayu-1974 cage when the inversion was eliminated. Thus, the frequency changes of these two allozymes in the cages during the elimination of *In(2L)t* varied. It neither followed the regression line calculated from natural populations nor reached a cage-specific equilibrium. However, it seems that each natural population has an equilibrium frequency of each allozyme when the associated inversions are lost. Standard (*ST*) chromosomes carrying Adh^S or $\alpha Gpdh^F$ should have responded to the cage-specific selection that eliminated inversions.

DISCUSSION

Nonrandom associations between *In(2L)t* and allozymes such as Adh^S or $\alpha Gpdh^F$ have been reported for several natural populations, and the present results from Japanese populations confirm these findings. Table 4 shows the frequencies of the exceptional chromosomes having Adh^F or $\alpha Gpdh^S$ on *In(2L)t*. The association of *In(2L)t*-Adh^S or *In(2L)t*-$\alpha Gpdh^F$ was very tight, so the frequency of the exceptional combinations such as *In(2L)t*-Adh^F and *In(2L)t*-$\alpha Gpdh^S$ was 1.5–3% on the average. This phenomenon, as MUKAI and VOELKER (1977) suggested, might be the result of founder effect due to a single origin of *In(2L)t* combined with a lack of recombination between the centromere and the distal breakpoint of *In(2L)t* where *Adh* and *αGpdh* are located. Since the frequency of the exceptional chromosomes was low, we can indirectly estimate the frequency of the standard chromosome with Adh^S (*ST*-Adh^S) or with $\alpha Gpdh^F$ (*ST*-$\alpha Gpdh^F$), as follows:

$$Frequency^{ST\text{-}Adh^S} = Frequency^{Adh^S} - Frequency^{2Lt}$$

$$Frequency^{ST\text{-}\alpha Gpdh^F} = Frequency^{\alpha Gpdh^F} - Frequency^{2Lt}$$

The correlation between the latitude and the frequencies of *In(2L)t*, Adh^S, *ST*-Adh^S, $\alpha Gpdh^F$ and *ST*-$\alpha Gpdh^F$ were calculated as shown in Table 5 using these formulas. Significant negative correlations were observed in the two cases for *In(2L)t* and Adh^S but not for *ST*-Adh^S. The latitudinal cline of Adh^S frequency might be attributed to linkage with *In(2L)t*, since the frequency cline of *ST*-Adh^S itself was not latitudinal. On the other hand, significant positive correlations were observed in all cases for *ST*-$\alpha Gpdh^F$ but not for $\alpha Gpdh^F$, for which the correlations were negative. The cline of *ST*-$\alpha Gpdh^F$ itself was clearly latitudinal, but the association with *In(2L)t* made the cline somewhat vague. These results suggest that the two allozymes respond to latitudinal natural selection in different ways. VOELKER *et al.* (1978) calculated that linkage with *In(2L)t* explains 23% of the *Adh* cline and 34% of the *αGpdh* cline from eastern United States populations. On the other hand, ANDERSON (1981) calculated that 51% of the variation in *Adh*, and 15% of that in *αGpdh*, can be explained by latitudinal clines in the Australian populations.

What factor(s) caused these clinal variations? Temperature may be a major factor. The Adh^S and $\alpha Gpdh^F$ were found to be more heat stable than the corresponding alleles (DAY, HILLIER and CLARKE 1974; ALAHIOTIS, MILLER and BURGER 1977), which is in accord with the fact that the frequencies of Adh^S and $\alpha Gpdh^F$ increase toward the equator. Recently, OAKESHOTT *et al.*

TABLE 4. *The number of* In(2L)t *chromosomes with* Adh^F *or* $\alpha Gpdh^S$ *in several populations*

Population	(A) No. of *2Lt(Adh)*^{F}	(B) No. of *2Lt* tested	(A)/(B)	(C) No. of *2Lt*(α*Gpdh*^{S})	(D) No. of *2Lt* tested	(C)/(D)	Reference
Katsunuma, Japan, 1969	1	29	0.034	2	27	0.077	KOJIMA, GILLESPIE and TOBARI (1970)
Katsunuma, Japan, 1970	0	47	0	5	43	0.116	LANGLEY, TOBARI and KOJIMA (1974)
Katsunuma, Japan, 1972	0	32	0	0	32	0	WATANABE and WATANABE (1977)
Katsunuma, Japan, 1979	1	31	0.032	1	31	0.032	This study
Katsunuma, Japan, 1980	0	35	0	0	35	0	This study
Katsunuma, Japan, 1981	0	39	0	0	39	0	This study
Ishigaki-jima, Japan, 1973	2	41	0.049	0	41	0	WATANABE and WATANABE (1977)
Raleigh, North Carolina, 1968	0	24	0	2	24	0.83	MUKAI, METTLER and CHIGUSA (1971)
Raleigh, North Carolina, 1968	0	3	0	0	3	0	MUKAI, METTLER and CHIGUSA (1971)
Raleigh, North Carolina, 1970	2	40	0.05	4	40	0.100	MUKAI, WATANABE and YAMAGUCHI (1974)
Raleigh, North Carolina, 1974	0	43	0	3	43	0.070	VOELKER *et al.* (1978)
Carpenter, North Carolina, 1975	2	23	0.089	2	23	0.087	LANGLEY, ITO and VOELKER (1977)
Lake Wales, Florida	1	72	0.014	1	72	0.014	VOELKER *et al.* (1978)
Orlando, Florida	1	86	0.012	0	86	0	VOELKER *et al.* (1978)
Jacksonville, Florida	0	26	0	0	26	0	VOELKER *et al.* (1978)
Eugene, Oregon, 1973	0	3	0	0	3	0	VOELKER, MUKAI and JOHNSON (1977)
Bakersfield, California, 1972	0	4	0	0	4	0	VOELKER, MUKAI and JOHNSON (1977)
Brownsville, Texas, 1970	0	47	0	0	41	0	LANGLEY, TOBARI and KOJIMA (1974)
Cephalonia, Greece, 1973	0	3	0	0	3	0	ALAHIOTIS, MILLER and BERGER (1973)
Totals	10	628		20	616		
Mean ± SE			0.015 ± 0.006			0.030 ± 0.010	

TABLE 5

Correlation coefficients between latitude and frequencies of In(2L)t, Adh^S, ST-Adh^S, $\alpha Gpdh^F$ *and* ST-$\alpha Gpdh^F$ *in populations from the eastern United States, Australia and Japan, and their degrees of freedom (d.f.)*

	The eastern United States[a]	Australia[b]	Japan
In(2L)t	−0.920**	−0.917**	−0.917**
Adh^S	−0.880**	−0.830**	−0.929**
ST-Adh^S	−0.376	−0.454	−0.171
$\alpha Gpdh^F$	−0.560*	−0.435	−0.455
ST-$\alpha Gpdh^F$	+0.850**	+0.788**	+0.979**
d.f.	19	16	10

[a] Calculated from data of VOELKER *et al.* (1978).
[b] Calculated from data of KNIBB, OAKESHOTT and GIBSON (1981) and OAKESHOTT *et al.* (1982).
* and ** Significant at 0.05 and 0.01 levels.

(1982) showed that rainfall correlated strongly with Adh^S frequency in the United States, Mexico, Asia and Australia. They explained that temperature and humidity are effective microecological agents, acting in an indirect manner, on the type of vegetation, the composition of coexisting species and the rates of fermentation and decay of fruit.

In cage populations, *In(2L)t* was gradually eliminated, and the frequencies of Adh^S and $\alpha Gpdh^F$ also changed during the elimination of *In(2L)t*. The *ST-*Adh^S chromosome increased as if it made up for the decrease of *In(2L)t* which carried Adh^S in the Katsunuma and Ishigaki populations, but not in the Akayu populations. The *ST-*$\alpha Gpdh^F$ chromosome increased as if it balanced the decrease of *In(2L)t* which carried $\alpha Gpdh^F$ in the Ishigaki and Akayu populations. But *ST-*$\alpha Gpdh^F$ decreased drastically in the Katsunuma populations when $\alpha Gpdh^F$ linked with *In(2L)t* decreased. These rapid frequency changes continued up to the time that *In(2L)t* disappeared. Thereafter, the frequency of these allozymes remained relatively stable in the cages. The final frequencies of allozymes were not the same for all cages but depended on the populations sampled in nature that were used to found the cages. Thus, the selection under cage conditions seems to act on genes other than *Adh* or *αGpdh* alleles of the standard chromosomes, which are different from population to population. This might be termed the genetic background of the standard chromosomes. The size of genetic background can be speculated to be the size of *In(2L)t* or of the region from the distal breakpoint of the inversion to the centromere, since the inversion is the unit of selection in the cage populations.

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