THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER* XIII. FURTHER STUDIES ON LINKAGE DISEQUILIBRIUM[1]

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ABSTRACT

The Raleigh, North Carolina, population of *Drosophila melanogaster* was examined for linkage disequilibrium in 1974, several years after previous analyses in 1968, 1969, and 1970. αglycerol-3-phosphate dehydrogenase-1 (*αGpdh-1*), malate dehydrogenase-1 (*Mdh-1*), alcohol dehydrogenase (*Adh*), and hexokinase-C (*Hex-C*, tentative name, F. M. JOHNSON, unpublished; position determined by the present authors to be 2–74.5) were assayed for 617 second chromosomes, and esterase-C (*Est-C*) and octanol dehydrogenase (*Odh*) were assayed for 526 third chromosomes. In addition, two polymorphic inversions in the second chromosomes [*In(2L)t* and *In(2R)NS*] were examined, and the following findings were obtained: (1) No linkage disequilibrium between isozyme genes was detected. Significant linkage disequilibria were found only between the polymorphic inversions and isozyme genes [*In(2L)t* vs. *Adh*, and *In(2R)NS* vs. *Hex-C*]. Significant disequilibrium was not detected between *In(2L)t* and *αGpdh-1*, which is included in the inversion, but a tendency toward disequilibrium was consistently found from 1968 to 1974. The frequency of two-strand double crossovers within inversion *In(2L)t* involving a single crossover on each side of *αGpdh-1* was estimated to be 0.00022. Thus, the consistent but not significant linkage disequilibrium between the two factors can be explained by recombination after the inversion occurred. (2) Previously existing linkage disequilibrium between *Adh* and *In(2R)NS* (the distance is about 30 cM, but the effective recombination value is about 1.75%) was found to have disappeared. (3) No higher-order linkage disequilibrium was detected. (4) Linkage disequilibrium between *Odh* and *Est-C* (the distance of which was estimated to be 0.0058 ± 0.002) could not be detected ($\chi^2_{df=1} = 0.9$).—From the above results, it was concluded that linkage disequilibria among isozyme genes are very rare in *D. melanogaster*, so that the FRANKLIN-LEWONTIN model (FRANKLIN and LEWONTIN 1970) is not applicable to these genes. The linkage disequilibria between some isozyme genes and polymorphic inversions may be explained by founder effect.

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IN connection with the mechanisms for the maintenance of a large number of protein polymorphisms discovered in many species including man and Drosophila (LEWONTIN and HUBBY 1966; HARRIS 1966), FRANKLIN and LEWONTIN (1970) have proposed a heterotic model with epistasis. According to this model, a large amount of linkage disequilibrium is maintained at equilibrium, even if the distance between the genes in question is relatively large, if a number of overdominant loci with multiplicative gene action are tightly packed between them.

The results of extensive studies on linkage disequilibria between isozyme genes, *inter se*, and between isozyme genes and polymorphic inversions in the second and the third chromosomes of *Drosophila melanogaster* were reported by MUKAI, WATANABE and YAMAGUCHI (1974). In general, significant linkage disequilibria among isozyme genes were not consistently detected over years, but consistent linkage disequilibria were found between some isozyme genes and some polymorphic inversions, between which the recombination values appear small or effectively zero. Thus, the FRANKLIN-LEWONTIN model seems unlikely if the distance between the genes is not extremely small. However, in the case where the recombination values between factors are close to zero, it is impossible to conclude whether or not the FRANKLIN-LEWONTIN model can be applicable (MUKAI, WATANABE and YAMAGUCHI 1974).

There were two cases of special interest: (1) between *Adh* and *In(2R)NS* and (2) between *Est-C* and *Odh*. The linkage disequilibrium between the *Adh* locus, which is located at 50.1 on the left arm of the second chromosome (LINDSLEY and GRELL 1968), and *In(2R)NS*, the breakpoints of which are 52A and 56F in the salivary gland chromosomes, was detected for three consecutive years from 1968 to 1970. The effective recombination value between these two factors has been reported to be about 3.2% (*cf.*, LINDSLEY and GRELL 1968; MUKAI, METTLER and CHIGUSA 1971). This disequilibrium is most likely due to random historical accidents or epistasis and not to sampling error. A linkage disequilibrium was found between *Est-C* and *Odh* in the third chromosome for the 1970 population. The distance between these two loci has been reported to be less than 1% (LINDSLEY and GRELL 1968). Since only one sample (1970) was taken for the third chromosome, it is difficult to determine whether this disequilibrium is due to epistasis, genetic drift, or sampling error. If the above two cases of linkage disequilibria were the results of random genetic drift or sampling, it is highly probable that samples taken after an appreciable time would not show the same disequilibria. Thus, relatively large samples were taken in 1974 from the same population to discriminate between epistasis and chance events. Furthermore, recombination tests were conducted in order to obtain reliable recombination values between factors whose linkage disequilibria were in question, and the effective size of the population was estimated by the frequency of lethal-carrying chromosomes and their allelism rates.

MATERIALS AND METHODS

Materials: Six hundred and seventeen second chromosomes and 526 third chromosomes were isolated in the middle of July, 1974 from Reedy Creek State Park near Raleigh, North Carolina,

utilizing the marked inversion technique. This locality is the same as that from which the samples of 1968, 1969, and 1970 (*cf.*, MUKAI, WATANABE and YAMAGUCHI 1974) were taken. These sampled wild second ($+_i$; i = 1–617) and third ($+_j$; j = 1–526) chromosomes were replicated and frozen in the forms of $+_i/+_i$ (or *SM1(Cy)*/$+_i$ when $+_i$ chromosome carried a recessive lethal gene) for the second chromosomes and in the form of *Ubx*/$+_j$ for the third chromosomes.

Enzyme assays, examination of salivary gland chromosomes, and linkage disequilibrium tests: The following four enzyme loci, known to be located in the second chromosome (O'BRIEN and MACINTYRE 1969; LINDSLEY and GRELL 1968) and two loci, located in the third chromosome (LINDSLEY and GRELL 1968), were studied: αglycerol-3-phosphate dehydrogenase-1 (*αGpdh-1* [E.C.1.1.1.8], map position, 2–17.8); malate dehydrogenase-1 (*Mdh-1* [E.C.1.1.1.37], map position, 2–35.3); alcohol dehydrogenase (*Adh* [E.C.1.1.1.1.], map position, 2–50.1); hexokinase-C (tentative name, F. M. JOHNSON, unpublished, the map position was determined by the present authors to be 2–74.5; see the RESULTS AND ANALYSES); esterase-C (*Est-C* [E.C.3.1.1.2], map position, 3–49); and octanol dehydrogenase (*Odh*, map position, 3–49.2). These enzymes were assayed by starch-gel electrophoresis (*cf.*, STONE *et al.* 1968).

The 617 second chromosome lines were examined to determine whether they carried *In(2L)t* or *In(2R)NS*, which are known to be polymorphic in the present population, by observing the salivary gland chromosomes of the hybrids with WN16C, which carries the standard gene arrangement. For staining, 2% acetic-lactic orcein was used. The salivary gland chromosomes of the third chromosomes were not examined, since our main interest is the linkage disequilibrium between the *Est-C* and *Odh* loci and since we have found that the frequency of polymorphic inversions in the third chromosome is low (MUKAI, WATANABE and YAMAGUCHI 1974, who in their Figure 1 show the locations of these enzyme loci and polymorphic inversions).

Linkage disequilibria with respect to the four enzyme loci and the two polymorphic inversions in the second chromosomes were examined up to and including 4 factors, using the χ^2 method described in MUKAI, WATANABE and YAMAGUCHI (1974). The 5 and 6 factor interactions were not examined because of an insufficient number of chromosomes. The linkage disequilibrium between the *Est-C* and *Odh* loci in the third chromosome was examined by the 2 × 2 contingency table method.

Recombination tests: Four experiments were conducted: (1) Localization of hexokinase-C on the second chromosome. (2) Recombination test between *Est-C* and *Odh*. (3) Recombination test between Bristle (*Bl*: 2–54.8) and Lobe, (*L*: 2–72.0) in Standard/*In(2R)NS* heterozygotes. The purpose is to test how frequently recombination occurs between *In(2R)NS* and *Adh* (2–50.1), which is located to the left of *Bl*. The inversion is approximately marked by the *L* gene. (4) Recombination test between *αGpdh-1* and *Adh* in Standard/*In(2L)t* heterozygotes. The purpose is to test how frequently the *αGpdh-1* alleles included in *In(2L)t* are released from the inversion. The *Adh* locus approximately marks the right breakpoint of the inversion.

In all the above cases, the test cross (or backcross) method was employed in the second generation. The recombination values were estimated by counting flies for visible phenotypes or electrophoretic genotypes.

RESULTS AND ANALYSES

Gene and inversion frequencies: The isozyme allele and frequencies are shown in Table 1 in comparison with those obtained for the 1970 population. The *Adh*, *Mdh-1*, *αGpdh-1*, *Hex-C*, and *Odh* loci were found to be heteroallelic for the known alternative alleles Fast (F) and Slow (S) (LINDSLEY and GRELL 1968; O'BRIEN and MACINTYRE 1969), while the *Est-C* locus was triallelic (*Est-C*^{4}, *Est-C*^{6}, and *Est-C*^{2}). The most frequent allele was *Est-C*^{4}, which is labeled *F*. The other two are pooled and labeled *S* since *Est-C*^{2} was rare. From Table 1, it may be seen that the frequency of *F* alleles at the *αGpdh-1* locus and that in *In(2R)NS*

TABLE 1

Frequencies of Fast (F) alleles

	αGpdh-1	*Mdh-1*	*Adh*	*Hex-C*	*In(2L)t*†	*In(2R)NS*	*Est-C*	*Odh*
1970	0.795	0.028	0.291	——	0.057	0.114	0.892	0.941
	±0.015	±0.005	±0.013		±0.009	±0.012	±0.014	±0.011
1974	0.874	0.019	0.274	0.063	0.070	0.151	0.884	0.899
	±0.013	±0.006	±0.018	±0.010	±0.010	±0.014	±0.014	±0.013
Difference	***					*		

*** Significant at the 0.1% level.
* Significant at the 5% level.
† All extractions of this inversion in the 1974 collection have been verified to be *In(2L)t* rather than *In(2L)Cy*.

increased significantly in comparison with the results in 1970. All the other genes and inversions showed frequencies similar to those of 1970.

Location of hexokinase-C: From examination of the pattern of segregation of the hexokinase-C alleles in many lines, each line being isogenic for either the second or third chromosome but not both, the locus was located on the second chromosome. A precise map location was determined by a three point test employing the L^2 and *bw* markers in the cross

$$\frac{L^2\ Hex\text{-}C^4\ bw}{+\ Hex\text{-}C^2\ +}(\varphi) \times \frac{+\ Hex\text{-}C^4\ bw}{+\ Hex\text{-}C^4\ bw}(\delta)$$

with the following results:

nonrecombinant chromosomes	2381
chromosomes recombinant between L^2 and *Hex-C*	76
chromosomes recombinant between *Hex-C* and *bw*	965
chromosomes doubly recombinant	9

The recombination fraction between L^2 and *Hex-C* is estimated at 0.025 ± 0.003, and it is inferred that *Hex-C* is located to the right of L^2 at 2–74.5 ± 0.003.

Distance between the Odh *and the* Est-C *locus*: It has been reported that the distance between *Est-C* and *Odh* is 0.001 ± (LINDSLEY and GRELL 1968). The distance was measured by the following cross:

$$\frac{Est\text{-}C^F\ Odh^S}{Est\text{-}C^S\ Odh^F}(\varphi) \times \frac{Est\text{-}C^S\ Odh^F}{Est\text{-}C^S\ Odh^F}(\delta)$$

In the offspring, the number of recombinant gametes was 7 out of 1200. Thus, the distance between the two loci is estimated to be 0.0058 ± 0.0022.

Heterozygous effect of In(2R)NS *on recombination*: Alleles at the *Adh* locus have shown consistent nonrandom association with the polymorphic inversion *In(2R)NS* (significant disequilibrium was found in Raleigh population samples for the years 1968, 1969, and 1970). The map distance between these factors is approximately 30 cM and the effective recombination value has been estimated at about 3.2% (*cf.*, MUKAI, METTLER and CHIGUSA 1971). In order to check the

occurrence of crossovers in the region between *Adh* and *In(2R)NS* in inversion heterozygotes, two markers were used: *Bl*, which maps at *2*–54.8, close (but proximal) to *Adh* at 2–50.1, and *L* which maps at *2*–72.0, close to *In(2R)NS*. The following table shows the observed recombination rates for a control cross (ST/ST) and an experimental cross (ST/*In(2R)NS*). Recombination between *Bl* and *L* is suppressed, but not eliminated, in the *In(2R)NS* heterozygote; the observed population genetic effective rate of 1.75% (3.5 × 0.5, since there is no crossing over in males) is somewhat over one-half the previous estimate of 3.2% between *Adh* and *In(2R)NS*.

♀♀ Parents	♂♂ Parents	Total no. of flies counted	Recombination value between *Bl* and *L*
$\frac{Bl\ \ L\ \ +}{+\ \ +\ \ +} \times$	$\frac{+\ \ +}{+\ \ +}$	4491	0.154
$\frac{Bl\ \ L\ \ +}{+\ \ +\ \ In(2R)NS} \times$	$\frac{+\ \ +}{+\ \ +}$	2510	0.035

Two-strand double crossovers within In(2L)t: The polymorphic inversion *In(2L)t* has shown consistent nonrandom association with alleles in the *αGpdh-1* and *Adh* systems. *Adh* is located very close to the right breakpoint of *In(2L)t* and, therefore, no recombination has been observed between *In(2L)t* and *Adh*. *αGpdh-1* is located approximately in the middle of the inversion. Inversion heterozygote females were utilized in the following test cross:

$$\frac{[In(2L)t\ \alpha Gpdh\text{-}1^{F}]Adh^{S}}{[\ +\ \ \alpha Gpdh\text{-}1^{S}]Adh^{F}}(♀) \times \frac{[+\ \alpha Gpdh^{S}]Adh^{F}}{[+\ \alpha Gpdh^{S}]Adh^{F}}(♂)$$

The occurrence of a two-strand double crossover inside the inversion loop, one on each side of *αGpdh-1*, is expected to result in the recombinant progeny types

$$\frac{\alpha Gpdh^{F}\ Adh^{F}}{\alpha Gpdh^{S}\ Adh^{F}} \text{ or } \frac{\alpha Gpdh^{S}\ Adh^{S}}{\alpha Gpdh^{S}\ Adh^{F}}$$

Only one such crossover product was detected in 4,377 progeny gametes, a frequency of 0.00022.

Effective size of the 1974 Raleigh population: In considering linkage disequilibrium, it is important to know the effective size of the population. On the basis of the frequency of lethal-carrying second chromosomes and their allelism rate, the effective size of the population (N_e) was estimated by using NEI's (1968) formula:

$$\hat{N}_e = (1-\hat{I}_g)/[4(I_gU-\mu)]$$

where I_g stands for the allelism rate of lethal genes (and can be estimated by $-\log_e(1-I_cQ^2)/[\log_e(1-Q)]^2$ in which I_c stands for the allelism rate of lethal chromosomes and Q is the frequency of lethal-carrying chromosomes); U is the

total lethal mutation rate; and μ is the lethal mutation rate per locus. Two hundred and sixty out of 617 second chromosomes extracted from the 1974 population were homozygous lethal, *i.e.*, $\hat{Q} = 0.42$. The allelism rate between lethal-carrying chromosomes was 0.0081 (= 33/4068), and U and μ were assumed to be 0.005 and 10^{-5}, respectively. The effective size of the population was estimated to be *ca.* 18,000. This estimate is sufficiently close to the estimates for the 1968, 1969, and 1970 populations which were *ca.* 24,000, *ca.* 22,000, and *ca.* 115,000, respectively, (MUKAI and YAMAGUCHI 1974).

Analyses of linkage disequilibria: The raw data with respect to *αGpdh-1* (=A), *Mdh-1* (=B), *Adh* (=C), *Hex-C* (=H), *In(2L)t* (=I), and *In(2R)NS* (=J) on the second chromosome are presented in Table 2, together with the expected frequencies of isozyme genotypes. From this table, it can be seen clearly that isozyme genes are in approximate linkage equilibrium.

A χ^2 analysis was conducted up to and including 4 factor interactions, although in some classes expected values were less than 5 (*cf.*, LEWONTIN and FELSENSTEIN 1965). The results are given in Table 3. In the two-factor linkage disequilibria, only IC and HJ were significant. Both involve polymorphic inversions. The Adh^S allele continues to show a strong association with *In(2L)t* as in previous studies (MUKAI, METTLER and CHIGUSA 1971; MUKAI, WATANABE and YAMAGUCHI 1974). The hexokinase-C locus is either included in *In(2R)NS* or located just outside the inversion (see the result of recombination test). Indeed, the S allele and this polymorphic inversion were perfectly linked (see Table 2).

TABLE 2

Frequencies of second chromosome haplotypes for four isozyme loci and two polymorphic and unique inversions

Genotype ABCH*	Normal	*In(2L)t* I	*In(2R)NS* J	*In(2L)t* and *In(2R)NS*	Unique	Unique and *In(2L)t*	Unique and *In(2R)NS*	Total	Expected
FFFF	0	0	0	0	0	0	0	0	0.18
FFFS	3	0	0	0	0	0	0	3	2.68
FFSF	1	0	0	0	0	0	0	1	0.48
FFSS	4	0	0	0	1	0	0	5	7.11
FSFF	7	0	0	0	1	0	0	8	9.15
FSFS	112	0	23	0	3	0	2	140	135.62
FSSF	23	2	0	0	0	0	0	25	24.25
FSSS	258	31	51	6	6	2	3	357	359.53
SFFF	0	0	0	0	0	0	0	0	0.03
SFFS	1	0	0	0	0	0	0	1	0.39
SFSF	1	0	0	0	0	0	0	1	0.07
SFSS	0	0	1	0	0	0	0	1	1.03
SSFF	1	0	0	0	0	0	0	1	1.33
SSFS	15	0	1	0	0	0	0	16	19.62
SSSF	3	0	0	0	0	0	0	3	3.51
SSSS	46	2	6	0	1	0	0	55	52.02
Total	475	35	82	6	12	2	5	617	(617)

* I = *In(2L)t*, A = *αGpdh*, B = *Mdh*, C = *Adh*, H = *Hex-C*, J = *In(2R)NS*.

TABLE 3

The result of the test for linkage disequilibrium (1974 Raleigh, N.C., population)

Component	$\chi^2_{df=1}$	Component	$\chi^2_{df=1}$	Component	$\chi^2_{df=1}$
AB	1.69	ABC	0.09	ABCH	2.92
AC	0.84	ABH	5.52*	IABC	0.05
AH	0.00	ACH	0.00	IABH	0.40
BC	0.22	BCH	2.26	IACH	0.00
BH	2.21	IAB	0.02	IBCH	0.14
CH	0.39	IAC	1.57	ABCJ	1.71
IA	2.67	IAH	0.01	ABHJ	2.54
IB	0.92	IBC	0.21	ACHJ	0.05
IC	17.44***	IBH	0.12	BCHJ	0.59
IH	0.22	ICH	0.21	IABJ	0.19
AJ	1.62	ABJ	3.14	IACJ	0.12
BJ	0.43	ACJ	0.32	IAHJ	0.04
CJ	0.02	AHJ	0.10	IBCJ	0.03
HJ	7.39**	BCJ	0.51	IBHJ	0.04
IJ	0.04	BHJ	0.21	ICHJ	0.06
		CHJ	0.05		
		IAJ	0.00		
		IBJ	0.04		
		ICJ	0.01		
		IHJ	0.06		

I = *In(2L)t*, A = *αGpdh*, B = *Mdh*, C = *Adh*, H = *Hex-C*, J = *In(2R)NS*.

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

The IA component showed a rather large (2.67 with df = 1) but not significant χ^2 value. However, if we pooled the results of 1970 and 1974 (the tendencies of both years are very similar with correlations between gene and inversion frequencies of 0.0638 in 1970 and 0.0658 in 1974) we find a significant linkage disequilibrium ($z \cong -2.37$, using $z \cong \frac{\Sigma x}{\sqrt{n}}$ where n is the number of x's employed, $P < 0.05$). Thus, it may be speculated that a weak linkage disequilibrium exists between *αGpdh-1* and *In(2L)t*, which cannot be detected using a single year's result because of insufficient sample size.

The most significant finding is that the linkage disequilibrium between *Adh* (C) and *In(2R)NS* (J) disappeared. The observed correlation of frequencies between these two factors were: +0.118 in 1968, +0.141 in 1969, +0.074 in 1970 and −0.005 in 1974. These values are graphically presented in Figure 1. Starting from the observations in 1970, the expected correlation in 1974 (assuming neutrality) was calculated to be +0.036, which is not significantly different from the observed value (−0.005). Thus, the loss in disequilibrium is about that expected on the basis of recombination. The initial disequilibrium was thought to be induced by random genetic drift or mass migration, but it is difficult to choose one of them.

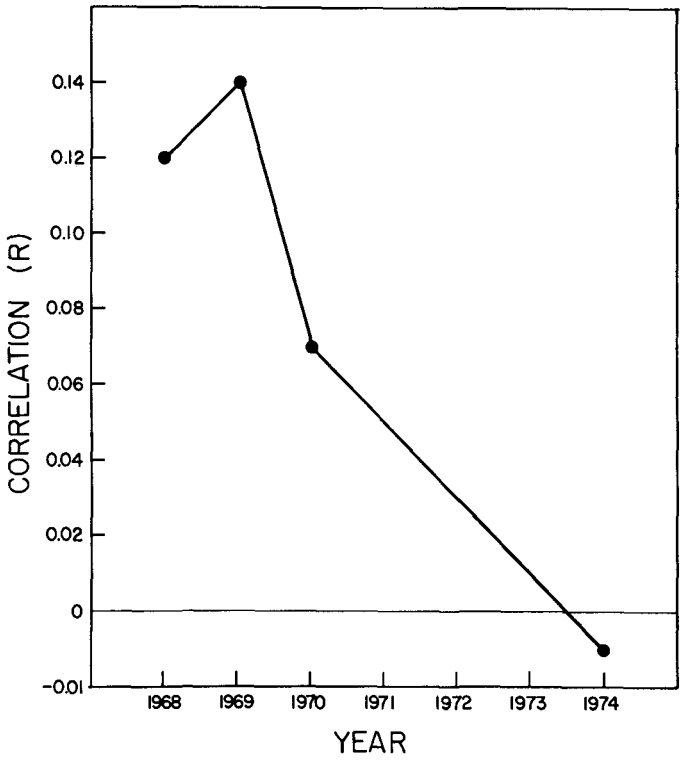

FIGURE 1.—Correlation between *Adh* and *In(2R)NS* for four samples from the Raleigh, North Carolina, population.

In the pooled data of 1968, 1969, and 1970 (MUKAI, WATANABE and YAMAGUCHI 1974), the AB disequilibrium was significant but thought still possibly due to sampling. The result of 1974 was not significant.

Only one component (ABH) was significant among 20 three-factor disequilibrium components, but this is probably due to small numbers of the expected values in some classes. In fact, the gene frequencies of B and H were very small (the former 0.019 ± 0.006 and the latter 0.063 ± 0.010). There was no significant component among 15 four-factor disequilibrium components.

In summary, significant linkage disequilibria were found only between isozyme genes and polymorphic inversions and no higher-order disequilibrium was found. These findings are in contrast with previous results and suggest that those previous disequilibria among isozyme genes (see MUKAI, WATANABE, and YAMAGUCHI 1974) were due to sampling.

The *Odh* and *Est-C* loci on the third chromosome were investigated, since they showed a linkage disequilibrium in the 1970 sample. Chromosome polymorphisms were not considered since the polymorphic inversion [*In(3R)P*] with the largest frequency (0.088) is low and it is a relatively large distance from the *Odh* and *Est-C* loci. The observed and the expected joint frequencies for the *Odh* and *Est-C* loci are shown in Table 4. The $\chi^2_{df=1}$ value of 0.9 is not significant

TABLE 4

Frequencies of third chromosome haplotypes for two isozyme loci (Est-C *and* Odh)

Genotypes	FF	FS	SF	SS	Total
Frequency	416	49	57	4	526
Expected frequency	418.0	47.0	54.9	6.1	526
		$\chi^2_{df=1} = 0.9$			

$(0.30 < P < 0.50)$. Thus, the linkage disequilibrium detected in the 1970 sample was not found in the 1974 sample. The correlation of the frequencies of these two genes in 1970 was 0.163. The expected correlation after 40 generations (in 1974) is 0.145 for a recombination value of 0.0058 and no recombination in males, using the relationship $D_n=D_0(1-r)^n$ where D_0 and D_n are the linkage disequilibria at the starting generation and generation n, respectively, and r is the recombination value. The estimated value is -0.042, which is significantly different from 0. Thus, it may be reasonable to conclude that the disequilibrium detected in 1970 was due to sampling.

DISCUSSION

No evidence for linkage disequilibrium due to epistasis and overdominance: In the present experiment, linkage disequilibrium between any two isozyme genes investigated could not be detected, even in the case where the map distance between the two genes (*Odh* and *Est-C*) is 0.0058. This result supports the speculation made previously, namely, that the disequilibria detected in 1968 (*Mdh-1* vs. *Amy*) and in 1970 (*αGpdh-1* vs. *Mdh-1*) must be due to sampling, since the detection of these disequilibria was not repeatable in the succeeding years and since the gene frequency of the *Mdh-1* locus is very low. Similar results have been reported in *D. subobscura* by ZOUROS, KRIMBAS, TSAKAS, and LOUKAS (1974). Repeated samplings over an extended period of time are most necessary in this kind of experiment. According to a recent investigation (UNITED NATIONS PUBLICATIONS 1972), 20 cistrons can be expected in the distance of 1 cM in the chromosome. Thus, there may be about 10 cistrons between the *Est-C* and the *Odh* locus. If the distance becomes shorter than the above, linkage disequilibria might be seen (MUKAI, WATANABE, and YAMAGUCHI 1974).

The linkage disequilibrium detected between the *Adh* locus and *In(2R)NS* (the map distance between them is about 30 cM, but the effective recombination value is about 1.75%) in 1968, 1969, and 1970 was not found in 1974. It has been reported that the heterozygote for *In(2R)NS* shows a slight heterosis with respect to viability (MUKAI and YAMAGUCHI 1974). If overdominance is manifest at the *Adh* locus as well as at many intervening loci between these two factors and all with multiplicative effects, linkage disequilibrium would be maintained according to the FRANKLIN-LEWONTIN model (FRANKLIN and LEWONTIN 1970). Thus, it may be concluded that the FRANKLIN-LEWONTIN theory is not applicable to these loci.

Linkage disequilibria between isozyme genes and polymorphic inversions: Linkage disequilibria were consistently detected between *Adh* and *In(2L)t*, and between *In(2R)NS* and *Amy* (or *Hex-C*) (the *Amy* and *Hex-C* loci are closely linked). These phenomena can be explained by the small amount of recombination between the two factors, after these polymorphic inversions occurred uniquely. The respective distances between the breakpoints of inversions and the loci in question are very small (*cf.* Figure 1 of MUKAI, WATANABE, and YAMAGUCHI 1974). In addition, the inversion heterozygotes suppress the recombination even outside the inversions themselves in *D. melanogaster*.

For *In(2L)t* and *αGpdh-1*, consistent but not significant linkage disequilibrium was detected in 1968, 1969, 1970, and 1974. The pooled result is significant. This weak disequilibrium can be explained as a process of approaching linkage equilibrium due to two-strand double crossing over within the inversion loop. The effective recombination value when accounting for no recombination in males is estimated to be 0.00011. It is possible to speculate about the number of generations, since *In(2L)t* originated. If, after its unique occurrence, it reached approximately the current equilibrium frequency with little recombination, the approximate *In(2L)t F* gamete frequency would be that (0.070) of the inversion. Assuming also that the *F* frequency (0.874) was the same, the linkage disequilibrium at this time would have been $D_0 = 0.0088$. With the current linkage disequilibrium, $D_n = 0.0056$, it is calculated from $D_n = (1-.00011)^n D_0$ that approximately 4100 generations intervened. Of course, this is a rough estimate, but it may indicate that *In(2L)t* occurred a long time ago, and it is understandable that this inversion is now cosmopolitan (LANGLEY, TOBARI, and KOJIMA 1974; MUKAI, METTLER, and CHIGUSA 1971). However, this time is much shorter than the time from the speciation between *D. pseudoobscura* and *D. persimilis* (of the order of a million years) (*cf.*, PRAKASH and LEWONTIN 1968).

For the reasons that specific inversions in the third chromosomes of *D. pseudoobscura* and *D. persimilis* carry specific alleles at the *Amy-1* and *Pt-10* loci and that there is a correlation in alleles between the above two closely related species which originated from the common ancestor some million generations ago, PRAKASH and LEWONTIN (1968) claimed coadaptation among isozyme genes within inversions. However, NEI and LI (1975) have criticized this hypothesis since, according to their calculation, the probability that there is a correlation in the types of alleles between the above two species is not small. Thus, we hypothesize that the tight linkages between *In(2L)t* and *Adh* and between *In(2R)NS* and *Amy* (or *Hex-C*) are due to founder effect.

In conclusion, it may be said that linkage disequilibria among isozyme genes are very difficult to find, which may well indicate that they rarely exist. However, it should be noted that what we treated as allelic frequencies in the present report might be the frequencies of groups of alleles of the same electrophoretic mobility according to the recent findings of BERNSTEIN, THROCKMORTON and HUBBY (1973) and others. It is interesting to examine linkage disequilibria among such alleles.

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