THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. XII. LINKAGE DISEQUILIBRIUM IN A LARGE LOCAL POPULATION[1]

TERUMI MUKI, TAKAO K. WATANABE[2] AND OSAMU YAMAGUCHI[3]

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607

Manuscript received August 3, 1973
Revised copy received March 22, 1974

ABSTRACT

Seven hundred and three second chromosomes were extracted from a Raleigh, North Carolina population of *Drosophila melanogaster* in 1970. Additionally, four hundred and eighty-nine third chromosomes were extracted from a large cage population founded from the flies in the 1970 Raleigh collection. The α glycerol-3-phosphate dehydrogenase-1, malate dehydrogenase-1, alcohol dehydrogenase, and α amylase loci were studied from the second chromosomes, and the esterase-6, esterase-C, and octanol dehydrogenase loci were analyzed from the third chromosomes. Inversions, relative viability and fecundity were studied for both classes of chromosomes. The following significant findings were obtained: (1) All loci examined were polymorphic or had at least two alleles at appreciable frequencies. Analysis of the combined data from this experiment with that of MUKAI, METTLER and CHIGUSA (1971) revealed that the frequencies of the genes in the second chromosomes collected in early August were approximately the same over three years. (2) Linkage disequilibria between and among isozyme genes *inter se* were not detected except in a few cases which can be considered due to non-random sampling. (3) Linkage disequilibria between isozyme genes and polymorphic inversions were detected when the recombination values between the breakage points of the inversions and the genes in question were small. In only a few cases, were second and third order linkage disequilibria including polymorphic inversions detected. (4) Evidence for either variation among genotypes within loci or cumulative effects of heterozygosity was found for viability and fecundity. As a result of these findings, it was tentatively concluded that although selection might be perceptibly operating on some polymorphic isozyme loci, most of the polymorphic isozyme genes are selectively neutral or near-neutral in the populations studied.

A very high level of genic heterozygosity discovered by LEWONTIN and HUBBY (1966) in *Drosophila pseudoobscura* populations was a revolutionary finding in population genetics, although it had already been theoretically predicted by KIMURA and CROW (1964). Two main hypotheses have been proposed to describe

[1] Paper number 4139 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina, and Contribution number 930 from the National Institute of Genetics, Mishima, Japan. This investigation was supported by NIH Research Grant Number GM-11546 from the National Institute of General Medical Sciences. The computing was supported by Public Health Service Grant FR-00011.

[2] Present address: National Institute of Genetics, Mishima, Japan.

[3] Present address: Tokyo Metropolitan University, Tokyo, Japan.

the mechanisms for maintenance of such large amounts of genic variability (isozyme or allozyme variability). First, there is the selection hypothesis (PRAKASH, LEWONTIN and HUBBY 1969; AYALA, POWELL and DOBZHANSKY 1971; and others) in which heterotic selection or some type of balancing selection is thought to maintain isozyme variability. The second hypothesis is the neutrality-random genetic drift hypothesis (KIMURA and OHTA 1971) in which polymorphic isozyme variability in random-maitng populations is attributed to random genetic drift of neutral genes.

Although much has been published concerning the mechanisms for maintaining high genic variability, it is impossible at present to determine which of the two hypotheses best describes the mechanism. It is known, however, that *even if* selection is operating on individual polymorphic loci, the selection effects must be extremely small (CROW 1968; MUKAI 1968, 1969; MUKAI, METTLER and CHIGUSA 1971; YAMAZAKI 1971; MUKAI *et al.* 1974).

The importance of linkage in the maintenance of genetic variability and the evolution of organisms has been theoretically discussed by KIMURA (1956), LEWONTIN and KOJIMA (1960), LEWONTIN (1964) and others. In very large populations, epistasis is required for linkage to be an important factor in natural selection (e.g., LEWONTIN 1964). However, random genetic drift alone can contribute to creating linkage disequilibrium in small populations (HILL and ROBERTSON 1968; OHTA and KIMURA 1969).

Only a few experimental investigations have been undertaken to ascertain levels of linkage disequilibrium in populations. An almost complete association between certain alleles and specific inversions for the α amylase locus and a larval protein locus (*Pt-10*) was found in *D. pseudoobscura* by PRAKASH and LEWONTIN (1968). Similar cases in *D. melanogaster* were detected by MUKAI, METTLER and CHIGUSA (1970, 1971) and KOJIMA, GILLESPIE and TOBARI (1970).

The presence of permanent linkage disequilibrium in very large populations indicates that epistasis is present or that selection is operating on the loci in question. However, linkage equilibrium does not imply that selection is not operating, since permanent linkage disequilibrium will not result if selection is operating additively on many loci. For this reason, MUKAI, METTLER and CHIGUSA (1971) investigated the linkage disequilibria between isozyme genes *inter se* and between isozyme genes and polymorphic inversions in addition to studying the relative viability of isozyme genotypes for the 1968 and 1969 Raleigh, North Carolina population of *D. melanogaster*. The effective size of this population is very large (much greater than 10,000; MUKAI and YAMAGUCHI 1974). The same population was analyzed on a large scale in 1970 and the results together with those of the previous analyses are reported herein.

MATERIALS AND METHODS

Preparation of materials: Seven hundred and three second chromosomes were extracted (cf., MUKAI and YAMAGUCHI 1974) in the summer of 1970, using the *Cy–Pm* inversion method. These chromosomal lines were maintained at 19°. Each chromosomal type was maintained in balance with *SM1(Cy)* chromosome, which does not recombine with its homologue and helps to maintain the less viable and lethal chromosomes.

Four hundred and eighty-nine third chromosomes were extracted from a large cage population initiated from 660 isofemale lines established from the same Raleigh, North Carolina population described above. Extraction of the chromosomes by the *Sb–Pr* inversion method was done 2–7 months after the initiation of the cage population. *Stubble* (*Sb*) is included in *TM3* (cf., LINDSLEY and GRELL 1967). Each chromosomal type was maintained in balance with *TM3(Sb)*. These chromosomes were assayed for isozyme variation and inversions.

Enzyme assays 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 three loci, located in the third chromosome (LINDSLEY and GRELL 1967), were studied α glycerol-3-phosphate dehydrogenase-1 (*α Gpdh-1* [EC 1.1.1.8]; map position, 2-17.8), malate dehydrogenase-1, (*Mdh-1* [EC 1.1.1.37], map position, 2–35.3), alcohol dehydrogenase (*Adh* [EC 1.1.1.1], map position, 2–50.1), α amylase (*Amy* [EC 3.2.1.1], map position, 2–77.3), esterase-6 (*Est-6* [EC 3.1.1.2], map position, 3–36.8), esterase-C (*Est-C* [EC 3.1.1.2], map position 3-49), and octanol dehydrogenase (*Odh*, map position, 3-49.2). The four dehydrogenases and two esterases were assayed by starch-gel electrophoresis (cf. STONE *et al.* 1968), while α amylase was assayed by acrylamidegel electrophoresis (cf., PRAKASH, LEWONTIN and HUBBY 1969).

For the tests of two-locus linkage disequilibria the usual 2×2 contingency χ^2 method was employed. For the examination of linkage disequilibria including higher order interactions, a method suggested by DR. PETER M. BURROWS was used: We supposed (1) that there are four loci, which can be described as *A*, *B*,*C*, and *D*, under consideration, and (2) that there are two alleles per locus (*F* and *S*). Thus, there are sixteen types of gametes. The total χ^2 for deviations from random associations is calculated as

$$\chi^2_{T(ABCD),\ df=11} = \sum_{i=1}^{16} \frac{(O_i - E_i)^2}{E_i} \tag{1}$$

with 11 degrees of freedom, where O_i and E_i are observed and expected frequencies, respectively, of gamete type *i*. E_i is calculated as a product of the sample size and observed allelic frequencies at the 4 loci. Then, $\chi^2_{T(ABCD),\ df=11}$ is partitioned into components of two-factor linkage disequilibria (*AB*, *AC*, *AD*, *BC*, *BD*, and *CD*), three-factor linkage disequilibria (*ABC*, *ABD*, *ACD*, and *BCD*), and four-factor linkage disequilibrium (*ABCD*); each component having one degree of freedom.

The simplest arithmetic is as follows: (1) Each of the χ^2's corresponding to six two-factor linkage disequilibria are calculated from the usual 2×2 contingency tables. (2) Considering loci *A*, *B*, and *C* marginally, the total χ^2 value with 4 degrees of freedom is calculated analogously. The χ^2 value for the specific three-factor linkage disequilibrium ($\chi^2_{ABC,df=1}$) is obtained as

$$\chi^2_{ABC} = \chi^2_{T(ABC)} - \chi^2_{AB} - \chi^2_{AC} - \chi^2_{BC} \tag{2}$$

(3) In exactly the same way, χ^2_{ABD}, χ^2_{ACD}, and χ^2_{BCD}, each with one degree of freedom are calculated. (4) The $\chi^2_{df=1}$ due to the specific four-factor linkage disequilibrium is calculated as follows:

$$\chi^2_{ABCD,df=1} = \chi^2_{T(ABCD)} - \sum_{i=1}^{4} \chi^2_{(3\text{ factor combination})i} - \sum_{j=1}^{6} \chi^2_{(2\text{ factor combination})j} \tag{3}$$

Obviously this method can be extended to cases of more than four loci. Using the same method, calculations of χ^2 values up to specific four-factor interactions were carried out including polymorphic inversions and isozyme genes.

Estimations of viability and fecundity: As in the case of the 1969 sample (cf., MUKAI *et al.* 1972), the average degrees of dominance of viability genes were estimated by the *Cy* method (cf., WALLACE 1956). The cross 5 *Cy*/+$_i$ (♀♀) × 5 *Cy*/+$_i$ (♂♂) was made to estimate homozygous viability for line *i* and the cross 5*Cy*/+$_i$ (♀♀) × 5 *Cy*/+$_j$ (♂♂) was used to determine the viability of heterozygotes between line *i* and line *j*. From these crosses *Cy* and wild-type flies segregate. Relative viability of chromosomal homozygotes or heterozygotes was

expressed by the ratio, (number of wild-type flies)/(number of *Cy* flies + 1.0), following HALDANE (1956). If the isozyme genotypes of each chromosomal type are known and there is no linkage disequilibrium, the relative viability of each isozyme genotype can be estimated on the average genetic background.

Fertility of $Cy/+_i$ females was estimated by counting the number of offspring of the $Cy/+_i$ (♀♀) × $Cy/+_j$ (♂♂) cross. If there is no viability variation among genotypes within the isozyme loci, it is possible to obtain an estimate of the relative fecundity of parental *Cy* flies with respect to isozyme genotypes.

For the third chromosomes, the same experiment was conducted using *TM3(Sb)*, and relative viability and fertility were estimated for isozyme genotypes.

RESULTS AND ANALYSES

Frequencies of isozyme genes and inversions

Inversions

The frequencies of inversion-carrying second chromosomes were 0.2000, 0.1370 (L. E. METTLER, S. I. CHIGUSA and T. MUKAI unpublished; MUKAI, METTLER and CHIGUSA 1971), and 0.1850 (MUKAI and YAMAGUCHI 1974) for the 1968, 1969, and 1970 samples. These frequencies were not found to differ significantly ($\chi^2_{df=2} = 2.80$, $0.20 < P < 0.30$). As previously reported (MUKAI and YAMAGUCHI 1974), two polymorphic inversions [*In(2L)t* and *In(2R)NS*] were detected in the 1968, 1969 and 1970 samples of the second chromosomes. Several unique inversions were also detected. *In(2L)t* was previously labeled Inversion C and *In(2R)NS* was called Inversion A (MUKAI, METTLER and CHIGUSA 1971). It was previously though that Inversion C was *In(2L)Cy* (MUKAI, METTLER and CHIGUSA 1971), but it is more probable that Inversion C is *In(2L)t*.

In the third chromosomes, three polymorphic inversions (*In(3L)P*, *In(3R)C*, and, *In(3R)P*) were found in addition to two unique inversions (one is located in the left arm of a chromosome which carries *In(3R)P* in the right arm, and the other is in the right arm).

The breakage points of these polymorphic inversions (cf. LINDSLEY and GRELL 1967; WATANBE 1967) and their frequencies are described below. The frequencies in the samples of 1968 and 1969 are from MUKAI, METTLER and CHIGUSA (1971).

In(2L)t: A single paracentric inversion between 22D and 34A with a frequency of 0.0762 (=24/315), 0.0205 (=3/146), and 0.0569 (=40/703) in 1968, 1969, and 1970, respectively. There is no significant heterogeneity among the frequencies of the three years ($\chi^2_{df=2} = 5.66$, $0.05 < P$).

In(2R)NS: A single paracentric inversion between 52A and 56F with a frequency of 0.0952 (=30/315), 0.0959 (=14/146), and 0.1138 (=80/703) in 1968, 1969, and 1970, respectively. There is no significant heterogeneity among the frequencies of the three years ($\chi^2_{df=2} = 0.92$, $0.60 < P < 0.70$).

In(3L)P: A single paracentric inversion between 63A and 72E (corrected by WATANABE [1967]) with a frequency of 0.0123 (=6/489) in 1970. One chromosome with this inversion also included *In(3R)P*.

In(3R)P: A single paracentric inversion between 89C and 96A with a frequency of 0.0879(=43/489) in 1970. As noted above, one chromosome with this inversion also carried *In(3L)P*.

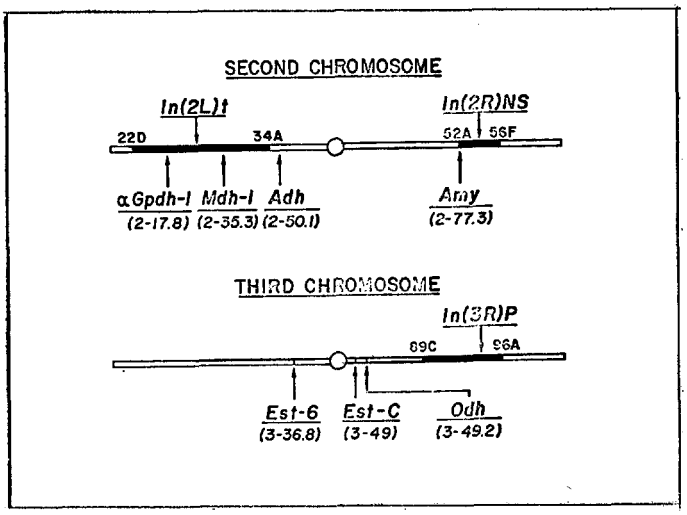

FIGURE 1.—Approximate locations of isozyme genes and polymorphic inversions investigated.

In(3R)C: A single paracentric inversion between 92D and 100F with a frequency of 0.0102 (=5/489).

Since the frequencies of *In(3L)P* and *In(3R)C* were very low, these inversions were not considered in the present investigation. Approximate locations of isozyme genes and polymorphic inversions investigated are shown in Figure 1.

Gene Frequency

The *Adh*, *Mdh-1*, *αGpdh-1*, and *Odh* loci were found to be heteroallelic for the known alternative alleles Fast (*F*) and Slow (*S*) (LINDSLEY and GRELL 1967; O'BRIEN and MACINTYRE 1969), while the *Amy*, *Est-6*, and *Est-C* loci were polymorphic.

At the *Amy* locus, four alleles Amy^1, $Amy^{1,3}$, Amy^3 and $Amy^{1,6}$ were found in the 1968 sample, three alleles Amy^1, $Amy^{1,3}$ and Amy^3 were identified in the 1969 sample (in a previous report [MUKAI, METTLER and CHIGUSA 1971], Amy^3 was confounded with $Amy^{1,3}$) and seven alleles Amy^1, $Amy^{1,3}$, $Amy^{1,6}$, $Amy^{1,2,3}$, $Amy^{2,3}$, Amy^3 and Amy^6 were observed in the 1970 sample. Amy^1 was the most frequent allele whereas $Amy^{1,3}$ had the second highest frequency. All other alleles were rare. For the sake of convenience, the Amy^1 allele is labeled *F*, and all other alleles labeled *S*. The *Amy* locus is a complex locus (BAHN 1967), so the presence of many alleles was not unexpected.

The *Est-6* locus had three alleles ($Est\text{-}6^4$, $Est\text{-}6^6$ and $Est\text{-}6^8$ (cf., STONE *et al.* 1968). The second allele occurred most frequently and is labeled *S*, while the first allele and $Est\text{-}6^8$, which is a rare allele, are labeled *F*.

The *Est-C* locus also had three alleles ($Est\text{-}C^4$, $Est\text{-}C^6$ and $Est\text{-}C^2$). The most frequent allele was $Est\text{-}C^4$ which is labeled *F*. The other two are labeled *S* since $Est\text{-}C^2$ was rare.

The gene frequency of the fast allele for each locus examined in the 1970 sample is presented in Table 1, together with the results obtained in the 1968

TABLE 1

Frequencies of Fast (F) *alleles*

Year	Enzyme (location)						
	αGpdh-1 (2–18.7*)	*Mdh-1* (2–35.3*)	*Adh* (2–50.1*)	*Amy* (2–77.3†)	*Est-6* (3–36.8†)	*Est-C* (3–49†)	*Odh* (3–49.2†)
1968‡	0.825 ±0.021	0.032 ±0.010	0.260 ±0.025	0.879 ±0.018	——	——	——
1969‡	0.788 ±0.034	0.021 ±0.012	0.349 ±0.040	0.863 ±0.029	——	——	——
1970	0.795 ±0.015	0.028 ±0.006	0.293 ±0.017	0.885 ±0.012	0.358 ±0.022	0.892 ±0.014	0.941 ±0.011
Pooled	0.802 ±0.012	0.028 ±0.005	0.291 ±0.013	0.881 ±0.010	0.358 ±0.022	0.892 ±0.014	0.941 ±0.011

* From O'BRIEN and MACINTYRE (1969).
† From LINDSLEY and GRELL (1967).
‡ From MUKAI, METTLER and CHIGUSA (1971).

and 1969 samples. The 1970 results for the *Adh*, *αGpdh-1* and *Mdh-1* loci were calculated on the basis of 703 second chromosomes, whereas the frequencies for the *Amy* locus are based upon 698 chromosomes (5 were missing before the enzyme assays). For all loci on the third chromosome, observations on 489 chromosomes served as a basis for calculating gene frequencies. The gene frequencies for Amy^1, $Amy^{1,3}$, $Amy^{1,2,3}$, $Amy^{1,6}$, $Amy^{2,3}$, Amy^3 and Amy^6 were 0.8854, 0.0745, 0.0014, 0.0129, 0.0029, 0.0215 and 0.0014, respectively. Those for $Est\text{-}6^4$, $Est\text{-}6^6$ and $Est\text{-}6^8$ were 0.3497, 0.6421 and 0.0082, respectively. Frequencies for $Est\text{-}C^2$, $Est\text{-}C^4$ and $Est\text{-}C^6$ were 0.0184, 0.8916 and 0.0900, respectively. The frequencies of *F* alleles are presented in Table 1.

Gene and inversion frequencies for second chromosomes were homogenous over the three years studied. Thus, heterogeneity in gene frequency between inversion-carrying and inversion-free chromosomes was studied using data pooled over the three years. Only at the *Adh* and *Amy* loci were significant heterogeneities detected. For the *Adh* locus, frequencies of *F* alleles (q_F) were 0.3218 ± 0.0151 in the inversion-free chromosomes but $\hat{q}_F = 0.1542 \pm 0.0248$ in those carrying inversions ($\chi^2_{df=1} = 23.47$, $P < 0.0005$). At the *Amy* locus $\hat{q}_F = 0.8647 \pm 0.0111$ in inversion-free chromosomes and $\hat{q}_F = 0.9258 \pm 0.0146$ in chromosomes having inversions ($\chi^2_{df=1} = 12.81$, $P < 0.0005$). Therefore, some linkage disequilibria between inversions and alleles at these loci are to be expected. Approximate constancy of the frequencies of isozyme genes and inversions suggests that the Raleigh population is in an approximate genetic equilibrium, as reported by MUKAI and YAMAGUCHI (1974).

For the third chromosomes, heterogeneities in gene frequencies between inversion-carrying and inversion-free chromosomes were studied, but no significant heterogeneities were detected (*Est-6*: $\chi^2_{df=1} = 2.57$; *Est-C*: $\chi^2_{df=1} = 0.28$; *Odh*: $\chi^2_{df=1} = 0.02$). Furthermore, no significant heterogeneities in gene frequencies between *In(3R)P* carrying chromosomes and all the other chromosomes were detected.

Linkage disequilibrium

Second chromosomes

Data for chromosomes for which all four enzymes (*αGpdh-1*, *Mdh-1*, *Adh* and *Amy*) and the two polymorphic inversions [*In(2L)t* and *In(2R)NS*] were assayed and those from the 1968 and 1969 samples are presented in Table 2. When a unique inversion and a polymorphic inversion were carried by a single chromosome, the chromosome was classified as having only a polymorphic inversion. (The third chromosomes carrying polymorphic and unique inversions were classified in the same manner [Table 4]). There were 314, 146 and 698 chromosomes in the 1968, 1969 and 1970 samples, respectively.

Even if in some chromosome lines the assays could not be completed for some loci because of the losses of the lines in the process, all available data were used to compare the 1970 results for two-factor linkage disequilibria with those of the previous two years (cf., MUKAI, METTLER and CHIGUSA 1971). The following combinations showed significant linkage disequilibria in the 1970 sample: *αGpdh-1–Mdh-1* ($\chi^2_{df=1} = 19.61, P < 0.001$), *In(2L)t–Adh* ($\chi^2_{df=1} = 12.09, P < 0.001$), and *In(2R)NS–Amy* ($\chi^2_{df=1} = 9.28, P < 0.001$). The test statistic for *In(2R)NS* and *Adh* combination nearly achieved significance ($\chi^2_{df=1} = 3.77$, $0.05 < P < 0.10$). Except for the *αGpdh-1–Mdh-1* and the *Mdh-1–Amy* combination (cf., MUKAI, METTLER and CHIGUSA 1971), similar tendencies were observed in all three years. The heterogeneities across years for these two combinations will be discussed later.

Many chromosomes are needed to test higher-order linkage disequilibria. The analysis used in this study was done on data pooled over years because the number of chromosomes sampled in each year alone was not sufficient to provide an adequate test. Bias due to pooling (cf., NEI and LI 1973) was probably very small since gametic frequencies with respect to the four loci and polymorphic inversion frequencies were not heterogeneous over years ($\chi^2_{df=14} = 10.19$, $0.70 < P < 0.80$ for the former; see above for the latter). Pooled data, together with the expected frequencies of all isozyme genotypes, are shown in Table 2. From this table, it is seen that the expected and observed frequencies agree well. These results differ from those of ALLARD *et al.* (1972) for *Avena barbata*, but this is most probably due to the difference in mating systems between the two species—*Avena barbata* is a predominantly selfing species.

For the sake of simplicity, *In(2L)t*, *αGpdh-1*, *Mdh-1*, *Adh*, *Amy* and *In(2R)NS* were designated as *I*, *A*, *B*, *C*, *D*, and *J*, respectively. Due to the restricted number of chromosomes available, analyses were only due for disequilibria up to and including four factors. Total numbers of two-, three-, and four-factor combinations were 15, 20, and 15, respectively. For a few combinations, the expected frequencies were less than 1, but a compromise was made, and the χ^2 analysis was used (cf., LEWONTIN and FELSENSTEIN 1965). A χ^2 value for each disequilibrium component with one degree of freedom was calculated. In addition, the square of the correlation of gene frequencies for each *two-factor combination* [$R^2 = D^2/p(1-p)q(1-q)$ was calculated, where D is a measure of linkage disequilibrium or covariance between gene frequencies of different loci (KIMURA 1956; LEWONTIN and KOJIMA 1960) and p and q are frequencies

TABLE 2

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

Isozyme genotype	Chromosome structure: Normal '68	Normal '69	Normal '70	*In(2L)t* '68	*In(2L)t* '69	*In(2L)t* '70	*In(2R)NS* '68	*In(2R)NS* '69	*In(2R)NS* '70	*In(2L)t* and *In(2R)NS* '68	*In(2L)t* and *In(2R)NS* '69	*In(2L)t* and *In(2R)NS* '70	Others† '68	Others† '69	Others† '70	Total within year '68	Total within year '69	Total within year '70	Grand total	Expected
*FFFF**	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	3	6.8
FFFS	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0.9
FFSF	4	0	5	0	0	0	1	0	0	0	0	0	0	0	0	5	0	5	10	16.4
FFSS	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	4	2.2
FSFF	54	30	125	0	0	1	3	2	14	0	0	0	1	0	4	58	32	144	234	231.8
FSFS	6	5	17	0	0	1	0	0	0	0	0	0	0	0	1	6	5	19	30	31.4
FSSF	122	50	264	16	3	28	14	10	38	4	0	4	9	3	4	165	66	338	569	562.3
FSSS	17	10	42	2	0	2	0	0	1	0	0	0	1	0	1	20	10	46	76	76.1
SFFF	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	1.7
SFFS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2
SFSF	0	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	0	10	10	4.2
SFSS	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.6
SSFF	10	12	31	0	0	0	0	0	2	0	0	0	2	0	2	12	12	35	59	57.2
SSFS	3	0	4	0	0	0	0	0	0	0	0	0	0	0	0	3	0	4	7	7.8
SSSF	26	12	62	1	0	3	6	2	16	1	0	0	1	0	2	35	14	83	132	139.3
SSSS	5	4	8	0	0	1	0	0	0	0	0	0	0	0	0	5	4	9	18	18.9
Total	252	126	568	19	3	36	24	14	76	5	0	4	14	3	14	314	146	698	1158	

Data from the 1968, 1969, and 1970 samples collected from a Raleigh, North Carolina population.

* The order of the loci is *αGpdh-1*, *Mdh-1*, *Adh* and *Amy* from left to right.

† Frequencies of the chromosomes carrying unique inversions alone. Chromosomes carrying polymorphic inversions together with unique inversions are classified on the basis of the polymorphic inversions alone.

of F alleles in the respective loci (HILL and ROBERTSON 1968)]. Values for R^2 were obtained from the formula $R^2 = \chi^2_{df=1}/N$, where N represents the sample size (YAMAZAKI 1973). The results are presented in Table 3.

From these data, the following results were observed: (1) Only one significant linkage disequilibrium was detected between isozyme genes *inter se* (*AB*). This result was undoubtedly due to disequilibrium in the 1970 sample, since no significant linkage disequilibrium was found between *A* and *B* in the 1968 and 1969 samples. Accordingly, the linkage disequilibrium found between *A* and *B* may be considered to be due to sampling error. Since the effective size of the 1970 population was estimated to be much greater than 10^4, even for recessive lethal genes (cf., KIMURA and MARUYAMA 1971), as was true for the 1968 and 1969 populations (MUKAI and YAMAGUCHI 1974), it cannot be expected that the disequilibrium was created by random genetic drift. (2) Significant linkage disequilibria occurred between polymorphic inversions and isozyme genes in the *IA*, *IC*, *CJ*, and *DJ* combinations. Indeed, the same results were obtained when independent χ^2 values of the three years were pooled using the appropriate relationship $z \doteq \Sigma\chi/\sqrt{n}$, where χ is the square root of χ^2 in a 2×2 contingency table (positive or negative depending on the sign of the difference in cross products), and n is

TABLE 3

Value of $\chi^2_{df=1}$ for each component of linkage disequilibrium up to and including four factor interactions for the αGpdh-1 (=A), Mdh-1 (=B), Adh (=C) Amy (=D) *loci, and the* In(2L)t (=I) *and* In(2R)NS (=J) *inversions on the second chromosomes*

Disequilibrium component	$\chi^2_{df=1}$	$R^2(\times 10^6)$	Disequilibrium component	$\chi^2_{df=1}$	Disequilibrium component	$\chi^2_{df=1}$
AB	10.8638***	9382	*ABC*	0.6304	*ABCD*	0.2817
AC	0.0915	79	*ABD*	2.8588	*IABC*	0.0001
AD	0.1027	89	*ACD*	0.0777	*IABD*	0.0935
BC	0.4020	347	*BCD*	0.0507	*IACD*	0.4618
BD	2.7958	2414	*IAB*	0.1781	*IBCD*	0.0635
CD	0.0652	56	*IAC*	1.2757	*ABCJ*	8.0919**
IA	5.3147*	4589	*IAD*	0.5486	*ABDJ*	1.6656
IB	2.0860	1801	*IBC*	0.9793	*ACDJ*	1.0762
IC	23.6240***	20401	*IBD*	0.0797	*BCDJ*	0.3249
ID	0.5943	513	*ICD*	1.4619	*IABJ*	0.9911
AJ	3.2748	2828	*ABJ*	16.2033***	*IACJ*	0.4888
BJ	2.0450	1766	*ACJ*	5.7970*	*IADJ*	0.0588
CJ	9.7727**	8439	*ADJ*	0.4924	*IBCJ*	0.2281
DJ	16.1651***	13959	*BCJ*	2.1143	*IBDJ*	0.2226
IJ	0.5919	511	*BDJ*	1.3290	*ICDJ*	0.0455
			CDJ	1.1002		
			IBJ	0.2363		
			ICJ	0.0081		
			IAJ	0.2117		
			IDJ	0.0081		

R^2 is the square of the correlation of gene frequencies (HILL and ROBERTSON 1968).
* Significant at the 5% level.
** Significant at the 1% level.
*** Highly significant.

the number of x's pooled (LEWONTIN and WHITE 1960). (3) The proportion of interacting combinations decreased with the increase in the number of factors considered simultaneously, i.e., 2 factors, 1/3; 3 factors, 1/10; 4 factors, 1/15). Even within the significant higher-order interactions, there were two suspicious combinations (*ABJ* and *ABCJ*), since a significant interaction, which can be considered to be due to non-random sampling, was discovered between the two factors *A* and *B*. It is probable, then, that the significance of both *ABJ* and *ABCJ* is also due to non-random sampling. (4) There was no significant interaction between the two inversions, and also no significant interaction component including these two inversions simultaneously. Therefore, *In(2L)t* and *In(2R)NS* do not seem to interact with each other. (5) Significant three- or four-factor interactions were always accompanied by significant interactions between some of the constituent factors. For example, in the case of *ACJ*, *CJ* was also significant. (6) *In(2R)NS* always occurred in the significant three- or four-factor interacting combinations. This suggests that *In(2R)NS* has a tendency to have genetic interactions with other loci, while *In(2L)t* does not.

From these findings, it is concluded that (1) linkage disequilibrium between isozyme genes is rare if the loci are not extremely closely linked and (2) higher-order linkage disequilibria do not occur frequently. It can also be speculated that interactions among more than four factors probably do not exist. This is inconsistent with the hypotheses proposed by WILLS, CRENSHAW and VITALE (1970) and FRANKLIN and LEWONTIN (1970) (See DISCUSSION).

A similar test was due for the four isozyme loci using inversion-free chromosomes. The result was very similar to that for chromosomes including both inversion-carrying and inversion-free chromosomes.

Third chromosomes

The gametic frequencies are presented in Table 4 for the three isozyme genes (*Est-6*, *Est-C*, and *Odh*), the polymorphic inversion, *In(3R)P*, and others (unique inversions, *In(3L)P*, and *In(3R)C*; their frequencies were pooled since

TABLE 4

Frequencies of the genotypes studied for the third chromosomes from a large cage population

	Chromosome structure			Total	
	Normal	*In(3R)P*	Others†	Observed	Expected
*FFF**	140	10	2	152	146.8
FFS	6	0	1	7	9.2
FSF	14	0	1	15	17.8
FSS	1	0	0	1	1.1
SFF	232	27	5	264	263.3
SFS	11	1	1	13	16.6
SSF	23	5	1	29	32.0
SSS	8	0	0	8	2.0
Total	435	43	11	489	

* The order of the loci is *Est-6*, *Est-C* and *Odh* from left to right.
† Includes chromosomes carrying unique inversion alone, *In(3L)P*, and *In(3R)C*.

they were extremely rare). Using the method previously described, the total $\chi^2_{df=11} = 28.5267$ was partitioned into two-factor, three-factor and four-factor interaction components. The results, along with the squares of the correlations of gene frequencies (R^2 values) for the two-factor combinations, are given in Table 5, where *Est-6*, *Est-C*, *Odh*, and *In(2R)P* are designated *E*, *F*, *G* and *K*, respectively. There were significant interactions for *FG* and *EFG* (the former: $\chi^2_{df=1} = 13.01$, $P < 0.001$; the latter: $\chi^2_{df=1} = 5.27$, $P < 0.05$).

Third chromosomes were sampled in only one year. Therefore, it is rather difficult to determine whether the linkage disequilibrium observed was due to sampling error, to random genetic drift or to epistasis. Since the effective size of the original natural population was large ($> 10^4$) and since the frequencies of second chromosome genotypes were homogeneous over years, it is unreasonable to assume that the disequilibrium was induced by random genetic drift. However, the map distance between the *Est-C* and *Odh* loci is only about 0.2 centimorgans, so the disequilibria could have been produced by epistasis. Nevertheless, it should be pointed out that the rarer alleles of both loci had very low frequencies, so there is a chance that the disequilibria were due to sampling error that occurred when chromosomes were sampled or when the cage population from which the chromosomes were drawn was initiated. The occurrence of this effect has some probability since this cage population originated from 660 isofemale lines. The latter contention is supported by studies involving two different populations (Brownsville in Texas and Katsunuma, Japan [LANGLEY, TOBARI and KOJIMA 1974]) where significant linkage disequilibria were not detected for these loci.

Similar results were obtained when only inversion-free chromosomes were analyzed.

Relative viability and fecundity

Relative viability

Three loci, in which the gene frequencies of rarer alleles were relatively high (*Adh*, *αGpdh-1*, and *Est-6*), were studied. Three independent experiments were conducted with the 1969 materials (MUKAI *et al.* 1972) whereas nine experi-

TABLE 5

Value of $\chi^2_{df=1}$ for each component of linkage disequilibrium for the Est-6 (=E), Est-C (=F), Odh (=G) *loci and the* In(3R)P (=K) *inversion on the third chromosome*

Disequilibrium component	$\chi^2_{df=1}$	R^2† ($\times 10^6$)	Disequilibrium component	$\chi^2_{df=1}$
EF	0.8103	1657	*EFG*	5.2718*
EG	0.9023	1845	*EFK*	1.0241
EK	3.2218	6589	*EGK*	0.0580
FG	13.0118***	26608	*FGK*	1.9587
FK	0.0304	62	*EFGK*	1.4718
GK	1.0982	2246		

† Square of the correlation of gene frequencies.
* Significant at the 5% level.
*** Highly significant.

TABLE 6

Mean relative viabilities of homozygotes of isozyme genes in homozygous genetic backgrounds

Genotype	*Adh* N	*Adh* Viability*	*αGpdh-1* N	*αGpdh-1* Viability*	*Est-6* N	*Est-6* Viability*
SS	581	0.4181±0.0159	172	0.4461±0.0293	314	0.4873±0.0282
	343	0.7030±0.0121†				
FF	254	0.4721±0.0241	663	0.4315±0.0149	175	0.5086±0.0378
	166	0.7180±0.0175†				

N = The number of independent lines.
* The average viability of chromosomal heterozygotes is assigned a value of 1.0000.
† Excluding lethal-carrying chromosomes.

ments were undertaken with the 1970 sample (cf., MUKAI and YAMAGUCHI 1974). Homozygote and heterozygote viabilities were simultaneously estimated. Within experiments, the average viabilities of heterozygotes were assigned a value of 1.000, and the homozygote and heterozygote viabilities of all lines or crosses were standardized.

1) *Homozygous genetic background:* The viabilities for 835 second chromosome lines collected in 1969 and 1970, and 489 third chromosome lines collected in 1970 were examined. The results on the basis of 10,592 vials (2 cm × 10 cm), are presented in Table 6. For the *αGpdh-1* and *Est-6* loci, no significant differences in viability between *FF* and *SS* genotypes were detected, but for the *Adh* locus, the t value is close to significance ($t \doteq 1.87$). Thus, for this locus, calculations were also done, excluding lethal-carrying lines. These results are also presented in Table 6. The average relative viabilities of *FF* and *SS* lines were then found to be very similar. Therefore, the above described difference must be due to differing frequencies of lethal-carrying chromosomes for the two classes, which itself is a product of linkage disequilibrium. It should be noted that, on the average, the frequency of lethal-carrying chromosomes in inversion-carrying chromosomes was greater than that found in inversion-free chromosomes (MUKAI and YAMAGUCHI 1974).

2) *Heterozygous genetic background:* In total, 831 crosses from both the 1969 and 1970 samples involving the second chromosomes and 489 crosses for the third chromosomes were investigated (the total number of vials used was 10,560). Approximate estimations of the effects of sperm competition and frequency dependent selection were made simultaneously. Using the second chromosomes as an example, the estimation procedure used can be described as follows: The *SM1(Cy)* chromosomes used for the estimation of viability, carry *F* alleles for both the *Adh* and *αGpdh-1* loci. Thus, the parental and offspring genotypes for these two loci are presented below:

	Parents: Female	Parents: Male	Genotype	Offspring Genotypic ratio *FF*	*FS*	*SS*	Frequency of *F*
(1)	$\frac{Cy(F)}{F}$	$\times \frac{Cy(F)}{F}$	$\frac{Cy(F)}{F}, \frac{Cy(F)}{F}, \frac{F}{F}$	3,	0,	0	1.0

(2)	$\frac{Cy(F)}{F} \times \frac{Cy(F)}{S}$	$\frac{Cy(F)}{F}, \frac{Cy(F)}{S}, \frac{F}{S}$	1 : 2, 0	$\frac{2}{3}$
(3)	$\frac{Cy(F)}{S} \times \frac{Cy(F)}{F}$	$\frac{Cy(F)}{F}, \frac{Cy(F)}{S}, \frac{F}{S}$	1 : 2, 0	$\frac{2}{3}$
(4)	$\frac{Cy(F)}{S} \times \frac{Cy(F)}{S}$	$\frac{Cy(F)}{S}, \frac{Cy(F)}{S}, \frac{S}{S}$	0, 2 : 1	$\frac{1}{3}$

Four types of matings are made. The expected segregation ratios with respect to enzyme alleles in respective matings and the gene frequencies in the offspring (within respective vials) are not the same. If meiotic drive is common and the segregation ratio is different in males and females, the viabilities of *FS* genotypes in the offspring of matings (2) and (3) determined in the manner described above will be different. If the effect of frequency dependent selection is appreciable as KOJIMA and YARBROUGH (1967) claimed, the viabilities of *FF*, *FS* and *SS* genotypes will be expected to be quite different. Similar arguments can be made for the third chromosome (the *TM3(Sb)* chromosome carries *S* allele in the *Est-6* locus).

The relative viabilities of the four genotypes (*SS*, *SF*, *FS* and *FF*) for the three typically polymorphic loci are presented in Table 7. F test from the analysis of variance for each locus indicate that there are no significant differences among the four genotypes. Thus, there is no evidence for meiotic drive (or sperm competition) or frequency dependent selection.

To increase the power of the tests for heterozygote superiority, viabilities were pooled for two types of homozygotes as well as for two types of heterozygotes. The pooled results are presented in Table 7. After pooling, the viabilities of the pooled homozygotes were assigned the value one. Subsequent tests revealed no evidence for heterozygote superiority.

TABLE 7

Mean relative viabilities of genotypes for isozyme genes in heterozygous genetic backgrounds

Genotype*	*Adh* N	*Adh* Viability†	*αGpdh-1* N	*αGpdh-1* Viability†	*Est-6* N	*Est-6* Viability†
SS	409	1.0042±0.0053	33	1.0051±0.0141	196	0.9896±0.0074
SF	169	0.9926±0.0079	138	0.9980±0.0084	105	0.9961±0.0089
FS	173	1.0048±0.0068	138	1.0063±0.0084	108	1.0174±0.0115
FF	80	0.9854±0.0104	522	0.9988±0.0046	62	1.0059±0.0059
Pooled homozygotes	489	1.0000	555	1.0000	258	1.0000
Pooled heterozygotes	342	0.9977±.0069	276	1.0030±.0074	213	1.0135±.0099

N = Number of crosses.

* The first genes are from the female parents and the second genes are from the male parents.

† For the first 4 rows, the average viability of chromosomal heterozygotes is assigned a value of 1.0000. For the last 2 rows, the average viability of pooled homozygotes is assigned a value of 1.0000.

TABLE 8

Cumulative effect of heterozygous loci on viability at the αGpdh-1, Mdh-1, Adh *and* Amy *loci*

Number of heterozygous loci	Number of crosses	Average viability (or fecundity)
0	264	1.0000
1	350	0.9991 ± 0.0081
2	185	1.0013 ± 0.0096
2 or more	215	0.9988 ± 0.0092
(3 or more)	30	0.9837 ± 0.0187

3) *Cumulative effect of heterozygosity:* To determine if heterozygosity has a cumulative effect on viability, the data were reanalyzed for the second chromosomes with heterozygous genetic backgrounds. All crosses except two outlying cases, whose viabilities were extremely low, were partitioned into 5 classes on the basis of the number of heterozygous loci existing for the four loci (*Adh*, *αGpdh-1*, *Mdh-1* and *Amy*). The average viability, relative to the average viability of the zero heterozygous class, and the number of crosses in each category are presented in Table 8. Since the numbers of crosses in the three and four heterozygous classes were small, these classes were pooled. These data did not contain any evidence for a cumulative heterozygous effect. Although a similar analysis can be conducted with the data from the third chromosomes, an analysis was not attempted since only the *Est-6* locus is typically polymorphic.

In summary, the analyses described yielded no evidence for *differential viabilities among the genotypes within loci.*

Relative fecundity

Similar analyses to the above viability analyses were done with respect to the number of progenies of *SM1(Cy)/F*(or *S*) and *TM3(Sb)/F*(or *S*) females. Since there are no perceptible differences in viability among the three genotypes for the isozyme loci, the number of progenies per genotype must have been proportional to female fecundity, if sperm were sufficiently supplied. For the three typically polymorphic loci described above, relative fecundities were calculated. The number of independent genotypes (or crosses) and the average relative fecundities of individual genotypes for each locus, where fecundity for the pooled homozygotes was assigned the value one, are shown in Table 9. Analyses of these data show that no significant difference exists between homozygotes and heterozygotes. It might be that *αGpdh-1* shows heterozygote superiority but this was not significant: $t = 1.42$, $0.05 < P < 0.10$ in one-tail test.

The cumulative effects of heterozygosity on fecundity were similarly investigated. The average relative fecundity and the number of independent crosses are listed in Table 10 for different numbers of heterozygous loci. From these data, it is evident that there are no perceptible differences in fecundity among genotypes with varying numbers of heterozygous loci.

It should be pointed out that the genetic background of *Cy/F*(or *S*) and *Sb/F*(or *S*) individualss is inter-populational. There still remains a possibility

TABLE 9

Mean relative fertilities (or fecundities) of homozygotes and heterozygotes for isozyme genes in heterozygous genetic backgrounds of inter-populational crosses

	*Adh**		*αGpdh-1**		*Est-6*†	
	N	Fertility‡	N	Fertility‡	N	Fertility‡
Homozygotes (*FF* or *SS*)	253	1.0000	660	1.0000	301	1.0000
Heterozygotes (*FS*)	578	0.9970±0.0092	171	1.0142±0.0100	170	1.0027±0.0147

N = The number of independent genotypes.
* *SM1(Cy)*/+ genetic background.
† *TM3(Sb)*/+ genetic background.
‡ The average fertility of homozygotes with respect to isozyme genes was assigned a value of 1.0000.

TABLE 10

Cumulative effect of heterozygous loci on fertility (or fecundity) of females at the αGpdh-1, Mdh-1, Adh *and* Amy *loci*

Number of heterozygous loci	Number of crosses	Average fertility (or fecundity)
0	174	1.0000
1	471	0.9894 ± 0.0111
2	161	1.0094 ± 0.0134
2 or more	186	1.0088 ± 0.0128
(3 or more)	25	1.0060 ± 0.0209

that the different isozyme genotypes have varying fecundities. This appears to be highly unlikely, however, since in these experiments no differences in average viabilities were found among genotypes within isozyme loci in intrapopulational genetic backgrounds.

DISCUSSION

Approximate genetic equilibrium of the population

MUKAI and YAMAGUCHI (1974) analyzed the Raleigh population in detail and concluded that it was approximately in genetic equilibrium. Estimates of some important genetic parameters of this population are very similar to those obtained from a large laboratory population that must be in approximate genetic equilibrium (cf., WALLACE 1956; GREENBERG and CROW 1960). Specifically, it was found that the frequencies of four isozyme genes and inversions are approximately constant over the three years. Furthermore, the gene frequency of the alcohol dehydrogenase locus was found to be approximately the same from the end of April to the end of November (F. M. JOHNSON, personal communication). The effective size of the population in question was estimated to be much greater than 10,000 in each of the three years (MUKAI and YAMAGUCHI 1974). In fact, the allelism rate of recessive lethals within and between two sites about 1.4 miles apart are approximately the same (at face value the former was smaller than the latter, but not significantly so). This suggests that flies migrate over a wide area.

Recently, NEI and LI (1973) warned that heterogeneous data and data from different populations should not be pooled in the study of linkage disequilibrium. However, this cautionary note is not applicable to the present studies (also see MUKAI, METTLER and CHIGUSA 1971).

Relative viability and fecundity

No evidence could be found for differential viability or fecundity among genotypes within three typically polymorphic isozyme loci. Furthermore, there was no evidence that the four isozyme loci in the second chromosome contained heterozygous cumulative effects for viability or fecundity. If fecundity or viability does vary among genotypes of polymorphic enzyme loci, the magnitude of the differences must be extremely small (probably of the order of 10^{-3} or less). This conclusion agrees with that obtained by variance component analysis of viability (MUKAI *et al.* 1974), and is consistent with that obtained from studies of the regression of heterozygote viability on the sum of the homozygote viabilities of component chromosomes (MUKAI and YAMAGUCHI 1974).

Recently, WILLS and NICHOLS (1972) reported "conditional heterosis" associated with two isoallele forms of octanol dehydrogenase in *D. pseudoobscura.* According to these authors, heterozygote superiority is detected at the autosomal *Odh* locus of flies with highly inbred genetic backgrounds that have been reared on media containing octanol. Although not indicated by the authors, the heterozygosities of the genetic backgrounds of homozygotes and heterozygotes are different (cf., YAMAZAKI 1972). Presumably the authors considered this fact as their reason for using the esterase-5 locus with highly inbred genetic background (unfortunately, this is located on the *X* chromosome) as a control. At this locus, heterozygote superiority was not manifest even when the culture medium contained octanol. Thus, they did not ascribe the above "conditional heterosis" to the difference in genetic backgrounds between homozygotes and heterozygotes for isozyme genes. However, it should be noted that the χ^2 test (Table 1 on page 324 of WILLS and NICHOLS 1972) used does not indicate heterozygote superiority at the *Odh* locus, but does show that there was a shortage of *SS* genotypes, which could have been due to the genetic background (Ratio of *FF* to *FS* averages approximately 1:2). Furthermore, according to their mating schemes, the inbreeding coefficient for the genetic background in the autosomes differs from that for that in the sex chromosomes (cf., YAMAZAKI 1972). As a result, there must have been greater heterozygosity around the *Odh* locus than around *Est-5*. An additional criticism holds, if functionally related genes are located in adjacent regions of the chromosomes (cf., JUDD, SHEN and KAUFMAN 1972). Then neighboring genetic background of the *Est-5* locus cannot be used as a control for the genetic background of the *Odh* locus.

Linkage disequilibrium between isozyme genes

In the present study, linkage disequilibria between isozyme genes *inter se* were detected in a few cases (*Mdh-1–Amy* in 1968 (MUKAI, METTLER and CHIGUSA 1971); *αGpdh-1–Mdh-1* in 1970; *Est-C–Odh* in 1970). Two general trends

became apparent. (1) There was an inconsistency in the results for the three years (nothing can be said conclusively about the linkage disequilibrium between *Est-C* and *Odh* since the test was carried out only in one year). (2) At least for one locus, the gene frequency was always low (*Mdh-1*, *Odh* and *Est-C*).

LANGLEY, TOBARI and KOJIMA (1974) investigated two populations: The Brownsville, Texas population and the Katsunuma population in Japan. They have not estimated effective sizes of these populations, but it appears that the Brownsville population had a large effective size. This population survives year-round (YANG and KOJIMA 1972) and the frequency of lethal-carrying chromosomes (Q) was estimated to be 0.249 ($= 58/233$). The effective size of the Katsunuma population has been estimated to be 1880 by YOSHIKAWA and MUKAI (1970), on the basis of the allelism rate and the frequency of lethal-carrying chromosomes ($\hat{Q} = 0.149$) reported by WATANABE (1969). Linkage disequilibria for the following pairs of isozyme genes have been found in the Katsunuma population: esterase-6–phosphoglucomutase, esterase-C–leucine aminopeptidase-D (map distance, 49.3 centimorgans), octanol dehydrogenase–aldehyde oxidase (map distance, 7.4 centimorgans). In contrast to the Katsunuma population, linkage disequilibria between isozyme genes *inter se* were not found in the Brownsville population (including the combinations, *Mdh-1–Amy*, *Mdh-1–αGpdh-1* and *Est-C–Odh* for which non-random associations were once detected in the Raleigh population). These results not only agree with the above trends, but also suggest the following additional trends: (3) There are inconsistencies among populations with respect to the presence of linkage disequilibrium and (4) the frequencies of linkage disequilibria between isozyme genes *inter se* are related to the effective sizes of populations although these were estimated using lethal genes, namely that small populations have more linkage disequilbria as theory predicts.

On the basis of trends (1), (2) and (3), it is concluded that the linkage disequilibria between isozyme genes found in the Raleigh population were due to non-random sampling in the process of extracting chromosomes rather than random genetic drift in the population. It cannot be determined whether the non-random associations in the Katsunuma population were due to non-random sampling or to random genetic drift since only one sample was extracted. It is concluded that linkage disequilibria between isozyme genes due to epistasis are rare events if the distances between genes are not extremely short. In the present studies, the shortest distance between pairs of isozyme loci was 0.2 centimorgans (the distance between *Odh* and *Est-C*) and significant linkage disequilibrium was detected. However, a similar disequilibrium was not found in the Brownsville population. If the distance between loci is much less, linkage disequilibria due to epistasis might be present. Unfortunately, there are no appropriate markers available to test this.

Recently, CHARLESWORTH and CHARLESWORTH (1973) reported some significant linkage disequilibria between isozyme genes in the third chromosomes of *D. melanogaster*, but it is probable that most of them were due to sampling error or non-random sampling. The reasons are that (1) the sample size is not large and (2) there is no clear relationship between the correlations of gene frequencies

and the distances between the isozyme genes in question. ZOURos and KRIMBAS (1973) also reported a significant linkage disequilibrium between the aldehyde oxidase locus and the xanthine dehydrogenase locus in *D. subobscura*. This may also be due to sampling error and/or to complicated interaction with polymorphic inversions, since the sample size is very small and there are several polymorphic inversions in the chromosome studied. The interaction between isozyme genes and polymorphic inversions can be seen often. Repeated sampling is most necessary for this kind of experiment.

WILLS, CRENSHAW and VITALE (1970) suggested that, even if two loci are relatively far apart, linkage disequilibrium can be found if there are overdominance and truncation selection, forces believed to be common in natural populations by several investigators (SVED, REED and BODMER 1967; KING 1967; MILKMAN 1967). Results from the present studies do not support this hypothesis. Indeed, MUKAI, SCHAFFER and COCKERHAM (1972) reported that the effect of phenotypic truncation selection is very similar to that of independent locus selection in *D. melanogaster*, since the heritability of viability is very low in this species (H^2 [broad sense] $\doteq 0.004$). Furthermore, the heterotic model of FRANKLIN and LEWONTIN (1970) seems inapplicable; it states that a large amount of linkage disequilibrium is maintained at equilibrium if a number of overdominant loci with multiplicative gene action are tightly packed in a chromosome.

Linkage disequilibria between isozyme genes and polymorphic inversions

Linkage disequilibrium of this type was first detected by PRAKASH and LEWONTIN (1968). Non-random associations for the *Adh–In(2L)t*, *Adh–In(2R)NS*, *αGpdh-1–In(2L)t*, *Amy–In(2R)NS* isozyme locus and polymorphic inversion combinations were found in the present investigation. The tendencies of these non-random associations were similar for the three years studied as well as over distant populations (cf., LANGLEY, TOBARI and KOJIMA 1974), namely that the R values are alike for each of the combinations. These results contrast with the findings for non-random associations between isozyme genes *inter se*. One of the reasons probably is that the heterozygotes for the polymorphic inversions express heterosis; [*In(2L)t*: about 1.2% over the normal heterozygotes in viability; *In(2R)NS*: about 1.3%. However, these figures are not significantly larger than zero. Heterosis may have been expressed in fecundity (MUKAI and YAMAGUCHI 1974)]. It has been reported that the squared standard linkage deviation (σ^2_d, a quantity similar to R^2) between a neutral locus and an overdominant one is expected to be approximately $1/(4N_ec)$ when $4N_ec$ is much larger than 1. In this formula N_e is the effective size of the population and c is the recombination value between the two genes (OHTA and KIMURA 1971; also see HILL and ROBERTSON 1968).

The polymorphic inversions undoubtedly occurred uniquely many years ago since they are now cosmopolitan (cf., OSHIMA, WATANABE and WATANABE 1964; WATANABE 1967; KOJIMA, GILLESPIE and TOBARI 1970), but it is not known when or where they first appeared. This makes speculation concerning the presence of epistasis hazardous. With one exception, all the above linkage disequi-

libria found between isozyme genes and inversions can be explained by the lack of recombination or by reduced recombination alone without assuming epistasis, provided that the inversions occurred relatively recently. The one exception is for linkage between *Adh* and *In(2R)NS*. The physical distance between the *Adh* locus and the left break-point of *In(2R)NS* is large. Indeed, they are located on different arms. However, crossing over is reduced to about 1.5% between the centromere and the inversion (LINDSLEY and GRELL 1967). As a result, the recombination value between *Adh* and the inversion becomes approximately 6.4%, or effectively 3.2% since there is no crossing over in Drosophila males. As described above, the frequency of *In(2R)NS* was stable over the three years.

Whether the isozyme-inversion linkage disequilibrium is due to random genetic drift alone can be tested. If random genetic drift created the disequilibrium, the correlation coefficient between the frequencies of the *Adh* alleles and *In(2R)NS* is expected to be about $\pm$ 0.02 under the formula given by OHTA and KIMURA (1971). The observed values (0.12 in 1968, 0.14 in 1969, and 0.07 in 1970) differ in the same direction from the expected absolute value, but only the absolute value for the 1968 sample is significantly larger than that of the expected value. Furthermore, the expected decay of linkage disequilibrium with generation was examined numerically under the assumptions of overdominance in the inversion heterozygote and neutrality at the *Adh* locus. The expected D value in 1970, on the basis of the observed D value in 1968, was compared with the observed value in 1970, but the deviation was not significant. Thus, it may be concluded that there is an interaction between the *Adh* alleles and *In(2R)NS*, but its magnitude is too small to be detected. The finding that *In(2R)NS* significantly interacted with *C*, *D*, *AB*, *AC*, and *ABC* (see Table 3) may support this speculation. It should be noted that linkage disequilibrium between the *Adh* alleles and *In(2R)NS* could not be found in either the Brownsville or the Katsunuma population.

DR. C. H. LANGLEY pointed out that the polymorphic inversions studied that are in linkage disequilibrium with isozyme genes, are always more associated with the alleles of higher frequencies than chance would predict. The only exceptions involve *In(3R)P* (LANGLEY, TOBARI and KOJIMA 1974). LANGLEY, TOBARI and KOJIMA (1974) have interpreted this result as evidence for directional epistatic interactions between the polymorphic inversions and the loci in question. However, it is questionable whether this hypothesis can be considered valid on the basis of the data from the present investigation. It is not known when these polymorphic inversions occurred uniquely, or the types of the isozyme alleles and their respective frequencies that existed at that time. If the gene frequencies were the same then as at present, the probability that these two polymorphic inversions [*In(2L)t* and *In(2R)NS*] occurred in the chromosomes carrying the alleles with the higher frequencies is approximately 0.34.

The difference in linkage relationships between isozyme gene–isozyme gene pairs and isozyme gene–inversion pairs can be explained in the following manner. Some isozyme genes appear to be potentially heterotic and epistatic when in combination with specific gene complexes (e.g., a polymorphic inversion) but otherwise have essentially no effect. This is similar to the manifestation of over-

dominance of viability polygenes when in a specific background (MUKAI, CHIGUSA and YOSHIKAWA 1965; MUKAI and YAMAZAKI 1968). This type of epistasis is rarely found for isozyme loci *inter se*, and no linkage disequilibria can be detected in a large population. In natural populations, only a few types of polymorphic inversions can be observed, although inversions are produced at a relatively high frequency per generation (WOODWARD 1971; O. YAMAGUCHI and T. MUKAI, unpublished; O. YAMAGUCHI, R. A. CARDELLINO and T. MUKAI, unpublished). This means that only a few well coadapted inversions out of a large number of newly arisen inversions are retained in populations in a polymorphic state. These few inversions probably survive because of favorable interactions with other genes or gene complexes. It follows then that uninverted well coadapted gene combinations must also be rare in populations of this species. It is possible that *some* potentially heterotic and epistatic nearly neutral isozyme genes interact with these well coadapted multi-genic polymorphic inversions, and consequently linkage disequilibria are formed. Thus, *the presence of permanent linkage disequilibria between isozyme genes and polymorphic inversions is not a general proof that appreciable selection pressure is exerted on individual isozyme loci.*

Environmental conditions and the polymorphisms of isozyme genes

It has been pointed out that genetic variability can be maintained in populations because of variable environmental condition (cf., LEVENE 1953). This argument may not be applicable to the maintenance of genetic variability for isozyme genes for the following reasons: (1) The isozyme gene frequencies are very stable from year to year (present investigation) and also from April to November (F. M. JOHNSON, personal communication), a period in which environmental conditions are extremely variable. Because of extremely low heritability of viability, a change in environmental conditions might not affect phenotypic selection coefficients greatly even if genotypic selection coefficients changed with environmental conditions. (2) Overdominance generally accelerates rather than retards fixation of segregating alleles when $2\,\bar{s} < V_s$, where $\bar{s}$ is the mean selection coefficient against either homozygote and V_s is the between-generation variance of the selection coefficient (OHTA and KIMURA 1972). If s fluctuates due to the changes in environmental conditions, the relationship of $2\,\bar{s} < V_s$ can easily be satisfied if s is close to zero. Therefore, the fluctuation of environmental conditions will cause a decrease in variability if slight overdominance exists and the size of the population is large.

It has been shown in the present investigation that the polymorphic isozyme genes are selectively neutral or nearly neutral for viability. In addition, the heritability of viability as a whole is extremely low under laboratory conditions (MUKAI *et al.* 1972). It may be argued that polymorphic isozyme genes are not near-neutral under natural conditions due to genotype–environment interactions. Not enough data are available to examine this hypothesis. However, (1) the constancy of isozyme gene frequencies (see above), (2) the similarity between the frequency of lethal-carrying chromosomes in the Raleigh population

($\hat{Q} \doteq 0.36$–0.39) and that of a large cage population of WALLACE (1956), and (3) the experimental results of O'BRIEN and MACINTYRE (1969) serve as arguments against this view. O'BRIEN and MACINTYRE analyzed a cage population which has been maintained for about 20 years, and found that the magnitude of heterozygosity with respect to isozyme genes was similar to that in the original natural populations. However, it is necessary to carry out experiments to clarify this problem.

It is tentatively concluded that most polymorphic isozyme genes are selectively neutral or near-neutral, although we must accept the importance of selection for some isozyme polymorphisms. Near-neutral genes can become *effectively neutral* in finite populations as described by KIMURA (1968).

We are grateful to DR. PETER M. BURROWS who suggested a statistical method for testing higher-order linkage disequilibrium and gave constructive criticisms and to DR. CHARLES H. LANGLEY, who gave permission to cite the paper to be published, of which he is the first author, and exchanged opinions about the present topic with the first author (T.M.).

LITERATURE CITED

ALLARD, R. W., G. R. BABBEL, M. T. CLEGG and A. L. KAHLER, 1972 Evidence for coadaptation in *Avena barbata*. Proc. Natl. Acad. Sci. U.S. **69**: 3043–3048.

AYALA, F. J., J. R. POWELL and TH. DOBZHANSKY, 1971 Enzyme variability in the *Drosophila willistoni* group. II. Polymorphisms in continental and island populations of *Drosophila willistoni*. Proc. Natl. Acad. Sci. U.S. **68**: 2480–2483.

BAHN, E., 1967 Crossing over in the chromosomal region determining amylase isozymes in *Drosophila melanogaster*. Hereditas **58**: 1–12.

CHARLESWORTH, B. and D. CHARLESWORTH, 1973 A study of linkage disequilibrium in populations of *Drosophila melanogaster*. Genetics **73**: 351–359.

CROW, J. F., 1968 The cost of evolution and genetic loads. pp. 165–178. In: *Haldane and Modern Biology*. Edited by K. R. DRONAMRAJU. The Johns Hopkins Press, Baltimore.

FRANKLIN, I. and R. C. LEWONTIN, 1970 Is the gene the unit of selection? Genetics **65**: 707–734.

GREENBERG, R. and J. F. CROW, 1960 A comparison of the effect of lethal and detrimental chromosomes from Drosophila populations. Genetics **45**: 1154–1168.

HALDANE, J. B. S., 1956 The estimation of viabilities. J. Genet. **54**: 294–296.

HILL, W. G. and A. ROBERTSON, 1968 Linkage disequilibrium in finite populations. Theoret. and Applied Genet. **38**: 226–231.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X* chromosome of *Drosophila melanogaster*. Genetics **71**: 139–156.

KIMURA, M., 1956 A model of a genetic system which leads to closer linkage by natural selection. Evolution **10**: 278–287. ——, 1968 Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles. Genet. Res. **11**: 247–269.

KIMURA, M. and J. F. CROW, 1964 The number of alleles that can be maintained in a finite population. Genetics **49**: 725–738.

KIMURA, M. and T. MARUYAMA, 1971 Pattern of neutral polymorphism in a geographically structured population. Genet. Res. **18**: 125–131.

KIMURA, M. and T. OHTA, 1971 Protein polymorphism as a phase of molecular evolution. Nature **229**: 467–469.

KING, J. L., 1967 Continuously distributed factors affecting fitness. Genetics **55**: 483–492.

KOJIMA, K., J. GILLESPIE and Y. N. TOBARI, 1970 A profile of Drosophila species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochemical Genet. **4**: 627–637.

KOJIMA, K. and K. M. YARBROUGH, 1967 Frequency-dependent selection at the esterase-6 locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **57**: 645–649.

LANGLEY, C. H., Y. N. TOBARI and K. KOJIMA, 1974 Linkage disequilibrium in natural populations of *Drosophila melanogaster*. Submitted for publication.

LEVENE, H., 1953 Genetic equilibrium when more than one ecological niche is available. Am. Naturalist **87**: 311.

LEWONTIN, R. C., 1964 The interaction of selection and linkage. I. General considerations of heterotic models. Genetics **49**: 49–67.

LEWONTIN, R. C. and J. FELSENSTEIN, 1965 The robustness of homogeneity tests in $2 \times N$ tables. Biometrics **21**: 19–33.

LEWONTIN, R. C. and J. L. HUBBY, 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amounts of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**: 595–609.

LEWONTIN, R. C. and K. KOJIMA, 1960 The evolutionary dynamics of complex polymorphisms. Evolution **14**: 458–472.

LEWONTIN, R. C. and M. J. D. WHITE, 1960 Interaction between inversion polymorphisms of two chromosome pairs in the grasshopper, *Moraba scurra*. Evolution **14**: 116–129.

LINDSLEY, D. L. and E. H. GRELL, 1967 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. 627.

MILKMAN, R. D., 1967 Heterosis as a major cause of heterozygosity in nature. Genetics **55**: 493–495.

MUKAI, T., 1968 Experimental studies on the mechanism involved in the maintenance of genetic variability in Drosophila populations. Japan J. Genet. **43**: 399–413. ——, 1969 Maintenance of polygenic and isoallelic variation in populations. Proc. XII Intern. Congr. Genet. **3**: 293–308.

MUKAI, T., R. A. CARDELLINO, T. K. WATANABE and J. F. CROW, 1974 The genetic variance of viability and its components in a local population of *Drosophila melanogaster*. Genetics (In press.)

MUKAI, T., S. I. CHIGUSA, L. E. METTLER and J. F. CROW, 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics **72**: 335–355.

MUKAI, T., S. CHIGUSA and I. YOSHIKAWA, 1965 The genetic structure of natural populations of *Drosophila melanogaster*. III. Dominance effect of spontaneous mutant polygenes controlling viability in heterozygous genetic backgrounds. Genetics **52**: 493–501.

MUKAI, T., L. E. METTLER and S. I. CHIGUSA, 1970 On the linkage equilibrium of isozymes in a Raleigh, N.C. population of *D. melanogaster*. Drosophila Inform. Serv. **45**: 77. ——, 1971 Linkage disequilibrium in a local population of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 1065–1069.

MUKAI, T., H. E. SCHAFFER and C. C. COCKERHAM, 1972 Genetic consequences of truncation selection at the phenotypic level in *Drosophila melanogaster*. Genetics **72**: 763–769.

MUKAI, T. and O. YAMAGUCHI, 1974 The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. Genetics **76**: 339–366.

MUKAI, T. and T. YAMAZAKI, 1968 The genetic structure of natural populations of *Drosophila melanogaster*. V. Coupling-repulsion effect of spontaneous mutant polygenes controlling viability. Genetics **59**: 513–535.

NEI, M. and W. H. LI, 1973 Linkage disequilibrium in subdivided populations. Genetics **75**: 213–219.

O'BRIEN, S. J. and R. J. MACINTYRE, 1969 An analysis of gene enzyme variability in natural populations of *Drosophila melanogaster* and *D. simulans*. Am. Naturalist **103**: 97–113.

OHTA, T. and M. KIMURA, 1969 Linkage disequilibrium due to random genetic drift. Genet. Res. **13**: 47–55. ——, 1971 Behavior of neutral mutants influenced by associated overdominant loci in finite populations. Genetics **69**: 247–260. ——, 1972 Fixation time of overdominant alleles influenced by random fluctuation of selection intensity. Genet. Res. **20**: 1–7.

OSHIMA, C., T. K. WATANABE and T. WATANABE, 1964 The persistence of some recessive lethal genes in natural populations of *Drosophila melanogaster*. IV. Proc. Japan Acad. **40**: 125–130.

PRAKASH, S. and R. C. LEWONTIN, 1968 A molecular approach to the study of genic heterozygosity in natural populations. III. Direct evidence of co-adaptation in gene arrangements of Drosophila. Proc. Natl. Acad. Sci. U.S. **59**: 398–405.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics **61**: 841–858.

STONE, W. S., F. M. JOHNSON, K. KOJIMA and M. R. WHEELER, 1968 Isozyme variation in island populations of Drosophila. I. An analysis of a species of the nasuta complex in Samoa and Fiji. Univ. of Texas Publ. **6818**: 157–170.

SVED, J. A., T. E. REED and W. F. BODMER, 1967 The number of balanced polymorphisms that can be maintained in a natural population. Genetics **55**: 469–481.

WALLACE, B., 1956 Studies on irradiated populations of *Drosophila melanogaster*. J. Genet. **54**: 289–293.

WATANABE, T., 1967 Chromosomal polymorphisms in natural populations of *Drosophila melanogaster*. Memoirs of the Faculty of Science, Kyushu University, Series E, (Biology) **4**(4): 159–178.

WATANABE, T. K., 1969 Frequency of deleterious chromosomes and allelism between lethal genes in Japanese natural populations of *Drosophila melanogaster*. Japan. J. Genet. **44**: 171–187.

WILLS, C., J. CRENSHAW and J. VITALE, 1970 A computer model allowing maintenance of large amounts of genetic variability in Mendelian populations. I. Assumptions and results for large populations. Genetics **64**: 107–123.

WILLS, C. and L. NICHOLS, 1972 How genetic background masks single-gene heterosis in Drosophila. Proc. Natl. Acad. Sci. U.S. **69**: 323–325.

WOODWARD, D. L., 1971 The nature of unique and polymorphic inversions in experimental populations of *Drosophila melanogaster*. M.S. Thesis, North Carolina State University Library.

YAMAZAKI, T., 1971 Measurement of fitness at the esterase-6 locus in *Drosophila pseudoobscura*. Genetics **67**: 579–603. ——, 1972 Detection of single gene effect by inbreeding. Nature New Biology **240**: 53–54. ——, 1973 Computer Uses in Biology (in Japanese). The Heredity (Iden) **27**: 102–111

YANG, H. Y. and K. KOJIMA, 1972 Chromosomal polymorphism and lethal alleles in a southwest Texas population of *Drosophila melanogaster*. Univ. of Texas Publ. **7213**: 229–236.

YOSHIKAWA, I. and T. MUKAI, 1970 Heterozygous effects on viability of spontaneous lethal genes in *Drosophila melanogaster*. Japan. J. Genet. **45**: 443–455.

ZOUROS, E. and C. B. KRIMBAS, 1973 Evidence for linkage disequilibrium maintained by selection in two natural populations of *Drosophila subobscura*. Genetics **73**: 659–674.

Corresponding editor: T. PROUT