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Original article

Linkage disequilibrium in French natural populations of *Drosophila melanogaster*

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Summary — Seventeen French natural populations of *Drosophila melanogaster* were analyzed to detect linkage disequilibrium between pairs of 6 polymorphic allozyme loci. The estimates of linkage disequilibrium were made from azygotic frequencies using both Burrows' and Hills's methods. No difference between these 2 methods was found. The amount of significant linkage disequilibrium detected was small and similar to those in other natural populations of *D. melanogaster*. Out of the 15 combinations examined, only 2 pairs, *Adh-α-Gpdh* and *Est-C-Est-6*, showed a consistent significant linkage disequilibrium in the populations studied. However, for the first pair, the result was probably due to an association between the loci and the inversion *(2 L) t* of the second chromosome. For the *Est-C-Est-6* pair, the disequilibrium detected might result from an interaction effect between the 2 genes *inter se*. These results again show the difficulties in detecting linkage disequilibrium due to epistasis between allozyme genes in natural populations.

***Drosophila melanogaster* – linkage disequilibrium – enzymatic loci – French natural populations**

Résumé — Déséquilibre de liaison dans des populations naturelles françaises de *Drosophila melanogaster*. *Une analyse du déséquilibre de liaison a été effectuée pour 6 locus enzymatiques dans 17 populations naturelles de* Drosophila melanogaster. *Les estimations de ce déséquilibre ont été faites, à partir des fréquences zygotiques, en utilisant les méthodes de Burrows et de Hill. Aucune différence n'a été observée entre ces deux méthodes. La quantité de déséquilibre décelée est faible et comparable à celle trouvée dans d'autres populations naturelles de* D. melanogaster. *Sur les 15 combinaisons examinées, seules les associations* Adh-α-Gpdh *d'une part,* Est-C-Est-6 *d'autre part, montrent un déséquilibre significatif dans les populations étudiées. Le déséquilibre* Adh-α-Gpdh *est probablement dû à la liaison entre les gènes correspondants et l'inversion* (2 L) t *du second chromosome. Au contraire, le déséquilibre* Est-C-Est-6 *pourrait être la conséquence d'interactions entre les 2 gènes eux-mêmes. Ces résultats soulignent à nouveau les difficultés rencontrées dans la mise en évidence d'un déséquilibre de liaison véritablement dû à une épistasie entre locus enzymétiques.*

Drosophila melanogaster – *déséquilibre de liaison – locus enzymatiques – populations natu-*

Introduction

Population studies of genetic variation are classically discussed in terms of single-locus variability measures, such as heterozygosities and changes in gene frequencies. However, there is much interest in knowing the genetic structure of populations at the multilocus level. The application of electrophoretic techniques to analyze genetic variation (Harris, 1966; Hubby and Lewontin, 1966) provides much information at the multilocus level, because a large number of genetic markers can be studied simultaneously in a single individual. Therefore, investigations made on allozyme polymorphism involve the estimation of linkage disequilibrium in natural and experimental populations of a variety of organisms (*see* Hedrick *et al.*, 1978, for a review).

Various authors (*e.g.*, Lewontin, 1974) have suggested that information about linkage disequilibrium among allozymes might be useful to explain the adaptive value of biochemical polymorphism. But unfortunately, the results obtained by the authors studyng linkage disequilibrium at electrophoretically variable loci in natural populations of *Drosophila melanogaster* (Mukai and Voelker, 1977; Voelker *et al.*, 1977; Langley *et al.*, 1978; Inoue *et al.*, 1984; Yamazaki *et al.*, 1984) are reconcilable with several models of population genetics. Consequently, even in the absence of inversion, it is difficult to determine whether these results are due to epistatic natural selection or to random genetic drift. However, we think that it is important to determine the nature and magnitude of linkage disequilibrium in natural populations, because the investigations may perhaps help in the study of interactions between genes and in developing new hypotheses about the mechanisms involved in the maintenance of allozyme polymorphism.

In this paper we report a study of linkage disequilibrium among 6 polymorphic allozyme loci in 17 natural populations of *D. melanogaster* collected from different regions of France.

Materials and Methods

Collections

Wild *Drosophila melanogaster* adults were collected and brought to the laboratory for electrophoresis. All collections were made during the annual demographic burst of the species (between August and October).

Populations studied

The populations studied are distributed from the North to the South of France (Fig. 1); their origins are listed below : (1) Venteuil near Epernay; (2) Verneuil near Epernay; (3) Vincennes near Paris; (4) Sèvres near Paris; (5) Ivry-sur-Seine near Paris; (6) Sainte-Geneviève-des-Bois near Paris; (7) Rannée near Rennes; (8) Nevez near Quimper; (9) Chateaubriant; (10) Ménétréol-sous-Sancerre near Sancerre; (11) Bonnac-la-Côte near Limoges; (12) Chessy-les-Mines near Villefranche-sur-Saône; (13) Beynost near Montluel; (14) Le Curtelod near Yenne; (15) Montauban; (16) Tautavel near Perpignan; (17) Port-Vendres. Only populations (1) and (2) were captured in wine-cellars; the others originated from fruits of the localities studied. Two collections were made for populations (6) and (9), the first in 1983 and the second in 1984. Populations (1)–(5) and (17) were collected in 1984 and the others in 1983.

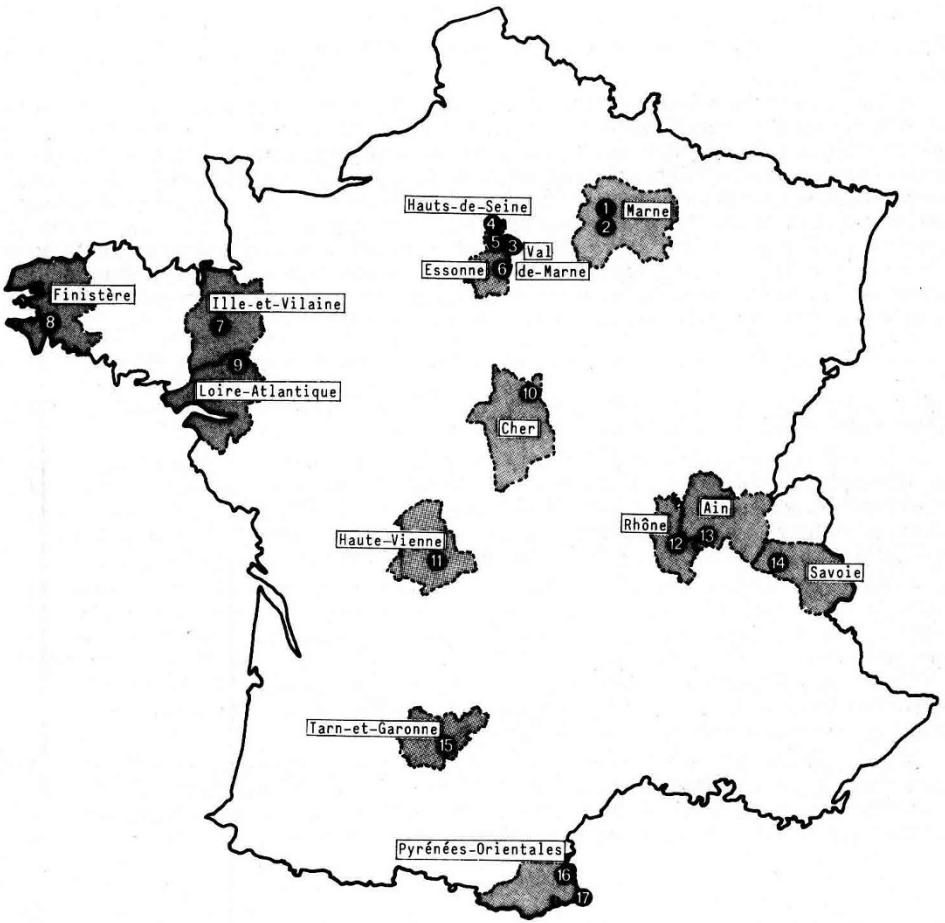

Fig. 1. Geographical location of the 17 French natural populations of *Drosophila melanogaster* studied.

Electrophoresis

Electrophoresis was performed in horizontal starch gel with Poulik's discontinuous buffer system. Six polymorphic enzyme loci were studied, according to the techniques described by Charles-Palabost (1986) : acid phosphatase (*Acph;* 3:101.4), alcohol dehydrogenase (*Adh;* 2:50.1), esterase-C (*Est-C;* 3:47.6), esterase-6 (*Est-6;* 3:36.8), α-glycerophosphate dehydrogenase (*α-Gpdh;* 2:20.5), and phosphoglucomutase (*Pgm;* 3:43.4).

Estimation of linkage disequilibrium

In this study almost all the data were analyzed by a 2-allele system. If more than 2 alleles exist at a locus, they have been grouped in 2 classes: the most frequent allele corresponding to the first class, and the others to the second.

Let us consider loci *A* and *B*, each having, respectively, 2 alleles. *A-a* (frequency of *A* : *p*) and *B - b* (frequency of *B: q*), 4 gametes are possible : *AB, Ab, aB,* and *ab.* If the gametic frequencies are,

respectively, f_{11}, f_{12}, f_{21}, and f_{22}, the linkage disequilibrium D is given by : $D = f_{11}.f_{22} - f_{12}.f_{21} = f_{11} - pq$.

In order to make the values of the parameter *D* less sensitive to change in gene frequency, several other measures of gametic disequilibrium are useful in various contexts. The correlation coefficient $R = D/\sqrt{pq\,(1-p)\,(1-q)}$ was used by Hill and Robertson (1968) and by Franklin and Lewontin (1970). However, in a sample of individuals taken from a population, the degree of linkage disequilibrium cannot be estimated directly from the genotypic frequencies when the coupling and repulsion heterozygotes cannot be distinguished. In this case, estimation of linkage disequilibrium can be done in several ways. Hill (1974) provides a maximum-likelihood method where the population is assumed to be random mating and in Hardy–Weinberg equilibrium at each locus. In the case of 2 codominant alleles per locus, the frequency of one gamete (for example *AB)* estimated by the maximum-likelihood method (f_{11}) is given by a cubic equation :

$$f_{11} = \{ 2N_{11} + N_{12} + N_{21} + N_{22}\, f_{11}\,(1-p-q+f_{11})/\,[f_{11}\,(1-p-q+f_{11}) + (p-f_{11})\,(q-f_{11})]\}/2N, \qquad (1)$$

with N_{11}, N_{12}, N_{21}, N_{22}, and N corresponding, respectively, to the observed numbers of *AABB, AABb, AaBB, AaBb,* and total individuals in the sample.

In Eq. (1) the only unknown is f_{11}. Hill suggests that an initial value : $f_{11} = (4N_{11} + 2N_{12} + 2N_{21} + N_{zz})/2N$-*pq* can be substituted into the right-hand side of (1) and the resulting expression regarded as an improved estimate and itself substituted into the right-hand side of (1). The iterative process is continued until stability is reached and *D* obtained as : $D = f_{11} - pq$. A test for $D = O$ is given by : $K = N\,D^2/pq\,(1-p)\,(1-q)$, with *K* following the chi-square distribution with one degree of freedom.

A second approach, suggested by Burrows (*see* Cockerham and Weir, 1977 and Langley *et al.*, 1978), is simply used to estimate the overall covariance of non-allelic genes in individuals. This method does not require that one distinguish between the 2 types of double heterozygotes and know the mating system. Burrows's parameter is estimated by : $\Delta = 1/2\,(4N_{11}/N + 2N_{12}/N + 2N_{21}/N + 2N_{22}/N) - 2pq$. A test for $\Delta = 0$ is given by : $\chi^2 = N\Delta^2/pq\,(1-p)\,(1-q)$, where χ^2 is approximately a χ^2 distribution with one degree of freedom (Cockerham and Weir, 1977). The correlation coefficient based on Burrows's estimation is : $R = \Delta/2\sqrt{pq\,(1-p)\,(1-q)}$.

In any population, all the loci are not necessarily in Hardy–Weinberg equilibrium. Therefore, we used not only Hill's method, which assumes that the loci are in accordance with the Hardy–Weinberg law, but also Burrows's estimation. Moreover, it was interesting to compare the results obtained by both methods because this was done only in few cases.

Results

Table I gives, for each population, the number of flies analyzed per locus and the frequencies of the most common allele at each locus. With regard to the distribution of allelic frequencies, the populations collected in 1983 were analyzed in another paper (Charles-Palabost *et al.*, 1985), and those of 1984 will be analyzed later. Concerning the goodness of fit to Hardy–Weinberg equilibrium, the use of the χ^2 test is not appropriate in some cases, since the expected numbers of genotypes are too small. Therefore, each α value given in Table I is the probability that the genotypic frequencies distribution of a random sample are farther from the expected Hardy–Weinberg model than the corresponding observed distribution. These values were obtained by means of Monte-Carlo simulations, using the observed allelic frequencies as the real frequencies and under the null hypothesis in which the populations are in Hardy–Weinberg equilibrium. This test is consequently frequency independent. We observe that 21 α values out of 101 are significant and among these 21 significant values, 10 are due to the presence of a rare genotype in the samples. It means that generally, the observed frequencies of heterozygotes

Table I. Frequencies of the most common allele at 6 polymorphic loci in 17 French natural populations of *Drosophila melanogaster.*

Populations	$Acph^F$	Adh^F	$Est\text{-}C^F$	$Est\text{-}6^S$	$\alpha\text{-}Gpdh^F$	Pgm^F
Venteuil	152 0.987 0.38	150 0.953 0.54	147 0.962 0.48	146 0.711 0.65	150 0.586 0.25	126 0.898 0.32
Verneuil	117 1.000 —	115 0.956 0.48	114 0.956 0.51	111 0.629 0.04 *	115 0.639 0.78	116 0.895 0.58
Vincennes	95 1.000 —	95 0.879 0.71	95 0.889 0.19	93 0.666 0.49	94 0.633 0.16	96 0.906 0.01 *
Sèvres	166 0.994 0.001 **	167 0.904 0.16	152 0.964 0.52	166 0.608 0.54	167 0.569 0.00 ***	167 0.871 0.01 *
Ivry-sur-Seine	100 1.000 —	99 0.803 0.60	100 0.910 0.26	97 0.660 0.23	99 0.571 0.17	96 0.984 0.33
Sainte-Geneviève-des-Bois (A)	82 1.000 —	76 0.960 0.42	81 0.957 0.03 *	77 0.779 0.70	82 0.622 0.90	82 0.951 0.05 *
Sainte-Geneviève-des-Bois (B)	175 0.997 0.25	168 0.923 0.27	175 0.897 0.48	175 0.754 0.07	166 0.470 0.92	175 0.928 0.89
Rannée	108 0.958 0.47	108 0.986 0.34	108 0.995 0.26	108 0.963 0.45	107 0.430 0.06	108 0.838 0.91
Nevez	108 1.000 —	108 0.995 0.26	108 0.995 0.26	108 0.949 0.01 *	108 0.380 0.55	107 0.953 0.003 **
Chateaubriand (A)	106 1.000 —	106 0.991 0.00 ***	105 0.952 0.48	106 0.566 0.42	105 0.419 0.17	106 0.882 0.15
Chateaubriand (B)	108 1.000 —	108 0.958 0.47	— — —	58 0.578 0.83	101 0.530 0.88	107 0.939 0.48
Ménétréol-sous-Sancerre	108 1.000 —	108 0.949 0.50	108 0.958 0.002 **	108 0.616 0.46	100 0.530 0.12	108 0.856 0.54

Line 1 : number of individuals analyzed; *Line 2 :* frequencies of the most common allele; *Line 3 :* α values for goodness of fit to Hardy–Weinberg equilibrium; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; populations classified according to latitude from north to south; A : collections of 1983; B : collections of 1984.

Table I *(continued).* Frequencies of the most common allele at 6 polymorphic loci in 17 French natural populations of *Drosophila melanogaster.*

Populations	*Acph*^{F}	*Adh*^{F}	*Est-C*^{F}	*Est-6*^{S}	α-*Gpdh*^{F}	*Pgm*^{F}
Bonnac-la-Côte	108 0.995 0.26	105 0.938 0.002 **	108 0.926 0.37	105 0.657 0.006 **	108 0.648 0.74	108 0.894 0.18
Chessy-les-Mines	104 0.981 0.41	108 0.940 0.02 *	112 0.897 0.00 ***	113 0.619 0.00 ***	112 0.540 0.00 ***	113 0.907 0.94
Beynost	103 1.000 —	103 0.888 0.75	103 0.913 0.05	103 0.597 0.50	103 0.621 0.37	102 0.848 0.62
Le Curtelod	111 1.000 —	110 0.941 0.21	111 0.986 0.001 **	111 0.734 0.37	110 0.504 0.42	83 0.958 0.47
Montauban	107 0.991 0.32	108 0.954 0.51	108 0.935 0.33	108 0.695 0.23	108 0.569 0.70	108 0.949 0.50
Tautavel	108 1.000 —	107 0.977 0.38	108 0.931 0.39	108 0.829 0.01 *	108 0.574 0.34	108 0.940 0.49
Port-Vendres	96 1.000 —	91 0.994 0.26	96 0.995 0.26	94 0.803 0.06	94 0.553 0.03 *	96 0.839 0.01 *

Line 1 : number of individuals analyzed; *Line 2 :* frequencies of the most common allele; *Line 3 :* α values for goodness of fit to Hardy–Weinberg equilibrium; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; populations classified according to latitude from north to south; A : collections of 1983; B : collections of 1984.

per locus in each population are in good agreement with those expected under the Hardy–Weinberg law. A significant excess of heterozygotes was found only at the α-*Gpdh* locus of the Sèvres population.

Table II shows the frequencies of the observed heterozygotes for each locus and population. Classically, the amount of variation differs greatly from one locus to another. The average heterozygosity over the 6 loci analyzed ranges from 0.092 in the Nevez population, to 0.250 in the Ivry-sur-Seine and Sèvres populations. Except for Nevez, the mean heterozygosities obtained are similar to those estimated previously in other French natural populations of *D. melanogaster* (Girard and Palabost, 1976).

The values of linkage disequilibrium estimated by Burrows' (Δ and R_b) and Hill's methods (D and R_h) are given in Table III for the unlinked loci (located on different chromosomes) and in Table IV for those linked (located on the same chromosome). The use of the χ^2 distribution in order to determine the significance level of a linkage disequilibrium implies that in a sample of 100 individuals, the frequencies of the most common

Table II. Frequencies of heterozygotes at each locus and mean heterozygosities (H) over the 6 loci surveyed in the 17 French natural populations studied.

Populations	*Acph*	*Adh*	*Est-C*	*Est-6*	α-*Gpdh*	*Pgm*	*H*
Venteuil	0.026	0.093	0.075	0.400	0.440	0.167	0.200
Verneuil	—	0.087	0.088	0.370	0.443	0.172	0.193
Vincennes	—	0.223	0.221	0.383	0.394	0.125	0.224
Sèvres	0.012	0.156	0.072	0.428	0.654	0.180	0.250
Ivry-sur-Seine	—	0.330	0.178	0.394	0.560	0.041	0.250
Sainte-Geneviève-des-Bois (A)	—	0.075	0.062	0.351	0.469	0.062	0.170
Sainte-Geneviève-des-Bois (B)	—	0.130	0.194	0.400	0.494	0.131	0.225
Rannée	0.083	0.028	0.009	0.074	0.579	0.269	0.174
Nevez	—	0.009	0.009	0.037	0.444	0.056	0.092
Chateaubriand (A)	—	0.000	0.095	0.434	0.552	0.236	0.219
Chateaubriand (B)	—	0.076	—	0.500	0.505	0.121	0.240
Ménétréol-sous-Sancerre	—	0.102	0.046	0.420	0.420	0.231	0.203
Bonnac-la-Côte	0.009	0.066	0.148	0.314	0.472	0.213	0.202
Chessy-les-Mines	0.035	0.083	0.116	0.336	0.348	0.168	0.181
Beynost	—	0.204	0.118	0.427	0.427	0.245	0.237
Le Curtelod	—	0.100	0.009	0.396	0.536	0.063	0.184
Montauban	0.009	0.055	0.110	0.367	0.504	0.101	0.191
Tautavel	—	0.047	0.139	0.204	0.444	0.111	0.157
Port-Vendres	—	0.011	0.010	0.212	0.383	0.198	0.136

A : Collections of 1983; B : Collections of 1984.

alleles at each of the 2 loci must be smaller than 0.85 (Montchamp-Moreau, 1985). Thus, the significance levels in Tables III and IV correspond to the probability that the linkage disequilibrium estimated from a random sample is greater than the linkage disequilibrium estimated from the sample analyzed. These probabilities were obtained using Monte-Carlo simulations, under the null hypothesis of a disequilibrium equal to 0. This test is independent of the distribution, but assumes that the observed allelic frequencies are the real frequencies in the populations. We can note that the values of D and Δ are very similar for unlinked as for linked loci. By contrast, the correlation coefficients R_h (Hill's estima-

tion) and R_b (Burrows's estimation) are different and, in most cases, R_b is smaller in absolute values than R_h (161 cases out 216 values). When $R_b = R_h$ (in 55 cases), no double heterozygotes are present in the samples and $\Delta = 2D$; this result is particularly evident for unlinked loci. With Hill's method, 23 out of the 216 comparisons made between pairs of loci are significant, which represents a percentage of 10.6. The percentages obtained, respectively, for the unlinked and linked loci are 10.5 (13/124) and 10.9 (10/92). With Burrows's method, these values are 15.3% (33/216) for all the loci, 11.3% (14/124) and 20.6% (19/92), respectively, for unlinked and linked loci.

In the present study, out of the 15 combinations between allozyme loci, only the pair *Est-C-Est-6* shows a significant linkage disequilibrium in most of the populations : 4 *D* values out of 18 populations sampled (22%) and 8 Δ values (44%) are significant (Table IV). Using combined data of all the populations, a significant deviation was obtained only in 2 cases : for the *Est-C-Est-6* pair and also for *Adh-α-Gpdh.* With Hill's estimation, the values are, respectively, for *Adh-α-Gpdh* and *Est-C-Est-6* pairs : $D = 0.0116$ ($P < 0.01$), $R_h = -0.0991$, and $D = -0.0097$ ($P < 0.01$), $R_h = -0.0943$. The corresponding values with Burrows's estimation are : $\Delta = -0.0129$ ($P < 0.01$), $R_b = -0.0548$, and $\Delta = -0.0132$ ($P < 0.01$), $R_b = -0.0643$.

Discussion

The results of the present study are not essentially different from those obtained by other investigators in natural populations of *D. melanogaster.* The amount of linkage disequilibrium detected in the French populations surveyed is small, but nevertheless higher than the amount reported in other natural populations of *D. melanogaster,* which reveal a significant linkage disequilibrium of around 5–9% of the analyzed pairs of loci (see, for example, Mukai *et al.,* 1971, 1974; Mukai and Voelker, 1977; Yamaguchi *et al.,* 1980; Yamazaki *et al.,* 1984). But in the studies previously mentioned, the method used to detect linkage disequilibrium is the extraction of whole chromosomes by the marked inversion technique. Therefore, our results are more strictly comparable to the data reported by Langley *et al.,* 1978), because they calculate Burrows's estimation R_b using genotypic data obtained in natural populations of *D. melanogaster.* However, they also report a small proportion of significant linkage disequilibrium (5.1% for linked loci and 6.7% for those unlinked).

Among the 15 combinations between the 6 enzymatic loci studied, the data provide clear evidence of a significant linkage disequilibrium for only 2 pairs of linked loci : *Adh-α-Gpdh* and *Est-C-Est-6.* The same result was obtained by Triantaphyllidis *et al.* (1981) for the *Adh-α-Gpdh* pair in Greek populations. This may suggest consistent epistatic interactions between these pairs of genes (Lewontin, 1974). But another explanation is possible in the case of *Adh-α-Gpdh;* the linkage disequilibrium detected in our populations might be due to an association between these 2 loci and the inversion *(2L)t* in the same chromosome arm. In effect, the inversion *(2L)t* is located on the left arm of chromosome 2 and contains the *α-Gpdh* locus, while the *Adh* locus is outside and very near to the breakpoint of this inversion (Lindsley and Grell, 1968). Unfortunately, the frequencies of inversions were not analyzed in our populations. However, data of natural populations collected in the Northern hemisphere show a significant negative gametic disequilibrium

Table III. Values of linkage disequilibrium estimated by Hill's and Burrows' methods for unlinked loci.

Populations	*Adh-Acph*		*Adh-Est-C*		*Adh-Est-6*	
Venteuil	− 0.001	− 0.001	0.003	0.003	0.001	0.001
	− 0.026	− 0.026	0.086	0.039	0.016	0.008
Verneuil	———		0.001	0.001	− 0.004	− 0.003
	———		0.023	0.011	− 0.039	− 0.014
Vincennes	———		− 0.014	− 0.011	0.000	0.000
	———		− 0.132	− 0.055	0.001	0.001
Sèvres	0.002	0.002	− 0.004	− 0.004	− 0.002	− 0.003
	0.091	0.040	− 0.066	− 0.038	− 0.020	− 0.012
Ivry-sur-Seine	———		− 0.017	− 0.015	0.014	0.011
	———		− 0.155	− 0.066	0.076	0.031
Sainte-Geneviève-des-Bois (A)	———		0.003	0.003	0.007	0.008
	———		0.083	0.038	0.099	0.054
Sainte-Geneviève-des-Bois (B)	———		− 0.004	− 0.007	0.000	0.000
	———		− 0.052	− 0.045	0.001	0.000
Rannée	− 0.001	− 0.001	− 0.001	0.000	0.001	0.001
	− 0.024	− 0.024	− 0.008	− 0.008	0.023	0.023
Nevez	———		0.000	0.000	0.000	0.000
	———		− 0.005	− 0.005	0.010	0.010
Chateaubriant (A)	———		0.000	− 0.001	0.004	0.008
	———		− 0.022	− 0.022	0.087	0.087
Chateaubriant (B)	———		———		0.011	0.017 *
	———		———		0.139	0.101
Ménétréol-sous-Sancerre	———		0.000	0.000	− 0.013	− 0.008
	———		− 0.016	− 0.016	− 0.126	− 0.040
Bonnac-la-Côte	0.000	0.000	0.007	0.001	0.021	0.022 *
	− 0.017	− 0.017	0.013	0.005	0.183	0.095
Chessy-les-Mines	− 0.001	− 0.002	0.001	0.001	− 0.002	− 0.003
	− 0.030	− 0.030	0.013	0.010	− 0.017	− 0.014
Beynost	———		− 0.009	− 0.013	− 0.022	− 0.022
	———		− 0.104	− 0.075	− 0.154	− 0.078
Le Curtelod	———		0.000	0.000	0.014	0.019
	———		− 0.015	− 0.015	0.140	0.094
Montauban	0.000	0.000	0.006	0.009 *	0.005	0.008
	− 0.013	− 0.013	0.131	0.098	0.058	0.048
Tautavel	———		0.001	0.001	0.004	0.008
	———		0.040	0.018	0.069	0.069
Port-Vendres	———		0.000	0.000	0.001	0.002
	———		− 0.005	− 0.005	0.035	0.035

Line 1 : values of *D* (Hill's method) and then Δ (Burrows' method); *Line 2 :* values of R_H and then R_a; A : collection of 1983; B : collections of 1984; * $P < 0.05$; ** $P < 0.01$.

Adh-Pgm		α-*Gpdh-Acph*		α-*Gpdh-Est-C*		α-*Gpdh-Est-6*		α-*Gpdh-Pgm*	
0.000	0.000	0.002	0.002	0.007	0.008	− 0.013	− 0.014	0.002	0.011
0.001	0.000	0.039	0.020	0.075	0.041	− 0.057	− 0.031	0.015	0.038
0.016**	0.014*	———		0.006	0.008	− 0.004	− 0.005	− 0.014	− 0.012
0.265	0.113	———		0.060	0.041	− 0.020	− 0.012	− 0.094	− 0.039
− 0.004	− 0.007	———		− 0.032*	− 0.047**	0.031	0.041	0.003	0.004
− 0.045	− 0.037	———		− 0.216	− 0.159	0.137	0.091	0.024	0.015
− 0.004	− 0.003	0.003	0.001	0.007	0.006	0.001	0.001	− 0.024*	− 0.015
− 0.045	− 0.017	0.088	0.010	0.084	0.031	0.004	0.001	− 0.164	− 0.050
0.001	0.002	———		− 0.039**	− 0.032*	0.028	0.032	0.003	0.003
0.019	0.019	———		− 0.273	− 0.114	0.121	0.067	0.040	0.020
0.003	0.003	———		0.003	0.004	0.012	0.009	− 0.017	− 0.021
0.083	0.038	———		0.029	0.020	0.060	0.022	− 0.017	− 0.105
0.005	0.004	———		0.015	0.014	− 0.049**	− 0.035*	− 0.023*	− 0.019*
0.074	0.031	———		0.097	0.045	− 0.238	− 0.086	− 0.188	− 0.077
0.000	0.000	0.004	0.003	0.002	0.004	− 0.004	− 0.004	0.000	0.000
0.004	0.001	0.044	0.017	0.060	0.060	− 0.048	− 0.022	0.003	0.001
0.000	0.000	———		− 0.003	− 0.001	0.012	0.005	− 0.009	− 0.016
− 0.015	− 0.015	———		− 0.087	− 0.017	0.188	0.041	− 0.087	− 0.078
− 0.001	− 0.002	———		0.020	0.012	− 0.036	− 0.034	0.000	0.000
− 0.036	− 0.036	———		0.192	0.056	− 0.148	− 0.070	− 0.001	− 0.001
− 0.003	− 0.005	———		———		0.006	0.006	− 0.020	− 0.022
− 0.053	− 0.053	———		———		0.026	0.012	− 0.167	− 0.093
0.016*	0.013	———		− 0.009	− 0.013	− 0.015	− 0.018	− 0.037*	− 0.056**
0.215	0.085	———		− 0.101	− 0.070	− 0.061	− 0.038	− 0.210	− 0.160
0.012	0.010	0.003	0.001	− 0.026*	− 0.019	0.029	0.035	0.015	0.014
0.165	0.068	0.094	0.023	− 0.206	− 0.076	0.133	0.079	0.103	0.048
− 0.001	− 0.002	− 0.002	− 0.003	− 0.030*	− 0.046**	0.003	0.005	0.038**	0.040**
− 0.018	− 0.018	− 0.029	− 0.029	− 0.199	− 0.150	0.013	0.010	0.258	0.137
0.007	0.009	———		0.023	0.029*	− 0.004	− 0.004	0.046**	0.038*
0.006	0.039	———		0.177	0.111	− 0.017	− 0.009	0.263	0.107
− 0.002	− 0.004	———		0.007	0.009	0.033	0.030	− 0.005	− 0.004
− 0.045	− 0.045	———		0.119	0.079	0.155	0.069	− 0.059	− 0.024
− 0.002	− 0.004	0.003	0.001	− 0.013	− 0.009	0.000	0.000	0.010	0.007
− 0.045	− 0.045	0.079	0.010	− 0.106	− 0.037	0.000	0.000	0.095	0.034
0.007	0.007	———		0.001	0.001	− 0.023	− 0.026	− 0.024*	− 0.020
0.210	0.096	———		0.012	0.004	− 0.128	− 0.071	− 0.210	− 0.086
0.004	0.009*	———		0.003	0.006	− 0.026	− 0.004	− 0.025	− 0.036
0.167	0.167	———		0.081	0.081	− 0.141	− 0.106	− 0.133	− 0.096

Table IV. Values of linkage disequilibrium estimated by Hill's and Burrows' methods for linked loci.

Populations	*Adh-Est-C*	*Adh-Est-6*	*Acph-Pgm*
Venteuil	0.000 − 0.001 − 0.023 − 0.023	− 0.004 − 0.002 − 0.080 − 0.023	− 0.002 − 0.003 − 0.043 − 0.043
Verneuil	—— ——	—— ——	—— ——
Vincennes	—— ——	—— ——	—— ——
Sèvres	0.000 0.000 − 0.016 − 0.016	− 0.004 − 0.002 − 0.103 − 0.021	− 0.001 − 0.001 − 0.026 − 0.026
Ivry-sur-Seine	—— ——	—— ——	—— ——
Sainte-Geneviève-des-Bois (A)	—— ——	—— ——	—— ——
Sainte-Geneviève-des-Bois (B)	—— ——	—— ——	—— ——
Rannée	0.000 0.000 − 0.014 − 0.014	− 0.002 − 0.002 − 0.044 − 0.020	− 0.007 − 0.014 * − 0.092 − 0.092
Nevez	—— ——	—— ——	—— ——
Chateaubriant (A)	—— ——	—— ——	—— ——
Chateaubriant (B)	—— ——	—— ——	—— ——
Ménétréol-sous-Sancerre	—— ——	—— ——	—— ——
Bonnac-la-Côte	− 0.001 − 0.001 − 0.019 − 0.019	0.002 0.003 0.047 0.047	− 0.001 − 0.001 − 0.023 − 0.023
Chessy-les-Mines	− 0.002 − 0.004 − 0.046 − 0.046	0.006 0.013 * 0.102 0.102	− 0.002 − 0.003 − 0.043 − 0.043
Beynost	—— ——	—— ——	—— ——
Le Curtelod	—— ——	—— ——	—— ——
Montauban	0.000 0.000 − 0.018 − 0.018	− 0.003 − 0.007 * − 0.105 − 0.105	0.000 0.000 − 0.016 − 0.016
Tautavel	—— ——	—— ——	—— ——
Port-Vendres	—— ——	—— ——	—— ——

Line 1 : values of *D* (Hill's method) and then Δ (Burrow's method); *Line 2 :* values of R_H and then R_a; A : collection of 1983; B : collections of 1984; * $P < 0.05$; ** $P < 0.01$.

Est-C-Est-6		*Est-C-Pgm*		*Est-6-Pgm*		*Adh-α-Gpdh*	
0.001	0.001	0.006	0.005	− 0.022	− 0.030 *	− 0.008	− 0.009
0.016	0.008	0.107	0.046	− 0.162	− 0.113	− 0.074	− 0.044
− 0.018	− 0.026 **	− 0.005	− 0.005	− 0.007	− 0.011	− 0.001	− 0.001
− 0.176	− 0.128	− 0.075	− 0.040	− 0.050	− 0.037	− 0.009	− 0.004
− 0.047 **	− 0.049 **	− 0.010	− 0.020 *	− 0.021	− 0.030 *	− 0.043 **	− 0.054 **
− 0.339	− 0.143	− 0.110	− 0.111	− 0.153	− 0.107	− 0.278	− 0.174
− 0.024 **	− 0.019 *	− 0.004	− 0.004	− 0.013	− 0.013	− 0.029 *	− 0.012
− 0.254	− 0.103	− 0.068	− 0.039	− 0.088	− 0.046	− 0.199	− 0.041
− 0.025	− 0.039 **	− 0.002	− 0.004	− 0.006	− 0.006	− 0.020 *	− 0.017 *
− 0.185	− 0.174	− 0.045	− 0.045	− 0.090	− 0.048	− 0.104	− 0.044
− 0.005	− 0.006	− 0.002	− 0.004	− 0.007	− 0.002	− 0.006	− 0.009
− 0.058	− 0.036	− 0.045	− 0.045	− 0.090	− 0.016	− 0.066	− 0.053
− 0.004	− 0.004	0.004	0.003	− 0.008	− 0.008	− 0.018	− 0.020
− 0.034	− 0.017	0.046	0.019	− 0.079	− 0.039	− 0.136	− 0.076
0.000	0.000	− 0.001	− 0.002	0.006	0.007	− 0.008	− 0.007
0.013	0.013	− 0.029	− 0.029	0.086	0.053	− 0.137	− 0.057
− 0.005 **	− 0.005 **	0.000	0.000	0.001	0.001	− 0.003	− 0.006
− 0.496	− 0.243	− 0.015	− 0.015	0.027	0.027	− 0.087	− 0.087
− 0.022 *	− 0.032 **	0.004	0.003	− 0.042 **	− 0.045 **	− 0.004	− 0.008
− 0.207	− 0.149	0.052	0.021	− 0.271	− 0.143	− 0.083	− 0.083
———		———		0.009	0.023	− 0.004	− 0.002
———		———		0.230	0.091	− 0.038	− 0.011
0.002	0.002	0.003	0.006	− 0.006	− 0.006	− 0.006	− 0.007
0.018	0.014	0.047	0.047	− 0.036	− 0.016	− 0.057	− 0.029
− 0.008	− 0.011	− 0.008	− 0.006	0.006	0.006	− 0.022 *	− 0.029 *
− 0.061	− 0.043	− 0.098	− 0.040	0.044	0.020	− 0.187	− 0.125
− 0.006	− 0.011	− 0.008	− 0.006	0.018	0.021	− 0.001	− 0.001
− 0.045	− 0.041	− 0.090	− 0.034	0.129	0.074	− 0.005	− 0.005
− 0.002	− 0.003	− 0.007	− 0.005	− 0.008	− 0.007	− 0.030 *	− 0.026
− 0.018	− 0.013	− 0.073	− 0.026	− 0.042	− 0.019	− 0.129	− 0.086
− 0.005	− 0.007	− 0.001	− 0.001	− 0.006	− 0.006	− 0.022	− 0.022
− 0.095	− 0.066	− 0.021	− 0.021	− 0.080	− 0.043	− 0.181	− 0.094
− 0.014	− 0.025 *	0.003	0.003	0.015	0.026 **	0.001	0.001
− 0.124	− 0.111	0.057	0.025	0.152	0.129	0.007	0.004
− 0.007	− 0.010	0.007	0.006	0.009	0.009	− 0.010	− 0.010
− 0.080	− 0.055	0.120	0.052	0.108	0.053	− 0.133	− 0.071
− 0.005	− 0.010 **	0.004	0.004	− 0.009	− 0.011	0.003	0.001
− 0.173	− 0.173	0.165	0.067	− 0.065	− 0.042	0.082	0.007

between these 2 loci only when all the chromosomes (chromosomes with standard sequence and chromosomes with inversion *(2L)t*) are considered. This disequilibrium remains negative but not significantly different from 0 when the *In (2L)t* chromosomes are removed from the analysis (Mukai *et al.,* 1971; Langley *et al.,* 1974, 1978; Alahiotis *et al.,* 1976; Voelker *et al.,* 1977; Yamaguchi *et al.,* 1980; Yamazaki *et al.,* 1984). Consequently, in our opinion, the linkage disequilibrium currently observed between *Adh* and *α-Gpdh* is probably due to the association of these loci with *In (2L)t,* despite the well known interactions between these 2 enzymes (Geer *et al.,* 1983, 1985).

The result obtained for the *Est-C-Est-6* pair appears more interesting since, in this case, *Est-C* and *Est-6* are located in different arms of chromosome 3. Few cases of significant linkage disequilibrium between esterase loci have been previously reported in natural populations of *D. melanogaster* (see for example Johnson and Schaffer, 1973; Langley *et al.,* 1978; Laurie-Ahlberg and Weir, 1979), but such a result is known in other organisms such as salamander (*Plethodon cinereus;* Webster, 1974); and barley (*Hordeum spontaneum;* Kahler and Allard, 1970). In *D. melanogaster,* this linkage disequilibrium could be explained by interactions between the 2 loci themselves or by interactions between these loci and inversions located on the same or different arms of the chromosomes 3. This last hypothesis was tested by several authors (Kojima *et al.,* 1970; Mukai *et al.,* 1974; Langley and Ito, 1977; Yamazaki *et al.,* 1984). In most cases, when inversions *(3R)P* and *(3L)P* were analyzed simultaneously with the esterase loci, no evidence of linkage disequilibrium was found between these 2 inversions, or between them and the esterase loci. The physiological function of esterases remains unknown (Dickinson and Sullivan, 1975; Danford and Beardmore, 1980), but the esterase loci may code for a class of closed proteins, probably functionally related. Therefore, the significant gametic disequilibrium observed between *Est-C* and *Est-6* might be examined in terms of interactions between genes metabolically related, as suggested by Zouros and Krimbas (1973) and then by Zouros and Johnson (1976) for 2 other enzymes. However, in our populations, it is not possible to eliminate entirely the influence of inversions in the origin of linkage disequilibrium found between *Est-C* and *Est-6* loci. Thus, for a better and more extensive evaluation of this result, it is necessary to know the population size (since genetic drift could gives rise to an important disequilibrium; Montchamp-Moreau and Katz, 1986) and to verify if this linkage is maintained over time.

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