THE GENETICS OF *DROSOPHILA SUBOBSCURA* POPULATIONS. XIV. FURTHER DATA ON LINKAGE DISEQUILIBRIA

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ABSTRACT

Data coming from one natural population of *D. subobscura*, that of Crete, are presented in detail and examined for nonrandom associations of genes and gene arrangements. This population and four others previously studied are reanalyzed for the detection of higher than first-order interactions. Only first-order interactions are important and statistically significant, especially those concerning genes and inversions in which these genes are included. The paucity of linkage disequilibria detected is remarkable, and we argue that it does not depend on the methods of study, rather it is genuine. We further argue that most of the disequilibria detected are probably due to mechanisms based on epistatic selection.

THE study of nonrandom associations between genes in natural populations is a promising approach to the problem of the maintenance of genetic variability, especially in regard to the selectionist-neutralist controversy. Indeed, the existence of linkage disequilibria can be explained either by epistatic selection or drift and historical events. In a previous paper (LOUKAS, KRIMBAS and VERGINI 1979) we presented data concerning four natural populations of *Drosophila subobscura*. We found a limited number of such disequilibria: only between genes included within inversions, with those inversions and only between the very closely linked genes of the *Est-9* gene complex. Although a neutralist explanation could not be excluded, the evidence was in favor of a selection mechanism.

In this paper, we present data concerning another natural population, one from Crete. Furthermore, we reanalyze the previous data together with the new data for higher-order interactions between genes and between genes and inversions. These second-order and higher interactions have not been investigated in the data already published by us; only pairs of genes, or a pair consisting of one gene and one inversion were considered.

MATERIALS AND METHODS

A natural population in orange orchards near the village of Alikianou of the Canea Department in Crete was sampled in September 1977. (For a description of the site, see KRIMBAS

1954.) The pertinent crosses and the techniques for detecting allozymes, as well as the description of the sites of the Mt. Parnes, Preveza, Barcelona and Sussex populations, are found in LOUKAS, KRIMBAS and VERGINI 1979. The genetics of these loci and their genetic maps can be found in LOUKAS *et al.* 1979.

RESULTS AND ANALYSES

The Crete population: Only polymorphic genes located on chromosomes *O* and *J* were sampled since the situation was clear for chromosome *E* (LOUKAS and KRIMBAS 1975), and genes located in chromosomes *A* and *U* were few and did not display important polymorphism in the other four populations studied. Thus, 13 genes have been sampled, eight located on chromosome *O* (*Est-5*, *Odh*, *AO*, *ME*, *Xdh*, *Lap*, *Pept-1*, *Acph*) and five on chromosome *J* (*Aph*, *Est-3*, *Pgm*, *Est-7* and *Idh*). Allozyme frequencies are indicated in Table 1. Since isogenic strains for chromosome *O* have been derived, using a balanced strain, it was also possible to detect the presence of silent alleles. We found only one such allele, for *Pept-1*. Table 2 contains the frequencies of gene arrangements in chromosomes *O* and *J*. The study was made in such a way as to record the gene arrangement and the allele present at each locus for every chromosome sampled.

TABLE 1

Alleles (in the left columns) and their frequencies (in the right columns) for 13 polymorphic loci located on chromosomes O and J in the Crete natural population

Est-5 (chr. *O*)	
1.25	0.025
1.06	0.025
1.00	0.441
0.90	0.455
0.86	0.054

Odh (chr. *O*)	
1.14	0.040
1.00	0.950
0.86	0.005
0.58	0.005

AO (chr. *O*)	
1.10	0.005
1.08	0.040
1.05	0.163
1.00	0.663
0.95	0.079
0.91	0.030
0.82	0.020

ME (chr. *O*)	
1.10	0.010
1.00	0.955
0.94	0.035

Xdh (chr. *O*)	
1.08	0.005
1.06	0.010
1.04	0.124
1.00	0.738
0.96	0.074
0.92	0.029
0.86	0.020

Lap (chr. *O*)	
1.11	0.015
1.06	0.149
1.00	0.802
0.86	0.020
0.69	0.015

Pept-1 (chr. *O*)	
1.60	0.015
1.00	0.277
0.40	0.703
silent	0.005

Acph (chr. *O*)	
1.88	0.020
1.00	0.926
0.54	0.054

Aph (chr. *J*)	
1.00	0.554
0.73	0.431
0.46	0.015

Est-3 (chr. *J*)	
1.20	0.218
1.00	0.728
0.90	0.054

Pgm (chr. *J*)	
1.32	0.054
1.00	0.931
0.71	0.015

Est-7 (chr. *J*)	
1.04	0.020
1.00	0.980

Idh (chr. *J*)	
1.17	0.005
1.00	0.995

All samples contain 202 genes.

TABLE 2

Frequencies of gene arrangements for chromosomes O *and* J *in the Crete natural population*

O_{ST}	0.005	J_{3+4}	0.460
O_{3+4+8}	0.678	J_{1}	0.554
O_{3+4}	0.218	J_{ST}	0.040
O_{3+4+22}	0.040	$N = 202$	
O_{3+4+1}	0.054		
O_{3+4+2}	0.005		
$N = 202$			

We have performed all 20 possible tests for random associations between pairs consisting of one gene and gene arrangements located on the same chromosome, as well as all 34 possible tests between pairs of genes located on the same chromosome. In these tests, chromosome *O* has been divided in two segments, Segment I and Segment II, since inversions of the two segments do not overlap. The genes *Est-5*, *Odh*, *AO*, *ME* and *Xdh* are located on Segment II, whereas *Pept-1*, *Lap* and *Acph* are located on Segment I. Statistically significant tests only at a probability less than 0.05 are reported in Table 3; that is, four tests between genes and inversions and four tests between pairs of genes. In Table 3, for every test we indicate the alleles considered, as well as the gene arrangements, the chi-square testing homogeneity, the degrees of freedom and the significance. By *r* we symbolize the remaining alleles, grouped in one class, and by *inv* the inversions (all gene arrangements, except the Standard one, grouped in one class). Probabilities, indicated by an asterisk on its left, are Fisher's exact probabilities calculated by a method invented by J. SOURDIS and briefly described in our previous paper (LOUKAS, KRIMBAS and VERGINI 1979). This method was used only when one expected class contained less than five genes or chromosomes.

From the four significant tests between genes and gene arrangements, three were not unexpected, the genes *Pept-1* and *Acph* show nonrandom associations

TABLE 3

Tests for random associations between genes and inversions of the same chromosome and between pairs of genes located on the same chromosome

		χ^2	d.f.	*P*
1. *Xdh* (*1.04, 1.00, r*)	*versus* *O* chr. SI(O_{3+4+8}, *r*)	6.371	2	<0.05
2. *Pept-1* (*0.40, r*)	*versus* *O* chr. SI($O_{3+4+8} + O_{ST}, O_{3+4}$)	13.256	1	<0.001
3. *Pept-1* (*0.40, r*)	*versus* *O* chr. SII(*ST, inv*)	6.611	1	<0.02
4. *Acph* (*1.00*, r)	*versus* *O* chr. SI($O_{3+4+8} + O_{ST}, O_{3+4}$)	11.691	1	<0.001
5. *Est-5* (*0.90, r*)	*versus* *Lap* (*1.00, r*)	4.532	1	<0.05
6. *Odh* (*1.00, r*)	*versus* *AO* (*1.00, r*)			*0.033
7. *AO* (*1.05, 1.00, r*)	*versus* *Xdh* (*1.04, 1.00, r*)	16.018	4	<0.01
8. *Est-3* (*1.00, r*)	*versus* *Aph* (*1.00, r*)	6.350	1	<0.02

Inversions of the *O* chromosome are considered separately according to their position in Segment I (SI) or Segment II (SII) of this chromosome. Only the eight statistically significant tests at the 0.05 level are reported out of 54 tests performed. Futher explanation in the text.

with gene arrangements of Segment I (No. 2 and No. 4) exactly in the same direction as found in the other four populations studied previously. These genes, as well as *Lap*, are included in these inversions. *Lap* does not show a departure from random association because both predominant gene arrangements in Crete, namely O_{3+4+8} and O_{3+4}, harbor mostly the same allele of *Lap* (*1.00*). This is not the case with O_{ST}, but this arrangement is rare in Crete. O_{3+4} and O_{3+4+8} differ from one another by a single inversion (O_8) and thus by a single step in the gene arrangement phylogeny. It is easily conceivable that, whatever is the original gene arrangement (either O_{3+4} or O_{3+4+8}), the derived one (O_{3+4+8} or O_{3+4}) would most probably include the same allele found in the original one. This, however, has not been the case for *Pept-1* and *Acph*. Although this is not an airtight argument for selection, it constitutes evidence in favor of it. For a more detailed discussion of these associations see LOUKAS, KRIMBAS and VERGINI (1979).

The nonrandom association between alleles of *Pept-1* and inversions of Segment II (No. 3) on which it is not located can also be explained from the nonrandom associations between *Pept-1* and inversions of Segment I. It is due to the well-known nonrandom association between inversions of Segment I and those of Segment II; most of these inversions (actually all of them in Crete) are found associated with the $O_{3}{}^{4}$ gene arrangement of Segment I.

The nonrandom association of *Xdh* alleles with inversions of Segment I (No. 1) was not expected, since this gene lies on Segment II. However, the level of significance is not high, and in 20 tests, one is expected to be significant at the 0.05 level. The most probable explanation is sampling error. In general, no associations were detected in Segment II between genes located in that segment and the respective inversions. It is true that in Crete such inversions are not very frequent, about 0.10. Furthermore, in the tests performed, different inversions are grouped into one class. However, inspection of the detailed results did not show any evidence for association, and this is the same pattern reported in our previous paper.

Four of 34 tests were significant when tested for associations of pairs of genes located in the same chromosome. The first deals with *Est-5* and *Lap* (No. 5). Although these genes are far apart in the chromosome and the level of significance is not high, we tend to believe that it is a genuine association because it was previously found (ZOUROS *et al.* 1974). The second is between *Odh* and *AO* (No. 6). Both genes are located in Segment II, near to each other (their effective separation is 1.7 centimorgans). This association was found for the first time; it could be due to a sampling error, but it should be noted that the distance between these two genes is the least among all other pairs of genes studied. It is not due to a mechanical linkage by inversions; if we test the allelic combinations of *AO* and *Odh* (*1.00* and *1.00 versus r* and *r*) with the gene arrangement in Segment II (*ST*, *inv*), we do not find a nonrandom association ($P = {}^{*}0.299$).

The third case is found between alleles of *AO* and *Xdh* (Segment II of chromosome *O*, No. 7). ZOUROS and KRIMBAS (1973) also found significant nonrandom association between *AO* and *Xdh*, but in a different direction, in the same popu-

lation as the one reported here. It is possible that the variation in the electrophoretic techniques does not permit a complete cross-identification of *Xdh* and *AO* alleles between the study of ZOUROS and KRIMBAS and the present one.

Xdh and *AO* are not closely linked, but they seem to be physiologically related, as pointed out by ZOUROS and KRIMBAS (1973). Recently, FINNERTY and JOHNSON (1979) formulated an hypothesis according to which the genetic variability of both *Xdh* and *AO* in *D. melanogaster* could result from recessive modifiers at the *lxd* (and *mal*) loci in natural populations. If this hypothesis is correct for *D. subobscura*, our findings could be explained without the invocation of a selective mechanism. However, the extraction technique and the use of a balanced strain reduces such a possibility since the modifiers should be located on the same chromosome with the markers. Furthermore, the mapping data reported in LOUKAS *et al.* (1979) make probable that at least some variants of *Xdh* and *AO* should be alleles of the structural genes. This question needs a more detailed investigation before a definite judgment can be formulated.

Finally, there is a case of nonrandom association between *Est-3* and *Aph* (No. 8), both located on chromosome *J* far apart in the map. However, the Crete population harbors, at a significant frequency, the gene arrangement J_{3+4}, which is located between these two loci; for J_{3+4}, heterozygotes the genetic distance (remembering zero male recombination) is reduced to five centimorgans.

In general, we can state that there is a paucity of nonrandom associations between genes. When these disequilibria are found, the genetic distances tend to be small or reduced by the presence of inversions in the population studied.

Likelihood analysis of multiple-locus gametic disequilibria in the five natural populations: SMOUSE (1974) presented the methodology for the likelihood analysis of multiple-locus gametic disequilibria. The present multi-locus analysis utilizes the same methods and approach that were utilized by CHARLESWORTH, CHARLESWORTH and LOUKAS (1979). Although our analyses involve contingency tables of nine dimensions, a brief description of log-linear models, which we have drawn from FIENBERG (1977), is given for the simpler situation of a three-dimensional contingency table.

Let each gamete be categorized according to the following three variables: gene arrangement of a chromosome segment (dimension 1, $i = 1, \ldots, I$), allele at enzyme locus *A* (dimension 2, $j = 1, \ldots, J$) and allele at enzyme locus *B* (dimension 3, $k = 1, \ldots, K$). Then, x_{ijk} is the count of gametes in the i^{th} row, j^{th} column and k^{th} layer of the table of observations, and m_{ijk} is the corresponding expected value for that cell for some specified model. We can express the natural logarithms of the expected values, $\log m_{ijk}$, for each model as a linear combination of terms (u-terms) involving deviations from a grand mean. Each model is subject to analysis-of-variance-like constraints in which successively higher-order u terms measure deviations from lower-order terms.

For example, the model of complete independence of the three variables is given by:

$$\log m_{ijk} = u + u_{1(i)} + u_{2(j)} + u_{3(k)}$$

subject to the constraints

$$\sum_{i}^{I} u_{1(i)} = \sum_{j}^{J} u_{2(j)} = \sum_{k}^{K} u_{3(k)} = 0 \ ,$$

where

$$u = \frac{1}{IJK} \sum_{i}^{I} \sum_{j}^{J} \sum_{k}^{K} \log m_{ijk}$$

$$u_{1(i)} = \frac{1}{JK} \sum_{j}^{J} \sum_{k}^{K} \log m_{ijk} - u$$

$$u_{2(j)} = \frac{1}{IK} \sum_{i}^{I} \sum_{k}^{K} \log m_{ijk} - u$$

$$u_{3(k)} = \frac{1}{IJ} \sum_{i}^{I} \sum_{j}^{J} \log m_{ijk} - u \ .$$

Under multinomial sampling of gametes, the maximum likelihood estimates of the expected values for this model are:

$$\hat{m}_{ijk} = \frac{x_{i++}\, x_{+j+}\, x_{++k}}{N^2} \ ,$$

where + in place of an index denotes the sum over the appropriate dimension of the table of gametes.

We are interested only in a set of hierarchical log-linear models in which higher-order terms are included only if the related lower-order terms are included. For the three-dimensional contingency table, there are eight hierarchical models that can be specified. Further, we specify a nested hierarchy of models in which each model contains the previous ones in the hierarchy as special cases. Thus, the following sequence of models for the logarithms of the expected values forms a nested hierarchy:

(a) $u + u_{1(i)} + u_{2(j)} + u_{3(k)}$,

(b) $u + u_{1(i)} + u_{2(j)} + u_{3(k)} + u_{12(ij)} + u_{13(ik)}$,

(c) $u + u_{1(i)} + u_{2(j)} + u_{3(k)} + u_{12(ij)} + u_{13(ik)} + u_{23(jk)}$.

For models (a) and (b), there are closed-form estimates of m_{ijk} utilizing appropriate marginals of x_{ijk} as complete minimal sufficient statistics. However, for model (c) it is necessary to use an alternative, proportional-fitting algorithm to obtain maximum likelihood estimates of the expected gametic counts. In practice, the expected counts for each model were computed by using this algorithm. The goodness-of-fit of each model was assessed by the statistic

$$G^2 = 2 \sum_{i}^{I} \sum_{j}^{J} \sum_{k}^{K} x_{ijk} \ln \left[\frac{x_{ijk}}{\hat{m}_{ijk}} \right] \ ,$$

where ln is the natural logarithm. G^2 is minus twice the logarithm of the likelihood-ratio test statistic. If the model fitted is correct and the total sample is large,

G^2 is approximately distributed as χ^2 with degrees of freedom equal to the number of cells minus the number of parameters fitted (including the single parameter for the grand mean, u). A "large" sample size is considered to be 10 times the number of cells in the table.

We are primarily interested in partitioning the total chi-square value for a nested hierarchy of log-linear models into several components due to differences between models. The differences of goodness-of-fit statistics between adjacent models in the hierarchy are interpreted as tests of effects of nonrandom associations of alleles among loci or alleles with gene arrangements.

In the analysis of the three-dimensional contingency table we proceed as follows: (1) test of total effects: G^2(a), with degrees of freedom equal to d.f.(a); (2) test of gene arrangement × locus effects: G^2(a) − G^2(b), with d.f(a) − d.f.(b); (3) test of two-locus effects, after adjustment for gene arrangement × locus effects: G^2(b) − G^2(c), with d.f.(b) − d.f.(c); 4. test of the residual two-locus × gene arrangement effect, after adjustment for all effects above: G^2(c) with d.f.(c). In practice the test of total effects was obtained as the sum of the tests of the components comprising estimable parameters (see below).

To test randomness of associations between two or more genetic markers (genes, gene arrangements), we have used data dealing only with the *O* chromosome for the five natural populations studied. The table of gametes for each of the five populations was a nine-dimensional set of dichotomies. A dichotomy represents the most common class *versus* all other types combined of a particular variable. The nine variables were allozymes for each of seven enzyme loci and chromosome arrangements for each of two segments of the *O* chromosome. Because of the large number of cells and moderate sample sizes, the tables of counts are sparse. Therefore, certain higher-order interaction terms could not be estimated because of the persistence of zero values in the configurations of marginals. The approach adopted was to exclude from a model any parameter that could not be estimated because of zeros in the configuration of margins corresponding to that particular term. Also, because of the restriction to hierarchical models, those higher-order terms were excluded that had a related lower-order term excluded from a previous model in the nested hierarchy.

It is important to note that it was not possible to obtain a uniform degree of convergence in some samples for successively higher-order models. Therefore, the fit of the models will become poorer for this reason alone. In particular for the Preveza population, it would not be safe to attribute biological significance to the apparent significance of effects of higher-order interactions. The detailed results are too voluminous to be included and are deposited with the Department of Genetics of the Agricultural College of Athens.

The results of partitioning the likelihood-ratio goodness-of-fit statistics for the multi-locus gametic distribution of a sample from each of five populations are presented in Table 4. In this table, tests of the statistical significance of effects are adjusted for those terms included in effects higher in the table and all higher-order effects are assumed to be nil. One should exercise caution in interpreting

TABLE 4

Loglikelihood analyses for O *chromosome data*

Source of variation	Sussex ($N = 200$) d.f.	G^2	Parnes ($N = 190$) d.f.	G^2	Preveza ($N = 202$) d.f.	G^2	Barcelona ($N = 187$) d.f.	G^2	Crete ($N = 202$) d.f.	G^2
Arrangements SI × arrangements SII	—	—	—	—	1	27.99***	1	22.75***	—	—
Locus × arrangements SI	7	58.78***	6	42.67***	7	38.61***	7	55.48***	7	37.15***
Locus × arrangements SII	5	6.76	7	13.09	7	13.32	7	7.74	6	14.29*
Locus × arrangements SI × arrangements SII	—	—	—	—	—	—	3	0.82	—	—
2-locus effects	21	6.59	21	28.56	21	17.66	21	34.81*	20	23.73
2-locus × arrangements SI	21	16.86	12	9.76	21	15.42	17	20.57	14	13.92
2-locus × arrangements SII	—	—	16	16.12	18	17.51	14	6.02	5	3.65
3-locus effects	33	48.62*	19	28.43	35	36.01	29	51.07**	19	11.63
3-locus × arrangements SI	18	14.98	6	4.27	20	28.30	8	14.50	4	2.51
3-locus × arrangements SII	—	—	8	7.50	13	22.45*	6	12.46	—	—
4-locus effects	12	20.27	6	7.87	20	36.56*	7	7.64	2	0.94
4-locus × arrangements SI	1	0.22	—	—	—	—	—	—	—	—

Five different populations are examined for different kinds of interactions. Further explanations in the text (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

these results because of the relatively small number of observations and relatively large number of parameters that have been fit.

In terms of overall effects, we have the following sums for each population (where z is the approximate standard normal deviate):

Sussex, England . . . . . $G^2 = 173.08$, d.f. $= 118$, $z = 3.28$
Mt. Parnes, Greece . . . . . $G^2 = 158.27$, d.f. $= 101$, $z = 3.61$
Preveza, Greece . . . . . $G^2 = 253.82$, d.f. $= 163$, $z = 4.50$
Barcelona, Spain . . . . . $G^2 = 233.85$, d.f. $= 120$, $z = 6.17$
Crete, Island of . . . . . $G^2 = 107.82$, d.f. $= 77$, $z = 2.32$.

The sum of the effects for each population is highly significant except for the sample from the Crete population ($P < 0.05$).

The results are clear with regard to at least two major points: the significance of nonrandom associations between inversions of Segment I and Segment II of the *O* chromosome, which is already well known and documented (for a review see KRIMBAS and LOUKAS 1980), and the most statistically significant interactions, which are those between loci and chromosome *O* arrangements of Segment I. This last result is consistent with that found previously for the chromosome *O* data in LOUKAS, KRIMBAS and VERGINI (1979) and mentioned above in this paper. The present analyses further suggest that there is no significant additional interaction of arrangements of Segment II with loci after adjusting for the effects involving those of Segment I. Furthermore, there are a few statistically significant higher-order locus interactions in the present data, in agreement with the results of a similar analysis performed on the British populations (CHARLESWORTH, CHARLESWORTH and LOUKAS 1979). However, in neither set of data is there consistent evidence for strong interactions among all pairs (or greater n-tuples) of loci.

DISCUSSION

The result of these studies, concerning five natural populations of *D. subobscura*, is the detection of a very limited number of linkage disequilibria; the only clear-cut cases being the associations of some genes with the inversions in which these genes are located. However, the contrary is not true, *e.g.*, every gene located within an inversion does not necessarily display a nonrandom association with the inversion.

This is contrary to the expectations from the model proposed by FRANKLIN and LEWONTIN (1970), but is in agreement with similar studies performed on natural populations of species of the genus Drosophila (for a review see LOUKAS, KRIMBAS and VERGINI 1979). The paucity is probably genuine and not due to the restricted power of discrimination between allozyme variants by the usual electrophoretic techniques used (for the loss of discrimination due to the pooling of alleles into electrophoretic classes, see ZOUROS, GOLDING and MACKAY 1977; WEIR and COCKERHAM 1978). The additional use of methods based on sensitivity to urea and on changes in electrophoretic conditions permits a better allozyme discrimination, but does not seem to increase the number of these disequilibria

(unpublished). This is partially expected to be the case from the already published pattern of allelic frequencies determined by such methods (COYNE, FELTON and LEWONTIN 1978); the number of alleles is increased, but common alleles remain common and their frequencies do not change considerably, whereas most of the new alleles detected display rather low frequencies. With such changes, the homogeneity tests performed by us (where rare alleles are grouped in one class) would not be greatly affected. Taken at face value, the experimental data available so far do not show such disequilibria. Of course these studies are in progress and the final results are not yet available.

We have taken care to sample our populations in such a way as to minimize the possibility of creation of spurious disequilibria by the inclusion in the same sample of flies originating from two micro-populations differing in their allelic frequencies. This was achieved by sampling in one of two consecutive days under one and the same tree for each population. Furthermore, the dispersal behavior of this species (LOUKAS and KRIMBAS 1979) is such as to exclude such a possibility. Flies can move about 50m per day in summer, and this results, under neutrality conditions, in complete panmixia for very large areas populated by the species. Moreover, three different lines of evidence point nearly to the conclusion that at least two of our populations, the only ones studied in these respects, have an effective size not essentially different from infinite. We refer to studies performed on lethal allelism for the populations of Mt. Parnes and Crete (LOUKAS, KRIMBAS and SOURDIS, in submission), to studies dealing with the estimation of population size by the usual ecological methods during the period of bottleneck and that of high density, and to studies based on the temporal method (of temporal changes of allozyme frequencies) BEGON, KRIMBAS and LOUKAS, in submission) for the population of Mt. Parnes. The obvious conclusion is that the linkage disequilibria detected are not due to drift. They could, however, be due to our sampling errors. This can be excluded for most of the disequilibria detected since they are repeatedly found in the same direction in all populations, especially when we examine the combinations of genes and inversions including these genes. The pattern is such that simple neutralist explanations are not completely convincing (for a detailed discussion of this subject in regard to the middle-gene explanation, see LOUKAS, KRIMBAS and VERGINI 1979).

There is, however, another possibility. When the inversions were created, certain alleles were "tied up" with these inversions. Not enough time has elapsed since that event to destroy completely the original disequilibrium that has been conserved by purely mechanical means. In this case, an estimation of the age of inversions is possible. Simple calculations would set a rather recent origin for these inversions (of some ten thousand to at most a hundred thousand years) (KRIMBAS and LOUKAS 1980). From the actual evidence available and for other reasons mentioned in the previously cited paper, it seems that inversions are much more ancient; thus, this explanation would not be satisfactory. It is probable that most of the disequilibria detected by us between genes and inversions are maintained by epistatic interactions at the fitness level. However, it should

be noted that the argument concerning the ancientness of inversions is based on rather arbitrary mutation rate values.

The possibility of alternative explanations for the linkage disequilibria observed in this and previous studies and especially the paucity of the linkage disequilibria would plead in favor of the view that epistatic interactions on the fitness level are not so important, contrary to what is actually believed (FALCONER 1960).

We would be inclined to conclude that epistatic interactions do not seem to be important for electrophoretically detected polymorphic genes, although they could exist in some specific cases. However, this does not provide a final argument against a panselectionist view, since frequency-dependent selection mechanisms or mechanisms based on niche variations in selection coefficients would not necessarily be detected when studying linkage disequilibria.

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