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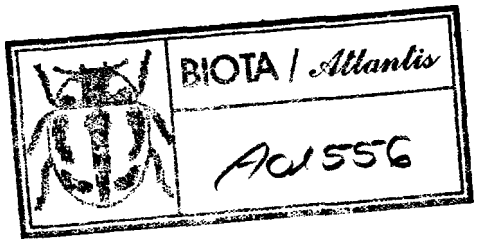


# ASSOCIATION BETWEEN ALLOZYME ALLELES AND CHROMOSOMAL ARRANGEMENTS OF THE O CHROMOSOME IN *DROSOPHILA SUBOBSCURA*. I. DATA OF NATURAL POPULATIONS (1)

by

*María del Pilar GARCIA and Antonio PREVOSTI*

*Department of Genetics. Faculty of Biology. University of Barcelona. Barcelona, Spain*

The classical work of Dobzhansky and other authors on the chromosomal polymorphism in Drosophila established the adaptive value of chromosomal arrangements and, consequently that different arrangements have different gene content. However, a direct proof of this differentiation was not obtained until Prakash and Lewontin (1968) found qualitative differences in some allozyme alleles between different chromosomal arrangements in *Drosophila pseudoobscura*.

After the publication of this paper and specially after the publication of the theoretical paper by Franklin and Lewontin (1970) on linkage disequilibrium, in many Drosophila species much work has been carried out, with the purpose of investigating the existence of associations between allozyme loci and chromosomal arrangements and also the linkage disequilibrium between different allozyme loci in natural populations. Research on this subject has been carried out on *Drosophila melanogaster*

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by Kojima, Gillespie and Tobari (1970), Mukai, Metler and Chigusa (1971), Charlesworth and Charlesworth (1973), Mukai, Watanabe and Yamaguchi (1974), Langley, Tobari and Kojima (1974), and Mukai and Voelker (1977); on *D. pseudoobscura* and *D. persimilis* by Prakash and Lewontin (1971), Prakash (1974 and 1976), Prakash and Merrit (1972); on *D.* robusta by Prakash and Levitan (1973 and 1974); on *D. pavani* by Nair and Brncic (1971); and on *D. subobscura* by Zouros and Krimbas (1973); Zouros, Krimbas, Tsakas and Loukas (1974); Loukas and Krimbas (1976); Pinsker, Lankinen and Sperlich (1978); Charlesworth, Charlesworth, Loukas and Morgan (1979); Loukas, Krimbas and Vergini (1979) and Pinsker and Sperlich (1973)).

*D. subobscura* is an autochthonous european species, whose geographical distribution does not seem to have been much affected by man, at least until recently. It has a rich polymorphism in all the chromosomes and many arrangements show latitudinal clines, which in general are thought to be adaptive. All this, makes of *D. subobscura* an ideal material for the analysis of the association between chromosomal arrangements and allozyme alleles or electromorphs. For this reason, since 1974 we began an analysis of this subject in natural populations of this species. In this paper we present the results obtained on the 0 chromosome, the most polymorphic in the species. Three allozyme systems controlled by genes of this chromosome, also highly polymorphic, have been analysed. According to Gillespie and Langley (1974) the allozymes can be separated into two categories, those transformimg substrates external to the organism and those acting on intracellular substrates. Usually, enzymes of the first group are active on a variety of external substrates and this seems to be correlated with a higher polymorphism in these enzymes. The allozymes studied in this paper are included in this group.



## MATERIAL AND METHODS

Samples of *D. subobscura* from 16 different localities have been studied (see *fig. 1*).

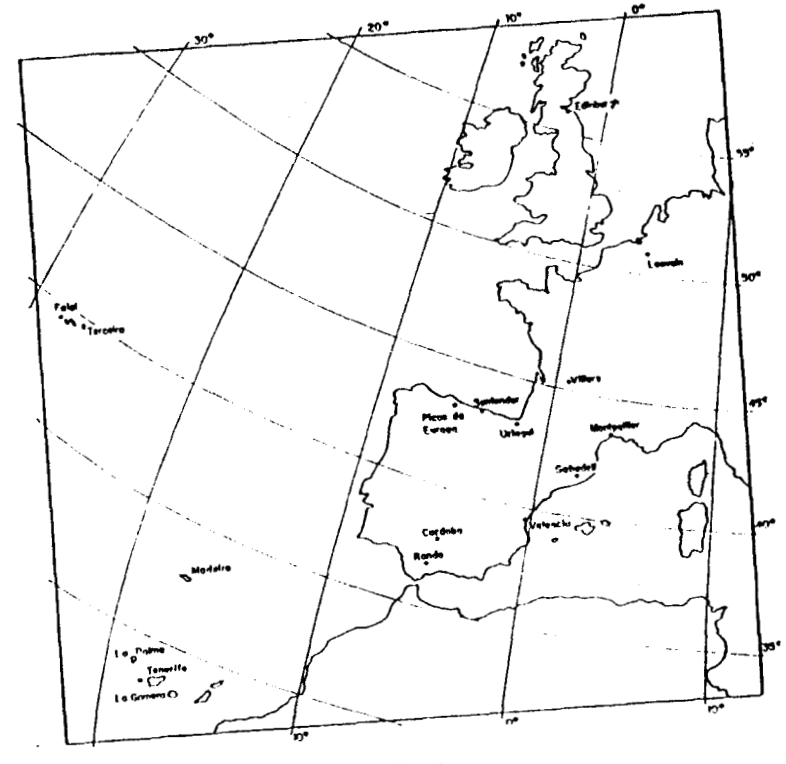


*Fig. 1*

Wild males or males obtained from wild females progeny reared in single cultures, were crossed with females of the *ch cu* stock. Single males obtained in the progeny of these crosses

were backcrossed with *ch cu* females, with the purpose of extracting in each backcross one single 0 chromosomes of the wild male used in the first cross. The *ch cu* is homozygous for the $0_{s+4}$ arrangement and for one allele of each of the studied allozyme systems; it is also homozygous for the recessive visible mutants of the 0 chromosome cherry (ch) and curled (cu), serving as markers for this chromosome.

Three allozyme systems controlled by genes of the 0 chromosome have been analysed: Leucineaminopeptidase (Lap) and Peptidase-1 (Pept-1) both located in the region of the $0_{s+4}$ system of inversions and Esterase-5 (Est-5) located in the region of the inversion $0_t$. Loukas *et al.* (1979 a) give for these genes positions 213.5, 181.8 and 13.5 respectively, in the 0 chromosome.

The analysis of the chromosomal arrangements has been performed in third instar larvae obtained in the progeny of backcrosses between $F_1$ males (obtained by crossing males trapped in the wild with *ch cu* females) with *ch cu* females. The squash technique and staining with lactoacetic orcein have been used to prepare the chromosomes. Seven larvae of the progenies of the backcrosses were analysed, in order to have a low probability ($0{,}5^7 = 0{,}0078$) of not detecting the chromosome that the $F_1$ male did receive from its wild father.

In the progeny of the above mentioned backcrosses, the individuals with the wild phenotype are carriers of one chromosome coming from a wild male, whose alleles for the allozyme systems can be identified. Adults have been used in the analysis of the Pept-1 and Est-5 systems and pupae for the Lap, since the activity of this system is greater in this developmental stage. The identification of the wild type individuals is possible in advanced pupae by observing the eye colour visible throughout the pupal envelope.

The alleles of the allozyme systems studied in this paper are named according to the terminology of Loukas *et al.* (1979).

The analysis of the Lap allozymes has been carried out with starch gel electrophoresis according to Poulick (1957) and incubating according to Beckman and Johnson (1964) with the modification introduced by Sakai *et al.* (1969); for the Est-5 the method of Zouras *et al.* (1974) has been used and for the Pept-1 that of Loukas *et al.* (in press, personal communication).



## RESULTS

### Chromosomal polymorphism

*Table 1* presents the frequency of the 0 chromosome arrangements in the sampled populations. These data agree with those previously found by several authors (Prevosti, 1964 a, 1964 b, 1966, 1971, 1972; Frutos, 1972). $0_n$ has its maximum frequency in the north, decreasing gradually southwards and disappearing completely in some populations of the extreme south range of the distribution of the species, in the Canary Islands. This latitudinal cline of $0_n$ is also found in the standard arrangements of the other chromosomes (Sperlich and Kunze-Mühl, 1963; Sperlich, 1964; Prevosti, 1964 a, 1964 b, 1966 and 1974).

$0_{s+4}$ is ubiquotous in the area of the species distribution, with the exception of some marginal populations of North Europe (Sperlich, 1964). This arrangement is present in all the populations here analysed, being the most frequent in the Azores (Faial and Terceira) and the unique present in some populations of the Canary Islands, as was already indicated by Prevosti (1971).

$0_{s+4+7}$ is an arrangement characteristic of the Iberian Peninsula, although it is also rather frequent in some Anatolian populations (Junger, 1968). In *table 1* there is seen to be an increase of the frequency of this arrangement from the North-East of Spain, towards the North-West and the South; its maximal values are found in Ronda (73,5 %) and Picos de Europa (64 %). North of the Pyrenées its frequency decreases steeply, in Montpellier it is of 3,3 %, in Villiars of 1,8 % and is lacking completely in Louvain. Its distribution in the Iberian Peninsula agrees with the radial clines described by de Frutos (1972).

Another arrangement frequent in the populations here studied

*Table 1. Frequencies of the 6 chromosome arrangements*

| | ED | LO | VI | MO | ST | PE | UZ | SA | VA | TE | FA | CO | RO | LP | TF | GO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $0_{st}$ | 0.420 | 0.708 | 0.506 | 0.398 | 0.294 | 0.114 | 0.278 | 0.196 | 0.155 | 0.0[illegible] | 0.397 | 0.079 | 0.066 | | | |
| $0_{3+4}$ | 0.320 | 0.108 | 0.143 | 0.209 | 0.113 | 0.131 | 0.130 | 0.255 | 0.190 | 0.[illegible] | 0.807 | 0.121 | 0.120 | 1.00 | 1.00 | 0.024 |
| $0_{3+4+5}$ | | | 0.018 | 0.033 | 0.397 | 0.640 | 0.252 | 0.190 | 0.419 | | | 0.679 | 0.733 | | | 0.976 |
| $0_{3+4+6}$ | | 0.149 | 0.327 | 0.309 | 0.118 | 0.044 | 0.183 | 0.125 | 0.069 | 0.[illegible] | 0.126 | 0.082 | | | | |
| $0_{3+4+7}$ | | | 0.006 | 0.013 | | 0.018 | 0.035 | 0.070 | 0.058 | 0.006 | | 0.010 | | | | |
| $0_{3+4+8}$ | | 0.014 | | 0.011 | 0.029 | | 0.017 | 0.080 | 0.024 | | | 0.005 | | | | |
| $0_{3+4+10}$ | | 0.027 | | 0.016 | 0.015 | 0.018 | 0.026 | 0.025 | 0.048 | | | 0.032 | 0.024 | | | |
| $0_{3+4+11}$ | 0.220 | | | 0.004 | | | | 0.005 | | | | | | | | |
| $0_{3+4+12}$ | 0.040 | | | | | | | 0.005 | | | | | | | | |
| $0_{3+4+15+8}$ | | | | 0.002 | | | | | | | | | | | | |
| $0_{3+4+5}$ | | | | | | | | | 0.014 | | | | | | | |
| $0_{3+4+9}$ | | | | | | | 0.005 | | | | | | | | | |
| $0_{3+4+6}$ | | | | | | 0.009 | | | | | | | | | | |
| $0_{3+4+15+8}$ | | | | | | | | | | | | | | | | |
| $0_{3+4+7+8}$ | | | | | | | | 0.014 | | | | | | | | |
| $0_{7}$ | | | | | | | | | | | | | | | | |
| $0_{23}$ | | | | | M.029 | 0.028 | 0.070 | 0.035 | 0.014 | | | 0.005 | | | | |
| N | 50 | 74 | 168 | 450 | 68 | 114 | 115 | 200 | 291 | 168 | 135 | 190 | 82 | 178 | 294 | 122 |

ED Edinburgh
LO Louvain
VI Villars
MO Montpellier
ST Santander
PE Picos de Europa
UZ Uztegui
SA Sabadell
VA Valencia
TE Terceira
FA Faial
CO Córdoba
RO Ronda
LP La Palma
TB Tenerife
GO Gomera



is $0_{st,23}$. It is an arrangement rather frequent in the West Mediterranean area (Kunze-Mühl and Sperlich, 1962; Prevosti, 1964) and it is the prevailing one in North Africa (Gotz, 1965; Jungen, 1968; Prevosti, 1974). It has also been found in the Azores.

## Enzyme polymorphism

### *Lap System*

According to the data in *table 2*, in the analysed populations there is no uniformity in the frequencies of the alleles of this system. A latitudinal cline is observed in these frequencies. The $Lap^{1.00}$ allele is the most frequent in all the populations, but its frequency increases from the North to the South. The $Lap^{1.11}$ allele behaves inversely.

### *Pept-1 System*

As in the Lap system the alleles of Pept-1 show a regular geographical variation. The $Pept\text{-}1^{0.40}$ allele increases from the North to the South and the reverse is true of the $Pept\text{-}1^{1.00}$ allele. However, in this case there is an exception: the populations from the Canary Islands have a high frequency of the $Pept\text{-}1^{1.00}$ allele, in spite of its situation in the South-West boundary of the distribution of the species.

### *Est-5 System*

Est-5 do not show a clear geographical differentiation, in distinction from the Lap and Pept-1. The latitudinal cline found by González-Duarte *et al.* (1973) in Spanish and North African populations is not found in the present data. The frequencies of the more common alleles, $Est\text{-}5^{1.00}$ and $Est\text{-}5^{0.90}$, have intermediate values in most populations, but in the Canary Islands, Madeira and Azores the frequency of $Est\text{-}5^{1.00}$ is high.

*Table 2. Allozyme allele frequencies*

| | ED | LO | VI | MO | ST | PE | UZ | SA | VA | TE | FA | CO | RO | MA | LP | TF | GO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lap | | | | | | | | | | | | | | | | | |
| 1.18 | 0.020 | 0.081 | 0.048 | 0.017 | 0.022 | 0.018 | 0.017 | 0.025 | 0.031 | 0.043 | 0.104 | 0.005 | 0.012 | 0.028 | 0.012 | — | — |
| 1.11 | 0.220 | 0.324 | 0.304 | 0.249 | 0.206 | 0.259 | 0.313 | 0.210 | 0.192 | 0.080 | 0.200 | 0.205 | 0.193 | 0.104 | 0.157 | 0.133 | 0.205 |
| 1.00 | 0.660 | 0.581 | 0.619 | 0.673 | 0.691 | 0.673 | 0.652 | 0.750 | 0.742 | 0.859 | 0.631 | 0.758 | 0.735 | 0.779 | 0.779 | 0.823 | 0.788 |
| 0.86 | 0.100 | — | 0.030 | 0.024 | 0.059 | 0.018 | 0.017 | 0.010 | 0.021 | 0.018 | 0.015 | 0.028 | 0.060 | 0.065 | 0.041 | 0.041 | 0.027 |
| 0.69 | — | 0.014 | — | 0.007 | 0.013 | — | — | 0.005 | 0.014 | — | — | 0.005 | — | 0.028 | 0.012 | 0.003 | — |
| N | 50 | 74 | 168 | 450 | 68 | 124 | 115 | 200 | 391 | 198 | 135 | 190 | 98 | 72 | 172 | 294 | 112 |
| Pep-1 | | | | | | | | | | | | | | | | | |
| 1.60 | — | 0.095 | 0.082 | 0.028 | — | — | — | — | 0.044 | — | 0.017 | 0.085 | 0.034 | 0.097 | — | 0.030 | 0.029 |
| 1.00 | — | 0.486 | 0.456 | 0.443 | — | — | — | — | 0.300 | 0.206 | 0.254 | 0.271 | 0.253 | 0.250 | — | 0.720 | 0.803 |
| 0.40 | — | 0.419 | 0.513 | 0.528 | — | — | — | — | 0.656 | 0.794 | 0.729 | 0.644 | 0.713 | 0.653 | — | 0.250 | 0.168 |
| N | — | 74 | 158 | 458 | — | — | — | — | 270 | 160 | 118 | 118 | 87 | 72 | — | 256 | 208 |
| Est-5 | | | | | | | | | | | | | | | | | |
| 1.00 | 0.500 | 0.357 | 0.553 | 0.585 | 0.532 | 0.621 | 0.695 | 0.568 | 0.691 | 0.020 | 0.784 | 0.376 | 0.498 | 0.306 | 0.304 | 0.964 | — |
| 0.90 | 0.440 | 0.443 | 0.442 | 0.405 | 0.468 | 0.379 | 0.288 | 0.413 | 0.285 | 0.879 | 0.206 | 0.586 | 0.502 | 0.119 | 0.018 | 0.006 | — |
| 0.86 | — | — | — | 0.011 | — | — | 0.079 | 0.019 | 0.003 | — | — | 0.038 | 0.039 | 0.045 | 0.018 | — | — |
| N | 84 | 70 | 103 | 284 | 77 | 124 | 127 | 213 | 291 | 138 | 110 | 158 | 78 | 67 | 282 | 176 | — |

MA Madeira



The results obtained for tlic three allozyme systems agree with those of Zouros *et al.* (1974), Charlesworth *et al.* (1979) and Loukas *et* nl. (1979) in several Greek aiid British populations and iii one Spanish populatiou; there is also agreement witli tlic results of Martinez Cabrera (1980) in populations from the Canary Islands.

It is difficult to compare tlir results of Lakovaara aiid Saura (1971), Saura *et al.* (1973) and Pinsker *et* al. (1978) for the Lap systriii witli our data because of the different techniques and terminology used.

## Association between allozymes and chromosomal arrangements

$\chi^2$ tests have been performed for every population aiid allozyme system, iii order to prove whether the different alleles are randomly distributed among tiie different chromosomal arrangements. The association has not been tested in the popiilations from tlic Canary Islands and Madeira, practically monomorphic for the $0_{+}$ arrangement. The association between the alleles of the Est-5 system and the chromosomal arrangements has been studied only in the populations where $0_{+ , , ,}$ is present, because tlic locus for this esterase is located in tlir region of tlir $0_{t}$ inversion.

### *Association between Lap alleles and 0 chromosome arrangements*

The arrangements of tlir 0 chromosome have been sorted into two classes, $0_{+}$ and $0_{+ , , ,}$. In the $0_{+ , ,}$ class are included all the arrangements of the $0_{+ , ,}$ phylad; in the $0_{+}$ class the nrrangement $0_{+}$ plus all the arrangements being standard in the extreme of the chromosome, where tlir inversions $0_{+}^{a}$, are located.

The alleles of the Lap systriii have also been grouped into two classes, $Lap^{1.00}$ aiid $Lap^{1.11}$, the main alleles in the populations. Into the $Lap^{1.11}$ class are included tlir $Lap^{1.11}$ and $Lap^{1.18}$ alleles. The last one has low frequencies in most popiilations and it is associated witli $0_{+}$ like $Lap^{1.11}$. The $Lap^{1.00}$ class includes the $Lap^{1.00}$ alleles and those witli lower movilities, $Lap^{0.86}$ aiid $Lap^{0.69}$.

*Table 3. Association between 0 chromosome arrangements and Lap alleles*

| | ED | LO | VI | MO | ST | PE | UZ | SA | VA | TE | FA | CO | RO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lap** | | | | | | | $0_{st}$ | | | | | | |
| 1.18 | 1 | 6 | 7 | 19 | 2 | 2 | ? | 4 | 4 | 5 | 13 | 1 | 0 |
| 1.11 | 10 | 19 | 37 | 72 | 8 | 5 | 23 | 27 | 24 | 1 | 10 | 9 | 6 |
| 1.00 | 9 | 26 | 38 | 82 | 11 | 9 | 14 | 14 | 20 | 4 | 13 | 12 | 4 |
| 0.86 | 1 | 1 | 3 | 6 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 0.69 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | $0_{3+4}$ | | | | | | |
| 1.18 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 8 | 2 | 1 | 1 | 1 |
| 1.11 | 1 | 5 | 14 | 40 | 6 | 28 | 13 | 15 | 32 | 12 | 17 | 29 | 10 |
| 1.00 | 24 | 17 | 66 | 221 | 36 | 68 | 61 | 136 | 196 | 136 | 79 | 132 | 57 |
| 0.86 | 4 | 0 | 2 | 5 | 4 | 2 | 1 | 2 | 5 | 3 | ? | 5 | 5 |
| 0.89 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 1 | 0 |

$0_{st}$ ($0_{st}$, $0_t$, $0_{st}$)
$0_{3+4}$ (all the arrangements of the $0_{3+4}$ phylad are included)



Lap$^{0.86}$ has low frequencies in the analysed populations and Lap$^{0.69}$ very low. Lap$^{1.00}$ and both slower alleles are associated with $0_{3+4}$. *Table 3* presents the distribution of alleles and arrangements.

The $\chi^2$ values calculated in order to prove the association in the 13 populations analysed are given in *table 7*. A significative and strong association between Lap$^{1.00}$ and $0_{3+4}$ and between Lap$^{1.11}$ and $0_{st}$ is detected in all the populations, with the exception of Picos de-Europa. However, in this population there is a tendency to the same association, although it is not statistically significant.

### *Association between Pept-1 alleles and arrangements of the 0 chromosome*

The gene controlling Pept-1, as for the Lap, is located in the $0_{3+4}$ region of the 0 chromosome. For this reason, the 0 chromosome arrangements have been distributed into 2 classes, $0_{st}$ and $0_{3+4}$, being included in $0_{st}$ all the «standard» arrangements in the $0_{3+4}$ region. In the $0_{3+4}$ class are lumped together all the arrangements of its phylad. However, as can be seen in *table 4*, in the populations from Valencia and Terceira, $0_{3+4t}$ shows a different association. All the arrangements of the $0_{3+4}$ phylad contain in excess Pept-1$^{0.46}$, being deficient in Pept-1$^{1.00}$. However, in the above said populations $0_{3+4t}$ has an excess of Pept-1$^{1.00}$. As $0_t$ is overlapping with $0_{3+4}$, the recombination between $0_{3+4}$ and $0_{3+4t}$ is possibly restricted. For this reason, in the above mentioned populations, $0_{3+4t}$ has been considerated as a separate cl ss.

The alleles have been grouped into two classes, Pept-1$^{0.46}$ and Pept-1$^{1.00}$. The last class includes Pept-1$^{1.46}$, since from the point of view of the association it behaves like Pept-1$^{1.00}$ and it has a very low frequency *(table 4)*.

Seven among the eight populations analysed show a strong association between Pept-1$^{0.46}$ and the $0_{3+4}$ phylad and between Pept-1$^{1.00}$ and $0_{st}$. This can be seen in the $\chi^2$ values of *table 7*.

A tendency to the same associations is observed in the other population, but the $\chi^2$ value is not significant.

*Table 4. Association between 0 chromosome arrangements and Pept-1 alleles*

| | LO | VI | MO | VA | TE | FA | CO | RO |
|---|---|---|---|---|---|---|---|---|
| | | | | $O_{st}$ | | | | |
| Pept-1 | | | | | | | | |
| 1.60 | 6 | 4 | 8 | 4 | 0 | 2 | 1 | 2 |
| 1.00 | 28 | SI | 103 | 22 | 3 | 12 | 9 | 7 |
| 0.40 | 18 | 27 | 7u | 20 | 5 | 15 | 5 | 3 |
| | | | | $O_{3+4}$ | | | | |
| 1.60 | 0 | 0 | 4 | 7 | 0 | 0 | 9 | 1 |
| 1.00 | 4 | 10 | 46 | 48 | 18 | 16 | 22 | 15 |
| 0.40 | 6 | 14 | R7 | 148 | 95 | 59 | 68 | 69 |
| | | | | $O_{3+4+8}$ | | | | |
| 1.00 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 1.00 | 3 | 11 | 55 | 11 | 12 | 3 | 1 | 0 |
| 0.40 | 7 | 40 | 86 | 9 | 27 | 12 | 3 | 0 |

$0_{st}$ ($0_{st}$, $0_{7}$, $0_{22}$)
$0_{3+4}$ (with exception of $0_{3+4+8}$)

*Association between Est-5 alleles and chromosome 0 arrangements*

The locus of Est-5 is located in the region of the $0_7$ inversion. Two classes have been considered in order to analyse the association, $0_7$ and $0_{st}$. In the class $0_7$ are included the arrangements $0_7$ and $0_{3+4+8}$. All the remaining arrangements are standard in the region of the $0_7$ inversion and are included in the $0_{st}$ class.

The Est-5 alleles are also grouped into two classes, Est-$5^{1.00}$ and Est-$5^{0.90}$. This last class comprises the alleles with movilities 0.90 and 0.86. The frequency of Est-$5^{0.86}$ is low, or this allele is lacking in some populations *(Table 5)*.

Looking at *table* 7, it is seen that only in 2 populations, Uz-



tegui and Ronda, there is a significant deviation froiii a random combination of Est-5 alleles and chromosome 0 arrangements. These popiilatioiis show an association between Est-$5^{1.00}$ and $0_7$ and between Est-$5^{1.00}$ and $0_{st}$. With the exception of tlie population from Córdoba, in tlir other populations there is a tendency to the same association, but it is not statistically significant. If we add the $\chi^2$ values of tlie association tests of all the populations we obtain a value of $\chi^2 = 26{,}642$ for 7 degrees oí freedom, giving a significant association at ilir level of 0,001. Then, association is present in this case too, but less strong than in the previous ones.

*Table 5. Association between 0 chromosomes arrangements and Est-5 alleles*

| | ST | PE | UZ | SA | VA | CO | RO |
|---|---|---|---|---|---|---|---|
| | | | | $O_7$ | | | |
| Est-5 | | | | | | | |
| 1.00 | 13 | 40 | 15 | 18 | 66 | 58 | 18 |
| 0.90 | 15 | 32 | 15 | 18 | 55 | 67 | 38 |
| 0.86 | 0 | 0 | 4 | 3 | 1 | 7 | 3 |
| | | | | $O_{st}$ | | | |
| 1.00 | 22 | 26 | 54 | 81 | 109 | 24 | 13 |
| 0.90 | 17 | 11 | 17 | 55 | 60 | 27 | 4 |
| 0.86 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

$0_7$ ($0_7$, $0_{3+4+8}$)
$0_{st}$ (all the arrangements $0_{st}$ in the proximal region of the chromosome are included)

Our results on the association between the Lap and Pept-1 alleles and the 0 chromosome arrangements agree with those obtained by Charlesworth *et al.* (1979) and Loukas *et al.* (1979 b). These authors did not find an association between Est-5 alleles and chromosome 0 arrangements because the inversion $0_7$ is lacking in the populations they analysed. Only in one population from Barcelona studied by Loukas *et al.* are these arrangements present, but they only proved the association bet-

ween Est-5 and $0_{3,4}$. González-Duarte *et al.* (1973) did find the same association between 0, and Est-5$^{0.90}$.

*Linkage disequilibrium between alleles of different allozyme loci*

The linkage disequilibrium (1) between the alleles of the Lap, Pept-1 and Est-5 allozyme systems has also been tested. The

*Table 6. Linkage disequilibrium between alleles*

| | LO | VI | MO | VA | TR | FA | CO | RO | TF | GO |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lap 1.11 | | | | | |
| Pept – 1 | | | | | | | | | | |
| 1.00 | 11 | 26 | 60 | 20 | 1 | 9 | 11 | 7 | 18 | 26 |
| 0.40 | 10 | 19 | 50 | 30 | 10 | 14 | 14 | 11 | 4 | 6 |
| | | | | | Lap 1.00 | | | | | |
| Pept – 1 | | | | | | | | | | |
| 1.00 | 2 | 39 | 129 | 56 | 30 | 19 | 22 | 16 | 16 | 28 |
| 0.40 | 3 | 59 | 185 | 142 | 106 | 65 | 64 | 57 | 9 | 8 |
| | | | | | Est – 5 1.00 | | | | | |
| Pept – 1 | | | | | | | | | | |
| 1.00 | 16 | 6 | 76 | 47 | 19 | 25 | 13 | 6 | — | — |
| 0.40 | 16 | 9 | 83 | 109 | 79 | 58 | 30 | 29 | — | — |
| | | | | | Est – 5 0.90 | | | | | |
| Pept – 1 | | | | | | | | | | |
| 1.00 | 10 | 8 | 46 | 35 | 14 | 6 | 18 | 16 | — | — |
| 0.40 | 14 | 4 | 66 | 67 | 44 | 20 | 49 | 38 | — | — |
| | | | | | Pept 1.11 | | | | | |
| Est – 5 | | | | | | | | | | |
| 1.00 | 11 | 7 | 3 | 9 | 7 | 16 | 10 | 3 | — | — |
| 0.90 | 11 | 0 | 2 | 8 | 4 | 6 | 10 | 11 | — | — |
| | | | | | Lap 1.00 | | | | | |
| Est – 5 | | | | | | | | | | |
| 1.00 | 24 | 57 | 114 | 136 | 81 | 59 | 40 | 28 | — | — |
| 0.90 | 19 | 50 | 79 | 79 | 49 | 20 | 64 | 34 | — | — |

(1) Here we prefer to speak of linkage disequilibrium instead of association, because the association is due to the presence of inversions.



*Table 7. Values of $\chi^2$ tests*

| | Lap/ Chromosome 0 | Pept-1/ Chromosome 0 | Est-5/ Chromosome 0 | Est-5/ Lap | Est-5/ Pept-1 | Lap/ Pept-1 |
|---|---|---|---|---|---|---|
| Edinburgh | 15.99++ | | | 0.29 | 0.15 | 0.0001 |
| Louvain | 4.70+ | 3.50+ | | 0.23 | 3.94+ | 4.03+ |
| Villars | 20.93+++ | 22.96+++ | | 0.13 | 1.20 | 3.96+ |
| Montpellier | 63.54+++ | 24.10+++ | | | | |
| Santander | 3.68++ | | 0.05 | | | |
| Picos de Europa | 0.86 | | 2.22 | | | |
| Ustegui | 24.04+++ | | 6.50+ | | | |
| Sabadell | 64.02+++ | | 2.21 | | | |
| Valencia | 41.11+++ | 19.59+++ | 3.-0 | 1.73 | 0.49 | 2.53 |
| Terceira | 21.51+++ | • 3.87+ | | 0.007 | 0.49 | 1.08 |
| Faial | 29.10+++ | • 9.73+ | | 0.84 | 0.48 | 2.35 |
| Córdoba | 7.34++ | 7.23++ | 0.002 | 0.82 | 0.15 | 1.64 |
| Ronda | 8.20++ | 12.04+++ | 11.58++ | 2.86 | 1.78 | 1.61 |
| Tenerife | | | | | | 0.07 |
| Comen | | | | | | 0.14 |

■ P ⩽ 0.05
++ P ⩽ 0.01
+++ P ⩽ 0.001

• $\chi^2$ test for 2 d. g. f.

alleles with very low frequencies have been omited in these tests, the analysis having been carried out only with the 2 common alleles of each *(Table 6)*.

As in the study of the association between the alleles and the chromosomal arrangements, $\chi^2$ tests have been performed with the data of every population, taking the systems two by two. The values of these $\chi^2$ are given in *table 7*.

Association between $Lap^{1.00}$ and $Pept\text{-}1^{0.40}$ has been detected in two populations, and the same tendency is observed in most of the other populations, although the results are not statistically significant. Probably this association is due to the association of both, $Lap^{1.00}$ and $Pept\text{-}1^{0.40}$, with $0_{3+4}$. In fact when the association between these alleles is analysed separately in the $0_{3+4}$ and $0_4$ chromosomes, the significant association found in the populations of Villars and Montpellier disappears.

Between Est-5 and Lap and between Est-5 and Pept 1 no systematic deviations from a random combination of alleles have been found. In one population a significant association between $Pept\text{-}1^{0.40}$ and $Est\ 5^{0.00}$ was found, but in our opinion it is due to a random effect, since in other populations a constant tendency of deviations in the same sense, is not observed. When we do a high number of statistical tests, we expect to find some significant results just by chance.

Zouros *et al.* (1974), Charlesworth *et al.* (1979) and Loukas *et al.* (1979) did not find linkage disequilibrium either, between allozyme loci in *D. subsobscura* populations.

## DISCUSSION

### *Association between allozymes and chromosome 0 arrangements*

The alleles $Lap^{1.00}$ and $Pept\text{-}1^{0.40}$ are strongly associated with $0_{3+4}$ and $Lap^{1.11}$ and $Pept\text{-}1^{1.00}$ with $0_{st}$. Between $Est\text{-}5^{0.00}$ and $0_4$ and between $Est\text{-}5^{1.00}$ and $0_{st}$ there is a weaker association. All these associations seem to be general, at least in Western Europe. The data of Loukas *et al.* (1979) indicate the presence in Greece of the same association in the case of the Lap.



There are two general groups of hypotheses accounting for the associations between allozymes and chromosomal arrangements. As it is generally assumed that each different inversion has been produced only once in the history of one species, the association could be due to a founder effect, since when a new inversion appears, this bears only one allele in each of the loci included in it. Later on, all the gametes bearing the inversion will also bear the same alleles as the initial inversion and this will be maintained during a more or less large number of generations, depending on how strongly the recombination between the inverted segment and the other arrangements is blocked and how high the frequency of mutation is in the genes included in this inverted segment. Association will be found in populations in which this founder effect is still present.

An alternative explanation is adaptive selection. It is possible that some allozyme alleles are part of the complex of coadapted genes contained in the inversion giving to it an adaptive value.

Loukas *et al.* (1979) did find recombination values of 1,1 between 0, and the Est-5 locus. The presence of association in spite of this frequency of recombination, supports the adaptive value of this association, or at least a very strong linkage of the Est-5 gene with genes integrated in the group of coadapted genes contained in the inversion.

In fact, assuming the maximum linkage disequilibrium possible between two genes (0,25) and taking into account the frequency of recombination between the gene for Est-5 and the inversion $0_4$, which according to Loukas *et al.* (1979) is 0,011, it is possible to obtain an approximate estimate of the time necessary to arrive at the association value observed in natural populations. We can assume that the inversion 0, contained originally the allele $Est\text{-}5^{0.9}$. Afterwards, this initial association should decrease, because of recombination and mutation. Disregarding mutation, since its frequency is very small, the relation $(1 - c)^t = f$ (where $c$ is the recombination frequency and $f$ the fraction of linkage disequilibrium remaining after $t$ generations) allows us to estimate the number of generations necessary to pass from a maximum initial linkage

disequilibrium of 0,05 to the values observed in the populations analysed in this paper. In our case we have:

$$t = \frac{\log 1/16}{\log (1 - 0{,}0055)} = 502$$

We take $c = \frac{0{,}011}{2}$ because there is no recombination in the males of Drosophila; $f = 1/16$ is the fraction of linkage disequilibrium remaining after $t$ generations, if the initial value of D was 0,25 and the present is D = 0,015. This last figure corresponds to the lowest D values found in the populations here analysed. Supposing 5 generations per year in *D. subobscura*, the value of $t = 502$ would indicate that $0_7$ had originated only a century ago. This value is extraordinarily low. In these calculations we did not account for the effect of the variations in the frequencies of the Est-5$^{a,b}$ alleles and the inversion on the values of the linkage disequilibrium, after the appearance of $0_7$, during the period of increase of its frequency and after its definitive establishment in the populations. If we calculate $t$ by the formula $d_t = d_0 e^{-ct}$ (Ishii and Charlesworth, 1977; Nei and Wen-Hsiung Li, 1980), which gives a value independent of the frequencies of the alleles and the chromosomal arrangements, we obtain $t = 114$, a value much lower than the previous one. In this case $d_t$ is the difference between the frequency of the allele associated with the inversion in the inversion chromosome and the frequency of the same allele in the non-inversion chromosomes; $d_0$ is the same difference when the inversion was originated. To calcule $d_0$ we consider that initially the frequency of the associated allele in the inverted chromosomes was 1, and we assume that the frequency of this allele in the non-inverted chromosomes did not change much and was 0,4, since it oscillates around this value in present populations.

The previous calculations allow us to conclude that the observed association between $0_7$ and Est-5$^{a,b}$ can not be explained by an historical founder effect, even considering that these calculations are based on an important simplification of the reality. Then, it



seems that the alternative hypothesis should be accepted: the observed association has been maintained or originated because of selection. However, we are not able to discriminate between a possible direct effect of the selection on the Est-5$^{a,b}$ allele or a drawing effect on it by an allele of a strongly linked gene on which selection is acting.

On the other hand, in this case, the weakness of the association could be explained if «movility alleles» are actually electromorphs. In fact, in several papers, analysis on different esterase systems carried out in different species, has shown on introducing changes in the conventional electrophoretic techniques, or on submiting the enzymes to temperature shocks, that some of the supposed movility alleles are actually groups of alleles (Mc Dowell and Prakash, 1976; Cochrane, 1976; Cochrane and Richmond, 1979).

The data on recombination of the Lap and the Pept-1 loci with the $0_{3,4}$ arrangement are negative. Loukas *et al.* (1979) did not find any recombinant of these genes among 112 individuals analysed of the progeny of $0_{3,4}/0_{st}$ parents. This is not surprising since both loci are inside $0_{3,4}$ and this arrangement is the result of two overlapping inversions. In this case it is even more difficult than in the previous one, to favour one of the two alternative hypotheses, to explain the association. The latitudinal clines observed in Lap$^{1,00}$ and Pept-1$^{1,40}$ could be due to the association of these alleles with chromosomal arrangements, which show latitudinal clines. In this case, the cline should be an expression of the adaptive value of the inversions, but not of the allozyme alleles. This point will be more deeply discussed in a next paper.

In the populations from Valencia (Spain) and Terceira (Azores), $0_{3,4,5,6}$ has a different content of Pept-1 alleles than the other arrangements of the $0_{3,4}$ phylad. In these populations $0_{3,4,5,6}$ is associated with Pept-1$^{1,00}$. This association is statistically significant in both populations with $\chi^2$ values of 8,2 and 4,03 (1 d. f.) in Valencia and Terceira, respectively. Loukas *et al.* (1979 b) found similar results in a population from Barcelona. To explain this association, local factors should be taken into account.

However, local environmental conditions peculiar to these populations are difficult to ascertain. This makes it difficult to explain this association adaptively. It is easier to invoke some historical factors. Peculiarities of the process leading to the coadapted pool of genes of these populations could account for it. An alternative explanation would be a foundation effect in the introduction of the $0_{3+4L+8}$ arrangement in these populations, i. e. that all the $0_{3+4L+8}$ arrangements in these populations are derived from one or a few $0_{3+4L+8}$ arrangements initially introduced into the population. In a similar way this association could be explained by drift.

### *Association between alleles of different allozyme systems*

No systematic association has been detected between alleles of different allozyme systems, accounted for by interaction between only the alleles themselves. Between Est-5 and Lap, no cases of association have been found. Between Est-5 and Pept-1 only in one population linkage disequilibrium has been found, but in our opinion this is due to chance, since this result is not supported by deviations in the same sense in other populations. There is a consistent association between $Lap^{1.00}$ and $Pept\text{-}1^{0.40}$. In two populations this association is significant and in the other the same tendency is observed. However, in this case both alleles are associated with $0_{3+4}$ and its linkage disequilibrium is due to its common association with this arrangement.

In conclusion our data support the results obtained by most authors having analysed the existence of linkage disequilibrium in natural populations of Drosophila. Linkage disequilibrium between genes, seems to be much less frequent, in natural populations, from what could be predicted on the basis of theoretical results like those obtained by Franklin and Lewontin (1970).

## SUMMARY

In the analysis of 16 different populations of *D. subobscura*, a strong association has been detected between alleles of allozyme systems (Lap and Pept-1) and chromosomal arrangements of the 0 chromosome, in which the genes controlling these allozymes systems are located. The Est-5 alleles show a weak association with arrangements of the same chromosome. This last association is maintained in spite of a recombination frequency of 0,011 between the Est-5 locus and $0_{+}$, the inversion carrying this locus. This supports that selection is maintaining this association, whether acting directly on the Est-5 gene or on another gene strongly linked with it. With the present data we do not have a basis that allows us to discriminate between a selective or an historical explanation of the strong association detected for the other two allozyme systems.



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