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# Allozyme variation in fourteen natural populations of *Drosophila melanogaster* collected from different regions of France

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**Summary**

We analyzed allozyme variation of 6 gene loci in 14 populations of *Drosophila melanogaster* originating from different regions of France and captured from fruits of the localities studied (cellars excluded). With respect to these 6 loci the populations are not genetically homogeneous. Allelic frequencies at the *Adh* locus are correlated with latitude and at the *Est-6* with the kind of fruit in the habitats.

In spite of the heterogeneity of these 14 new populations, the comparison between them and 15 other French populations collected 9 or 10 years ago, in cellars, shows a remarkable similarity in the genetical composition of 4 loci among the 5 compared. Thus, the different habitats of these populations are not variable enough to enable much genetic differentiation.

*Key words :* Drosophila melanogaster, *biochemical polymorphism, French natural populations.*

**Résumé**

*Polymorphisme biochimique de quatorze populations naturelles de* Drosophila melanogaster, *capturées dans différentes régions de France*

Le polymorphisme de 6 locus enzymatiques a été analysé dans 14 populations de *Drosophila melanogaster* réparties du Nord au Sud de la France et capturées sur des fruits (caves à vin exclues). Un cline de fréquences avec la latitude a été mis en évidence au locus de l'*Adh*. Pour l'*Est-6*, les fréquences alléliques semblent dépendre de la nature du fruit de piégeage.

Malgré l'hétérogénéité de ces 14 nouvelles populations, la comparaison de nos résultats avec ceux concernant 15 autres populations françaises, capturées dans des caves à vin 9 ou 10 ans plus tôt, révèle une remarquable similitude dans la structure génétique de 4 des 5 locus comparés. Par conséquent, ces populations semblent vivre dans des habitats assez voisins pour ne pas entraîner de grandes différenciations génétiques.

*Mots clés :* Drosophila melanogaster, *polymorphisme biochimique, populations naturelles françaises.*

## I. Introduction

Generally, allozyme frequencies in natural populations of *Drosophila melanogaster* within the same geographical area differ slightly from one locality to another. On the contrary, when collections are made in different regions, the populations show a large-scale genetic differentiation (BERGER, 1971 ; DAVID, 1982 ; GIRARD & PALABOST, 1976 ; GIRARD *et al.*, 1977 ; JOHNSON & SCHAFFER, 1973 ; O'BRIEN & MacINTYRE, 1969 ; SINGH *et al.*, 1982). The first French populations analyzed, located in wine-cellars of the Saône and Rhône valleys (GIRARD & PALABOST, 1976 ; GIRARD *et al.*, 1977) showed that the frequencies of the most common alleles at some loci *(Est-C, Est-6* and *α-Gpdh)* fluctuate over wide limits ; on the contrary, at other loci *(Acph, Adh* and *Odh),* allele frequencies are very similar between populations. These results could be due, either to the particular habitat of wine-cellars, or to the specific geographical localization of the populations examined.

Consequently, for a better understanding of biochemical polymorphism in French populations, it was necessary to extend the analysis to populations exclusively collected on fruit, from the North to the South of France.

## II. Material and methods

### A. *Collections*

Wild *Drosophila melanogaster* adults were collected and brought to the laboratory. Flies were frozen and then used for electrophoresis. All the collections were made during the annual demographic burst of the species (August and September).

### B. *Populations studied*

Fourteen populations were studied ; their origins are listed below : (1) Tostes near Louviers ; (2) Le Haras du Pin near Argentan ; (3) Sainte-Geneviève-des-Bois near Paris ; (4) Rannée near Rennes ; (5) Nevez near Quimper ; (6) Chateaubriant ; (7 ) Ménétréol-sous-Sancerre near Sancerre ; (8) Bonnac-la-Côte near Limoges ; (9) Chessy-les-Mines near Villefranche-sur-Saône ; (10) Beynost near Montluel ; (11) Le Curtelod near Yenne ; (12) Allevard near Grenoble ; (13) Montauban ; (14) Tautavel near Perpignan.

Figure 1 gives the geographical localization and the fruit of the habitats.

### C. *Electrophoresis*

Allozyme variation was studied by starch gel electrophoresis using POULIK's discontinuous buffer system (POULIK, 1957). Six polymorphic enzyme loci were studied, according to the techniques described in AYALA *et al.* (1972) : *Acph* (acid phosphatase ; 3-101.4), *Adh* (alcohol dehydrogenase ; 2-50.1), *Est-C* (esterase-C ; 3-47.6), *Est-6* (esterase 6 ; 3-36.8), *α-Gpdh* (α-glycerophosphate dehydrogenase ; 2-20.5) and *Pgm* (phosphoglucomutase ; 3-43.4).

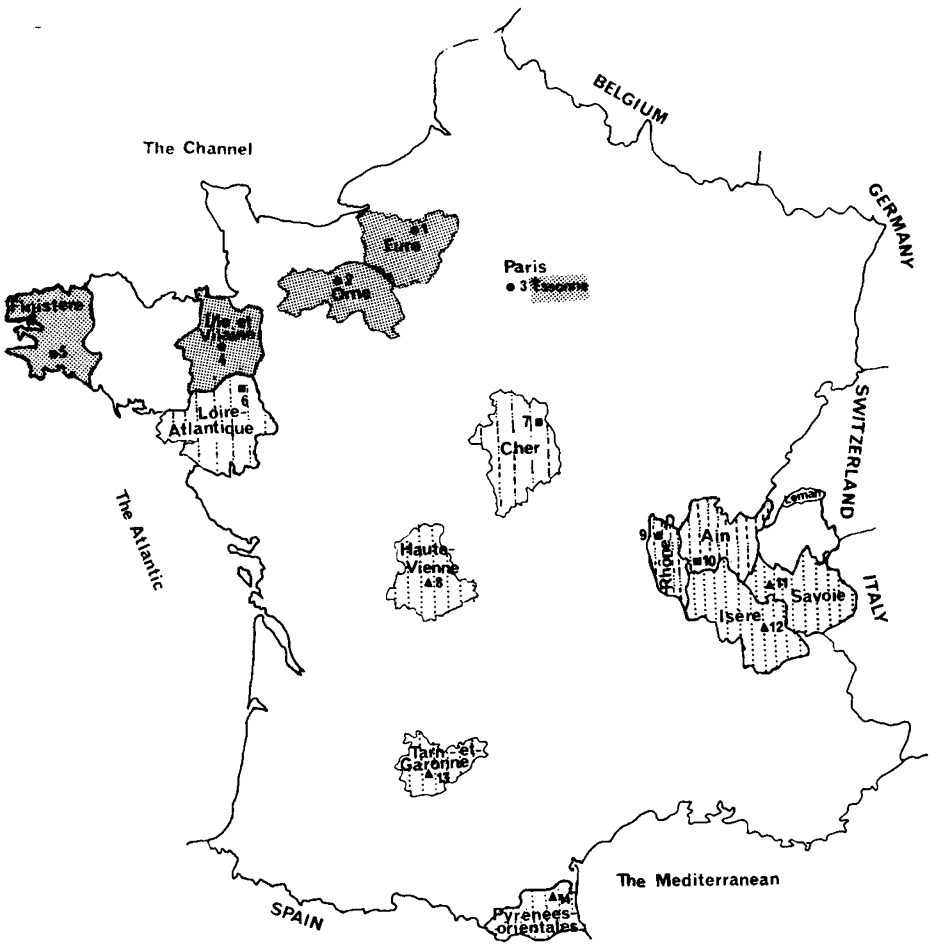


FIGURE 1

*Geographical localization and fruit in the biotopes of the 14 French populations of* Drosophila melanogaster.

*Situation géographique et fruits des biotopes de 14 populations naturelles françaises de* Drosophila melanogaster.

*apple / pommes.*

*plum / prunes.*

*others fruits (varied fruits such as tomatoes, melons, peaches) / autres fruits (tels que tomates, melons, pêches...).*

## III. Results

### A. *Allelic frequencies and comparison between the populations studied*

Table 1 gives for each population the number of genes sampled and the allelic frequencies. Homogeneity between populations was tested using a $\chi^2$ analysis (no $\chi^2$ value was available at the *Acph* locus, because the expected allelic numbers of the *S* allele were too small). Table 1 shows that some geographical heterogeneity exists in the frequency distribution of the alleles at every locus, i.e., local differences exist at highly polymorphic loci *(Est-6* and *α-Gpdh)* as is indeed the case at other loci.

### B. *Comparison between our results and those previously published by* GIRARD & PALABOST *(1976)*

Since neither the present populations nor those of GIRARD & PALABOST (1976) were homogeneous, it was not possible to pool them in each group for a comparison

TABLE 1

*Allelic frequencies at 6 polymorphic loci in 14 French populations of* Drosophila melanogaster.

*Fréquences alléliques à 6 locus dans 14 populations françaises de* Drosophila melanogaster.

| Populations | *Acph* | | | *Adh* | | | *Est-C* | | | Locus others |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | *F* | *S* | N | *F* | *S* | N | *F* | *S* | |
| Tostes | 360 | 1.000 | — | 360 | 1.000 | — | 360 | 0.994 | 0.006 | — |
| Le haras du pin | 256 | 0.977 | 0.023 | 256 | 0.984 | 0.016 | 256 | 0.922 | 0.051 | 0.027 |
| Sainte-Geneviève-des-Bois | 164 | 1.000 | — | 152 | 0.960 | 0.040 | 162 | 0.957 | 0.043 | — |
| Rannée | 216 | 0.958 | 0.042 | 216 | 0.986 | 0.014 | 216 | 0.995 | 0.005 | — |
| Nevez | 216 | 1.000 | — | 216 | 0.995 | 0.005 | 216 | 0.995 | 0.005 | — |
| Chateaubriant | 212 | 1.000 | — | 212 | 0.991 | 0.009 | 210 | 0.952 | 0.048 | — |
| Menetrol-sous-Sancerre | 216 | 1.000 | — | 216 | 0.949 | 0.051 | 216 | 0.958 | 0.042 | — |
| Bonnac-la-Côte | 216 | 0.995 | 0.050 | 210 | 0.938 | 0.062 | 216 | 0.926 | 0.074 | — |
| Chessy-les-Mines | 208 | 0.981 | 0.019 | 216 | 0.940 | 0.060 | 224 | 0.897 | 0.103 | — |
| Beynost | 206 | 1.000 | — | 206 | 0.888 | 0.112 | 206 | 0.913 | 0.078 | 0.009 |
| Le Curtelod | 222 | 1.000 | — | 220 | 0.941 | 0.059 | 222 | 0.986 | 0.014 | — |
| Allevard | — | — | — | 236 | 0.932 | 0.068 | 240 | 0.987 | 0.013 | — |
| Montauban | 214 | 0.991 | 0.009 | 216 | 0.954 | 0.046 | 216 | 0.935 | 0.065 | — |
| Tautavel | 216 | 1.000 | — | 214 | 0.977 | 0.023 | 216 | 0.931 | 0.069 | — |
| Total or mean | 2922 | 0.992 | 0.008 | 3146 | 0.962 | 0.038 | 3176 | 0.955 | 0.042 | 0.003 |
| $\chi^{2a}$ | | — | | | 80.00** (13) | | | 82.75** (13) | | |

N : number of genes sampled ; *F, S,* others : alleles ; populations classified according to their latitude from North to South ; a) $\chi^2$ values for homogeneity between the 14 populations studied ; degrees of freedom in brackets ; ** significant at $P < 0.01$.

using the $\chi^2$ analysis. Thus the comparison was carried out by the non-parametric MANN-WHITNEY U test on the common allele at every locus. Table 2 gives at 5 loci (*Pgm* was not studied by GIRARD & PALABOST) the number of populations, the mean allelic frequencies, the differences between the averages and the observed U values. Except for *Est-C*, the gene frequencies among the 5 loci studied are uniform between populations originating from the Saône and Rhône Valleys (wine-cellar populations) and populations more widely distributed throughout France (fruit populations). Fruit populations analyzed here appear to be characterized only by a higher frequency of the *F* allele at the esterase-C locus.

### C. *Correlation between gene frequencies and latitude*

Because of the overlap between the latitudinal distribution and the nature of the fruit in our populations (fig. 1), it was necessary to examine the latitudinal relationships of allelic frequencies before analyzing the role of the fruit in the differentiation of gene frequencies. Statistical analysis was carried out by SPEARMAN's rank correlation test on the common allele at each locus (table 3).

TABLE 1 (continuation)

| *Est-6* | | | | *α-Gpdh* | | | | *Pgm* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N | *F* | *S* | others | N | *F* | *S* | others | N | *F* | *S* | others |
| 360 | 0.122 | 0.878 | — | 360 | 0.797 | 0.203 | — | — | — | — | — |
| 256 | 0.316 | 0.684 | — | 256 | 0.492 | 0.508 | — | — | — | — | — |
| 154 | 0.214 | 0.779 | 0.007 | 164 | 0.622 | 0.378 | — | 164 | 0.951 | 0.049 | — |
| 216 | 0.037 | 0.963 | — | 214 | 0.430 | 0.570 | — | 216 | 0.838 | 0.162 | — |
| 216 | 0.023 | 0.949 | 0.028 | 216 | 0.380 | 0.620 | — | 214 | 0.953 | 0.047 | — |
| 212 | 0.415 | 0.566 | 0.019 | 210 | 0.419 | 0.581 | — | 212 | 0.882 | 0.118 | — |
| 216 | 0.361 | 0.616 | 0.023 | 200 | 0.530 | 0.470 | — | 216 | 0.856 | 0.144 | — |
| 210 | 0.290 | 0.667 | 0.043 | 216 | 0.648 | 0.347 | 0.005 | 216 | 0.894 | 0.106 | — |
| 226 | 0.372 | 0.619 | 0.009 | 224 | 0.540 | 0.460 | — | 226 | 0.907 | 0.089 | 0.004 |
| 206 | 0.369 | 0.597 | 0.034 | 206 | 0.621 | 0.379 | — | 204 | 0.848 | 0.152 | — |
| 222 | 0.257 | 0.734 | 0.090 | 220 | 0.504 | 0.496 | — | 166 | 0.958 | 0.042 | — |
| 238 | 0.160 | 0.840 | — | 238 | 0.714 | 0.286 | — | — | — | — | — |
| 216 | 0.296 | 0.695 | 0.009 | 216 | 0.569 | 0.431 | — | 216 | 0.949 | 0.051 | — |
| 216 | 0.157 | 0.829 | 0.014 | 216 | 0.574 | 0.426 | — | 216 | 0.940 | 0.060 | — |
| 3164 | 0.237 | 0.750 | 0.013 | 3156 | 0.570 | 0.430 | 0.0003 | 2266 | 0.905 | 0.095 | 0.0004 |
| | 270.12** (13) | | | | 186.39** (13) | | | | 49.79** (10) | | |

A correlation between *F* allelic frequency and latitude is observed at the *Adh* locus (r = 0.618). This latitudinal relationship is well known over small distances (GROSSMAN *et al.*, 1970 ; PIPKIN *et al.*, 1973) as over larger ones (ANDERSON, 1981 ; JOHNSON & SCHAFFER, 1973 ; OAKESHOTT *et al.*, 1982). The 5 other coefficients are not statistically significant. This means that for these loci *(Acph, Est-6, Est-C,* α*-Gpdh* and *Pgm)* a comparison between the populations, grouped according to the kind of fruit in the collecting localities, is available.

TABLE 2

*Allele frequencies at five polymorphic loci in populations studied by us and in studies by* GIRARD & PALABOST *(1976).*

*Comparaison entre nos résultats et ceux précédemment publiés par* GIRARD & PALABOST *(1976).*

| Locus | Alleles or number of populations | Populations of the Saône and Rhône valleys (wine-cellar populations, from GIRARD and PALABOST, 1976) | Populations originating from different regions of France (fruit populations ; present study) | Comparison | |
|---|---|---|---|---|---|
| | | | | d | U |
| *Acph* | n ..... | 15 | 13 | | |
| | *F* ..... | 0.991 ± 0.002 | 0.992 ± 0.003 | 0.001 | 77 |
| | *S* ..... | 0.009 ± 0.002 | 0.008 ± 0.003 | | |
| *Adh* | n ..... | 15 | 14 | | |
| | *F* ..... | 0.959 ± 0.005 | 0.962 ± 0.007 | 0.003 | |
| | *S* ..... | 0.040 ± 0.005 | 0.038 ± 0.007 | 0.002 | 99 |
| | others ..... | 0.001 ± 0.001 | — | 0.001 | |
| *Est-C* | n ..... | 15 | 14 | | |
| | *F* ..... | 0.883 ± 0.009 | 0.955 ± 0.007 | 0.072 | |
| | *S* ..... | 0.087 ± 0.008 | 0.042 ± 0.007 | 0.045 | 23** |
| | others ..... | 0.030 ± 0.005 | 0.003 ± 0.002 | 0.027 | |
| *Est-6* | n ..... | 15 | 14 | | |
| | *F* ..... | 0.272 ± 0.012 | 0.237 ± 0.015 | 0.035 | |
| | *S* ..... | 0.712 ± 0.012 | 0.750 ± 0.015 | 0.038 | 93.5 |
| | others ..... | 0.016 ± 0.003 | 0.013 ± 0.004 | 0.003 | |
| α*-Gpdh* | n ..... | 15 | 14 | | |
| | *F* ..... | 0.529 ± 0.014 | 0.570 ± 0.017 | 0.041 | |
| | *S* ..... | 0.468 ± 0.014 | 0.430 ± 0.017 | 0.038 | 87 |
| | others ..... | 0.003 ± 0.001 | — | 0.003 | |

n : number of populations ; *F, S,* others : alleles ; d : differences ; U : Mann and Whitney's variable ; ** significant at $P < 0.01$.

TABLE 3

SPEARMAN's *rank correlation coefficients (r) of $Acph^F$, $Adh^F$, $Est\text{-}C^F$, $Est\text{-}6^S$, $\alpha\text{-}Gpdh^F$, $Pgm^F$ frequencies with latitude.*

*Coefficients de corrélation de* SPEARMAN *(r) entre les fréquences des allèles communs et la latitude.*

| Locus | r | d.f. |
|---|---|---|
| *Acph* .... | — 0.209 | 11 |
| *Adh* ..... | — 0.618* | 12 |
| *Est-C* .... | — 0.297 | 12 |
| *Est-6* ..... | — 0.182 | 12 |
| *α-Gpdh* ... | 0.169 | 12 |
| *Pgm* ..... | 0.182 | 9 |

d.f. : degree of freedom ; * significant at $P < 0.05$.

TABLE 4

*Allele frequencies in populations classified by type of fruit.*

*Comparaison entre les populations classées selon la nature du fruit de piégeage.*

| Locus | Alleles or number of populations | Apple | Plum | Other Fruit | H |
|---|---|---|---|---|---|
| *Acph* | n ..... | 5 | 4 | 4 | |
| | *F* ..... | 0.988 ± 0.006 | 0.995 ± 0.005 | 0.997 ± 0.003 | 0.36 |
| | *S* ..... | 0.012 ± 0.006 | 0.005 ± 0.005 | 0.003 ± 0.003 | |
| *Adh* | n ..... | 5 | 4 | 5 | |
| | *F* ..... | 0.988 ± 0.006 | 0.942 ± 0.016 | 0.948 ± 0.013 | 6.12* |
| | *S* ..... | 0.012 ± 0.006 | 0.058 ± 0.016 | 0.052 ± 0.013 | |
| *Est-C* | n ..... | 5 | 4 | 5 | |
| | *F* ..... | 0.974 ± 0.009 | 0.930 ± 0.017 | 0.954 ± 0.012 | |
| | *S* ..... | 0.020 ± 0.008 | 0.068 ± 0.017 | 0.046 ± 0.012 | 3.54 |
| | others .... | 0.006 ± 0.004 | 0.002 ± 0.002 | — | |
| *Est-6* | n ..... | 5 | 4 | 5 | |
| | *F* ..... | 0.142 ± 0.020 | 0.379 ± 0.032 | 0.230 ± 0.025 | |
| | *S* ..... | 0.852 ± 0.020 | 0.600 ± 0.033 | 0.755 ± 0.025 | 10.99** |
| | others .... | 0.006 ± 0.004 | 0.021 ± 0.010 | 0.015 ± 0.007 | |
| *α-Gpdh* | n ..... | 5 | 4 | 5 | |
| | *F* ..... | 0.569 ± 0.028 | 0.527 ± 0.034 | 0.604 ± 0.029 | |
| | *S* ..... | 0.431 ± 0.028 | 0.473 ± 0.034 | 0.395 ± 0.029 | 1.62 |
| | others .... | — | — | 0.001 ± 0.001 | |
| *Pgm* | n ..... | 3 | 4 | 4 | |
| | *F* ..... | 0.911 ± 0.023 | 0.874 ± 0.022 | 0.934 ± 0.017 | |
| | *S* ..... | 0.089 ± 0.023 | 0.125 ± 0.022 | 0.066 ± 0.017 | 3.08 |
| | others .... | — | 0.001 ± 0.001 | — | |

n : number of populations ; *F*, *S*, others : alleles ; H : Kruskal and Wallis's variable ; * : significant at $P < 0.05$ ; ** : significant at $P < 0.01$.

D. *Influence of the type of fruit in the population studied*

The heterogeneity demonstrated in table 1 might be due to the kind of fruit on which the populations had been collected. So we have divided the populations into 3 groups : apples, plums and other fruit (varied fruits such as tomatoes, melons, peaches). Comparison between the populations of the 3 groups was carried out by the KRUSKAL-WALLIS H test on the common alleles (tables have been used for small samples : see BEYER, 1981). Table 4 gives the number of populations, the mean allelic frequencies in the 3 groups and the H values obtained after comparison between these groups. A survey of this table shows significant differences between the 3 groups in the cases of *Adh* and *Est-6* loci. Since for *Adh* a correlation with latitude was mentioned above (table 3) and since latitudinal and fruit distributions of the populations studied here are overlapping, the influence of fruit on the gene frequencies can be taken into account only for the case of *Est-6*. *The Est-6*$^S$ allele has the highest frequency ($0.852 \pm 0.020$) in apple populations, the smallest in plum populations ($0.600 \pm 0.033$) and an intermediate value ($0.755 \pm 0.025$) in the other fruit populations.

## IV. Discussion and conclusion

The results presented here provide a larger description of the biochemical polymorphism in French populations of *Drosophila melanogaster*.

The 14 new populations originating from different geographical areas (fig. 1) show a more or less high level of variability at the 6 loci studied (table 1). Curiously, in spite of this heterogeneity, the patterns of the allelic frequency distribution are similar between these 14 populations and those previously described by GIRARD & PALABOST in 1976 (table 2). Because all of GIRARD & PALABOST'S populations were sampled from the same geographical area, we can note that the extension of the geographical origin of the populations analyzed has not provided a differentiation in the allelic frequency distribution for four enzymatic loci out of the five compared. Moreover, as these new populations have been captured from fruit, it appears that habitats different from wine-cellars have not induced particular patterns of allelic frequencies. Thus, despite the small number of loci studied, it seems that temperate habitats in France do not vary enough to provide much genetic differentiation in the case of enzymatic polymorphism. DAVID (1982) has also analyzed 5 other French populations sampled in different habitats (wine-cellars, fruit or urban habitats) ; 4 of them were located in the same geographical area as those of GIRARD & PALABOST, the 5 th coming from Corsica. At the 4 loci commonly examined by DAVID and us (presently and in the note of 1976) the allelic frequencies are similar. DAVID'S data showed that *F* allele of the *Adh* locus was favored in wine-cellar populations. With our 14 new populations, no significant difference between wine-cellar and fruit populations is observed at *Adh*.

Nevertheless, in regard to the role of the nutritive resources some observations are noteworthy for the fruit populations. The 3 kinds of fruit (apples, plums and others) have induced a genetic differentiation at the *Adh* and *Est-6* loci (table 4), but the result is uncertain for *Adh* because a latitudinal cline is also observed (table 3).

In the case of *Est-6* it should be noted that the *S* allele is at the highest frequency in apple populations ($0.852 \pm 0.020$) and at the lowest in plum populations ($0.600 \pm 0.033$). As can be seen in figure 1, apple populations are all located in the same region of France (North-West) and plum populations in different regions : North-West (population 6), Center (population 7), and South-East (populations 9 and 10). Thus, because the resources have a geographical pattern, further studies are necessary before we can conclude unambiguously as to their role in the differentiation of allelic frequencies at the *Est-6* locus. Whatever the case, this result can argue in favor of the influence of selection on the *Est-6* locus, as has been demonstrated in the laboratory (DANFORD & BEARDMORE, 1980) and in natural (OAKESHOTT *et al.*, 1981) populations.

Up to now, despite some particular local situations, the different studies of French natural populations show that the distributions of the allelic frequencies at most loci are very similar, independently of geographical situation and habitats. However, they differ from populations originating in other continents (OAKESHOTT *et al.*, 1981, 1982, 1983). Different hypotheses can be suggested to explain this result relative to the French populations. First, if migrations are important between the numerous wine-cellar populations (wine-cellars are certainly the most common habitat of *Drosophila melanogaster* in France) and those living in other habitats (such as orchards and kitchen-gardens), this could explain the similarity in the distributions of allozyme frequencies. Secondly, the selective pressures in the different micro-habitats of *Drosophila melanogaster* in temperate countries like France would not be sufficient to allow a great between-populations differentiation at the enzymatic polymorphism level.

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