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Detection of individual variation in enzyme activity in natural populations of *Drosophila melanogaster*

KATALIN PECSENYE[1,2]

[1]*Department of Evolutionary Zoology and Human Biology, KLTE, Debrecen, Hungary*
[2]*Department of Genetics, Umeå University, Umeå, Sweden*

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The method allows the determination of the activity level of enzymes in a single fly and assessing the genetic composition of the given individual at these enzyme loci. Three isofemale lines were constructed which were monomorphic at several enzyme loci. Samples were prepared in two different ways: (i) individual samples — individuals were homogenised separately; (ii) collective samples — a common homogenate was prepared from several individuals. Oregon-R strain was also used to prepare a standard homogenate. The activities of alcohol dehydrogenase (ADH), α-glycerophosphate dehydrogenase (αGPDH), isocitrate dehydrogenase (IDH), and 6-phosphogluconate dehydrogenase (6PGDH), were measured in each sample on starch gel after the proteins were separated by electrophoresis. Enzyme activities were assessed by the optical density of the bands. Gel and position weights were estimated on the basis of the statistical analyses of the activities measured in the standard samples. Gel weights were then used to account for the activity differences among the gels while position weights were applied to correct for the general tendencies in the activities observed within the gels. The gel and position weighted activities of individual and collective samples were compared in the isofemale lines. The individual samples had approximately two times as much variation as the collective samples for all four enzymes. The electrophoretic method is sensitive enough to study the structure of the phenotypic variation in enzyme activity in the natural populations. The total variation among the standard samples was close to the within subline component of variation obtained for the collective samples (measurement error). This shows that the standard samples can be used to estimate the size of the measurement error.

Katalin Pecsenye, Department of Evolutionary Zoology, KLTE, Debrecen, Egyetem tér 1, HU-4010 Hungary

Enzyme activity is a phenotypic trait determined by different genetic and environmental factors. Different alleles at the structural gene and regulatory genes affect the amount or the stability of enzyme molecules (AYALA and MCDONALD 1980). An important environmental factor is the metabolic flux along the pathway which includes an enzyme. Metabolic flux, the efficiency of the conversion of the first substrate in the pathway to the end product, is influenced by the activities of the enzymes participating in the pathway (MIDDLETON and KACSER 1983; CLARK 1991). On the other hand, however, changes in the flux through one ore more regulatory enzymes of the pathway might have a general influence on the activities of the other participating enzymes (CLARK and KOEHN 1992). That is, there is a certain level of correlation among the activities of the enzymes involved in the same metabolic pathway.

In the study of enzyme activity variation in natural populations, one has to determine the proportion of the genetic and environmental components of variation. In order to do so, one must measure the variation in enzyme activity among the individuals (PIERCE and CRAWFORD 1994). Since *Drosophila melanogaster* adults and larvae are equally small, it has only been possible to study enzyme activity variation among different isogenic lines originating from natural populations. The results show that natural *D. melanogaster* populations exhibit a considerable amount of genetic variation in enzyme activity (LEWIS and GIBSON 1978; LAURIE-AHLBERG et al. 1980, 1982). For some enzymes (e.g., ADH), a large portion of this genetic variation could be attributed to regulatory genes (LAURIE and STAM 1994; MERCOT 1994). WILTON et al. (1982) and CLARK and KEITH (1988) have also detected correlations among the activities of enzymes which are involved in the same metabolic pathways.

Here I show the possibility of assessing individual variation in enzyme activity in a *D. melanogaster* population by using simple gel electrophoresis and measuring the activity of the enzymes on the gel after the separation of the protein molecules. Electrophoresis is traditionally used for qualitative purposes, i.e., to compare the patterns of enzyme variants in different individuals. It is, however, difficult to obtain accurate quantitative estimates of the activity of the enzymes on the gel. Nevertheless, MCINTYRE (1971) proved that gel assays can be used in the comparison of the activity levels of acid phosphatases in different *Drosophila* species. Our previous studies (PECSENYE et al. 1994a,b, 1996) on the alcohol induced changes in the activities of certain enzymes in laboratory strains of *D. melanogaster*

have also shown that, with internal controls in the experiments, the electrophoretic method of measuring enzyme activities can be satisfactorily used for comparative purposes. Is it possible to standardise the conditions of the whole set of procedures (sample preparation, electrophoresis, and staining) so that one may analyse the structure of variation in enzyme activity in a natural *D. melanogaster* population? The method I present here seems to be suitable both for measuring the activity level of particular enzymes in a single fly and to determine the genetic composition of an individual fly at these enzyme loci.

MATERIALS AND METHODS

Samples

Samples from a natural population. — Several isofemale lines (IFL) were established from a natural *Drosophila melanogaster* population (Tiszaszolos, Hungary, 1996). In the F_1 generation, five groups of inseminated females (2–4) were randomly chosen and used to form 5 sublines for each IFL. The genotypic constitution of all sublines were determined electrophoretically at certain enzyme loci: Alcohol dehydrogenase (*Adh*), α-Glycerophosphate dehydrogenase (*αGpdh*), Isocitrate dehydrogenase (*Idh*), and 6-Phosphogluconate dehydrogenase (*6Pgdh*). Three IFLs were found to have three sublines completely homozygous for the most common allele at these loci. The genotypic constitution of all nine sublines was: Adh^{FF}-$\alpha Gpdh^{FF}$-Idh^{SS}-$6Pgdh^{SS}$. In the F_6 generation, 6–8 days old females were collected from each of the 9 sublines in two different ways: (i) 6 females were put together to obtain the collective samples; (ii) 6 further females were taken separately to obtain the individual samples. All samples were then frozen until the electrophoresis.

Standard strains. — The Oregon-R strain kept in the Umeå Drosophila Stock Center was used as a standard strain in this study. The strain has the following allele combinations at the four investigated loci: Adh^{FF}-$\alpha Gpdh^{SS}$-Idh^{FF}-$6Pgdh^{SS}$.

Enzyme studies

Four enzymes were surveyed in all samples, alcohol dehydrogenase (ADH), α-glycerophosphate dehydrogenase (αGPDH), isocitrate dehydrogenase (IDH), and 6-phosphogluconate dehydrogenase (6PGDH). The enzyme activities were measured on starch gel after the proteins were separated by electrophoresis.

Sample preparation. — (1) Standard samples (Fig. 1A): 80 females (approx. 7 days old) were collected from the Oregon-R strain and homogenised in 2.4 ml (30 µl/individual) of the following buffer: 0.01M Tris/HCl (pH = 7.5) and 2 mg/ml Dithiotreitol. After homogenisation the standard sample was centrifuged (12000 rpm, 10 min) and the supernatant was distributed into several 60 ml aliquots. These aliquots were then separately frozen until electrophoresis. (2) Samples from the natural population (Fig. 1B): (a) Collective samples: the 6 females were homogenised in 300 µl (50 µl/individual) of the above buffer. Then the collective samples were centrifuged (12000 rpm, 10 min). (b) Individual samples: each female was separately homogenised in 50 µl of the homogenising buffer. The individual samples were also centrifuged (12000 rpm, 10 min).

Electrophoresis and enzyme assays. — One gel contained the 6 samples from one of the sublines of all three IFLs. These 18 samples were randomly arranged on each gel. In addition, every gel had 5 standard samples at permanent positions: 1, 7, 13, and 23. From all samples, 10 µl of the supernatant was applied on the gels. The starch gel electrophoresis was always carried out in exactly the same way throughout the whole study. After electrophoresis the gel was sliced and four different slices 5 were systematically stained for four different enzymes. The conditions of the staining procedures were strictly controlled. The buffer systems, the electrophoretic conditions, and the staining solutions are given in APPENDIX 1. After staining, the gels were immersed in distilled water and photographed immediately. The photographic negatives were scanned with Scanmaker E6 and the images of the gels were analysed by Gel Pro TM analyzer ver.2.0 programme package. For each sample, the enzyme activities were characterised by the sum of the total optical densities of the individual bands.

Statistical procedures

The data were analysed with generalised linear models under the assumption of constant coefficient of variation, i.e., I specified gamma error distribution coupled with identity link function (see McCULLAGH and NELDER 1989).

Standard samples. — Enzyme activities measured in the standard samples (standard activities) were used to standardise the activity values obtained for the collective and individual samples across the six gels and over the different sample positions within a gel. The first models constructed for the standard data only contained gel as main factor (CRAWLEY 1993; FRANCIS et al. 1994). Based on these models, special weights (gel weights) were calculated for the different enzymes to account for the variation among the gels.

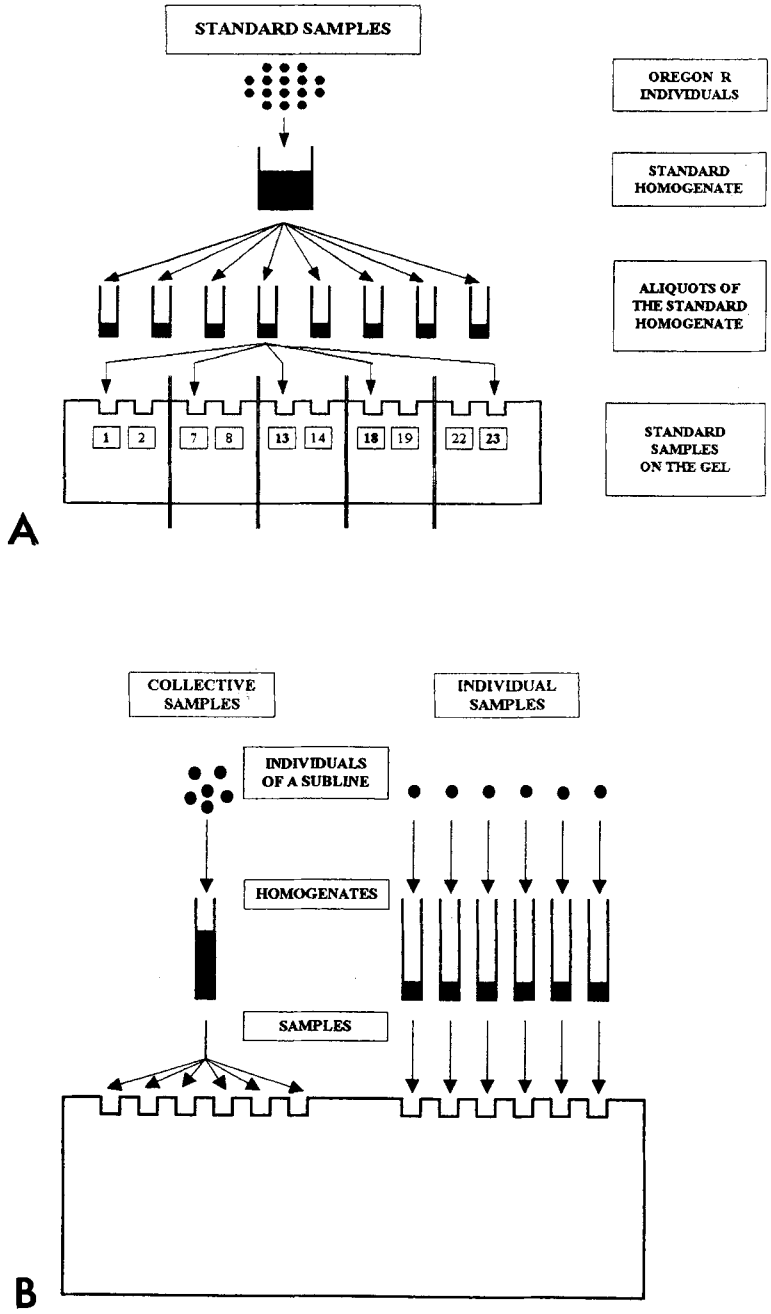

Fig. 1A and B. **A** Preparation of the standard samples. **B** Differences in the preparation between the collective and individual samples.

The grand mean of the standard activity values had 1.000 weight and the gel weight values were actually the ratios between the grand mean and the gel averages.

The next step was to test whether the position of the samples within the gels had any effect on enzyme activities. The scatter plots of the gel weighted standard activity values against the position of the samples on the gels showed that except for 6PGDH, there was a clear correlation between these variables. This correlation, however, was different for GPDH, ADH, and IDH. Consequently, different models were constructed to analyse the gel weighted standard activities of these three enzymes. All models were simple regression models with sample positions as independent variables and the gel weighted standard activity values as dependent variables. The slopes of the different regression lines were then used to calculate

the position weights for all three enzymes to account for the activity differences due to the positions of the samples on the gels (APPENDIX 2).

Samples from the natural populations. — The original activity values of all enzymes both for the collective and individual samples were first corrected for the differences among the gels using gel weights. The gel weighted activity data for αGPDH, ADH, and IDH were further corrected using position weights to account for the variation due to the position of the samples on the gel. These gel and position weighted data were then used in nested analyses of deviance. Separate models were constructed for the data obtained for the collective and individual samples. The models contained IFLs as main factor and the sublines (SL) were nested within the specific IFLs. In this way, the total variation in enzyme activity was partitioned into between IFL, within IFL (i.e., between SL), and within SL variation.

RESULTS AND DISCUSSION

Activity variation in the standard samples at different stages of the standardisation

In order to test the efficiency of the standardisation process, all original standard activity values were weighted in two consecutive steps: gel weights were applied first (gel weighted activities), and then position weights were used (gel and position weighted activities). These three sets of the standard data were used in separate deviance analyses. The total deviance of the standard activities of all four enzymes decreased considerably after accounting for the variation among the gels (Table 1: 46.5% decrease for αGPDH, 42.0% for ADH, 54.1% for IDH, and 63.5% for 6PGDH). The position of the samples on the gel did not influence the activities of 6PGDH. For the other 3 enzymes, however, the total deviance decreased further after accounting for the activity differences due to the sample positions on the gels (13.4% decrease for αGPDH, 27.6% for ADH, and 12.4% for IDH). I therefore concluded that both the gel weights and the position weights calculated on the basis of the statistical models constructed for the standard data were satisfactory to correct the enzyme activities obtained for the other samples.

Table 1. *Total deviance for the enzyme activities of the standard samples using the original and corrected data sets. Gel weighted data: the activities of the individual samples were corrected for the differences among the gels. Pos. & gel weighted data: the gel weighted data were further corrected for activity trends within the gels (for detais see the text). For 6PGDH activity no obvious trend was observed within the gels*

Total deviance	αGPDH	ADH	IDH	6PGDH
Original data	3.159	4.855	3.276	7.607
Gel weighted data	1.689	2.814	1.505	2.778
Pos. & gel weighted data	1.266	1.476	1.098	–

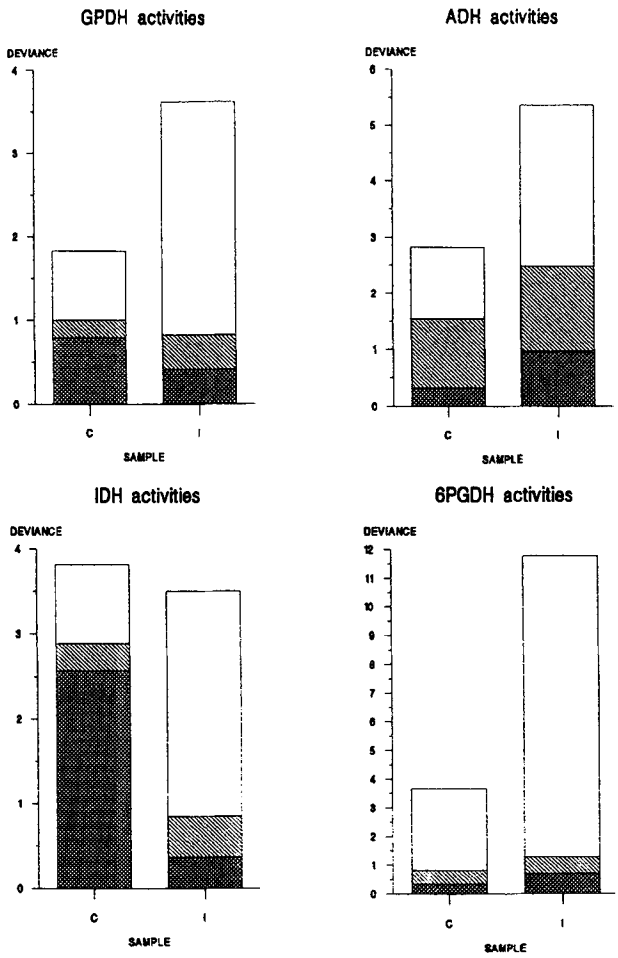

Fig. 2. Distribution of the total deviance at different levels of the hierarchy. C: collective samples; I: individual samples. Empty part of the column: within subline component; hatched part of the column: between subline component (i.e., within isofemale line component); cross-hatched part of the column: between isofemale line component.

The structure of variation in enzyme activities in the collective and individual samples

Except for IDH, the individual samples exhibited far larger variation in enzyme activity than the collective samples (Fig. 2 and Table 2: total deviance). It was especially clear for 6PGDH. For all enzymes, the within subline component of variation represented a greater portion of the total deviance in the individual samples compared with the collective samples (Fig. 1). Nevertheless, the structure of variation was simi-

Table 2. *Percent of the total deviance at different levels of the sample hierarchy for the enzyme activities using gel and position weighted data. IFL: isofemale line; SL: subline; Collective: analysis for the activities measured in the collective samples; Individual: analysis for the activities measured in the individual samples*

Enzyme	Sample	Total deviance	% of total deviance		
			Between IFL	Within IFL*	Within SL
αGPDH	Collective	1.830	43.2	11.6	45.2
	Individual	3.622	11.3	11.5	77.2
ADH	Collective	2.818	11.3	43.4	45.3
	Individual	5.355	18.0	28.2	53.8
IDH	Collective	3.822	67.2	8.4	24.4
	Individual	3.499	10.4	13.8	75.8
6PGDH	Collective	3.628	9.3	12.7	78.9
	Individual	11.763	5.8	5.1	89.1

* Within IFL = Between SL

lar in the collective and individual samples for ADH and 6PGDH (Fig. 2 and Table 2). In the collective samples, considerable activity differences were observed among the IFLs for all enzymes (Table 3). In contrast, there was no significant difference among the IFLs in their GPDH, IDH, and 6PGDH activities in the individual samples (Table 3).

When comparing the distribution of variation in the collective and individual samples, it is important to note the difference in the composition of the within subline component of variation between these two sets of samples. In the individual samples, this component consists of the true variation among the individuals originating from the same subline and the measurement error. At the same time, the within subline component of variation in the collective samples only represents the measurement error among the subsamples of the same homogenate prepared from a subline. The results of the deviance analyses for all enzymes show that both the amount of variation (total deviance) and the within subline component of the total variation were far greater in the individual samples than in the collective samples. This shows that the electrophoretic method, together with an appropriate standardisation process, is sensitive enough to study the individual variation in enzyme activity in natural populations.

Table 4. *Deviances for the four enzyme activities obtained in different analyses. Gel and position weighted data were used in all three analyses except for 6PGDH*

Samples, deviance	αGPDH	ADH	IDH	6PGDH
Standard samples total deviance	1.266	1.476	1.098	2.778
Collective samples within SL deviance	0.826	1.276	0.933	2.864
Individual samples within SL deviance	2.797	2.882	2.653	10.498

The results obtained for the individual samples show that individual variation makes up a considerably large component of the total activity variation. As a consequence, the differences among the families (IFL) compared with the variation within the subfamilies (i.e., among the individuals of the same subline) were only significant for ADH in the individual samples (Tables 2 and 3). At the same time, the within subline component of the collective samples (measurement error) was relatively smaller. The differences among the families (between IFL) therefore seemed to be significant for all four enzymes (Tables 2 and 3). These results clearly show that in the study of the structure of variation in enzyme activity in natural populations, the sampling method greatly influences the outcome of the analysis. It appears that

Table 3. *Predicted values of enzyme activities together with their approximate standard errors in the three isofemale lines. IFL: isofemale line; Coll.: activities in the collective samples; Ind.: activities in the individual samples. Bold characters indicate that the enzyme activity is significantly lower in the given IFL compared with the others*

IFL	αGPDH		ADH		IDH		6PGDH	
	Coll.	Ind.	Coll.	Ind.	Coll.	Ind.	Coll.	Ind.
1	**8.88** (0.33)	11.12 (0.67)	**7.46** (0.39)	**5.05** (0.35)	**14.15** (0.52)	14.48 (0.86)	2.465 (0.148)	1.950 (0.214)
2	9.86 (0.47)	9.95 (0.89)	8.93 (0.61)	6.98 (0.60)	22.22 (0.97)	12.70 (1.12)	**2.065** (0.193)	1.487 (0.269)
3	12.72 (0.54)	9.78 (0.86)	8.60 (0.59)	5.76 (0.53)	23.28 (1.01)	13.67 (1.18)	2.361 (0.205)	1.645 (0.280)

the collective way of sampling individuals within a subfamily biased the distribution of variation so that the standard errors were too small, and therefore the family means (mean enzyme activities of the IFLs) were distinguished as significantly different values.

Comparison of the standard and collective samples

When studying phenotypic variation in a population, it is of paramount importance to estimate the degree of measurement error. In this study, the within subline component of variation determined for the collective samples can be considered as an approximation of the measurement error. It is, however, rather laborious to work with collective and individual samples in parallel from all isofemale lines or sublines in a population study. Hence, I was looking for a more straightforward way of the estimation of measurement error in this survey. Comparing the total deviance obtained for the gel and position weighted standard data and the within subline component of variation among the collective samples, I found that they were very similar (Table 4). Actually, the total variation in the standard samples was slightly higher than the within subline component for the collective samples. It implies that the standard samples were not only essential for the standardisation of the activity values obtained on different gels and at various sample positions but could also be used for an approximate estimation of the measurement error.

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APPENDIX 1

Electrophoretic conditions

Electrode buffer: 0.28 M Tris – 86 mM citric acid (pH = 7.5); gel buffer: 1:28 dilution of the electrode buffer. Running conditions: 14 V/cm, 6-7°C, 3.5 h.

Staining conditions

ADH assay: 0.1 M Tris-HCl (pH = 8.5), 1 mM EDTA, 0.6 mM NAD, 0.25 mM nitro blue tetrazolium (NBT), 0.1 mM phenazine methosulfate (PMS), 2 v% ethanol, 2 v% butanol; 40 min at 37°C in the dark.

αGPDH assay: 0.1 M Tris-HCl (pH = 8.5), 1 mM EDTA, 0.6 mM NAD, 0.25 mM NBT, 0.1 mM PMS, 12 mM DL-α-glycerophosphate; 30 min at 37°C in the dark.

IDH assay: 0.1 M Tris-HCl (pH = 8.5), 5 mM $MgCl_2$, 0.25 mM NADP, 0.25 mM NBT, 0.1 mM PMS, 4 mM DL-isocitric acid; 40 min at 37°C in the dark.

6PGDH assay: 0.1 M Tris-HCl (pH = 8.0), 5 mM $MgCl_2$, 0.25 mM NADP, 0.25 mM NBT, 0.1 mM PMS, 2 mM 6-phosphogluconic acid; 60 min at 37°C in the dark.

APPENDIX 2

The formula for the calculation of the position weights (wp) was:

$$w_p = \frac{a}{a + x \cdot s}$$

where: a Predicted value of the enzyme activity at a specific position of the standard samples which was the starting point of the regression line. At this specific position, the value of the position weight was 1.000. For GPDH, this specific position was 13 (predicted activity: 14.38). For ADH and IDH, it was 7 (predicted ADH activity at this position was 9.014, while the predicted IDH activity was 21.827).

x Number of positions between the specific position (see above) and the sample position in question.

s Slope of the regression line.

REFERENCES

Ayala FJ and McDonald JF, (1980). Continuous variation: Possible role of regulatory genes. Genetica 52/53: 1–15.

Clark AG (1991). Mutation-selection balance and metabolic control theory. Genetics 129: 909–923.

Clark AG and Keith LE, (1988). Variation among extracted lines of Drosophila melanogaster in triacylglycerol and carbohydrate storage. Genetics 119: 595–607.

Clark AG and Koehn RK, (1992). Enzymes and adaptation. In: Genes in Ecology (eds RJ Berry, TJ Crawford and GM Hewitt) Blackwell Scientific Publ., London, p. 193–228.

Crawley MJ, (1993). GLIM for Ecologists. Blackwell Scientific Publications, London.

Francis B, Green M and Payne C, (1994). GLIM 4 The statistical system for generalized linear interactive modelling. Oxford University Press, New York.

Laurie CC and Stam LF, (1994). The effect of an intronic polymorphism on alcohol dehydrogenase expression in Drosophila melanogaster. Genetics 138: 379–385.

Laurie-Ahlberg CC, Maroni G, Bewley GC, Lucchesi JC and Weir BS, (1980). Quantitative genetic variation of enzyme activities in natural populations of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 77: 1073–1077.

Laurie-Ahlberg CC, Wilton AN, Courtsinger JW and Emigh TH, (1982). Naturally occurring enzyme activity

variation in Drosophila melanogaster. I. Sources of variation for 23 enzymes. Genetics 102: 191–206.

Lewis N and Gibson J, (1978). Variation in amount of enzyme protein in natural populations. Biochem. Genet. 16: 159–170.

McCullagh P and Nelder JA, (1989). Generalized Linear Models. Second edition, Chapman and Hall, London.

McIntyre RC (1971). A method for measuring activities of acid phosphatases separated by acrylamide gel electrophoresis. Biochem. Genet. 5: 45–56.

Mercot H (1994). Phenotypic expression of ADH regulatory genes in Drosophila melanogaster: a comparative study between a palearctic and a tropical population. Genetica 94: 37–41.

Middleton RJ and Kacser H, (1983). Enzyme variation, metabolic flux and fitness: Alcohol dehydrogenase in Drosophila melanogaster. Genetics 105: 633–650.

Pecsenye K, Lefkovitch LP, Giles BE and Saura A, (1994a). The influence of the Odh-Aldox region of the third chromosome on the response of Drosophila melanogaster to environmental alcohol. Hereditas 121: 225–236.

Pecsenye K, Lefkovitch LP, Giles BE and Saura A, (1994b). Does Drosophila melanogaster use ethanol as an energy source during starvation? Hereditas 121: 237–248.

Pecsenye K, Lefkovitch LP, Giles BE and Saura A, (1996). Differences in environmental temperature, ethanol and sucrose associated with enzyme activity and weight changes in Drosophila melanogaster. Insect Biochem. Mol. Biol. 26: 135–146.

Pierce VA and Crawford DL, (1994). Rapid enzyme assays investigating the variation in the glycolytic pathway in field-caught populations of Fundulus heteroclitus. Biochem. Genet. 32: 315–330.

Wilton AN, Laurie-Ahlberg CC, Emigh TH and Courtsinger JW, (1982). Naturally occurring enzyme activity variation in Drosophila melanogaster. II. Relationships among enzymes. Genetics 102: 207–221.