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Note

Adaptive significance of amylase polymorphism in *Drosophila*. Analysis of the association between tissue-specific expression and specific activity in Amy^S or Amy^F genotypes of *Drosophila subobscura*

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Abstract – The phenotypic variability at the level of the specific activity of α-amylases and their tissue-specific expression in the midgut of adult *Drosophila subobscura* flies, homozygous for the Amy^S or Amy^F allele, was analysed. The results indicate a homogeneous distribution of the phenotypes with a different numbers of α-amylase activity regions in the adult midgut between the lines homozygous for Amy^S and Amy^F alleles. The mean number of α-amylase midgut activity differs significantly only between the groups of lines homozygous for Amy^S, with the specific activity of the enzyme above the average, and the groups of Amy^F homozygote with a significantly lower mean specific activity of amylase. The analysis suggests the existence of compensation between the number of active regions and the specific activity of α-amylase within Amy^S and Amy^F lines. © Inra/Elsevier, Paris

***Drosophila* / amylase / tissue-specific expressions / specific activity of the enzymes / polymorphisms**

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Résumé – Signification adaptative du polymorphisme de l'amylase chez *Drosophila*. Analyse de l'association entre l'expression tissulaire et l'activité spécifique des génotypes Amy^S et Amy^F chez *Drosophila subobscura*. La variabilité phénotypique de l'activité amylasique dans l'intestin moyen de *Drosophila subobscura* a été analysée dans des lignées homozygotes pour l'allèle Amy^S ou Amy^F. Dans les deux lignées on observe les mêmes phénotypes comportant un nombre variable de régions où l'amylase est exprimée. Globalement, l'activité amylasique est significativement différente entre les lignées homozygotes pour Amy^S, activité spécifique supérieure à la valeur moyenne, et Amy^F, valeur inférieure à la valeur moyenne. L'analyse suggère l'existence d'une compensation entre le nombre de régions actives et l'activité enzymatique spécifique dans ces lignées.

***Drosophila* / amylase α / expression tissulaire / enzyme / polymorphisme**

1. INTRODUCTION

Besides structural gene polymorphism, analyses of enzyme systems in eukaryotes reveal the existence of polymorphism in tissue-specific enzyme expression. Various kinds of regulatory genes have different effects on tissue-specific, developmental and quantitative expression of the enzymes coded from structural genes. Because differences in morphological, biochemical and physiological characteristics, as well as differences between species, which appear despite similarities in the protein structure, originate from changes in the polygenic complex of regulatory genes, examination of their variability is of importance.

α-Amylase in *Drosophila*, active in the midgut and hemolymph, is a well-known model suitable for analysing the adaptations of organisms to different environmental conditions, and for examining the general biological significance of genetic diversity in natural populations of different organisms. α-Amylase polymorphism includes both the variability of the structural *Amy* locus and the variability of tissue-specific expression [9]. The latter type of variability is represented by the number and position of the amylase activity regions in the midgut [5]. Inter- and intrapopulation variability exists both for the number and position of the active regions in adult midgut [3, 12]. At the phenotypic level the *Amy* locus variability is associated with the specific activity of the enzyme α-amylase. Physicochemical conditions for the optimal activity of α-amylase are species-specific [8].

The present report gives an analysis of the phenotypic variability of genotypes homozygous for the Amy^S and Amy^F allele of the *Amy* locus at the level of tissue-specific expression, as reflected in the number of active midgut regions and the specific activity of amylase in *Drosophila subobscura* adults.

2. MATERIALS AND METHODS

Drosophila subobscura lines homozygous for the Amy^S (S) or Amy^F (F) alleles, inbred for 20 generations in optimal laboratory conditions en masse, were taken for dissection of the midgut and for the specific enzyme activity assay. Determination of the specific activity of α-amylase was carried out according to the method described by Noelting and Bernfeld [11]. Midgut dissection and α-amylase activity pattern were performed according to Abraham and Doane [1].

The results were analysed for each line of *Drosophila subobscura* homozygous for Amy^S and Amy^F. Midgut dissection was performed with 12 to 15 flies per line, and α-amylase activity pattern was analysed with 50 flies in three replicates per line.

The digestive function of the α-amylase enzyme is present in the AMG (anterior) and PMG (posterior) parts of the *Drosophila* midgut owing to suitable pH values in those parts. The α-amylase activity can be detected in a maximum of three AMG regions and two PMG regions.

Parametric tests (chi-square [χ^2] and Student's) and non-parametric tests (Mann-Whitney, Kruskal-Wallis analysis of variance and correlation) were used for the analysis of the results. In this way, the variability in the number of active regions and the specific activity of the enzyme, as parameters, were analysed within and between the Amy^S and Amy^F genotypes. Line grouping was performed according to deviations outside $\pm$ 2 standard errors (SE) from the mean value of the observed parameter. In this way, three categories of lines were made for the number of active regions and three for the specific activity of the enzyme.

3. RESULTS

Results of the analysis of 37 lines homozygous for the Amy^S allele and 19 lines homozygous for the Amy^F allele with respect to the phenotypic variability of the total number of active midgut regions are shown in *table I.* According to the previous results [2], there is no difference between the sexes in their MAP variability, so the data for sexes are pooled in this analysis.

Table I. Frequencies (P) of the number of α-amylase active regions of *Drosophila subobscura* midgut in the lines homozygous for the 'slow' (S) and 'fast' (F) amylase allele.

NAR	S/S		F/F	
	n	P	n	P
5	144	0.277	74	0.322
4	78	0.150	48	0.209
3	153	0.294	62	0.270
2	106	0.204	34	0.148
1	39	0.075	12	0.052
	520	1.000	230	1.000

NAR = number of active regions.

On average, lines homozygous for the Amy^F allele have more active regions (3.577 ± 0.109) than the group of S/S lines (3.318 ± 0.134). It is indicative that for the S/S genotype the most abundant phenotypes (29.4 %) are the ones with three active regions, while F/F genotypes have 32.2 % flies with five active regions. In the lines of both genotypes flies with only one active region are the least frequent (7.5 % for S/S and 5.2 % for F/F genotype).

Table II. The mean number ± 2 standard errors (SE) and the frequencies (P) of phenotypes with different numbers of active regions (NAR) in the midgut of the slow (S/S) and/or fast (F/F) genotype *Drosophila subobscura*.

	NAR (P)					
S/S line	5	4	3	2	1	NAR < NAR ± 2 SE
44	0.000	0.231	0.308	0.308	0.154	2.615 ± 0.290
40/10/8A	0.000	0.000	0.286	0.429	0.286	2.000 ± 0.210
33/2/13	0.000	0.000	0.533	0.333	0.133	2.400 ± 0.190
40/10/2/1	0.000	0.091	0.818	0.091	0.000	3.000 ± 0.135
87/7/9	0.214	0.143	0.143	0.429	0.071	3.000 ± 0.363
6/10	0.077	0.231	0.385	0.154	0.154	2.923 ± 0.329
64	0.250	0.063	0.188	0.375	0.125	2.938 ± 0.359
15	0.071	0.143	0.286	0.214	0.286	2.500 ± 0.344
11/2/9	0.000	0.000	0.429	0.357	0.214	2.214 ± 0.214
82	0.000	0.000	0.615	0.308	0.077	2.538 ± 0.183
38/8/13	0.000	0.286	0.071	0.500	0.143	2.500 ± 0.292
39/1	0.250	0.000	0.000	0.583	0.167	2.583 ± 0.434
87/7/2	0.091	0.091	0.091	0.727	0.000	2.545 ± 0.312
1	0.000	0.267	0.467	0.133	0.133	2.867 ± 0.256
28	0.200	0.000	0.267	0.467	0.067	2.800 ± 0.327
67	0.000	0.154	0.615	0.231	0.000	2.923 ± 0.178
46	0.077	0.154	0.231	0.385	0.154	2.615 ± 0.331
53	0.000	0.071	0.643	0.214	0.071	2.714 ± 0.194
						2.649 ± 0.076
						NAR ↔ NAR ± 2 SE
5/4/8/1	0.286	0.071	0.500	0.071	0.071	3.429 ± 0.327
68	0.429	0.143	0.071	0.143	0.214	3.429 ± 0.453
18	0.077	0.308	0.385	0.231	0.000	3.231 ± 0.257
27	0.067	0.133	0.600	0.200	0.000	3.067 ± 0.206
62	0.067	0.200	0.533	0.200	0.000	3.133 ± 0.215
6/1/9	0.143	0.071	0.571	0.214	0.000	3.143 ± 0.254
65	0.357	0.143	0.214	0.214	0.071	3.500 ± 0.374
						3.276 ± 0.030
						NAR > NAR ± 2 SE
22	0.583	0.000	0.250	0.167	0.000	4.000 ± 0.362
30/8/11	0.813	0.063	0.125	0.000	0.000	4.688 ± 0.176
7/3	0.533	0.400	0.067	0.000	0.000	4.467 ± 0.165
7/1	0.867	0.067	0.067	0.000	0.000	4.800 ± 0.145
40/10/7	0.333	0.556	0.111	0.000	0.000	4.222 ± 0.152
35	0.667	0.200	0.133	0.000	0.000	4.533 ± 0.192
39	0.615	0.154	0.154	0.077	0.000	4.308 ± 0.286
66	0.533	0.000	0.267	0.067	0.133	3.733 ± 0.396
45	0.733	0.133	0.133	0.000	0.000	4.600 ± 0.190
40/2	0.308	0.231	0.385	0.000	0.077	3.692 ± 0.328
13	0.667	0.267	0.067	0.000	0.000	4.600 ± 0.163
20	0.600	0.333	0.067	0.000	0.000	4.533 ± 0.165
						4.348 ± 0.134

Table II. (Contd.)

F/F line	NAR (P) 5	4	3	2	1	NAR < NAR ± 2SE
6/1/3	0.250	0.083	0.417	0.083	0.167	3.167 ± 0.405
12/2	0.000	0.143	0.714	0.143	0.000	3.000 ± 0.218
33/5/9	0.250	0.250	0.167	0.250	0.083	3.333 ± 0.396
33/5/11	0.300	0.000	0.200	0.400	0.100	3.000 ± 0.471
32/8	0.077	0.231	0.308	0.308	0.077	2.923 ± 0.309
29/5/3	0.143	0.214	0.286	0.214	0.143	3.000 ± 0.348
						3.071 ± 0.023
						NAR → NAR ± 2SE
35	0.000	0.600	0.300	0.100	0.000	3.500 ± 0.244
56	0.357	0.071	0.357	0.214	0.000	3.571 ± 0.327
33	0.385	0.231	0.077	0.231	0.077	3.615 ± 0.401
54	0.100	0.200	0.700	0.000	0.000	3.400 ± 0.221
33/5/16	0.100	0.400	0.300	0.200	0.000	3.400 ± 0.306
29/3/5	0.133	0.333	0.400	0.067	0.067	3.400 ± 0.273
59	0.308	0.308	0.231	0.154	0.000	3.769 ± 0.303
						3.522 ± 0.020
						NAR > NAR ± 2SE
22/6/15	0.462	0.231	0.077	0.231	0.000	3.923 ± 0.348
33/2/3	0.385	0.154	0.462	0.000	0.000	3.923 ± 0.265
56/5/7	0.667	0.083	0.000	0.167	0.083	4.083 ± 0.434
16/1	0.643	0.071	0.143	0.000	0.143	4.071 ± 0.399
6/1/3A	0.615	0.154	0.154	0.077	0.000	4.308 ± 0.286
36	0.667	0.250	0.083	0.000	0.000	4.583 ± 0.193
						4.149 ± 0.065

Homogeneity is found in the distributions of phenotypes with various numbers of active regions between the groups of S/S and F/F lines ($\chi^2 = 8.614$, df = 4, $P > 0.05$), although the differences between genotypes homozygous for either the S or F allele are not statistically significant for the average number of active regions ($t = 1.500$, df = 52, $P > 0.05$).

Regarding the specific activity of α-amylase, the group of lines homozygous for the S allele shows a higher activity (3.292 ± 0.154) than the group of lines homozygous for the F allele (3.042 ± 0.241). However, the mean specific activity values do not differ significantly between these genotypes ($t = 0.910$, df = 54, $P > 0.05$).

Differences between the lines characterised by extremely low, extremely high, or moderate average values for the number of active regions and specific activity are considered for additional analysis of the association between the phenotypic variabilities caused by the polymorphism of the structural and/or regulatory components of the α-amylase gene–enzyme system in *Drosophila subobscura* (*tables II* and *III*).

The results obtained indicate that a statistically significant difference in the variability of the mean number of α-amylase active regions exists only between

Table III. Specific amylase activity (SAA) ± 2 standard errors (SE) of *Drosophila subobscura* genotypes homozygous for the Amy^S and Amy^F alleles classified into three classes (the amylase enzyme activity is expressed in terms of specific activity; activity per mg of protein estimates from crude extracts).

	SAA				
S/S line	SAA < SAA ± 2SE	S/S line	SAA ↔ SAA ± 2SE	S/S line	SAA > SAA ± 2SE
22	2.89	66	3.02	28	4.16
44	2.98	64	3.15	65	4.02
5/4/8/1	2.52	15	3.58	67	6.08
40/10/8/A	2.95	45	3.31	68	4.28
33/2/13	2.63	62	3.18	46	3.96
40/10/2/1	1.92	11/2/9	3.58	53	4.21
87/7/9	2.90	82	3.58	13	5.39
30/8/11	2.66	38/8/13	3.07	20	3.84
27	2.36	40/2	3.27	18	5.25
7/3	2.18	6/1/9	3.60		
7/1	2.45	39/1	3.09		
40/10/7	1.96	87/7/2	3.16		
35	2.28	1	3.37		
39	2.79				
6/10	2.17				
Mean SAA	2.51		3.31		4.58
F/F line	SAA < SAA ± 2SE	F/F line	SAA ↔ SAA ± 2SE	F/F line	SAA > SAA ± 2SE
22/6/15	1.37	54	2.75	56	3.66
33/2/3	2.23	6/1/13	2.58	33	4.57
35	1.84	12/2	2.81	36	3.90
56/5/7	2.25	33/5/16	2.57	32/8	6.03
59	2.36	16/1	3.27	29/5/3	3.55
		33/5/9	2.73	29/3/5	3.55
		33/5/11	3.20		
		6/1/3A	2.57		
Mean SAA	2.01		2.81		4.21

the group of S/S lines, whose specific activities range within ± 2 SE of the mean, and the group of F/F lines which is at least ± 2 SE below the mean ($U = 7.00$, $P < 0.05$), as well as between the groups of lines of both genotypes within ± 2 SE of the mean ($U = 24.00$, $P < 0.05$).

The analysis of intergroup differences in the number of active regions for the six groups formed according to amylase-specific activity confirmed that the difference is not significant ($H = 8.424$, $P > 0.05$). It also confirmed the previously obtained results, i.e. the equivalence in the distribution of the number of active regions between S/S and F/F lines grouped in three categories.

When the groups are formed according to the number of active regions, the same test shows no statistically significant intergroup difference in the variability of the enzyme specific activity of either genotype ($H = 5.727$, $P > 0.05$).

Analyses of the association between the number of active regions and the enzyme-specific activity through compensation of the enzyme quantity in S/S and F/F genotypes, carried out by Spearman and Pearson tests of correlation on all categories, indicate statistically non-significant negative correlations in five cases. Such correlations are found mostly in the groups in which the number of active regions or enzyme specific activity fall below and within the mean value ± 2 SE.

Correlation tests applied to the ungrouped lines of S/S or F/F genotype, indicate possible compensation of deficiency or excess of the enzyme by the correspondingly higher or lower number of active regions, respectively (negative correlation without statistical significance; $r_{\text{Pearson}} = -0.139$, $r_{\text{Spearman}} = -0.178$ for the S/S genotype; for the F/F genotype, $r_{\text{Pearson}} = -0.279$, $r_{\text{Spearman}} = -0.309$).

4. DISCUSSION

The association between the genetic determination and the phenotypic functionality of the gene–enzyme system is still obscure. The functional relationship between the structural and regulatory genes, realised through complex and multiple interactions, leads to many hypotheses in the interpretation of the experimental data.

The structural and regulatory variability of α-amylase represents one of the most frequently analysed gene–enzyme systems in *Drosophila* (see [9]). The analysis of the enzyme activity represents a model for distinguishing between the effects of the structural and regulatory genes involved in the control of a particular gene–enzyme system. According to many authors, the variability of regulatory genes may have an evolutionary role, even more important than the structural gene polymorphism [7, 10]. Tissue-expressed polymorphism in *Drosophila* adult midgut exemplifies a specific determination of regulation [1, 12].

The absence of differences at the level of the mean tissue-specific expression and specific amylase activity between two *Amy* genotypes observed, along with the presence of differences among particular categories, may indicate the possible role of non-genetic effects. This could explain the existence of the intraline variability.

The analysis of *Drosophila subobscura* amylase activity shows that the individuals homozygous for the S allele generally have a higher amylase activity than those homozygous for the F allele, as reported for other *Drosophila* species [13]. Immunoelectrophoretic data reveal that different levels of amylase activity are a consequence of different quantities of amylase protein, which is directly related to the regulation at the transcriptional level [6]. The existence of different biochemical phenotypes in *Drosophila subobscura* may be the result of the genetic variability in the structural and/or regulatory genes responsible for the synthesis and expression of α-amylase.

In the work by Doane [4], a clear absence of dependence was found between the total amylase activity and the distribution of the active regions in *Drosophila melanogaster*. In the present paper the negative (although non-significant) correlation between the number of active regions and specific amylase activity within each of the genotypes, as well as between certain categories, suggest a compensatory effect between these two phenotype expressions.

The differences in the mean number of α-amylase active regions in adult midgut between the lines homozygous for the Amy^S allele with above average specific amylase activity and lines homozygous for the Amy^F allele with significantly lower enzyme activity may indicate additional genetic variability within the *Amy* locus. This possibility adds to the complexity of studying the degree of the functional significance of different types of genetic polymorphism in adaptation processes.

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