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Original article

Adaptative significance of amylase polymorphism in *Drosophila*. III. Geographic patterns in *Drosophila subobscura* tissue-specific expression of amylase in adult midgut

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Summary – Eight natural populations of *D subobscura* (Collin) were studied for genetically controlled variation in tissue-specific expression of α-amylase enzyme. Polymorphism for amylase tissue variation in the midgut was found to be present in natural populations. This type of phenotypic variability showed intra- and interpopulation variability. The geographic variation in α-amylase midgut activity patterns of gene expression was found to be uncorrelated with allozyme variation at the structural locus. There was no detectable correlation of activity patterns in the anterior midgut with those in the posterior midgut.

Drosophila subobscura / α-amylase / tissue-specific expression / inter- and intrapopulation variability

Résumé – Signification adaptive du polymorphisme de l'amylase chez *Drosophila*. III. Variation géographique de l'expression de l'amylase dans l'intestin moyen de l'adulte de *Drosophila subobscura*. *Huit populations naturelles de* Drosophila subobscura *ont été étudiées et se sont révélées polymorphes pour l'expression tissulaire de l'amylase α dans l'intestin moyen. Il existe un polymorphisme intra- et interpopulationnel. Il n'existe pas de corrélation entre l'activité amylasique dans la partie antérieure et postérieure de l'intestin moyen, pas plus qu'avec le polymorphisme au locus structural.*

Drosophila subobscura / amylase α / expression tissulaire / variabilité inter- et intrapopulation

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INTRODUCTION

A number of authors have suggested that changes in genetic regulation are of major importance in eukaryotic evolution (for references see Hedrick and McDonald, 1980; Templeton, 1981; McIntyre, 1982; Paigen, 1986). Many tissue-specific enzyme pattern differences may be due to the effect of variants at regulatory loci. One of the most extensively studied regulatory gene systems, that affects a tissue-specific enzyme pattern is α-amylase expression in *Drosophila* (Powell and Lichtenfels, 1979; Doane, 1980; Powell *et al*, 1980).

We have started to study intensively *Drosophila subobscura* for genic and phenotypic polymorphism of α-amylase and its tissue-specific expression in adult midgut; hence, in the present paper the geographic variation of these types of polymorphism is presented.

MATERIALS AND METHODS

An analysis of tissue-specific midgut α-amylase activity pattern (MAP-type) was undertaken in *D subobscura* adults from 8 natural populations (details on these collections are given in Andjelković *et al*, 1987). All flies were reared on standard cornmeal-sugar-agar-yeast food medium for one generation before determining midgut amylase activity. First generation fresh sampled flies from nature were studied.

The method described by Abraham and Doane (1978) was used to prepare dissected midguts of 3-5 d-old adults and to determine their amylase activity. The activity could be expressed in 3 regions of the anterior (AMG) and 2 regions of the posterior (PMG) midgut. The presence of activity is indicated by a number and absence by zero (*eg*, 123 10 means activity in the 3 regions of AMG and region 1 of PMG, but absence of activity in region 2 of PMG).

The range of intrapopulation phenotypic variability is given by Shannon's diversity index and for testing the significance of differences in phenotypic, frequency distributions between populations I-divergence analysis was used, derived from Shannon's entropy function (Orloci, 1970), given by :

$$I = \sum\sum f_{ij} \ln \frac{f_{\cdot\cdot} f_{ij}}{f_{i\cdot} f_{\cdot j}}$$

In this expression $f_{j\cdot}$, $f_{\cdot j}$ and $f_{\cdot\cdot}$ signify the ith phenotype (row) total, the jth locality (column) total, and the grand total for all phenotypes (or all localities), respectively. The number of species is r. Twice the value of I is an approximation to χ^2 with $(r-1)(c-1)$ degrees of freedom.

RESULTS

Table I presents the frequencies of midgut activity pattern (MAP-types) in 8 natural populations of *D subobscura*. In the data both sexes were combined, since no significant differences have been detected between male and female patterns (Stamenković-Radack *et al*, 1987).

The number of theoretically possible patterns of amylase midgut activity in *D subobscura* is 32, but the MAP-type AMG-000 PMG-00 has not been detected so far. Other midgut activity patterns occurred with different frequencies in populations analyzed in this paper. In 7 of them, the most frequent MAP-type was AMG-123 PMG-12, whose frequency was in the range from 33.3 – 62.6%. In the Kužni Do population, the most frequent MAP-type was AMG-100 PMG-00 (34.7%). Except for those 2 MAP-types, all others appeared with lower frequencies in populations studied. There were 5 MAP-types (123 10, 123 00, 120 12, 120 00, 100 10), with frequency of 5% and higher, in some populations and 3 MAP-types (123 00, 120 12, 100 12), which occurred only in a number of populations with a frequency of 10% and higher.

As the genotypes of different MAP types were not known, it was not possible to estimate gene frequencies or gene heterozygosity. Therefore, the Shannon diversity measure (diversity index) was used. The value of H showed that there were differences in the degree of polymorphism between the populations. These differences reached a ratio as great as 1.7 in the case of the Popovica population (the highest H value) with respect to the Zürich population (the lowest H value).

Because it was impossible to test the statistical significance of diversity index differences, we tested the significance of the difference in phenotypic frequency distribution among population applying the I-divergence analysis (Orloci, 1970). The analysis showed (table II) that significant differences in the type of diversity existed between the populations Popovica. Ravnište and Kužni Do, as well as between each of these populations and all the others. Statistically significant differences were also found between the Pomena populations and all others except Zürich and Raices. Shannon's index mainly represents qualitative variability according to the number of classes involved. On the other hand, the normalized Shannon's index (R) (Legendre and Legendre, 1983), better reflects quantitative variability according to the frequency of certain phenotypes. These values are given in table I, as well. The relations obtained were similar to the values of Shannon's indices of diversity, although certain discrepancies existed. The highest R value belonged to the Kužni Do population, which was the result of the fact that the frequencies of several phenotypes were nearly equal and relatively high.

Considering that the total number of amylase active regions in the midgut has a certain biological significance in a sense of phenotypic expression, we classified the phenotypes obtained according to that (NAR). The analysis showed a statistically significant difference ($\chi^2 = 303.094$, $df = 28$, $P < 0.001$), as the midgut patterns with varying number of active regions were differently represented in populations studied (table III).

Table I. Frequencies (p) of tissue-specific α-amylase midgut activity pattern (MAP-type) in 8 natural populations of *D subobscura**.

MAP type	*Sunne* (N = *92*)	*Zürich* (N = *99*)	*Popovica* (N = *89*)	*Beli Izvor* (N = *92*)	*Ravnište* (N = *414*)	*Kužni Do* (N = *101*)	*Pomena* (N = *320*)	*Raices* (N = *114*)
123 12	0.489	0.626	0.416	0.467	0.333	0.238	0.541	0.605
123 10	0.033	0.071	0.067	0.054	0.041	0.010	0.028	0.044
123 02			0.010		0.002			0.018
123 00	0.054	0.131	0.067	0.217	0.002	0.030	0.038	0.079
120 12	0.109	0.040	0.079	0.044	0.155	0.040	0.063	0.044
120 10	0.033	0.020	0.034		0.048	0.040	0.019	0.018
120 02			0.011	0.011	0.002			
120 00	0.087	0.010	0.034	0.033	0.002		0.022	0.026
103 12		0.010	0.034	0.011	0.063		0.019	
103 10	0.011		0.011		0.012			
103 00			0.034	0.011		0.010		
023 12			0.034		0.039		0.006	
023 10			0.011		0.017			
023 02			0.011		0.005			
023 00	0.022		0.011				0.003	
100 12	0.043	0.040	0.011	0.065	0.138	0.168	0.113	0.105
100 10	0.033				0.053	0.099	0.022	0.026
100 02			0.011		0.022			0.009
100 00	0.054	0.010		0.076		0.347	0.025	0.009
020 12		0.030			0.017		0.016	
020 10	0.011		0.011		0.012		0.006	
020 00				0.011				
003 12			0.023		0.007			
003 10	0.011				0.002			
003 02					0.002			
003 00			0.023					
000 12		0.010	0.011		0.010	0.020	0.072	0.018
000 10	0.011		0.023		0.010		0.009	
000 02			0.023		0.005			
H	1.871	1.377	2.382	1.665	2.253	1.766	1.763	1.503
R	0.708	0.574	0.748	0.694	0.708	0.767	0.636	0.605

* Localities are listed in north–south order starting at the left. H is the diversity index : $H = -\sum(p_i \ln p_i)$; where p_i is the frequency of the ith pattern in the population. When the population is monomorphic, $H = 0$ and when it is polymorphic, $H > 0$. The maximum value of H is obtained when all p_i (n_i/N) are equal. R is the normalized index $(H/H_{\max})$.

Table II. Comparisons of pairs of populations using independence components test (*I* – divergence).

	Zürich	*Popovica*	*Beli Izvor*	*Ravnište*	*Kužni Do*	*Pomena*	*Raices*
Sunne	39.159	48.929*	38.121	104.195*	71.941*	43.434*	31.006
Zürich		49.661*	24.019	117.546*	101.795*	36.849	19.930
Popovica			56.918*	85.206*	123.643*	86.278*	57.038*
Beli Izvor				78.385*	78.385*	66.618*	34.636
Ravnište					182.482*	154.827*	103.450*
Kužni Do						116.520*	84.085*
Pomena							30.939

* $P < 0.05$.

Table III. Frequencies of α-amylase active in the midgut of the 8 natural populations of *D subobscura*.

NAR	*Sunne*	*Zürich*	*Popovica*	*Beli Izvor*	*Ravnište*	*Kužni Do*	*Pomena*	*Raices*
5	0.489	0.626	0.416	0.467	0.333	0.238	0.541	0.605
4	0.141	0.121	0.225	0.109	0.300	0.050	0.116	0.105
3	0.141	0.222	0.180	0.294	0.294	0.238	0.184	0.202
2	0.163	0.020	0.112	0.044	0.104	0.129	0.125	0.079
1	0.065	0.010	0.067	0.087	0.015	0.347	0.034	0.009
H	1.377	1.008	1.436	1.305	1.370	1.463	1.270	1.106

NAR = number of active regions.

Table IV presents *H* values for several forms of amylase polymorphism in *D subobscura* amylase structural gene (H_{AMY}), MAP-types (H_{MAP}), number of active regions (H_{NAR}), MAP-types for AMG (H_{AMG}) and for PMG (H_{PMG}) separately. The correlations between these diversity indices were not statistically significant.

Table IV. The diversity index *H* of : *Amy* alleles (H_{AMY}), midgut activity patterns in the whole midgut (H_{MAP}), total number of active regions in the midgut (H_{NAR}), midgut patterns of the AMG region (H_{AMG}) and midgut patterns of the PMG region (H_{PMG}), for 8 *D subobscura* populations. The correlation coefficients between the diversities are given at the lower part of the table, critical value of $P = 0.05$ is 0.707; $df = 6$.

	Localities							
	Sunne	*Zürich*	*Popovica*	*Beli Izvor*	*Ravnište*	*Kužni Do*	*Pomena*	*Raices*
H_{AMY^*}	0.761	1.063	0.824	0.860	1.055	0.930	0.925	1.049
H_{MAP}	1.871	1.377	2.345	1.664	2.253	1.767	1.763	1.503
H_{NAR}	1.377	1.008	1.436	1.305	1.370	1.463	1.270	1.106
H_{AMG}	1.200	0.693	1.434	0.845	1.633	0.979	1.236	0.787
H_{PMG}	0.893	0.714	1.076	0.888	0.679	1.007	0.578	0.757

MAP:AMY $r = +0.3533$; NAR:AMY $r = -0.6262$; AMG:AMY $r = -0.2247$; PMG:AMY $r = -0.6020$; AMG:PMG $r = +0.0191$. * From data of Andjelković *et al* (1987).

DISCUSSION

Recent investigations have shown that variation in gene regulation is widespread in natural populations, although the evidence is limited to a few gene-enzyme systems (McIntyre, 1982). The variation in the regulation of α-amylase activity in *Drosophila* midgut is one such example.

Our results primarily showed that 29 detected MAP-phenotypes were not equally distributed among *D subobscura* populations (table I). The results on *D pseudoobscura* (Powell and Andjeljković, 1983; Powell and Amato, 1984) and on *D melanogaster* (Klarenberg *et al*, 1987) clearly reveal that different midgut activity phenotypes show selective differences in laboratory populations maintained on media with different starch concentrations. The results of Marinković *et al* (1984) on *D subobscura* have shown that there is a correlation between MAP-types and pre-adult developmental rate. All this confirms the assumption that intra- and interpopulation polymorphism for the tissue-specific expression of α-amylase in *D subobscura* adult midguts is probably controlled by selection. The absence of correlation among the activity patterns in the AMG and PMG gives evidence that these 2 midgut activity subpatterns are at least separately determined (Stamenković-Radak *et al*, 1987).

Natural populations of *D subobscura* are highly polymorphic for inversions in every chrosomome, with certain temporal and spatial patterns (Krimbas and Loukas, 1980). Different alleles could be associated with different gene arrangements and gives direct evidence in support of the coadaptation hypothesis, as has been shown for several *Drosophila* species (Sperlich and Pfriem, 1986). Such association could be responsible for the lack of correlation with geographic variation in α-amylase midgut activity pattern obtained in this paper, but this should be the subject of further investigations of different kind and approach.

A question arises of whether there could be any kind of correlation between the 2 types of polymorphism, *ie* whether the degree of polymorphic variability of gene regulation (MAP) varies independently of that of the structural gene polymorphism in the natural *D subsobscura* populations under study. A similar phenomenom was observed in some other *Drosophila* species (Powell, 1979; Powell *et al*, 1980). However, Klarenberg and Scharloo (1986) demonstrated linkage disequilibrium between *Amy* and *map* in *D melanogaster* populations of different geographic origin.

Besides these, laboratory studies on *Drosophila Amy* variants exist, which detect selective pressures on gene-structural polymorphism level (De Jong and Scharloo, 1976; Scharloo *et al*, 1977; Hickey and Benkel, 1982; Powell and Andjelković, 1983). Considering these data, the absence of correlation between these 2 types of polymorphism could not be explained by their adaptive neutrality. A possible explanation could be that both polymorphisms are controlled by selection, but are not under the influence of the same evolutionary–ecological forces.

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