Adaptive significance of amylase polymorphism in *Drosophila*

I. The geographical pattern of allozyme polymorphism at the amylase locus in *Drosophila subobscura*

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Abstract

Allelic variation at the *Amy* locus was studied in eight natural populations from the central and northern range of *D. subobscura*, and the geographical pattern of *Amy* polymorphism over the range of this species was described. Even though regional and local differences in gene frequencies were found, in general the same alleles occur at high, intermediate and low frequencies, in nearly all populations. There are no significant differences in allele frequencies, but there is significant difference in the degree of heterozygosity among groups of populations from the northern, central and southern range. An analysis of population subdivision indicates that heterogeneity within populations is higher than between populations. Genetic distance values indicate that there is a variable degree of geographical differentiation between local populations. Variability within and between continental and insular populations is also discussed.

Introduction

The role of enzyme variants (izozymes and allozymes) in environmental adaptation and evolution has been a major focus of recent evolutionary studies. Two decades of research have failed to shed much light on the adaptive significance of molecular variants of enzymes in different organisms.

Of all the native European *Drosophila* species, *D. subobscura* is genetically and ecologically the best known and extensively used for studies in population genetics. As most *Drosophila* species, it is polymorphic for the structural gene (*Amy*) producing α-amylase (E.C.3.2.1.1., α-1.4-glucan-4-glucanohydrolase) (Powell et al., 1980). The *Amy* locus is located on chromosome E in the region of chromosome arrangement E1-2,9 (Pinsker, pers. comm.). In adult *Drosophila* amylase activity is found primarily in the midgut and hemolymph and its major function is digestion of starch (Doane, 1969).

In addition to structural gene variants (allozymes), *Drosophila* amylase polymorphism includes the variation in tissue-specific expression of the gene, i.e., gene regulation (Abraham & Doane, 1978; Powell & Lichtenfels, 1979; Powell et al., 1980). This type of polymorphism gives an additional approach to explanation of the significance of polymorphism in the processes of adaptation and speciation. Regarding this point, we started to study the polymorphism α-amylase in *D. subobscura* extensively. This paper, as the first in a series, reports the results of our geographic survey of *Amy* alleles.

Material and methods

The eight natural populations of *D. subobscura* have been sampled in the following localities:

1. Sunne (Sweden): Bulk sample supplied by D. Sperlich (Univ. of Tübingen) 1979. The sample locality is at 300 m altitude in South Sweden. The vegetation of this locality is fir tree forests and
gardens around the village.

(2) Zürich (Switzerland): Bulk sample collected by G. Bächli (Univ. of Zürich) 1980.

(3) Popovica (Yugoslavia): Bulk sample collected 1980. The sampled locality is at Fruška gora mountain at 300 m above sea level in a forest with tree species *Picea abies*, *Robinia pseudoacacia* and *Sambucus nigra*.

(4) Beli izvor (Yugoslavia): Bulk sample collected 1981. The sampled locality is at Mt. Goč at 950 m above sea level. The vegetation of the study area is a forest with *Fagus moesiaca* as the dominant tree species.

(5) Ravnište (Yugoslavia): Bulk sample collected 1981. The sampled locality is at Mt. Jastrebars. The area where the flies were trapped was 600 m above sea level and overgrown with a forest vegetation where *Fagus moesiaca* is a dominant species.

(6) Kužni do (Yugoslavia): Bulk sample collected by V. Kekić (Univ. of Belgrade) 1983. The sampled locality is at Mt. Durmitor at the height of 1440 m above sea level. The vegetation of this area is a forest with dominant tree species *Picea abies* and *Abies alba*.

(7) Pomena (Yugoslavia): Bulk sample collected 1982. The sampled locality is on island Mljet (Adriatic Sea) at about 10 m above sea level, in a forest with dominant tree species *Pinus halepensis* and *Quercus pubescens*.

(8) Raices (Spain): Isofemale lines collected by J. M. Larruga (Univ. of Laguna) 1983. The sampled locality is at 900 m altitude on Tenerife, the biggest island of the Canary Islands (Atlantic Ocean). The vegetation of this locality is a sparse forest of *Pinus arborea* and *Myrica faya*.

All the flies were grown on cornmeal-sugar-agar food medium for one generation before electrophoresis. F₁ offsprings of fresh sampled flies from nature were studied. *Amy* genotypes were determined by disc polyacrylamide electrophoresis described by Doane (1967).

Adult flies were homogenized in 0.025 ml 0.05 M glycine-TRIS buffer. Electrophoresis was performed using 7.5% acrylamide gel. The electrode buffer was 0.05 M glycine-TRIS buffer, pH 8.4. It took 2 h at 4 °C with current intensity of 3 mA/gel. Gels were incubated with transparent starch-acrylamide film, containing suitable buffer, at 35 °C for 2 h. Staining was done in J-KJ solution (0.001 M) for a few minutes.

*Amy* alleles are designated according to the relative mobility of the corresponding allozyme so that the most common allele in *D. willistoni* (laboratory line 'Maya' supplied by J. R. Powell), is labeled as 1.00.

**Results**

The allele frequencies of the α-amylase structural gene *Amy* for eight different natural populations of *D. subobscura* are given in Table 1. Allelic frequencies (p) are given for each locality and for the whole species with all local samples pooled ('Total'). Individuals with three, even four alleles of *Amy* locus appear in some populations (Table 3). Such individuals were not taken in the calculations of allelic frequencies and other parameters.

According to the electrophoretical mobility four alleles have been detected: 0.48 (or VS – very slow), 0.58 (S – slow), 0.65 (F – fast) and 0.73 (VF – very fast). The data for females and males have been pooled, since gene frequency differences between sexes were not statistically significant.

All the analysed populations, except for Sunne, contain four alleles. Similarity among localities occurs not only in the amount of genetic variation, but also, and most interestingly in the pattern of variation. The allele 0.58 (S) is the most common allele in each population, with frequencies ranging from 0.49 at Raices (southern part of range) to 0.74 at Sunne (northern part of range). The next most frequent allele in the six geographically intermediate populations is 0.65 (F), whereas 0.48 (VS) is the next frequent in the other two populations (Sunne and Raices). In all populations the rarest allele is 0.75 (VF); it has not been detected in the Sunne population.

We can conclude that the configuration of allelic frequencies remains fairly constant over the area of study. The allele frequencies are by no means identical everywhere, although they fluctuate over wide limits. In other words, gene frequencies are not uniform throughout the whole species (G = 189.254; df = 21; p < 0.001).

Not all populations contribute equally to such