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Short communication

Alcohol dehydrogenase polymorphism in barrel cactus populations of *Drosophila mojavensis*

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Abstract

Starch gel electrophoresis revealed that the alcohol dehydrogenase (ADH-2) locus was polymorphic in two populations (from Agua Caliente, California and the Grand Canyon, Arizona) of cactophilic *Drosophila mojavensis* that utilize barrel cactus (*Ferocactus acanthodes*) as a host plant. Electromorphs representing products of a slow (S) and a fast (F) allele were found in adult flies. The frequency of the slow allele was 0.448 in flies from Agua Caliente and 0.659 in flies from the Grand Canyon. These frequencies were intermediate to those of the low (Baja California peninsula, Mexico) and high (Sonora, Mexico and southern Arizona) frequency *Adh*-2^S populations of *D. mojavensis* that utilize different species of host cacti.

Introduction

An interesting and well-documented case of genetic polymorphism in natural populations of *Drosophila* exists for electrophoretic variation at the alcohol dehydrogenase (ADH)[1] locus in adult *D. mojavensis* Patterson and Crow (Zouros, 1973; Heed, 1978). Endemic to the deserts of North America, *D. mojavensis* breeds in necrotic tissue of columnar cacti. In southern California, USA, *D. mojavensis* uses necrotic barrel cactus (*Ferocactus acanthodes*) as a host and is referred to as race A. Race B is found in Mexico and southern Arizona. On the Baja California peninsula in Mexico (which includes the states of Baja California Sur and Baja California Norte), agria cactus (*Stenocereus gummosus*) is the preferred host, whereas organpipe cactus (*S. thurberi*) is the preferred host in the state of Sonora, Mexico and southern Arizona. *Drosophila mojavensis* from Sonora, Mexico are designated subrace BI and those from the Baja California peninsula are designated subrace BII (Zouros, 1973).

Zouros (1973) first reported, and Heed (1978) later confirmed, that subraces BI and BII show striking and consistent differences in their frequencies of the fast (F) and slow (S) migrating *Adh* gene products expressed in adult flies. For subrace BI, the slow allele (Adh^S) is predominant, with frequencies ranging from 0.71 to 1.00 in 15 localities sampled. For subrace BII, the situation is reversed and the slow allele is either absent or reaches a frequency of only 0.09 across 20 separate localities. Fast and slow alleles of ADH also are known from race A (Batterham et al., 1983), but there is little detailed information on allele frequencies. An earlier study (Zouros, 1973) showed that the slow allele was fixed in race A, but only four individuals (three wild-caught and one from a laboratory stock) were analyzed. Here we report ADH allele frequen-

ADH – alcohol dehydrogenase (EC 1.1.1.1); Adh^F and Adh^S – alleles of alcohol dehydrogenase that produce fast (F) and slow (S) electromorphs of ADH. Batterham, Starmer, and Sullivan (1982) have shown the presence of two loci, *Adh-1* and *Adh-2*, in *Drosophila mojavensis*. *Adh-1* is expressed only in larvae and female adults. All results presented here refer to the *Adh-2* locus, which is expressed in adults of both sexes.

Table 1. Frequencies of the slow (S) and fast (F) alleles of alcohol dehydrogenase (*Adh-2*) in populations of adult *Drosophila mojavensis*

Locality	Race	n[1]	S	F	F'[2]	H(DC)[3]
Agua Caliente	A	124	0.448	0.552	0.0	0.363
Grand Canyon	?	41	0.659	0.341	0.0	0.195
Guaymas	BI	49	0.755	0.163	0.082	0.326
Mulegé	BII	50	0.130	0.870	0.0	0.060

[1]Number of individuals.
[2]A rare allele whose product migrates faster than the product of Adh^F.
[3]Heterozygosity determined by direct count.

cies from samples of adult flies from populations of *D. mojavensis* that represent all described races, including a new undescribed population from the Grand Canyon, Arizona found to use barrel cactus as a host. The results confirm earlier studies (Zouros, 1973; Heed, 1978) on ADH allele frequencies in subraces BI and BII and, in addition, document a high degree of polymorphism in both race A from Agua Caliente, California and the undescribed population from the Grand Canyon.

Materials and methods

Collection of D. mojavensis

Flies were collected from Agua Caliente, California; Mulegé, Baja California Sur; and Guaymas, Sonora. Flies from Agua Caliente and Mulegé were brought to the laboratory to establish mass cultures under uncrowded conditions; electrophoresis was conducted on the F_1 generation. Electrophoresis was performed on wild-caught flies from Guaymas. Flies from the Grand Canyon, Arizona were obtained from a laboratory mass culture started from a sample collected more than ten years ago by Richard Thomas (Heed & Mangan, 1986).

Agria and organpipe cactus, which could potentially be used as hosts, were absent from both the Agua Caliente and Grand Canyon collecting sites. Additionally, no evidence of sympatry could be detected between the races that utilize barrel cactus (race A and the undescribed Grand Canyon race) and race B. Adult flies from both race A and the population from the Grand Canyon are more yellowish than individuals from the darker race B.

Electrophoresis

Flies were homogenized in approximately two volumes of 10 mM Tris-HCl, 1.0 mM Na_2EDTA, and 0.05 mM NADP (pH 7.0). Paper wicks saturated with the homogenate were placed on 12.5% starch gels. Horizontal electrophoresis was performed at 4°C in the following buffer systems: 1) 0.04 M citrate buffer adjusted to pH 6.0 with N-(3-aminopropyl)morpholine (diluted 1 : 20 in the gel); 2) 0.135 M Tris, 0.045 M citrate, 1.3 mM Na_2EDTA (pH 7.0) (diluted 1 : 15 in the gel); 3) 0.50 M Tris, 0.65 M borate and 0.018 M Na_2EDTA (pH 8.0) (diluted 1 : 10 in the gel). ADH activity was visualized on gel slices using standard histochemical staining techniques (Murphy et al., 1990).

Results and discussion

ADH allele frequencies from *D. mojavensis* are shown in Table 1. Frequencies of fast and slow alleles for subraces BI (Guaymas) and BII (Mulegé) showed the same patterns as reported 20 years ago (Zouros, 1973; Heed, 1978). However, flies from Agua Caliente (race A) that breed in barrel cactus were not fixed for Adh^S. *Adh* also was polymorphic in laboratory-reared flies originally collected from the Grand Canyon. Frequencies of Adh^S differed in populations of flies from Agua Caliente and the Grand Canyon, but both were intermediate to those of subraces BI and BII (Table 1). Another interesting result was the high degree of genetic variability observed in flies from the Grand Canyon population that had been maintained in laboratory culture for more than ten years.

Heed (1978) mentions the presence of a rare third allele of ADH in subrace BII. Although we did not observe this allele in our subrace BII sample from Mulegé, a rare allele, here referred to as $Adh^{F'}$, was observed in the subrace BI sample from Guaymas (Table 1). The product of $Adh^{F'}$ migrated faster than the product of Adh^F on starch gels.

The remarkable consistencies in ADH allele frequencies of subraces BI and BII when compared with earlier work, and the discovery of a third pattern of polymorphism in groups of flies from two areas where barrel cactus is utilized, underscores the importance of obtaining additional information on genetic structure of populations breeding in barrel cactus. It is likely that the earlier report (Zouros, 1973) of fixation of Adh^S in race A was due to the small number of flies examined.

Starmer, Heed, and Rockwood-Sluss (1977) reported results of experiments that offer an explanation for ADH polymorphisms based upon characteristics of host plants, regional climatic differences, and ability of *D. mojavensis* to metabolize atmospheric ethanol. According to their study, Adh^F is favored on the Baja California peninsula because of moderate air temperatures and the combination of moderate pH values and the production of isopropanol in fermenting agria cactus. The higher temperatures in Sonora, Mexico and the lack of isopropanol in fermenting organpipe were suggested to favor Adh^S. These experiments are compelling because they provide a mechanism that links properties of the environment to physicochemical properties of the gene products to explain the polymorphism.

Our observations raise several questions. The first deals with the forces underlying the allele frequencies seen here in barrel cactus-breeding populations. It is unclear if the intermediate frequencies in Agua Caliente or the Grand Canyon reflect gene flow from subraces BI or BII, linkage disequilibrium, or if some feature of fermenting barrel cactus and local climatic conditions is shaping the polymorphism.

In addition, the relationships among different barrel cactus populations, as well as between barrel cactus populations and subraces BI and BII, need to be established. According to Heed and Mangan (1986), populations in southern California, as well as those in the Grand Canyon, exist in small isolated areas. That observation, coupled with differences in ADH allele frequencies, raises the question of the degree to which barrel cactus populations of *D. mojavensis* are genetically differentiated. We are currently addressing these questions.

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