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Note

Loss of Expression of Alcohol Dehydrogenase in Adult Males of *Drosophila pachea*

Edward Pfeiler[1,3] **and Therese A. Markow**[2]

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INTRODUCTION

Drosophila pachea, a member of the *nannoptera* species group, is a cactophilic species endemic to the Sonoran Desert of North America (Heed, 1978). *Drosophila pachea* breeds and feeds in necrotic tissue of a specific host cactus, senita (*Lophocereus schottii*), which produces phytosterols necessary for its normal development (Heed and Kircher, 1965; Fogleman *et al.*,1986). During the course of a study on the population genetics of Sonoran Desert *Drosophila* we noticed that the enzyme alcohol dehydrogenase (ADH; EC 1.1.1.1) was not expressed in mature adult males of *D. pachea*, although high activity was seen in females. The pattern was consistent in flies collected from widely separated localities.

In the present Note we describe the relative expression of ADH in different life history stages of *D. pachea* and the time course of suppression of enzyme activity in adult males. We also show that the sex-specific suppression in ADH activity is not a characteristic of the other members of the *nannoptera* species group, *D. nannoptera*, *D. acanthoptera*, and *D. wassermani*. Finally, we demonstrate that expression of ADH activity in adult females of *D. pachea* is not localized in the ovaries and, in addition, show that enzyme activity in adult males can be induced with short-term exposure to exogenous ethanol.

MATERIALS AND METHODS

Collection and Treatment of Flies. Drosophila pachea were obtained from both natural populations and from laboratory cultures. Adult *D. pachea* were collected

[1] Department of Biology, Arizona State University, Tempe, Arizona 85287. Fax: (480) 965-2519. e-mail: epfeiler@asu.edu.
[2] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721.
[3] To whom correspondence should be addressed.

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in northwestern Mexico (Guaymas, Sonora, and Mulegé, Baja California Sur) and southern Arizona [Organ Pipe Cactus National Monument (OPNM)], in association with necrotic tissue of the senita host cactus. Flies collected at Mulegé were frozen at −18°C for 2–3 months before analysis; all others were fresh when analyzed.

Laboratory cultures were maintained for *D. pachea* [from Guaymas (May 2000), OPNM (April 2000), and La Paz, Baja California Sur (November 1998)], *D. nannoptera* [from Tehuacán Valley, Puebla, Mexico (July 1998)], *D. acanthoptera*, and *D. wassermani* [from Oaxaca, Mexico (August 1990)]. Flies were reared on banana–*Opuntia* culture medium to which had been added a pinch of dry active yeast. A small piece of senita was added to the culture medium for *D. pachea*. From cultures of *D. pachea*, third-instar larvae and pupae were removed for analysis. Unlike larvae of *D. melanogaster*, it is not possible to differentiate gonadal size for determination of sex. Of the 11 larvae and 24 pupae screened, however, half were expected to be male. Adults of *D. pachea* were separated by sex and screened for ADH activity at 1, 2, 4, and 14 days after eclosion.

Electrophoresis. Individual flies were homogenized in 15 μl of grinding buffer (Cleland *et al.*, 1996). Homogenates were centrifuged for 5 min at 10,000*g* and the supernatants were analyzed by electrophoresis on either 12.5% starch gels (Starch Art Corp., Smithville, TX) or Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX). Horizontal starch gel electrophoresis was carried out at 4°C for approximately 5 hr in a buffer system of 40 m*M* citrate adjusted to pH 6.0 with *N*-(3-aminopropyl)morpholine (diluted 1 : 20 in the gel). Gel slices were stained for ADH activity using a standard recipe (Murphy *et al.*, 1990). Cellulose acetate electrophoresis was performed at room temperature (22°C) for 15–20 min at 200 V using Tris–glycine buffer (pH 8.0); ADH staining followed the method of Hebert and Beaton (1989).

In one experiment, ovaries from laboratory-cultured *D. pachea* from La Paz were dissected in *Drosophila* Ringer's. The ovaries and carcass from three individuals were immediately placed in separate centrifuge tubes containing 15 μl of grinding solution and prepared for electrophoresis. For two different groups of flies, combined ovaries of three individuals, and their corresponding combined carcasses, were also homogenized and analyzed separately.

Effect of Exogenous Ethanol. Eight mature, 17-day-old males of *D. pachea* from the Guaymas laboratory stock were placed in an 8-dram glass vial containing banana–*Opuntia* culture medium (equivalent to a volume of 5 ml) to which had been added 0.1 ml of commercial tequila (Cazadores Reposado; Tequila Cazadores, S.A. de C.V.; 38% ethanol, by volume). A small piece of Kimwipe tissue was then placed into the vial and one edge was pressed into the food. The tissue provided a relatively large surface area saturated with the ethanol solution. After 24 hr the flies were removed and prepared for electrophoresis as before. Untreated 17-day-old males and females of *D. pachea* from Guaymas were used as a control.

RESULTS AND DISCUSSION

Sexual dimorphism in ADH expression was evident in adult *D. pachea* from all localities sampled. For example, no ADH staining was evident after starch gel electrophoresis of frozen adult males of *D. pachea* from Mulegé ($N = 13$), whereas females showed high ADH activity ($N = 19$). The same pattern was seen with freshly caught males ($N = 20$) and females ($N = 18$) of *D. pachea* from Guaymas. Similar results were found after cellulose acetate electrophoresis using fresh, wild females from Guaymas ($N = 25$) and OPNM ($N = 13$) and males from the OPNM ($N = 17$). Three of 12 fresh males from Guaymas, however, showed very weak activity. Therefore, of a total of 62 wild-caught males of *D. pachea* analyzed, 59 showed no visible activity. In all female flies, and the three males that expressed weak activity, ADH was monomorphic.

The sex-specific suppression of ADH activity seen in natural populations of *D. pachea* was also found in laboratory stocks. Table I shows that no ADH activity was found in 14-day-old laboratory-reared males, whereas activity was high in females. All the preadult individuals analyzed ($N = 35$), however, expressed ADH activity. The youngest (1-day-old) eclosed males of *D. pachea* still expressed ADH activity, although levels were low. Activity decreased further in 2-day-old adult males and was absent by 4 days. ADH activity in adult females remained high during this time.

Because ADH suppression in mature adults was sex-specific, the possibility existed that the expression of activity in females might be linked to ovarian tissue.

Table I. Relative ADH Activity in Laboratory-Cultured *Drosophila* from the *nannoptera* Species Group Assessed by Degree of Staining on Cellulose Acetate Plates[a]

Species/stage	Male	Female	Sex not determined
D. pachea			
Third-instar larvae	—	—	++ (1); + + + (10)
Early pupae[b]	—	—	+ + + (12)
Advanced pupae[c]	—	—	++ (6); + + + (6)
Adults			
1 day old	++ (8)	++ (2); + + + (2)	—
2 days old	+ (3)	+ + + (4)	—
4 days old	0 (2)	+ + + (3)	—
14 days old	0 (8)	+ + + (4)	—
D. acanthoptera (adults)[d]	+ + + (10)	+ + + (10)	—
D. nannoptera (adults)[d]	+ + + (6)	+ + + (6)	—
D. wassermani (adults)[d]	+ + + (6)	+ + + (6)	—

[a] Number of individuals analyzed in parentheses. + + +, moderate to heavy staining; ++, light staining; +, staining evident but barely visible; 0, no staining.
[b] Pupated within previous 24 hr.
[c] Fully formed flies visible through pupal case.
[d] Adults of *D. acanthoptera*, *D. nannoptera*, and *D. wassermani* were at least 7 days old.

No ADH staining, however, was evident in any of the ovaries analyzed, whereas strong staining was always found in the carcasses (not shown). The lack of ADH expression in ovaries has also been noted in other species (Colón-Parrilla and Pérez-Chiesa, 1999). Even in the *hydei* and *mulleri* subgroups of the *repleta* group, in which the *Adh* locus has been duplicated, neither *Adh-1* nor *Adh-2* is expressed in ovaries of adult females, with the exception of *D. mojavensis*, *D. arizonae*, and *D. buzzatii*, where *Adh-1* is expressed (Batterham *et al.*, 1983; Alberola *et al.*, 1987).

In order to assess whether the sex-specific suppression of ADH activity was a characteristic of the *nannoptera* species group in general, we analyzed mature adults of the other three species that comprise this group, *D. nannoptera*, *D. acanthoptera*, and *D. wassermani*. In all three species, both males and females expressed ADH activity (Table I). The intensity of staining was usually somewhat lower in males compared to females, most likely owing to the generally smaller size of males. This pattern has also been reported in other species of *Drosophila* unrelated to *D. pachea* (Colón-Parrilla and Pérez-Chiesa, 1999). In two of these species, *D. acutilabella* and *D. belladunni*, ADH bands for males were barely visible compared to females, reminiscent of the sex-specific suppression in *D. pachea*.

Proposed phylogenetic relationships among the four species of the *nannoptera* group, based on mitochondrial DNA (cytochrome oxidase) sequence data, place *D. acanthoptera* as the primitive sister group of the other three, with *D. pachea* basal to the two most derived species, *D. nannoptera* and *D. wassermani* (Pitnick *et al.*, 1997). Thus no relationship between the proposed phylogeny and loss of ADH expression is evident.

Because the *Adh* gene appears to be down-regulated in adult males of *D. pachea*, we were interested to see what effect a short-term exposure to an ethanol environment would have on survival and enzyme activity. After 24 hr of ethanol exposure no mortality was observed, and all eight males now showed low, but detectable, ADH activity; the control males showed no activity as expected (Fig. 1). These results suggest that more detailed experiments on determining ethanol tolerances and threshold values for ADH induction are warranted. Ethanol induction of ADH activity in males may explain the very low activity found in the three wild-caught males of *D. pachea* described earlier. Although the average ethanol concentration is relatively low in senita rots, maximum ethanol concentrations can exceed 9% (Fogleman and Abril, 1990). Another possibility suggested by our results, however, is that these three males were recently eclosed and still expressing low ADH activity (see Table I).

The sex-specific loss of ADH expression in adults of *D. pachea* raises interesting questions concerning possible relationships with life history traits and fitness. Larvae are restricted to feeding on necrotic tissue containing variable amounts of ethanol (Fogleman and Abril, 1990). Thus, relatively high levels of ADH activity

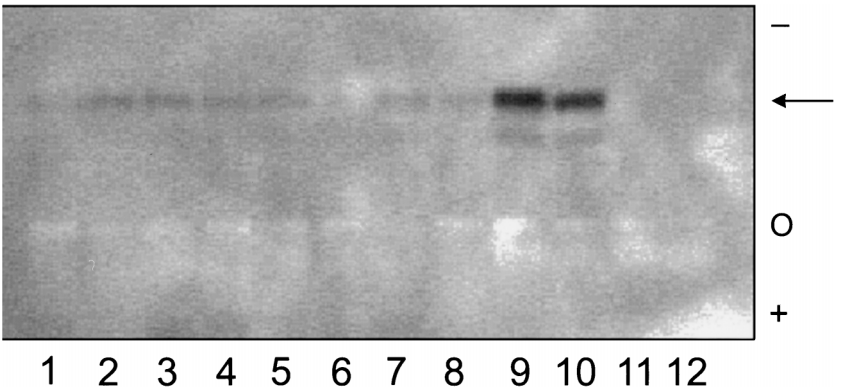

Fig. 1. Cellulose acetate electrophoresis plate showing the induction of ADH activity in eight laboratory-reared males of *Drosophila pachea* after 24 hr of exposure to an ethanol-rich environment (lanes 1–8). Female controls with high ADH activity are shown in lanes 9 and 10. Male controls (lanes 11 and 12) show the typical suppression of enzyme activity. The arrow shows the main ADH band; the slower, less intense band seen in lanes 9 and 10 is a satellite band and does not represent the product of a separate *Adh* locus. O, origin.

in larvae of both sexes increases their tolerance to ethanol by providing a means to metabolize and detoxify this substance (Merçot *et al.*, 1994; Colón-Parrilla and Pérez-Chiesa, 1999). Adults, however, have the option of finding and utilizing additional feeding sites. Whether the loss of ADH activity in males of *D. pachea* has any ecological significance regarding feeding preferences is unknown. Analyses of stable isotope ratios (δ^{13}C and δ^{15}N) in *D. pachea* and their senita host plant, however, suggest that adult flies of both sexes may be feeding on alternative hosts (Markow *et al.*, 2000). But the reason for the specific loss of ADH expression in adult-only males of *D. pachea*, and the controlling mechanisms involved, remain a conundrum.

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