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# Relationship Between ADH Activity and Behavioral Response to Environmental Alcohol in *Drosophila*

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*Three alcohol dehydrogenase genotypes, homozygous for either the electrophoretically fast, slow, or null allele at the* Adh *locus in* D. melanogaster, *were tested for relative larval alcohol preference behavior (APB) over a range of ethanol concentrations. Differences in behavior between genotypes were not significant at concentrations below 10%. At concentrations greater than 10%, avoidance behavior was negatively correlated with the relative ADH activity levels of the genotypes tested. A model based on the differential buildup of toxic acetaldehyde is proposed to explain the avoidance response.*

**KEY WORDS:** *Drosophila*; behavior; ADH activity; adaptation; evolution; alcohol avoidance; *Adh* genotypes.

## INTRODUCTION

For the past few years a number of laboratories have been involved in a detailed analysis of alcohol adaptation in *Drosophila* as a model system for the study of adaptive evolution (e.g., Clarke, 1975; McDonald and Ayala, 1978*a*). This system is ideally suited to evolutionary studies, for the adaptive process can be followed from the level of the relevant environmental stimulus through the level of the adaptive genetic response. Much of the work carried out to date has focused on the physiological ability of *Drosophila* to detoxify and utilize environmental alcohols. In this regard, the enzyme alcohol dehydrogenase (E.C. 1.1.1.1) has been found to play a central role. ADH activity consistently correlates with the ability of flies to exploit

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alcohol environments (e.g., Ainsley and Kitto, 1975; Kamping and Van Delden, 1978; McDonald and Avise, 1976) and this property has been found to be under "regulatory" as well as structural gene control (Hewitt *et al.*, 1974; Ward, 1975; McDonald *et al.*, 1977; McDonald and Ayala, 1978*a*; Ward and Hebert, 1972).

The possible adaptive significance of the behavioral response to *Drosophila* to alcohol environments and its relationship to alcohol tolerance and utilization have only begun to be studied (e.g., Cavener, 1979; King *et al.*, 1976; McKenzie and Parsons, 1972; Richmond and Gerking, 1979). Several recent investigations have explored the possibility of a causal relationship between ADH activity and relative alcohol preference. For example, Richmond and Gerking (1979) found a positive correlation between oviposition site preference and ADH activity between the *Drosophila* species groups they examined. Parsons (1977) and Parsons and Kind (1977) measured the relative alcohol preference behavior (APB) of first instar larvae of *D. melanogaster* and *D. simulans*—two sibling species known to differ significantly in their ADH activities (McDonald and Avise, 1976). Their finding that *melanogaster* displayed a significantly higher initial alcohol preference than *simulans* suggests a positive correlation between relative levels of ADH activity and interspecific variation in larval APB.

In this article we report the results of a series of alcohol preference tests carried out with *D. melanogaster* third instar larvae homozygous for different *Adh* alleles. The purpose of this study was to determine if consistent differences in larval APB exist between genetically homozygous strains of the same species and, if so, whether these differences correlate with the relative ADH activity differences known to be associated with different *Adh* genotypes. In addition, our tests were carried out over a range of alcohol and aldehyde concentrations in order to determine if relative APB of a fixed genotype is affected by such environmental variability.

## MATERIAL AND METHODS

Four strains of *D. melanogaster* were used in our study. Three of the strains—F1, F2, and S1—were made completely homozygous for their second and third chromosomes (McDonald and Ayala, 1978*b*). Strains F1 and F2 carried the electrophoretically detectable fast allele at the *Adh* locus. Strains S1 was fixed for the electrophoretically detectable slow allele. The fourth strain, N2 (null), had no detectable ADH activity. All stocks were maintained on standard cornmeal–molasses medium at 22°C unless otherwise specified.

Each experiment was conducted on a petri plate (3.5 inches diameter) in which half of the 1.5% agar medium (Difco) was supplemented with con-

trolled amounts of ethanol or acetaldehyde and half supplemented with equivalent volumes of $H_2O$. Each test consisted of randomly placing 15 early third instar larvae (90–100 hr after oviposition) on the plates and allowing them to distribute themselves according to their performance. It has previously been demonstrated that larval feeding rate reaches a maximum at early third instar (Burnet and Connolly, 1974). We found it necessary to add all alcohol and aldehyde supplements to the agar as it cooled (~48°C) to minimize evaporation. Failure to do this removed any effective control of test concentrations due to evaporation and resulted in nonreproducible results. Test plates were prepared with ethanol concentrations of 0%, 2%, 4%, 6%, 8%, 10%, and 15% by volume and aldehyde concentrations of 1%, 2%, and 3% by volume. Six plates were prepared simultaneously and tests were run in sequence 2 min apart with plates rotated 60° clockwise from the preceding plate. Rotation served to control any possible positional bias, as from uneven lighting. All tests were conducted in an enclosed chamber with overhead fluorescent lighting to ensure a standardized test environment. An average of 17 replicate tests were run for each strain at each concentration (0% tests and six replicates; all other concentrations had $>12$ replicates).

Larval response to the stimuli were recorded at 15, 30, and 60 min after test initiation. All tests were preceded by a 2 min lag to allow for larval readjustment to the transfer to test plates.

Pretreatment of *Drosophila* larvae with isopropyl alcohol results in a posttranslational modification of the ADH protein, causing an apparent reduction in the enzymes specific activity (Schwartz and Sofer, 1976). In order to test whether such a modification can influence behavior, F1 and S1 larvae were subjected to isopropanol-supplemented *Drosophila* culture medium (1%) for 48 hr prior to the testing of their APB as described above.

ADH activities of isopropanol-treated and control larvae were assayed according to the techniques of McDonald and Avise (1976). Starch gel electrophoresis was carried out according to the techniques of Ayala *et al.* (1972).

ANOVAs were carried out for each strain over all test concentrations, and the significance level of any difference between mean preference values at each concentration was determined by the least-squares method (Steel and Torrie, 1960).

## RESULTS

### Response to Ethanol with No Pretreatment

All strains exhibited essentially random behavioral response when tested on control plates containing no alcohol or aldehyde. Figure 1 graphically depicts the APB of the four strains tested relative to controls.

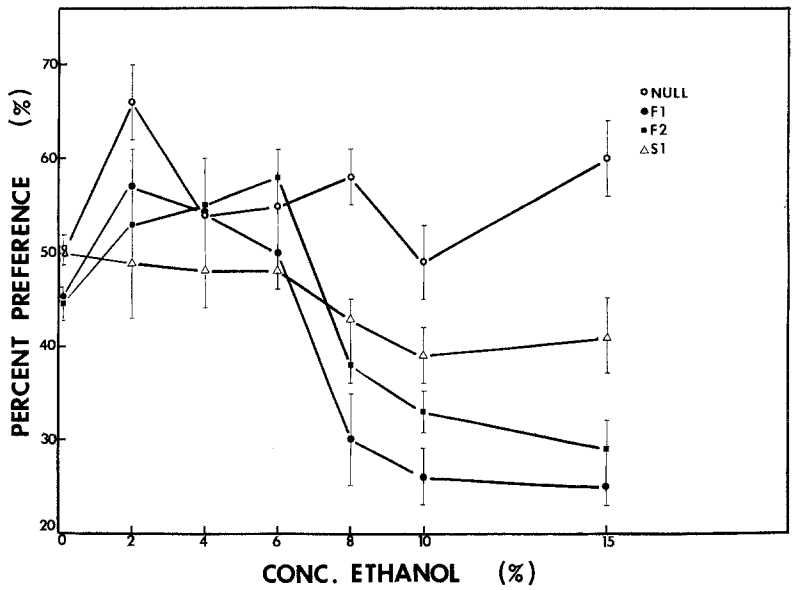


**Fig. 1.** Mean percentage of larvae preferring alcohol over range of ethanol concentrations for strains of *Drosophila melanogaster*.

The behavior of the $Adh^{Null}$ strains, which has no detectable ADH activity, was not significantly different from the control. At the 2% ethanol concentration, however, a marked but nonsignificant preference was observed. The $Adh^{Fast}$ strains (F1 and F2) displayed significantly different response profiles from that of the $Adh^{Null}$ strain. At alcohol concentrations $\leq 6\%$ the response was not significantly different from the control. However, at concentrations $>8\%$ we observed a significant decrease in alcohol preference ($P < 0.05$). This avoidance behavior was maximized at a concentration of 15% ethanol ($P < 0.01$), where avoidance was exhibited by 75% of the larvae tested. The slow genotype exhibited significantly different behavior from the Fast and Null strains. As is shown in Fig. 1, S1 larvae exhibited a random response at ethanol concentrations $<8\%$ and only a slight avoidance response at higher concentrations.

The influence of time of exposure of larvae to the test conditions on APB is presented in Fig. 2. In general, the relative preference patterns that were apparent at 15 min were the same patterns observed at 60 min. The one exception was the initial preference shown by F1 larvae for 4% ethanol. At 15 min 68% of the larvae were found on the alcohol-treated medium. By 30 min this initial preference was observed to decrease until at 1 hr no significant preference was observed. The average preference value of strain F1

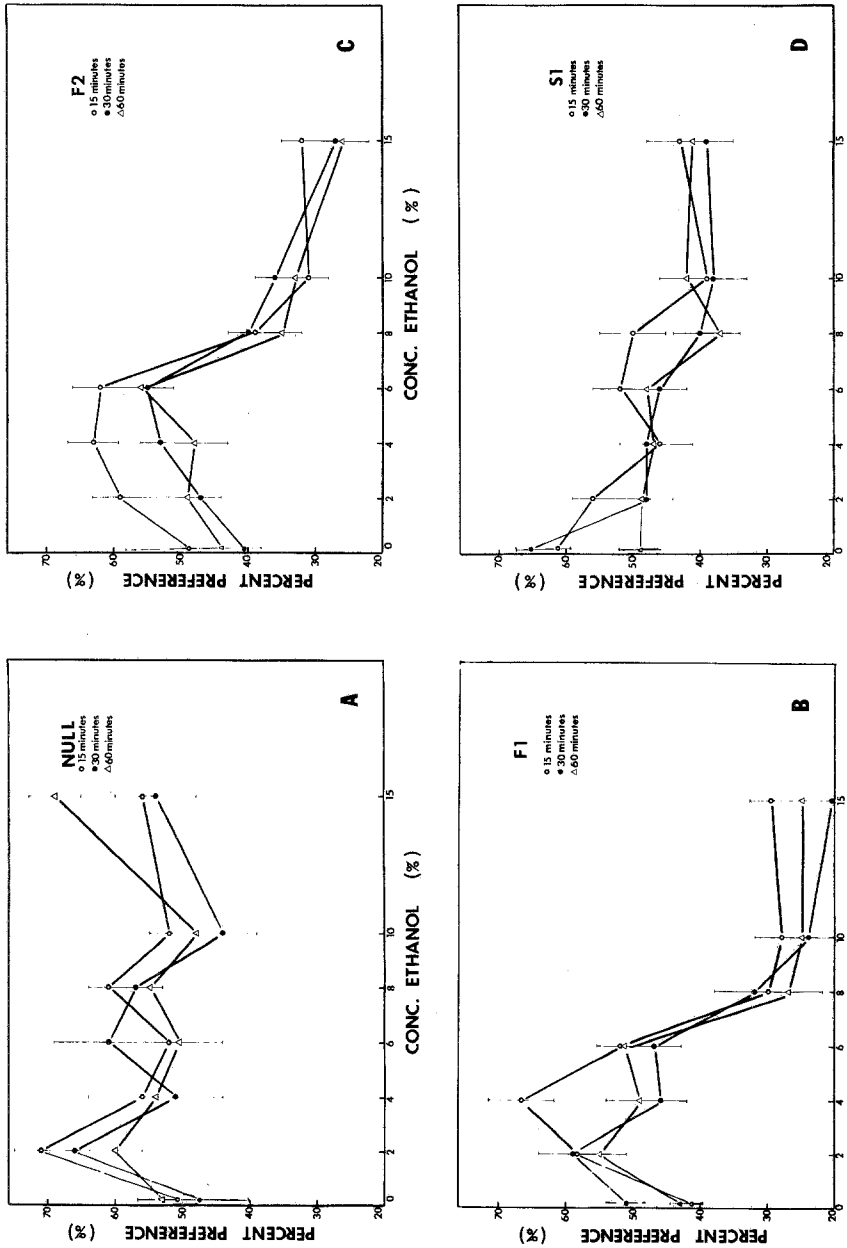


**Fig. 2.** Mean percentage of larvae perferring alcohol over a range of ethanol concentrations at 15, 30, and 60 min.

at 4% ethanol, however, was not significantly different from that of controls. No appreciable reduction in larval motility was observed in any strain over the 1-hr test period.

In general, consistent preference behavior was observed only in the $Adh^{Null}$ strain and then only at relatively low alcohol concentrations (2%). Avoidance behavior began to appear at 8% ethanol and was significant only for the $Adh^{Fast}$ strains. The $Adh^{Slow}$ strain demonstrated slight but nonsignificant avoidance at concentrations $>10\%$. The $Adh^{Null}$ strain failed to show avoidance at any of the test concentrations. ADH activities determined for the tested strains were as follows: Null = 0, F1 = 75.5 ± 2.3, F1 treated = 9.0 ± 1.3, F2 = 75.2 ± 2.1, S1 = 26.0 ± 2.1, S1 treated = 8.0 ± 0.8. When APB was correlated with total ADH activity, we observed a highly significant correlation with avoidance behavior at concentrations $>8\%$ ($P < 0.01$). No significant correlation between ADH activity and larval behavior existed below 8% ethanol. These results clearly demonstrate that the expression of genotypic differences in larval APB depend on the environment. That is, there is a definite difference in larval APB between the genotypes tested, but this difference is manifest only at ethanol concentrations $>8\%$.

### Response to Ethanol After Pretreatment with Isopropanol

If the correlation between ADH activity and larval avoidance behavior truly reflects a casual relationship, a posttranslational modification of the ADH protein that results in a reduction in the enzyme's specific activity should result in decreased avoidance behavior as well. In order to test this prediction two strains, F1 and S1, were pretreated with 1% isopropanol, which successfully induced a posttranslational modification. This shift or conversion was verified by electrophoresis of treated flies (Fig. 3). The isopropanol-mediated conversion lowered the specific activity of ADH by three-to-eightfold (see above).

Figures 4 and 5 compare the behavioral responses of the isopropanol-treated and control strains. In each case we observed a marked decrease in avoidance behavior at all concentrations. These results are consistent with the above findings that ADH activity is negatively correlated with alcohol preference behavior.

In order to ensure that the behavioral differences observed in pretreated larvae were indeed due to the posttranslational shift in the ADH enzyme and not the result of some nonspecific conditioned response, we pretreated the Null strain with isopropyl alcohol and observed no significant difference in APB between treated and untreated $Adh^{Null}$ larvae. In other words, isopropanol pretreatment has an influence on APB only when the

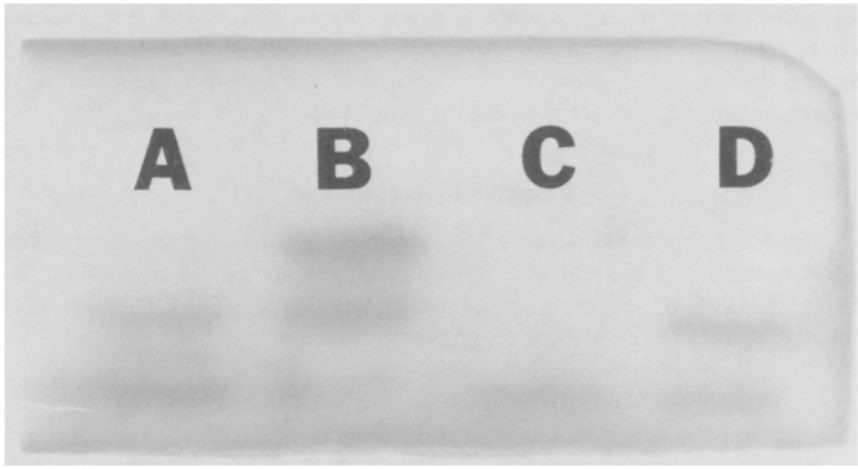


**Fig. 3.** Electrophoretic variants of the *Adh* locus on a starch gel. A: $Adh^F$ (F1). B: $Adh^F$ converted (F1 converted). C: $Adh^S$ (S1). D: $Adh^S$ converted (S1 converted).

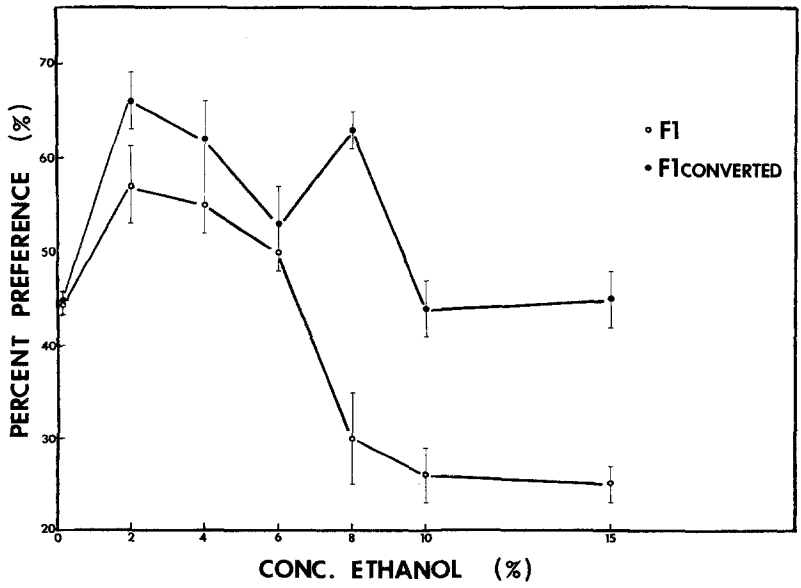


**Fig. 4.** Mean percentage of larvae preferring alcohol over a range of ethanol concentrations for F1 and F1 converted strains of *Drosophila melanogaster*.

larvae being treated are capable of producing an active *Adh* gene product. These results demonstrate that it is not the alcohol pretreatment of larvae *per se* that modifies larval APB but rather the isopropyl-induced ADH conversion which pretreatment brings about.

## Response to Acetaldehyde

One possible explanation of the observed negative correlation between ADH activity and alcohol preference is that the larvae are responding to a differential rate of accumulation of toxic acetaldehyde. The higher the ADH activity of a larva the greater its conversion rate of ethanol to acetaldehyde and thus the greater its avoidance behavior.

Although our data are fully consistent with this hypothesis, it is also possible that the observed behavioral differences are at least partially due to genetically determined variation in acetaldehyde sensitivity rather than to ADH-regulated rates of aldehyde buildup.

In order to test this possibility, we subjected our strains to a range of acetaldehyde concentrations and observed their relative preference behavior as described above. Acetaldehyde is an extremely toxic substance to *Drosophila* (David *et al.*, 1978), and we observed that larvae exposed to

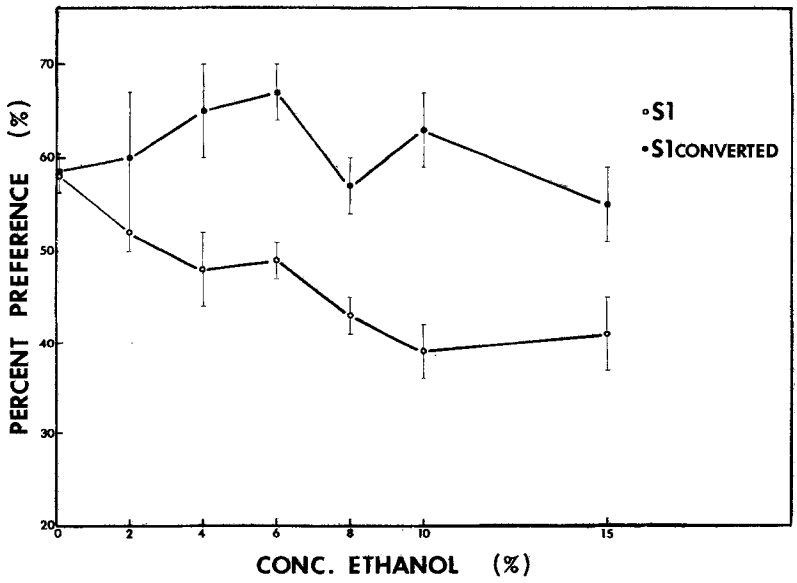


**Fig. 5.** Mean percentage of larvae preferring alcohol over a range of ethanol concentrations for S1 and S1 converted strains of *Drosophila melanogaster*.

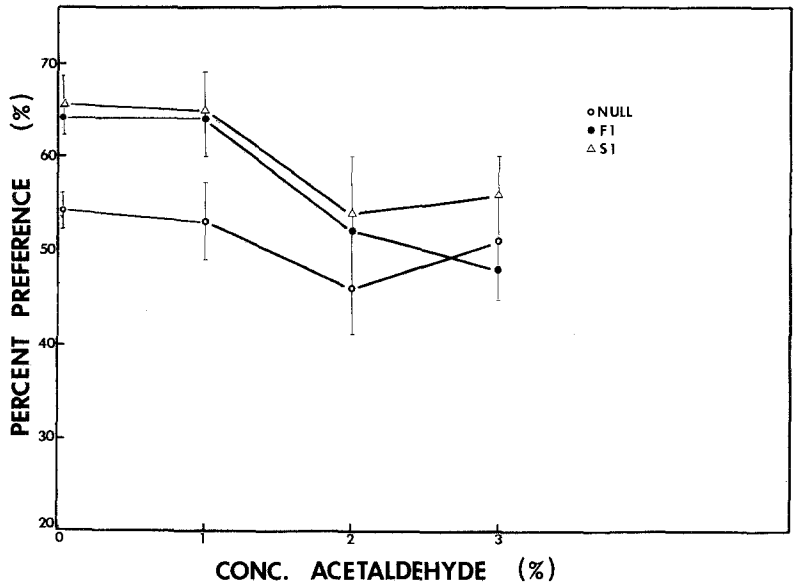


**Fig. 6.** Mean percentage of larvae preferring aldehyde over a range of acetaldehyde concentrations for strains of *Drosophila melanogaster*.

concentrations greater than 3% became sluggish and died. We therefore had to limit our behavior tests to concentrations <3%. The results of the study are presented in Fig. 6. All strains displayed a slight but nonsignificant preference behavior at 1% acetaldehyde followed by slight avoidance at higher concentrations. As in the ethanol studies, the avoidance response of all genotypes was observed to increase with time of exposure to the aldehyde environments. In no test did we observe significant differences in relative response to acetaldehyde between genotypes. These findings are not consistent with the view that our strains are differentially sensitive to acetaldehyde. The results are, however, consistent with the hypothesis that the negative correlation observed between ADH activity and avoidance to environmental ethanol is the direct result of ADH-regulated rates of acetaldehyde buildup.

## DISCUSSION

Three general conclusions come out of our study.

1. *Significant differences in larval APB exist between different Adh genotypes. Differences in avoidance of environmental alcohol have been associated with the product of a single structural gene.*

Although a number of studies have shown that taxes in *Drosophila* are under complex genetic control (e.g., Markow and Merriam, 1977; Dobzhansky and Spassky, 1967; Benzer, 1967), our results demonstrate that a specific gene may exert a significant effect. The action of such a gene need not be directly involved with the neural-physiological mechanisms of behavior (e.g., Pruzan and Bush, 1977; Fuyama, 1976, 1978, Metcalf *et al.* 1979), but may exert its influence by altering specific components of an organism's "internal environment."

Our data suggest that larval avoidance of alcohol environments is directly dependent on the rate of aldehyde buildup within the cell and that this buildup is positively correlated with ADH activity levels.

2. *The influence of the Adh genotype on larval APB is environmentally dependent.* Both inter- and intrastrain differences in APB were most significant at ethanol concentrations above 10%. This suggests that there exists an alcohol threshold above which further ingestion triggers an avoidance response. This threshold may serve to mediate alcohol consumption within physiologically tolerable limits and is correlated with type and amount of alcohol as well as *Adh* genotype.

The fact that the genetic component of APB is environmentally dependent advises against drawing general conclusions based on experiments carried out at only one or two alcohol concentrations. For example, if we had performed our tests at ethanol concentrations of 4% and 6%, we would not have observed significant differences in behavior and may have concluded that larval APB is independent of *Adh* genotype. The environmentally dependent nature of genetic differences in oviposition site preference *vis-à-vis* alcohol environments have previously been documented (Richmond and Gerking 1979).

3. *Larval preference for environmental alcohol appears to be independent of the Adh locus.* Although each of the strains tested displayed a slight initial (15 min) preference for low levels of environmental ethanol (<4%), this initial preference was not significantly correlated with relative ADH activity. In addition, the attraction of larvae to low levels of ethanol was not a stable phenomenon but was found to wane substantially by 30-min exposure to the test conditions. We conclude, therefore, that initial preference of third instar *D. melanogaster* larvae for ethanol environments is an ephemeral phenomenon and is apparently independent of *Adh* genotype.

These conclusions are in apparent contrast to those studies carried out on adult *Drosophila* in which the relative preferences in different species to oviposit on alcohol-treated medium were found to correlate directly with relative ADH activities (Richmond and Gerking, 1979). It is important to note, however, that the behavioral responses to larvae and adult *Drosophila*

to the same environmental stimuli need not be identical and indeed have at times been observed to be quite different (Cooper, 1960). In addition, it is unclear whether ADH activity–APB relationships which are observed between species will hold up within species as well. For example, interspecific studies of larval APB suggest a positive correlation between ADH activity and initial alcohol preference (e.g., Parsons, 1977). As discussed above, our intraspecific results do not support such a conclusion. A series of interspecific larval APB studies employing the same experimental procedures as were used in this study are presently under way in our laboratory and should help clarify this issue.

In a recently published survey of larval APB, Cavener (1979) presents results which, on the surface at least, contradict our own. Cavener found a significant positive correlation between larval APB and relative ADH activity between the strains he tested. This discrepancy could be due to intragenotypic variation among the strains being analyzed or perhaps, what is more likely, to differences in experimental technique. As mentioned above, we found it necessary to add alcohol supplements to cooling agar in order to avoid significant evaporation. In his technique, Cavener added the alcohol supplement prior to the boiling of his alcohol–agar mixture. It is likely that this method of preparation results in an effective test alcohol concentration considerably reduced from that present in the preboiled mixture. If we are correct in this suspicion, Cavener's observation of a positive APB-ADH activity correlation may be analogous to the relationship (nonsignificant in our case) observed between our $Adh^{Fast}$ and $Adh^{Slow}$ strains at low test alcohol concentrations.

The adaptive significance of the interrelationship of physiology and behavior is an area long neglected by evolutionary biologists. The reason for this neglect is not so much a lack of interest as a scarcity of experimental systems in which these two aspects of adaptive response can be studied simultaneously. The ability of *Drosophila* to adapt to alcohol environments provides a model system to investigate a number of facets of the adaptive process including the significance of the relationship between behavior and physiology.

The present study represents a first step in a series of experiments designed to shed light on the interplay between behavior and physiology *vis-à-vis* the ability of *Drosophila* to exploit alcohol environments. We have established a definite connection between larval avoidance response to alcohol and the relative catalytic efficiency of different *Adh* genotypes. Further studies carried out at different life stages and over a broad spectrum of environmental challenges are required before definitive conclusions can be drawn concerning adaptive significance. We are, nevertheless, encouraged with our preliminary results and feel optimistic that this line of

experimentation will render valuable insight into the holistic nature of adaptive evolution.

## ACKNOWLEDGMENTS

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