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# ALCOHOL TOLERANCE, ADH ACTIVITY, AND ECOLOGICAL NICHE OF *DROSOPHILA* SPECIES

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*Abstract.*—In vitro alcohol dehydrogenase (ADH) activity was measured in adults of species belonging to *Drosophila* and to the related genus *Zaprionus*. Data were analyzed according to the known breeding sites and the level of ethanol tolerance of these species. Alcohol dehydrogenase activity was assayed with both ethanol (E) and isopropanol (I). Our results show a very broad range of activities among the 71 species investigated, the ratio of the highest value observed (*D. melanogaster*) to the lowest (*D. pruinosa*) being 65:1. A general positive correlation was found between the level of ADH activity and the capacity to detoxify ethanol. Nevertheless, many species show exceptions to this rule. Contrary to a logical expectation, adaptation to high alcoholic resources, which has been a recurrent evolutionary event, was not mediated by a more efficient use of ethanol, that is, an increase of the E/I ratio. This ratio seems to be quite variable according to the phylogeny and is especially low in the subgenus *Sophophora* as well as in *Zaprionus*. Alcohol tolerance clearly is related to the larval habitat of the species and shows that adaptation to alcoholic resources has been a major evolutionary challenge in drosophilids. This adaptation is not related to phylogeny, having occurred independently several times during the evolution of the group. Finally, it should be borne in mind that, besides metabolization and detoxification, other physiological processes such as nervous-system tolerance or ethanol excretion may be involved in ethanol tolerance, and such functions also should be investigated. Environmental ethanol, which is certainly a major ecological parameter for many drosophilids, has selected a diversity of physiological adaptations, all related to the *Adh* locus, but presumably much more complicated than was previously believed.

*Key words.*—Alcohol dehydrogenase (ADH), *Drosophila*, ethanol tolerance, habitat.

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The evolutionary success of angiosperms and the concomitant production of sweet fruits have resulted in a wide variety of sugar-rich resources. When decaying, these resources are mainly attacked by yeasts, which excrete alcohol into the environment. Alcohol may be considered primarily as a toxic compound that deters most potential consumers from these sites.

Drosophilid species are saprophagous and their basic ecological niche consists of decaying plant materials and fungi (David et al. 1983). Numerous species, however, belonging to various evolutionary radiations in the family have adapted to decaying sweet resources and are known as fruit breeders.

If the presence of ethanol in fermenting fruits is a significant selective pressure, a higher tolerance to ethanol is expected in fruit breeders than in nonfruit breeders. Experimental studies have confirmed this expectation (David and Van Herrewege 1983). The amount of alcohol found in fermenting fruits remains generally low, less than 4% (Gibson et al. 1981; McKechnie and Morgan 1982; Oakeshott et al. 1982; Capy et al. 1988). However, in some manmade environments, resources resulting from artificial fermentations may contain more than 10% alcohol (Briscoe et al. 1975; McKenzie and McKechnie 1979). *Drosophila melanogaster* is well known for its capacity to proliferate in wine cellars during vintage time and shows much geographic variability with respect to alcohol tolerance (David et al. 1986). Two other species are also known to exhibit a high alcohol tolerance and breed under artificial fermentation conditions: these are *D. virilis*, found in breweries (David and Kitagawa 1982) and *D. lebanonensis*, the most tolerant species, which is found in Spanish wine cellars (David et al. 1979).

In *D. melanogaster*, 90% of ethanol is degraded using the metabolic pathway of ADH (alcohol dehydrogenase) (Geer et al. 1985; Heinstra et al.

1987; Heinstra and Geer 1991). In adults, the main metabolic function of this enzyme seems to be to allow the use of ethanol as a resource (Van Herrewege and David 1980).

If ADH plays a key role in the adaptation of other drosophilid species to substrates undergoing alcoholic fermentation, we expect to find (1) a higher ADH activity in fruit breeders than in nonfruit breeders, and (2) an interspecific correlation between the ADH activity and ethanol tolerance.

The present work was undertaken to check these expectations. To test these hypotheses, in vitro ADH activity in species belonging to *Drosophila* and the related genus *Zaprionus*, was analyzed and compared according to their known breeding sites and to their level of ethanol tolerance. Alcohol dehydrogenase activity was assayed with both ethanol (a primary alcohol) and isopropanol (a secondary alcohol), which is known to be a better substrate, at least in *D. melanogaster* (Winberg et al. 1982).

## Materials and Methods

*Species Studied.*—Altogether 67 *Drosophila* and 9 *Zaprionus* species have been studied (table 1). For practical reasons, alcohol dehydrogenase (ADH) activity and ethanol tolerance were jointly determined for only 67 species. The strains studied came from populations collected in different places in the world. The present study has been limited to species that can be reared in laboratory conditions, that is, on a killed yeast medium (David 1962). In *D. melanogaster*, two $Adh^{FF}$ and two $Adh^{SS}$ strains, using populations established from the Netherlands and Congo, and a strain ($Adh^{n4}$) with a null ADH activity (David et al. 1976) have been analyzed.

*Habitats.*—*Drosophila* habitats, especially for the larval stages, are quite diverse and more or less specific. Data for this study have been taken from several papers (Barker and Starmer 1982; David and Van Herrewege 1983; Lachaise and Tsacas 1983) and from numerous unpublished observations. Species may be characterized by their main larval habitat, corresponding to the following categories: breweries and cellars (BAC), fermented fruit (FFT), cacti (CAC), fungi (FUN), flowers (FLO), and decaying plant material (DPM). However, considering the presence of alcohol in the resources, we combined some of these categories to form just three groups.

Group 1 corresponds to species able to breed in artificial, man-made, high alcoholic resources (i.e., BAC). This group consists of three species: *D. melanogaster* (from temperate countries), *D. lebanonensis* (David et al. 1979), and *D. virilis* (David and Kitagawa 1982). We have added *D. hydei* to this group, a species that often proliferates, together with *D. melanogaster*, in wine exudates or grape residues in Spanish and French wine cellars (unpubl. data).

Group 2 corresponds to species breeding in sweet fermenting fruits (i.e., FFT) with a significant, but not very high, amount of alcohol. We include in this group the tropical *D. melanogaster* populations because they breed mainly in various cultivated fruits. *Drosophila arizonae* is also included here, as this cactophilic species breeds in organ-pipe cacti such as *Lemaireocereus thurberi* and *Machaerocereus gummosus* (Heed 1982; Ruiz and Heed 1988) whose decomposing tissues produce ethanol (Starmer et al. 1986; Fogleman and Heed 1989). We have also included *D. buzzatii* in this group. In its countries of origin (South America), the species breeds in rotting cladodes of *Opuntia*. But for the present study, the strain analyzed was collected in a Tunisian oasis, breeding in prickly pears, associated with *D. melanogaster* and *D. simulans* (Haouas et al. 1984).

Group 3 includes species using nonsweet substrates, that is, categories FLO, FUN, and DPM.

*Alcohol Dehydrogenase (ADH) Activity.*—The in vitro ADH activity was assayed, following the procedure described in Merçot and Higuet (1987), by monitoring the rate of NADH production in crude extracts of adult flies. Two substrates were used: ethanol and isopropanol. Three or four samples of 30 males (6–8 d old), obtained from uncrowded cultures at 25°C, were weighed and homogenized in 0.1 M tris HCl buffer, pH 8.6 (1 mL of buffer for 30 mg of fresh weight). After centrifugation, the supernatant was taken and kept at −70°C for 1 to 9 wk before ADH activity was measured. The assay mixture was composed of 0.8 mL of supernatant, 0.1 mL of 10% alcoholic substrate, 90% of 0.1 M Tris HCl buffer, and 0.1 mL of 0.02M $\beta$NAD. The measurements were made using a Perkin-Elmer Lambda 1 spectrophotometer at 25°C, for 90 s with ethanol and 30 s with isopropanol. One unit of ADH activity is defined as an increase in absorbance of 0.001 per minute at 340 nm. The ADH activity is expressed in $\Delta OD \times 10^3$ per mg of fresh weight. This protocol for ADH activity assays has been established for *D. melanogaster*. Although it may not be optimal for all species, it appeared reliable enough to be used for the species studied here.

*Ethanol Tolerance.*—Ethanol tolerance of adult flies was measured according to the method described by David and Van Herrewege (1983). Larvae were grown at 25°C on a killed yeast medium (David 1962), which minimizes crowding effects and produces healthy adult flies. After light etherization, the adults were aged on vials with the same medium. For toxicity tests, samples of 20 4–6-d-old males or females were transferred in airtight plastic vials in the presence of 2 mL of an ethanol solution of a given concentration. In all concentrations, 3% sucrose was added to prevent any mortality caused by starvation. This may be important, especially for species very sensitive to ethanol. With this technique, mortality under control conditions (without ethanol) must be null. This was verified during the aging process. In a few cases, a significant mortality was observed before the treatment. In such cases, the flies were discarded and the assay repeated in another experiment. For each assay we used three or four concentrations. For each ethanol concentration, at least four vials (80 adults) of both sexes were used. Dead flies were scored after two d of treatment. Mortality was plotted against concentration and the LD50 estimated graphically. Toxicity studies must show a very clear correlation between concentration and mortality, that is, almost no dead flies at the lowest concentration. This was regularly verified. In all investigated species, sex differences were very small and the data were pooled before calculation of the LD50.

## Results

The basic data for the 76 species investigated are given in table 1, according to the three ecological groups previously defined. Table 1 also shows the taxonomy, the origin of the strains, and their main food resource. The data were analyzed in several ways.

### *ADH Activity on Ethanol*

The frequency distributions of alcohol dehydrogenase (ADH) activity on ethanol for the three groups are shown in figure 1, and the mean values are given table 1.

Species of the first group clearly have the highest average ADH activities (3.953 ± 0.604). For species living on rotting fruit (group 2), the mean activity (2.031 ± 0.194) is almost four times that of species that do not live on such sweet substrates (group 3; 0.586 ± 0.139), the difference being highly significant (Wilcoxon's test, $E_w$ = 7.981; $P < 0.001$). Even if the species of group 1 are not taken in account, there is a clear average difference in ADH activity between species confronted by alcoholic fermentation and those that grow preferably, or exclusively, in resources without fermentation.

Apparently no relationship exists between ADH activity and phylogeny. In the *melanogaster* subgroup, the three following cases coexist: (1) species with a high ADH activity such as *D. melanogaster,* (2) species with a low ADH activity such as *D. orena,* and (3) species with intermediate activities. In the *montium* subgroup, *D. burlai* and *D. bocqueti* have a high activity level, which is four times that of the eight other investigated species. In the subgenus *Drosophila,* as well as in the *melanogaster* subgroup, some species with high ADH activity are found (e.g., *D. tsigana* and *D. virilis*), whereas others have a very low activity, such as *D. arawakana, D. iri,* and *D. ornatipennis.* These observations suggest that the interspecific variability in ADH activity level may change rapidly. However, it is not possible to say whether such variability results from changes in the specific activity of the protein coded by the *Adh* structural gene or from changes in regulatory genes controlling the amount of ADH.

Many species of the *repleta* group harbor a functional duplication of the *Adh* gene (Batterham et al. 1984; Yum et al. 1991). Such is the case for four of the five species tested in this group (*D. arizonae, D. hydei, D. repleta,* and *D. buzzatii*), but not for *D. mercatorum.* The fact that the latter presents the lowest activity is probably not related to the presence of only a single gene copy in this species. Indeed, in the other species (except *D. hydei*) the two genes do not function at the same time: one is expressed during the larval stages, the other in the adults (Fisher and Maniatis 1986). Moreover, differences may exist between strains of different origins because Batterham et al. (1984) observed a higher adult specific activity in males of *D. mercatorum* than in males of *D. buzzatii* and *D. hydei.*

### *ADH Activity on Isopropanol*

The in vitro ADH activities on isopropanol are also given in table 1. They are strongly correlated to those on ethanol ($r = 0.952$, df = 71, $P < 0.001$). If a preponderance of ethanol in the resources has selected a higher ADH activity, we could expect this increase to result from a modification of the active site of the enzyme, favoring

TABLE 1. Relationship between larval habitat, ADH activity, and ethanol tolerance (DL50%) among drosophilid species. BAC, breweries and cellars; CAC, cacti; DPM, decaying plant material; FFT, fermented fruits; FLO, flowers; FUN, fungi; NT, not tested.

| Genus Subgenus | Group | Subgroup | Species | Geographic origin | Habitat | ADH activity Ethanol (E) | ADH activity Isopropanol (I) | E/I | DL50 (%) |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 (species living in artificial, man-made alcoholic environment). | | | | | | | | | |
| *Drosophila* | | | | | | | | | |
| *Scaptodrosophila* | *victoria* | | *lebanonensis* | Spain | BAC | 5.378 ± 0.589 | 19.664 ± 2.538 | 0.273 | 22.5 |
| *Sophophora* | *melanogaster* | *melanogaster* | *melanogaster* $Adh^{SS}$ | Netherlands | BAC | 2.544 ± 0.254 | 9.544 ± 0.405 | 0.267 | 13.8 |
| | | | *melanogaster* $Adh^{FF}$ | Netherlands | BAC | 5.419 ± 0.306 | 26.571 ± 1.122 | 0.204 | 15.6 |
| *Drosophila* | *repleta* | *hydei* | *hydei* | France | BAC | 3.071 ± 0.089 | 8.588 ± 0.254 | 0.358 | 10.0 |
| | *virilis* | | *virilis* | Japan | BAC | 3.353 ± 0.090 | 8.301 ± 0.323 | 0.404 | 11.6 |
| **Mean** | | | | | | **3.953 ± 0.604** | **14.534 ± 3.676** | **0.301** | **14.7** |
| Group 2 (species breading on rotting sweet fruits and exposed to alcoholic fermentation). | | | | | | | | | |
| *Drosophila* | | | | | | | | | |
| *Scaptodrosophila* | *latifasciaeformis* | | *deflexa* | France | FFT | 3.210 ± 0.037 | 13.672 ± 0.088 | 0.235 | NT |
| | | | *finitima* | Madagascar | FFT | 1.341 ± 0.154 | 4.063 ± 0.720 | 0.330 | 5.0 |
| | | | *latifasciaeformis* | Reunion | FFT | 0.903 ± 0.094 | 2.932± 0.254 | 0.308 | 3.7 |
| | *victoria* | | *rufifrons* | Greece | FFT | NT | NT | | 4.3 |
| *Sophophora* | *melanogaster* | *melanogaster* | *erecta* | Ivory Coast | FFT | 1.935 ± 0.085 | 8.757 ± 0.656 | 0.221 | 3.9 |
| | | | *mauritiana* | Mauritius | FFT | 1.711 ± 0.160 | 8.576 ± 0.815 | 0.200 | 3.2 |
| | | | *melanogaster AdhSS* | Congo | FFT | 2.644 ± 0.170 | 9.737 ± 0.568 | 0.272 | 6.3 |
| | | | *melanogaster AdhFF* | Congo | FFT | 5.793 ± 0.330 | 27.738 ± 2.514 | 0.209 | 7.3 |
| | | | *orena* | Cameroun | FFT | 0.791 ± 0.119 | 3.148 ± 0.529 | 0.252 | 1.4 |
| | | | *sechellia* | Seychelles | FFT | 1.140 ± 0.084 | 5.363 ± 0.680 | 0.213 | 1.8 |
| | | | *simulans* | France | FFT | 1.860 ± 0.140 | 7.009 ± 0.377 | 0.265 | 4.1 |
| | | | *teissieri* | Congo | FFT | 1.781 ± 0.121 | 7.610 ± 0.713 | 0.234 | 3.4 |
| | | | *yakuba* | Congo | FFT | 1.787 ± 0.159 | 8.103 ± 0.903 | 0.220 | 3.7 |
| | | *montium* | *bakoue* | Ivory Coast | FFT | 0.665 ± 0.073 | 2.950 ± 0.272 | 0.225 | 3.3 |
| | | | *burlai* | Congo | FFT | 2.497 ± 0.192 | 8.718 ± 0.830 | 0.286 | 3.2 |
| | | | *bocqueti* | Congo | FFT | 1.478 ± 0.195 | 4.667 ± 0.358 | 0.317 | 4.6 |
| | | | *chauvacae* | Congo | FFT | 0.750 ± 0.056 | 2.948 ± 0.223 | 0.254 | NT |
| | | | *davidi* | Congo | FFT | 0.644 ± 0.068 | 1.503 ± 0.073 | 0.428 | 1.8 |
| | | | *dossoui* | Benin | FFT | 0.721 ± 0.038 | 2.083 ± 0.155 | 0.346 | 1.9 |
| | | | *kikkawai* | Madagascar | FFT | 0.607 ± 0.076 | 2.143 ± 0.319 | 0.283 | 2.3 |
| | | | *malagassya* | Madagascar | FFT | 0.683 ± 0.044 | 3.080 ± 0.215 | 0.222 | 2.8 |
| | | | *serrata* | Australia | FFT | 0.728 ± 0.032 | 2.797 ± 0.189 | 0.260 | 2.5 |
| | | | *vouidibioi* | Congo | FFT | 1.041 ± 0.135 | 4.278 ± 0.456 | 0.243 | 2.8 |

TABLE 1. Continued.

| Genus Subgenus | Group | Subgroup | Species | Geographic origin | Habitat | ADH activity Ethanol (E) | Isopropanol (I) | E/I | DL50 (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | *ananassae* | *ananassae* | Tahiti | FFT | 0.994 ± 0.059 | 3.820 ± 0.367 | 0.260 | 4.0 |
| | | | *bipectina* | New Caledonia | FFT | 2.185 ± 0.183 | 7.439 ± 0.540 | 0.294 | 2.1 |
| | | | *ercepeae* | Reunion | FFT | 5.743 ± 0.417 | 17.629 ± 0.326 | 1.372 | 2.1 |
| | | | *malerkotliana* | Seychelles | FFT | 2.556 ± 0.246 | 8.694 ± 0.894 | 0.294 | 4.0 |
| | | | *parabipectina* | Mauritius | FFT | 2.718 ± 0.077 | 9.391 ± 0.385 | 0.289 | 3.0 |
| | *obscura* | *obscura* | *obscura* | France | FFT | 2.424 ± 0.013 | 8.864 ± 0.068 | 0.273 | NT |
| | | | *subobscura* | France | FFT | NT | NT | | 2.8 |
| | *saltans* | *saltans* | *prosaltans* | Guadeloupe | FFT | 1.821 ± 0.176 | 7.380 ± 0.662 | 0.247 | 3.6 |
| | | *sturtevanti* | *sturtevanti* | Guadeloupe | FFT | 3.142 ± 0.101 | 11.707 ± 0.735 | 0.268 | 4.0 |
| | *willistoni* | *bocainensis* | *nebulosa* | Guadeloupe | FFT | 2.248 ± 0.083 | 7.776 ± 0.427 | 0.289 | 4.0 |
| | | *willistoni* | *equinoxialis* | Brazil | FFT | NT | NT | | 4.0 |
| | | | *tropicalis* | Mexico | FFT | 3.250 ± 0.134 | 11.732 ± 0.413 | 0.277 | 3.5 |
| | | | *willistoni* | Guadeloupe | FFT | 2.043 ± 0.129 | 6.387 ± 0.806 | 0.320 | 3.8 |
| *Drosophila* | *cardini* | | *caribiana* | Martinique | FFT | 0.165 ± 0.032 | 0.681 ± 0.057 | 0.242 | NT |
| | | | *arawakana* | Guadeloupe | FFT | 0.256 ± 0.041 | 0.815 ± 0.120 | 0.314 | 1.5 |
| | *immigrans* | | *immigrans* | Tunisia | FFT | NT | NT | | 2.0 |
| | | | *sulfurigaster* | Moorea | FFT | 1.678 ± 0.185 | 7.705 ± 0.596 | 0.218 | 2.0 |
| | | | *trilimbata* | Moorea | FFT | 0.232 ± 0.034 | 0.354 ± 0.054 | 0.655 | 1.3 |
| | | *nasuta* | *nasuta* | Reunion | FFT | 2.131 ± 0.262 | 8.686 ± 0.996 | 0.245 | 3.0 |
| | *melanica* | | *tsigana* | France | FFT | 3.100 ± 0.332 | 14.193 ± 1.321 | 0.218 | 6.8 |
| | *polychaeta* | | *polychaeta* | Guadeloupe | FFT | 0.869 ± 0.106 | 2.539 ± 0.225 | 0.342 | 2.4 |
| | *repleta* | *mulleri* | *arizonae* | Arizona | CAC | 3.495 ± 0.083 | 8.958 ± 0.215 | 0.390 | 5.9 |
| | | | *buzzati* | Tunisia | FFT | 1.016 ± 0.079 | 2.905 ± 0.165 | 0.350 | 6.4 |
| | undetermined | | *pruinosa* | Madagascar | FFT | 0.089 ± 0.006 | 0.166 ± 0.022 | 0.536 | 1.3 |
| *Zaprionus* | | | *ghesquieri* | Madagascar | FFT | 2.106 ± 0.096 | 7.764 ± 0.224 | 0.271 | 3.0 |
| | | | *indianus* | Congo | FFT | 1.754 ± 0.202 | 6.105 ± 0.879 | 0.287 | 3.3 |
| | | | *inermis* | Congo | FFT | 4.032 ± 0.213 | 11.454 ± 0.814 | 0.352 | 4.1 |
| | | | *aff. inermis* | Madagascar | FFT | 5.094 ± 0.548 | 21.829 ± 1.106 | 0.233 | 5.6 |
| | | | *kolodkinae* | Madagascar | FFT | 5.044 ± 0.097 | 12.732 ± 0.749 | 0.396 | 5.0 |

TABLE 1. Continued.

| Genus Subgenus | Group | Subgroup | Species | Geographic origin | Habitat | ADH activity Ethanol (E) | ADH activity Isopropanol (I) | E/I | DL50 (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | *mascariensis* | Reunion | FFT | 1.896 ± 0.197 | 7.534 ± 0.965 | 0.252 | 4.8 |
| | | | *sepsoides* | Congo | FFT | 4.239 ± 0.344 | 15.848 ± 1.192 | 0.267 | NT |
| | | | *tuberculatus* | Congo | FFT | 2.484 ± 0.363 | 11.033 ± 1.440 | 0.225 | 4.5 |
| | | | *vittiger* | South Africa | FFT | 2.099 ± 0.779 | 7.525 ± 2.254 | 0.279 | 3.8 |
| **Mean** | | | | | | **2.031 ± 0.194** | **7.530 ± 0.750** | **0.289** | **3.6** |
| Group 3 (species using nonsweet substrates). | | | | | | | | | |
| *Drosophila* | | | | | | | | | |
| *Sophophora* | *melanogaster* | *ananassae* | *monieri* | Tahiti | FLO | 1.639 ± 0.059 | 5.994 ± 0.330 | 0.273 | 1.5 |
| *Drosophila* | *bromeliae* | | *bromeliae* | Guadeloupe | FLO | 0.481 ± 0.025 | 0.947 ± 0.106 | 0.508 | 1.5 |
| | *funebris* | | *funebris* | France | DPM | 0.135 ± 0.026 | 0.549 ± 0.072 | 0.246 | 4.2 |
| | *quinaria* | | *kuntzei* | France | FUN | 0.235 ± 0.037 | 0.344 ± 0.069 | 0.683 | 1.1 |
| | | | *limbata* | France | FUN | 0.185 ± 0.023 | 0.172 ± 0.050 | 1.076 | 1.6 |
| | | | *nigromaculata* | Japan | DPM | 0.203 ± 0.042 | 0.384 ± 0.136 | 0.529 | 1.8 |
| | | | *phalerata* | France | FUN | 0.148 ± 0.029 | 0.211 ± 0.062 | 0.701 | 1.6 |
| | | | *transversa* | France | FUN | NT | NT | | 1.7 |
| | *repleta* | *repleta* | *repleta* | Guadeloupe | DPM | 2.251 ± 0.225 | 5.743 ± 0.890 | 0.392 | NT |
| | | *mercatorum* | *mercatorum* | Colombia | DPM | 0.826 ± 0.042 | 2.400 ± 0.138 | 0.344 | 4.2 |
| | *tripunctata* | | *crocina* | Guadeloupe | FLO | 0.559 ± 0.024 | 0.762 ± 0.133 | 0.734 | 1.5 |
| | | | *metzei* | Guadeloupe | FLO | 0.346 ± 0.069 | 0.503 ± 0.080 | 0.688 | 1.1 |
| | *virilis* | | *fraburu* | Congo | DPM | 0.656 ± 0.111 | 1.806 ± 0.303 | 0.363 | 2.0 |
| | | | *iri* | Congo | DPM | 0.404 ± 0.044 | 1.009 ± 0.137 | 0.400 | 1.8 |
| | | | *littoralis* | France | DPM | 0.558 ± 0.072 | 1.325 ± 0.190 | 0.421 | 3.8 |
| | | | *ornatipennis* | Guadeloupe | DPM | 0.432 ± 0.057 | 1.489 ± 0.171 | 0.290 | 0.9 |
| *Drosophila* | | | *buschii* | France | DPM | 0.196 ± 0.031 | 0.504 ± 0.065 | 0.389 | 3.2 |
| *Hirtodrosophila* | | | *confusa* | France | FUN | NT | NT | | 1.1 |
| **Mean** | | | | | | **0.586 ± 0.139** | **1.535 ± 0.450** | **0.504** | **2.0** |

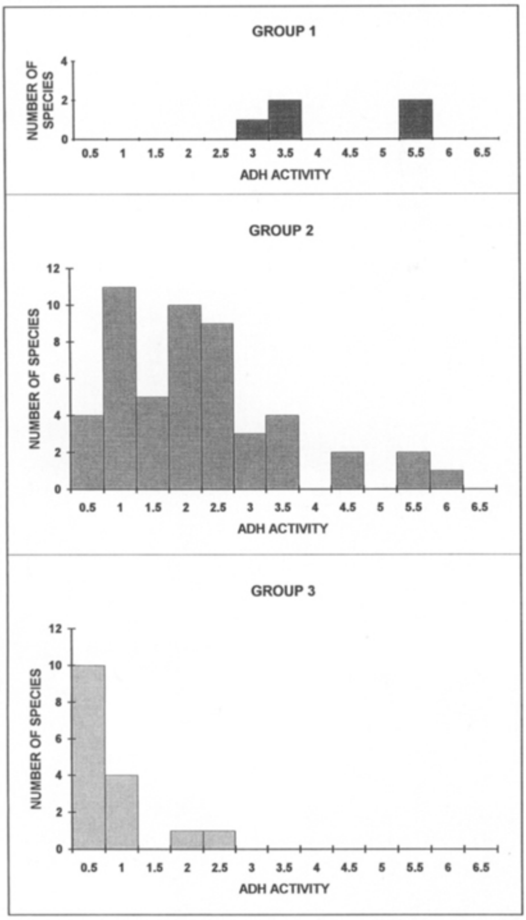


FIG. 1. Frequency distribution of in vitro ADH activity on ethanol in males among the three groups of drosophilid species. Group 1, species living in artificial, man-made alcoholic environments. Group 2, species living on fermenting fruits. Group 3, species living on nonsweet substrates.

a better affinity of the enzyme for a primary alcohol (such as ethanol) relatively to a secondary alcohol. To check this hypothesis, the following ratio (E/I) was calculated:

$$E/I = \text{ethanol ADH activity}/\text{isopropanol ADH activity}.$$

Under this hypothesis, the E/I ratio should be positively correlated to the ADH activity on ethanol. The E/I ratios (table 1), with one exception (*Drosophila limbata*), are lower than unity, thus confirming the higher specificity of ADH on secondary alcohols, which is well documented in *D. melanogaster* (Winberg et al. 1982; Hovik et al. 1984). The average ratio is 0.289 ± 0.011 in group 2 and is very similar (0.301 ± 0.036) in

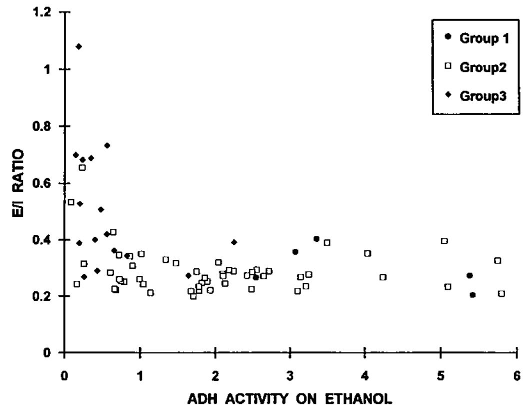


FIG. 2. Relationship between ADH activity on ethanol and E/I ratio (E, ADH activity on ethanol; I, ADH activity on isopropanol) among the three groups of drosophilid species as listed in figure 1 and table 1.

group 1. By contrast, a significantly much higher value (0.504 ± 0.055) is found in species of group 3, which do not face alcoholic fermentation.

Considering the whole set of 73 species, a negative correlation is observed between E/I ratio and ADH activity on ethanol ($r = -0.377$, df $= 71$, $P < 0.001$; fig. 2). The correlation remains negative, although becoming nonsignificant, when calculated separately in group 2 ($r = -0.173$, df $= 50$, NS). The same result is obtained for the species of group 3 ($r = -0.390$; df $= 14$, NS). All these observations clearly demonstrate that adaptation to alcoholic resources does not increase the specificity of ADH towards ethanol.

Because the enzyme specificity is not related to the larval ecological niche, it could be related to phylogeny. Among the 73 species investigated, 33 belong to the subgenus *Sophophora*, whereas the 40 others are distributed in various other subgenera of *Drosophila* and in *Zaprionus*. The distributions of the E/I ratio in these two groups is shown in figure 3. Obviously, these two distributions are not gaussian, especially that of the non-*Sophophora* flies. A $\chi^2$ test showed that they are significantly different ($\chi^2 = 17.49$, df $= 2$, $P < 0.001$). The average value of the ratio in *Sophophora* is 0.268 ± 0.008 with a coefficient of variation of 17%, whereas in other drosophilids it is 0.394 ± 0.029, with a coefficient of variation of 46%. Indeed, the nonsophophoran flies are a heterogeneous group. For example in *Zaprionus*, which is certainly a monophyletic genus, the average ratio is 0.285 ± 0.019 (CV = 18%), that

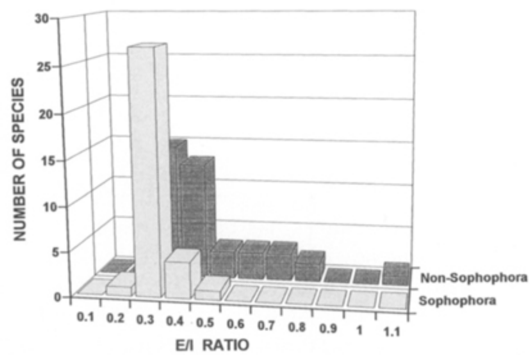


FIG. 3. Distribution of E/I ratio (E, ADH activity on ethanol; I, ADH activity on isopropanol) among species of the subgenus *Sophophora* and non*Sophophora* species.

is, very similar to that found in the sophophoran lineage.

### *Relationship with Ethanol Tolerance*

The distributions of the ethanol LD50 values for the three ecological groups are shown in figure 4. These groups are obviously different, with mean values of 14.7 ± 2.2% alcohol in group 1; 3.6 ± 0.2% in group 2; and 2.0 ± 0.3% in group 3, this last value being significantly lower than the value of the group 2 (Wilcoxon's test, $E_w$ = 3.746; $P < 0.01$). Such a conclusion extends the results of David and Van Herrewege (1983), which were based on fewer species: alcohol tolerance is, on average, related to the larval ecological niche.

In the 64 species tested (67 values, because the four *D. melanogaster* strains were considered separately), an overall correlation between ADH activity and ethanol tolerance (fig. 5) is observed ($r = 0.700$, $P < 0.001$, df = 65). However, some exceptions are found. Several species showing a low ADH activity, such as *D. funebris, D. littoralis,* and *D. mercatorum,* are fairly tolerant to ethanol. The ADH of *D. funebris* has a high specific activity although the enzyme protein is present at low concentrations in the flies (Atrian and Gonzalez-Duarte 1982). A possible explanation for this is that this high specificity may improve the ethanol tolerance, thanks to a rapid metabolic flux. Alternatively, it may be that the low concentration of enzyme prevents accurate measurement of the activity in crude extracts, even if, in this species, the enzymatic expression is optimal at the same pH (8.6) used in our assays. In contrast to the above cases, some species show a high ADH activity but a low ethanol tolerance.

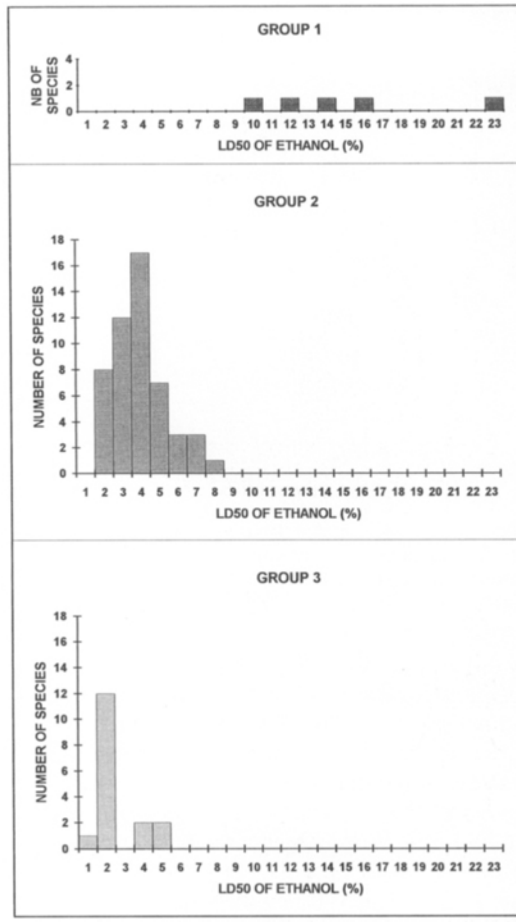


FIG. 4. Frequency distribution of ethanol tolerance (LD50 of ethanol for adult flies after 2 d of treatment) among the three groups of drosophilid species as listed in figure 1 and table 1.

Such is the case for *D. bipectinata, D. parabipectinata,* and *D. ercepeae.* This last species is remarkable because it ranks second for ADH activity (5.173) but only 50th for ethanol tolerance (LD50 = 2.1%).

*Drosophila melanogaster* is a good example of the relative independence between ADH activity and ethanol LD50. Only the geographic origin of the four strains studied is discriminant for ethanol tolerance. Thus, the two $Adh^{SS}$ strains have similar ADH activities (2.644 and 2.544) but their LD50 values are very different, one of them being twice that of the other (6.3 versus 13.8). The two $Adh^{FF}$ strains also have similar ADH activities (5.793 and 5.419) but very different LD50 values (7.3 versus 15.6). By contrast, the two dutch strains have similar LD50

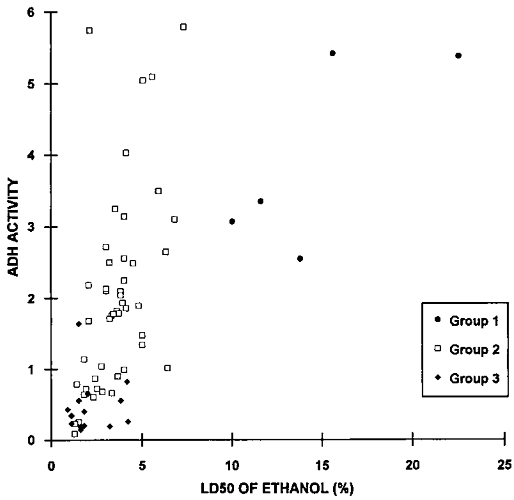


FIG. 5. Relationship between ADH activity on ethanol and tolerance to ethanol (LD50) among the three groups of drosophilid species as listed in figure 1 and table 1.

values (15.6 and 13.8), though the ADH activity of $Adh^{FF}$ strains (5.419) is 2.13 times that of the $Adh^{SS}$ strain (2.544). The same phenomenon is observed in the two afrotropical strains, which have comparable LD50 values (7.3 and 6.3), whereas the ADH activity of the $Adh^{FF}$ strain (5.793) is 2.19 times higher than that of the $Adh^{SS}$ strains (2.644). Last the $Adh^{n4}$ *D. melanogaster* strain (ADH activity = 0.014) has a low LD50 (2%). However, this LD50 is higher than the LD50 of 16 species (Table 1). The ADH activity values of these 16 species vary from 0.089 (*D. pruinosa*) to 1.140 (*D. sechellia,* living on fruits) and to 1.639 (*D. monieri,* breeding in decaying flowers).

All these results suggest that other factors may affect ethanol tolerance and that the ADH enzymatic activity cannot in itself explain the interspecific diversity observed for this physiological trait.

We therefore decided to test if the relationship between ADH activity and LD50 remained valid when the species ecology is considered. For species breeding on alcoholic substrates (groups 1 and 2), these two variables are correlated ($r$ = 0.749, df = 49, $P < 0.001$). This is true even when the five values for the species of group 1 are discarded ($r$ = 0.603, df = 45, $P < 0.001$). Conversely, there is no correlation for the species of group 3 ($r$ = 0.037, df = 13, NS). The positive correlation observed between ADH activity and ethanol tolerance in groups 1 and 2 appears to be an adaptation to the presence of ethanol in the larval food sources.

## DISCUSSION

Alcohol tolerance is clearly related to the larval habitat of the species and shows that adaptation to alcoholic resources has been a major evolutionary challenge in drosophilids. This adaptation is not related to phylogeny, having occurred independently several times during the evolutionary process and probably arises rapidly. This is best demonstrated for those species that can breed in artificial man-made fermenting resources because they belong to three different subgenera. If, however, we compare fruit-breeding and nonfruit-breeding species, a broad overlap exists between the two groups (fig. 5). For example, eight fruit breeding species have tolerances less than 2 and are therefore very sensitive to ethanol. This observation may be explained in two ways. First, the amount of alcohol in natural fruits must be likely very low in many cases, especially when the ambient temperature is low (temperate species) and the resource is small in size. Second, all the measurements of alcohol toxicity have been made on adults, because they are easier to test, whereas in nature environmental alcohol mainly affects larvae. Further investigations should be carried out on the larval tolerances, for which we have few data. In *Drosophila melanogaster* we found that larval and adult tolerances were highly correlated (David et al. 1986) but this may not be the case for all species. However, *Drosophila* sugar-cornmeal medium, when seeded with live yeast, undergoes an alcoholic fermentation. The amount of ethanol that is produced is not known, but may exceed 2%. We found that all nonfruit-breeding species for which adult tolerance is less than 2% could not be grown in such conditions, that is, with live yeast. Even more interestingly, the eight fruit-breeding species that exhibit an adult tolerance of less than 2, also cannot be grown on such fermenting food: low adult tolerance is thus correlated with an obvious larval sensitivity. In nature, these species presumably survive because they use small fruits, or fruits with a low sugar content. Another observation is that larval and adult preferences are generally related, that is, their ecological niches are similar. More precisely, adults of all fruit-breeding species are attracted by fermenting baits. However most adults of the nonfruit-breeding species do not come to these baits, although there are some exceptions, such as *Scaptomyza pallida* (David and Van Herrewege 1983). Altogether, it seems that larval and adult tolerances are positively correlated, although significant exceptions are found. Again, extensive investigations on the larvae should be undertaken.

Ethanol is the main alcohol found in drosophilid resources, and alcohol dehydrogenase (ADH) is the key enzyme for ethanol metabolization and detoxification (Van Herrewege and David 1980). Our results show a very broad range of activities among the 71 species investigated, the ratio of the highest value observed (*D. melanogaster*) to the lowest (*D. pruinosa*) being 65:1. All species exhibit significant ADH activity when compared to the ADH-null mutant of *D. melanogaster*. As shown in figure 4, a positive correlation is found between ADH activity levels and the capacity to detoxify ethanol. As pointed out in the results section, a broad range of variations is observed. Some species, such as *D. funebris, D. littoralis,* and *D. mercatorum,* are quite tolerant to ethanol but have a low ADH activity. Others, like *Drosophila ercepeae,* are very sensitive to alcohol, in spite of a high level of enzyme activity. Also in *D. melanogaster,* a well-known difference exists between the two common allozymes (Day et al. 1974; Lewis and Gibson 1978; McDonald et al. 1980; Maroni et al. 1982), but alcohol tolerance in this species depends almost exclusively on the geographic origin (tropical Africa versus Europe) rather than the ADH activity or *Adh* genotype of the strain. Other pathways for ethanol degradation are known in *D. melanogaster,* such as catalase and MEOS (Van der Zel et al. 1991). The amount of fatty acid in the food may also increase ethanol tolerance (McKechnie and Geer 1993) and modify the sensitivity of cell membranes to the disordering effects of ethanol. These observations show that alcohol tolerance is a complex phenotypic trait that may be influenced by several physiological functions leading to ethanol degradation, as well as by other processes, such as the sensitivity of the target organs (in this case the nervous system) or the capacity to excrete ethanol (Geer et al. 1993). Further investigations on some conveniently chosen species should lead to a better understanding of these processes.

*Drosophila melanogaster* ADH is a generalist enzyme known to act on a broad range of natural and artificial substrates, but is most efficient at breaking down molecules with more than two carbon atoms and secondary alcohols, like isopropanol rather than primary alcohols such as n-propanol (Winberg et al. 1982; Hovik et al. 1984; Eisses 1989). However, the biological efficiency, measured by comparing ADH$^+$ and ADH$^-$ flies, is maximal on ethanol (David et al. 1976). Moreover, isopropanol, one of the best substrates, is converted into acetone, a metabolic "cul de sac" for a fly, which is more toxic than the alcohol (Papel et al. 1979; David et al. 1981). These observations have now been extended to many other species, and in particular a higher activity is found on isopropanol than on the more abundant ethanol. Contrary to logical expectations, adaptation to high alcoholic resources, which has been a recurrent evolutionary event, is not mediated by a better use of ethanol, that is, an increase of E/I ratio. However, this ratio seems to be quite variable phylogenetically and is especially low in the subgenus *Sophophora* and the genus *Zaprionus.* Such a conclusion is not unexpected. Changing the substrate properties by natural selection would imply a modification of the active site of the enzyme, that is, a change in the amino-acid sequence of the protein, and this would require a very long evolutionary time scale. As has often been argued, short-term adaptation is more likely to occur by changing gene regulation than gene structure (McDonald et al. 1977).

The occurrence of an active ADH enzyme in all species investigated confirms the hypothesis that this enzyme has a general function in the fly, presumably acting on internal, unknown substrates, independent of environmental ethanol. It is surprising that nonfruit breeders have a higher average E/I ratio and, other things being equal, should be better able to use ethanol, though this substrate is not found in their food sources. Indeed, the very high ratio found in the four species of the *quinaria* group ($0.747 \pm 0.201$) suggests further investigations should be undertaken at the molecular level.

Finally, it should be borne in mind that, besides metabolization and detoxification, other physiological processes such as nervous-system tolerance and ethanol excretion may be involved in ethanol tolerance (Geer et al. 1993). Environmental ethanol, which is certainly a major ecological parameter for many drosophilids, has produced a diversity of physiological adaptations, all related to the *Adh* locus, but which are apparently much more complicated than previously believed (Clarke 1975).

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