Van Herrewege, J.*and J.R. David.+ *-Université Claude Bernard, Villeurbanne, France. +-C.N.R.S., Gif-sur-Yvette, France. Ethanol tolerance in **D.melanogaster:** parallel variations in larvae and adults from natural populations.

Drosophila melanogaster is known for its high ethanol tolerance, which in turn is related to a high alcohol dehydrogenase (ADH) activity. This physiological property correlates with the ecological niche of the species: adults are abundant in wine cellars, and larvae thrive in fermenting jars or grape residues, in which the ethanol concentration may exceed 10%.

There are genetic variations between geographic populations, both as to frequencies of ADH alleles and as to ethanol tolerance. Tolerance data, however, are greatly influenced by the technical procedure used to estimate tolerance since ethanol is highly volatile. For example, when alcohol is incorporated into a food medium, on which the adults oviposit, the alcohol concentration steadily decreases, becoming almost negligible after 10 days, i.e., at the end of the development.

Many published data obtained with this procedure are difficult to compare, since they depend on the rate of alcohol evaporation. To overcome this technical imprecision, we decided, some years ago (David et al. 1974) to use adults for toxicity tests, putting them in air tight-vials in the presence of various concentrations of ethanol. This procedure has given a large amount of reproducible results, showing in particular that the adults of European populations are highly tolerant to alcohol, having an LC 50 (lethal concentration that kills 50% of the flies) above 16%, while Afrotropical flies, which correspond to the ancestral populations of the species, are much more sensitive (L.C.50 about 7%) (David & Bocquet 1975).

Recently, a comparative study (David & Van Herrewege 1983) of numerous **Drosophila** species showed that the ethanol tolerance of adults correlated with the amount of alcohol in larval breeding sites: the adults of species breeding in non-fermenting resources, such as fungi

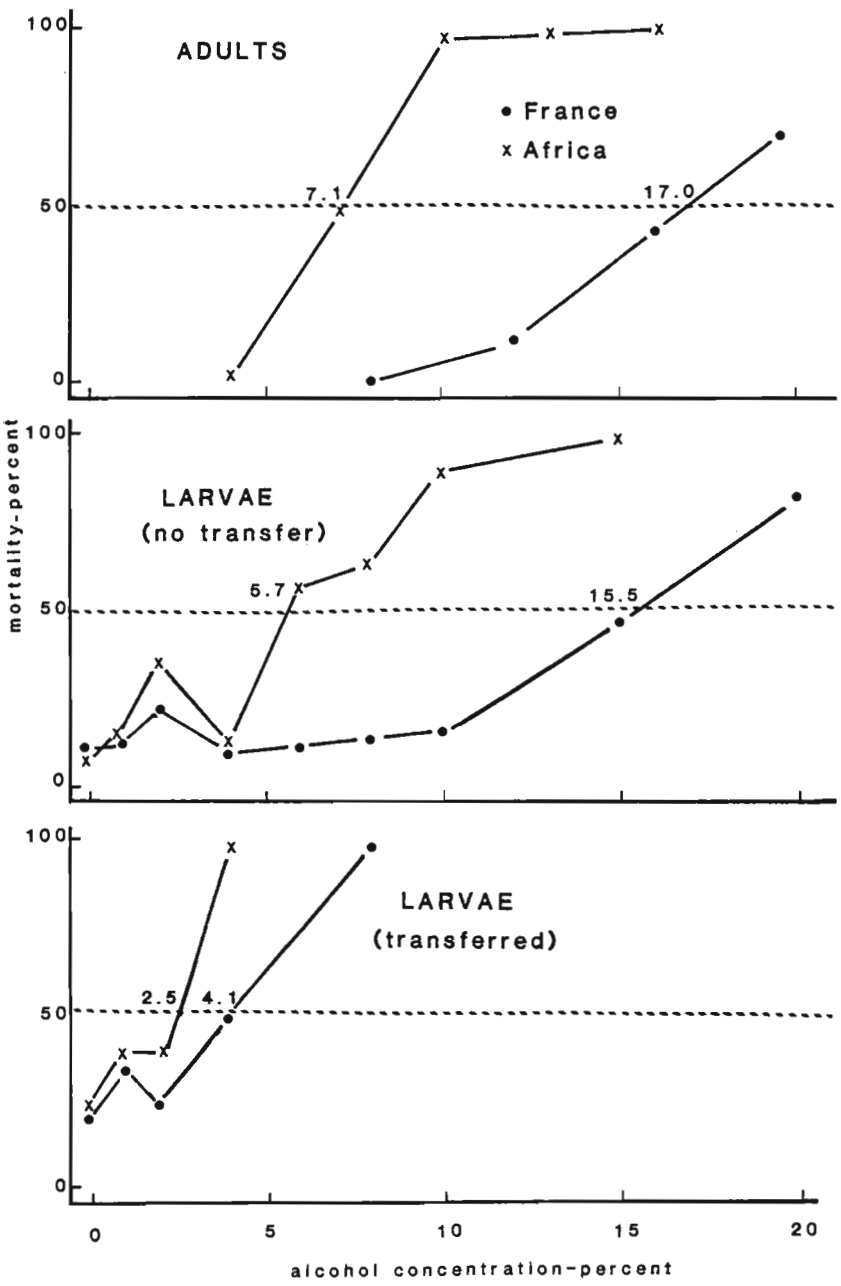

Figure 1. Influence of ethanol concentration upon **D.melanogaster** mortality: comparison of an Afrotropical and a French strain. **TOP:** mortality of adults after two days of ethanol treatment in air tight vials. **MIDDLE:** egg to adult mortality when development took place on the same medium without any additions and with a naturally declining ethanol concentration. **BOTTOM:** egg to adult mortality when developmental stages, larvae or pupae, were transferred every day to fresh medium in order to keep the ethanol concentration constant. [The numerical values on each curve indicate the LC 50 in percent of ethanol.]

or flowers, were found to be very sensitive to alcohol, whereas species breeding in sweet, fermenting fruits were found to be more tolerant. This suggests that, if environmental ethanol is really a selective factor it is more likely to act upon larvae than on adults. If that is the case, ecological genetics studies should preferably be carried out on larvae rather than on adults.

Of course, a possibility remained that larval and adult tolerances were highly correlated, so that adult tolerance would be mainly a by-product of larval adaptation.

To test this last hypothesis, we have compared larval and adult ethanol sensitivies in European and Afrotropical strains of **D.melanogaster.** Because of the difficulty arising from ethanol evaporation, we tried to keep constant the alcohol concentration during whole development. To this end we worked out a powdered killed yeast medium (formula to be published elsewhere) which can be prepared with cold water. Every day the larvae were sieved out of the medium and transferred to a fresh one containing the appropriate ethanol concentration. The results obtained for the transferred larvae, for adults of the same strains, and also for larvae kept in the same medium without any transfer during their whole development are shown in Figure 1.

When the alcohol concentration during the development was kept constant (i.e., when larvae were transferred every day) the LC 50 values were quite low, being 2.5 and 4.1% ethanol for African and French flies, respectively. But when the larvae were kept in the same medium, from which the alcohol progressively evaporated, the LC 50 values were much higher (5.7 and 15.5%). Interestingly, these latter values were very close to those (7.1 and 17.0) found for adults of the same strains.

From these observations we can conclude that, at least in **D.melanogaster**, variations of larval and adult tolerance are highly correlated. As had been assumed previously (David & Bocquet 1975), the divergence between European and Afrotropical populations may be attributable to different amounts of alcohol in the resources.

References: David, J.R., P. Fouillet & M.F. Arens 1974, Arch. Zool. exp. gen. 115: 401-410; David, J.R. & C. Bocquet 1975, Nature 257: 588-590; David, J.R. & J. Van Herrewege 1983, Comp. Biochem. Physiol. 74: 283-288.

Van Zijll Langhout, B.W. and F.M.A. van Breugel. University of Leiden, Netherlands. Cytological localization of the Aldox gene of **Drosophila melanogaster** in the region 3R 89A1.2.

For successful microdissection of genes from salivary gland chromosomes, exact cytological localization of the gene in question is required. With the ultimate aim of cloning the well-studied and histochemically interesting Aldox gene, we tried to locate the gene as accurately as possible. So far Spillmann & Nothiger (1978) assigned the locus to the region 88F-89A1 to 89B1-4 on chromosome 3R. Dickson Burkhart (1984) recently isolated from a North Carolina population two Aldox-null alleles associated with inversion breakpoints close to the 89A bands. While studying $Aldox^{n1}$ heterozygotes with various wildtype 3R chromosomes, we discovered that the $Aldox^{n1}$ mutation, originally isolated from an Urbana-S wild type strain (Dickinson 1970), in fact might be a small one-band or intraband deficiency. F1 larvae from a cross Aldox sbd x wildtype (Leiden) consistently showed much less stainable material in 89A (Figure 1) on one of the two homologous chromosomes. More proximal and distal regions fitted exactly with the Bridges (1935) map. Our conclusion is that the Aldox gene must be located in the double band 89A1.2. Upon inspection of the paper of Ashburner (1967), we found a very similar situation of unequal banding on the two homologous chromosomes on at least one of his photographs (viz. Figure 14A) of the Oregon-R wildtype strain. This suggests, that this wildtype strain could have been polymorphic for the small Aldox deficiency we have described here.

References: Spillmann, E. & R. Nöthiger 1978, DIS 53:124; Dickson Burkhart, B. et al. 1984, Genetics 107:295-306; Dickinson, W.J. 1970, Genetics 66:487-496; Bridges, C.B. 1935, J. Heredity 26:60-64; Ashburner, M. 1967, Chromosoma 21:398-428.

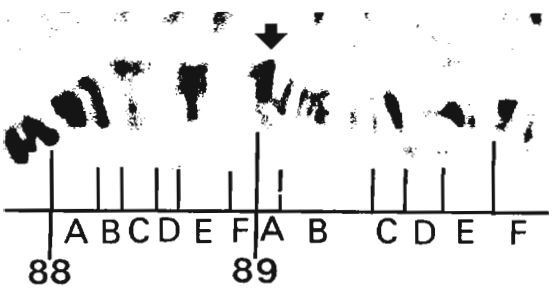

Figure 1. Part of chromosome 3R showing a deficiency for the Aldox locus in the lower part of band 89A.