a statistical analysis showed only slight differences (in relative length of the No. 1 chromosome and arm ratios of Nos 3 and 6. $p < 0.05$) between the corresponding measurement values. This is cytogenetic evidence supporting the close relationship of *R. kuhlii* and *R. namiyei* previously claimed from morphological aspects[8,9].

K. picta has 2n=28 chromosomes as in 2 other species reported in this genus, *K. borealis*[10] and *K. pulchra*[11]. The karyotype of *K. picta* differs from that of *K. pulchra* in the positions of the secondary constrictions.

Heteromorphic chromosomes (sex chromosomes) were reported in the males of *Hyla japonica*[12] and the females of *Xenopus laevis*[13], but these results have been rejected[14-16]. It is suspected that the sex chromosomes cannot be discriminated from the autosomes because the former are not visibly differentiated from the latter[14]. However, it is possible that some anuran species have heteromorphic sex chromosomes and, since male heterogamety is suggested in the genus *Rana*[17], the heteromorphic pair No. 8 in *R. narina* may be sex chromosomes. If the pair No. 8 are the sex chromosomes, *R. narina* is exceptional in having a Y-chromosome which is larger than the X-chromosome. A problem remains to be clarified, however: No. 1 of all examined males also showed a slight heteromorphism. A part on one arm of No. 1 seems to be deleted in the smaller component. The fact that the sum of relative lengths of the 2 male-specific components (the smaller component of No. 1 and the larger component of No. 8) roughly equals that of non-specific components may indicate a simple translocation. Further cytological examination is needed to confirm the nature of the heteromorphic pairs of this species.

1 Supported in part by a grant from the Japan-American Scholarship Foundation.
2 I wish to thank Dr H.K. Kim, Dr Y.-S. Liang, Dr V. Samson-Carino, Mr C.-S. Wang, Mr P.S. Lin and Mr B. Durusan for their aid in every aspect during the collecting trips.
3 T. Seto, Cytologia *30*, 437 (1965).
4 M. Kuramoto, Caryologia *25*, 547 (1972).
5 M. Kuramoto, Caryologia *30*, 333 (1977).
6 T. Omura, Zool. Mag., Tokyo *76*, 239 (1967).
7 A. Levan, K. Fredga and A.A. Sandberg, Hereditas *52*, 201 (1964).
8 J. Van Denburgh, Proc. Calif. Acad. Sci. ser. 4, *3*, 187 (1912).
9 R.F. Inger, Fieldiana: Zool. *32*, 297 (1947).
10 I. Sato, Zool. Mag., Tokyo *48*, 958 (1936).
11 A. Morescalchi, Experientia *24*, 280 (1968).
12 T.H. Yosida, J. Fac. Sci. Hokkaido Univ., Ser. 6, *13*, 352 (1957).
13 C. Weiler and S. Ohno, Cytogenetics *1*, 217 (1962).
14 T. Seto, J. Fac. Sci. Hokkaido Univ., Ser. 6, *15*, 366 (1964).
15 K. Mikamo and E. Witschi, Cytogenetics *5*, 1 (1966).
16 J. Tymowska and H.R. Kobel, Cytogenetics *11*, 270 (1972).
17 T. Kawamura and M. Nishioka, in: The Reproductive Biology of Amphibians, p. 103. Ed. D.H. Taylor and S.I. Guttman. Plenum, New York and London 1977.

Effect of ethanol and isopropanol on the activity of alcohol dehydrogenase, viability and life-span in *Drosophila melanogaster* and *Drosophila funebris*

Ll. Vilageliu Arqués and R. Gonzalez Duarte

Departamento de Genética, Facultad de Biología, Universidad de Barcelona, Gran Via, 585, Barcelona 7 (España), 31 October 1979

Summary. The effects caused by the addition of 2 alcohols to the culture medium of 2 species of *Drosophila, D. melanogaster* and *D. funebris,* are compared. Ethanol at 1% concentration causes slight and tolerable changes in both species. 1% isopropanol is lethal in 1 species and causes drastic changes in the other.

Much work has been devoted to studying the effects, in different *Drosophila* species, of some alcohol dehydrogenase in vitro substrates added to the control medium, in order to understand the biological function of this enzyme and its response to various environments[2-6]. Resistance to ethanol in *Drosophila* has been widely studied[4,7-10] and the interconversion of different molecular forms of this enzyme in the presence of alcohols, ketones and NAD has been reported[11-13]. The aim of the present investigation has been to compare the effects of 2 alcohols, ethanol and isopropanol added to the medium in 2 *Drosophila* species: *D. melanogaster* (slow allele) ADH^s/ADH^s and *D. funebris* (monomorphic in all the populations studied). Ethanol, at low concentration, has no toxic effect in *D. melanogaster* because the acetaldehyde produced is rapidly degraded[4], whereas isopropanol produces acetone, which probably accumulates in the fly and is a highly toxic compound[4]. The parameters chosen for comparison between the species are: the activity of the enzyme, viability, the effect on development and the banding pattern on thin layer electrofocussing polyacrylamide gels.

180 eggs were collected and transferred to bottles containing the usual corn meal-agar medium. This medium contains 10 ml of ethanol per 1800 ml of water. 1% v/v ethanol or 1% v/v isopropanol was added to each bottle except to the controls and the resulting concentration was measured in a Varian 3700 Gas Chromatograph (column 2 m × 3.2 mm 4% Hallcomid CH. G. AW 100/120). Samples of equal weight of larvae, not less than 50 mg, were taken after 8 and 14 days, in *D. melanogaster* and after 11 and 18 days in *D. funebris.* The surviving larvae were counted each time a sample was taken and compared to the control bottle to estimate the viability of the flies in the presence of alcohol. Samples were also taken to measure the changes in alcohol and acetone concentration in the medium during the time of the experiment. The results are illustrated in the table. Analytical thin layer electrofocussing polyacrylamide gels were prepared according to Karlsson et al.[14] to compare the banding pattern of alcohol dehydrogenase with and without alcohol.

When samples of 8- and 14-days larvae of *D. melanogaster* were taken it was found that the viability had increased 26% and 31%, compared with the controls. This is not surprising because besides not being toxic at that concentration, ethanol is used as food and can increase the life-span of *D. melanogaster* adults[15]. *D. melanogaster* larvae develop frequently on the surface of fermenting fruits, and are found in cellars living on residues containing high concentrations of ethanol. The alcohol dehydrogenase activity of larvae grown on 1% ethanol decreases to 90.2%, at 8 days and to 69.5% at 14 days. Considering that a slight retardation of the development of larvae reared in ethanol

Ethanol, isopropanol and acetone concentrations measured chromatographically at different times, as indicated. Concentrations are given in ml/100 g of culture medium

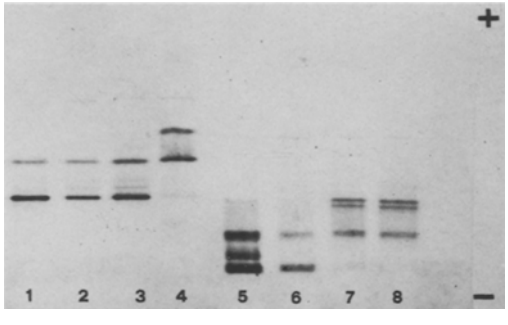
Days	*Drosophila melanogaster*					*Drosophila funebris*	
	Control (no alcohol added)	Experiment 1 1% (v/v) ethanol added	Experiment 2 1% (v/v) isopropanol added			Control (no alcohol added)	Experiment 1 1% (v/v) ethanol added
	Ethanol	Ethanol	Ethanol	Isopropanol	Acetone	Ethanol	Ethanol
0	0.720	1.360	0.610	0.780	–	0.60	1.51
4	0.014	0.040	–	0.082	0.319	0.007	0.008
8	0.005	0.032	–	0.200	0.125	0.005	0.003
14	–	0.020	–	0.038	0.178	0.019	0.013
17	–	–	–	0.047	0.204	–	–
18	–	–	–	0.050	0.181	–	–

Alcohol and acetone concentrations were determined in 25–30 mg of culture medium dissolved in 0.5 ml of water and centrifuged at 6000 rpm; 150 µl of supernatant was taken for analysis. The same volume of internal standard, tert.-butanol, at 0.2% (v/v) in water was added. 1-µl samples were injected in a 2 m×3.2 mm 4% Hallcomid CH.C.AW 100/120 column. Each value is a average of a minimum of 2 samples.

is observed and that the ADH activity rises sharply in 3rd instar larvae of *D. melanogaster* it could well be that larvae in 1% ethanol are also retarded with respect to the enzyme activity which will eventually attain the maximum value corresponding to this stage. The presence of 1% ethanol in the medium has a slight effect on the banding pattern in the acrylamide gels (figure). When ethanol is present in the medium there is a slight increase in intensity of the most cathodal band which has a pI value of 7.32.

The presence of 1% isopropanol in the medium has a greater effect in retarding the development of larvae of *D. melanogaster*. The alcohol dehydrogenase activity has gone down to 13% at 14 days and the viability to 26%. This decrease in activity is associated with a marked change in the banding pattern in the electrofocussing polyacrylamide gels: the more cathodally migrating band, with the highest pI, almost disappears whereas the more anodally migrating band in the control flies, very often non-detectable in the gels, stains very intensely in this sample (see figure). It seems that there is a correlation between the decrease in activity and the appearence of electronegative bands which has already been described[13]. This decrease in activity is also in our case related to the presence of acetone in the medium (table). Acetone is a highly toxic compound and a decrease in the activity of alcohol dehydrogenase prevents a rapid oxidation of isopropanol avoiding further toxicity. It could well be that the reverse reaction, reduction of acetone, is increased.

When *D. funebris* larvae reared with 1% ethanol added to the medium are compared to the control larvae the same trends are observed. Laboratory analysis showed that only concentrations of ethanol above 2% affected the mortality of the flies. The 11-day larvae retain 98.5% of the alcohol dehydrogenase activity, the viability is increased 42.8% and the effect of ethanol on the retardation of development is very small. In the 18-day larvae the viability seems unaffected compared with the control; there is a decrease in alcohol dehydrogenase activity of 23.5%, but in this case there is not a consistent change in the banding pattern because a slight increase in intensity of the more cathodally migrating band has been observed only in some of the gels. If the alcohol added to the medium is isopropanol instead of ethanol at the same concentration, drastic effects are observed. It inhibits the development of *D. funebris* completely and not even small larvae are observed. An additional experiment shows that if isoproponal concentration is decreased to 0.5% there is a marked retardation of the development of the larvae and the same change in the banding pattern as in *D. melanogaster* is observed (figure).



Thin layer electrofocussing polyacrylamide gels were made according to Karlsson et al.[14]. Gels were incubated for ADH activity. From left to right, *D. melanogaster: 1* adults. From *2* to *4* 14-day larvae: *2* control, *3* 1% (v/v) ethanol, *4* 1% (v/v) isopropanol. *D. funebris,* 18-day larvae: *5* control, *6* 1% (v/v) ethanol, *7* and *8* 0.5% (v/v) isopropanol.

The different effects produced by isopropanol in the 2 species are not surprising. The toxicity of this alcohol in *D. melanogaster* is possibily balanced by a regulatory mechanism which is linked to the fact that the most anodally migrating band is favoured and to a partial inhibition of the alcohol dehydrogenase activity. The same effect, that is, a change in the relative proportion of the isozymes and the appearance of new electronegative forms, has been reported in *D. melanogaster*[12] and observed in *D. buzzatii*[13]. *D. melanogaster* lives frequently in rich alcoholic media so has adapted to high concentrations of alcohols including isopropanol. *D. funebris* has not specifically adapted to alcohol-rich environments because it is the only species found in cellars which does not seem to be specifically attracted either by cellars or human habitations[16].

The mechanism of adaptation of *D. melanogaster* to isopropanol seems remarkable. As the chromatographic data reveal (table) the isopropanol concentration decreases and acetone appears in the medium after 4 days. This is associated with a decrease in alcohol dehydrogenase activity, keeping oxidation of isopropanol at a lower rate, but when the acetone concentration reaches a determinate level of toxicity, which is reflected by 0.319 and 0.204 ml of acetone per 100 g of food medium, the reverse reaction seems to be favoured, probably by the same enzyme. Studies on this topic are being carried out at present.

As is illustrated in the table, the concentration of both alcohols decreases markedly after 4 days. Obviously part of it evaporates and the rest is degraded in the cell. There must then be an accumulation of a degradation product which, especially in the case of isopropanol, has drastic effects on the individuals.

1 The authors acknowledge Dr A. Prevost for continuous support and E. Juan and Ll. Serra for their helpful suggestions.
2 J. Gibson, Nature *22*, 959 (1970).
3 W. van Delden and A. Kamping, Experientia *31*, 418 (1975).
4 J.R. David, Ch. Boquet, M.F. Arens and P. Fouillet, Biochem. Genet. *14*, 989 (1976).
5 A. Kamping and W. van Delden, Biochem. Genet. *16*, 541 (1977).
6 W. van Delden, A.C. Boerema and A. Kamping, Genetics *90*, 160 (1978).
7 D.A. Briscoe, A. Robertson and J.M. Malpica, Nature *255*, 148 (1975).
8 J. David and Ch. Boquet, Nature *257*, 588 (1975).
9 J. David and Ch. Boquet, Genetica *47*, 43 (1977).
10 J. David, J. Van Herrewege, M. Monclus and A. Prevosti, Comp. Biochem. Physiol. *63C*, 53 (1978).
11 K.B. Jacobson, J.B. Murphy, J.A. Knopp and J.R. Ortiz, Achs Biochem. Biophys. *149*, 22 (1972).
12 M. Schwartz and W. Sofer, Nature *263*, 129 (1976).
13 A. Fontdevila, M. Santos and R. Gonzalez, in preparation.
14 Ch. Karlsson, H. Davies, J. Ohman and U. Andersson, application note LKB, 1973.
15 J. Van Herrewege and J.R. David, Experientia *34*, 163 (1978).
16 M. Monclus and A. Prevosti, Genet. iber., in press (1980).

Spectral sensitivity in a fresh-water Gastrotrich (*Lepidodermella squamatum* Dujardin)[1]

M. Balsamo[2]

Istituto di Zoologia dell'Università di Modena, via Università 4, I-41100 Modena (Italy), 1 November 1979

Summary. Confronted with a series of alternative choices between environmental lights of different wavelengths, *Lepidodermella squamatum* Dujardin (Gastrotricha) shows a selective sensitivity towards coloured lights, especially blue, in preference to white light.

The fresh-water forms of phylum Gastrotricha are lacking in eyes and definite photoceptors. The cephalic refractile bodies or 'eyespots' described in some species probably have a static rather than a photoceptor function.

Until now nothing was known about the responses of fresh-water Gastrotrichs to differing conditions of environmental light. Adult samples of *L. squamatum* were used with the aim to clarify this aspect of the physiology of these organisms. Their behaviour was tested by confronting them with a choice between 2 different coloured lights. The alternative choices were: white/red, white/green, white/blue, white/no light, red/green, red/blue, green/blue.

The behaviour of the animals was observed by using a transparent cylindrical plexiglass container, 3 mm deep, 8 mm in diameter. The container was illuminated from below by reflected light from a tungsten lamp, diffused by means of an emery screen. In order to colour 1 of the 2 half-fields, colour filters (Kodak gel No.29, red; No.47a, green; No.61, blue) of defined wavelength (figure 1) were inserted between the light source and the bottom of the container. The intensity of energy of the light in the 2 half-fields was measured on the vertical of the container by means of a Kipp & Zonen CA1 type compensated thermopile connected to a Hewlett Packard voltmeter mod. 419 DC Null, and rendered equal with neutral filters. In this way the animals were confronted with the choice between 2 half-fields illuminated by 2 lights of different wavelengths but equal energy intensity (except, of course, in the case of the choice between white and no light). All tests were performed in a darkened room in order to exclude influences from environmental light.

During the tests slight variations may have been found in the temperature of the water in the container, in spite of the use of reflected and diffused light and the brief duration of the individual tests: however, they should not have influenced the results, since all the filters employed were completely transparent to IR-rays.

7 series of different alternative choices were offered, each series consisting of 50, 30 or 20 tests. The differences in the numbers of tests were owing to the difficulty of collecting the Gastrotrichs, and their fragility, which ruled out excessive handling. Each test lasted 5 min: 5 Gastrotrichs were placed on the border between the 2 half-fields and the number of animals present in each half-field was recorded every min.

The sign test was carried out on the distribution values of the animals in the 2 half-fields measured after the 1st and 5th min of each observation.

In 3 of the series of choices shown in the table (Nos 1, 2 and 6) the distribution appears casual after 1 min, but no longer so after 5 min. In the first 2 cases the half-field preferred is the coloured one as against the white-lit one; in the 3rd, on choosing between red and blue, after 5 min the Gastrotrichs are observed to prefer the blue area (figure 2). However, in the choice between white and blue light and green and blue light the distribution after 1 min is found not to be casual, and in both cases the preference is for the blue half-field.

Lastly, the tests performed on the choices between white light and no light, and between red and green light, showed no significant differences in the distribution of the animals at the beginning and the end of the tests.

Taken together these results would seem to show that *L. squamatum* has a selective sensitivity to coloured lights, especially to blue light (400–500 nm) as opposed to white light: they point to a tendency on the part of the animals to

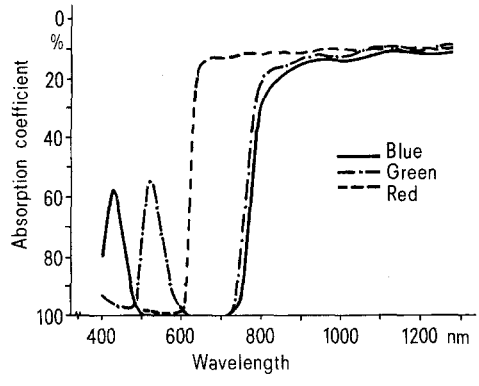


Fig. 1. Absorption curves of filters as a function of the wavelength of light.