fluorometer with 375 nm and 425 nm as the excitation and emission wavelengths respectively. Fluorescence values due to endogenous thiamine were subtracted before percentages were calculated. Absorbance measurements, for inner-filter-effect, were carried out on a Perkin-Elmer Coleman 55 spectrophotometer.

Results and discussion. Table 1 shows the comparative results from assays using $K_3Fe(CN)_6$, $HgCl_2$ and CNBr on extracts of kapok seeds, tomato, lemon, peanut, betel nut, betel leaves, rice bran and *Neptunia oleracea,* Lour (a local edible plant). Without pre-extraction by isobutanol the percentage fluorescence readings were generally lower than those obtained from pure thiamine solutions. This is partly attributable to the inner-filter-effect because the OD_{375} of the isobutanol layer was quite high (> 0.1) in all cases. When the extracts were pre-washed by isobutanol, CNBr gave excellent percentage fluorescence yields in all cases followed by $K_3Fe(CN)_6$ and $HgCl_2$. Not only do the data show the superiority of CNBr in thiochrome formation but also the relatively low solubility of acidified thiamine in isobutanol. With pre-extraction and the resulting insignificant OD_{375} and OD_{425} of the final isobutanol extract used for fluorescence measurements, the lower percentage fluorescence in $HgCl_2$ and $K_3Fe(CN)_6$ assays indicate interference from redox and other causes in the aqueous layer.

In order to identify some compounds in plant extracts for their interfering ability, we focused our attention on known polyhydric phenolic compounds and reducing agents found in plants. Table 2 shows the approximate concentrations of these compounds that began to interfere with the 3 reagents used to assay thiamine. Again, CNBr is the least susceptible, followed by $HgCl_2$ and $K_3Fe(CN)_6$. To provide some idea of the extent of interference, tannic acid at 1 mM gave 0.7% relative fluorescence with $K_3Fe(CN)_6$, 1.3% with $HgCl_2$ and 100% with CNBr. The same order of percentage fluorescence was given by many other polyphenols at 1 mM.

To demonstrate the considerable significance of the results obtained above, we present below our attempted reproduction of a reported experiment, which previously led to the conclusion that brewing of tea could significantly lower the added thiamine contents over the first 10 min[8]. In our hands, with the loading of smaller and smaller amounts of the infusion on to the column, the isobutanol extract of the eluate gave higher and higher percentage fluorescence with all 3 reagents. The figure shows the results from an experiment in which the CNBr and $HgCl_2$ assays show hardly any change in thiamine with time, whereas the $K_3Fe(CN)_6$ assays indicate an artifactual rapid initial change[2]. Thus, contrary to previous findings, our conclusion is that in fortified tea infusion only slight thiamine modification occurred and this was caused by heat and not by the polyphenols in the tea.

Despite interference, added amounts of thiamine in known polyphenol solutions or tea extract showed a linear fluorescence vs concentration relationship with all 3 reagents. The fluorescence/concentration slopes follow the order CNBr > $HgCl_2$ > $K_3Fe(CN)_6$. Finally, although the CNBr reagent gives consistent and reproducible results, more care should be exercized in its preparation and handling.

Table 2. Approximate concentration of polyphenols and reducing agents at which interference began to develop with $K_3Fe(CN)_6$, $HgCl_2$ and CNBr as the assay reagents

Polyphenol and reducing agent	Reagent CNBr	$HgCl_2$	$K_3Fe(CN)_6$
Caffeic acid	80 mM	100 µM	5 µM
Catechol	1 mM	50 µM	
Catechin	20 mM	100 µM	
Chlorogenic acid	1 mM	200 µM	
Tannic acid	5 mM	20 µM	
Methylgallate	2 mM	100 µM	
Hydroquinone	20 mM	100 µM	20 µM
Ascorbic acid	40 mM	2 µM	200 µM
Cysteine	50 mM	600 µM	60 µM

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2 B. Panijpan, P. Vilartsakdanon, K. Rungruangsak and S.L. Vimokesant, Int. J. Vit. Nutr. Res. *48,* 262 (1978).
3 B. Panijpan and P. Detkriangkraikun, Am. J. clin. Nutr. *32,* 723 (1979).
4 W.N. Pearson, in: The Vitamins VII, 2nd edn, p.60. Ed. P. Györgyi and W.N. Pearson. Academic Press, New York 1967.
5 A.O.A.C., Official Methods of Analysis, 12th edn. Association of Official Analytical Chemists, Washington 1975.
6 M. Morita, T. Kanaya and T. Minesita, J. Vitam. *15,* 116 (1969).
7 M. Fujiwara and K. Matsui, Analyt. Chem. *25,* 810 (1953).
8 R.B.H. Wills and M.A. Dwyer, Int. Nutr. Rep. *18,* 197 (1978).

Karyotypes of several frogs from Korea, Taiwan and the Philippines[1]

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Summary. Rana amurensis coreana, R. plancyi chosenica, R. latouchii, R. narina and *Ooeidozyga laevis* have 2n=26 chromosomes, *R. kuhlii* has 2n=22, and *Kaloula picta* has 2n=28. Males of *R. narina* have a conspicuous heteromorphic pair, No. 8, which might play a rôle in sex-determination.

Karyological data on the anurans of the Far East are very scarce, except for Japanese species[3-5]. The present paper reports the karyotypes of 7 anuran species belonging to the genera *Rana, Ooeidozyga* and *Kaloula.* The former 2 genera belong to the family Ranidae and *Kaloula* to the Microhylidae.

The chromosome spreads were prepared from bone marrow cells according to Omura's method[6]. Relative length and arm ratio of chromosomes were measured on each of 10 metaphase figures. Secondary constrictions were included in, and small satellites were excluded from, the measurements. Chromosome pairs were numbered in order of decreasing mean relative length. Chromosome morphology was described as by Levan et al[7].

Rana amurensis coreana (Seoul, Korea): 2n=26 with 5 large and 8 small pairs. Nos 4, 8, 10, 12 and 13 are submedian and the other pairs median. No. 9 has a secondary constriction on the long arm.

Fig. 1. Karyotypes of *Rana amurensis coreana* (Ra), *R. plancyi chosenica* (Rp), *R. latouchii* (Rl), *R. narina* ♀ (Rn, upper) and *R. narina* ♂ (Rn, lower).

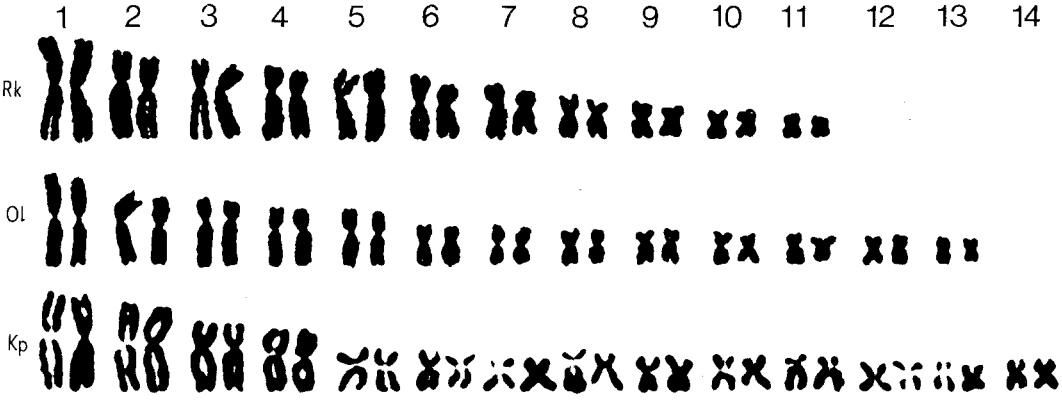

Fig. 2. Karyotypes of *Rana kuhlii* (Rk), *Ooeidozyga laevis* (Ol) and *Kaloula picta* (Kp).

Rana plancyi choseinica (Suweon, Korea): 2n = 26 with 5 large and 8 small pairs. Nos 3, 7, 10 and 13 are submedian and the other pairs are median. No. 9 has a secondary constriction on the long arm.

Rana latouchii (Mucha, Taiwan): 2n = 26 with 5 large and 8 small pairs. Nos 3, 7, 9, 11, 12 and 13 are submedian and the other pairs are median.

Rana narina (Kueshangli and Fualien, Taiwan): 2n = 26 with 5 large and 8 small pairs. Nos 3, 6, 8 and 12 are submedian and the other pairs median. Nos 6, 8 and 10 have a secondary constriction on the long arm.

All 4 males of *R. narina* used in this study have a conspicuous heteromorphic pair, No. 8. In this pair the smaller component corresponds to the female homologue, No. 8, both in size and shape. The larger component is subterminal and comparable to the large chromosomes in length. Its long arm has a secondary constriction which is located in the same position (relative to the centromere) as in the smaller components. The short arms of both components are nearly the same in length. Although not so remarkable as in No. 8, No. 1 is also heteromorphic with the smaller submedian component reduced in one of the homologues.

Rana kuhlii (Kueshangli, Taiwan): 2n = 22. The 11 pairs cannot be divided into distinct size groups. No. 3 is submedian and the other pairs are median. No. 10 has a secondary constriction on the long arm.

Ooeidozyga laevis (Binangonan, Philippines): 2n = 26 with 5 large and 8 small pairs. No. 3 is submedian and the other pairs are median.

Kaloula picta (Binangonan, Philippines): 2n = 28with 4 large and 10 small pairs. No. 11 is submedian and the other pairs are median. Nos 6 and 14 have a small satellite on the short arm.

The typical karyotype of the Ranidae, consisting of 5 large and 8 small chromosome pairs, was observed in 4 species of the genus *Rana*, and in *O. laevis*. The karyotype of *R. kuhlii* with 2n = 22 chromosomes is exceptional in the Ranidae; only 1 species, *R. namiyei* of the Ryukyu Islands, has been reported to have the same number of chromosomes[4]. The karyotypes of *R. kuhlii* and *R. namiyei* are very similar and

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.

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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*

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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