sess clear telomeric bands too. The microchromosomes are banded although in some plates they appear only slightly positive to the C-banding. Finally, in *Takydromus sexlineatus*, there is a difference in the C-banding pattern between the two sexes, as was already reported[11]. In fact, both in the male and the female the C-bands are rather small and may be localized on the centromeres and telomeres; the microchromosomes are labeled only on the centromere. However, in the female, one of two homologous chromosomes of the 13th pair is completely C-band positive. This led us to suggest that they are sex chromosomes and that in this species a primitive and little differentiated female heterogamety of the type ZW is present[11].

The results of our study provide evidence that the alleged karyological conservativeness in lacertid lizards may be essentially due to inadequate resolution by the conventional cytological techniques.

Inter- and intraspecific variability in the amount and localization of the C-bands has been observed in several organisms[12], and it has been found also in some reptilian groups[7,8,13-15]. Generally, this variability is related to variations in highly repeated DNA fractions[12]; this may be true also for lizards. In fact the percentage of the heterochromatin in *Podarcis sicula sicula* (25%) corresponds approximately to the percentage of palindromic and highly repetitive DNA (22%)[16].

Several investigators suggest that inter- and intraspecific variations of the heterochromatin are connected with speciation phenomena[12,17,18]. It is therefore possible that these variations played an important role during the evolution of the present lacertid species.

Cobror[5] has suggested that some of the telomeric C-bands of *Lacertidae* result from a translocation of heterochromatic microchromosomes to the macrochromosomes. This translocation took place during the evolution of ancestral karyotypes richer in microchromosomes to the present lacertid karyotypes[2,5]. However, it is also possible that the quantitative variations of heterochromatin in the species studied, especially in the centromeric C-bands, result from amplification of pre-existing heterochromatic blocks. This phenomenon has been observed in several rodents[17,19] and in snakes[7]. It would seem to play an important role in transforming, through the addition of heterochromatin at the centromeric level, uni-armed chromosomes into bi-armed ones[17] and microchromosomes into macrochromosomes[7]. In this regard, it is noteworthy that our preliminary results show that in the two lacertid species *Gallotia galloti* and *Lacerta viridis*, some of the largest chromosomes possess a minute short arm, which seems to be C-banding positive (Olmo et al., unpublished).

Finally, the heterochromatinization of the W-chromosome seen in *Takydromus sexlineatus* might have an important function (unpublished observations made by us seem to show an analogous situation in *Gallotia galloti*). In fact, it has been suggested that, as in snakes[20,21], in this species also this phenomenon is connected with the accumulation of a specific sex-linked satellite DNA, and that it might represent a primary event in the differentiation of the sex-chromosomes[11].

Heterochromatin amount of the four species studied, expressed as a percentage of the total chromosomal area, positive to the C-banding. A statistical analysis, based on the Snedecor's F test, shows that the heterochromatin content of *L. trilineata* differs significantly from those of the other species.

| Species | Heterocr. amount (%) | SE |
|---|---|---|
| *Lacerta dugesii* | 25.99 | ± 2.11 |
| *Lacerta trilineata* | 9.09 | ± 1.08 |
| *Podarcis sicula* | 25.53 | ± 3.17 |
| *Takydromus sexlineatus* | 22.33 | ± 4.82 |

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## Acetaldehyde oxidation in *Drosophila* null-mutants for alcohol dehydrogenase[1]

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*Summary.* Aldehyde dehydrogenase (ALDH) activity is demonstrated in four strains of *D. melanogaster* lacking active alcohol dehydrogenase (ADH-null mutants). In the four strains, ALDH activities are similar to those found in a wild strain. It is concluded that ADH-null flies are able to detoxify acetaldehyde. This finding is discussed in relation with the dual function of ADH proposed recently.

*Key words.* Acetaldehyde oxydation; alcohol dehydrogenase; aldehyde dehydrogenase; *Drosophila melanogaster; Drosophila simulans;* ethanol catabolism; null-mutants for alcohol dehydrogenase.

In *Drosophila*, like many other animal species, the catabolism of ethanol (ETOH) proceeds in a two-step chain of reactions including the oxidation of ETOH into acetaldehyde and the oxidation of acetaldehyde into acetate. For the first step, the role of alcohol dehydrogenase (ADH) is very well established. Numerous studies have demonstrated the physiological role of this enzyme as well as its adaptative value for species living in environments where ETOH is produced[2-5].

In contrast, investigators have not been able to agree on the identity of the enzyme involved in the second step. Aldehyde oxidase (ALDOX) was proposed as the active enzyme by several[6,7]. But it soon appeared as an unlikely candidate since strains of *D. melanogaster* lacking ALDOX activity (ALDOX-null mutants) exhibit comparable levels of tolerance to ETOH as wild strains[8], and ALDOX activities show no correlation with the level of ETOH tolerance and the ability of flies to use it for energy production[3,9]. Another candidate is the $NAD^+$-dependent aldehyde dehydrogenase (ALDH), an enzyme shown to play a major role in acetaldehyde detoxication in human and other mammals[10-12]. Several recent papers report the presence of ALDH activity in *Drosophila*[13-17]. It appears that *Drosophila* ALDH is kinetically very similar to the mitochondrial high-affinity ALDH found in man[14]. This similarity can be explained by a very large mitochondrial contribution (more than 70%) to the total enzyme activity[18]. Nevertheless, Liétaert and coworkers have reported recently a mitochondrial contribution of only 30% of the total activity[17]. The last candidate is ADH itself. In a recent challenging paper Heinstra and co-workers have proposed a dual function for ADH[19]. According to these authors, ADH would be involved in both steps of ETOH catabolism: ETOH oxidation as well as acetaldehyde oxidation.

The present study was initiated on the assumption that ADH would not be a likely candidate for acetaldehyde oxidation in *Drosophila*, at least in vivo. We have used four strains of ADH-null mutant flies as well as the wild strain for the determination of ALDH activity. We show that ALDH activities in these strains are quite comparable to those of the wild strain.

The *Drosophila* flies used in this study are four mutants strains $Adh^{n1}$, $Adh^{n2}$, $Adh^{n4}$ and $Adh^{n5}$ kindly made available by Prof. D. Suzuki (University of Vancouver) and the two sibling species *D. melanogaster* from Colmar (France) and *D. simulans* from Villeurbanne (France) kindly made available by Prof. J. David (Gif-sur-Yvette, France). The flies were grown in low-density populations on yeast-free *Drosophila* instant medium (Carolina Formula 4-24). Adult flies, 5-6 days old, were immobilized by cooling, rapidly frozen by immersion in a bath of liquid nitrogen, vortexed to remove the legs and wings and homogenized with polytron (Brinkman) in 10 vols of 10 mM Tris-HCl buffer at pH 7.0 containing 0.25 M sucrose, 1 mM dithiothreitol, 1 mM EDTA. The homogenates were centrifuged at 80,000 × g for 30 min at 4 °C. The resulting supernatants were carefully pipeted out avoiding the lipid layer, mixed with deoxycholic acid, sodium salt (2 mg/ml) and centrifuged at 80,000 g for 30 min at 4 °C. The clear supernatants obtained were stored at − 80 °C until used. ADH and ALDH activities were measured spectrophotometrically at 25 °C by monitoring the formation of NADH at 340 nm (20-sec intervals) with a Beckman DU 8 UV/VIS computerized spectrophotometer equiped for enzyme kinetics. The assay conditions were those described by Garcin et al.[14] for ALDH and by Sofer and Ursprung[20] for ADH. Soluble proteins were determined according to the technique of Bradford[21].

Alcohol dehydrogenase and aldehyde dehydrogenase activities in *D. simulans* and in several strains of *D. melanogaster (D.m.)*

| Strain/species | ADH activity mU/assay* | mU/mg protein | ALDH activity mU/assay | mU/mg protein |
|---|---|---|---|---|
| *D. simulans* | 0.85 ± 0.03 | 23.0 ± 0.8** | 0.42 ± 0.02 | 11.3 ± 0.6 |
| *D.m.* wild | 3.62 ± 0.33 | 74.0 ± 4.0 | 1.23 ± 0.10 | 25.7 ± 1.5 |
| *D.m.* $Adh^{n1}$ | nil | nil | 1.07 ± 0.10 | 38.5 ± 3.3 |
| *D.m.* $Adh^{n2}$ | nil | nil | 1.29 ± 0.11 | 35.7 ± 2.9 |
| *D.m.* $Adh^{n4}$ | nil | nil | 1.35 ± 0.12 | 32.7 ± 3.0 |
| *D.m.* $Adh^{n5}$ | nil | nil | 1.14 ± 0.14 | 31.9 ± 2.8 |

* 1 mU is defined as 1 nmole NADH produced per min. ** Each value represents the mean (± SE) of four independent experiments including each 3-4 determinations.

The table shows the data obtained in the comparative study of ADH and ALDH activities in *D. melanogaster*, *D. simulans* and four ADH-null strains. As expected, no ADH activity was found in any of the mutant strains. ADH activities in *D. melanogaster* and *D. simulans* were recorded for control of our experimental conditions. The values obtained are in concordinance with previous data. A surprising finding was the presence of high ALDH activities in all mutant strains. As one can see, the activities are much higher than those found in *D. simulans*, a species able to use low concentrations of ETOH for energy production[22]. On the basis of specific activity (mU/mg protein) ALDH in mutants appears to be higher than in the wild strain. But the intrinsic activity (mU/wet weight) shows no significant differences. This discrepancy is due to a lesser content in soluble proteins in the mutants than in the wild strain.

Our biochemical data show that ADH-null mutant flies are able to detoxify acetaldehyde in the same extent as the wild species. This is at variance with the data of Heinstra and co-workers who showed that ADH-mutant strains do not oxidize acetaldehyde[19]. These authors failed to detect ALDH activity in all the strains tested (ADH-null, ALDOX-null, wild). Though their data on the wild strain and on the ALDOX-null strains can suggest the contribution of ADH in acetaldehyde oxidation, one might be concerned with the methodological approach taken by the authors. For instance, the high substrate concentration used for the semiquantitative histochemical assay of ADH could have very little relevance to enzyme kinetics in in vivo conditions[23-25].

Our own data, nevertheless, raise the question of the biological significance of a highly active ALDH in flies showing no ADH activity. Experiments in progress in our laboratory on acetaldehyde toxicity and acetaldehyde utilization in several ADH-null mutant strains should soon provide a rational explanation of this paradox[26].

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26 Since submission of this manuscript, our in vivo data have shown that the ADH-null mutant strain $Adh^{n4}$ (which also lacks ALDOX activity) is indeed able to use low acetaldehyde concentrations as food for energy production.

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## Vitellin is the nutrient reserve during starvation in the nymphal stage of a tick

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*Summary.* In the tick *Ornithodorus moubata,* the major protein of egg yolk, vitellin, was conserved in the guts of larvae and nymphs in aggregated forms for over four months after hatching. Vitellin was the nutrient which supported tick survival until the nymph could obtain a blood meal. This adds to the known role of yolk protein as the nutrient reserved for embryos a new role as a reserve for post-embryonic development and during starvation in the nymphal stage.
*Key words.* Tick; *Ornithodoros moubata;* embryogenesis; vitellin; egg yolk; nutrient reserve; nymphal stage; starvation.

The major proteins of egg yolk – vitellin (Vn) in insects and other invertebrates, lipovitellin and phosvitin in oviparous vertebrates – are generally considered to be nutrient reserves for embryonic development. The synthesis of their precursors, vitellogenins (Vg) in the fat body or liver, respectively, provides good material for the study of the control of specific gene activation by hormones[1-4]. The physiological roles and eventual fate of these proteins, on the other hand, has been relatively little investigated. In certain insects, Vn, which is a lipoglycoprotein, has been shown to be mostly consumed before hatching[5,6]. In Drosophila, three yolk polypeptides are digested during embryogenesis and are not detectable in the first instar larva[7]. In the silkworm, *Bombyx mori,* most of the Vn is used in the final stage of embryonic development and only a little remains in the first instar larva[8]. On the other hand, recently Vn was reported not to be an essential protein for silkworm embryogenesis[9]. To examine the role of Vn in another class of arthropod, I undertook a study of the fate and the essentiality of Vn during embryogenesis and further development in the soft tick, *Ornithodoros moubata* (sensu Walton 1962). I found that Vn is conserved over four months after hatching for use as the nutrient during starvation until the nymph can obtain a blood meal.

In this tick, the adult female synthesizes Vg in the fat body (Chinzei, unpublished data) and produces about 200 eggs after the stimulus of a blood meal. A female tick utilizes 75% of the blood meal protein for synthesis of Vg[10]. Vg purified from hemolymph of reproductive females was separated into two components (Vg1 and Vg2), identified as monomer (mol. wt 300,000) and dimer (600,000), respectively, by polyacrylamide

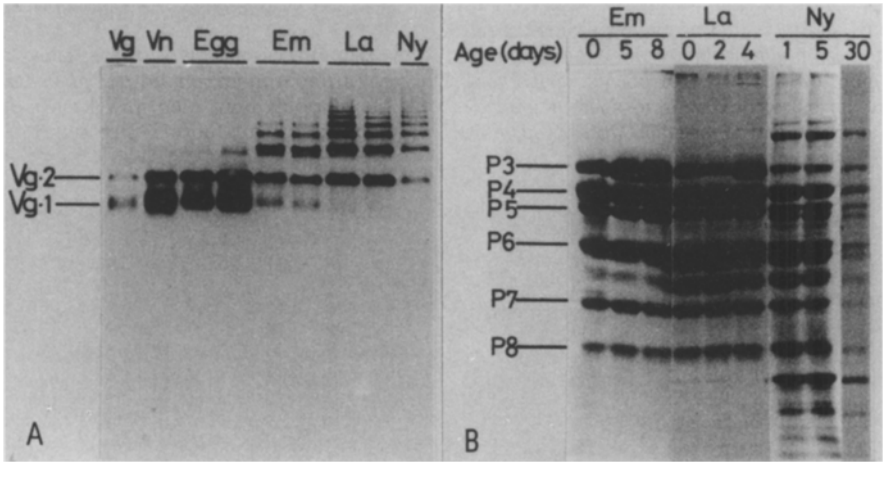


Figure 1. Polyacrylamide gel electrophoresis (PAGE) of soluble proteins from eggs, embryos (Em), larvae (La) and nymphs (Ny). Ticks were reared as described previously[11]. Eggs were collected within 24 h after oviposition and kept at 30 °C in batches laid on the same day. Ten eggs, larvae or nymphs were removed at definite intervals and were homogenized with a glass-teflon homogenizer in 5 vol. of extraction buffer (0.02 M Tris-HCl (pH 8.5), 0.4 M NaCl, 0.2% Triton X100, 0.2% SDS, 0.2% sodium deoxycholate, and 1 mM phenylmethyl sulfonyl fluoride). The homogenate was centrifuged at 5000 × g for 15 min at 4 °C. The supernatant crude extracts were analyzed by A) PAGE in 2.5 to 15% acrylamide gradient without SDS, and B) PAGE in 7% acrylamide with 0.1% SDS. Gels were stained with Coomassie blue R 250. Vg, Vn: vitellogenin and vitellin purified by anti-Vn-IgG affinity chromatography. See text for explanation of Vg1, Vg2 and P3 to P8.