elsewhere[10]. One set of animals was starved for a month and another set was aestivated for 1 year. Freshly-collected snails acclimated to laboratory conditions for a week were used as controls. The tissues viz. hepatopancreas, foot and mantle were isolated in the cold. 10% tissue homogenates were prepared in cold 0.2 M succinate Butter, centrifuged at 1000 × g for 15 min and the supernatants were used as enzyme source. AMP deaminase activity was estimated by the method of Weil-Malherbe[11] with slight modifications[12] and adenosine deaminase by the modified method of Glanti and Ginseppe[13].

The activity levels of AMP deaminase and adenosine deaminase were significantly decreased in all the tissues of snails during aestivation, but during starvation the AMP deaminase activity was increased and the activity of adenosine deaminase was decreased. The changes were relatively larger in the hepatopancreas as might be expected, being the metabolic centre in these animals[14]. The decreased activity of the 2 enzymes during aestivation might suggest decreased ammonia production which is in good agreement with previous reports[15]. The decrease in AMP deaminase and adenosine deaminase might also be attributed 1. to the decreased levels of adenosine nucleotides during aestivation[16] and 2. to the diminished energy state of the animals[17], since AMP deaminase requires ATP as an activator. The decrease in AMP deaminase during aestivation has an adaptive value for conserving precious energy which can be stored for revival of the animal when the environment turns favorable. As aestivation is a process for escaping drought, the aestivating animals either has to develop methods for detoxifying ammonia, or somehow restrict its production. There was indeed a decrease in the activity of glutamate dehydrogenase, which produces ammonia by the oxidative deamination of glutamate during aestivation[8]. Thus *Pila globosa*, which is ammonotelic during active life shifted to urecotelism during aestivation by lowering the ammoniagenesis and enhancing uric acid synthesis[18]. The increased activity of AMP deaminase during starvation suggests that the animal was still producing ammonia. Under these conditions its elimination is not a problem for the animal, since water is not a limiting factor during starvation.

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Lactate dehydrogenase isozymes of two new *Xenopus* species[1]

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Summary. The lactate dehydrogenase (LDH) isozyme patterns of *Xenopus epitropicalis* and *Xenopus amieti* have been characterized by means of polyacrylamide gel electrophoresis and isoelectric focusing and compared with those of their closely related species. The LDH-A and B genes differ in *X. epitropicalis* and *X. tropicalis* but are the same in *X. amieti* and *X. ruwenzoriensis.*

Lactate dehydrogenase (LDH), a tetrameric enzyme, is in most vertebrates composed of the 2 subunits A and B which are coded by separate genes[2,3]. When both subunits are synthesized 5 isozymes are built by random aggregation.

Although in amphibians various isozyme bands ranging from 2 to 25[4-12] have been reported, there exist in amphibians only the 2 genes which code for the A and B subunits[13]. Patterns of more than 5 bands are either the result of allelic polymorphism[13-19], gene duplication[19] or the existence of secondary isozymes[19]. The occurrence of only 2 bands has been clarified at least in 1 case; in *Triturus vulgaris* they have been identified as A_4 and B_4 polymers respectively[20]. No heteropolymers are formed in vivo.

The genus *Xenopus* contains diploid and polyploid species[21,22]. Except for *X. fraseri* the electrophoretic mobility of the A_4 polymer is the same for all diploid species ($2n = 36$ chromosomes) and *X. tropicalis* ($2n = 20$)[19]. In contrast, the gene which codes for the B subunit differs in all but 1 case. As a result, species-specific isozyme patterns are formed. There exist simple patterns with 5 isozymes, and more complex ones as well. One main cause for a complex pattern is the occurrence of secondary isozymes.

Recently, new *Xenopus* species have been discovered[23,24]. In the present paper the LDH isozyme patterns of 2 new species are characterized.

Materials and methods. The LDH isozymes were separated

by means of polyacrylamide gel electrophoresis and isoelectric focusing. As electrophoresis system the disc system of Ornstein[25] and Davis[26] was used. Either cylindrical gels or slab gels were employed. The slab apparatus described by Studier[27] was used.

Isoelectric focusing was carried out in the LKB2117 Multiphor apparatus. Either ready-made LKB ampholine PAG plates, pH 3.5–9.5 or self-made plates were used. The self-made plates consisted of 5% polyacrylamide (cross linkage 3%), 12% sucrose, and 2.42% LKB ampholines (1.9% pH 3.5–10, 0.13% pH 4–6, 0.13% pH 5–7, 0.26% pH 9–11). Polymerization was realized with ammonium persulfate (45 µl 10% solution for 60 ml solution) and TEMED (30 µl for 60 ml gel solution). Focusing was across the width with a constant power of 25 W for 1 h 45 min. Focusing was interrupted after 45 min to remove the sample application papers.

Isozymes were visualized by incubating the gels at 37 °C in the dark for about 30 min in a slightly modified staining solution given by Dietz and Lubrano[28].

Results and discussion. X. epitropicalis (2n = 40 chromosomes[29]) is as a first characterized by the appearence of more than 5 bands (up to 9 in some organs; fig. 1). The related species *X. tropicalis* (2n = 20[21]) exhibits only 5 LDH isozymes[19]. The isoelectric points of the A_4 polymers of the 2 species are slightly different (fig. 2). It is a suprising finding that *X. epitropicalis* and *X. tropicalis* differ in their A subunit since the A gene behaves rather conservatively in *Xenopus*[19]. It is not easy to identify the B_4 tetramer in *X. epitropicalis*. In heart tissue 5 isozymes are visible from which the isozymes with an intermediate electrophoretic mobility are more intensively stained (fig. 1). The offspring of a single pair mating shows the same patterns as their parents (not shown). These 2 findings allow me to speculate that in *X. epitropicalis* possibly a B-gene duplication is expressed. 2 B_4 tetramers would thus exist (the fastest and slowest migrating bands in heart tissue) from which the electrophoretic mobility is different from the *X. tropicalis* B_4. In *X. tropicalis* × *X. epitropicalis* hybrids at least the A gene behaves as a codominant (fig. 2). This is true for both reciprocal crosses and is in agreement with what is known about LDH gene expression in interspecies hybrids in *Xenopus*[31].

X. amieti (2n = 72[29]) shows clearly tissue-specific isozyme patterns (fig. 3). In heart and ovary tissues the B_4 homopolymer can be easily identified. In isoelectric focusing, the B_4 splits in various bands (fig. 4). The isoelectric points of this bands are identical with the isoelectric points of the *X. ruwenzoriensis* (2n = 108[30]) B_4 bands. Thus, the 2 species possess the same B gene. In skeletal muscle, and less intensively in other tissues, 5 cathodal bands occur (fig. 3).

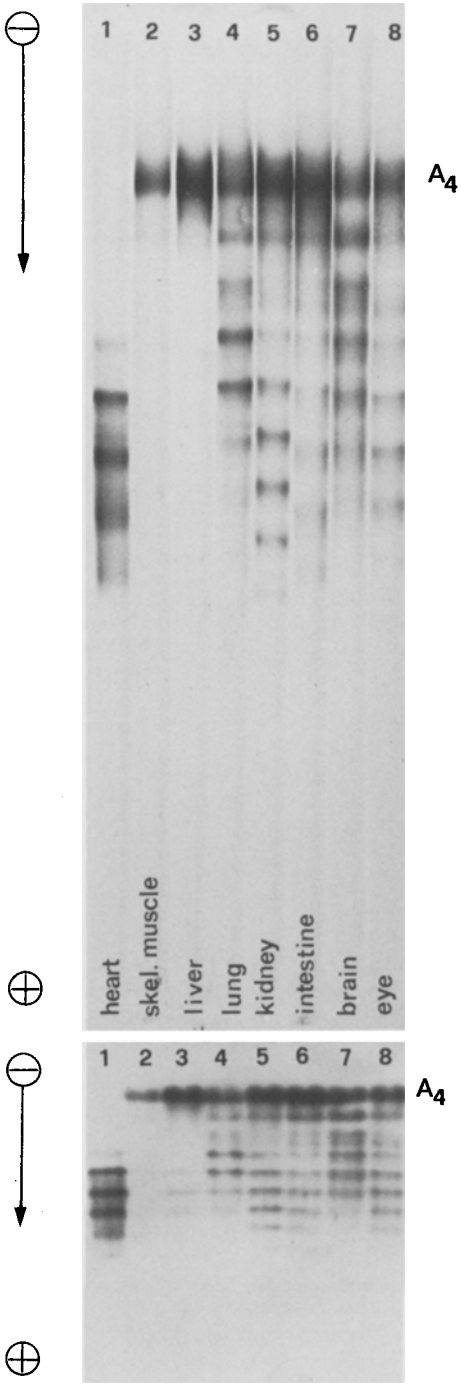

Figure 1. LDH isozyme patterns of *X. epitropicalis*. Isozymes were separated in cylindrical (above) and slab (below) 5.5% polyacrylamide disc gels.

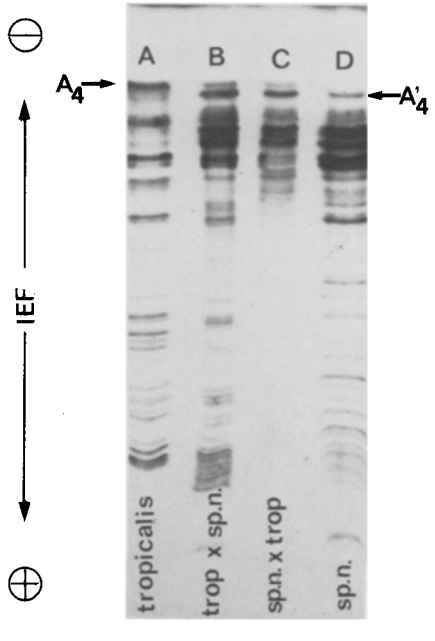

Figure 2. Comparison of LDH isozyme patterns of *X. epitropicalis* (X.sp.n.), *X. tropicalis* and their hybrids. Isozymes were separated by means of isoelectric focusing in polyacrylamide gel plates. Intestine extract was used.

The focusing mobility is the same for these bands as for the cathodal isozymes in *X. ruwenzoriensis* for which evidence exists that they are the result of an A gene duplication[32] (fig. 4). Both species thus have this duplication in common. Some additional bands exist in *X. amieti*. I interprete them as being secondary isozymes. The fact that the A and B genes of these 2 species are identical is not surprising, although they differ in chromosome number, the 2 species are morphologically very similar[24].

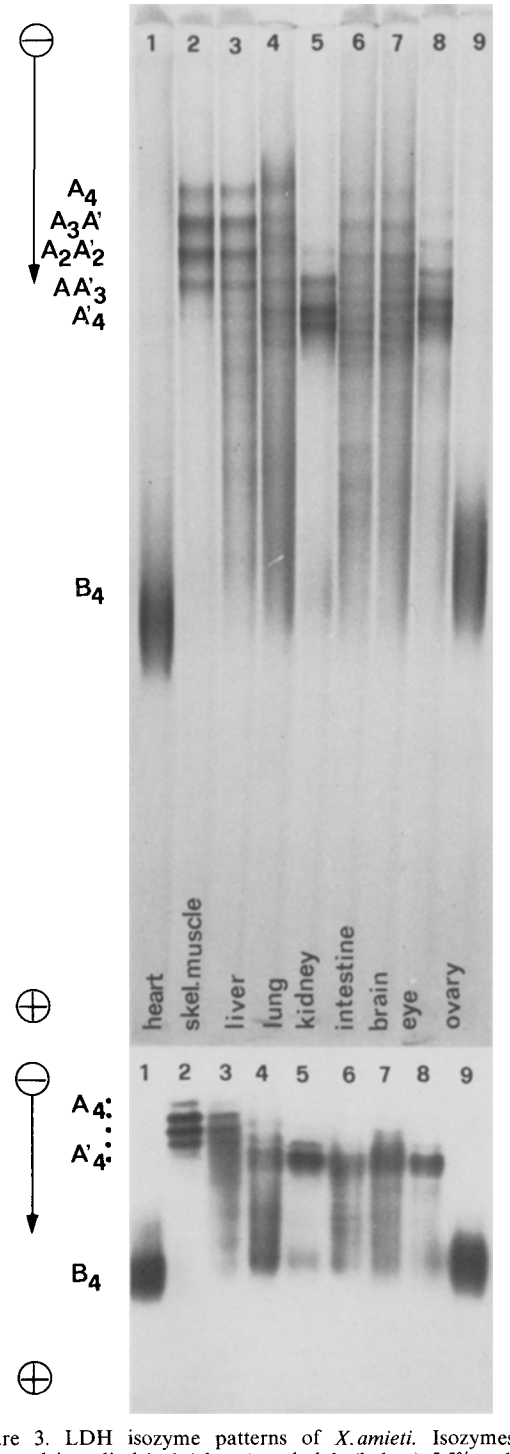

Figure 3. LDH isozyme patterns of *X. amieti*. Isozymes were separated in cylindrical (above) and slab (below) 5.5% polyacrylamide disc gels.

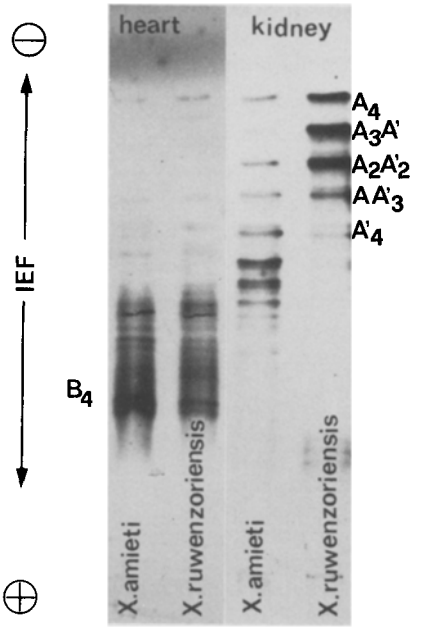

Figure 4. Comparison of LDH isozyme patterns of *X. amieti* and *X. ruwenzoriensis*. Isozymes were separated by means of isoelectric focusing in polyacrylamide gel plates.

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