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Biochemical variability between two Egyptian *Stenodactylus* species (Reptilia: Gekkonidae) inhabiting North Sinai

Mohamed A. M. Kadry[1*], Sayed A. M. Amer[1,2]

[1]Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt
[2]Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia
Email: *ecokadry@yahoo.com

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ABSTRACT

Polyacrylamide gel electrophoreses for malate dehydrogenase (*Mdh*) and beta-esterase (*β-Est*) isoenzymes were conducted for biochemical differentiation between two *Stenodactylus* gekkonid species inhabiting North Sinai of Egypt. Total lipids and proteins of liver and muscle tissues in both species were also analyzed. A total of three *Mdh* isoforms were recorded in the analysis, in which the activity of *Mdh*-2 and *Mdh*-3 seemed to be higher in *S. petrii* than in *S. sthenodactylus*. This high activity could be supported by the significant increase in the total lipids and proteins in liver and muscle tissues of the species. It may thus be reasonable to suppose that *S. petrii* is more active, energetic and adaptable in the desert habitat than *S. sthenodactylus*. *β-Est* showed six fractions in *S. petrii* and only one fraction in *S. sthenodactylus*. It is therefore noticeable that *β-Est* is more highly expressed in *S. petrii* than in *S. sthenodactylus*.

Keywords: Electrophoreses; Physiological Ecology; Geckos; Isoenzymes; Lipids; Proteins

1. INTRODUCTION

Gekkonidae are among the lizards known for their striking range of morphological characteristics, ecological habitats and body sizes. In Egypt, most of the gekkonid species are found in and around human habitation. Some, however, live in Egyptian deserts [1].

Many studies determined the relationships among members of the family Gekkonidae on the basis of morphological and environmental characteristics [2,3], karyotyping [4,5], and biochemical [6-8] and molecular variations [9-19]. The genus Stenodactylus contains 13 recognized species. The species *Stenodactylus sthenodactylus* and *S. petrii* are distributed in Egypt, Iran, Iraq, Syria, Jordan and Arabian Peninsula [1], and areas from Sudan to Mauritania [20].

Isoenzymes are multiple forms of a single enzyme. The forms are often marked by different isoelectric points and hence separable by electrophoresis. Malate dehydrogenase (*Mdh*) is considered as one of the most extensively studied isozyme systems [21]. This enzyme with lactate dehydrogenase constitutes a very suitable system for studying several metabolic, genetic, ecological features, and they are very useful in systematic studies. As a homodimeric enzyme, *Mdh* is well known for the many cell compartment-specific isoenzymes that characterize various organisms. There is a mitochondrial *Mdh* functioning in the tricarboxylic acid cycle which is usually NAD^+-dependent. Most eukaryotes that have been studied also have a cytosolic *Mdh* isoform. The cytosolic *Mdh*, also known as NADP-malic enzyme (*ME*), catalyzes the NADP dependent oxidative decarboxylation of malate into pyruvate and carbon dioxide to generate NADPH. *ME* is thought to be a key enzyme in lipid biosynthesis [22,23]. Esterase isoenzymes (*Est*)—as one kind of the lipid-hydrolyzing enzymes—possess high significance in genetics and toxicology [24]. The present study aims to investigate the patterns of the interspecific biochemical variations between two common gekkonid species (*S. petrii* and *S. sthenodactylus*) inhabiting the Sinai desert of Egypt.

2. MARERIALS AND METHODS

2.1. Taxon Sampling and Study Area

Collected for this project were a total of 12 individuals

*Corresponding author.

from, firstly, 2 Egyptian gekkonid species of *S. sthenodactylus* and *S. petrii* and, secondly, Beer El-Abd (North Sinai) [31˚01'2.46"N 33˚00'40.35"E] (**Figure 1**).

2.2. Sample Preparation and Isoezyme Assay

Tissue samples of liver and heart were taken to the lab immediately after their removal and stored at −80˚C for further laboratory use. For isoenzyme extraction, approximately 0.5 g of tissue was homogenized in 1 mL saline solution NaCl (0.9%) using a manual Homogenizer. The homogenates were centrifuged at 5000 rpm for 10 minutes and the supernatants were kept at −20˚C until use. For electrophoresis, 30 µL of the extract was mixed with 10 µL of treatment buffer and 35 µL of this mixture was applied to the well. Isoenzymes were electrophorased in 10% native polyacrylamide gel as described by Stegemann *et al.* [25]. After electrophoresis, the gels were stained according to their enzyme system, which was followed by the incubation of the appropriate substrate and chemical solutions at room temperature in dark for complete staining. In most cases an incubation of about 1 to 2 hours would be enough.

For *Mdh*, after the completion of electrophoresis, the gel was soaked in 100 mL of 0.05 M Tris-HCl (pH 8.5) containing 25 mg NBT, 25 mg EDTA, 25 mg NAD, 10 mg malic acid and 3 mg PMS. 0.05 M Tris-HCl pH 8.5 was prepared by dissolving 0.605 g Tris in 50 mL distilled water. The pH was adjusted to 8.5 by HCl. Then the solution was completed to 100 ml by using distilled water [26].

(a) (b)

Figure 1. Photos of *S. sthenodactylus* (a) and *S. petrii* (b) inhabiting Beer Al-Abd in North Sinai.

Regarding *β-Est*, after electrophoresis, the gel was soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4˚C. (This procedure would lower the pH of the gel from 8.8 to about 7, at which the reaction would proceed readily. The low temperature would minimize the diffusion of the protein within the gel). After being rinsed rapidly in two changes of double distilled water, the gel then stained for esterase activity and incubated at 37˚C in a substrate solution of 100 mg *β*-naphthyl acetate (*β-Est*) and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 [27].

After the appearance of the enzyme bands, the reaction was stopped by washing the gel two or three times with tap water. This was followed by adding the fixative solution, which consists of ethanol and 20% glacial acetic acid (9:11 v/v). The gel was kept in the fixative solution for 24 hours and then was photographed.

2.3. Metabolic Reserve Study

Immediately after collection, geckos were weighted to the nearest 0.01 - 0.1 g and dissected. Pieces of liver and thigh muscles were removed and immediately weighted to the nearest 0.01 g. They were stored frozen at −20˚C till use. Livers and thigh muscles were processed for the estimation of total lipids according to the method of Zöllner and Kirsch [28] and total proteins according to the method of Gornall *et al.* [29] using a kit of Biodiagnostics Company.

2.4. Statistics

All gels were scanned using Gel Doc-2001 Bio-Rad system. For isoenzymes, the bands of enzyme activity were designated using the known system of nomenclature [30]. Each locus was assigned with an abbreviation corresponding to the name of the enzyme. When multiple loci were involved, the fastest anodal protein band was designated as Locus One, the next as Locus Two and so on. Student t-test in the PASW package v. 20 was used to calculate the significance difference of total lipids and total proteins within and between species.

3. RESULTS AND DISCUSSION

Three *Mdh* isoforms were recorded in the two species of Stenodactylus. The activity of *Mdh*-2 and *Mdh*-3 isoforms seemed to be higher in *S. petrii* than in *S. sthenodactylus*. Such higher activity was reflected in the thicker and denser bands of *Mdh*-2 and *Mdh*-3 in *S. petrii* (**Figure 2**). The cytosolic *Mdhs* catalyzed the NADP dependent oxidative decarboxylation of malate into pyruvate and carbon dioxide to generate NADPH [22,23]. Due to its ability to produce NADPH, this enzyme is thought to be a key enzyme in lipid biosynthesis [23].

The apparent increase in the activity of *Mdh* in liver

tissues of *S. petrii*, in the present study, could be supported by the significant increase in the total lipids and proteins in liver and muscle tissues of this species. This species is also shown to be fattier than *S. sthenodactylus*. It is thus possibly reasonable to consider *S. petrii* more active, energetic and adaptable in the desert habitat than *S. sthenodactylus*.

β-Est showed six fractions in *S. petrii* and only one fraction in *S. sthenodactylus*. These fractions were denser and thicker in *S. petrii* (**Figure 3**). The first fraction in the *S. sthenodactylus* was the only clear fraction in the electrophoretic pattern, while the second fraction was recorded only in two samples of this species. It is therefore noticeable that *β-Est* is highly expressed more in *S. petrii* than in *S. sthenodactylus*. No reasonable explanation has been found as regards why many bands disappeared in the pattern of *S. sthenodactylus*. But factors can be supposed, such as the staining reaction, sampling storage or enzyme activity.

The present results revealed higher activity of esterases in the examined tissues of *S. petrii* than in *S. sthenodactylus*. Esterases are used as bio-indicators to measure the toxic potency of pesticide residues [31]. The presence of only one isoform of esterases, *β-Est*-1, in heart tissue of *S. sthenodactylus* may—to some extent—reflect the safety of the diet applied to this animal in the field , which is unlike the case with that for *S. petrii* [31].

Table 1 records the mean and standard error values of the total lipids and proteins in the liver and muscle tissues of both *Stendodactylus* species. By comparing the total lipids and total proteins of liver and muscle tissues in the two *Stenodactylus* species, we found more significant increase in the total lipids in the liver ($P < 0.001$) and muscle ($P < 0.01$) tissues of *S. petrii* than in those of *S. sthenodactylus*. *S. petrii* also showed more significant increases ($P < 0.01$) in total proteins in liver and muscle

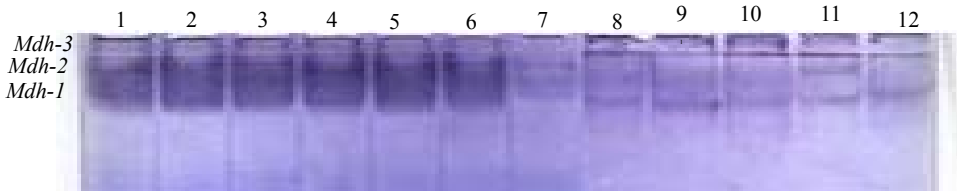

Figure 2. The electrophoretic profile of *Mdh* isoenzymes in liver tissues. Lanes are as follows: 1-6 (*S. petrii*), 7-12 (*S. sthenodactylus*).

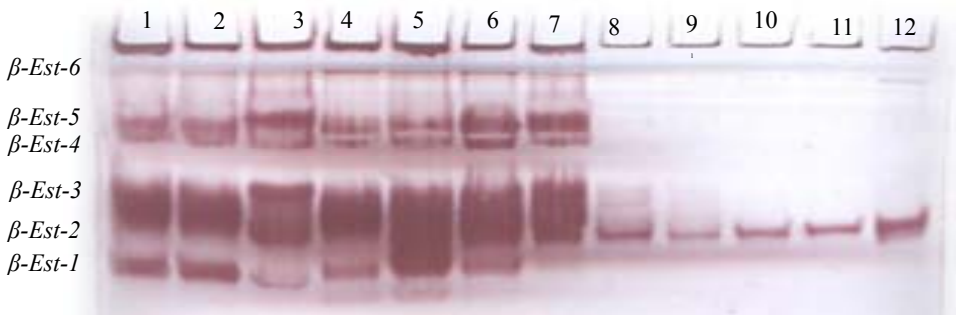

Figure 3. The electrophoretic profile of *β-Est* isoenzymes in heart tissues. Lanes are as follows: 1-6 (*S. petrii*), 7-12 (*S. sthenodactylus*).

Table 1. Comparison of total lipids and total proteins in liver and muscle tissues of *S. petrii* and *S. sthenodactylus*. Data are expressed as mean ± standard error. Number of individuals between parentheses.

Parameters	*S. sthenodactylus*	*S. petrii*	*t-test*
Liver total lipids (mg/100mg)	8.518 ± 1.209 (6)	10.384 ± 2.265 (6)	7.526^{***}
Thigh muscle total lipids (mg/100mg)	4.250 ± 0.738 (6)	8.033 ± 3.820 (6)	3.165^{**}
t-test	6.845^{***}	4.290^{**}	
Liver total proteins (mg/100mg)	96.095 ± 31.717 (6)	216.909 ± 31.717 (6)	4.617^{**}
Thigh muscle total proteins (mg/100mg)	57.154 ± 32.605 (6)	134.735 ± 27.767 (6)	4.078^{**}
t-test	3.411^{**}	5.804^{***}	
Body weight (g)	2.083 ± 0.098 (6)	4.750 ± 0.414 (6)	7.589^{***}

**Highly significant at $P < 0.01$. ***Very highly significant at $P < 0.001$.

tissues than *S. sthenodactylus*. Within each species, total lipids and proteins were significantly higher in liver ($P < 0.01$, $P < 0.001$) tissues than in muscle tissues.

4. CONCLUSION

In conclusion, *S. petrii* displayed higher physiological performance and activity than *S. sthenodactylus*, while isoenzyme expression was higher in the first species than in the second. The accumulation of total lipids and proteins was also significantly higher in the first species than in the second. Data analysis in the present research project does not support what is concluded in the study of Amer [6] for both species and within *S. petrii*. We therefore can affirm that the identification of *S. petrii* is incorrect in the study by Amer [6], while the research results point to the identity of other haplotypes of *S. sthenodactylus* than that of *S. petrii*.

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