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RESEARCH ARTICLE

ZOOLOGY

GENETIC VARIABILITY OF ISOZYME LOCI IN INDIAN GARDEN LIZARD,*CALOTES VERSICOLAR*

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ABSTRACT

The expression pattern of 5 metabolically important enzymatic systems in blood of Indian garden lizard *Calotes versicolar* were studied from four different regions of Agra. A total of 19 alleles at ten protein coding loci were identified. The degree of heterozygosity of 0.180 with an average of 1.59 alleles per locus was observed. The average proportion of polymorphic loci per population was estimated to be 1.70.

KEYWORD

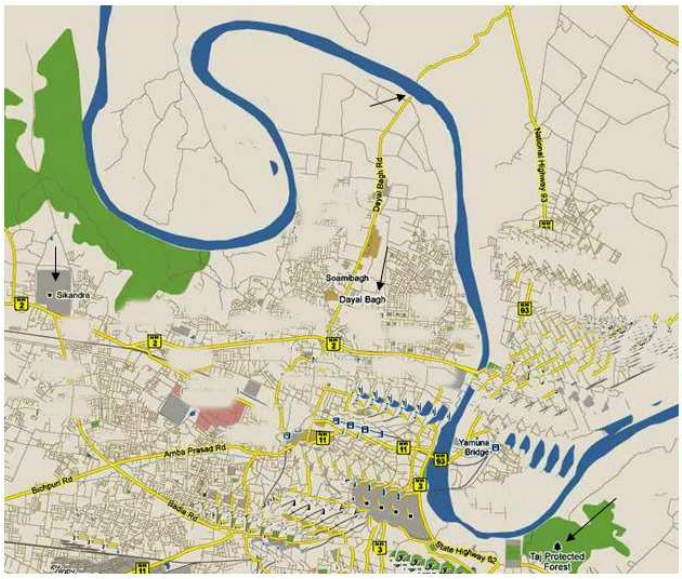
Isozymes, Allozymes, Genetic divercity, *Calotes versicolar*

INTRODUCTION

Isozymes (multiple molecular forms of enzymes encoded by separate genetic loci) have been found to be ideal gene product in studies of conservation genetics. Multiple molecular forms of proteins are important not only in determining intra- specific relationship; within the populations of same specie (Markert and Moller 1959), also in inter specific relationship. Such genetic variant provide marker for investigations of relationships of populations races and natural hybrids. Heterozygosity in species explains the heterozygous loci obtained in species among the population (Hartl and Clark 1997). In addition,studies revel average heterozygocity and genetic distance can be estimated from a small number of individuals per locus (Nei, M.1978). Biochemical studies provide that what is the main cause of decreasing in genetic diversity and resistance of biodiversity is more valuable because its presence form phylogeny of system (Frankham 1995). Three major categories of isozymes characters have been recognized (Buth, 1981) as (i) the number of gene controlling a multilocus enzymes system, (ii) the regulation of enzyme expression and intensity of expression (i.e. qualitative and quantitative differentiation at the regulatory level), and (iii) the ability or lack thereof of heteropolymer assembly in multimeric enzymes (due to interaction at the intralocus or interlocus level). These characters have been used in systematic contexts in various groups of organisms (Buth 1984). In the present studies efforts have been made to develop and establish the genetic database using isozymal analysis in *Calotes versicolar* from different regions of semiarid area of Agra.

MATERIAL AND METHOD

120 Individuals of *Calotes versicolar were* sampled from populations of various region of Agra viz (27º 18 º north and 78 02º in east) during a period of two year (2009-2011). About 50µl blood was collected from the cadual vein of *Calotes versicolar* (Jeffrey *et al*., 1980). Blood was stored in 250 µl EDTA buffers in plastic vials and frozen until further processing. Protein electrophoresis (Native PAGE) was performed on 5 enzymes lactate dehydrogenase (LDH, 1.1.1.27, malate dehydrogenase (MDH, 1. 1. 1. 37) Glucose 6-phosphate dehydrogenase (G6PDH, 1.1.1.49), alcohal dehydrogenase (ADH, 1.1.1.1), and non specific esterase. Protein estimation was done by dying assay method (Bradford, 1976). Equal amount of protein samples were loaded on native PAGE in the form of continuous vertical slab gel and electrophoreses at 120V in 1X TBE at 4ºC was performed to resolve all the isoforms of enzymes for complete visualization. After electrophoresis the gel was transferred to enzyme staining recipes (Shaw and Prasad, 1970). Statistical analysis was done by computer programs GENEPOP (Raymond and ROUSSET, 1995) and CERVUS (Marshall *et al*., 2000), SAS (SAS Institute, 1987).



Four sampling sites in Agra

RESULTS

Isozymes and allozymes polymorphisms at different enzyme coding loci

To avoid animal killing only plasma proteins were used for all the different populations of *Calotes versicolar.*

(A) Lactate dehydrogenase (LDH):

The lactate dehydrogenase polymorphism was recorded on 6% polyacylamide gel. A total of three bands were visualized in plasma of heterozygous individuals. Only one broad band was observed in homozygous individuals. The different allozymes of LDH, encoded by LDH-1 locus, were designated as LDH-1-100, LDH-1-90 and LDH-1-80.

(B) Malate dehydrogenase (MDH):

A total of five bands of MDH isozymes were observed, which could be divided into three zones based on patterns of appearance on polyacrylamide gel. The first zone consisted only of single band, observed in both heterozygotes and homozygotes individuals, encoded by MDH-1 locus and designated as MDH-1-100, showed monomorphism. Other two loci, MDH-2 and MDH-3 were polymorphic due to their co-dominant patterns in individuals of the four populations. At MDH-2-100 and MDH-2-90 in heterozygotes and single band in the homozygotes individuals. Similarly, MDH-3 locus encoded two bands MDH-3-100 and MDH-3-90.

(C) Glucose 6- phosphate dehydrogenase (G6PDH):

One broad band of activity was observed. No allelic variation was observed at this locus and designated as G6PDH-1-100. Thus, the enzymes (G6PDH) exhibited monomorphism.

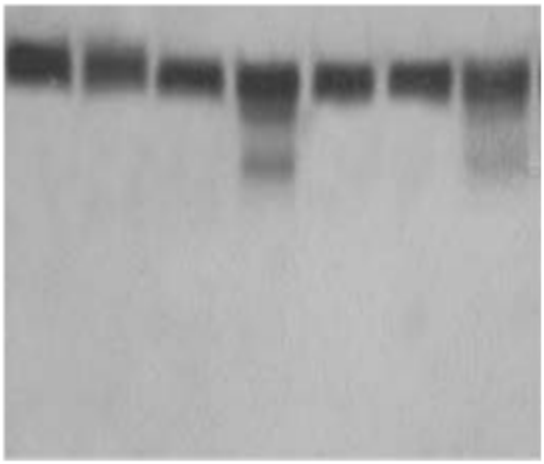
(d) ***Alcohal dehyrogenase (ADH)***

The ADH was observed (on 6% polyacrylamide) to be polymorphic in all the individuals of the four populations. A total of three loci ADH -1, ADH -2 and ADH -3 encoding five bands in heterozygotes were observed in three distinct zones of activity and designated as ADH- 1-100, ADH-2-100, ADH-2-90, ADH-3-100 and ADH-3-90

ADH-1 and ADH-3 showed polymorphism, while ADH -2 locus encoded only one band, both in homozygotes and heterozygotes.

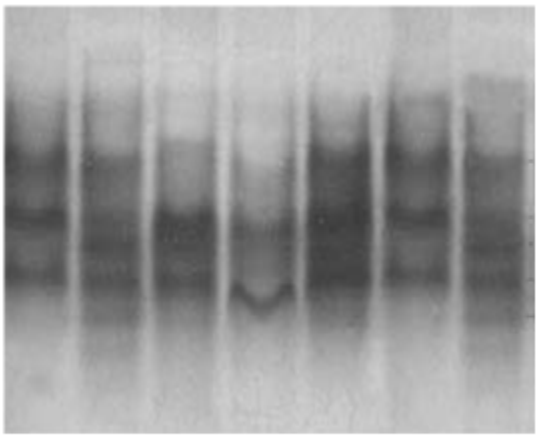
(e) ***Estrase (EST)***

The pattern of esterase bands were visualized on the polyacrylamide gels in blood plasma. These bands were divided into two zones of activity, which could be related to two enzyme-coding loci, designated as EST-1 and EST-2. This allele was designated as EST-1-100 and was monomorphic. The second locus EST-2 showed two zones of activity or total bands were present in heterozygous individuals and designated as EST -2-100 or EST-2-90.

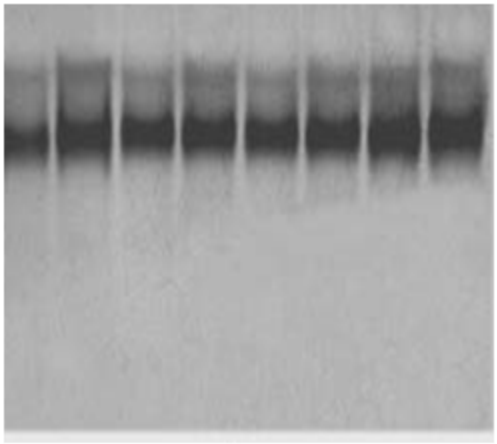


LDH in *Calotes versicolar* (A)

ADH in *Calotes versicolar (B)*



MDH in *Calotes versicolar (C)*



G6PDH in *Calotes versicolar (D)*

Isozymal analysis in blood of *Calotes versicolar*

Table 1
Measures of genetic variations in four populations of Calotes versicolar

Parameter of variation	Sikandra	Dayalbagh	Puiaghat	Tajganj
Polymorphic loci	50	50	50	50
Polymorphism	1.70	1.70	1.70	1.70
Number alleles	1.59	1.59	1.59	1.59
Observed Heterozygocity	0.241	0.289	0.250	0.224
Average Heterozygocity	0.250	0.210	0.219	0.232
Expected Heterozygocity	0.490	0.489	0.491	0.490

Table -2
Analysis the total genetic diversity in Calotes vercicolar

Locus	Total genetic--diversity	Average hetero--zygosity between populations	heterozygocity within population	interpopulation differentiation
EST-2	0.735	0.490	0.245	0.333
LDH-1	0.623	0.474	0.149	0.239
MDH-2	0.766	0.495	0.271	0.353
MDH-3	0.766	0.495	0.271	0.353
ADH-1	0.754	0.572	0.182	0.241
ADH-3	0.754	0.572	0.182	0.241
Average value	0.733	0.516	0.216	0.293

DISCUSSIONS

It has been observed that the relationship of enzymes present or absent in a particular enzyme is considered as a biochemical marker. Polymorphism, number of allele, heterozygocity/locus, and observed heterozygocity reveal the genetic variation within

populations (table 1) these are similar to those observed in several othere vertebrate (Driesel *et al* 2003) which were analyzed by the native PAGE. All polymorphic loci were studied more than one allele, Out of ten protein coding loci, only six were polymorphic loci. Which were analyzed on the basis of gel observation (EST-2, LDH-1, MDH-2, MDH-3, ADH-1, ADH-3). Our result correlates with the study of Nevo *et al.*, 1984 i.e. in the *Calotes* species the average degree of polymorphism is about 50% and the average degree genetic heterogygocity is close to 5% (using the average value for isozymal loci). The remaining 4 loci (EST-1, G6PDH, MDH-1, and ADH-2) show the monomorphism as no allelic changes were considered in all four population of *Calotes versicolar*.

Isozymal analysis in lizard shows the similar frequency as in other vertebrates (Nevo *et al.*, 1984). The effects of genetic variability of isozyme loci on the fitness of individuals and population have been largely discussed. Genetic heterogeneity can have a effect on the fitness of individuals (Berger, 1976, Charlesworth, 1991, Ohta, 1971, Turelli and Ginzburg 1983). Allendorf and Leary 1986 demonstrated that heterogygocity at the single or multilocus is interlinked to fitness of the species. According to Kimura (1968) genetic variation analysed by the heterozygocity included its expectation and observed population of genetic parameters. Ohta (1997, 1998) found that size of population can be easily analyzed by the isozymal analysis which include what pattern would be observed by the alleles. Genetic variation increase with,by the fitness of the population that means fitness increase with heterozygocity (Ginzburg 1979, 1983, Turelli and Ginzburg 1983). The present study reveal that the population of size is not interlinked to generation to generation (Gillespie and Guess, 1978). Hardy–Weinberg expectation shows the ($P < .001$) decreasing in biodiversity of gel analysis. Estimates of genetic variability e.g. percentage of polymorphism loci (P) and heterozygocity (H), are important prarameter for analysis of genetic variability in individuals. It shows the interconnection of individuals in population which plays a crucial role in conservation.

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