ISSN 2449-8955

European Journal of Biological Research

Research Article

Isozyme variants in two natural populations of *Lymnaea luteola*

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Received: 02 September 2017; **Revised submission:** 02 November 2017; **Accepted:** 10 November 2017

DOI: http://dx.doi.org/10.5281/zenodo.1045133

ABSTRACT

Lymnaea luteola is a fresh water gastropod snail, inhabiting ponds and lakes of different parts of India. Two populations of *L. luteola* were collected from fresh water ponds of district Varanasi (Uttar Pradesh) and analysed for their isozyme variants of *Xanthine dehydrogenase (Xdh)* and *Aldehyde oxidase (Ao)* enzymes loci. Both enzymes were found to be represented by two distinct loci and each locus of an enzyme showed polymorphic appearance. Based on the electrophoretic variant data, level of heterozygosity was computed for each enzyme locus. Our analysis clearly reveals that *L. luteola* inhabiting in these two ponds have undergone enough genetic differentiation.

Keywords: Isozyme polymorphism; Natural populations; *Lymnaea luteola*.

1. INTRODUCTION

Analyzing genetic polymorphisms of a species is the only way to decipher the level of genetic variation in that species. Measures which have been adopted for this purpose can be computing genetic variation at the level of phenotypic, chromosomal, protein and nucleotide [1-7]. A number of phenotypic features are well defined to be single gene inherited traits that follow Mendelian pattern of inheritance in a large number of sexually breeding organisms. Chromosomal polymorphisms have been used as a tool to measure genetic polymorphisms in Dipteran insects, particularly in *Drosophila*, due to presence of Polytene chromosomes in them [1, 2]. At molecular level, protein and nucleotide polymorphisms have been undertaken to see genetic variation among the different populations of a species [6, 9-11]. Study on isozyme polymorphisms started during 1960s [12, 13] and for the period of thirty years since then a large number of invertebrate and vertebrate species were involved for the perusal of their genetic profile based on allozyme/isozyme polymorphisms. It has been reported that invertebrates show more genetic differentiation than the vertebrates particularly, higher vertebrates [6, 14, 15]. Molluscs, both marine and fresh water have also been the focus of this kind of study [16-19]. The freshwater snails are of immense importance and have a useful status in the pond ecosystem. They are bio-indicators and being saprophytic animals help to clean water bodies as they consume algae, zooplanktons, diatoms and organic waste [20, 21]. They also form food of animals like fishes, birds and mammals even humans.

Lymnaea luteola is a fresh water gastropod mollusc. It is distributed across all the states of India. Its presence is also recorded from other neighboring countries of India [22]. This species is

often found in ponds, lakes and even in temporary water bodies, which may dry up in the summer months. It can withstand even unfavorable conditions by burying itself in the mud [23]. This species has also been reported to exist in water bodies that have a meager salinity [24, 25]. Its existence has fairly been recorded from different parts of Uttar Pradesh, one of the larger states of India. The main objective of our study was to observe allozyme/ isozyme polymorphism in two natural populations of *L. luteola*. To fulfill this aim, specimens were collected from two places of district Varanasi and in gel assay was performed to see whether the two populations differ from each other, on the basis of their enzyme variants. Results obtained in this regard are being presented in this paper.

2. MATERIALS AND METHODS

Allozyme polymorphism was studied in two natural populations of *L. luteola* which were collected from a small pond located in the close vicinity of Swtantrata Bhavan (SB), Banaras Hindu University, Varanasi and another pond situated outside the boundary wall of Diesel Locomotive Works (DLW), Varanasi. The distance between these two ponds was approximately six kilometers and the area in between is inhabited by thickly populated human population. During rains the two ponds do overflow but the organisms inhabiting them (especially molluscs) never come in contact to each other.

Genetic polymorphism in this invertebrate species was assessed by analyzing two enzyme systems i.e. Xdh (xanthine dehydrgenase) and Ao (aldehyde oxidase). For isozyme analysis, a small portion of visceral mass of the animal was homogenized in 50 µl of 20 mM Tris buffer (pH 7.4) and the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes. The supernatant was equally divided into two aliquots to scrutinize allelic arrangements of two enzyme systems at a time. Supernatant was separated and subjected to 8% native polyacrylamide gel electrophoresis in 25 mM Tris and 250 mM Glycine electrode buffer (pH 8.2) at 100V for 4 hours at 4°C. In-gel staining for a specific enzyme was made by adopting the procedure suggested by Ayala and his coworkers [26]. The locus and allele designations were decided by expression of enzyme bands. A single locus was marked by the appearance of its variants separated by meager distance, whereas, two loci of a gene were seen to be separated by marked distance.

The electrophoretic variants (alleles) of aldehyde oxidase and xanthine dehydrogenase observed in *L. luteola* are shown in Figure 1. A total of 4 enzyme loci (2 for *Xdh* and 2 for *Ao)*, corresponding to these two enzymes were ascertained. Based on the number of different genotypes of the four gene loci, frequency of allozyme variants were computed. By using Hardy-Weinberg equilibrium, the number of expected genotypes for their respective observed genotype was also computed. Chi-square analysis was performed to test the difference between observed and expected values. A significant deviation from expectation ($p<0.05$) indicated that the enzyme locus is under the influence of evolutionary force/s.

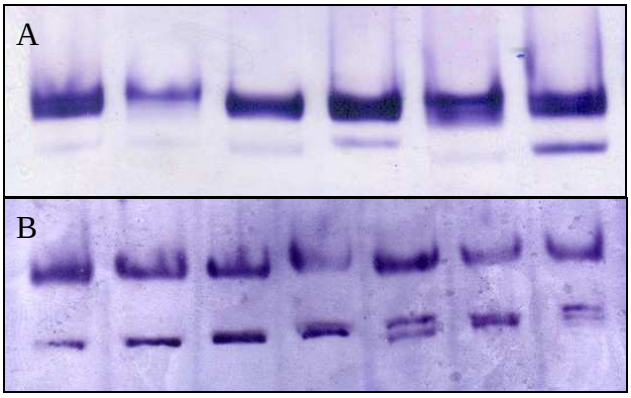

Figure 1. Electrophoretic variants (alleles) of aldehyde oxidase (A) and xanthine dehydrogenase (B) observed in *L. luteola*.

3. RESULTS

The frequency of different enzyme variants (alleles) of four gene loci of *L. luteola* is presented in Table 1. In SB population, the xanthine dehydrogenase *(Xdh)* enzyme was found to be represented by two distinct loci, *Xdh1* and *Xdh2* and each enzyme locus was expressed into two electrophoretic variants. *Xdh1* allele designated as 1.00 was in highest frequency being 0.75 whereas the same allele of *Xdh* 2 was 0.72 in this population. A measure of heterozygosity at its both loci was found to be same (0.38) in this population. Chi square analysis based on the observed and expected

numbers of genotypes of *Xdh1* and *Xdh2* revealed that the two loci are in perfect Hardy-Weinberg equilibrium. The same enzyme observed in DLW population showed the frequency of 0.61 and 0.39 for alleles 1.00 and 1.20 respectively for *Xdh1* whereas 0.45 and 0.55 for alleles 0.98 and 1.00 respectively for *Xdh2 locus*. Hardy-Weinberg equilibrium tested for these two loci revealed that they are in equilibrium.

Table 1. Frequencies of xanthine dehydrogenase (*Xdh*) and aldehyde oxidase (*Ao*) enzyme variants in two natural populations of *Lymnaea luteola.*

Enzyme locus	Alleles	Swtantrata Bhavan (SB)	Diesel Locomotive Works (DLW)
		Number 31	Number 32
Xdh1	1.00	0.75	0.61
	1.20	0.25	0.39
	$\chi 2$	0.00	0.007
Xdh2	0.98	0.28	0.45
	1.00	0.72	0.55
	$\chi 2$	0.167	3.014
		Number 31	Number 34
Ao1	1.00	0.53	0.53
	1.20	0.47	0.47
	$\chi 2$	0.057	1.106
Ao2	0.98	0.24	0.49
	1.00	0.76	0.51
	$\chi 2$	0.403	4.21*

*P<0.01

Aldehyde oxidase (*Ao*) enzyme was also studied for the same purpose and was found to be represented by two distinct polymorphic loci, i.e., *Ao1* and *Ao2* in the two natural populations. Each locus of this enzyme was expressed by two electrophoretic variants. The most common variant of each locus designated as 1.00 was 0.53 and 0.76 in their frequency in SB population. The other variant 1.20 for Ao1 and 0.98 for Ao2 were found to be 0.47 and 0.24 respectively in the same population. A study on Hardy-Weinberg equilibrium in this population for *Xdh* loci indicated that both the loci were in Hardy-Weinberg equilibrium. Aldehyde oxidase (*Ao*) enzyme considered for similar investigation in DLW population revealed that its two loci, *Ao1* and *Ao 2* were polymorphic, *Ao1* represented by variants 1.00 and 1.20 and *Ao2* by 1.00 and 0.98. The frequency of allele 1.00 and 1.20 was found to be 0.53 and 0.47 respectively. In this population another enzyme locus, *Ao2* showed frequency 0.51 and 0.49 for their respective alleles 1.00 and 0.98. Ao2 locus did not show Hardy-Weinberg equilibrium ($p<0.01$) indicating that this locus may be under the effect of some evolutionary forces.

Figure 2 is presented here to depict the frequency of heterozygotes for four gene loci studied in two different natural populations of *L. luteola.* The frequency of heterozygotes is quite high in DLW population (more than fifty percent) for Ao1 and the same enzyme was also found to be in higher heterozygosity in SB population. Overall heterozygosity was recorded to be more than thirty percent for all the loci examined. Although the two populations are completely different and exist as allopatric populations but exhibit similar pattern of evolutionary alterations depicting that similar ecological condition prevail in the area.

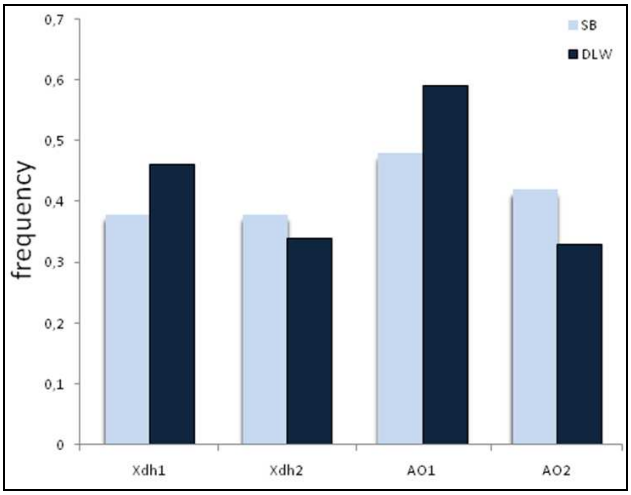

Figure 2. Bar diagram showing frequency of heterozygotes for four gene loci studied in two different natural populations of *L. luteola.*

4. DISCUSSION

The main identifying features of Lymnaeid snails are based on traits like shell morphology, structural peculiarity of radula, characteristics of renal and reproductive organs. The genus *Lymnaea*

Lamarck, includes some freshwater snails that harbours the larval stages of liver-fluke, *Fasciola hepatica*, a helminth parasite which causes fascioliasis in grazing animals and humans. Allozyme polymorphism has been studies in land snails and the significance of such studies have been used for the conservation of snails [27, 28]. Genetic variation in *Lymnaea luteola* can be studied only by following both protein or nucleotide polymorphisms and the results of such studies can be extrapolated to know genetic profile of a species. Carvalho et al. adopted polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) techniques to genetically characterize *Lymnaea columella*, *L. viatrix*, and *L. diaphana* collected from Brazil, Argentina, and Uruguay [29].

Ao and Xdh are well studied enzymes for their polymorphic status in a number of organisms particularly in different species of *Drosophila* [6, 7]. Such studies have not been undertaken in fresh water gastropods, especially in genus *Lymnaea*, from the perspective of Indian regions. We found abundant occurrence of *L. luteola* in two ponds of southern end of Varanasi City and decided to see isozyme variations in the individual of these two separate populations. Isozyme analysis clearly reveals that these two populations are genetically differentiated from each other. Since both the enzymes were represented by two loci and were polymorphic in appearance, the allelic frequencies were computed based on their genotypic frequencies and then a comparative analysis was made. A comparison made on level of heterozygosity for all the four loci studied, indicated variation between the two populations giving an idea that the two populations are genetically different from each other.

L. luteola and other species of this genus are mainly hermaphrodite mollusk species and exhibit self as well as cross fertilization [30]. Since high level of heterozygosity has been observed in both the natural populations of this species, the present study is a testimony to explain that this can happen only if individuals opt to cross fertilization. To maintain genetic heterogeneity is of prime significance to every sexually reproducing species, because species with substantial genetic variation can be better thriving in changing environmental conditions. All the four enzyme loci in the present case, in both populations show more than thirty percent heterozygosity, indicating that during breeding two individuals with varying genetic constitution get involve in reproduction.

Animal species which are migratory in nature get mixed with neighboring populations and as a result of it little genetic differences are expected to exist among the neighboring populations. Thus migration results into gene flow among the populations and consequently no substantial genetic differences can be recorded between the adjacent populations. Gastropod mollusks which remain confined in local ponds do not find it possible to get merged with other populations of neighboring water bodies until they are assisted by some other animal and therefore remain intact as a single population. Gene flow in such gastropods does not occur at all and thus their populations remain as allopatric populations. Fresh water mollusks are therefore expected to be represented by more number of species than those where substantial gene flow do occur. We could witness the existence of more than one species of snails in a single pond indicating that gastropods can be one of the best examples of sympatric speciation.

AUTHORS' CONTRIBUTION

AKS: Manuscript writing and statistical calculation; NY: Conducted experiments and literature survey; GS: Designed and conducted experiments. The final manuscript has been approved by all authors.

ACKNOWLEDGEMENT

The present work has been the part of M. Sc. dissertation of Mr. Naveen Yadav, which could be accomplished by the financial assistance provided by Centre of Advance Study, Department of Zoology, Banaras Hindu University, Varanasi.

TRANSPARENCY DECLARATION

The authors declare that they have no conflict of interest.

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