GENETIC STUDIES ON *Rana limnocharis*

ABSTRACT

SANT PRAKASH
DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES

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In recent years, there has been an increased interest in the study of genetic variation and its application in population genetics to problems of speciation. This interest reflects a variety of ideas but in particular these ideas reflect the potential importance of random and adaptive events in the development of reproductive isolation. The 'strategy of adaptation' in such a changing environment consists in the formation of labile genetic systems capable of responding adaptively to a wide range of variable environmental conditions. Molecular genetics and certain technical developments have made it possible in recent years to answer questions concerning the amount of genetic differentiation in species both at inter and intra population levels, at least to a first approximation. Genetic variation is the basic premise upon which our study has been based. Traditionally, such studies have been based on the geographic classification of the populations. We have discussed here two types of genetic changes, chromosomal and molecular, that have been advocated as important factors in the evolutionary process.

India on the whole and its north eastern part in particular show greatest diversity in form and number in the amphibian fauna. Preliminary survey has revealed that the streaked frog, <u>Rana limnocharis</u> Wiegmann, is the commonest species available in the state of Meghalaya, and its neighbourhood. Moreover, this frog has been also identified to be Oriental in origin, while most other frogs are palearctic. Subsequently, we have used *R. limnocharis* as ou experimental model because of its abundance and high rat of genetic polymorphism. We have undertaken in our laborator a detailed study on the molecular and chromosomal genetic of this group and keeping this in view, the present wor has been carried out and the findings are presented i two sections (A and B).

SECTION - A : ISOZYMIC STUDIES

The usefulness of an enzyme system as a geneti marker in differentiating between geographically separate populations is determined by its variability, i.e., displaying either the presence of allozymes or difference i the number of bands on the gels. If the enzyme does no show variability, then its isozyme pattern is of littl use for comparison among populations. The biochemical techniques of polyacrylamide gel electrophoresis coupled wit specific enzyme staining procedures become an effectiv tool for such analysis.

The whole project has been accomplished in tw phases. In phase I six tissues of *Rana limnocharis* fro nine different localities have been electrophoreticall surveyed to provide data necessary to make an appropriat selection as to which tissue and enzymes should be analyse for genetic differentiation. In phase II the genetic variation has been studied with emphasis on the tissue and th and the enzymes indicated from phase I data.

It appears on comparison from all the populations that this frog like most vertebrates possesses a simple pattern of five LDH isozymes. Normal polarity has been observed in the two tetramers A_4 and B_4. Tissue specific LDH isozyme expression is observed due to the inconsistency of LDH B_4, in contrast to the relatively constant A_4.

The tissue MDH isozyme specificity has been placed under two groups - one having the single band and the other with the three banded phenotype. The two loci also express differential activity.

Maximum activity of the ADH isozyme has been observed in the liver tissue while the other tissues failed to show significant activity. The greatest number of ADH isozymes bands resolved is four. This frog carries three separate loci coding for ADH but the product of only two can form heterodimers. The third locus is monomorphic with no identification of a heterozygous combination between the other two ADH loci.

Two different forms of G6PD isozymes in the liver tissue, called A and B have been confirmed. No heterozygous combination is observed between these two forms, thus leaving some doubt on their genetic compatibility.

The preliminary findings from phase I indicated that the above enzymes in the liver tissue by large warranted extensive investigation because of their more variable phenotypes across the whole population range of Rana limnocharis in Meghalaya.

Under phase II the allozymic variation encoded by nine loci from nine widely separated localities ranging across the whole of Meghalaya has been analysed. These different populations occupy increasingly varying and unpredictable environments. Genetic variation increased in the following order: Tura, Nongstoin, Williamnagar > Cherrapunjee, Mawsynram >Dawki > Nongpoh , Shillong, Jowai.

The mean number of alleles per locus (A), the mean number of loci polymorphic per population (P) and the mean number of loci heterozygous per individual (H) based on the 9 shared loci, were - A = 1.03±0.4, P = 0.48±0.15 and H = 0.08±0.02.

The average number of alleles is low in comparison to th other vertebrates but the mean number of polymorphic loci and heterozygosity is comparable to some of the vertebrates. Low values of alleles may be the characteristic of such populations due to specific environmental conditions. The high levels of polymorphism and heterozygosity are typical for this species when compared with other amphibians.

The expected Hardy-Weinberg proportions have been observed in majority of the populations with each locus.

The values have been fairly correlated with the environmental - variability model. The genetic variations increased in a definite order, showing that the frogs living in a relatively constant and predictable environment are less genetically variant in contrast to the populations which have distinct spatial and temporal variations. Ecologically two similar populations have the commonest factor that they receive the highest rainfall than any part of the world. But the electrophoretic results do not fit the predicted similarity value. The frogs from one population showed similarity to a ecologically different population. We could not explain this phenomenon on the basis of existing data. Another samples from a population also unequivocally stands out in many respects. However, it is concluded that protein polymorphism is adaptively important and is maintained in the species by natural selection.

Roger's coefficient of genic similarity and Nei's genetic distance formulae were utilized to detect genetic relatedness between the nine populations. The average genetic distance value was 0.11±0.04. The similarity index averaged to 0.96±0.23. The values obtained are difficult to interpret because the approximate or standard values for amphibian species are not available. Our average D value falls approximately in the range of the value obtained in a salamander species. This could possibly be the confirmation of a standard value in amphibia.

SECTION - B : KARYOLOGICAL STUDIES

The overall karyology is consistent with that of the earlier report and also with the well known homogeneity among the Asiatic Rana. The present report can be considered a satisfactory confirmation of the conclusion shared by several cytogeneticists that the karyotypes of Rana are conservative. This fact attests to the attainment of the analogous level of karyological differentiation by several families of this order, and may be to their common origin as well; nevertheless, it also provides additional evidence of the large number of parallelisms which also at the karyological level have characterized the evolution of Rana.

The sex determining mechanism of amphibia in general is still a controversial issue. Our observations indicate the lack of heteromorphic sex chromosomes and when both the male and female were available the karyotype was identical. Sex determination in this frog could be the task of one pair of chromosomes still morphologically undifferentiated. An accumulation of heteromorphic sex determining genes could be occuring in the chromosome by decreasing the rate of crossing over in a given pair, the morphological differentiation of this chromosome having not begun. It can also be suggested that the sex in this frog is determined by one or several pairs of male and female determining genes distributed among several chromosomes.

The Nucleolus Organizing Regions (NORs) are located in the homologues of the 10th pair of chromosomes as is true in other Rana. This again points out the fact that the localization of the NOR in Rana karyotype is also very conservative. Additional NOR sites have also been observed in the chromosomes as well as in the nucleoli. Secondary constrictions are acclaimed to be the characteristic feature of the Rana chromosomes but we have not observed secondary constrictions in the karyotypes, which makes this frog to be the third in the list of cytogenetically investigated Rana to lack secondary constrictions.

An overview of the karyological studies shows the limnocharis to possess all the characteristics of Rana except the secondary constrictions on the chromosomes.

Comparative studies made between the other Rana endemic to this region and the Rana limnocharis shows a low similarity value. This could be due to high level of differentiation in this frog. Comparison between the populations of this species shows variations in arm ratios and relative chromosomal lengths. In terms of geographic variations these variations could be considered as 'cytologically diagnostic'.

Some efforts have been made in establishing the evolutionary link of this frog in this region with the data available on other *Rana* species from the south-east Asian countries. *Rana limnocharis* has been identified to be Oriental in origin, while most of other frogs are palearctic. Its place of origin in south-east Asia has been fairly established to be Taiwan (Kuramoto, 1985, personal communication). From Taiwan the migration path of *Rana limnocharis* could be traced easily to the Meghalayan populations. This must have been possible only due to the land and water connections which were established later.

Present evidence relating genetic and environmental variations is still inconclusive and needs critical analytical studies to unveil the potential relationship between the ecological and genetical profiles. Attempts have to be made to compare populations of this species in other localities or with other *Rana* species in the same localities. As mentioned before there is a relative abundance of *Rana limnocharis* in whole of south east Asia as compared to other *Rana* species. It is therefore necessary to study the possible linkage of this frog of this region with other regions. It is evident that time is rapidly approaching when a major effort has to be made towards obtaining complete and reliable estimate of these critical factors to provide a base to build such studies.

We anticipate that exciting development in the next few decades will result from using the new techniques of DNA sequencing and gene cloning. These methods will reveal substitutions and gross variability. This may also permit regulatory gene evolution to be examined directly. Finally, these approaches may provide a high resolution method for studying evolutionary changes in gene arrangement.

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