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Polyacrylamide gel electrophoretic analysis of some species specific proteins in six Indian rodent genera (Subfam.: Gerbillinae and Murinae: Fam: Muridae)

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Abstract. Studies on polyacrylamide gel electrophoresis were undertaken for analysing haemoglobins and eye lens proteins in 6 Indian rodent genera from chaemotaxonomic point of view. The report reveals a distinct segregation of *Rattus* and *Bandicota* from *Mus* and other two murine genera. Revisionary studies of the entire subfamily Murinae for reconsidering the taxonomic placement of *Rattus* and *Bandicota* in the subfamily has been suggested.

Keywords. Polyacrylamide gel electrophoresis; haemoglobins; eye lens proteins; genetic identities; rodent genera; dendograms.

1. Introduction

The Indian rodent genera, particularly the commensal forms like *Rattus*, *Bandicota* and *Mus*, present a most complicated classification. Laborious work of Marshall (1977) for the reconstruction of the taxonomy of *Mus* gives an idea of magnitude of the problem. Raman and Sharma (1977) and Yosida (1973) have reported chromosomal polymorphism in the species of *Rattus*. Selander and Yang (1969) have analysed polymorphism in about 36 proteins including haemoglobin, plasma proteins, specific enzymes etc. and studied genetic heterozygosity in *Mus*. Chromosomal polymorphism is also well known in *Mus* (Marshall 1977). Sharma and Raman (1973), Avirachan *et al* (1971) and Pradhan *et al* (1985) have traced variations in the chromosomes and some of the species specific proteins such as haemoglobins, haptoglobins and transferrins in *Bandicota*. Hence, a necessity is now being felt to compare and study the relationships between the different genera of commensal rodents and confirm their taxonomic placement in the family.

This communication reports the relationship of haemoglobins and eye lens proteins amongst *Rattus*, *Mus*, *Bandicota*, *Tatera*, *Golunda* and *Vandeleuria* (Fam: Muridae) after reviewing the results of polyacrylamide gel electrophoresis (PAGE).

2. Materials and methods

Figure 1 and table 1 show the sampling areas of 6 different rodent genera and the genera-wise samples pooled from the populations. The rodents were collected in the sampling area during extensive surveys carried over a period of 4 years (1982–86). The collection of live specimens from the wild populations was made by using wonder and sherman traps and/or by escavating live burrows. The animals were sacrificed and the haemoglobins in solution were separated from red blood

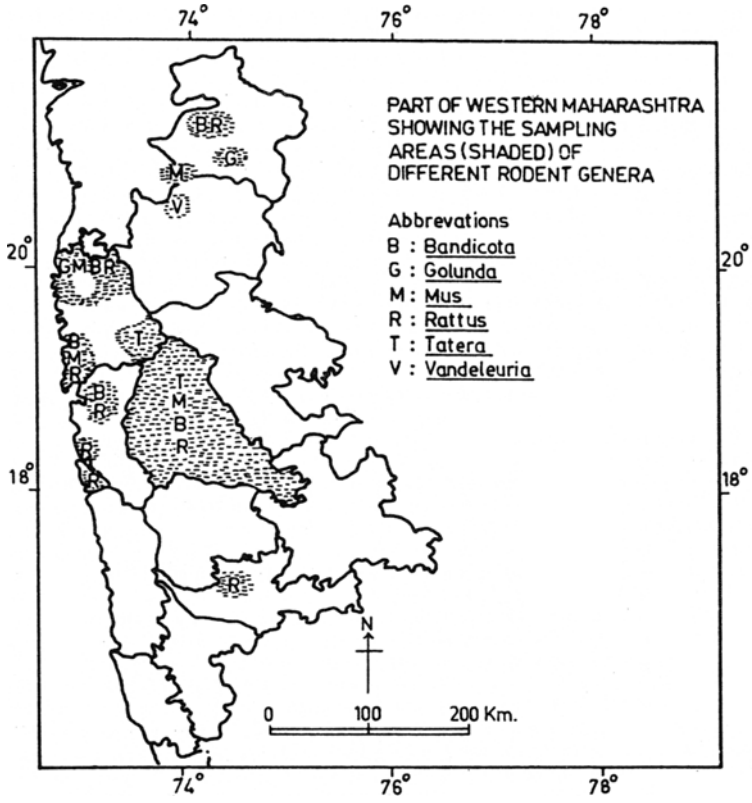

Figure 1. Part of western Maharashtra showing the sampling areas (shaded) of different rodent genera.

Table 1. Sample size of 6 rodent genera pooled from different localities.

Names of genera	No. of species (subspecies studied)	Sampling areas	Sample size
Rattus	2(4)	Dhulia Dist., Thane Dist., Greater Bombay, Pune Dist., Raigad Dist., Sangli Dist.	75
Bandicota	3(3)	Dhulia Dist., Thane Dist., Greater Bombay, Pune Dist., Raigad Dist.	51
Mus	5(8)	Dhulia Dist., Thane Dist., Dang Dist., Greater Bombay, Pune Dist.	25
Golunda	1(2)	Dhulia Dist., Thane Dist.	3
Tatera	1(1)	Thane Dist., Pune Dist.	6
Vandeleuria	1(1)	Nasik Dist.	1

corpuscles as per the methods given by Wright (1974). The methodology used for electrophoretic analysis of both the proteins was mostly based on methods described by Whitaker (1967) and Wright (1974). However, detailed account of the modified methodology adopted is given in the following paragraphs.

Haemoglobins were separated by lysing them in distilled water from isolated red blood corpuscles that were washed to remove plasma contamination and the

mixture was then centrifuged to separate solution containing dissolved haemoglobins and cell debris. The eye lenses were extracted essentially by the method of Smith (1971). Eye lenses of individual specimen were removed from the eye balls immediately after killing the animal. Using a glass homogeniser and 0·012% NaCl solution the soluble lens proteins were extracted. The homogenate was maintained at 0°–4°C for 24 h with intermittent stirring. The extract was then centrifuged at 5000 rpm for 20 min in a refrigerated centrifuge (Temp. 4°C ± 1°C) to obtain a clear extract containing soluble eye lens proteins. The protein extracts, after treatment of sodium dodecyl sulphate (SDS), in the presence of mercaptoethanol were used for SDS-PAGE.

Polyacrylamide gel (7·5%), stacked at pH 8·3 and running at pH 9·5, was used for the separation of haemoglobins and eye lens proteins. The gel solutions were cast in neutral glass tubes (size: 0·6 × 10 cm). Ammonium per sulphate (0·14%) freshly prepared and added to gel solution before casting in tubes served as an additional catalyst. Each glass tube contained 2 ml of running gel and 0·1 ml of 5% spacer gel. The buffer with 8·3 pH used in the system contained 6 g of Tris and 28·8 g of glycine dissolved in one litre of distilled water. Before filling the chambers of electrophoretic assembly the buffer solution was diluted 10 times with distilled water. Haemoglobin solutions/eye lens extracts were loaded on the gel columns in each tube after mixing the sample solution with marker, bromophenol blue. The electrophoretic run was carried out at 4°C ± 1°C at a constant voltage (200 V) and current (3–4 mA) per tube. The separation was terminated after 3 h, the time by which marker migrated to the other end of the tube. After completion of the electrophoretic runs, the gel columns were stained by benzedene coupled reaction (Ornstein 1967) for identifying the haemoglobin bands, while the lens proteins were stained with 0·1% methanolic Coomassi brilliant blue R-250 (Gordon 1980). Consolidated protein profiles were prepared by analysing each sample in several replicates and averaging the electrophoretic mobilities with reference to the marker (R_m values) for individual specimens. The final R_m values obtained for the individuals were clubbed together to obtain characteristic profiles for the 6 genera under investigation.

The identification of the individual specimen was done and confirmed by one of the authors as per Ellerman (1961), Marshall (1977) and Roonwal and Agarwal (1962). The voucher specimens have been registered with Western Regional Station, Zoological Survey of India, Pune.

3. Results and discussion

Figure 2 shows a diagrammatic representation of the consolidated electrophoretic patterns of the selected rodent genera. It depicts the patterns for haemoglobins (6 genera) and eye lens proteins (3 genera). The polymorphism at the gene locii regulating the synthesis of both these proteins in *Rattus* and *Bandicota* have been reported elsewhere (Pradhan *et al* 1989). In spite of the polymorphism at the gene locii for haemoglobin, a single band haemoglobin pattern representing homozygosity was observed in individuals of atleast 4 genera. However, Pradhan *et al* (1989) have reported minimum expression of 3 band patterns for eye lens proteins in *Bandicota*. Three basic band patterns of eye lens proteins were also recorded from some of the *Rattus* and *Mus* samples pooled for the current studies.

The haemoglobin profiles (figure 2) of *Rattus* and *Bandicota* show bands

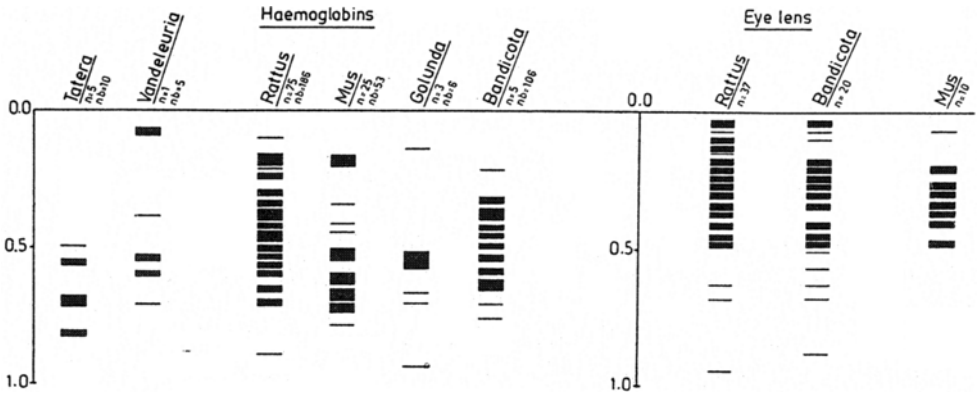

Figure 2. Diagrammatic representation of the electrophoretic patterns of the species specific proteins in the 6 rodent genera.

distributed over the entire R_m range, whereas the profile for *Mus* haemoglobins has a restricted distribution of bands (either mostly below 0·5 or around 0·1). The polyacrylamide gel electrophoretic runs of individual *Mus* specimens were very characteristic and distinctly different. With the exception of members from *Mus booduga*, *Mus saxicola* and *Mus musculus urbanus*, large number of *Mus musculus* samples showed total absence of haemoglobin bands in the R_m range between 0·1–0·5. The lighter fractions were seen either singularly or in combination with the heavier fractions depicting characteristic *Mus* patterns. Similar observations were made in the specimens pooled from *Tatera*, *Golunda* and *Vandeleuria* populations also.

Though extensive variations are obvious in the eye lens protein profiles of *Rattus* and *Bandicota*, variations appear to be limited in *Mus*. As stated earlier, the basic 3-band pattern in an individual, was seen in all the 3 genera.

Thus, a careful comparison of both the profiles in all the 6 genera suggests the presence of two separate groups. *Rattus* and *Bandicota* fall in one group with almost identical pattern of variations, while *Mus*, *Tatera*, *Golunda* and *Vandeleuria* show significant resemblances amongst each other in the haemoglobin profiles. *Mus* also exhibits a distinctly different eye lens protein profile when compared to *Rattus* and *Bandicota*.

For better insight of the relationship of these rodents, genetic identities and distances (Nei 1972) in both the proteins were calculated and compared. Table 2 shows that *Tatera*, *Mus*, *Golunda* and *Vandeleuria* fall in one group with absolute genetical identity for haemoglobin locii. It further shows that *Rattus* is closely related to *Bandicota*, the genetic identity being 0·90. Similarly, locii for *Mus* eye lens proteins show maximum genetic distance from those for *Rattus* and *Bandicota*. Based on these values (table 2) the UPGMA method of Sneath and Sokal (1973) was applied to construct a dendogram for the 6 rodent genera (figure 3). It portrays a clear separation of *Rattus* and *Bandicota* from *Mus* and its associates forming independent groups. The level of similarity for *Mus*, *Tatera*, *Golunda* and *Vandeleuria* was absolute for the haemoglobin locii. Moreover *Rattus*, *Bandicota* and *Mus* are not at all closely related when levels of similarities for eye lens proteins were considered, *Mus* being placed further (I = 0·44) from *Rattus* and *Bandicota*.

Table 2. Estimates of genetic identity (above diagonal) and genetic distance (below diagonal) among the members of 6 genera of Murinae (Fam: Muridae, order: Rodentia) based on haemoglobins and eye lens proteins analysis on PAGE.

Haemoglobins			Eye lens proteins		
Tatera, Vandeleuria, Golunda, Mus	*Rattus*	*Bandicota*	*Rattus*	*Bandicota*	*Mus*
—	0·62	0·62	—	0·48	0·41
0·49	—	0·9	0·47	—	0·47
0·49	0·11	—	0·91	0·76	—

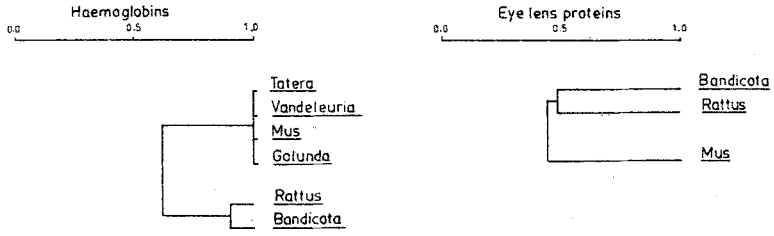

Figure 3. Dendograms showing the relationships of 6 rodent genera generated according to UPGMA method.

The above results would raise some doubts and speculations. However, a second thought will help to resolve some problems in the taxonomic placements of these genera in the family. On the basis of Goodman's (1976) data on phylogeny of various organisms, Fergusson (1980) has doubted the placement of *M. musculus* and *Rattus norvegicus* in the same subfamily, Murinae. Jacobs (1978) and Jacobs and Pilbeam (1980) on the basis of immunological data and information derived from analysis of amino acid sequences do not feel that *Rattus* and *Mus* are phylogenetically close. Musser and Newcomb (1983), after examining the native murid material from Malaya and Sumatra have reached a conclusion that the relationship of *Rattus* with other genera is obscure. The present observations also clearly show that a revision of the taxonomic placements of some of the murine rodent genera has, now, become essential. Though, Cain (1968) has rightly pointed out that the taxonomic placement of any category should not be based only on the biochemical analysis, it is certain that genus *Mus* is distinctly different in all respects from the remaining murine rodent genera studied for the present work. *Tatera* belongs to an independent subfamily, *Gerbillinae*, though during the present investigations it has shown an absolute genetic identity for haemoglobin locii with the other 3 genera (*Mus*, *Golunda* and *Vandeleuria*). Thus, it is suggested that *Mus*, *Golunda* and *Vandeleuria* be retained in the subfamily, Murinae, and the taxonomic placement of Indian genera *Rattus* and *Bandicota* in the subfamily should be reconsidered. A definite conclusion on the placement of *Rattus* and *Bandicota* in the subfamily Murinae can be drawn only after comparing these two genera with those of murine genera which have not been examined during the present investigations.

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