Allozymic variation in four Indian species of genus *Mus*: a comparative analysis

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Abstract

The present study focusses on allozyme variation in the commensal house mouse *Mus musculus*, the pygmy field mice *M. booduga* and *M. terricolor*, and the spiny mouse *M. platythrix.* Genetic heterozygosity was estimated using a set of 24 polymorphic biochemical genetic markers. The extent of variability present in *M. booduga, M. terricolor* and *M. platythrix* has been compared with that in the *M. musculus* complex. Levels of allozyme variation at species level indicate that *M. musculus* has the maximum heterogeneity, followed by *M. booduga* and *M. terricolor*, while *M. platythrix* shows comparatively homogeneous genetic make-up. Gene frequency data have been used to trace phylogenetic relationships among these four species.

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Introduction

The Indian subcontinent is considered to be the main hub of the genus *Mus* as evident from the large number of species present in this part of the world. Marshall (1977) identified many morphological groups, designated as 'species' within the genus *Mus*, and described 16 species from Asia alone based on quantitative differences in morphology and karyotype. These were grouped under three main subgenera: *Pyromys, Coelomys* and *Mus*.

Mice belonging to subgenus *Pyromys* are commonly known as spiny mice because of the presence of trough-shaped spines mixed in the fur and are represented by *M. platythrix* and *M. saxicola*. Though sometimes included in the subgenus *Mus*, they are quite divergent from *Mus* as they show distinct karyotypes ($2n = 22$–26).

The subgenus *Mus* has four lineages. *M. booduga* and *M. terricolor* (previously known as *M. dunni* till Musser and Carleton (1993) replaced the nomenclature), which form one of the lineages, are the Indian pygmy field mice, characterized by their small size. These are endemic to the Indian subcontinent, and are morphologically very similar. These mice also inhabit the same ecological fields. Like *M. musculus, M. booduga* has 40 all acrocentric chromosomes. *M. terricolor* on the other hand shows variation in its karyotype. In different nonoverlapping populations of *M. terricolor*, three divergent karyotypes (types I, II and III) are found (Sharma 1996; Sharma and Sharma 1998).

M. musculus is the true house mouse, showing a karyotype of 40 acrocentric chromosomes. On the Indian subcontinent a large number of species of the subgenus *Mus* exist which prevent the house mouse from occupying outdoor habitats. The Indian house mouse is a purely commensal form found exclusively in association with man-made structures (Marshall 1977).

Since electrophoretic methods were first applied to determine the extent of allozyme variation in population samples, a major confusion over the classification of *Mus* has been cleared. Genetic polymorphisms have contributed significantly to our understanding of intraspecific and interspecific

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systematic relationships since they allow measurements of the extent of gene exchange. The first phylogenetic tree of the subgenus *Mus* was obtained from allozyme data (Sage 1981; Bonhomme *et al.* 1984). This was followed by sequence information and restriction fragment length polymorphism data for various genes (Jouvin-Marche *et al.* 1988), mitochondrial DNA analysis (Fort *et al.* 1984), and DNA/DNA hybridization of single-copy nuclear DNA (scn DNA) (She *et al.* 1990). All these data were used to draw a synthetic tree and to infer different levels of quasi-synchronous speciation events (Boursot *et al.* 1993). The first level corresponds to the separation of the subgenus *Mus* from other subgenera *Nannomys, Pyromys* and *Coelomys.* Yet another level is the separation of the Indian pygmy mice *M. terricolor* and *M. booduga*, which is correlated with the appearance of a 40-acrocentric-chromosome karyotype characteristic of the whole subgenus *Mus*. The final level of radiation corresponds to the individualization of the main subspecies of *M. musculus* which took place half a million years ago. The house mouse is the most recent phylogenetic offshoot of the genus *Mus*.

The Indian *M. musculus* species has recently been the focus of interest primarily because of its high genetic heterogeneity on the basis of which it was proposed that the Indian subcontinent is the cradle from where the entire *M. musculus* species originated and colonized the world (Bonhomme *et al.* 1994; Boursot *et al.* 1996; Din *et al.* 1996). This hypothesis is supported by studies of mice from different localities of India which have shown high levels of gene variation at 15 polymorphic biochemical genetic markers (Awasthi *et al.* 1998).

Whereas *M. musculus* has been in the major limelight of research, its distant relatives have been relatively neglected. There has been a solitary report on the Asian species *platythrix* and *pahari*, which were examined at 28 protein loci (Bonhomme *et al.* 1984). Allelic variation in five individuals of *M. terricolor* trapped from India was examined at 22 protein loci, and phylogenetic trees within the genus *Mus* were proposed (Bonhomme *et al.* 1984). Chatterjee *et al.* (1994) published the mitochondrial DNA restriction maps of *M. booduga, M. terricolor* and *M. m. tytleri.* Singh and Sharma (1996) reported the use of superoxide dismutase (*Sod-1*) as a diagnostic marker for the identification of *M. booduga.* Singh and Sharma (1997) studied genetic variation in the pygmy mice *M. booduga* and *M. terricolor* complex and in the house mouse *M. m. tytleri* trapped from Varanasi and Delhi.

Whereas there are exhaustive reports on *M. musculus* from all over the world, there has not been much work done on the biochemical genetic structure of other closely related species. It would be interesting to examine them and to observe whether they also exhibit high genetic diversity. In this paper we report allozyme variation in different species of *Mus*, compare their population structure and heterozygosity levels, and establish phylogenetic relationships linking them.

Table 1. Populations used in the study.

Locality	Sample size
Mus musculus	
1. Coimbatore (CO)	3
2. Delhi (DEL)	10
3. Guwahati (GU)	10
Mus booduga	
1. Chennai (CN)	10
2. Coimbatore (CO)	5
Mus terricolor III	
1. Chennai (CN)	10
Mus platythrix	
1. Coimbatore (CO)	4
2. Hosur (HO)	4
3. Ooty (OT)	2

Material and methods

Animals: Mice were trapped live from six Indian localities (Coimbatore, Chennai, Delhi, Guwahati, Hosur and Ooty; table 1 shows sample sizes). Random samples from these localities were identified on the basis of their morphology by Zoological Survey of India, Calcutta. In addition to this, aboriginal *M. platythrix* was distinguished on the basis of its large size, eyes, short tail, and presence of 26 acrocentric chromosomes. These animals shed off the entire caudal integument when picked up by the tail. *M. musculus* and *M. booduga* showed $2n = 40$ chromosomes. *M. terricolor* type III trapped from Chennai was identified on the basis of its large submetacentic X, large acrocentric Y, and three pairs (1, 3 and 6) of biarmed autosomes with prominent heterochromatic short arms (Sen and Sharma 1983).

Biochemical genetic analysis: Blood and tissues of freshly dissected animals were stored at -70°C. RBC lysates, plasma, kidney and liver homogenates were used for biochemical analysis. A total of 24 variable biochemical genetic loci were analysed. Electrophoresis was performed on TITAN III cellulose acetate plates (Helena Laboratories, Beaumont, Texas, USA), with each marker having its own unique buffer system (Langley and Roderick 1984). The grading of the alleles was done by comparing the electrophoretic mobility profile with that of C57BL/6J inbred strain of mouse, whose alleles were considered as standard allele 100, except for *Hbb* and *Ldr-1* for which the conventional alphabetic allele designation was used.

Data analysis: (i) Allelic frequencies at the polymorphic loci were calculated. (ii) Using the gene frequency data Nei's genetic distance matrix was constructed (Nei 1978). (iii) PHYLIP package (version 3.5c, Felsenstein 1993) was used for phylogenetic analysis. One thousand replicates of the original data set were constructed by bootstrap resampling and Nei's genetic distance was computed for all data sets

using the GENDIST program. These were further used to construct phylogenies using UPGMA and NEIGHBOR JOINING implemented by NEIGHBOR program. CONSENSE program selects the best possible phylogeny and this was used to construct an unrooted tree by DRAWTREE.

Results

Biochemical-genetic profile of M. musculus

The Indian populations of *M. musculus* are highly heterogeneous. This contributes to increased polymorphism. The three localities chosen for the study were Delhi, Guwahati and Coimbatore, which represent the north, east and south of India respectively. Out of the 24 loci studied, 13 (*Amy-1, Apoa-1, Car-2, Es-1, Es-3, Es-10, Got-1, Gpd-1, Gpi-1, Hbb, Idh-1, Np-1, Pgm-1*) were found to be variable and segregated for two or more alleles (table 2). Certain loci such as *Idh-1* were highly polymorphic, showing the presence of five alleles. *Es-3, Gpi-1* and *Pgm-1* showed three alleles each. A total of 11 novel alleles, *Es-1*^{98} (figure 1), *Es-3*^{105}, *Gpi-1*^{110}, *Gpi-1*^{130} (figure 2), *Idh-1*^{80}, *Idh-1*^{90}, *Idh-1*^{120}, *Np-1*^{90}, *Np-1*^{95}, *Pgm-1*^{85} and *Pgm-1*^{105}, were observed. These have not been reported earlier in any of the inbred strains of mice. As earlier studies (Awasthi *et al.* 1998) have shown, these populations do not show any subspecies-specific diagnostic profile and hence cannot be grouped as belonging to a particular subspecies. A detailed analysis of the allelic profile shows that though these *M. musculus* populations show a bias towards the '*castaneus*' lineage, they also show many different alleles such as *Es-1*^{b} of the '*domesticus*' lineage, *Es-1*^{98} observed in the '*bactrianus*' lineage, *Idh-1*^{120} reported earlier in Tehran (Din *et al.* 1996), and *Np-1*^{90} and *Pgm-1*^{b} found in '*musculus*' and '*molossinus*' subspecies.

Biochemical-genetic profile of M. booduga and M. terricolor type III

This is the first report on allozyme variation in the Indian pygmy mice using cellulose acetate electrophoresis. Due to lack of established inbred strains for use as controls, allelic profile was determined using mice from laboratory strains of *M. musculus* as controls. Both the species are polymorphic at nine genetic loci, out of which six loci (*Apoa-1, Car-2, Es-1, Es-3, Np-1, Trf*) are common. In addition, *M. booduga* is polymorphic at *Amy-1, Pgm-1 and Pgm-2*, and *M. terricolor* type III is variable at *Akp-1, Alb-1* and *Es-10* (table 2).

M. booduga was found to be more heterogeneous and certain loci such as *Car-2, Es-1* and *Np-1* were polymorphic with three or four alleles. *M. terricolor* type III on the other hand is less polymorphic; only *Apoa-1* shows three variant alleles, while two alleles were seen at the remaining polymorphic loci.

A comparison of the allelic profiles indicates that the pygmy field mice are closely related to each other. According to Marshall (1977) the karyotype of *M. terricolor* has evolved as a device to preclude interbreeding with *M. booduga*. Compared to the Indian *M. musculus* populations, these animals show a lower level of genetic polymorphism.

Biochemical-genetic profile of M. platythrix

On cellulose acetate electrophoresis these animals show distinct patterns that easily discriminate them from other members of the subgenus *Mus*. Of the 24 loci studied, nine loci, *Amy-1, Apoa-1, Es-1, Es-10, Idh-1, Ldh-1, Pgm-1, Pgm-2* and *Trf*, were variable and the remaining 15 were invariant (table 2). Though these animals were trapped from three different localities, their level of polymorphism and degree of heterogeneity is low as seen in the segregation of only two alleles for each of the nine polymorphic markers. The animals do not show significant geographical variation in their allelic profiles.

Unique alleles of the aboriginal species

M. booduga shows nine novel alleles at seven loci: *Amy-1*^{80}, *Apoa-1*^{90}, *Car-2*^{90}, *Car-2*^{130}, *Es-1*^{90}, *Np-1*^{70}, *Pgm-2*^{116}, *Trf*^{95} and *Trf*^{106}. *M. terricolor* type III on the other hand has only two species-specific alleles, *Akp-1*^{90} and *Es-10*^{145}. These two species also show certain alleles such as *Es-3*^{110}, *Np-1*^{60}, *Np-1*^{85}, *Got-1*^{a}, *Hbb*^{p} and *Mod-1*^{a} that can be considered as characteristic of pygmy mice. *M. platythrix* also shows the presence of eight species-specific alleles: *Apoa-1*$^{(PaV1)}$ (PaV1: Platythrix variant 1, bands exactly at position 100 of C57BL/6J, but it is identified on the basis of its dark staining intensity and thick band size), *Es-3*^{75}, *Es-10*^{160}, *Idh-1*^{95}, *Ldh-1*^{110}, *Ldr-1*^{110}, *Mod-1*^{125} and *Pgm-2*^{120}. The three species *booduga, terricolor* and *platythrix* showed monomorphic *Gpd-1*^{120}, *Gpi-1*^{68} (figure 2) and *Got-2*^{b} and polymorphic *Es-1*^{92} (figure 1), which can be considered as alleles characteristic of the aboriginal populations.

Genetic distance and cluster analysis

The gene frequency data (table 2) were used to trace phylogenetic relationships among the four species by constructing Nei's genetic distance matrix (Nei 1978) (table 3). If we average the genetic distances by clubbing the populations of the species together, five levels of divergence can be worked out on the basis of the genetic distances. At level I, a distance of the order of 0.1067 separates the populations of *M. booduga* and a distance of 0.1776 the *M. musculus* populations. These interpopulation genetic distances can roughly be correlated with geographic distances between the populations (Awasthi *et al.* 1998). However, no general mechanism by which geographic distance can produce genetic isolation has been identified till now. Level II is of the order of 0.277 between the *booduga* and *terricolor* lineages. These two species are closely related to each other as seen by the number of common alleles. The genetic

Table 2. Alletic frequencies of mouse populations at 24 biochemical genetic loci.

Gene	Allele	*M. m.* CO	*M. m.* DEL	*M. m.* GU	*M. b.* CN	*M. b.* CO	*M. ter.*	*M. pla.*
Akp-1	b	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	90	0.00	0.00	0.00	0.00	0.00	0.90	0.00
	100	0.00	0.00	0.00	0.00	0.00	0.10	1.00
Alb-1	95	0.00	0.00	0.00	1.00	1.00	0.90	0.00
	100a	1.00	1.00	1.00	0.00	0.00	0.10	1.00
Amy-1	80	0.00	0.00	0.00	0.10	0.00	0.00	0.00
	100a	0.00	0.10	0.00	0.85	1.00	1.00	0.95
	120b	1.00	0.90	1.00	0.05	0.00	0.00	0.05
Apoa-1	PaV1	0.00	0.00	0.00	0.00	0.00	0.10	0.90
	80	0.00	0.00	0.00	0.20	0.60	0.80	0.00
	85b	1.00	1.00	0.20	0.00	0.00	0.00	0.10
	90	0.00	0.00	0.00	0.80	0.00	0.00	0.00
	100a	0.00	0.00	0.80	0.00	0.40	0.10	0.00
Car-2	70	0.00	0.00	0.00	0.00	0.40	0.90	1.00
	90	0.00	0.00	0.00	0.00	0.20	0.00	0.00
	100a	1.00	1.00	0.80	0.80	0.40	0.10	0.00
	110b	0.00	0.00	0.20	0.00	0.00	0.00	0.00
	130	0.00	0.00	0.00	0.20	0.00	0.00	0.00
Es-1	90	0.00	0.00	0.00	1.00	0.20	0.00	0.00
	92	0.00	0.00	0.00	0.00	0.40	0.10	0.90
	95b	0.85	0.00	0.00	0.00	0.40	0.90	0.10
	98	0.15	1.00	1.00	0.00	0.00	0.00	0.00
Es-3	75	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	a	0.00	0.40	0.00	0.00	0.00	0.00	0.00
	90c	0.00	0.20	0.10	0.00	0.00	0.00	0.00
	100b	0.00	0.40	0.90	0.65	0.60	0.60	0.00
	105	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	110	0.00	0.00	0.00	0.35	0.40	0.40	0.00
Es-10	100a	1.00	0.00	0.80	1.00	1.00	0.95	0.25
	102b	0.00	1.00	0.20	0.00	0.00	0.00	0.00
	145	0.00	0.00	0.00	0.00	0.00	0.05	0.00
	160	0.00	0.00	0.00	0.00	0.00	0.00	0.75
Got-1	90b	0.00	0.00	0.30	0.00	0.00	0.00	1.00
	100a	1.00	1.00	0.70	1.00	1.00	1.00	0.00
Got-2	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gpd-1	92c	1.00	0.80	1.00	0.00	0.00	0.00	0.00
	110b	0.00	0.20	0.00	0.00	0.00	0.00	0.00
	120	0.00	0.00	0.00	1.00	1.00	1.00	1.00
Gpi-1	68	0.00	0.00	0.00	1.00	1.00	1.00	1.00
	85a	1.00	1.00	0.90	0.00	0.00	0.00	0.00
	110	0.00	0.00	0.05	0.00	0.00	0.00	0.00
	130	0.00	0.00	0.05	0.00	0.00	0.00	0.00
Hbb	d	1.00	0.80	0.95	0.00	0.00	0.00	1.00
	p	0.00	0.20	0.05	1.00	1.00	1.00	0.00
Idh-1	80	0.00	0.00	0.05	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.10	0.00	0.00	0.00	0.00
	95	0.00	0.00	0.00	0.00	0.00	0.00	0.40
	100a	1.00	0.10	0.85	1.00	1.00	0.00	0.00
	110b	0.00	0.60	0.00	0.00	0.00	1.00	0.60
	120	0.00	0.30	0.00	0.00	0.00	0.00	0.00
Ldh-1	100a	1.00	1.00	1.00	1.00	1.00	1.00	0.10
	110	0.00	0.00	0.00	0.00	0.00	0.00	0.90
Ldr-1	100a	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	110	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Mod-1	100a	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	125	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Mor-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00

(*continued*)

Table 2. (*continued*)

Gene	Allele	*M. m.* CO	*M. m.* DEL	*M. m.* GU	*M. b.* CN	*M. b.* CO	*M. ter.*	*M. pla.*
Mpi-1	90	0.00	0.00	0.00	1.00	1.00	0.00	0.00
	100b	0.00	0.00	0.00	0.00	0.00	1.00	1.00
	110a	1.00	1.00	1.00	0.00	0.00	0.00	0.00
Np-1	60	0.00	0.00	0.00	0.10	0.20	0.20	0.00
	70	0.00	0.00	0.00	0.20	0.20	0.00	0.00
	85	0.00	0.00	0.00	0.70	0.60	0.60	0.00
	90	1.00	0.60	0.90	0.00	0.00	0.00	0.00
	95	0.00	0.00	0.10	0.00	0.00	0.20	1.00
	100a	0.00	0.40	0.00	0.00	0.00	0.00	0.00
Pep-3	100a	0.00	0.00	0.00	1.00	1.00	1.00	1.00
	104b	1.00	1.00	1.00	0.00	0.00	0.00	0.00
Pgm-1	80	0.00	0.00	0.00	0.85	0.60	1.00	0.80
	85	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	90	0.00	0.00	0.00	0.15	0.40	0.00	0.20
	95b	0.00	0.40	0.25	0.00	0.00	0.00	0.00
	100a	0.00	0.20	0.75	0.00	0.00	0.00	0.00
	105	0.00	0.40	0.00	0.00	0.00	0.00	0.00
Pgm-2	100a	1.00	1.00	1.00	0.85	1.00	1.00	0.90
	116	0.00	0.00	0.00	0.15	0.00	0.00	0.00
	120	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Trf	70	0.00	0.00	0.00	0.00	0.00	0.10	0.90
	95	0.00	0.00	0.00	0.20	0.20	0.00	0.00
	100b	1.00	1.00	1.00	0.00	0.00	0.00	0.00
	103a	0.00	0.00	0.00	0.00	0.80	0.90	0.10
	106	0.00	0.00	0.00	0.80	0.00	0.00	0.00

distance between the *musculus* and *booduga* species is of the order of 0.86 while that between *musculus* and *terricolor* is 1.09 (level III), indicating that *M. booduga* is closer to *M. musculus* on the evolutionary chart compared to *M. terricolor*. This observation is also in accordance with the karyotypic constitutions of these species: *M. booduga* shows 40 all acrocentric chromosomes like *M. musculus*. Level IV indicates the genetic distance between the spiny and pygmy mice, which is 0.87. The greatest divergence (level V) is seen between the spiny and house mice (genetic distance = 1.48). Phylogenetic trees (figure 3 and figure 4; Felsenstein 1993) constructed from the genetic distance matrix show clear divergence of the *M. musculus* populations from the aboriginal populations of *booduga, terricolor* and *platythrix*. The *booduga* and *terricolor* populations tend to cluster together while the species *M. platythrix* shows greatest divergence.

Allozyme variation at the species level

Table 4 gives a comparative analysis of allozyme variation at the species level. The three parameters used are: average number of alleles per locus, percentage of polymorphic loci, and heterozygosity. *M. musculus* has the highest polymorphic loci (62%), highest average number of alleles per locus (2.08), and highest heterozygosity level (0.19). The other three species together had nine polymorphic loci (41%), but the number of alleles per locus is at most 1.59 and heterozygosity level 0.17 in *M. booduga*. Much of the variation at the species level is due to the proportion of polymorphic loci and the mean number of alleles per locus.

Discussion

This is essentially the first comprehensive study of different species of *Mus* at the population level using cellulose acetate electrophoresis to assess the levels of allozyme variation and determine their evolutionary relationships. Cellulose acetate electrophoresis offers many advantages over conventional starch and polyacrylamide gel electrophoretic techniques. The system is very quick and easy to handle, and hence many samples can be analysed, which is particularly important when multiple markers are to be studied in populations. The interpretation of bands is easier and the plates can be stored indefinitely.

Our results show that the pygmy field mice have many alleles that are also present in the house mouse, although they also have certain unique alleles that can be considered to be species specific. Compared to *M. terricolor* (type III), *M. booduga* showed a high level of diversity in terms of number of alleles per locus and mean heterozygosity. The variability in pygmy mice is however lower than that in *M. musculus* populations.

In a recent study (Singh and Sharma 1997) electrophoretic analysis of 20 enzymatic and nonenzymatic proteins was carried out on a panel of house mice (*M. m.*

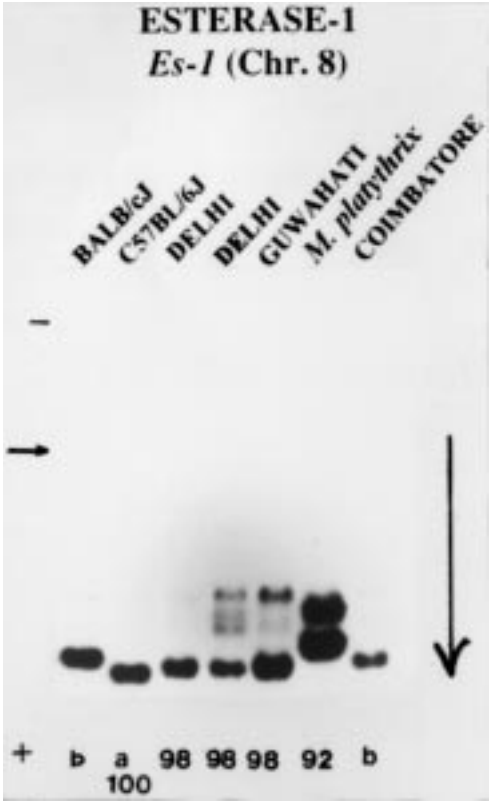

Figure 1. Cellulose acetate plate showing allelic variation at the *Es-1* locus. Lanes contain samples of the following: 1, BALB/cJ; 2, C57BL/6J; 3, *M. musculus* (Delhi); 4, *M. musculus* (Delhi); 5, *M. musculus* (Guwahati); 6, *M. platythrix*; 7, *M. musculus* (Coimbatore). The arrow on the left indicates the sample loading point, and the downward arrow indicates direction of electrophoresis from cathode to anode.

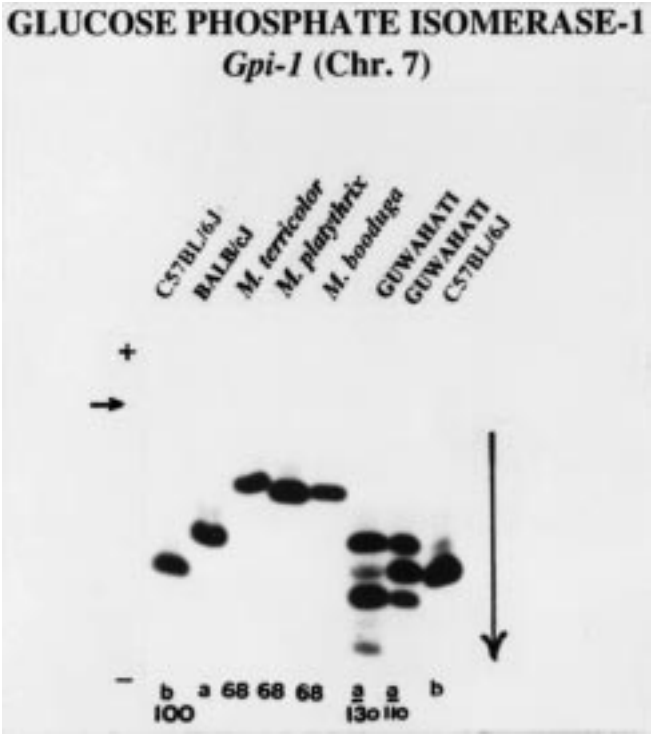

Figure 2. Cellulose acetate plate showing allelic variation at the *Gpi-1* locus. Lanes contain samples of the following: 1, C57BL/6J; 2, BALB/cJ; 3, *M. terricolor*; 4, *M. platythrix*; 5, *M. booduga*; 6, *M. musculus* (Guwahati); 7, *M. musculus* (Guwahati); 8, C57BL/6J.

tytleri trapped from Delhi and Varanasi) and pygmy field mice (*M. booduga* collected from Mysore and Varanasi from burrows in cultivated fields, and *M. terricolor* types I, II and III similarly collected from Varanasi, Mysore and Chennai respectively). Of the 20 loci studied nine are common with the present study (*Alb-1, Trf, Hbb, Ldh-1, Mod-1, Idh-1, Es-1, Es-3 and Got-1*). The paper reports very high levels of heterozygosity for all the populations; the difference in the level of diversity observed in the house mouse and the pygmy field mice was not statistically significant. On the other hand, we have observed (Awasthi *et al.* 1998) that the loci *Alb-1, Trf* and *Mod-1* are completely monomorphic in 10 populations of the house mouse trapped from various Indian localities, in contrast to the present report which describes them as polymorphic. A similar contradiction is noticed in the genetic profiles of the pygmy field mice, where comparatively lower levels of gene variation have been recorded by us in the loci studied.

Nei's genetic distances calculated for pairwise comparisons was estimated to be 0.277 between *booduga* and *terricolor* III, while between the house and pygmy mice it is much higher, of the order of 0.97. Our results further contradict the data of Singh and Sharma (1997), where the closeness of pygmy mice to house mice has been shown on the basis of genetic distance to be of the order of 0.285, against 0.97 estimated in the present study.

Bonhomme *et al.* (1984) studied *M. platythrix* along with other *Mus* species at various loci and showed that the species belongs to an independent genus, *Pyromys*. Apart from this there are no reports on the biochemical-genetic characterization of these animals. In the present study, the

Table 3. Nei's genetic distance matrix for *M. musculus, M. booduga, M. terricolor* and *M. platythrix* populations from various localities based on 24 biochemical genetic markers.

M. musculus CO	0.0000						
M. musculus DEL	0.2145	0.0000					
M. musculus GU	0.1730	0.1453	0.0000				
M. booduga CN	0.8577	0.9571	0.8194	0.0000			
M. booduga CO	0.8280	0.9736	0.7878	0.1067	0.0000		
M. terricolor III	1.0800	1.0949	1.1085	0.3516	0.2031	0.0000	
M. platythrix	1.5326	1.4810	1.4443	1.0826	0.9164	0.7480	0.0000

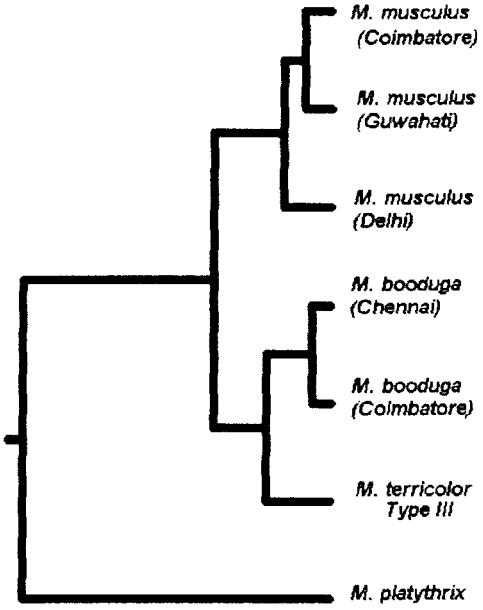

Figure 3. UPGMA phenogram of different species of *Mus* based on Nei's genetic distance matrix at 24 gene loci treated with NEIGHBOR program of PHYLIP package (version 3.5c).

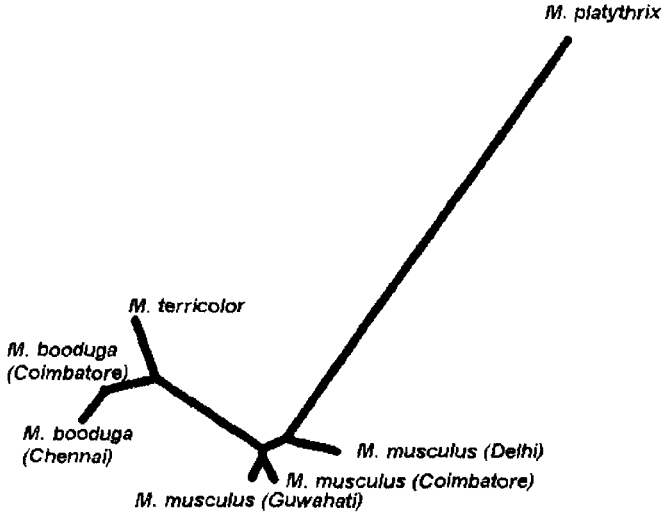

Figure 4. Phylogenetic tree constructed using NEIGHBOR JOINING program implemented by NEIGHBOR program of PHYLIP package (version 3.5c).

genetic profile of *M. platythrix* shows the least variation. Though the animals were trapped from three widely separated localities they show a low level of polymorphism. The allelic profile of these animals is very distinctive and characteristic of the species. It is possible to identify them on the basis of just a few genetic loci. From an evolutionary viewpoint it would be interesting to determine what factors contribute to high levels of divergence in some species while others are stable.

The results of qualitative analysis based on cladistic principles also detect synapomorphies (degree of shared characters) which are very useful for grouping of species. Three genetic loci, *Gpi-1*, *Gpd-1* and *Got-2*, which are monomorphic and conserved in all the three aboriginal populations, can also be used as diagnostic markers for the identification of these species.

Table 4. Allozyme variation at the species level.

Species	Percentage polymorphic loci	Mean number of alleles per locus	Mean genetic diversity
M. musculus	0.62 ± 0.10	2.08 ± 0.21	0.19 ± 0.04
M. booduga	0.41 ± 0.10	1.59 ± 0.17	0.17 ± 0.05
M. terricolor	0.41 ± 0.10	1.50 ± 0.14	0.11 ± 0.03
M. platythrix	0.41 ± 0.10	1.14 ± 0.05	0.09 ± 0.03

Phylogenetic trees were constructed using UPGMA (figure 3) and NEIGHBOR JOINING (figure 4) algorithms. Both methods show similar phenograms depicting the relatedness between the different species. If we consider *M. musculus* as the most recent phylogenetic offshoot of the genus *Mus* the tree could be considered as a representation of historical branching events. Among the four species *M. platythrix* is the most widely separated, next to appear is *M. terricolor*, followed by *M. booduga*, and finally *M. musculus*.

To summarize: Among the four species *M. musculus* shows the highest level of allozyme variation at both the population and the species level. *M. booduga* also shows considerable genetic variability, which is higher than that observed in *M. terricolor* type III. This could also be due to the fact that *M. terricolor* type III has been trapped from just one locality while *M. booduga* were obtained from two. *M. platythrix* on the other hand appears to be genetically the most stable. The three aboriginal species endemic to India have been studied in depth morphologically and karyotypically by researchers in India and abroad. Our study fills major gaps with their biochemical-genetic profile and thus provides useful insights into better understanding of *Mus*.

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