

Temporal variation of allele frequencies in four population samples of *Akodon montensis* (Rodentia, Cricetidae)

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ABSTRACT

Protein variation at 22 loci was studied in four population samples of *Akodon montensis* investigated at different times in Torres, RS, Brazil, (29°20'S; 49°45'W). Fourteen loci were monomorphic and the other eight showed polymorphism in at least one sample. Differences between samples in allele frequencies were found at all polymorphic loci and occurred in two general patterns: the first was a wide variation in allele frequencies as well as the appearance or disappearance of alleles in some samples (Aat, Alb, Glo, Pgi, Pgm-1, Pgm-2, Pgm-3, Sod-1), and the second involved a shift from one predominant allele to another (Glo, Pgi, Pgm-2, Pgm-3). There was also a decline in the degree of polymorphism and mean heterozygosity from the first to the last sample. Gene frequency fluctuations may be due to reproductive variation in terms of population density, genetic drift, selection and migration or recolonization. We obtained indications that recolonization is a very important factor in gene frequency fluctuations.

INTRODUCTION

Fluctuations in gene frequencies in rodent populations have been reported by several authors. This variation has been explained by density-dependent selection (Semeonoff and Robertson, 1968; Tamarin and Krebs, 1969; Gaines and Krebs, 1971), as well as by survival rate (Tamarin and Krebs, 1969). Zimmerman (1988) assigned the high level of temporal gene variation observed in fossorial rodents to isolation and small effective size of populations, these groups being

subjected to genetic drift, bottleneck and inbreeding, factors which propitiate rapid speciation. Apfelbaum and Blanco (1985) point out that the temporal variation could be due to changes in age-structure.

In order to provide more information which may help the understanding of temporal genetic variation in rodent populations we studied the variability at 22 protein loci in four samples of *Akodon montensis* collected at different times and at the same site over a three-year period.

MATERIAL AND METHODS

Animals were live-trapped at Faxinal, 3 km southwest of Torres, a city in Rio Grande do Sul State, Brazil (29°20'S; 49°45'W). Four samples, comprising a

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total of 91 individuals, were obtained in three different years as follows: sample I in April (fall), 1986, N=28; sample II in February (summer), 1988, comma N=34; sample III in March (summer), 1989, N=11 and sample IV in September (winter), 1989, N=18. The sampling area consisted of a highly fragmented swamp forest and shrubland mosaic. Between the third and fourth collection periods a small part of the area was burned.

Specimens were killed by ether inhalation and tissues were dissected out, washed quickly in 0.9% NaCl solution, wrapped in air-tight plastic bags and stored at -20°C until utilization for electrophoresis, no longer than six months. Under these conditions, most proteins remain undenatured in the intact organs for several months (Selander *et al.*, 1971). All samples were karyotyped, showing polymorphism due to B chromosomes and 2n=23-26. (E.C. Castro and A.U. Christoff, personal communication). All individuals are preserved as skins and skulls at the Mammal Collection of the Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Liver and kidney extracts were prepared by homogenizing 50 mg of tissue in 0.15 ml distilled water and 0.05 ml 2% β -mercaptoethanol. Homogenates were centrifuged at 2,000 rpm (750 g) for 10 minutes, at 4°C and the clear supernatant was used for electrophoresis.

Genetic variation at 22 structural loci encoding 14 proteins was assayed by horizontal starch gel electrophoresis, except for glyoxalase, for which 1:2 agarose-starch gel was used. The proteins and loci investigated and the tissues and methods employed are presented in Table I.

Alleles were designated alphabetically in order of increasing mobility. The genotype and gene frequencies were analyzed using the BIOSYS-1 computer program (Swofford and Selander, 1981). Polymorphism (P) was calculated directly from the data and the average heterozygosity per locus (H) was calculated on the basis of allele frequencies according to Nei (1978).

RESULTS AND DISCUSSION

Fourteen of the 22 loci analyzed were monomorphic, with all individuals showing the same phenotype in the four samples. The other eight loci were polymorphic in at least one sample (Table II). Figures 1 to 7 show the electrophoretic patterns observed in the polymorphic systems. Differences between samples in allele frequencies were found at all

Table I - *Akodon montensis* proteins and loci investigated and laboratory methods employed.

| Proteins | Loci | Tissue extracts | Methods | |
|--------------------------------|------------------|-----------------|---------|---|
| | | | a | b |
| Acid phosphatase | Acp | Kidney | 2 | 2 |
| Albumin | Alb | Liver | 1 | 1 |
| Aspartate aminotransferase | Aat1, Aat2 | Liver | 4 | 2 |
| Glyoxalase | Glo | Kidney | 5 | 3 |
| Isocitrate dehydrogenase | Idh1, Idh2 | Kidney | 2 | 2 |
| Lactate dehydrogenase | Ldh1, Ldh2 | Kidney | 4 | 2 |
| Malate dehydrogenase | Mdh1, Mdh2 | Kidney | 2 | 2 |
| Malic enzyme | Me1, Me2 | Kidney | 4 | 2 |
| Nucleoside phosphorylase | Np | Liver | 2 | 2 |
| Phosphoglucomutase | Pgm1, Pgm2, Pgm3 | Liver | 5 | 5 |
| Phosphogluconate dehydrogenase | Pgd | Kidney | 2 | 2 |
| Phosphoglucose isomerase | Pgi | Liver | 4 | 2 |
| Superoxide dismutase | Sod1, Sod2 | Kidney/Liver | 2 | 2 |
| Xantine dehydrogenase | Xdh | Kidney | 4 | 4 |

a, Buffer systems; b, staining methods; 1, Bowman and Bearn (1965); 2, Harris and Hopkinson (1976); 3, Parr *et al.* (1977); 4, Selander *et al.* (1971); 5, Spencer *et al.* (1964).

Table II - Allele frequencies (x 100) at eight polymorphic loci and genetic variation parameters (P,H) in four *Akodon montensis* samples.

| Loci | Alleles | Samples | | | |
|-------|---------|-------------------|-------------------|--------------------|-------------------|
| | | I fall/86 N=28 | II sum/88 N=34 | III sum/89 N=11 | IV win/89 N=18 |
| Aat-1 | a | 8 | 0 | 0 | 0 |
| | b | 90 | 94 | 100 | 100 |
| | c | 2 | 6 | 0 | 0 |
| Alb | a | 0 | 23 | 0 | 0 |
| | b | 56 | 54 | 100 | 100 |
| | c | 28 | 0 | 0 | 0 |
| | d | 16 | 23 | 0 | 0 |
| Glo | a | 63 | 3 | 31 | 39 |
| | b | 37 | 97 | 56 | 61 |
| | c | 0 | 0 | 13 | 0 |
| Pgi | a | 11 | 0 | 0 | 0 |
| | b | 31 | 47 | 67 | 0 |
| | c | 58 | 53 | 33 | 100 |
| Pgm-1 | a | 0 | 5 | 0 | 0 |
| | b | 100 | 95 | 100 | 100 |
| Pgm-2 | a | 68 | 48 | 100 | 100 |
| | b | 32 | 52 | 0 | 0 |
| Pgm-3 | a | 32 | 3 | 70 | 100 |
| | b | 68 | 97 | 30 | 0 |
| Sod-1 | a | 96 | 100 | 100 | 100 |
| | b | 4 | 0 | 0 | 0 |
| | P | .32 | .32 | .14 | .04 |
| | H | .12 | .09 | .06 | .02 |

N - sample number; sum - summer; win - winter.

eight polymorphic loci occurring in two general patterns.

The first was a wide variation in allele frequencies as well as the appearance (detection) or disappearance (no detection) of alleles in some samples (Aat, Alb, Glo, Pgi, Pgm-1, Pgm-2, Pgm-3, Sod-1). This might be due to bottleneck or genetic drift, as a consequence of different sample size, specimen extraction or habitat destruction. In *A. cursor*, a sibling species of *A. montensis*, population size is strongly reduced each year (Fonseca and Kierulff, 1989); therefore only a part of the genetic diversity is found in the next generation. However, genetic drift alone may not account for the major shifts in allele frequency such as the disappearance of an allele with 50% frequency observed in our samples. Selection and migration or recolonization of the area may also intervene in this process. This last hypothesis is supported by the

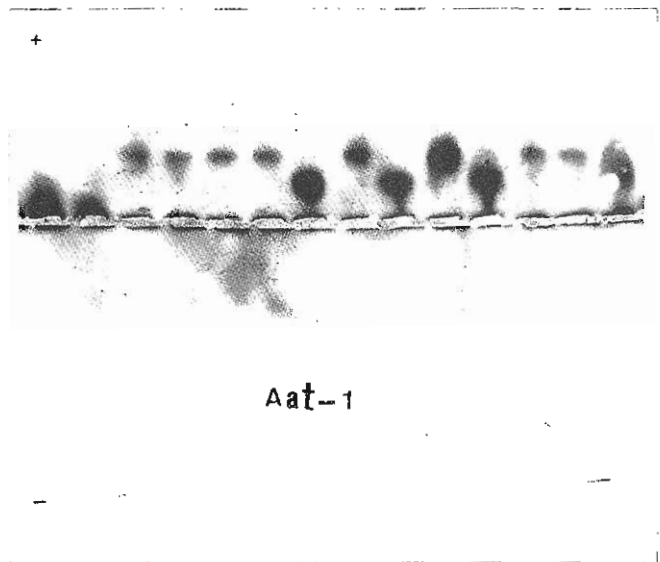


Figure 1 - Aspartate aminotransferase-1 electrophoresis patterns. Lanes 1, 2 and 15: Aat-1 aa; 3 to 6, 8, 10, 12 and 13: Aat-1 cc; 7, 9 and 11: Aat-1 bb; 14: Aat-1 bc.

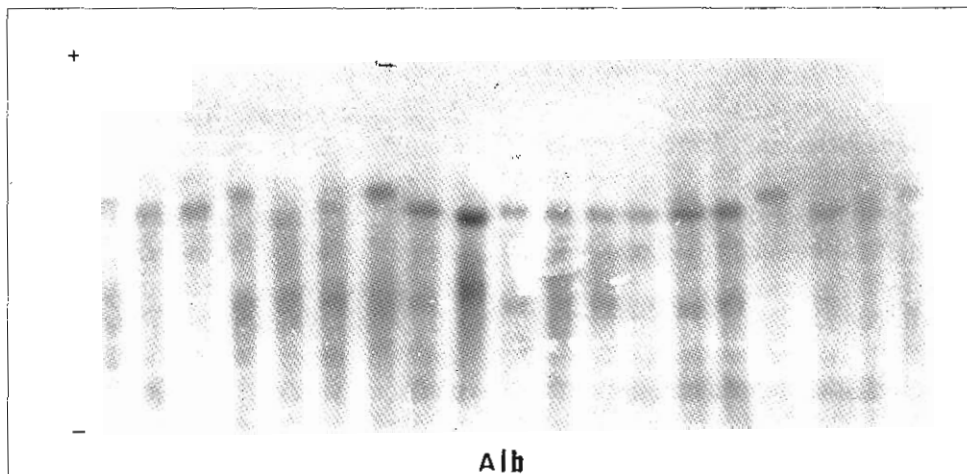


Figure 2 - Albumin electrophoresis profile. Lanes 1, 16 and 19: Alb cc; 2, 3, 6, 8, 10 to 15 and 17: Alb bb; 4 and 7: dd; 5 and 9: aa; 18: bc.

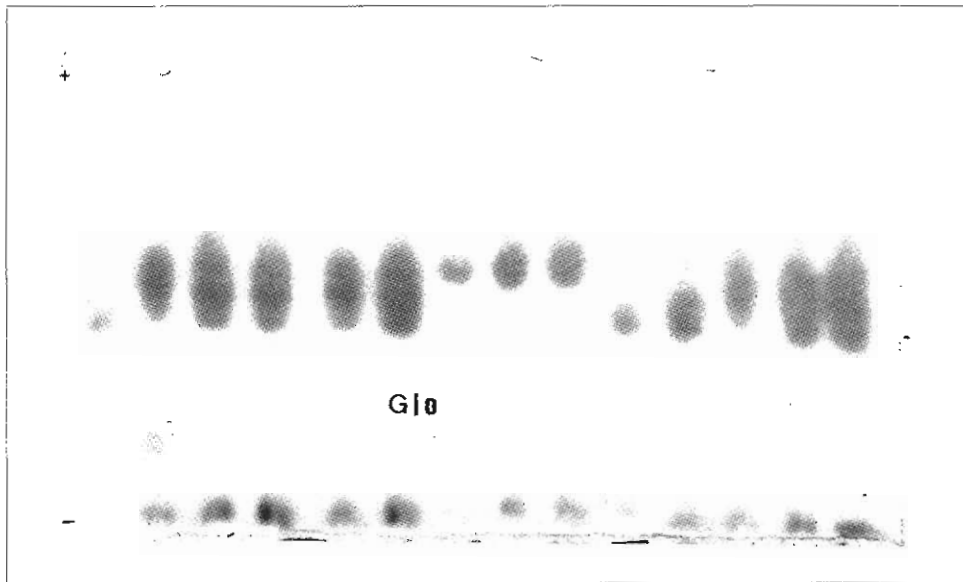


Figure 3 - Glyoxalase electrophoresis patterns: Lanes 1, 10 and 11 *Glo* aa; 2 to 6 and 12 to 14 *Glo* ab; 7 to 9: *Glo* bb.

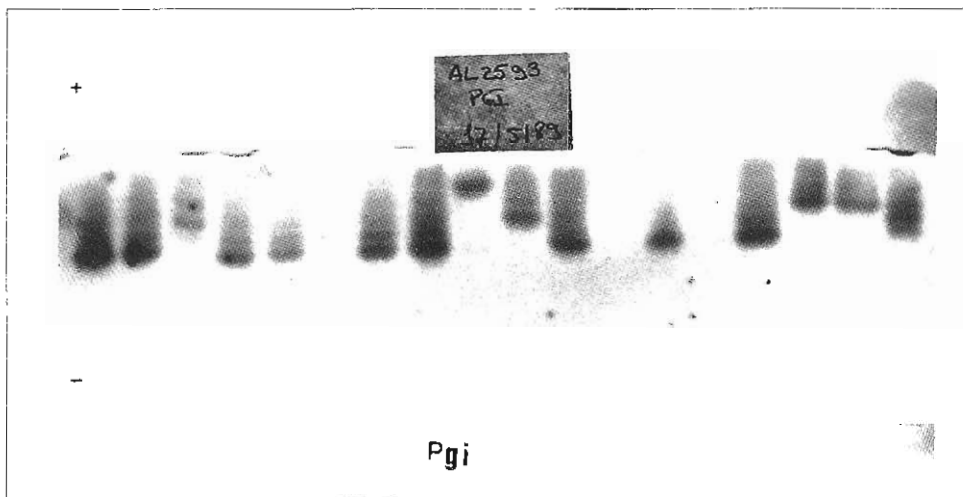


Figure 4 - Phosphoglucose isomerase electrophoresis profile. Lanes 1, 2, 4, 5, 7, 8, 11, 13 and 15 *Pgi* cc; 3, 10, 16 and 17: *Pgi* bb; 9: *Pgi* aa; 18 *Pgi* ac.

occurrence of some alleles exclusively in certain samples (*Aat-1^A* and *Alb^C* in I, *Alb^A* in II and *Glo^C* in III).

The second kind of variation involves the shift from one predominant allele to another (*Glo*, *Pgi*, *Pgm-2*, *Pgm-3*). In this case the main source of variation might also be migration, although selection or genetic drift could not be excluded. However no seasonal fluctuation pattern or any other indication of selection was found.

These allele frequency changes are reflected in the estimates of genetic variation parameters (Table II). Average heterozygosity (*H*) decreased from 0.12 in the first sample to 0.02 in the last one; polymorphism (*P*) was highly correlated with heterozygosity and followed a similar pattern.

An important source of allele frequency changes is variation in population density. The main source of density changes is the annual reproductive cycle of populations. Sample extraction and habitat destruction may also influence density. As Fonseca and Kierulff (1989) have shown for *A. cursor*, captures may approach zero in the rainy season and the peak of population density is found between May and July, in the middle of the dry season. The population turnover of *A. cursor* was found to be very high by these authors, 78% of individuals being recorded during a single trapping session. We do not have data about population density for our samples but a similar pattern would be expected. All of these ecological factors favor the high genetic variation among samples reported in this paper.

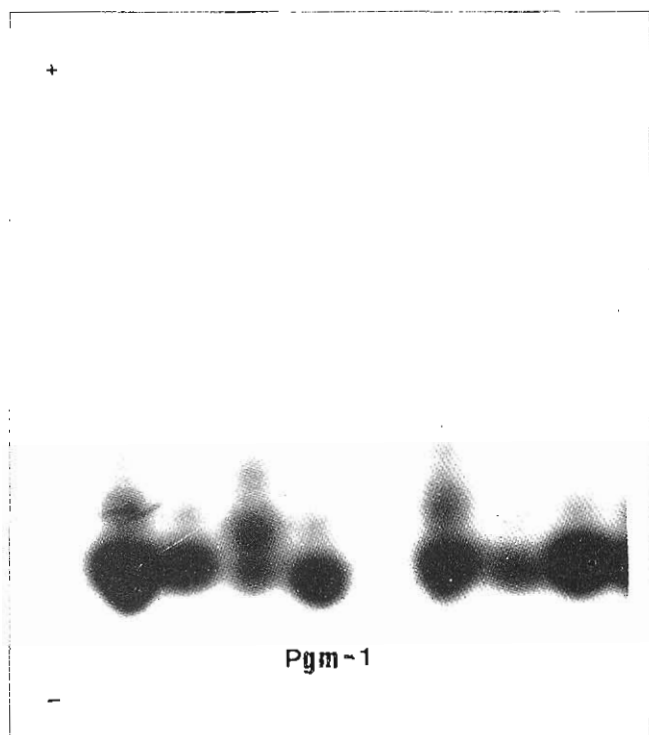


Figure 5 - Phosphoglucomutase-1 electrophoresis patterns. Lanes 1, 2, 4 and 6 to 8: Pgm-1 bb; 3: Pgm-1 bc. (*Pgm-1^c* was not verified in this investigation).

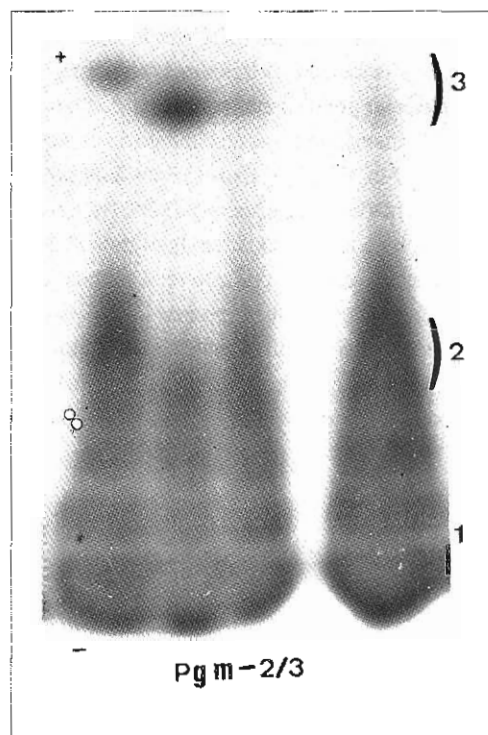


Figure 6 - Phosphoglucomutase-2 and Pgm-3 electrophoresis profiles: Pgm-2: Lane 1: bc; 2: aa; 3: ab. Pgm-3: Lane 1: bb; 2 and 3: aa.



Figure 7 - Superoxide dismutase-1 electrophoresis patterns (negative stain). Lanes 1 to 9 and 12 to 20: Sod-1 aa; 10 and 11: Sod-1 bb.

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RESUMO

A variabilidade genética, em 22 loci proteicos, foi investigada em quatro populações de *Akodon montensis*, obtidas em diferentes épocas, em um mesmo local (Torres, RS, Brasil, 29°20'S; 49°45'W). Quatorze loci mostraram-se monomórficos; os oito restantes apresentaram polimorfismo em pelo menos uma

amostra. Observou-se variação nas frequências gênicas entre populações em todos os locos polimórficos; tais variações foram de dois tipos principais: grandes alterações nas frequências alélicas, bem como aparecimento ou desaparecimento de alelos em algumas amostras (Aat-1, Alb, Glo, Pgi, Pgm-1, Pgm-2, Pgm-3 e Sod-1) e troca de um alelo predominante por outro (Glo, Pgi, Pgm-2, Pgm-3). Observou-se também uma redução no grau de polimorfismo e na heterozigose média entre a primeira e a última amostra. As flutuações nas frequências gênicas podem ser devidas a variações na densidade populacional, deriva genética, seleção e migração ou recolonização do local. Há indicações de ser este último fator bastante importante como causa de alteração nas frequências alélicas.

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