Temporal variation of allele frequencies in populations of *Akodon dolores* (Rodentia, Cricetidae)

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Summary. Six population samples of the South American cricetid rodent *Akodon dolores* were collected at the same site at six-month intervals over a three year period. Changes in density were detected. Seven out of 18 loci analyzed by means of starch gel electrophoresis were polymorphic. Only two of these loci (*Est-4* and *G6pdh*) showed statistically significant variation in allele frequencies following a seasonal pattern. There was no correlation between allele frequencies and population density. When animals were grouped into two classes according to body weight, a clear difference in allele distribution at the *Est-4* and *G6pdh* loci was observed between individuals 39 g or less and those heavier than 39 g. As the first group comprises predominantly younger animals, the data indicate that changes in the age-structure of population, rather than density variations, are responsible for the cyclic pattern of allele frequencies fluctuations.

Introduction

During the three year period 1979–1981, we studied certain aspects of genetic polymorphism in populations of rodents of the *Akodon* genus (Apfelbaum and Blanco 1984, 1985). We found high values for the proportion of polymorphic loci (P) and for the average heterozygosity per locus (H). In addition, we observed temporal variation in allele frequencies at two of the polymorphic loci.

Temporal patterns of variation in protein polymorphisms have been previously detected in rodent populations un-

dergoing cyclic fluctuations in density (Semeonoff and Robertson 1968; Tamarin and Krebs 1969; Gaines and Krebs 1971; Canham and Cameron 1972). The finding of a correlation between changes in allelic frequencies and population density has been interpreted by those authors as evidence for the alternating action of selective forces during population cycles. This interpretation supports Chitty's hypothesis (1960), which proposed that natural selection, together with changes in gene frequencies, are the forces responsible for population density fluctuations.

An alternative view of the same problem is that of Charlesworth and Giesel (1972), who postulated that changes in allele frequencies may be the expression of demographic changes rather than the cause of density variation. Although some data support this hypothesis (Gaines et al. 1978; Mihok et al. 1983), the question has not yet been elucidated.

In order to provide information which may help in understanding temporal genic variation, we present data on population samples of the cricetid rodent *Akodon dolores*.

Materials and methods

Animals

Animals were collected with live-traps set in 100 m long lines of 33 traps each, in a rural area located 60 km Southeast of Córdoba city (Argentina). Six samples, comprising a total of 194 animals, were obtained in the same site at about 6-month intervals: I. May 1979, 42 individuals; II. October 1979, 43 individuals; III. May 1980, 25 individuals; IV. November 1980, 16 individuals; V. May 1981, 50 individuals; VI. November 1981, 18 individuals. Seventy per cent of the animals were captured in an area of xerophytic wood next to a cultivated field and the rest in high grass of road and field borders.

The animals were killed by ether inhalation, weighed and shipped to the laboratory frozen in containers with liquid air.

Tissue homogenates, electrophoresis and staining

Extracts of liver, kidney and heart, electrophoresis and specific staining were performed as indicated by Apfelbaum and

Table 1. Allele frequencies of polymorphic loci in samples of *Akodon dolores*

Locus	Alleles	Samples					
		I May 79	II Oct 79	III May 80	IV Nov 80	V May 81	VI Nov 81
Est-3	a	0.24	0.25	0.22	0.29	0.35	0.19
	b	0.62	0.50	0.68	0.45	0.47	0.62
	c	0.14	0.25	0.10	0.26	0.18	0.19
Est-4[a]	a	0.27	0.14	0.26	0.08	0.23	0.11
	b	0.64	0.48	0.54	0.61	0.59	0.53
	c	0.09	0.38	0.20	0.31	0.18	0.36
Est-5	a	0.11	0.23	0.08	0.17	0.15	0.06
	b	0.57	0.45	0.70	0.50	0.54	0.70
	c	0.32	0.32	0.22	0.33	0.31	0.24
G6pdh[b]	a	0.18	0.56	0.26	0.42	0.35	0.42
	b	0.82	0.44	0.74	0.58	0.65	0.58
H6pdh	a	0.81	0.74	1.00	1.00	1.00	1.00
	b	0.19	0.26	0	0	0	0
Acp-2	a	0.32	0.37	not	0.19	not	0.30
	b	0.68	0.63	scored	0.81	scored	0.70
Hb	a	0.26	0.21	0.20	0.10	0.12	0
	b	0.74	0.79	0.80	0.90	0.88	1.00

[a] Comparison of alleles *a*, *b* and *c*: G=23.32; d.f. 10; $P<0.005$
[b] Comparison of alleles *a* and *b*: G=21.44; d.f. 5; $P<0.001$
Differences in all other loci are non-significant

Blanco (1984). The following loci were studied: soluble esterases (*Est-3*, *Est-4* and *Est-5*), glucose-6-phosphate (*G6pdh*), hexose-6-phosphate (*H6pdh*), malate (*Mdh-1* and *Mdh-2*) and lactate (*Ldh-1* and *Ldh-2*) dehydrogenases, acid phosphatases (*Acp-2* and *Acp-3*), malic enzyme (*Mod-1*), aspartate aminotransferases (*Aat-1* and *Aat-2*) and hemoglobin (*Hb*).

Calculations

Genotype distribution and allele frequencies were estimated by means of Levene's formula (1949) for small samples. Sokal's homogeneity G test was used to compare allele frequency values.

Results

Temporal changes of allele frequencies

Seven out of the 18 loci analyzed were polymorphic: *Est-3*, *Est-4*, *Est-5*, *G6pdh*, *H6pdh*, *Acp-2* and *Hb*. Allele frequencies for these loci in all samples are shown in Table 1. Only two of the polymorphic loci (*Est-4* and *G6pdh*) show statistically significant variation in allele frequencies over the period of this study. For *Est-4*, the frequency of the most common allele (*b*) was rather stable, while those of alleles *a* and *c* showed a seasonal pattern of fluctuations. The frequency of allele *a* was always higher in the autumn (May) than in the spring (October–November); the opposite is true for allele *c*. At the *G6pdh* locus, alleles *a* and *b* also showed sig-

nificant variation following a cyclic pattern which resembles that for *Est-4 a* and *c* alleles.

Population density

Population density was estimated by the number of animals captured per 100 traps/day (Table 2). Highest density was recorded in 1979, and values fell in the following years. However, within each year the values for autumn were always higher than those for spring.

No correlation could be detected between density values and allele frequencies, but if autumn/spring ratios for density and for allele frequencies are analyzed, the correlation is good for $Est-4_c$ (r=0.998).

Table 2. Population density of *Akodon dolores*

Sample	Density[a]	$\frac{\text{Autumn}}{\text{Spring}}$ ratio
I. May 1979	7.071	
II. Oct. 1979	3.030	2.33
III. May 1980	1.263	
IV. Nov 1980	0.299	4.22
V. May 1981	2.778	
VI. Nov. 1981	0.789	3.52

[a] Values indicate number of animals captured per 100 traps/day

There was no such correlation for the other changing frequencies.

Body-weight of animals

Animals have been grouped into two categories according to corporal weight: Class I, comprising individuals of 39 g or less. The smallest specimen was collected in October 1979 and weighed 11 g, and class II, corresponding to animals of more than 39 g (Table 3). It is evident that smaller animals are more abundant in samples obtained in autumn, while heavier specimens predominate in the spring samples.

If the categories are analyzed separately, allele frequencies are similar for all samples within each class; no temporal differences are detected. This similarity allows pooling of all animals from the six samples belonging to the same category. Allele frequencies for *Est-4* and *G6pdh* loci in class I are significantly different from those in class II (Table 4). Alleles $Est-4_c$ and *G6pdh* are more frequently found in class I individuals.

No differences in allele frequencies between sexes could be detected. No significant differences in allele frequencies were observed among animals captured in different lines within a sample as to suggest possible spatial subdivisions.

Table 3. Distribution of animals according to body weight

	Samples					
	I	II	III	IV	V	VI
	May 79	Oct 79	May 80	Nov 80	May 81	Nov 80
Class I[a]	83.72[c]	28.57	84.0	21.43	86.0	38.89
Class II[b]	16.28	71.43	16.0	78.57	14.0	61.11

[a] Animals weighing 39 g or less
[b] Animals heavier than 39 g
[c] Values indicate percentage, taking as 100% the total number of animals in each sample

Table 4. Allele frequencies at the *Est-4* and *G6pdh* loci for animals from all samples grouped according to body weight

	Est-4[c]			*G6pdh*[d]	
	a	b	c	a	b
Class I[a]	0.24	0.58	0.18	0.28	0.72
Class II[b]	0.14	0.55	0.31	0.43	0.57

[a] Animals weighing 39 g or less
[b] Animals heavier than 39 g
[c] For alleles of *Est-4*: G = 8.52, d.f. 2, $P < 0.05$
[d] For alleles of *G6pdh*: G = 7.0, d.f. 1, $P < 0.05$

Discussion

In six population samples of the cricetid rodent *Akodon dolores* collected at the same site at six-month intervals over a three-year period, analysis of allele frequencies for polymorphic proteins showed that only two out of the seven polymorphic loci investigated present statistically significant variations following a seasonal pattern.

Similar temporal fluctuations have been reported in other species (Semeonoff and Robertson 1968; Krebs 1969; Gaines and Krebs 1971; Gaines et al. 1978; Mihok et al. 1983). In studies of one or two loci in populations of the *Microtus* genus, a good correlation between changes in population density and in allele frequencies was found. Although these findings could be considered as evidence supporting the behavioral-genetic hypothesis advanced by Chitty in 1960, no clear demonstration of selective advantages of one genotype over the other/s in different stages of the population cycle has been presented.

We have observed marked variations in the density of *A. dolores* populations during the period covered by the study. In populations of *A. azarae*, another species from the same genus inhabiting a vast region to the southeast of that sampled here, Crespo et al. (1970) found variations in density similar to those of *A. dolores*: maximal values are attained from April to June each year. Although our values are higher in the autumn than in the spring within each year, differences in absolute values from year to year may reflect the existence of longer population cycles which could be detected if studies were prolonged for longer periods.

We could not demonstrate a direct correlation between density values and allele frequencies even with the relatively large sample of polymorphic loci being explored. Some sort of correlation could be detected only for allele $Est-4_c$, when the relative change within each year (autumn/spring ratios) for density and gene frequency was considered. The lack of correlation between density and gene frequency fluctuations has also been indicated by experiments altering gene frequencies in fenced or field populations of *Microtus* (Gaines et al. 1971; LeDuc and Krebs 1975). Perturbation of allele frequencies by incorporating animals with a particular genotype, did no produce significant alterations of density patterns.

In some cases frequency-dependent selection can be expressed by correlations with other parameters. Soulé (1976) found a positive correlation between mean heterozygosity per locus (H) and population density. Values of H should be higher when density is greater. The action of selective forces could also be expressed by an excess or a deficit of heterozygotes in samples at different stages of the cycle.

Values of H for our samples were high (Apfelbaum and Blanco 1985) and did not show any correlation with density. The distribution of genotypes did not depart significantly from that expected according to the Hardy-Weinberg equilibrium (Apfelbaum and Blanco 1985) in any of the samples. Non-significant excesses or deficits of heterozygotes did not follow a defined trend in relation to seasonal changes.

Charlesworth and Giesel (1972) showed, by means of computer simulation, that temporal variation in gene frequencies of populations with overlapping generations may be only side-effects of demographic changes.

It has been repeatedly observed that density changes also imply modifications in the age-structure of populations. We have appraised the age-structure of the samples of *A. dolores* population by separating the individuals into two groups according to their body-weight. We have used this parameter as a rough estimate, since there are no reliable indexes to assess age in *A. dolores*. Class I, comprising animals of 39 g or less, presumably includes younger individuals, while class II would be mostly adult and old animals. It appears evident that "young" specimens are predominant in the samples taken in the autumn, while spring samples are composed of a higher proportion of adults. This is in agreement with the known reproductive pattern for *A. azarae* (Pearson 1967; Crespo et al. 1970), which presents a population cycle similar to that of *A. dolores*.

There is a clear difference in allele distribution at the *Est-4* and *G6pdh* loci in the two weight-classes, which can explain the seasonal gene fluctuations. For those two loci, changes in the age-structure of populations, rather than density variations, are responsible for the cyclic pattern of modifications observed in allele frequencies. This result supports the Charlesworth and Giesel assumptions. However, if changes in genetic frequencies were just a by-product of density fluctuations, one would expect a larger proportion of loci showing cyclic variations. Instead, our data, as well as those of Gaines et al. (1978) and of Mihok et al. (1983), show that only a reduced proportion of loci present those changes.

The observations presented here indicate that individuals carrying allele *Est-4$_c$* or *G6pdh$_b$* live longer than those with *Est-4$_a$* or *G6pdh$_a$*, respectively. On the other hand, *Est-4$_a$* and *G6pdh$_a$*, or some gene/s linked to them, must afford reproductive advantages, since they predominate in young individuals. This phenomenon is of interest and deserves further investigation.

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