Journal of Mammalogy, 87(5):848–854, 2006

TEMPORAL FLUCTUATION OF EFFECTIVE SIZE IN POPULATIONS OF *CALOMYS MUSCULINUS* (MURIDAE: SIGMODONTINAE)

MARINA B. CHIAPPERO,* BEATRIZ A. GARCÍA, GLADYS E. CALDERÓN, AND CRISTINA N. GARDENAL

Cátedra de Genética de Poblaciones y Evolución, FCEFyN, Universidad Nacional de Córdoba, Córdoba, Argentina (MBC, CNG)
Cátedra de Bioquímica y Biología Molecular, FCM, Universidad Nacional de Córdoba, Córdoba, Argentina (BAG)
Instituto Nacional de Enfermedades Virales Humanas ''Dr Julio I. Maiztegui,'' Pergamino, Argentina (GEC)

Calomys musculinus is the natural reservoir of Junin virus, the etiological agent of Argentine hemorrhagic fever. In this paper we measure the effective size of 2 populations of the rodent over a 2-year period. Twenty enzyme-coding loci were analyzed using vertical starch gel electrophoresis. Effective population sizes (N_es) were estimated by the pseudolikelihood method in 2 populations 280 km apart in central Argentina. Both populations experienced marked seasonal changes in relative density and in N_e (between 19.8 and infinity). Changes in percentage of polymorphic loci and mean number of alleles per locus were statistically significant and were roughly correlated with density and N_e. Observed changes in heterozygosity, in contrast, were not significant. After low-density periods, mixing of surviving individuals coming from different demes may play an important role in the maintenance of variability and recovery of N_e in populations of *C. musculinus*.

Key words: Argentine hemorrhagic fever, *Calomys musculinus*, effective population size, heterozygosity, relative density

Calomys musculinus (Muridae, Sigmodontinae) is one of the most abundant native rodent species in agroecosystems in central Argentina. The study of the ecology and genetics of this species is significant because of its role as the reservoir of Junin virus, the etiological agent of Argentine hemorrhagic fever. This serious endemic disease affects mostly farm workers in a large part of central Argentina, where most of the agricultural and cattle production activities of the country are concentrated (Sabattini and Contigiani 1982; Sabattini et al. 1977).

Previous studies in our laboratory found remarkably high levels of allozyme genetic variability and many alleles maintained in frequencies between 0.01 and 0.05 in natural populations of *C. musculinus* throughout central Argentina (Chiappero et al. 2002; Gardenal and Blanco 1985); levels of polymorphism were even higher when random amplified polymorphic DNAs were used as genetic markers (Chiappero and Gardenal 2003). Lack of correlation between pairwise genetic and geographic distances at a macrogeographic scale (>10 km) and the phylogeographic pattern observed indicate a relatively recent range expansion with current moderate to low levels of gene flow. These results suggest that *C. musculinus* maintains population sizes large enough to increase the time needed to reach a migration–drift equilibrium and to avoid the loss of genetic variability due to genetic drift (Chiappero and Gardenal 2003; Chiappero et al. 2002; González Ittig and Gardenal 2004).

Populations of *C. musculinus* undergo seasonal fluctuations in density as measured by trapping success. Minimum density, including zero values, occurs around the end of winter and the beginning of spring (August–September), whereas maximum density is reached in autumn (May–June). These variations could be due, in part, to weather conditions that affect the availability of food and refuge, and in part to the particular ecological characteristics of agroecosystems, which are highly unstable habitats that offer adequate conditions for rodents only during certain periods of the year. The magnitude of density fluctuations also is variable, not only in space, but also in time. Some years are characterized by relatively low densities, whereas in other years great population explosions occur (Calderón 2004; Crespo et al. 1970; de Villafañe and Bonaventura 1987; de Villafañe et al. 1977; Ellis et al. 1997; Mills et al. 1991). If populations of *C. musculinus* experience large fluctuations in census size as indicated by ecological data, genetic variability should be lost due to the effect of genetic drift.

* *Correspondent: mchiappero@efn.unc.edu.ar*

The magnitude of genetic drift can be quantified by the parameter known as effective size (N_e). Usually, N_e is lower than the census size (N) because various demographic factors such as unequal sex ratio, nonrandom distribution of family size, and fluctuating population size result in loss of genetic variation passed from one generation to the next.

Knowledge of N_e in natural populations is important because of the key role that this parameter plays in the maintenance of genetic variability, which, in turn, provides the basis for natural populations to respond to environmental challenges such as the presence of an infectious agent (Frankham 1996). In *C. musculinus*, infection with Junin virus may have 2 outcomes. Infection with the virus can result in an acute infection, characterized by antibody development and a subsequent clearing of the infection, or it can lead to a chronic carrier state, in which the rodents shed the virus into the environment through saliva, urine, and feces for extended periods. This chronic state is crucial in the transmission of virus to other rodents and to humans. It has been suggested that the course that the infection takes may depend not only on dose and route of infection and virulence of the Junin virus strain but also on the genetics of the host (Childs et al. 1995). In addition, the maintenance and spread of viruses may be facilitated in large populations that do not experience great fluctuations, given that the probability of infected rodents to become in contact with noninfected ones will increase (André and Hochberg 2005).

The purpose of the present study was to estimate the effective size of 2 natural populations of *C. musculinus*, using the pseudolikelihood method of Wang (2001). We investigated the following questions. Do N_e values of *C. musculinus* populations vary along the year as density does? What are the effects of N_e changes on genetic variability levels? Are changes in N_e similar in different spatial and temporal populations?

Materials and Methods

Samples.—We studied 2 populations of *C. musculinus* from Córdoba Province, Argentina, separated by 280 km. The 1st population is located inside the endemic zone of Argentine hemorrhagic fever, near Melo (34°20′S, 64°26′W), in the phytogeographic region called ''Humid Pampa,'' which is characterized by a humid, temperate climate. The original prairies have been replaced almost completely by crop and grazing fields (Cabrera 1976; Crespo et al. 1970). Samples from this population, referred to here as Melo, were taken in February, May, and September 1992, March and August 1993, and March 1994. The 2nd population is located near Manfredi (31°50′S, 63°45′W), outside the endemic zone of Argentine hemorrhagic fever in the phytogeographic zone called ''Espinal,'' which is characterized by small wooded areas alternating with crop and grazing fields, and with most rainfall concentrated in the summer months (Cabrera 1976; Polop and Sabattini 1993). Samples from this population, referred to as Manfredi, were obtained in May, August, and November 1982, January, April, May, August, and November 1983, and March 1984.

Live traps similar to Sherman traps (7.5 × 9.5 × 25 cm; Moller, Buenos Aires, Argentina) were set in cultivated fields and tall weeds of field borders in eight 100-m-long lines of 33 traps each for 3 consecutive nights and were inspected daily. Specimens from Manfredi were sacrificed by ether inhalation; those from Melo by using methoxyflurane. Liver and kidneys were removed and conserved in liquid nitrogen. Field procedures agreed with the guidelines for the capture, handling, and care of mammals of the American Society of Mammalogists (Animal Care and Use Committee 1998). Tissues of animals from Melo were submitted to gamma radiation to inactivate potential viral particles. We have observed that this treatment does not affect the activity and electrophoretic mobility of the enzymes analyzed.

Table 1.—Enzyme and loci analyzed in 2 populations of *Calomys musculinus* from central Argentina. Details for electrophoresis systems are given in footnotes.

Enzyme	Locus	ECN[a]
Aspartate aminotransferase[b]	*Aat-1*	2.6.1.1
	Aat-2	
Alcohol dehydrogenase[b]	*Adh*	1.1.1.1
Malic enzyme[b]	*Me*	1.1.1.40
Esterases[b]	*Est-1*	3.1.1.1
	Est-2	
	Est-4	
	Est-5	
	Est-6	
Acid phosphatase[b]	Acp_l	3.1.3.2
	Acp_k	
Glycerophosphate dehydrogenase[b]	*Gpdh*	1.1.1.8
Lactate dehydrogenase[b]	*Ldh-1*	1.1.1.27
	Ldh-2	
Nicotinamide adenine dinucleotide–linked nonspecific dehydrogenase[b]	*Ndh*	
Catalase[b]	*Cat*	1.11.1.6
Phosphoglucomutase[b]	*Pgm-1*	2.7.5.1
	Pgm-2	
6-Phosphogluconate dehydrogenase[b]	*6-Pgdh*	1.1.1.44
Leucine aminopeptidase[b]	*Lap*	3.4.1.1
Superoxide dismutase[b]	*Sod*	1.15.1.1
Malate dehydrogenase[c]	*Mdh-1*	1.1.1.37
	Mdh-2	
Isocitrate dehydrogenase[c]	*Idh-1*	1.1.1.42
	Idh-2	

[a] Enzyme Commission number.
[b] Tris borate ethylenediaminetetraacetic acid, pH 8.6 (Markert and Faulhaber 1965).
[c] Tris citrate, pH 6.3 (Gardenal and Blanco 1985).

Protein electrophoresis.—Vertical starch gel electrophoresis of 15 enzymatic proteins, which gave information on 25 loci, was performed (Table 1). Details of preparation of homogenates, electrophoresis, and staining to reveal enzyme activity are given by Gardenal et al. (1980) and Gardenal and Blanco (1985). Genetic control of electrophoretic phenotypes in *C. musculinus* and in *Calomys laucha* (a sympatric and closely related species) was demonstrated by García and Gardenal (1989) and Gardenal et al. (1980).

Data analysis.—Allele frequencies, mean number of alleles per locus, mean observed heterozygosity, percentage of polymorphic loci using the 99% and 95% criteria (a locus was polymorphic if the frequency of the most common allele was lower than 99% or 95%, respectively), and conformance to Hardy–Weinberg equilibrium were calculated using the program TFPGA (*Tools for population genetic analyses (TFPGA): a Windows program for the analysis of allozyme and molecular population genetic data,* version 1.3—M. Miller 1998; available at http://www.marksgeneticsoftware.net/tfpga.htm; last accessed 24 May 2006). Differences in mean number of alleles per locus and in mean observed heterozygosity between samples were tested using a Friedman test with the program InfoStat (Grupo InfoStat 2004). Population density of each temporal sample was estimated by

TABLE 2.—Pseudolikelihood effective population size estimates (N_es) for 2 populations of *Calomys musculinus* in Argentina in consecutive temporal samples (t = number of generations between temporal samples; *CI* = confidence interval).

Sample period	t	N_e	95% *CI* for N_e
Manfredi			
May–August 1982	1	∞	44.0–∞
August–November 1982	1	60.8	13.2–∞
November 1982–January 1983	1	20.5	9.6–400.1
January–May 1983	1	47.6	23.8–253.3
May–August 1983	1	45.8	24.6–205.4
August–November 1983	1	116.8	27.8–∞
November 1983–March 1984	1	35.3	11.3–∞
Melo			
February–May 1992	1	18.8	12.5–32.7
May–September 1992	1	28.5	10.1–∞
September 1992–March 1993	2	38.6	16.6–∞
March–August 1993	2	∞	26.6–∞
August 1993–March 1994	2	19.8	7.1–∞

trapping success as: D = [(number of animals)/(number of traps × number of days)] × 100.

Approximate time to sexual maturity in *C. musculinus* is 1 month, and pregnancy lasts 21 days. The maximum lifespan is 6–8 months. Given these data, generation length in this species can be roughly approximated to be a minimum of 2 months and a maximum of 4 months (Buzzio and Castro-Vázquez 2002; de Villafañe and Bonaventura 1987). N_es were estimated for periods comprising 1 generation using the method of Wang (2001), which uses a pseudo–maximum likelihood approach to estimate N_e from the temporal change in allele frequencies at multiallelic loci. This method performs nearly as well as ''full'' likelihood methods (such as that of Williamson and Slatkin [1999]) but is less computationally intensive (Wang 2001). The program NeML (Wang 2001) was used to calculate point estimates of N_e and their 95% confidence intervals (95% *CI*s).

Two tests to detect recent bottlenecks were performed on each temporal sample, using the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). The 1st test is based on the principle that in populations that experienced a recent bottleneck, allele diversity is reduced faster than heterozygosity. Therefore, the expected heterozygosity (H_e) calculated from allele frequencies of the population is higher than the expected equilibrium heterozygosity (H_{eq}), which is computed from the observed number of alleles assuming an infinite allele mutation model and a constant-size (equilibrium) population (Luikart et al. 1998). In nonbottlenecked populations, half of the loci will show $H_e < H_{eq}$ and half $H_e > H_{eq}$, on average. A Wilcoxon signed rank test is used to determine if the population exhibits an overall significant excess of heterozygosity with respect to H_{eq}. The 2nd test examines if the distribution of allele frequencies significantly deviates from an L-shaped distribution (characteristic of populations of approximately constant size) to one with fewer low-frequency alleles (characteristic of bottlenecked populations). This test was not applied to samples consisting of <10 individuals (Luikart et al. 1998). Loci with null alleles were not taken into account for these calculations.

RESULTS

In both populations studied, allele frequencies were determined for 20 of the 25 loci assayed. In the Melo population, loci *Aat-2, Adh, Gpdh, Idh-2,* and *Ndh* were discarded because the zymograms were not clear enough to allow an unequivocal interpretation of phenotypes in all cases. Loci *Lap, Cat, Pgm-1, Pgm-2,* and *6Pgdh* were not analyzed in the Manfredi population for the same reason. Allelic frequencies for polymorphic loci are available from the authors. In Manfredi, a null allele was detected in loci *Est-1* and *Est-6*; therefore, frequencies shown in the appendices are maximum likelihood estimates calculated using the EM algorithm (Dempster et al. 1977) implemented in the program GENEPOP (Raymond and Rousset 2000). Genotypes at all other loci were at Hardy–Weinberg equilibrium in all samples.

Point estimates of N_e and 95% *CI*s are shown in Table 2, and N_e and density estimates are represented graphically in Fig. 1. Both populations showed marked temporal changes in N_e. In Manfredi, N_e varied between 20.5 and infinity, whereas in Melo, it ranged from 18.8 to infinity. Upper limit of the 95% *CI*s was infinity for 4 of 7 N_e estimates in Manfredi, and for 4 of 5 estimates in Melo. In both populations, point estimates of infinite N_e values were obtained in autumn months, in which maximum densities usually occur. In both localities, N_e

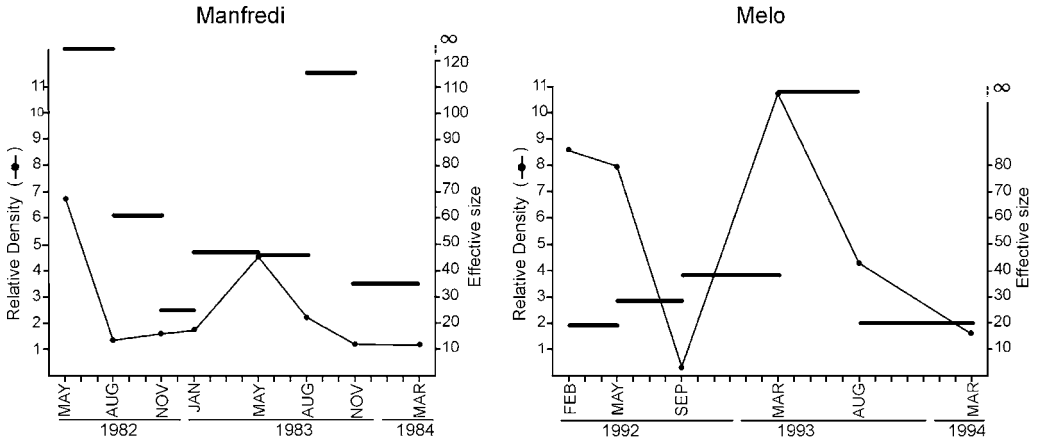

FIG. 1.—Relative density estimated as trapping success, shown as thin curve, and effective population size (N_e), shown as thick horizontal line, in temporal samples of *Calomys musculinus* from 2 populations (Manfredi and Melo) from central Argentina.

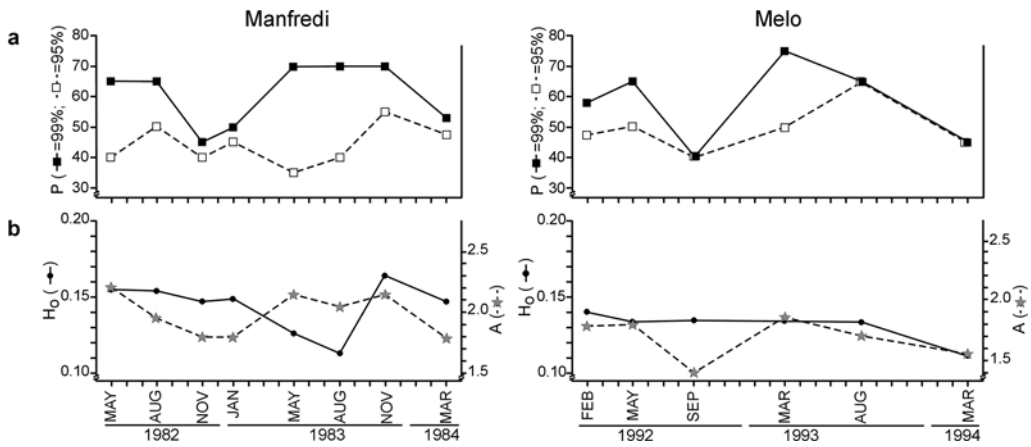

FIG. 2.—Genetic variability in temporal samples of *Calomys musculinus* from 2 populations (Manfredi and Melo) from central Argentina. a) Percentage of polymorphic loci (P) using the 99% and 95% criteria (a locus was polymorphic if the frequency of the most common allele was lower than 99% or 95%, respectively); b) mean observed heterozygosity (H_o) and mean number of alleles per locus (A).

followed approximately the density curve, with 2 exceptions: between August and November 1983 in Manfredi, density decreased but N_e increased to 116.8; and in Melo, density between February and May 1992 was very high but N_e was low.

Mean number of alleles per locus, percentage of polymorphic loci, and mean observed heterozygosities are shown in Table 3 and represented graphically in Figs. 2a and 2b. Mean observed heterozygosities did not differ significantly among temporal samples in either populations (Friedman test: $P = 0.34$ for Manfredi, $P = 0.93$ for Melo). Mean number of alleles per locus varied significantly in the period studied in both populations (Friedman test: $P = 0.021$ for Manfredi, $P = 0.010$ for Melo; Fig. 2b), as did the proportion of polymorphic loci. As the percentage of polymorphic loci decreased the range of values between 99% and 95% narrowed, as is expected when lost alleles are primarily those of low frequency (Chiappero et al. 2002; Fig. 2b). Decreases in percentage of polymorphic loci and in mean number of alleles per locus are coincident with periods of low density and low N_e in both populations (Fig. 1).

All samples exhibited an L-shaped distribution of allele frequencies and none of them showed a significant excess of heterozygosity; therefore, there was no evidence of a recent bottleneck. On the contrary, some samples presented a significant deficiency of heterozygosity, indicating a population expansion, recent immigration, or population substructure (Table 4) (Cornuet and Luikart 1996; Luikart et al. 1998). The possibility of a Wahlund effect can be discarded because all loci are in Hardy–Weinberg equilibrium.

TABLE 3.—Mean number of alleles per locus, mean observed heterozygosity (H_o), and proportion of loci that were polymorphic in temporal samples of 2 populations of *Calomys musculinus*.

Sample period	Mean no. alleles per locus	H_o	Proportion of polymorphic loci	
			95% criterion	99% criterion
Manfredi				
May 1982	2.20	0.155	65.0	40.0
August 1982	1.95	0.154	65.0	50.0
November 1982	1.80	0.147	45.0	40.0
January 1983	1.80	0.149	50.0	45.0
May 1983	2.15	0.126	70.0	35.0
August 1983	2.05	0.113	70.0	40.0
November 1983	2.15	0.164	70.0	55.0
March 1984	1.79	0.147	52.6	47.4
Melo				
February 1992	1.79	0.14	57.9	47.4
May 1992	1.80	0.133	65.0	50.0
September 1992	1.40	0.135	40.0	40.0
March 1993	1.85	0.134	75.0	50.0
August 1993	1.70	0.133	65.0	65.0
March 1994	1.55	0.121	45.0	45.0

DISCUSSION

The effective population size parameter (N_e) is crucial to understanding the degree of influence that genetic drift has on

TABLE 4.—Probabilities for heterozygosity deficiency or excess obtained from the Wilcoxon signed rank test in temporal samples of *Calomys musculinus* in Argentina.

Sample period	Probability		Status of population
	Heterozygote deficiency	Heterozygote excess	
Manfredi			
May 1982	0.002	0.998	Expanding
August 1982	0.001	0.999	Expanding
November 1982	0.054	0.96	Stable
January 1983	0.234	0.469	Stable
April 1983	0.095	0.916	Stable
May 1983	0.001	0.999	Expanding
August 1983	0.007	0.995	Expanding
November 1983	0.001	0.999	Expanding
March 1984	0.006	0.996	Expanding
Melo			
February 1992	0.485	0.545	Stable
May 1992	0.084	0.927	Stable
September 1992	0.68	0.371	Stable
March 1993	0.036	0.968	Expanding
August 1993	0.002	0.998	Expanding
March 1994	0.285	0.752	Stable

the levels of genetic variability maintained in a population. In previous papers, we found remarkably high levels of genetic variability and an important proportion of alleles maintained in low frequencies in natural populations of *C. musculinus* (Chiappero and Gardenal 2003; Chiappero et al. 2002; Gonzalez Ittig and Gardenal 2004). These observations posed the question of whether the populations do experience great fluctuations in N_e, despite the large seasonal variations in density indicated by trapping success (Crespo 1966; Crespo et al. 1970; de Villafañe and Bonaventura 1987; Mills et al. 1991).

In this paper, we found that 2 populations separated by 280 km, from different phytogeographic regions and sampled 10 years apart, have similar patterns in temporal fluctuations in N_e (Fig. 1). In most time intervals, N_e and genetic variability measured as percentage of polymorphic loci and mean number of alleles per locus decreased after a decrease in population relative density, and recovered when density increased. Observed changes in heterozygosity, in contrast, were not significant (Fig. 2).

The relationship between N_e and different measures of population genetic variability has been treated by several authors. In experimentally bottlenecked populations of mosquitofish, Leberg (1992) demonstrated that percentage of polymorphic loci and mean number of alleles per locus were better indicators of a reduction in N_e than was heterozygosity. Nei et al. (1975) established by simulation experiments that the amount of reduction in average heterozygosity depends not only on the magnitude of reduction in N_e, but also on the rate of population growth after it. If the population recovers quickly, the reduction in H may be small, even with an extreme bottleneck. *C. musculinus* is characterized by a high reproductive rate, determined by a large litter size, sexual maturity at an early age, long reproductive season, and high frequency of postpartum estrus (Crespo et al. 1970; de Villafañe et al. 1977), and a promiscuous mating system (Laconi and Castro-Vazquez 1999). These characteristics would allow a rapid recovery of populations after a decrease, thereby diminishing the effects of population crashes on the decrease of heterozygosity. However, given the few generations that comprise this study, the fast growth of populations of *C. musculinus* only explains the low impact of drift on H, and not the recovery of percentage of polymorphic loci and mean number of alleles per locus.

Calomys musculinus is an opportunistic species, inhabiting a wide variety of habitats in Argentina. In the Humid Pampa, it occupies the weedy borders of roads and field borders throughout the year and periodically colonizes cultivated fields when the crops have grown enough to offer good cover (Ellis et al. 1997). When the fields are harvested or plowed, individuals leave them and take refuge in the borders. If the recolonization of cultivated fields takes place with a mixture of individuals from diverse origins, this may have the effect of small-scale migration and may explain the recovery of alleles shown by the values of percentage of polymorphic loci and mean number of alleles per locus. It could also explain the high heterozygosity values and the deficiency of heterozygosity with respect to that expected under mutation–drift equilibrium, shown by the bottleneck test, despite the small N_e that the populations maintain during unfavorable periods.

Similar results were reported in other rodent species. Matocq (2004) found that in a population of the big-eared woodrat (*Neotoma macrotis*), N_e was 2 orders of magnitude higher than census size, which was attributable to a large neighborhood size, that is, more individuals contribute to the maintenance of genetic variability than those included in the study. Zenger et al. (2003) did not find reduced genetic variability in introduced populations of the European rabbit (*Oryctolagus cuniculus*) in Australia, compared to European populations; rabbits would have experienced a rapid population expansion at the time of their establishment in Australia. Matocq et al. (2000) estimated the genetic structure in *Proechimys steerei*, an Amazonian small rodent inhabiting a region that experiences seasonal floodings that produce significant decreases in population size every year. These authors did not detect any reduction in mitochondrial haplotype diversity; indeed, it was higher in those downriver areas that experience more extensive and annual floods, compared to upriver areas where inundations were rare. Genetic diversity downriver would be maintained by rapid population expansions after flooding and an intermixing of individuals from different populations that survived in more elevated areas and recolonized lowlands.

One of the characteristics of Argentine hemorrhagic fever is its changing incidence. When the disease appears in a new area it remains high for 5–10 years and then declines gradually or disappears (Maiztegui et al. 1986). Studies by Calderón (2004) between 1991 and 1994 showed that the infection in rodent host populations is often focal and varies in both time and space. In some localities Junin virus reduced its prevalence, in others it was maintained, and in yet others the virus became extinct. Minimum density of *C. musculinus* occurs by the end of winter (August–September) and sometimes reaches zero values. In this study, N_e values during winter were low, but not zero. Although this would imply fewer infected individuals in the population and also less opportunity to infect a new host, there is still a probability of transmission throughout winter. The repetition of this phenomenon every year would cause the random extinction of Junin virus in some demes.

In conclusion, populations of *C. musculinus* experience fluctuations in density during the year, which are accompanied by fluctuations in N_e. This was verified for 2 populations situated in different phytogeographic regions and with sampling periods separated by 10 years. Heterozygosity levels did not change significantly; on the contrary, percentage of polymorphic loci and mean number of alleles per locus dropped significantly when N_e decreased and recovered when N_e increased. Mixing of surviving individuals would play an important role in the maintenance of genetic variability and recovery of N_e in populations of *C. musculinus* after population decreases.

Resumen

Calomys musculinus es el reservorio natural del virus Junin, el agente etiológico de la fiebre hemorrágica Argentina. En el

presente trabajo se estima el tamaño efectivo de 2 poblaciones del roedor a lo largo de un período de 2 años. Se analizaron veintidós loci que codifican para enzimas por medio de electroforesis vertical en gel de almidón. Los tamaños efectivos (N_e) de 2 poblaciones del centro de Argentina (separadas por 280 km) se estimaron utilizando el método de pseudo-probabilidad. Ambas poblaciones experimentaron cambios notables de densidad relativa y N_e (entre 19.8 e infinito). El porcentaje de loci polimórficos y el nùmero medio de alelos por locus mostraron cambios estadísticamente significativos, concordantes con los cambios de densidad y de N_e. Por el contrario, los cambios observados en los niveles de heterocigosis no fueron significativos. Después de los períodos de baja densidad, la mezcla de individuos procedentes de diferentes demos puede tener un papel importante en el mantenimiento de la variabilidad y en la recuperación de N_e en las poblaciones de *C. musculinus*.

Acknowledgments

We thank H. López, D. Olivera, G. O'Duyer, C. Polidoro, and V. Vega for their assistance in fieldwork, and Drs. A. Blanco and M. Sabattini for their useful comments and suggestions on the manuscript. This work was supported in part by grants from Consejo Nacional de Investigaciones Científicas y Técnicas, the Agencia Córdoba Ciencia, and the Secretaría de Ciencia y Tecnología (Universidad Nacional de Córdoba) of Argentina.

Literature Cited

André, J. B., and M. E. Hochberg. 2005. Virulence evolution in emerging infectious diseases. Evolution 59:1406–1412.

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Buzzio, O. L., and A. Castro-Vázquez. 2002. Reproductive biology of the corn mouse, *Calomys musculinus*, a neotropical sigmodontine. Journal of Neotropical Mammalogy 9:135–158.

Cabrera, A. L. 1976. Territorios fitogeográficos de la Repùblica Argentina. Acme, Buenos Aires, Argentina.

Calderón, D. E. 2004. Desarrollo de indicadores de riesgo de contraer la FHA por medio del estudio de los roedores que actuán como reservorio de los arenavirus en Argentina. Ph.D. dissertation, Universidad Nacional del Litoral, Santa Fe, Argentina.

Chiappero, M. B., and C. N. Gardenal. 2003. Restricted gene flow in *Calomys musculinus* (Rodentia, Muridae), the natural reservoir of Junin virus. Journal of Heredity 94:490–495.

Chiappero, M. B., M. S. Sabattini, A. Blanco, G. E. Calderón, and C. N. Gardenal. 2002. Gene flow among *Calomys musculinus* (Rodentia, Muridae) populations in central Argentina. Genetica 114:63–72.

Childs, J. E., J. N. Mills, and G. E. Glass. 1995. Rodent-borne hemorrhagic fever viruses: a special risk for mammalogists? Journal of Mammalogy 76:664–680.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Crespo, J. A. 1966. Ecología de una comunidad de roedores silvestres en el partido de Rojas, provincia de Buenos Aires. Revista del Museo Argentino de Ciencias Naturales e Instituto Nacional de Investigación en Ciencias Naturales, Ecología 1:79–134.

Crespo, J. A., M. S. Sabattini, M. J. Piantanida, and G. de Villafañe. 1970. Estudios ecológicos sobre roedores silvestres. Observaciones sobre densidad, reproducción y estructura de comunidades de roedores en el sur de Córdoba. Ministerio de Bienestar Social, Secretaría de Estado de Salud Pùblica, Buenos Aires, Argentina.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the *EM* algorithm. Journal of the Royal Statistical Society, Series B (Statistical Methodology) 34:1–38.

de Villafañe, G., and S. M. Bonaventura. 1987. Ecological studies in crop fields of the endemic area of Argentine hemorrhagic fever. *Calomys musculinus* movements in relation to habitat and abundance. Mammalia 51:233–248.

de Villafañe, G., et al. 1977. Dinámica de las comunidades de roedores en agroecosistemas pampásicos. Medicina (Buenos Aires) 37, suplemento 3:128–140.

Ellis, B. A., et al. 1997. Structure and floristics of habitats associated with five rodent species in an agroecosystem in central Argentina. Journal of Zoology (London) 243:437–460.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

García, B. A., and C. N. Gardenal. 1989. Enzyme polymorphism and inheritance of allozymic variants in *Calomys laucha* (Rodentia, Cricetidae). Comunicaciones Biológicas 8:1–10.

Gardenal, C. N., and A. Blanco. 1985. Polimorfismo enzimático en *Calomys musculinus*: nueva estimación. Mendeliana 7:3–12.

Gardenal, C. N., M. S. Sabattini, and A. Blanco. 1980. Enzyme polymorphism in a population of *Calomys musculinus* (Rodentia, Cricetidae). Biochemical Genetics 18:563–575.

González Ittig, R. E., and C. N. Gardenal. 2004. Recent range expansion and low levels of contemporary gene flow in *Calomys musculinus*: its relationship with the emergence and spread of Argentine haemorrhagic fever. Heredity 93:535–541.

Grupo InfoStat. 2004. InfoStat versión 1.1. Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina.

Laconi, M. R., and A. Castro-Vazquez. 1999. Nest building and parental behaviour in two species of *Calomys* (Muridae, Sigmodontinae): a laboratory study. Mammalia 63:11–20.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46: 477–494.

Luikart G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequencies provides a test for recent population bottlenecks. Journal of Heredity 89:239–247.

Maiztegui, J. I., M. R. Feuillade, and A. M. Briggiler. 1986. Progressive extension of the endemic area and changing incidence of Argentine hemorrhagic fever. Medical Microbiology and Immunology 175:149–152.

Markert, C. L., and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. Journal of Experimental Zoology 159:319.

Matocq, M. D. 2004. Reproductive success and effective population size in woodrats (*Neotoma macrotis*). Molecular Ecology 13:1635–1612.

Matocq, M. D., J. L. Patton, and M. N. F. da Silva. Population genetic structure of two ecologically distinct Amazonian spiny rats: separating history and current ecology. Evolution 54:1423–1432.

MILLS, J. N., B. A. ELLIS, K. T. MCKEE, J. I. MAIZTEGUI, AND J. E. CHILDS. 1991. Habitat associations and relative densities of rodent populations in cultivated areas of central Argentina. Journal of Mammalogy 72:470–479.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

POLOP, J. J., AND M. S. SABATTINI. 1993. Rodent abundance and distribution in habitats of agrocenosis in Argentina. Studies of Neotropical Fauna and Environment 1:39–46.

RAYMOND, M., AND F. ROUSSET. 2003. GENEPOP 3.4, updated version of: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249 (1995).

SABATTINI, M. S., AND M. S. CONTIGIANI. 1982. Ecological factors influencing the maintenance of arenaviruses in nature with special reference to the agents of Argentinean hemorrhagic fever. Pp. 251–262 in International Symposium on Tropical Arenaviruses and Hemorrhagic Fevers (F. P. de Pinhiero, ed.). Academia Brasileira de Ciencias, Rio de Janeiro, Brasil.

SABATTINI, M. S., L. E. GONZÁLEZ DE RÍOS, G. DÍAZ, AND V. R. VEGA. 1977. Infección natural y experimental de roedores con virus Junin. Medicina (Buenos Aires) 37:149–161.

WANG, J. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. Genetical Research 78:243–257.

WILLIAMSON, E. G., AND M. SLATKIN. 1999. Using maximum likelihood to estimate population size from temporal changes in allele frecuencies. Genetics 152:755–761.

ZENGER, K. R., B. J. RICHARDSON, AND A. M. VACHOT-GRIFFIN. 2003. A rapid population expansion retains genetic diversity within European rabbits in Australia. Molecular Ecology 12:789–794.

Submitted 25 November 2005. Accepted 6 March 2006.

Associate Editor was Eric A. Rickart.