Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Temporal fine-scale genetic variation in the zoonosis-carrying long-tailed pygmy rice rat in Patagonia, Argentina

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Keywords

Oligoryzomys longicaudatus; temporal genetic structure; Argentinean Patagonia; microsatellites; Hantavirus Pulmonary Syndrome; migration; population genetics; zoonosis.

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Editor: Jean-Nicolas Volff

Received 18 April 2013; revised 10 February 2015; accepted 16 February 2015

doi:10.1111/jzo.12238

Abstract

Factors modeling the population genetic structure are of major importance when species involved in the transmission of zoonoses are considered. The long-tailed pygmy rice rat or 'colilargo' *Oligoryzomys longicaudatus* is a highly vagile rodent species which acts as the reservoir of the Andes hantavirus in southern Argentina and Chile. To contribute to the knowledge of the processes determining the microgeographical genetic structure of populations of this species, we used 10 microsatellite loci to estimate the levels of polymorphism, individual relatedness and the population effective sizes through time and to explore if the effective size correlated with density. Individuals were sampled seasonally during a 25-month period in a population from the Argentinean Patagonia located in the endemic area of Hantavirus Pulmonary Syndrome. We detected three genetic clusters. An important temporal change in cluster prevalence was detected after a population bottleneck. Individuals of the same period presented higher mean relatedness values than between consecutive periods. Density and effective population size estimations were correlated. All analyses performed in this study are in line with the conclusion that high levels of gene flow encompassing different habitats would be a major process producing fine-scale temporal changes in the genetic composition of the studied population.

Introduction

The non-random distribution of genetic variation in space and time, known as the genetic structure of a population, has received particular attention of ecologists interested in the study of population dynamics. Most experimental studies concerning spatial genetic variability among populations are based on samples collected in one time period and results are often interpreted assuming that population structure and genetic diversity are stable over time (Eckert, Samis & Lougheed, 2008). However, temporal restrictions to gene flow can also affect the population structure. Small mammals with temporal and spatial variability in parameters such as density, dispersal and space use provide a good opportunity to investigate the relative impact of these demographic characteristics on the dynamics of temporal variation in genetic diversity. In addition, in species acting as reservoirs of etiological agents of human diseases, the knowledge of the processes shaping the distribution of allele frequencies in space and time is crucial to predict the spread and persistence of zoonoses (Guivier *et al.*, 2011).

In this work, we focus on the temporal genetic structure of the rodent *Oligoryzomys longicaudatus* (long-tailed pygmy rice rat or 'colilargo'; family Cricetidae, subfamily Sigmodontinae). This species is the reservoir of the Andes hantavirus (Levis *et al.*, 1998), the etiological agent of the Hantavirus Pulmonary Syndrome (HPS) disease. It is common in temperate *Nothofagus* forests and bushy areas of the eastern and western slopes of the Andes mountains in the Patagonia (Carbajo & Pardiñas, 2007; Palma *et al.*, 2012). The genetic structure of *O. longicaudatus* has been studied at a macrogeographic scale in Chile (Palma *et al.*,

2012) and Argentina (González-Ittig *et al.*, 2010). Besides, Torres-Pérez *et al.* (2011) explored the co-divergence between *O. longicaudatus* and Andes virus in Chile at a macro-geographic scale and found a different phylogeographic history in both virus and host. However, these studies are not focused in the analysis of the dynamics of the virus–host system involved in the HPS disease at a local and short-term scale.

Several studies have reported that *O. longicaudatus* presents high vagility, as inferred from the low success in the recapture of marked specimens and that populations undergo fluctuations in density showing predictable increases during autumn or winter (April–June) and sharp declines mainly in summer (December–February) (Contreras, 1972; Pearson, 1983; Murúa, González & Meserve, 1986; Polop *et al.*, 2010). The reproductive period extends from September (spring) to March (late summer); juveniles (with *c.* 1 month old) are promptly incorporated into the breeding population. The Andes virus is maintained in nature by transmission among rodents mainly through social grooming among adult males and is transmitted via saliva or aerosols rather than feces, urine and wounds (Padula *et al.*, 2004). In periods of population growth, *O. longicaudatus* invades human settlements, increasing the probability of human infection with Andes virus (Calderón *et al.*, 1999). In addition, this species experience occasional explosive population irruptions over short periods of time (locally known as 'ratadas'), triggered by exceptionally warm winters and rainfall peaks such as those detected during El Niño events (Jaksic & Lima, 2003; Murúa, González & Lima, 2003) or massive flowering of native bamboo plants (*Chusquea quila*, *C. coleou* and *C. valdiviensis*) (Gallardo & Mercado, 1999). Boric-Bargetto *et al.* (2012) studied the genetic composition of a population in Chile before, during and after an outbreak, revealing important differences in mtDNA haplotype and nucleotide composition between each temporal phase. The authors proposed that gene flow would be the main mechanism underlying changes at the local scale, which is in line with the high vagility detected in the species.

In previous works in Cholila (Andean region of Argentinean Patagonia, where several cases of HPS occur yearly), Polop *et al.* (2010) and Polop (2011) described the temporal and spatial dynamics of the Andes virus/*O. longicaudatus* system to find ecological and demographic features associated with infection. The authors detected significant changes in the relative population density and in the number of antibody-positive animals in different seasons and habitats. They observed a delayed density peak in shrublands with respect to forest, suggesting seasonal movements of *O. longicaudatus* between these habitats in search of better microhabitat conditions and resources. To provide insight about the processes causing the observed patterns of population structure at a microgeographical scale, we present here a fine-scale temporal genetic structure analysis of *O. longicaudatus* from Cholila using microsatellite allelic data. We estimate levels of polymorphism and individual relatedness, the effective population sizes through time and explore if the effective size is correlated with density.

Material and methods

Fieldwork

This study was conducted in Villa Lago Rivadavia valley (42° 33' S, 71° 37' W), in the locality of Cholila (Chubut Province, south-western Argentina). The mean annual precipitation is about 1000 mm on average and occurs as rainfall or snowfall mainly from April to September when the climate is cold. From October to May, the climate is temperate and droughts are common (less than 100 mm rainfall on average). The vegetation structure is quite unstable because of human activities (wood extraction, logging, fire, exotic species introduction, cattle farming and tourism). The valley presents four types of habitats: pastures, peridomestic areas, forests and shrublands; however, *O. longicaudatus* is much more abundant in the last two, according to the results of Polop *et al.* (2010) who trapped rodents in every season from November 2003 to July 2006 in the four habitats of Cholila. The dominant species of shrublands include native species such as 'calafate' *Berberis microphylla*, 'romerillo' *Senecio filaginoides*, 'espino negro' *Discaria articulata* and the exotic species 'rosa mosqueta' *Rosa rubiginosa*. This last species has been found to release numerous seeds and offer protection favoring the presence of hantavirus-infected rodents (Andreo *et al.*, 2012). Fragmented remains of forest are composed of species such as 'ñire' *Nothofagus antarctica*, 'lenga' *N. pumilio*, 'coihue' *N. dombeyi*, 'maitén' *Maytenus boaria*, 'arrayán' *Luma apiculata* and the conifer 'ciprés de la cordillera' *Austrocedrus chilensis* in lower numbers, with patches of 'caña coligüe' *C. culeou* in the understory.

Rodent trapping was conducted seasonally from August 2006 to September 2008, except in summer 2007, when two samples were obtained: one in February and one in March. Trapped rodents were removed. Four to eight lines were set in shrublands, each consisting of 40 traps placed at 5-m intervals (20 live-capture traps and 20 snap traps alternating). The minimum distance between trapping lines was 200 m. Additional lines were placed in two sites of forest habitats using 13 to 24 traplines per season. Each trapline consisted of 20 traps (10 live-capture traps and 10 snap traps alternating) placed at 5-m intervals. Traps were baited with a mixture of peanut butter and cow fat. All traps were checked daily for 3 consecutive days. Differences in trapping effort between forests and shrublands were given by a pre-sampling work performed in the area, which yielded more constant abundances in shrublands than in forests. Despite this, capture success was very low in forests in most of the periods, except in February and March 2007. In these two months, forest traplines were separated from shrublands, on average by 5600 and 2100 m, respectively. Even though the removal trapping procedure used is considered an invasive technique that could alter the population structure (Kelt, Meserve & Lang, 1994), in our present study, in each season and habitat, traplines were placed in sites with more than 200 m apart from those of the previous season, in order to minimize possible biases derived from specimen removal.

To estimate rodent abundance, we used the relative density index (RDI), which weights the relationship between the number of animals captured and the number of trap-nights used: $RDI = \frac{number\ of\ captures}{(number\ of\ traps \times number\ of\ nights) - a} \times 100$, where a is the number of shut traps without captures (Polop *et al.*, 2010). We assumed that this index is correlated to the population abundance and that it is useful for comparing different sites and moments.

Tail tips of specimens were conserved in 85% ethanol for DNA analysis. Blood and liver samples were sent to the Instituto Nacional de Enfermedades Virales Humanas (Pergamino, Argentina) where the infection status of individuals was assessed using an enzyme-linked immunosorbent assay. Specific prevalence of infection for *O. longicaudatus* was calculated as the proportion of antibody-positive individuals divided by the total number tested. Research on live animals was performed in a humane manner and followed guidelines for the care and use of animals approved by the American Society of Mammalogists (Sikes *et al.*, 2011). Handling of rodents followed standardized safety guidelines recommended by the US Centers for Disease Control and Prevention (Mills *et al.*, 1995).

Microsatellite genotyping

Tail tips were manipulated in a vertical laminar flow cabinet of biosafety level 1 to deal with potentially infected animals. DNA was extracted from each individual according to standard phenolic methods. Proteinase K and RNAse were used in the extraction procedure and helped to inactivate virus particles potentially present in the samples. Ten microsatellite loci were used in this study: nine loci specific for *O. longicaudatus* (Olong_1, Olong_4, Olong_5, Olong_6, Olong_7, Olong_9, Olong_10, Olong_12 and Olong_13; González-Ittig *et al.*, 2008) and the locus Nec_24 isolated in *Nectomys squamipes* (Maroja *et al.*, 2003). Amplifications were performed as described in those two studies and alleles were separated by electrophoresis using tris glycine buffer system (White, Sahota & Edes, 2002) on 20-cm long and 6% or 8% polyacrylamide gels according to the length of the polymerase chain reaction product. Gels were run at 280V for 3.5 h and stained with silver nitrate (Neilan *et al.*, 1994). Allele sizes were determined by comparison with a molecular size standard (10 bp Ladder, Invitrogen Life Technologies, Carlsbad, CA, USA). To confirm genotypes and to minimize allele scoring errors, all amplifications were electrophoresed a second or a third time, running together those individuals that appeared to have similar genotypes in the first run.

Statistical analyses

Microsatellite data were checked for potential stuttering, large allele drop out or presence of null alleles using Micro-Checker 2.2.3 (van Oosterhout *et al.*, 2004).

We examined the genetic structure using the Bayesian algorithm implemented in Structure 2.3 (Falush, Stephens & Pritchard, 2007) assuming a model of population admixture and correlated allele frequencies among groups; we used the temporal population membership of each individual as prior information. We performed preliminary analyses with K ranging from 1 to 13 to detect the most representative number of groups and final analyses for a number of K ranging from 1 to 5, with a burn-in of 4 000 000 iterations and 12 000 000 iterations. We used the parameter Alphapropsd = 0.125 to improve mixing. The procedure was repeated five times for each K to check for consistency; all analyses were run twice in different computers at our laboratory. The optimal value of K was calculated according to the ΔK method described by Evanno, Regnaut & Goudet (2005) using the online Structure Harvester 0.6.7 software (http://taylor0.biology.ucla.edu/struct_harvest/). Replicated runs of Structure were analyzed using CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007) to summarize each individual's membership across runs using the Greedy algorithm. We used these averaged results to build summary bar plots for each individual using the program DISTRUCT v 1.1 (Rosenberg, 2004).

Genetic variability was estimated as individual-observed heterozygosity (IH; Coulson *et al.*, 1998); a locus was scored as 0 if the individual was homozygous or 1 if it was heterozygous and these values were averaged over all loci. Then, the mean IH was obtained for rodents from each period, using the macro provided by Bill Amos at the website http://www.zoo.cam.ac.uk/directory/william-amos. Significance of IH differences between periods was obtained with the Friedman test using the program InfoStat v. 2013 (Di Rienzo *et al.*, 2013).

Relatedness (r) between pairs of individuals was calculated according to Queller & Goodnight (1989) with GENALEX 6.5 (Peakall & Smouse, 2012). Mean values of r were calculated using InfoStat with 5000 bootstrap replicates to obtain a 95% confidence interval around each mean. We calculated: (1) the mean relatedness between pairs of individuals within each sampling period, (2) the mean relatedness of pairs of individuals from consecutive sampling periods and (3) the mean relatedness of individuals presenting the same genetic cluster according to the Structure results. Positive mean values indicate that the individuals analyzed are related, while negative mean values or means whose confidence interval encompasses zero are considered as not significantly related.

We also included population-based analyses, considering each sampling period as a population. Agreement with Hardy-Weinberg equilibrium was proved using the sequential Bonferroni correction for multiple tests for each population. Allelic richness (AR) was calculated with Fstat 2.9.3.2 (Goudet, 2001), while observed (Ho) and expected heterozygosities (He) were calculated with Genalex. Differences among populations were tested using a Friedman test with the program InfoStat.

The effective population size (N_e) was estimated using two different approaches: (1) the temporal method, which estimates N_e from the standardized variance in allele frequency between samples separated by a known number of generations. The generation time was set in 6 months based on the population dynamics described by Polop (2011). Calculations were performed using TempoFs (Jorde & Ryman, 2007),

which produces relatively unbiased estimates also when based on a modest number of alleles and loci. We used the sampling plan 1 (Waples, 1989), which considers overlapping generations. (2) OneSamp 1.2 (http://genomics.jun.alaska.edu/asp/Default.aspx; Tallmon *et al.*, 2008), the method is based on summary statistics of a single population using an approximate Bayesian framework. Runs were performed in a preliminary analysis with priors of 2 for the lower limit and of 100, 500, 1000 and 2000 for the upper limit or maximum effective population size. After analyzing how N_e changed with those priors and the amplitude of the 95% confidence limit, we decided to continue with the prior limit of 500.

Finally, we tested if the RDI correlated with the TempoFs N_e and with the OneSamp N_e using the program InfoStat and if the RDI and the effective size correlated with antibody prevalence. We used the Spearman's non-parametric rank-order correlation coefficient; the statistical significance was tested by 9999 Monte Carlo permutations.

Results

We analyzed 166 individuals from shrublands belonging to 11 temporal populations and 30 individuals of two temporal populations from forests. All 10 microsatellite loci were polymorphic, with the number of alleles per locus ranging from 7 to 22. Mean values for allelic richness, expected, observed and individual heterozygosities are shown in Table 1; none of the values were significantly different among samples (Friedman test: $P = 0.54$ for AR, $P = 0.17$ for He, $P = 0.94$ for Ho and $P = 0.57$ for IH). All loci were in Hardy-Weinberg equilibrium after Bonferroni corrections.

Analyses performed with the program Structure revealed three genetic clusters (Fig. 1), with log posterior probabilities of -8671 ± 33 for individuals from shrublands and from the two sampling periods of forest. The genetic composition of individuals within most sampling periods was quite homogeneous. The frequency of each of the three clusters showed

Table 1 Allelic richness (AR), observed (Ho), expected (He) and mean internal (IH) heterozygosities in each sampling period of *Oligoryzomys longicaudatus*. None of the values were significantly different among samples. All loci were in Hardy-Weinberg equilibrium after Bonferroni corrections

	Shrubland											Forest	
	Winter 2006 (August) $n = 17$	Spring 2006 (November) $n = 17$	Summer 2007A (February) $n = 14$	Summer 2007B (March) $n = 15$	Fall 2007 (May) $n = 16$	Winter 2007 (August) $n = 16$	Spring 2007 (November) $n = 17$	Summer 2008 (February) $n = 8$	Fall 2008 (April) $n = 15$	Winter 2008 (June) $n = 15$	Spring 2008 (September) $n = 16$	Summer 2007A (February) $n = 14$	Summer 2007B (March) $n = 16$
Ho	0.78	0.77	0.76	0.80	0.77	0.81	0.74	0.76	0.81	0.80	0.79	0.80	0.79
He	0.78	0.79	0.77	0.80	0.77	0.80	0.80	0.76	0.78	0.78	0.80	0.81	0.81
IH	0.78	0.77	0.76	0.80	0.78	0.81	0.74	0.76	0.81	0.80	0.79	0.80	079
AR	7.36	7.11	6.79	7.34	7.11	7.06	7.19	7.6	6.98	7.01	7.18	7.57	7.34

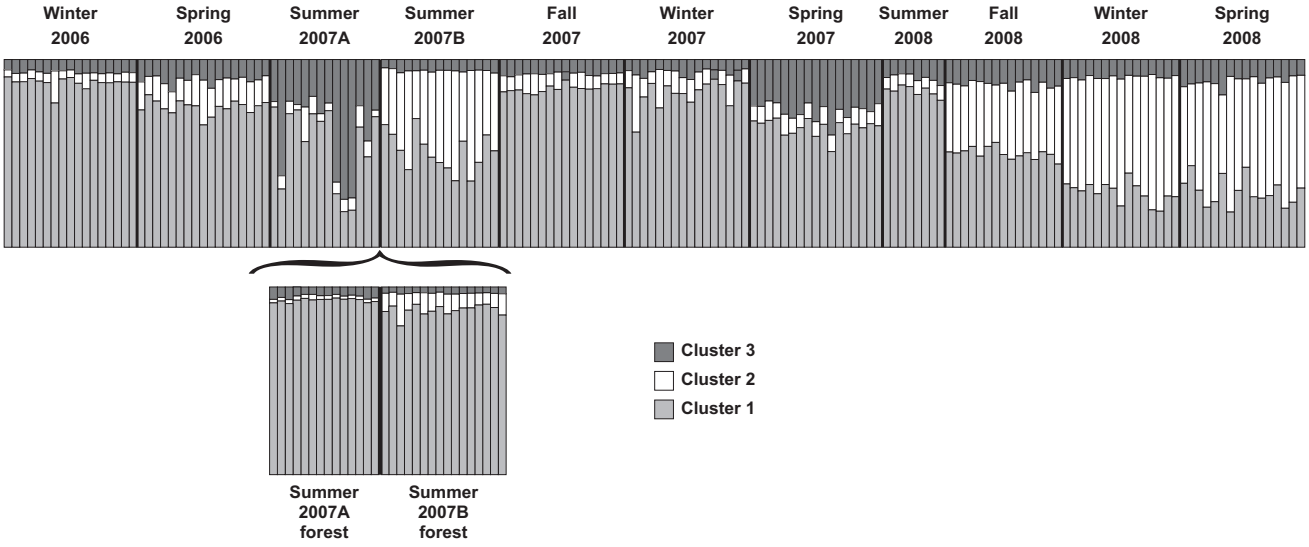

Figure 1 Temporal variation of Bayesian cluster analysis of multilocus microsatellite genotypes of *Oligoryzomys longicaudatus* specimens from shrublands and the two sampling periods from forest of summer 2007. Each individual within a sampling period is represented by a thin vertical line, partitioned into segments that represent the probability of an individual to belong to one of the three estimated genetic clusters.

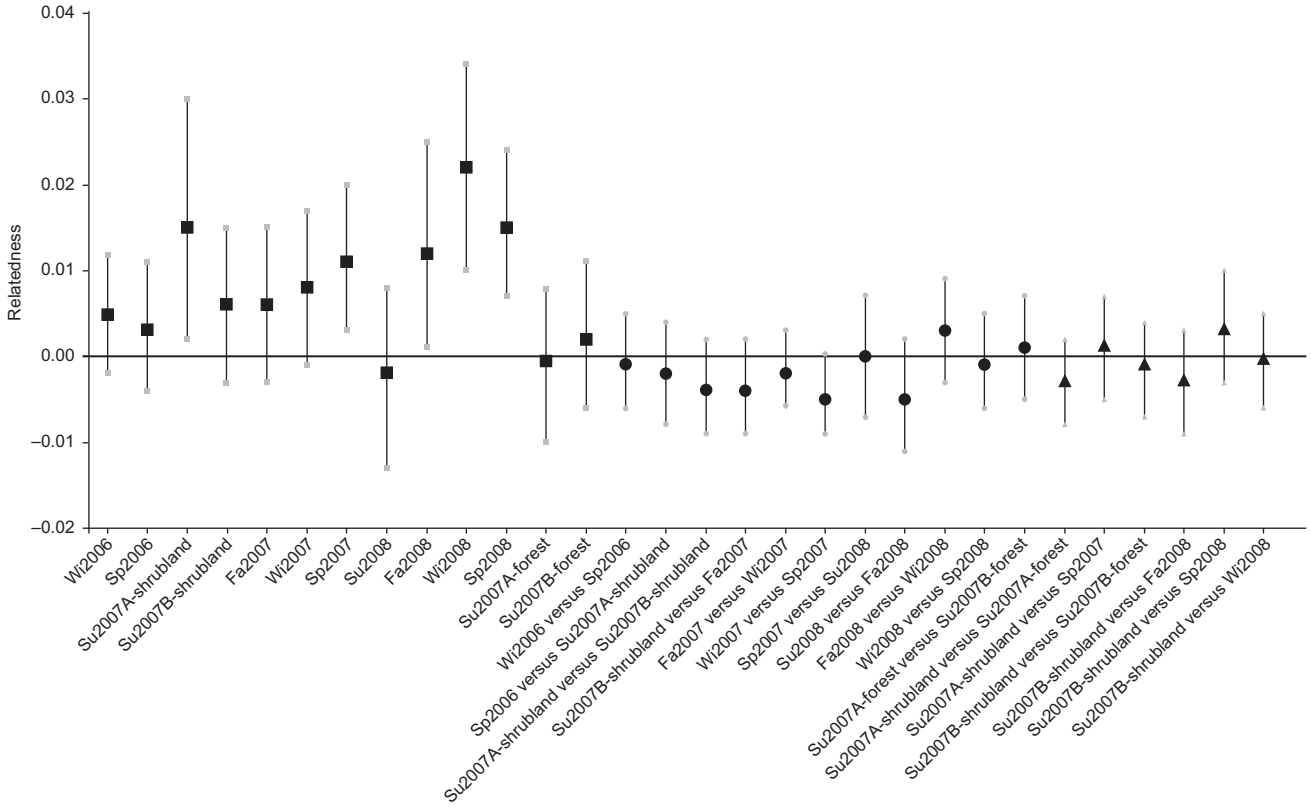

Figure 2 Mean relatedness 'r' of individuals of *Oligoryzomys longicaudatus* within a sampling period (squares), of individuals of consecutive sampling periods (circles) and of individuals from sampling periods presenting the same genetic cluster as shown in Fig. 1 (triangles). Bars represent the 95% confidence interval of r obtained by 5000 bootstrap replicates.

temporal changes. Until summer 2008, most individuals presented a high membership in cluster 1 (light grey), but in some periods, individuals showed admixed ancestry between cluster 1 and 3 (dark grey; summer 2007A) or between cluster 1 and 2 (white; summer 2007B). Only four individuals from summer 2007A showed a high membership in cluster 3; otherwise, this cluster was only present as part of admixed individuals. It is interesting to note that after summer periods, individuals presented a different genetic composition. In 2008, cluster 2 gradually increased its frequency, displacing cluster 1 as the dominant in the population. Individuals from forest captured in summer 2007A and summer 2007B showed a high membership to cluster 1; they were different from those of the same sampling period from shrublands, which presented admixture of two clusters.

Almost all seasonal mean r values from shrubland were positive and 5 out of 11 were significantly different from 0 (squares in Fig. 2). In contrast, the majority of mean r values from consecutive periods were negative, indicating that individuals are less related than expected by chance (circles in Fig. 2). Individuals from different temporal samples that were classified by Structure in the same cluster were not related (triangles in Fig. 2). In addition, individuals from forest showed non-significant relatedness values and comparisons among individuals from forest and those from shrublands obtained in summer 2007 showed no relatedness. When we compare the results of Structure with the mean values of relatedness, we observe that individuals from cluster 1 present low values of r within period (Fig. 2; e.g. winter 2006 and spring 2006). For clusters 2 and 3, results are different. Cluster 3 is present only in two periods, summer 2007A and spring 2007, as part of admixed individuals; these two periods show high r values. Similar results are obtained for individuals from cluster 2: fall, winter and spring 2008 present high values of r.

Table 2 shows estimations of N_e using two different methods: TempoFs and OneSamp. The plotting of these values along with the RDIs (Fig. 3) showed no significant correlation ($r = 0.2014$, $P = 0.55$) for OneSamp N_e versus RDI but significant correlation ($r = 0.6444$, $P < 0.05$) for TempoFs N_e versus RDI. The RDI reflected a severe bottleneck in summer 2008 (in this period very few individuals were captured). This phenomenon was also evident, although not in the same order of magnitude, in the reduction of N_e compared with the precedent period: the point estimations of OneSamp revealed a low reduction of N_e from 21 (spring 2007) to 16 (summer 2008) and the TempoFs computed allele frequencies changes from spring 2007 to summer 2008 leading to a

Table 2 Effective population sizes of *Oligoryzomys longicaudatus* calculated with OneSamp and with TempoFs. In parenthesis, the 95% confidence limits

Habitat	Temporal sample	OneSamp N_e	Period	TempoFs N_e
Shrubland	Winter 2006	15 (13–22)	Winter 2006–Spring 2006	20 (13–44)
	Spring 2006	21 (16–32)	Spring 2006–Summer 2007A	12 (7–37)
	Summer 2007A	18 (14–30)	Summer 2007A–Summer 2007B	10 (7–21)
	Summer 2007B	17 (13–24)	Summer 2007B–Fall 2007	13 (6–34)
	Fall 2007	17 (13–26)	Fall 2007–Winter 2007	15 (9–40)
	Winter 2007	18 (15–29)	Winter 2007–Spring 2007	11 (8–20)
	Spring 2007	21 (16–35)	Spring 2007–Summer 2008	12 (6–40)
	Summer 2008	16 (13–26)	Summer 2008–Fall 2008	8 (5–23)
	Fall 2008	22 (17–38)	Fall 2008–Winter 2008	14 (9–41)
	Winter 2008	7 (6–10)	Winter 2008–Spring 2008	9 (6–22)
	Spring 2008	12 (10–18)		
Forest	Summer 2007A	15 (12–22)	Summer 2007A-forest–Summer 2007B-forest	13 (8–42)
	Summer 2007B	18 (14–28)		

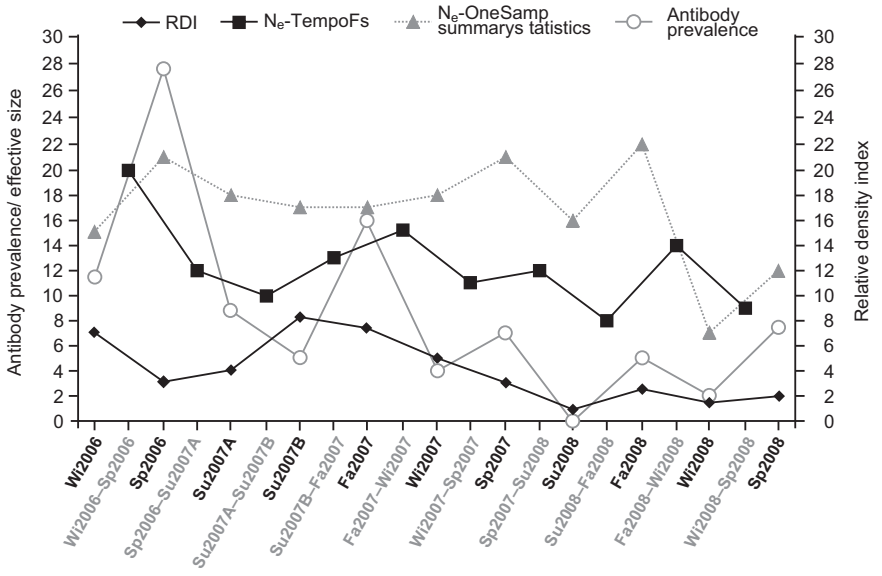

Figure 3 Plot reflecting temporal changes in the relative density index (RDI), in the antibody prevalence and in the OneSamp and TempoFs N_e estimations calculated for *Oligoryzomys longicaudatus* populations. Fa, fall; Sp, spring; Su, summer; Wi, winter.

reduction of N_e to 8. After this bottleneck, both One Samp and TempoFs showed a recovery of the N_e from summer 2008 to fall 2008, which was not so evident in the RDIs values and a new reduction in winter 2008.

Antibody prevalence changed throughout the sampling period, with one peak in spring 2006 and one in fall 2007. In summer 2008, no infected animals were detected, coincidently with the severe bottleneck that affected the population.

Correlations of prevalence versus TempoFs N_e and prevalence versus RDI were not significant ($r = 0.39$, n.s.; $r = 0.35$, n.s., respectively); plots are shown in Fig. 3.

Discussion

In the present study, we explored how the genetic structure of *O. longicaudatus* changed in a 25-month period. The Bayesian individual-based analysis identified three clusters whose frequencies changed during the study period (Fig. 1). Rodents from summer 2007A and spring 2007 showed admixed ancestry in clusters 1 and 3, being their genetic composition different from those in the previous and in the following temporal populations. This result suggests that these temporal populations may be formed by individuals from different geographic origins. Murúa *et al*. (1986), Polop *et al*. (2010), Polop (2011) and Andreo *et al*. (2012), based on the very low levels of recapture, the high proportion of transient individuals throughout the year and the continuous recruitments (number of new animals added to the trappable population either born *in situ* or migrants from surrounding areas) concluded that individuals of *O. longicaudatus* are highly vagile, which is in line with our present results.

After summer 2008, the frequency of cluster 2 and the levels of relatedness increased. In that period, the sampling area (Cholila) suffered a major drought and a fire affected the vicinity of Villa Lago Rivadavia valley, producing a severe bottleneck. The recovery of the population by migration of individuals with another genetic composition could explain the maintenance of high levels of polymorphism (Ho, He and IH) over the period covered by this study (Table 1). Polop (2011) reported delayed density peaks in forests and shrublands populations from Cholila and proposed that rodents would be moving between these habitats. We observed that individuals collected in shrublands presented admixed ancestry (clusters 1 and 3 for summer 2007A and 1 and 2 for summer 2007B) and were genetically different from those obtained in forest in the same period (Fig. 1). But later on, in fall 2007, shrublands presented the same genetic cluster composition detected in forests of summer 2007A and B, which supports the idea that the entire population is moving and mixing with populations from surrounding habitats, reducing the levels of relatedness. These results suggest that a metapopulation dynamic system could be present in Cholila. This system was also proposed for the bank vole *Myodes glareolus*, reservoir of the Puumala hantavirus, in which large-sized forests would act as a source and fragmented habitats would be the sinks (Guivier *et al*., 2011). Notwithstanding, this hypothesis for *O. longicaudatus* deserves further research involving more individuals from forests obtained in different sampling periods to identify if forest or shrublands are acting as source or as sink in this dynamic scenario.

We also detected that the RDI and the N_e were positively correlated in the 25-month period of this study (Fig. 3), when considering the TempoFs N_e estimation. However, the correlation involving the OneSamp N_e estimation was non-significant. This method uses eight summary statistics and an approximate Bayesian computation to estimate N_e from a single sample of microsatellite data and does not include temporal variation between consecutive samples, as does the TempFs N_e; this variable should be considered in our case, given the important genetic changes detected in *O. longicaudatus*. Therefore, we only took into account the results obtained using the TempFs method. There are several species in which a positive correlation between density, effective size and genetic diversity (percentage of polymorphic loci, mean number of alleles per locus, N_e or haplotype frequencies) were reported. For example, in *Microtus californicus* (Bowen, 1982), the genetic differentiation was relatively high during phases of low density when the effects of genetic drift was evidenced by the loss of alleles. Chiappero *et al*. (2006) found that, in the 'vesper mouse' *Calomys musculinus*, important changes in the percentage of polymorphic loci and mean number of alleles per locus correlated with density and N_e in populations from central Argentina. In populations of this species, mixing of surviving individuals coming from different demes would play an important role in the maintenance of variability and recovery of N_e after low-density periods. Similar results were reported in *Arvicola terrestris*, a fossorial water vole (Berthier *et al*., 2006) and in *Tscherskia triton*, a hamster (Xie & Zhang, 2006). All these small rodents presented cyclic population dynamics, with medium to small home range areas and moderate levels of dispersion. Species inhabiting unstable environments usually present adaptations to prevent the negative effects of crashes: high reproductive rate, sexual maturity at an early age, long reproductive season and high migration rates, all features that would favor a rapid recovery of their populations (Ims & Andreassen, 2005). Several ecological studies reported most of the characteristics mentioned earlier in *O. longicaudatus* (Contreras, 1972; Pearson, 1983; Murúa *et al*., 1986; Polop *et al*., 2010), which would allow a rapid recovery in unstable human modified habitats such as those of Cholila. The high vagility of the species would produce changes in the genetic composition without fixation of alleles, preventing the negative effects of bottlenecks by maintaining relatively high N_e values, even in low-density periods. In that sense, it is important to note that, after the bottleneck detected in summer 2008, the whole population changed, probably leading to positive values of relatedness and to the observed cluster replacement (Figs 1 and 2).

Another observation emerges from our present results: cold and snowy winters seem not to affect N_e and RDI, as sharp declines in both values are not observed (Fig. 3); this is in line with the important proportion of overwintering individuals reported by Polop *et al*. (2010) and Polop (2011) using capture-recapture methods. On the other hand, dry summers tend to reduce both the RDI and the N_e values, as occurred in summer 2008 in Villa Lago Rivadavia valley. In another valley (El Blanco) of the locality of Cholila, which was also affected by particularly dry conditions in summer 2008, N_e showed a threefold reduction compared with summer 2007 (results not shown). The very low capture success and the low effective sizes estimated are evidence that *O. longicaudatus* is very sensitive to hot and dry weather conditions; however, after crashes, the level of genetic variability seems to recover very fast.

Hantavirus prevalence and density were not correlated. According to Mills (2005), the unclear relationships between the prevalence of infection and rodent abundance could be attributed to the manner in which prevalence is estimated. In high-density periods, if the number of infected individuals is low, a 'dilution effect' in the calculation of prevalence could occur. Conversely, in low-density periods, the infected individuals will produce an overestimation effect. It is interesting to note that the infection present in the population disappeared in summer 2008 (Fig. 3), coincidently with the drought and the population bottleneck here reported. The infection was detected again in the next season, which is in line with the arrival of infected specimens from surrounding areas.

In summary, in this study, we focused our attention in the fine-scale temporal genetic structure of *O. longicaudatus*. Results of all analyses are in line with the conclusion that high levels of gene flow would be a major process producing fine-scale temporal changes in the genetic composition of populations of *O. longicaudatus* from Cholila, in the Argentinean Patagonia. New studies performing spatial autocorrelation analyses at different geographical scales to define the size of the genetic patches have been initiated to get a better understanding of the movements of this Hantavirus reservoir.

Acknowledgments

We are very grateful to the Mundo Sano Foundation for supporting the field work. This study was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP 11420090100289), Secretaría de Ciencia y Tecnología (SECYT), Universidad Nacional de Córdoba and FONCYT (PICT 2008-0458, Préstamo BID) all from Argentina. R.E.G.-I., M.B.C. and C.N.G. are career researchers of CONICET.

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