# Genetic population structure of the long-tailed pygmy rice rat (Rodentia, Cricetidae) at different geographic scales in the Argentinean Patagonia

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## Abstract

The population genetic structure of *Oligoryzomys longicaudatus* colilargo was examined at two geographical scales: (a) regional, including five populations of the Argentinean Patagonia separated by 60–315 km and (b) landscape scale, using five populations from different valleys of the locality of Cholila in the subantarctic forest separated by 6–27 km, and a nearby locality of the Patagonian steppe, with an average distance from Cholila of 33 km. Eight microsatellite loci specific for *O. longicaudatus* were used as genetic markers. At the regional scale, four genetic clusters were detected by the Geneland software. The genetic structure was found to follow a latitudinal pattern. This result was supported by the $F_{ST}$ statistic, indicating low levels of current gene flow within the region. At the landscape level, genetic differentiation among the five populations was also found. Estimated migration rates were, in general, low and asymmetrical between nearby populations. Using a causal modeling approach, we detected that the combination of landscape features such as lakes, rivers, urban settlements and roads appear to constrain the dispersal of *O. longicaudatus* at this scale. This result would explain why nearby populations were so different in their genetic composition. The information about geographic features limiting rodent dispersal provided here could help to design more accurate prevention measures against the expansion of hantavirus pulmonary syndrome.

## Introduction

Species are rarely fully panmictic, but are often divided into genetic subgroups. This non-random distribution of genetic variation in space and time is referred to as the genetic structure of a population (Wright, 1938). Several methods have been developed to combine geographic and genetic information to estimate the number of populations in a dataset and to delineate their spatial organization (Guillot *et al.*, 2009). Small mammals having an extensive geographic distribution and variable density, dispersal and space use at different scales provide a good opportunity to investigate the relative impact of spatial scale in the genetic structure, since it reflects the influence of both historic and recent evolutionary processes. 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 is very important to predict the spread and persistence of zoonoses (Guivier *et al.*, 2011).

The long-tailed pygmy rice rat *Oligoryzomys longicaudatus* (Rodentia, Cricetidae) is the major reservoir of Andes hantavirus

genotype, associated with hantavirus pulmonary syndrome (HPS) in southern Argentina and Chile (Levis *et al.*, 1998; Padula *et al.*, 2004). The species inhabits environments that include rivers, lakes and mountains which could reduce the connectivity among populations; therefore, it is a good model to study this phenomenon at various geographic scales. *O. longicaudatus* is common in *Nothofagus* temperate forests and shrubland areas at both sides of the Patagonian Andes range in Argentina and Chile. In Argentina, it is distributed from 36°S to 51°S in both the Eastern slope of the Andes Mountains and the Patagonian steppe (Carbajo & Pardiñas, 2007; Andreo *et al.*, 2011). The species is characterized by a high vagility and flexibility in habitat use, which causes important fluctuations in its home range along the year (Murúa, González & Meserve, 1986); it experiments density fluctuations among seasons, which increases during autumn/winter (April/June) and decreases in summer (December/February) (Contreras, 1972; Murúa *et al.*, 1986; Polop *et al.*, 2010, 2014a). The generation time of *O. longicaudatus* is approximately 1 year, but it varies according to environmental conditions, climatic factors and

intraspecific competence (Pearson, 1983; Murúa *et al.*, 1986). Another phenomenon observed in this species is the explosive outbreaks or 'ratadas' generated by several conditions such as warm winters, rainfall peaks and the cyclic blooming and seeding of bamboo plants (*Chusquea quila, C. coleou* and *C. valdiviensis*) (Gallardo & Mercado, 1999; Lima, Marquet & Jaksic, 1999). As a consequence, *O. longicaudatus* invasions to human settlements are more likely to occur, increasing the probability of infection with the Andes hantavirus (Calderón *et al.*, 1999). The study of the genetic structure of *O. longicaudatus* would allow the evaluation of the degree of contact between populations which, in turn, would lead to the estimation of the risk of virus dispersion.

Concerning the genetic structure, several studies were carried out at macrogeographic scale. Palma *et al.* (2005) and González-Ittig *et al.* (2010) proposed that *O. longicaudatus* would have undergone a recent geographic range expansion. Torres-Pérez *et al.* (2011) and Palma *et al.* (2012) detected a strong north–south geographic structuring in populations of *O. longicaudatus* from Chile, while on the Argentinean side, González-Ittig *et al.* (2010) and Lessa, D'Elía & Pardiñas (2010) reported high genetic similarity among localities. However, González-Ittig *et al.* (2010) found private haplotypes in several populations, suggesting low to moderate current gene flow among them. To elucidate micro-evolutionary processes like dispersal, mitochondrial markers are not the most appropriate, since they usually reveal long time processes comprising large spatial scales (Avise, 2000). Microsatellites instead, have proved to be more accurate to detect short time changes in the genetic composition, given their high mutation rate (De-Xing & Godfrey, 2003; Selkoe & Toonen, 2006; González-Ittig *et al.*, 2015). In this study, we use microsatellites to characterize the population genetic structure of *O. longicaudatus* at different spatial scales: (1) regional, in different localities of the Argentinean Patagonia and (2) a finer scale, using a landscape genetics approach. This last discipline is based on the detection of genetic discontinuities and the correlation of these discontinuities with landscape and environmental features, such as barriers to gene flow (e.g. mountains, rivers, gradients of humidity, etc.) (Manel *et al.*, 2003). The analyses proposed here would provide useful information to understand the dispersal patterns of the natural host of Andes hantavirus.

## Materials and methods

### Study area, habitat description and sample collection

The study area ranges between 39°56′07.62″ S, 71°05′21.84″ W and 42°33′27.90″ S, 73°08′03.42″W (Fig. 1), encompassing two main phytogeographic regions: subantarctic forest and Patagonian steppe. To get a better understanding of dispersal patterns, two geographic scales were used: (1) a regional scale, including five populations separated from each other by 60–315 km: Junín de los Andes (Neuquén province), Bariloche and El Bolsón (Río Negro province), Leleque and the valley El Cajón of the locality of Cholila (Chubut province); (2) a landscape scale, including four valleys of Cholila separated by an average distance of 14.48 km: El Cajón, El Blanco, El Rincón, Villa Lago Rivadavia and Leleque, a nearby location in the Patagonian steppe separated from the valleys by an average distance of 32.85 km (Fig. 1; Table 2). Junín de los Andes, Bariloche, El Bolsón and Cholila are located in the deciduous district of the subantarctic forest region.
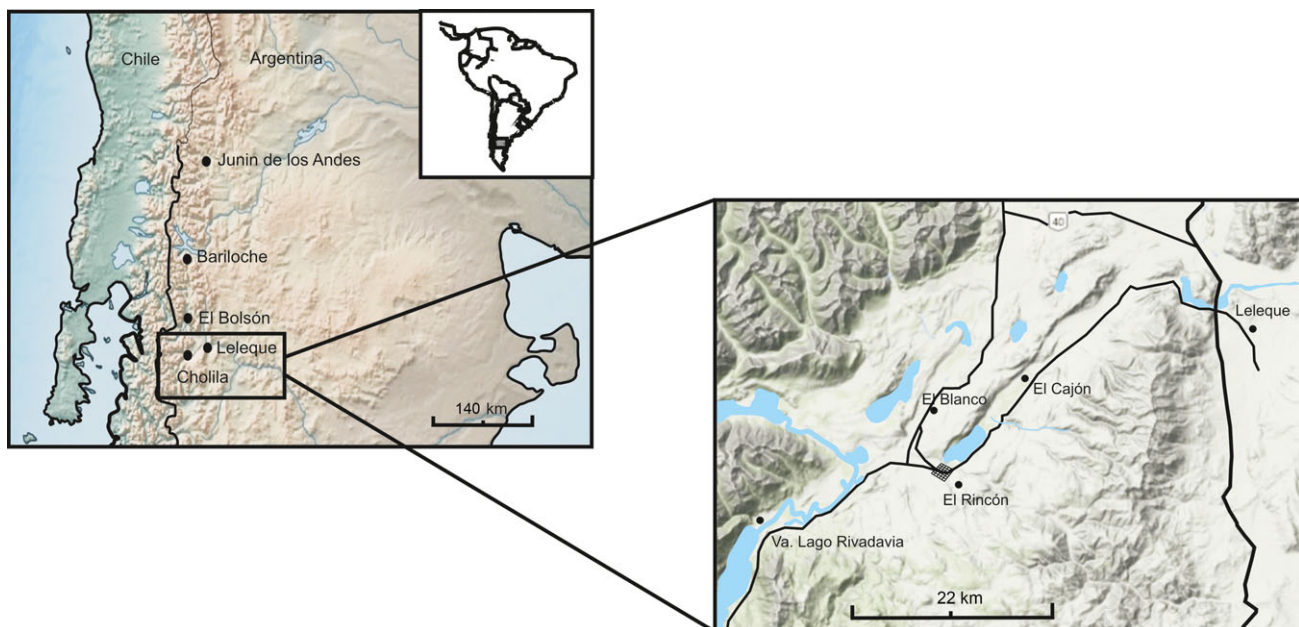


**Figure 1** Geographic distribution of sampled *Oligoryzomys longicaudatus* populations at regional (left) and landscape (right) scales.

In Cholila, Junín de los Andes, Bariloche and El Bolsón sampling was performed in the shrublands with predominance of arbustive specimens (mainly the exotic sweet briar, *Rosa rubiginosa*), whereas in Leleque, specimens were obtained from its unique component: the Patagonian steppe. Sampling was performed between 2006 and 2009 using a removal system, in lines separated by about 200 m; each consisted of 40 traps (20 live capture and 20 snap traps alternated) with a distance of 5 m between them. The baiting mixture was peanut butter and cow fat. The procedure was performed for 3 consecutive nights. Individuals were transferred in hermetic bags to the field laboratory for processing. Tail tips were conserved in 85% ethanol for DNA analysis. Rodent handling followed standardized safety guidelines recommended by the U.S. Centers for Disease Control and Prevention (Mills *et al.*, 1995).

## Microsatellite genotyping

DNA from each individual was extracted using standard phenol/chloroform methods. Samples were manipulated in a biosafety level 1 cabinet to deal with potentially infected animals. Eight microsatellite loci specific for *O. longicaudatus* were amplified as described in González-Ittig *et al.* (2008): Olong_1, Olong_4, Olong_5, Olong_6, Olong_7, Olong_9, Olong_10 and Olong_12. Procedures for allele separation by electrophoresis were performed according to González-Ittig *et al.* (2015). To confirm genotypes and to minimize allele scoring errors, all amplifications were run a second and/or a third time, together with individuals presenting 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). HW equilibrium was tested by estimating the differences between observed and expected heterozygosities using the program Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). Different indexes estimating the degree of polymorphism in each population were calculated with the program Genalex 6.41 (Peakall & Smouse, 2006) (Table 2).

### Regional scale

To evaluate the genetic differentiation among populations, an analysis of molecular variance (AMOVA) was performed and population pairwise $F_{ST}$ were calculated with the program Arlequin 3.5.1.2. For this purpose, 10 000 permutations and a statistic significance level of 0.05 were used.

To detect possible correlations between genetic ($F_{ST}/[1-F_{ST}]$) and the natural logarithm of geographic distances (Rousset, 1997) in *O. longicaudatus* populations, we performed a Mantel tests using TFPGA 1.3 (Miller, 1997); to assess the significance of the test, 10 000 permutations were computed.

To infer the number of genetic clusters ($K$) and their boundaries taking into account geographic information, we used the Bayesian approach implemented in Geneland 3.0

(Guillot, Mortier & Estoup, 2005). Preliminarily, we estimated the number of $K$ from 1 to 10, using 10 000 000 MCMC iterations and 1000 thinnings. For the final results, five independent runs with fixed $K$ (to avoid ghost populations) were performed for the spatially explicit and correlated allelic frequencies model. For each run, the posterior probability of subpopulation membership was computed for each pixel of the spatial domain (20*120 pixels) using a burn-in of 1000 iterations. Additionally, to visualize each individual's membership to the assigned genetic clusters, we built a bar plot in Excel with the summarized individual-based output given by Geneland.

### Landscape scale

We performed all the analyses already described for the regional scale and we added two extra analyses. With BayesAss 1.3 (Wilson & Rannala, 2003), we estimated symmetric and asymmetric migration rates in the last three generations between pairs of populations. Analyses were performed with 100 000 000 iterations, sampled every 100 000. We used a burn-in of 25 000 000 iterations and the estimator values for migration rate ($m$), allele frequency ($a$) and endogamy coefficient ($f$) were fixed at 0.5. Migration rates that exceeded 20% were considered important. Convergence was checked with Tracer 1.6. (Rambaut *et al.*, 2014).

The effect of both geographic distance and different elements of the landscape (lakes, rivers, mountain ranges, roads, urban zones) on genetic differentiation between *O. longicaudatus* individuals were evaluated with a causal modeling approach using partial Mantel tests (Cushman *et al.*, 2006, 2013). Maps were drawn at the scale 1:1250 from a Google Earth image using the OpenLayer plugin within QGIS 2.14 (QGIS Development Team, 2014). The geographic elements of the landscape were classified in classes such as lakes, rivers, mountain ranges [on average, 2000 meters above sea level (masl)], hills (on average 900 masl), main (paved) and secondary (dirt) roads, forests and urban zones (including houses and peridomestic settlements) (Fig. 2a). Resistance maps of the study site were generated, representing different hypotheses about the difficulty of *O. longicaudatus* individuals to disperse through landscape elements. Different resistance values were assigned to lakes, rivers, mountains, hills, roads and urban zones in order to generate hypotheses about their relative influence on populations' connectivity in the landscape. Different scenarios were tested: (1) Measuring separately the effect of each geographic feature, in which a resistance value was assigned to the evaluated landscape element, and a value of 1 (no resistance) was assigned to the rest of the elements. (2) Combining resistance values of the different elements in the same hypothesis. For this last group, five hypotheses were generated considering combined geographic features (Table 1). In the first hypothesis, high resistance values were assigned to lakes and urban zones, while intermediate values were given to rivers, mountain ranges and main roads. In the second hypothesis, the same resistance values were used for lakes and urban zones, but mountain ranges and roads received lower values. Hypothesis 3 was similar to hypothesis 1, except for the urban zone,
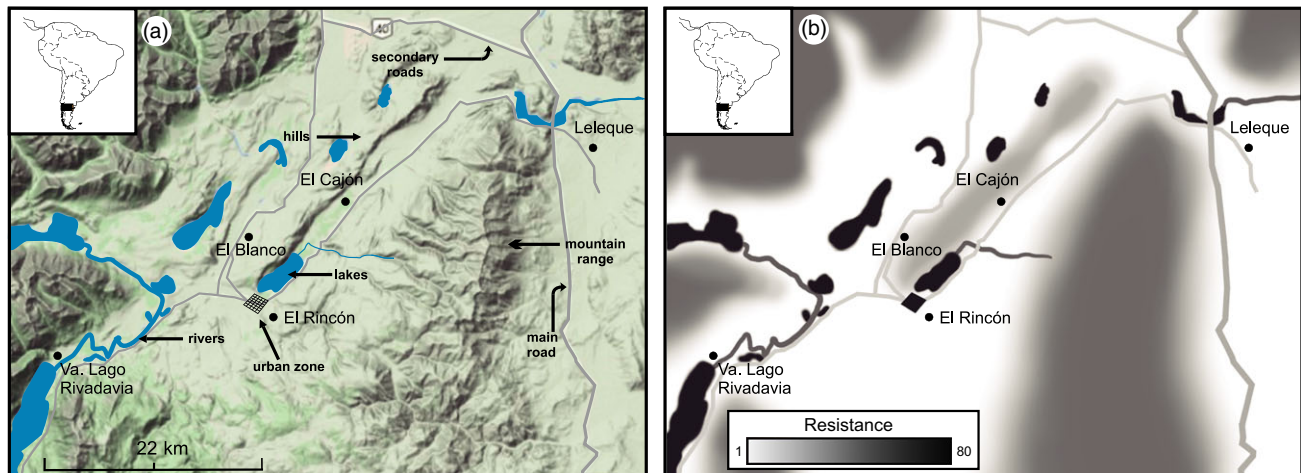
**Figure 2** (a) Geographic features and (b) their relative resistance values for Hypothesis 1, the only significant, tested in the causal modeling analysis.

to which a lower value was assigned. In hypothesis 4, all resistance values were the same as in hypothesis 1, but higher values were given to rivers. Lastly, in hypothesis 5, high resistance values were assigned to rivers and an intermediate value to the urban zone, being the rest as in hypothesis 1. Forests and shrublands presented the minimum resistance in all models, due to the strong preference of the species for these types of habitats (Polop *et al.*, 2010, 2014b). Finally, the null model considered only Euclidean geographic distances as predictor. The program Circuitscape 4.0 (Mcrae, Shah & Mohapatra, 2014) was employed to transform resistance maps into resistance distance matrices between pairs of individuals using a four-neighbor connection scheme. Genetic and geographic distances between pairs of individuals were estimated using Genalex 6.41. With the program zt (Bonnet & Van De Peer, 2002), a simple Mantel test was conducted between genetic distance and each of the distance matrices (null and landscape resistance hypotheses). For each of the five landscape resistance hypotheses, two partial Mantel tests were performed with the same program: one to test for the correlation between genetic distance and the resistance hypothesis, partialing out the effect of the null hypothesis (geographic distance) and, the other between genetic distance and geographic distance, discounting the effects of the resistance hypotheses. Results were interpreted according to Cushman *et al.* (2013) who provided a guidance to interpret the outcomes of the diagnostic partial Mantel tests and advices to avoid Type I errors or spurious correlations.

## Results

Micro-Checker analysis showed no evidence of scoring errors due to stuttering, large allele dropouts and null alleles for all loci and all populations. Observed and expected heterozygosities did not differ significantly from zero, indicating that all populations were at Hardy–Weinberg equilibrium (Table 2). The average number of different alleles ranged from 7.375 to 11.5, the average number of private alleles ranged from 0.125

to 1.125 (Table 2). The locus by locus observed and expected heterozygosities and allele number are shown in Supporting Information Table S1.

### Regional scale

The average $F_{ST}$ value was moderate (0.067), with values ranging from 0.015 to 0.097 (all were significant at $P < 0.05$). The lowest differentiation occurred between Leleque and El Cajón valley from Cholila, whereas the highest was between Bariloche and Leleque (Table 3). The AMOVA analysis revealed that 94.84% of the variance occurred within populations and 5.16% among them ($P < 0.05$). The isolation by distance test revealed a non-significant correlation ($r = 0.0105$; $P = 0.4150$) between genetic and geographic distances among all *O. longicaudatus* populations.

The combination of genetic and spatial data performed by the program Geneland revealed the existence of four genetic clusters; each $K$ was represented by a single population except for the last one, which comprised Leleque and El Cajón valley (Fig. 3). Posterior probabilities to belong to each cluster of Junín de los Andes, Bariloche, El Bolsón, and Leleque plus El Cajón were 0.7, 0.6, 0.8 and 0.9, respectively. The individual-based plot showed a similar result: individuals from Leleque and El Cajón presented a very similar genetic composition; individuals from Junín de los Andes, Bariloche and El Bolsón showed admixed ancestry but with high membership to cluster 2, 4 and 1, respectively (Fig. 4).

### Landscape scale

As stated above, this scale comprised the four valleys in Cholila (separated by 14.48 km on average) and Leleque population in the steppe, 32.85 km on average away from them (Fig. 1). The overall $F_{ST}$ value was moderate: 0.056. Pairwise values between populations ranged from 0.01 to 0.1; the former corresponded to the comparison between Leleque and El Cajón valley, and the latter to El Blanco-Villa Lago Rivadavia comparison (Table 4).

**Table 1** Resistance values to gene flow among *Oligoryzomys longicaudatus* populations for each landscape hypothesis

| | Hypothesis 1 | Hypothesis 2 | Hypothesis 3 | Hypothesis 4 | Hypothesis 5 | Null |
|---|---|---|---|---|---|---|
| Lakes | 80 | 80 | 80 | 80 | 80 | 1 |
| Rivers | 50 | 50 | 50 | 80 | 80 | 1 |
| Mountain ranges | 40 | 20 | 40 | 40 | 40 | 1 |
| Hills | 20 | 10 | 20 | 20 | 20 | 1 |
| Main roads (paved roads) | 30 | 15 | 30 | 30 | 30 | 1 |
| Secondary roads (dirt roads) | 10 | 5 | 10 | 10 | 10 | 1 |
| Forests and shrublands | 1 | 1 | 1 | 1 | 1 | 1 |
| Urban zone | 80 | 80 | 40 | 80 | 60 | 1 |

The AMOVA analysis revealed that 94.39% of the variance occurred within populations, while 5.61% occurred among them ($P < 0.05$). The Mantel test showed absence of isolation by distance: $r = 0.1754$; $P = 0.3651$.

The Geneland analysis revealed the existence of five clusters; each of them represented by a single population, with posterior probabilities ranging from 0.8 to 0.9 (Fig. 5). The individual assignments supported this result; where individuals from Va. Lago Rivadavia and Leleque showed high membership to clusters 4 and 5, respectively. In contrast, individuals from El Blanco, El Cajón and El Rincón presented admixed ancestry with predominance of clusters 1, 2 and 3, respectively (Fig. 6).

The program BayesAss 1.3 reached convergence and found, in general, low migration rates between population pairs. In general, migration was found to occur between nearby populations and to follow an asymmetrical direction. Leleque and Villa Lago Rivadavia showed mixed ancestry, with important migration rates. In the case of Leleque, the population was composed by migrants coming from El Blanco and from El Cajón, while Villa Lago Rivadavia received migrants from El Rincón (Fig. 7). All the results of BayesAss are shown in Supporting Information Table S2.

Regarding the causal modeling analysis, none of the hypotheses in which the landscape elements were evaluated separately was significant (results not shown). When evaluating the effect of combined geographic features on gene flow, simple Mantel tests between genetic distance and resistance hypotheses, as well as partial Mantel tests between genetic distance and geographic distance or resistance hypotheses were non-significant for the null hypothesis and for hypothesis 2, 3, 4 and 5. On the contrary, both the simple Mantel test between genetic distance and hypothesis 1, and the partial Mantel test between genetic distance and hypothesis 1, partialing out the effects of geographic distance, were significant, with correlation coefficients of 0.1665 and 0.1669, respectively, while the partial Mantel test between genetic distance and geographic distance, partialing out hypothesis 1 was not significant (Table 5).

## Discussion

### Regional scale

We used two different geographic scales to characterize the genetic structure of *O. longicaudatus*. At the regional scale, pairwise $F_{ST}$ values were all significant (average $F_{ST} = 0.067$),

indicating a restricted gene flow among populations in the area. The analysis performed with Geneland revealed a total of four latitudinal groups ($K = 4$). Our results show a significant genetic differentiation of populations with increasing latitude at a regional scale, reinforcing the idea of low current dispersal levels among populations in the region.

Torres-Pérez *et al.* (2011) found significant geographic structure in Chilean *O. longicaudatus* populations at a macrogeographic geographic scale (over 600–800 km); the pattern was attributed to reduced gene flow among Pleistocene glacial refuges in the western Andes. Moreover, in a study comprising most of the species geographic distribution in Chile, Palma *et al.* (2012) detected three clusters using the mtCyt-b gene, which mostly agreed with the three major Chilean ecogeographic regions: Mediterranean, Valdivian and Patagonian Forests. In the Argentinean Patagonia, on the other hand, *O. longicaudatus* is strongly associated with *Nothofagus* species of the subantarctic forests. If both (*Nothofagus* species and the rodent) experienced a similar evolutionary history, a comparable pattern in their genetic structure would be expected. In fact, for Argentinean *Nothofagus pumilio* and *N. antarctica*, different clusters were found along latitudinal regions in Patagonia (Soliani, 2012; Soliani, Gallo & Marchelli, 2012). Both chloroplastic and microsatellite markers revealed a north–south pattern of genetic variation, which could be associated with the effect of glaciers on a particular landscape configuration (a north–south-oriented mountain chain at 42–43°S and west–east orientated watershed at 44°S). The latitudinal genetic pattern found over the sampled *O. longicaudatus* populations in this study and the similar pattern found in *Nothofagus* species support the assumption that similar processes have shaped the genetic structure in these species.

González-Ittig *et al.* (2010) proposed that during the Last Glacial Maximum, *O. longicaudatus* would have survived on a putative south ice-free Pacific coast refuge, then expanding its range from that refuge. Although the authors reported a weak genetic divergence among populations, several private haplotypes were detected in most localities, suggesting spatial restriction to gene flow in recent times. The strong genetic differentiation found here in populations from the same area, would confirm this assumption.

### Landscape scale

In this study, we also compared the genetic structure of *O. longicaudatus* populations from four valleys in the vicinity

**Table 2** Indexes revealing the degree of polymorphism in *Oligoryzomys longicaudatus* populations

| Population | Latitude | Longitude | N | Na | Ne | Ho | P-value | He | UHe | N° Priv. alleles | %P | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Junín de los Andes | 39°56'07.6"S | 71°05'21.8"W | 14 | 11.250 ± 1.359 | 7.688 ± 1.196 | 0.857 ± 0.027 | 0.410 | 0.842 ± 0.029 | 0.873 ± 0.030 | 1.125 ± 0.295 | 100 | −0.021 ± 0.011 |
| Bariloche | 41°08'60.0"S | 71°27'00.0"W | 6 | 7.375 ± 0.9740 | 6.050 ± 0.974 | 0.792 ± 0.121 | 0.480 | 0.786 ± 0.047 | 0.858 ± 0.051 | 0.250 ± 0.164 | 100 | 0.041 ± 0.147 |
| El Bolsón | 41°59'20.7"S | 71°33'39.9"W | 14 | 10.500 ± 1.086 | 6.935 ± 0.937 | 0.813 ± 0.062 | 0.383 | 0.828 ± 0.031 | 0.859 ± 0.032 | 0.375 ± 0.183 | 100 | 0.029 ± 0.050 |
| Leleque | 42°24'35.0"S | 71°04'16.7"W | 20 | 10.625 ± 1.523 | 6.860 ± 0.933 | 0.825 ± 0.043 | 0.420 | 0.808 ± 0.054 | 0.828 ± 0.056 | 0.625 ± 0.498 | 100 | −0.038 ± 0.043 |
| El Blanco | 42°28'17.9"S | 71°26'55.1"W | 15 | 10.000 ± 1.069 | 6.527 ± 1.225 | 0.783 ± 0.080 | 0.382 | 0.771 ± 0.071 | 0.797 ± 0.074 | 0.250 ± 0.250 | 100 | −0.010 ± 0.043 |
| El Cajón | 42°26'55.1"S | 71°20'43.0"W | 14 | 9.250 ± 1.306 | 6.345 ± 1.306 | 0.741 ± 0.0359 | 0.205 | 0.796 ± 0.051 | 0.826 ± 0.053 | 0.375 ± 0.263 | 100 | 0.095 ± 0.085 |
| El Rincón | 42°29'42.7"S | 71°23'16.4"W | 19 | 11.500 ± 0.945 | 7.744 ± 0.849 | 0.894 ± 0.050 | 0.414 | 0.859 ± 0.016 | 0.883 ± 0.017 | 0.625 ± 0.375 | 100 | −0.037 ± 0.044 |
| Va. Lago Rivadavia | 42°33'27.9"S | 71°38'03.4"W | 14 | 8.250 ± 1.114 | 5.347 ± 0.792 | 0.705 ± 0.068 | 0.486 | 0.757 ± 0.057 | 0.785 ± 0.059 | 0.125 ± 0.125 | 100 | 0.065 ± 0.059 |

Na, average of the N° of alleles; Ne, average of the N° of effective alleles; Ho, observed heterozygosity; P-value, statistical significance between Ho and He; He, expected heterozygosity; UHe, unbiased expected heterozygosity; N° Priv. alleles, N° of alleles unique to a single population; %P, percentage of polymorphic loci; F, fixation index.

**Table 3** Pairwise $F_{ST}$ values among *Oligoryzomys longicaudatus* populations at a regional scale

| | Junín | Bariloche | Bolsón | Leleque | Cajón |
|---|---|---|---|---|---|
| Junín | 0.00000 | | | | |
| Bariloche | 0.02708 | 0.00000 | | | |
| Bolsón | 0.02814 | 0.02843 | 0.00000 | | |
| Leleque | 0.05887 | 0.09737 | 0.08045 | 0.00000 | |
| Cajón | 0.04161 | 0.06940 | 0.06533 | 0.01507 | 0.00000 |

of Cholila separated, on average, by 15 km. We included a sample from the Patagonian steppe (Leleque), about 33 km aside from Cholila (Fig. 1). Geneland assigned each population to a different cluster (Fig. 5) and individuals from the two most distant localities presented high membership to clusters 4 and 5, while individuals from intermediate localities presented admixed ancestry (Fig. 6) indicating restricted gene flow at the geographic scale here considered.

In a previous study performed in one of the valleys of Cholila (Villa Lago Rivadavia), González-Ittig *et al.* (2015) found that high levels of gene flow encompassing different habitats (forests and shrublands) would be a major process producing fine-scale temporal changes in the genetic composition of the studied population. However, in this study using a wider geographic scale, we detected restricted gene flow; migration rates proved to be higher between closer populations and to follow an asymmetrical direction (from el Blanco and El Cajón to Leleque and from El Rincón to Villa Lago Rivadavia) (Fig. 7). In contrast, migration rates between El Rincón and El Blanco (which are also nearby populations) were very low, reinforcing the idea of local barriers preventing gene flow between these populations. Thus, it is highly probable that the lake named Mosquito, the urban zone and the secondary road located between El Rincón and El Blanco, act as barriers, limiting individual exchange between the two populations (Fig. 2a). Regarding the genetic differences found between El Rincón and El Cajón, which are also nearby locations, the combination of features like the lake Mosquito and the secondary road would be restricting gene flow levels. This road and the river that coasts Villa Lago Rivadavia population could be exerting the same effect, at least under normal population density levels. All these results were supported by the causal modeling analysis, which showed significant correlation between genetic distance and hypothesis 1 (Table 5), where lakes and the urban zone have high resistance values to gene flow, mountain ranges, rivers and main roads have intermediate resistance values, and hills together with secondary roads, low resistance values (Fig. 2b). Cushman *et al.* (2013) provided a guide, with four steps, to interpret the results of the diagnostic partial Mantel tests. Our results are in accordance with step 1, this is: '…*when one finds that a particular resistance hypothesis is supported independently of alternative models using causal modeling with partial Mantel tests, this is likely to be correct, given that the elevated Type I error is a bias in the opposite direction.*' The other three steps are applied when there is more than one significant resistance hypothesis, which is not our case. Besides, Cushman *et al.* (2013) proposed two additional methods to reduce Type I error in the implementation of
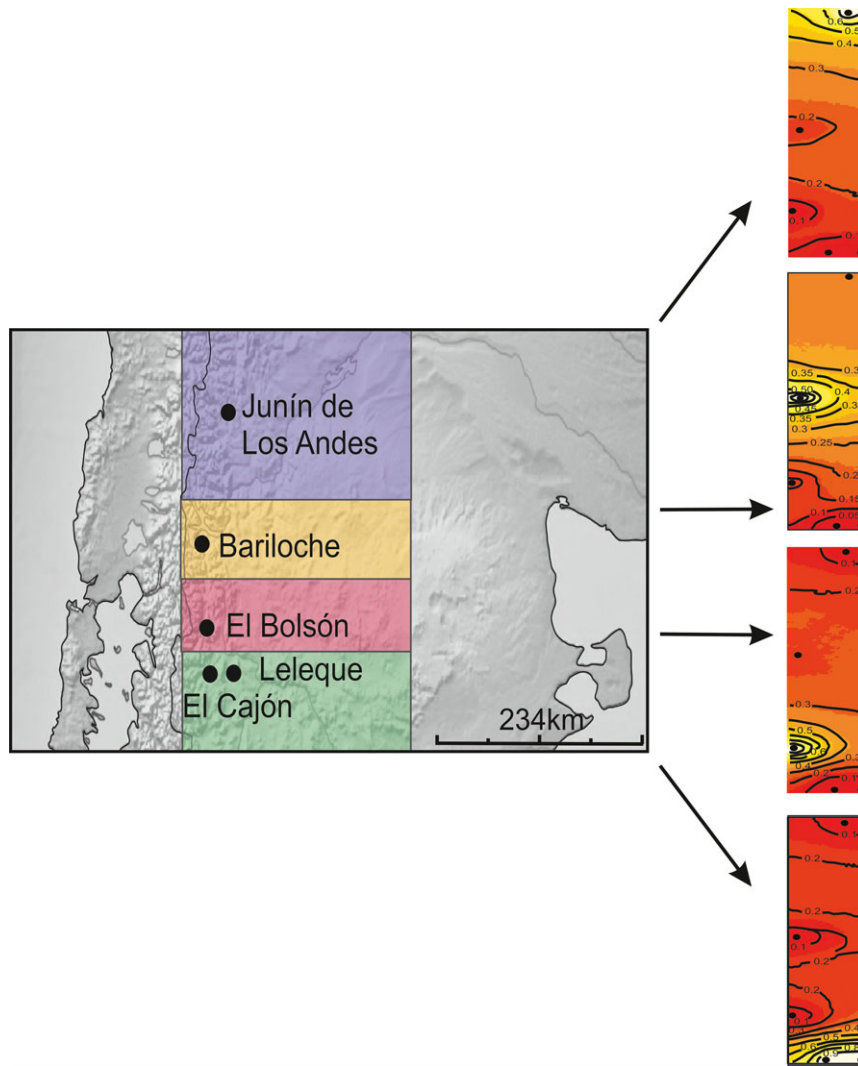
**Figure 3** Spatial distribution of *Oligoryzomys longicaudatus* genetic clusters estimated by Geneland at a regional scale and their posterior belonging probabilities. The highest membership values are in white.
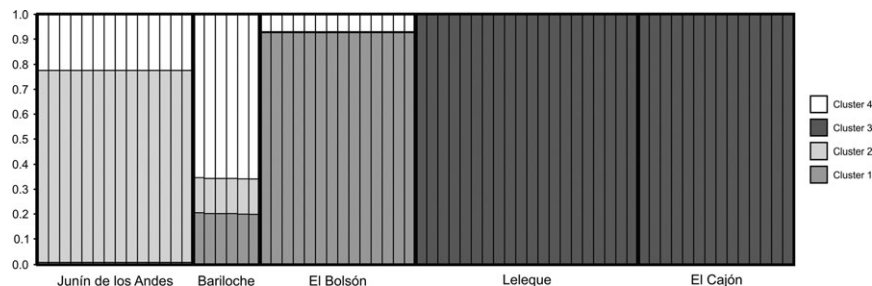


**Figure 4** Geneland Bayesian cluster analysis of multilocus microsatellite genotypes of *Oligoryzomys longicaudatus* specimens at a regional scale. Each individual within a population is represented by a thin vertical line, partitioned into segments that represent the probability of an individual to belong to one of the four estimated genetic clusters.

the causal modeling analysis: (a) To use a more stringent alpha level to evaluate significance of hypotheses; in our study, the 'P' value of hypothesis 1 is 0.0001, which means that even using a lower alpha level it will still be significant. (b) To compare the relative magnitude of the Mantel *r* values from the different hypotheses rather than the *P*-values; here, the

**Table 4** Pairwise $F_{ST}$ values among *Oligoryzomys longicaudatus* populations at a landscape scale

|           | Leleque | Blanco  | Cajón   | Rincón  | Rivadavia |
|-----------|---------|---------|---------|---------|-----------|
| Leleque   | 0.00000 |         |         |         |           |
| Blanco    | 0.03053 | 0.00000 |         |         |           |
| Cajón     | 0.01507 | 0.02892 | 0.00000 |         |           |
| Rincón    | 0.06434 | 0.06114 | 0.05439 | 0.00000 |           |
| Rivadavia | 0.07754 | 0.10668 | 0.09152 | 0.03330 | 0.00000   |

$r = 0.1665$ for hypothesis 1 (Table 5) is an order of magnitude or a 2.5-fold higher than the second highest $r$ ($r = 0.0675$ for hypothesis 5). Hence, hypothesis 1 best explains the restrictions to *O. longicaudatus* dispersal at a landscape scale. None of the other partial Mantel tests were significant (Table 5).

In periods of population outbreaks ('ratadas'), most barriers are easily surpassed (V. Andreo & F. Polop, pers. obs.); however, these phenomena occur occasionally and last for very short periods (Sage *et al.*, 2007), probably having a weak
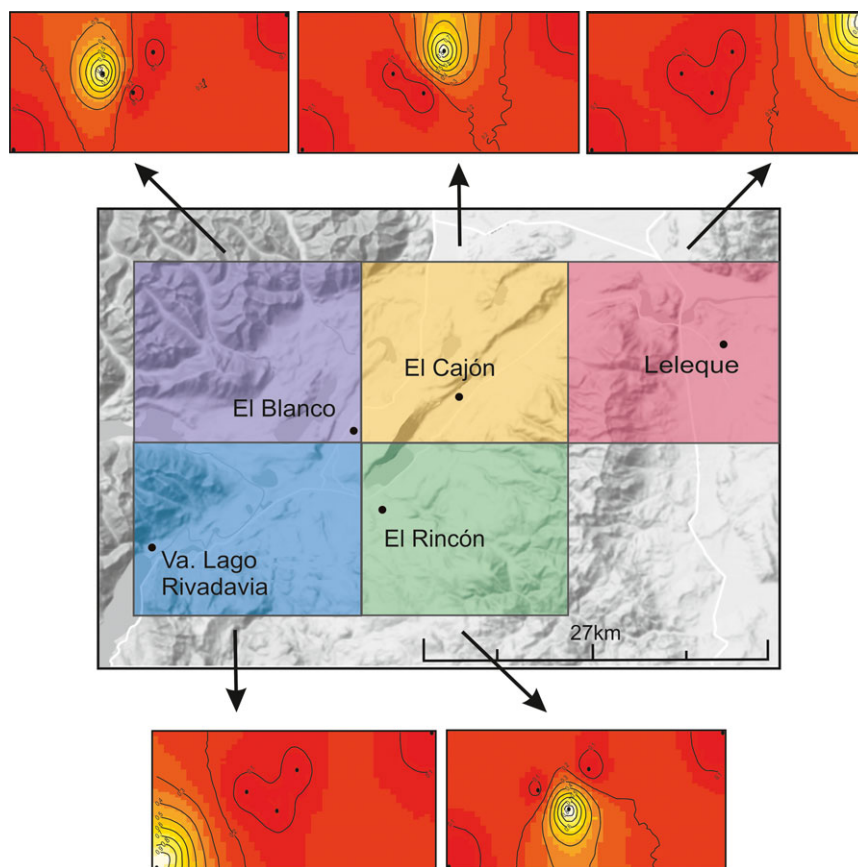


**Figure 5** Spatial distribution of *Oligoryzomys longicaudatus* genetic clusters estimated by Geneland at a landscape scale and their posterior belonging probabilities. The highest membership values are in white.
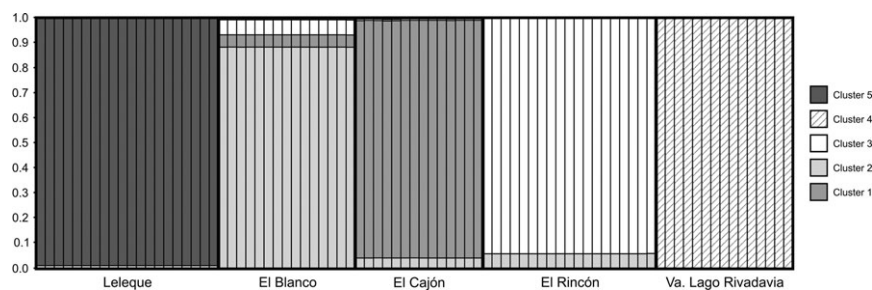


**Figure 6** Geneland Bayesian cluster analysis of multilocus microsatellite genotypes of *Oligoryzomys longicaudatus* specimens at a landscape scale. Each individual within a population is represented by a thin vertical line, partitioned into segments that represent the probability of an individual to belong to one of the five estimated genetic clusters
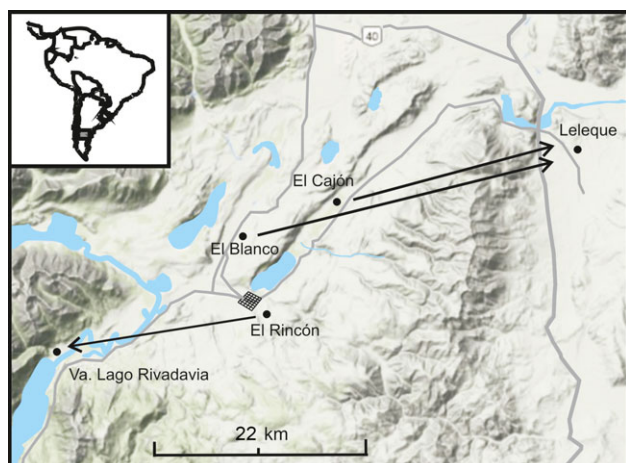
**Figure 7** Representation of migration rates between pairs of *Oligoryzomys longicaudatus* populations at a landscape scale. Each arrow represents the direction of the migration, and its width is proportional to the magnitude of the estimated rate. Only migration rates higher than 0.20 are represented: El Blanco-Leleque (0.2613), El Cajón-Leleque (0.2606) and El Rincón-Va Lago Rivadavia (0.2192). All the results of BayesAss are shown in Supporting Information Table S2.

effect on the genetic population structure of the species in the area. As a conclusion of the overall causal modeling analysis, the urban zone and lakes were identified as the most important barriers to gene flow (Fig. 2b); hypothesis 1 was the only significant (where resistance for both the urban zone and lakes had a value of 80). Nevertheless, it is important to bear in mind that the mentioned features may act in combination to model the population genetic structure of *O. longicaudatu*s. The effect of the combination of certain geographic features over population genetic structure was also examined by Coulon *et al.* (2006), which studied the roe deer *Capreolus capreolus* on a landscape containing several putative barriers to dispersal. The authors found that the combination of features such as highways, rivers and canals with different permeability values can lead to population differentiation. Regarding rodents, Garrido-Garduño *et al.* (2016) studied the genetic structure of the Painted spiny pocket mouse *Liomys pictus* and found that populations were clustered according to clear landscape boundaries, mainly defined by precipitation and stream channels. Furthermore, Gerlach & Musolf (2000) found the same pattern for the bank vole *Clethrionomys glareolus*, where rivers and highways acted as strong barriers to gene flow.

In conclusion, a latitudinal genetic structure was found at the regional scale, indicating low levels of current gene flow among *O. longicaudatus* populations. The high resolution of microsatellites markers enabled the detection of genetic gaps among Patagonian populations. Besides, it also allowed performing the study with a landscape genetic approach. At this scale, gene flow followed an asymmetrical direction, and was of variable magnitude among geographically nearby populations. We tested the effect of certain geographic features with different permeability to gene flow and found that the urban zone and lakes restricted gene flow in the area, while rivers would exert an intermediate resistance. Hills would be the less restrictive barriers to gene flow on *O. longicaudatus* populations. This result would explain why populations separated by few kilometers were so different in their genetic composition.

**Table 5** Results of the Mantel and partial Mantel tests for the analyses of isolation by resistance to gene flow among *Oligoryzomys longicaudatus* populations

| Matrix 1 | Matrix 2 | Partialled out | $r$ | $P$-value |
|---|---|---|---|---|
| Simple Mantel test between genetic distance and all hypotheses | | | | |
| Genetic distance | Null hypothesis | | −0.0220 | 0.1035 |
| Genetic distance | Hypothesis 1 | | 0.1669 | 0.0001* |
| Genetic distance | Hypothesis 2 | | −0.0155 | 0.1956 |
| Genetic distance | Hypothesis 3 | | 0.0658 | 0.1305 |
| Genetic distance | Hypothesis 4 | | 0.0623 | 0.1444 |
| Genetic distance | Hypothesis 5 | | 0.0676 | 0.1256 |
| Partial Mantel tests between genetic distance and resistance hypotheses, discounting the effect of geographic distance | | | | |
| Genetic distance | Null hypothesis | Geographic distance | −0.0303 | 0.0603 |
| Genetic distance | Hypothesis 1 | Geographic distance | 0.1665 | 0.0001* |
| Genetic distance | Hypothesis 2 | Geographic distance | −0.0005 | 0.4906 |
| Genetic distance | Hypothesis 3 | Geographic distance | 0.0658 | 0.1289 |
| Genetic distance | Hypothesis 4 | Geographic distance | 0.0621 | 0.1429 |
| Genetic distance | Hypothesis 5 | Geographic distance | 0.0675 | 0.1271 |
| Partial Mantel tests between genetic distance and geographic distance, discounting the effect of resistance hypotheses | | | | |
| Genetic distance | Geographic distance | Null hypothesis | −0.0193 | 0.1320 |
| Genetic distance | Geographic distance | Hypothesis 1 | −0.0188 | 0.1423 |
| Genetic distance | Geographic distance | Hypothesis 2 | −0.0157 | 0.2012 |
| Genetic distance | Geographic distance | Hypothesis 3 | −0.0219 | 0.1043 |
| Genetic distance | Geographic distance | Hypothesis 4 | −0.0216 | 0.1067 |
| Genetic distance | Geographic distance | Hypothesis 5 | −0.0219 | 0.1034 |

$P < 0.05$ are shown with an asterisk.

As mentioned before, *O. longicaudatus* is the natural reservoir of Andes hantavirus, which produces HPS, a serious disease in humans. According to our results, the probability of transmission of this virus among rodents is expected to be low from local emergence sites, at least in the area comprised by this study. Andreo *et al.* (2014) used species distribution modeling techniques to examine the potential distribution of HPS caused by Andes virus in southern Argentina. The authors found a high and positive correspondence among HPS occurrence and environmental features such as high annual mean precipitation, dry summers, low percentages of bare soil and low temperatures in the coldest month. The additional spatial and mechanistic information provided here, about landscape features limiting rodent dispersal, should be taken into account when building models to predict the expansion direction of a disease outbreak. These results will then help to enhance and focus surveillance efforts and prevention measures.

## Acknowledgements

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Allelic range, observed and expected heterozygosities for each locus in all populations of *O. longicaudatus*.

**Table S2.** Pairwise migration rates (in parenthesis, the 95% confidence interval) among *O. longicaudatus* populations at landscape scale.