Genetica (2017) 145:575–591
DOI 10.1007/s10709-017-9983-9

ORIGINAL PAPER

Landscape genetics in the subterranean rodent *Ctenomys* "*chasiquensis*" associated with highly disturbed habitats from the southeastern Pampas region, Argentina

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Received: 17 December 2015 / Accepted: 7 September 2017 / Published online: 13 September 2017

Abstract Studies of genetic differentiation in fragmented environments help us to identify those landscape features that most affect gene flow and dispersal patterns. Particularly, the assessment of the relative significance of intrinsic biological and environmental factors affecting the genetic structure of populations becomes crucial. In this work, we assess the current dispersal patterns and population structure of *Ctenomys* "*chasiquensis*", a vulnerable and endemic subterranean rodent distributed on a small area in Central Argentina, using 9 polymorphic microsatellite loci. We use landscape genetics approaches to assess the relationship between genetic connectivity among populations and environmental attributes. Our analyses show that populations of *C.* "*chasiquensis*" are moderately to highly structured at a regional level. This pattern is most likely the outcome of substantial gene flow on the more homogeneous sand dune habitat of the Northwest of its distributional range, in conjunction with an important degree of isolation of eastern and southwestern populations, where the optimal habitat is surrounded by a highly fragmented landscape. Landscape genetics analysis suggests that habitat quality and longitude were the environmental factors most strongly associated with genetic differentiation/uniqueness of populations. In conclusion, our results indicate an important genetic structure in this species, even at a small spatial scale, suggesting that contemporary habitat fragmentation increases population differentiation.

Keywords *Ctenomys* "*chasiquensis*" · Population genetics · Landscape genetics · Dispersal patterns · Subterranean rodents

Introduction

Gene flow and dispersal patterns may be affected by both landscape features (e.g. patchy habitats resulting from contemporary habitat fragmentation) and particular biological attributes of the species (e.g. age, sex, ecological niche constrains, habitat preferences), which directly affect the demography and population genetic structure (Foll and Gaggiotti 2006; Kittlein and Gaggiotti 2008; Gómez Fernández et al. 2012). Furthermore, gene flow and landscape fragmentation at different geographical scales are two opposing mechanisms determining the population structure of species. While the first homogenizes the genetic background across populations, the second one promotes their differentiation (see Garant et al. 2007 and references therein). Habitat fragmentation eventually leads to the loss of genetic variants, which increases the probability of inbreeding among individuals of each patch and threatens the viability of fragmented populations, particularly those with small sizes and low rates of dispersal (Foll and Gaggiotti 2006; Willi et al. 2006). Since natural landscapes have become progressively

Electronic supplementary material The online version of this article (doi:10.1007/s10709-017-9983-9) contains supplementary material, which is available to authorized users.

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smaller and more fragmented, understanding the ecological and evolutionary dynamics of small populations is increasingly important (Kittlein and Gaggiotti 2008).

In mammals, habitat loss and fragmentation are among major threats for survival of natural populations. Assessing the extent of these threatening processes is crucial in conservation management and has become a priority in studies of conservation biology (Ciofi et al. 1999). Given their low rate of dispersal, patchy distribution and high specificity to sandy soils (Mora et al. 2010, 2016), subterranean rodents are interesting models to test hypotheses about how the landscape attributes at different spatial scales can affect the movements of individuals. This is the case of the genus *Ctenomys* (Busch et al. 2000; Lacey 2000; Mirol et al. 2010; Mora et al. 2010; Mapelli et al. 2012a) which is distributed throughout the southern cone of South America and constitutes the most speciose group of all subterranean rodents (Reig et al. 1990; Cook and Lessa 1998; Lessa 2000).

Ctenomys "*chasiquensis*" (or Chasicó's tuco–tuco) is an entity of undefined status, originally reported for the Chasicó Lake in Buenos Aires Province by Contreras and Maceiras (1970) and Contreras (1973). However, it lacks a proper taxonomic description, since there is no formal designation of a holotype, diagnosis and description. Therefore, the name "*chasiquensis*" does not meet the criteria established by the International Code of Zoological Nomenclature (ICZN 1999: Art. 8) and is appropriately regarded as *nomen nudum* (see also Mora et al. 2016). Particularly, *C.* "*chasiquensis*" is included in the "*mendocinus* phylogenetic group", which is a monophyletic group within *Ctenomys* currently distributed from the western portion of Argentina to the southeast of Brazil. The seven distinctive species that integrate this group live in well-drained sandy soils, mostly linked to the recent Holocene sand dunes on the coastal plains of Southeastern Brazil, Southwestern Uruguay and Eastern Argentina, as illustrated respectively by *Ctenomys flamarioni, Ctenomys rionegrensis* and *Ctenomys australis* (Wlasiuk et al. 2003; Mora et al. 2006; Fernández-Stolz et al. 2007; Kittlein and Gaggiotti 2008); or associated with the Pleistocene continental dunes and paleo-dunes across the provinces of Mendoza and Buenos Aires in Argentina for *C. azarae, C. mendocinus, Ctenomys porteousi* and *C.* "*chasiquensis*" (Massarini et al. 1991; D'Elía et al. 1999; Mapelli and Kittlein 2009; Mapelli et al. 2012a; Mora et al. 2016). The suggested distribution for *C.* "*chasiquensis*" follows a highly patchy landscape of sand dunes extending from the Southwest of Buenos Aires to the Southeast of La Pampa in Central Argentina (Mora et al. 2016).

As other species of *Ctenomys*, the Chasicó's tuco–tuco is strictly associated with sandy and friable soils on median altitude dunes, avoiding low elevation areas prone to flooding. To the East, they have a fragmented distribution on dunes surrounded by lagoons, while the distribution of the species becomes more continuous and homogeneous westward. The landscape is also interrupted by the presence of some inland salt flats such as Salina Chica, Salina Colorada Grande and some streams such as Arroyo Chasicó, from which the homonymous lagoon arises (Mora et al. 2016; Fig. 1). In particular, the Chasicó Lake is the major lagoon in the area, and divides the sand dune system in three conspicuous sandy diagonals, two running west and one to the east (Fig. 1).

However, the structure of this landscape is currently being affected by forestry and the progressive advance of farming (e.g. soybean) in the region; these factors associated with the particular biological attributes of this species, could be causing a negative impact on their populations. Therefore, the persistence of this endemic species is also related to the preservation of the entire sand dune ecosystem. In order to design and implement actions for the conservation of this rodent and its particular habitat it is necessary to increase the knowledge about how habitat loss affects its genetics and population dynamics. Within this framework, the study of the boundaries and extension of populations is crucial for an accurate determination of the conservation and management scale.

Here we use multilocus genotype data in combination with landscape genetics tools to assess the population structure and gene flow patterns of *C.* "*chasiquensis*". One of the focal objectives of this research is to determine the potential effects of environmental and landscape features on the population genetic structure of the species at regional level. Like other species of *Ctenomys* (e.g. Mapelli et al. 2012a), we predict that some environmental attributes such as habitat quality (e.g. the normalized vegetation index) and the connectivity among sampling sites explain considerably the genetic differentiation among populations of this species. We also assess whether the most isolated and strongly impacted sampling sites in our study area show lower levels of gene flow between them than those populations with greater connectivity through suitable sand dune habitat.

Materials and methods

Study area and habitat characteristics

Sampling of populations was conducted in the distinctive sand dune habitat of this species which extends from Lihuel Calel National Park (in the Southwest of La Pampa Province) to Salitral de la Vidriera, close to Bahia Blanca (Southeast of Buenos Aires Province; see Fig. 1). The sampling area is included in the Pampas region, Argentina (Zárate and Tripaldi 2012) and is composed by sandy and silty deposits (loess).

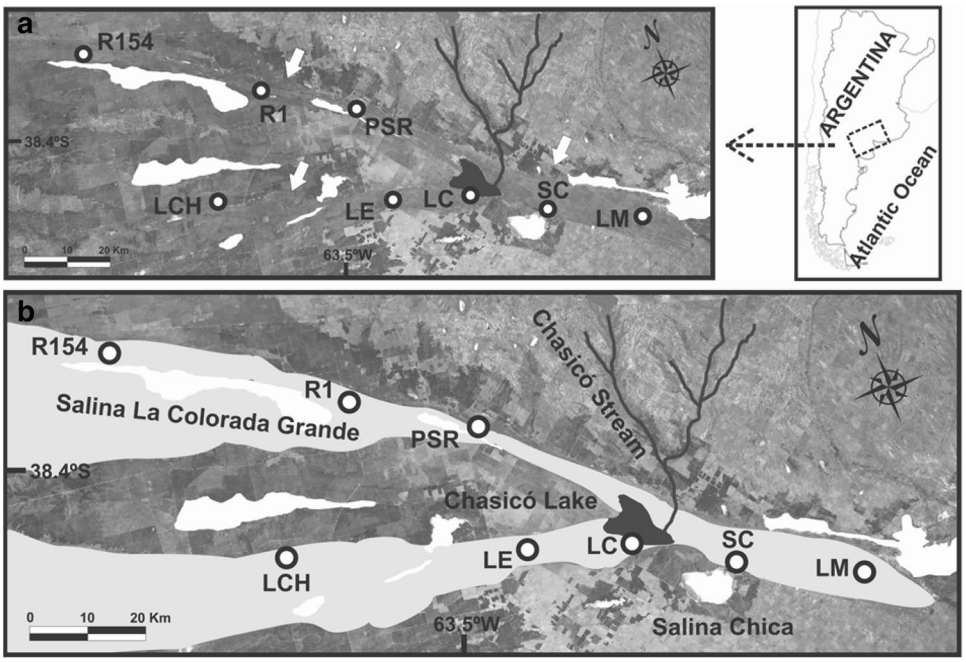

Fig. 1 Distribution of *Ctenomys* "*chasiquensis*" from the southwest of Buenos Aires and southeast of La Pampa (Argentina). References: Chasicó Lake (LC; 38°39′S, 63°5′W), La Chufla (LCh; 38°33′S, 63°44′W), La Equidad (LE; 38°38′S, 63°17′W), La Mascota (LM; 38°47′S, 62°38′W), Pulpería San Rosario (PSR; 38°23′S, 63°22′W), Ruta1 (R1; 38°18′S, 63°36′W), Ruta154 (R154; 38°8′S, 64°4′W), and Salina Chica (SC; 38°44′S, 62°52′W). The figure was edited using Landsat 7 ETM+ satellite images (three concatenated satellite images), available at the US Geological Survey repository (http://earthexplorer.usgs.gov). **a** Polygons in white represent the most important salt inland flats in the region. White arrows denote the three sandy diagonal where this species is more likely to be found. **b** Polygons in light gray represent the most suitable habitat for this species on sandy diagonals. Digitalization of these polygons was completed from an habitat supervised classification using satellite images (Landsat 7 ETM+), and were processed using the package "raster" in R (Hijmans and Etten 2012). For this approach, information about both 300 georeferenced points of mounds (product from digging activity) and captured individuals in their sampling sites were considered. Chasicó Lake and Chasicó Stream indicate the most significant barriers within the sandy landscape

The origin of this sand dune landscape (known as "Aeolian Pampean System") can be traced to the Quaternary period, when climatic perturbations led to cooler and drier conditions in the eastern and central portions of Argentina (Tonni et al. 1999; Quattrocchio et al. 2008; Zárate and Tripaldi 2012). Two sandy paleo-drainage basins that integrate this major aeolian unit, running in northwest-southeast and east–west directions, converge near Chasicó Lake. These basins correspond to beds of paleo streams that were filled with sands blown from western regions. Except for some landscape depressions and brackish lagoons (e.g. depressions of Salinas Grandes, Chasicó Lake and Chasicó stream), this landscape is characterized by the presence of sand dunes located along the side valleys of northwest-southeast direction. Particularly, populations of *C.* "*chasiquensis*" are almost continuous on three visible sand dune diagonals along these basins (Fig. 1). Their higher densities occur along the central dunes but they are also present along the outer borders. These sandy diagonals (sediments in the diagonals are conformed by slime-sand material of eolian origin; additional data are given in Online Resources 1 and 2) are surrounded by a highly fragmented landscape where the sandy environments with better aptitude for the occupation of this species disappear considerably (Mora et al. 2016). To the East, as the basins enter the Pampas grassland, plant cover increases and tuco-tucos are restrained to the less vegetated sandy patches.

Distance between sampling sites ranged from 20 to 40 km (geographic location of sampling sites are also shown in Online Resource 2). The Chasicó Lake and the Chasicó stream constitute the most important natural barriers to dispersal of this species and basically divide their distribution between two groups of populations: the westernmost sampling sites (LCh, R1, R154, PSR, LE, LC) located at the West of Chasicó Lake (included into two sand dune diagonals), and the easternmost sampling sites (SC, LM) located at the East of this lagoon (included into the eastern sand dune diagonal; Fig. 1). The eastern unit presents more percentage of loess and silt and higher levels of precipitation resulting in a higher diversity of grassland species in comparison to the West (Zech et al. 2009; see Fig. 1). It should be noted that vegetation in the eastern unit (Online Resource

1) has been highly modified by clearings, cultivation, introduction of domestic livestock (especially cattle and sheep) and the occurrence of accidental and prescribed fires during the last two centuries (Zárate and Tripaldi 2012). In contrast, the western unit is less affected, resulting in a continuous sand dune habitat (Fig. 1).

Sample collection and DNA extraction

Tissue samples were obtained from a total of 114 individuals from 8 sampling sites distributed across the study area (Fig. 1). Oneida Victor Nº0 snap traps were used to live trap the individuals, using a rubber cover to avoid any damage (Mora et al. 2006, 2016). Individuals were released back within the same burrow system where they had been initially captured. Sampling was conducted during 2009 and 2010. Positions of captures were recorded with a GPS.

Because of the difficulties for procuring blood samples in tuco-tucos, tissue samples were obtained from the first phalange from the little finger of the left foot (<1.5 mm), and were preserved in 95% ethanol at −70 °C. Our experience indicates that this method neither affects survival nor digging performance of the individuals (Mora et al. 2006, 2007, 2010, 2016; Cutrera et al. 2010; Mapelli et al. 2012a). All individuals captured were treated with lidocaine and disinfectant in order to avoid pain and prevent possible infections. All parts of this study concerning the handling of animals in the field followed guidelines of the American Society of Mammologists (Sikes et al. 2011), and were also approved by the "Dirección de Flora y Fauna, Ministerio de Asuntos Agrarios, Provincia de Buenos Aires, Argentina" (file 22,500–24,372/13). Tissue vouchers were prepared and deposited in the collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Ciudad Autónoma de Buenos Aires, Argentina (Online Resource 2). Genomic DNA was isolated following a protocol modified from Miller et al. (1988), involving treatment with sodium-dodecyl-sulphate (SDS) and digestion with proteinase-K, NaCl precipitation of proteins, and subsequent isopropyl alcohol precipitation of DNA, as was described in Mora et al. (2006, 2007, 2016).

Microsatellite amplification

Molecular analyses were performed using 9 polymorphic microsatellite loci, isolated from the Argentinean species *Ctenomys sociabilis* (Soc 1, Soc 2, Soc 5, Soc 7 and Soc 8, Lacey 2001) and *Ctenomys haigi* (Hai 2, Hai 5, Hai 9 and Hai 10, Lacey et al. 1999). Microsatellite loci were amplified either as multiplex PCR or individually using fluorescently labeled primers. Multiplex PCR amplifications were carried out in a reaction volume of 12 µl and the individual amplifications in a reaction volume of 10 µl, each containing 30 ng of DNA as template. We used the following reagents and final concentrations: 0.1 units of Taq Polymerase, 10× Taq Polymerase buffer, dNTPs 0.8 mM, primers at 0.2 mM each and $MgCl_2$ at 2.5 mM. The thermocycling profile included an initial denaturing step at 94 °C for 1 min, followed by 25–35 cycles of 94 °C for 30 s, annealing at 56–62 °C (depending on multiplex and locus, see Online Resource 3) for 30 s followed by a second annealing step at 60 °C for 30 s, and a final extension at 72 °C for 5 min. Negative controls were included in all PCR runs to check for contamination. Genotypes were subsequently analyzed in a MegaBace 1,000 automated sequencer (GE Healthcare). Resulting patterns were scored and analyzed using the MegaBACE Fragment Profiler 1.2 software (Amersham Biosciences).

Statistical analysis for microsatellites

Null alleles, Hardy–Weinberg, linkage disequilibrium and genetic diversity

Genetic diversity at each sampling site (N_i) and observed (H_o) and expected (H_e) heterozygosity (Nei 1978) were calculated using ARLEQUIN 3.0 (Excoffier et al. 2005). The characterization of the genetic variability of each locus through the polymorphic information content ($PIC = 1 - \sum (P_i)^2$, where P_i corresponds to the proportion of samples carrying the ith allele at a particular locus) was obtained using GenAlEx 6.5 (Peakall and Smouse 2012). Analysis of linkage disequilibrium between pairs of loci and possible deviations from Hardy–Weinberg equilibrium were carried out using ARLEQUIN 3.0 (Excoffier et al. 2005). We used 1000 dememorization steps and 100,000 iterations for the Markov chain implemented by the method of Guo and Thompson (1992). Sequential Bonferroni corrections for tests of linkage disequilibrium were applied to compensate for an inflated type-one-error (Rice 1989). Cervus 3.0 (Kalinowski et al. 2007) was used to evaluate the existence of null alleles within each sampling site, with a cut-off point of 0.1.

Population structure and gene flow

We computed pairwise R_{ST} (Slatkin 1995) between sampling sites. This parameter takes into account the size of the alleles and assumes a stepwise mutation model, and proved to perform better for small sample sizes than the analogous measure of genetic differentiation of Weir and Cockerham (1984; see Slatkin 1995; Goodman 1997). These analyses were also implemented using ARLEQUIN 3.0 (Excoffier et al. 2005).

In order to infer the partitioning of genetic variance within and among populations three Analyses of Molecular Variance (AMOVAs) were performed using ARLEQUIN 3.0

(Excoffier et al. 2005). AMOVAs were carried out considering (1) the eight sampling sites as independent units, (2) the differentiation between the western and eastern sampling units (defined on the base of the geographic position of these populations relative to the Chasicó Lake and the Chasicó stream), and (3) comparing the three sandy diagonals of suitable habitat for this species.

A Mantel test (Mantel 1967) between pairwise estimates of R_{ST} and linear geographic distances among localities was used to check the fit of the data to a model of isolation by distance (IBD, Slatkin 1993). IBD pattern is expected in this species due to its limited dispersal abilities (Mora et al. 2016) and the assumption of migration-drift equilibrium. Also, we correlated pairwise estimates of R_{ST} and geographic distances based on the most suitable sand dune habitat (along the sandy diagonals). Thus, we determined the configuration and extension of the most suitable habitat for *C*. "*chasiquensis*" using the package "raster" in R (Hijmans and Etten 2012). The analysis of 2 LANDSAT ETM+ images (30 m of resolution) covering the study area of the species (from R154 to LM; Fig. 1) helped us to determine the extent of the most suitable habitat for *C*. "*chasiquensis*". The information on the geographic position (recorded with GPS) of more than 100 individuals collected in the studied locations were used to obtain the spectral signature for the 7 ETM+ bands and determine the total area that potentially could be occupied by the species. Using a maximum likelihood method available in the same package, we determined areas statistically equivalent to those where the presence of individuals of this species was recorded. Potential barriers to gene flow (e.g. salt flats) were taken into account in the construction of the distance matrix of unsuitable habitat for this species. Because the use of Mantel tests in spatial analysis has been recently questioned (Legendre et al. 2015), we applied a spatial principal components analysis using functions available in the R-package "adegenet" (sPCA; Jombart 2008). In our analysis the sPCA yielded scores summarizing both genetic variability and the spatial structure among localities using Moran's eigenvector maps (MEMs). Spatial variation is represented by a connection network using a K-nearest neighbors approach. Global structure is evaluated by assessing if geographically close populations are genetically more similar than expected under a random spatial distribution of genetic variation. The observed test statistic (comparable to a r-square statistic, depicting associations of alleles to vectors in the matrix of global MEMs) is compared to the distribution of test statistics obtained through a Monte Carlo randomization procedure using 9999 permutations (Jombart 2008; Vonhof et al. 2016).

In addition, the hierarchical Bayesian routine reported in Foll and Gaggiotti (2006) and implemented in GESTE 2.0 was run in order to estimate local F_{STs}. This method uses the approach proposed by Balding and Nichols (1995) to estimate individual F_{ST} values for each local sampling site. Basically, this fixation indexes can be understood as a measure of genetic differentiation of each local population relative to the entire metapopulation (Foll and Gaggiotti 2006). The higher values of local F_{ST}s denote greater differentiation of a particular population compared to all sampling sites (or the entire study area) as a whole. We used 10 pilot runs of 5×10^4 iterations to obtain the parameters of the proposal distributions used by the MCMC algorithm. After a burn in of 1×10^5 iterations, the estimates of F_{ST} local values were obtained using 2×10^7 additional iterations with a thinning interval of 20. Two independent runs with identical setting values were performed to check for consistency of the estimates.

We used the Bayesian clustering method implemented by STRUCTURE 2.1 to estimate the number of genetic populations and assign individuals to them (Pritchard et al. 2000). We assumed the model with correlated allele frequencies and population admixture. Five independent runs were conducted for each K value (which varied from one to eight). We used 1.5×10^6 iterations as burn-in and consider 1×10^6 additional iterations for the final estimations of parameters. Detection of the number of genetic groups (K) that best fit the data was carried out using both the logarithm of the posterior probability [LnP(D)] as a function of k, and the method proposed by Evanno et al. (2005), based on ad hoc statistic Δk which depends on the rate of change in the log probability of the data between successive values of K. After assessing the value for K, we chose the run with the higher posterior probability and lower variance for interpreting results.

Landscape data acquisition

We assessed the potential effects of eight environmental variables on the genetic structure of *C*. "*chasiquensis*" using environmental variables that express landscape traits at a local level as well as habitat features that are related to connectivity among sampling sites (see Mapelli et al. 2012a). Environmental variables included Latitude (Lat) and Longitude (Long), both in UTM coordinates; mean distance from the focal site to all other sites (MDist); mean distance from the focal site to all other sites considering the most suitable habitat for this species (MDist2); habitat suitability (%hab), expressed as the proportion of suitable habitat around the sampling site (see Mapelli and Kittlein 2009 for further reference); average (Elav) and standard deviation (DSElav) of elevation; and the normalized vegetation index (NDVI). The last four variables were recorded in a 1-km-radius circle centered at each sampling location. Variables were first extracted from digital elevation models and Landsat images available at the United States Geological Survey repository (http://earthexplorer.usgs.gov); then calculated from the

digital media using the package "raster" in R (Hijmans and Etten 2012). Values of these variables are detailed in Online Resource 4.

Given that some sampling sites have a more isolated position in relation to the rest within the study area, MDist was used as a variable controlling for the possible effects of sampling design in the differentiation among localities. This variable takes into consideration the average distance of each site to all other sampling sites. On the other hand, we also calculated an additional variable which considers the average distance of each site to all other sampling sites on the most suitable habitat for *C*. "*chasiquensis*" (MDist2). Here, some barriers such as salt flats, the Chasicó Lake and the Chasicó Stream were taken into account for dispersal (movements only occur on the sandy diagonals).

The basin formed by the Chasicó Lake and the Chasicó stream, with an area of approximately 4400 km^2, is located in a region of lowland plains, subject to regular flooding periods. Accordingly, the surface of the Chasicó Lake fluctuates periodically in relation to rain, affecting habitat availability for tuco-tucos (Mapelli and Kittlein 2009; Mora et al. 2016). For this reason, variables denoting elevation of the sampling sites (Elav and DSElav) were considered as a proxy for the effects of flooding in the study area, which can lead to local population bottlenecks and increased genetic drift, as it was suggested for other species of tuco-tucos by Kittlein and Gaggiotti (2008) and by Mapelli et al. (2012a).

For tuco-tucos, vegetation cover is expected to be negatively correlated with habitat suitability, since subterranean rodents, in general, are restricted to friable soils with low vegetation cover where the cost of burrowing activities is lower and aeration of soils is higher (Busch et al. 2000; see also; Mapelli and Kittlein 2009). For this reason, NDVI was calculated defining a buffer zone of 1 km surrounding each population sampled (see Mapelli and Kittlein 2009 for further reference). Low values of NDVI correspond to poorly vegetated or bare soils whereas high values indicate an important vegetation cover. For the purposes of this work, NDVI (proxy of plant cover) is a reflection of the habitat quality, with higher values of the NDVI negatively correlated to population density and patch occupancy (Mapelli and Kittlein 2009; Mapelli et al. 2012a).

Landscape genetic analysis

In order to assess the potential effect of the eight different environmental and landscape variables (previously described in the ''Landscape data acquisition'' section) on the population genetic structure, we used the hierarchical Bayesian method implemented in GESTE 2.0 (Foll and Gaggiotti 2006). This method uses a generalized linear model and a reversible jump MCMC routine to correlate local F_{ST} values to landscape factors. The posterior probability of each environmental characteristic, inferred from the number of times the algorithm visited each model, is used to identify those factors that most influence population genetic structure. GESTE run settings were the same as in local F_{ST} estimation.

Additionally, the relationship between genetic differentiation ($F_{ST}/(1 - F_{ST})$, Rousset 1997) and environmental factors was tested using Mantel and partial Mantel tests (Mantel 1967). A permutation approach available in PASSage v. 2 (Rosenberg and Anderson 2011) were used to test the significance of the Mantel and partial Mantel tests. Spatial structure was taken into account using Moran's eigenvector maps (MEMs) as outlined above in isolation by distance analyses.

Historical demography

We used 2MOD (Ciofi et al. 1999), in order to examine the demographic history at each sampling site. The probability that two alleles are identical by descent is denoted by the frequency distribution of *F* values, which can be interpreted as a relative measure of the effect of genetic drift and dispersal on individual localities (Ciofi et al. 1999). The MCMC simulation was run for 1×10^5 iterations, and the initial 10% of data was discarded in order to obtain a reliable posterior distribution of *F*. Three independents runs were used to check the consistency of estimates.

Furthermore, we used the 2MOD program (Ciofi et al. 1999) to evaluate the fit of the data to a model of equilibrium between local genetic drift and gene flow (required for the establishment of an IBD pattern). In this approach two models of population structure are tested computing their relative likelihoods, considering either migration-drift equilibrium or pure drift. A model of pure genetic drift, for instance, takes into consideration that allele frequencies in each locality were only shaped by random changes, where the effect of dispersal between populations is negligible. On the other hand, in a situation of migration-drift equilibrium, the current allele frequencies in the populations have been the result of a balance between gene flow and local genetic drift. 2MOD compares the likelihoods under both scenarios. The run settings for 2MOD were the same as those for the estimation of *F* values.

Results

Null alleles, Hardy–Weinberg, linkage disequilibrium and genetic diversity

Across loci, null allele frequencies were low (range 0.01–0.09) and below the 0.1 threshold that would significantly bias population genetic studies. Only two loci (Hai 9 and Soc 2) showed a significant probability to present high

frequency of null alleles considering all populations as a whole, although these values were in the limit regarding the cut-off point of 0.1 (P = 0.11 and 0.1, respectively). These could be due either to a homozygote excess resulting from genetic structure among sampling sites or to non-amplifying alleles in a particular locus (Kalinowski et al. 2007). However, most sampling sites did not present high probabilities of null alleles for these two loci, and consequently, they were included for the analyses.

Genetic diversity parameters for each population are shown in Table 1. Departures from Hardy–Weinberg equilibrium were found in different loci and different sampling sites: Hai 2 in PSR, Hai 9 in LM, Hai 10 in LE, Soc 2 in LE, PSR and R154, Soc 5 in LCh and R154, and Soc 7 in LCh and R1 (Table 1). Linkage disequilibrium tests revealed linkages between pairs of loci, but these linkages were not consistent across populations (data not shown). As in previous studies in other species of the genus where no physical linkage among these loci was identified (see Wlasiuk et al. 2003; Cutrera et al. 2005; Mora et al. 2010; Mirol et al. 2010; Gómez Fernández et al. 2012; Mapelli et al. 2012a), we therefore considered all loci as independent. The nine loci used in this study were polymorphic (PIC > 0.70; Table 1), with more than 11 alleles per locus. Allele richness and the number of alleles per locus increased towards the west of the species's distribution (LCh, R154 and R1 presented the highest levels).

Population structure and gene flow

Most R_{ST} comparisons showed significant differences, except for the following pairs of sampling sites: LC/R1, LCh/R1, and R1/R154 (Table 2). R_{ST} values varied between 0.007 (e.g. between R1 and R154) and 0.23 (between LC and SC). LM/SC and LC/SC were the most differentiated populations, while LC and the northwestern sampling sites showed the highest values of gene flow (Table 2).

When all sampling sites were considered without any kind of grouping, differentiation was significant (AMOVA, $\Phi_{ST} = 0.15$, $p < 0.001$; Table 3). On the other hand, neither

Table 1 Genetic diversity parameters in *C.* "*chasiquensis*" from microsatellite loci

Sample sites (N)			LC (15)				LCh (14)				LM (15)				LE (14)			
Loci	At	PIC	N_i	H_o	H_e	r	N_i	H_o	H_e	r	N_i	H_o	H_e	r	N_i	H_o	H_e	r
Hai 2	12	0.88	6	0.69	0.83	5.88	7	0.70	0.77	6.70	6	0.71	0.67	5.13	5	0.75	0.72	4.69
Hai 5	12	0.73	6	0.67	0.71	4.99	7	0.71	0.65	5.53	5	0.50	0.48	4.16	7	0.87	0.85	6.41
Hai 9	15	0.89	10	0.77	0.88	8.35	9	0.92	0.88	8.09	10	0.5*	0.89	8.87	8	0.79	0.79	7.18
Hai 10	14	0.86	7	0.73	0.85	6.47	7	0.79	0.81	6.13	6	0.71	0.65	5.15	7	0.53*	0.72	5.93
Soc 1	15	0.92	11	0.8	0.89	9.10	10	0.74	0.82	8.10	8	0.71	0.73	6.49	5	0.67	0.71	4.64
Soc 2	14	0.9	8	0.80	0.86	7.18	9	0.69	0.89	8.10	6	0.85	0.76	5.30	7	0.71*	0.79	6.12
Soc 5	13	0.88	8	0.93	0.88	7.35	10	0.64*	0.90	8.60	6	1.00	0.80	5.49	9	0.93	0.89	8.01
Soc 7	13	0.87	9	0.93	0.87	7.52	5	0.46*	0.73	4.70	6	0.85	0.81	5.80	7	0.62	0.80	6.21
Soc 8	14	0.88	8	0.87	0.88	7.60	11	1.00	0.91	9.48	6	0.83	0.82	5.50	6	0.82	0.81	5.79
Mean	13.33	0.87	8.11	0.80	0.85	7.16	8.33	0.74	0.82	7.27	6.56	0.74	0.73	5.77	6.78	0.74	0.79	6.11
Sample sites (N)			PSR (13)				R1 (14)				R154 (17)				SC (12)			
Loci	At	PIC	N_i	H_o	H_e	r	N_i	H_o	H_e	r	N_i	H_o	H_e	r	N_i	H_o	H_e	r
Hai 2	12	0.88	5	0.58*	0.79	4.98	8	0.93	0.89	7.64	8	0.67	0.86	7.27	8	0.91	0.85	7.22
Hai 5	12	0.73	4	0.77	0.66	3.89	4	0.57	0.55	3.63	7	0.65	0.75	5.46	4	0.58	0.63	3.70
Hai 9	15	0.89	5	0.64	0.70	4.79	7	0.77	0.89	7.49	9	0.57	0.83	7.53	5	0.70	0.86	6.88
Hai 10	14	0.86	7	0.92	0.82	6.58	8	0.92	0.86	6.79	9	0.88	0.85	7.14	6	0.58	0.70	4.84
Soc 1	15	0.92	9	0.91	0.92	8.71	8	0.85	0.87	7.41	9	0.88	0.87	7.53	6	0.82	0.78	5.76
Soc 2	14	0.9	8	0.55*	0.89	7.74	8	0.83	0.85	7.33	10	0.53**	0.86	7.95	7	0.92	0.86	6.82
Soc 5	13	0.88	6	0.58	0.72	5.39	9	0.92	0.89	8.10	12	0.71*	0.91	9.72	9	0.75	0.85	7.92
Soc 7	13	0.87	5	0.69	0.68	4.60	10	0.64*	0.90	8.62	10	0.82	0.85	7.84	5	0.73	0.71	4.79
Soc 8	14	0.88	7	0.56	0.79	7.00	9	0.86	0.87	7.80	11	0.82	0.89	8.43	8	1.00	0.86	7.40
Mean	13.33	0.87	6.22	0.69	0.77	5.96	7.89	0.81	0.84	7.20	9.44	0.73	0.85	7.65	6.56	0.78	0.79	6.15

Significant deviations between observed and expected levels of heterozygosity in each geographical sample and locus by locus are shown.

N number of individuals per population, *At* number of alleles per locus, *N* number of alleles per population of each loci, *PIC* polymorphic information content, H_o observed heterozygosity, H_e expected heterozygosity, *r* allele richness

* $0.001 < P < 0.05$; and ** $P < 0.001$. Abbreviations for locations are defined in Fig. 1

Table 2 Pairwise R_{ST} estimates (Slatkin 1995) from microsatellite loci showing the genetic differentiation in *C.* "*chasiquensis*" amongst sampling sites, assuming a stepwise mutation model

Sandy diagonals									
	Northwest			Southwest			East		Local F_{ST} estimates
	R154	R1	PSR	LCh	LE	LC	SC	LM	
R154 (N = 17)	–								0.03 (0.01–0.045)
R1 (N = 14)	0.007	–							0.04 (0.02–0.072)
PSR (N = 13)	0.09**	0.07*	–						0.12 (0.074–0.17)
LCh (N = 14)	0.08*	0.04	0.14**	–					0.05 (0.02–0.072)
LE (N = 14)	0.1**	0.08*	0.07*	0.22**	–				0.12 (0.085–0.17)
LC (N = 15)	0.12**	0.04	0.14**	0.12**	0.12*	–			0.06 (0.03–0.083)
SC (N = 12)	0.13**	0.12**	0.06*	0.14**	0.16**	0.23**	–		0.13 (0.08–0.018)
LM (N = 15)	0.07*	0.07*	0.18**	0.21**	0.08*	0.16**	0.23**	–	0.15 (0.096–0.2)

Local F_{ST} estimates (right column) between each sampling site and the entire metapopulation (according to Foll and Gaggiotti 2006) and number of individuals per population (N) are also given

Values in parenthesis correspond to the 95% highest posterior probability interval (HPDI)

Abbreviations for sampling sites are defined in Fig. 1

* $0.001 < P < 0.05$; and ** $P < 0.001$

Table 3 Hierarchical analysis of molecular variance (AMOVA) using the sum of squares differences of R_{ST} for microsatellite data (Slatkin 1995)

Source of variation	Level of subdivision	Φ_{CT}	*P*	Φ_{ST}	*P*
No clustering of sampling sites into groups	[LCh] [R1] [R154] [PSR] [LE] [LC] [SC] [LM]			0.12	< 0.001
Among two groups of populations defined by their geographic location relative to the Chasicó Lake	[LCh, R1, R154, PSR, LE, LC] [SC, LM]	0.001	0.67		
Among three groups defined by the major continuous sandy diagonals	[PSR, R1, R154] [LCh, LE, LC] [SC, LM]	0.001	0.83		

The fixation indices (Φ-statistics) and their corresponding significance were tested by 100,000 permutations according to Excoffier et al. (1992). The respective levels of subdivision of each sampling site are shown in brackets

Abbreviations for sampling sites are defined in Fig. 1

the AMOVA performed on localities assembled into the East and West groups nor the AMOVA implemented on localities grouped according to the three sandy diagonals showed significant population substructure ($\Phi_{CT} = 0.001$, $p = 0.67$, $\Phi_{CT} = 0.001$, $p = 0.83$, respectively; see Table 3). Considering both, the results of the analyses of variance and the R_{ST} comparisons, it seems that differentiation among localities has no apparent regional pattern: isolation among local populations (most pronounced in the easternmost populations) has been more significant than the effect of the major natural barriers considered in this study.

Genetic differentiation in *C.* "*chasiquensis*" using microsatellite loci was not consistent with a simple IBD pattern. Mantel tests did not show a significant association either between pairwise estimates of R_{ST} and linear geographic distances or between estimates of R_{ST} and distances based on the suitable sand dune habitat ($R^2 = -0.11$, $P = 0.69$; $R^2 = -0.3$, $P = 0.10$, respectively; Fig. 2a, c). The tests for global structure using Moran's eigenvector maps in the sPCA analyses did not indicate spatial structure in the allele data ($R^2 = 0.26$, $P = 0.52$ and $R^2 = 0.18$, $P = 0.69$, respectively; Fig. 2b, d).

While the differentiation among populations seems not to be related to geographical distances (linear or considering the unsuitable habitat for this species), local F_{ST} values were larger in the most isolated populations from the east part of the species's distribution (Table 2). PSR, LE and the eastern peripheral populations were the most differing sites when compared to the whole metapopulation (LM, $F_{ST} = 0.15$; LE, $F_{ST} = 0.12$; PSR, $F_{ST} = 0.12$; and SC, $F_{ST} = 0.13$), while local F_{ST} values for some localities from the northwest (R154, R1) and southwest (LC, LCh) were smaller ($F_{ST} = 0.03$–0.06). Like 2MOD analysis and the R_{ST} estimations, results of local F_{ST} values suggest more limited gene flow and a stronger effect of genetic drift near the eastern boundaries of the distribution of *C.* "*chasiquensis*".

STRUCTURE results showed a high degree of admixed genotypes among northwestern and some central localities. According to the results shown previously, eastern (LM, SC) and some southwestern sampling sites (LE and LCh) were

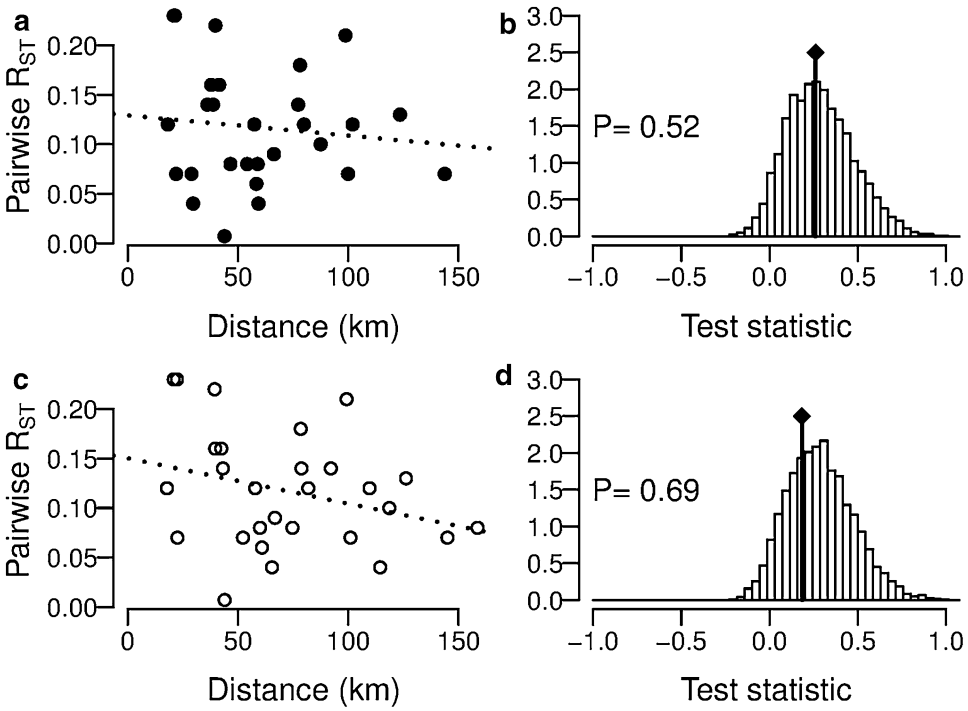

Fig. 2 Relationship between pairwise genetic differentiation among sampling sites (measured by R_{ST} estimates) and both lineal pairwise geographical distances (black circles, top panels) and pairwise geographical distances based on the most suitable sand dune habitat for the species (white circles, bottom panels). Histograms show the distribution of the test statistic for global spatial structure using Moran's eigenvector maps in sPCA; see Sect. "Material and methods"

highly differentiated from the other sites, showing the highest degree of population isolation (Fig. 3a, b; Fig. S1a, b). The logarithm of the data probability [LnP(D)] as a function of k reached a peak for k = 4 (Fig. S2a) and was consistent with the highest Δk value using the Evanno method (Fig. S2b). All runs at k = 4 showed similar values of cluster membership (Q) for all multilocus genotypes and formed equal clustering solutions. Therefore four genetic clusters were detected, being two of them strongly associated to SC and LM (Fig. 3a), and one of them geographically related to both LE and PSR sampling sites. These unique genetic clusters showed percentages of individual membership higher than 86%. The fourth genetic cluster included individuals from the westernmost sampling sites (LCh, R1 and R154); however, these localities were the most admixed sampling sites, with percentages of individual memberships to sampling locality lower than 70% (Fig. 3a, b). LC presented the highest level of admixture and was not clearly associated to any genetic cluster within our study area (Fig. 3a, b). This situation, most likely, is a result of its geographical location at the confluence of the three sandy diagonals.

While the most isolated populations LM and SC have not received migrants from other geographical areas, the westernmost sampling sites have maintained a continuous, contemporary gene flow among them. The isolated SC locality only showed one individual belonging to the neighbor sampling site LM. On the other hand, LM has not received migrants from other localities, denoting the highest degree of isolation in the easternmost part of the species's distribution. Although PSR and LE were sampled in different sand dune diagonals, these locations were genetically associated to the same genetic cluster. PSR showed several individuals with mixed ancestry, while at least one individual sampled in this locality was genetically assigned to LM (Fig. 3a). Some degree of admixed genotypes is also observed between the westernmost sampling sites and PSR.

Overall, these analyses showed higher levels of gene flow among the northwestern localities and restricted genetic connection and strong population structure towards the East. This genetic pattern does not seem to be associated with an obvious landscape factor such as major landscape barriers.

Landscape genetic analysis

We tested the relationship between local F_{ST}s and environmental factors using Generalized Linear Models (GLM) implemented in GESTE. Values for these factors are shown in Online Resource 4. The variables with higher posterior probabilities and consequently identified as related to population structure were NDVI (a landscape variable representing a measure of the vegetation cover, P = 0.43) and Long (variable denoting geographic position, P = 0.29).

In order to assess the strength of the influence of these two variables on population structure we correlated them with the local F_{ST} values estimated with GESTE (see Mapelli et al. 2012a). Correlation was significant in both cases (R = 0.72, P < 0.05, R = 0.91, P < 0.01, from Long and NDVI, respectively; Fig. 4a, b). A partial Mantel test between local F_{ST} values and NDVI (with geographical longitude as the constant matrix) was also significant

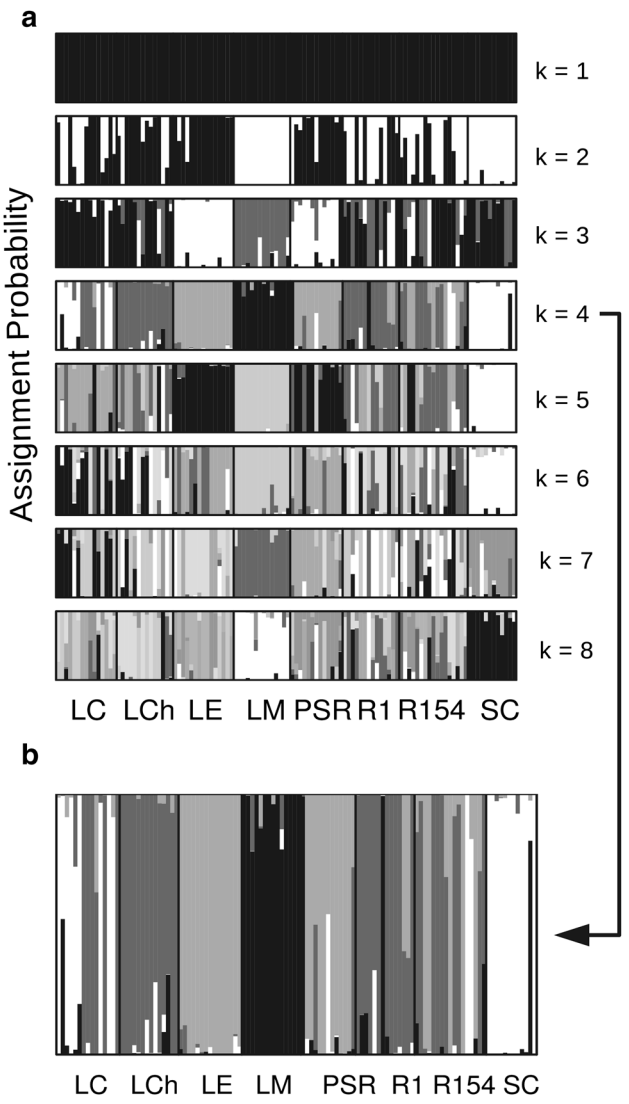

Fig. 3 Assignment probabilities (Q) to different genetic clusters identified by STRUCTURE considering k values from 1 to 8 (**a**), and with hypothesis of k = 4 (**b**). Each individual is represented by a vertical bar and each population is labeled as in Fig. 1 separated by black lines. Patterns in color are shown in Figure S1 (**a, b**; Supplementary Material). The logarithm of the probability of the data [LnP(D)] and Δk values (following Evanno et al. 2005), both as a function of K (defined as the number of putative genetic clusters according to STRUCTURE; Pritchard et al. 2000), are showed in Figure S2 (**a, b**; Supplementary Material)

(R = 0.82, P < 0.01), denoting the importance of the habitat quality on the genetic population structure. For these analyses, the test for global structuring in the sPCA also showed significant results (P = 0.012). In conclusion, lower F_{ST} values were obtained in the West, where habitat is more continuous, while the highest ones resulted in the East, where plant cover is higher and there is higher degree of habitat discontinuity (as a proxy of the consequences of habitat fragmentation in this area; see Mapelli and Kittlein 2009; Mapelli et al. 2012a; Mora et al. 2016).

Historical demography

Results from 2MOD show high contemporary gene flow among populations, but not following an isolation by distance pattern. In this sense, the relative likelihoods of the two alternative demographic scenarios were higher for the migration-drift equilibrium model (0.988) than for the model that considers only genetic drift (0.012). This situation reveals that *C.* "*chasiquensis*" populations have been at migration-drift equilibrium for a substantial period of time. It should be noted, however, the whole data set shows higher values of gene flow among populations in the West, and an important effect of genetic drift in the eastern sampling sites (see also pairwise estimates of R_{ST} in Table 2), possibly breaking the equilibrium between gene flow and genetic drift across the whole distribution of the species at recent times.

In addition, results of *F* values agreed with local F_{ST} inferences obtained with GESTE and varied across populations. Frequency distributions of *F* values (Fig. 5) at the eastern (LM and SC) and some central (LE and PSR) localities had higher median values (stronger effect of genetic drift) than the most western localities (R1, R154, LCh). Particularly, LC represents the conjunction of the three sandy diagonals (Fig. 1) and due to its high population density and its high availability of sandy habitat (Chasicó Lake has currently a status of natural reserve; Mora et al. 2016), is expected to show high degree of gene flow. On the other hand, PSR, in spite of being located at the center of the study area and being less distant with the western sampling sites, showed a high mean value of *F*. LE showed lower values of *F* than those of eastern localities, but higher than those of the other central and western localities. In agreement with STRUCTURE (see above), the other sampling sites (R154, R1, LCh and LC) showed the lowest mean values of *F*, denoting high levels of gene flow among them. In sum, the distribution of the genetic variability among the studied populations seems to be strongly influenced by landscape attributes instead of geographical distance.

Discussion

Here, we characterize the gene flow and population structure of *C.* "*chasiquensis*" from Argentina, considering the effects of geographic distance and landscape configuration on the genetic structure and connectivity of this vulnerable and endemic subterranean rodent. This is the first study to assess the population genetics of this tuco–tuco using nine highly polymorphic microsatellite loci over its most likely entire distribution, shedding light into the relationships

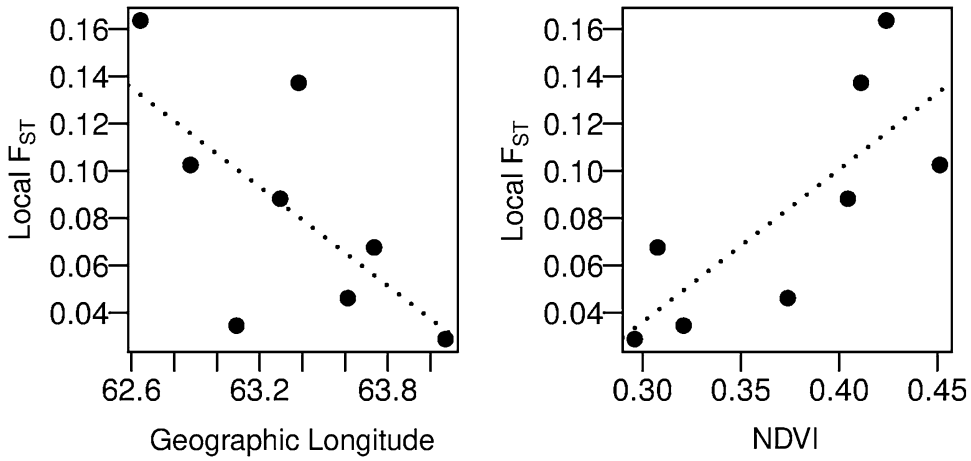

Fig. 4 Correlations between local F_{ST} values relative to geographic longitude (left) and NDVI (right)

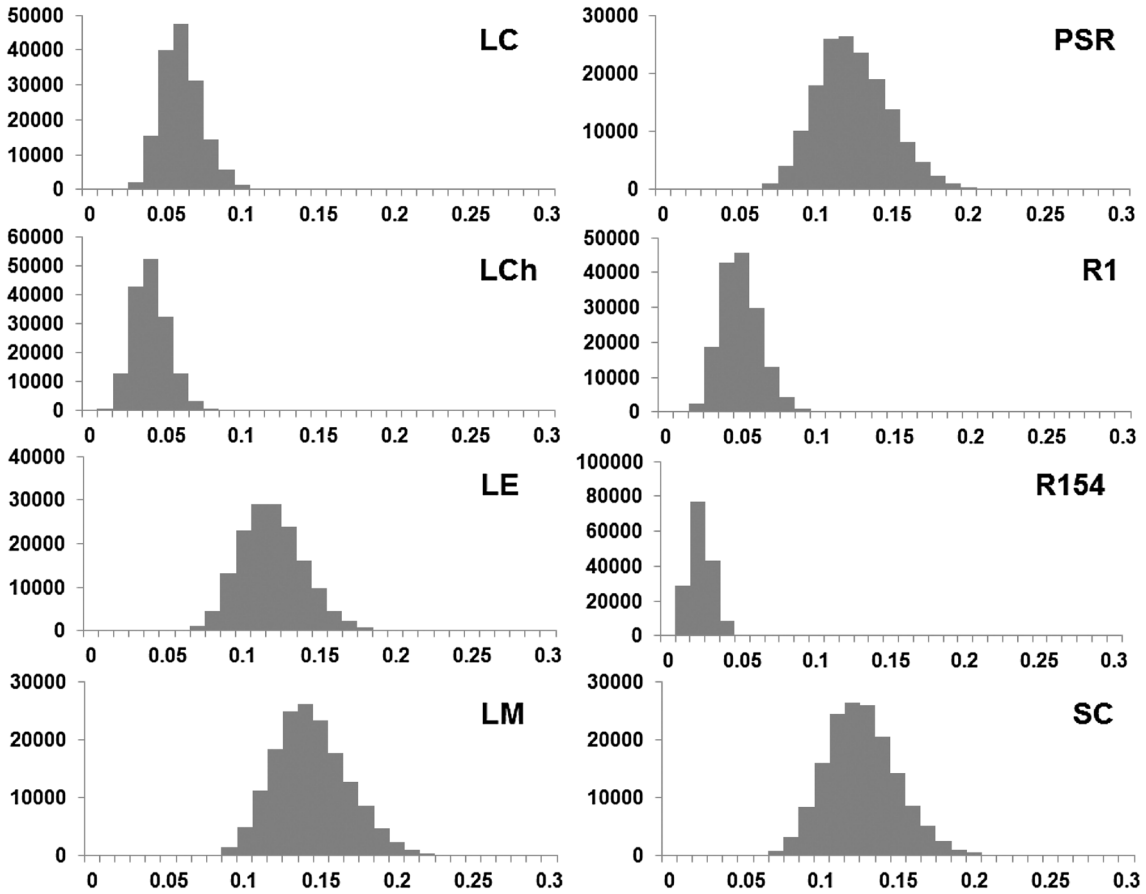

Fig. 5 Density plots of *F*, the probability that two alleles are identical by descent (Ciofi et al. 1999), for each sampling site of *C.* "*chasiquensis*". Abbreviations as in Fig. 1

among currently isolated populations, and providing important insights about this species in relation to current changes in its habitat. Moreover, some environmental and landscape factors that potentially may have affected the population genetic structure in this species were individually studied with the use of a landscape genetic approach.

Our analyses show that populations of *C.* "*chasiquensis*" are moderately to highly structured at the regional level and this pattern is the result, primarily, of important gene flow amongst northwestern populations together with restricted dispersal in the East and the Southwest. Despite the geographic proximity among localities (Fig. 1) four genetic

clusters were observed. These results suggest that higher dispersal rates on the western side of the distribution are not sufficient to avoid substructuring at the scale of this study.

Population structure and isolation by distance pattern

According to mitochondrial DNA data (Mora et al. 2016), this study also showed that the eastern and some southwestern populations had the lowest levels of genetic diversity. Currently, asymmetric migration amongst different populations breaks the equilibrium between gene flow and genetic drift, leading to a situation where the nearest populations are not necessarily the most similar at the genetic level.

Bayesian methods showed high degree of admixed genotypes among localities from the Northwest, indicating that the highest isolation has occurred between the East (LM, SC) and to a lesser extent in the central sampling sites (LE and PSR; see Fig. 3a, b). These latter localities were associated with three genetic clusters detected in this work with most of their individuals strongly assigned (the highest values of membership) to the locality where they originally were sampled. Here, genetic drift within local populations has played a key role in fixing and redistributing the allelic variants, being more intense than the homogenizing effect of gene flow (see Mapelli et al. 2012a). Although the westernmost sampling sites were assigned to an additional fourth independent genetic cluster (LCh, R1 and R154), these localities have received migrants from other isolated populations (such as the genetic cluster conformed by PSR and LE), showing an important degree of admixed multilocus genotypes.

Accordingly with Bayesian analyses, *F* frequencies clearly showed higher effects of genetic drift on the PSR, SC, LM and LE populations, denoting the highest degree of isolation in the easternmost part of the species's distribution. Stronger effects of genetic drift at local level are expected to occur in populations with high values of both *F* frequencies (considering their average value) and local F_{ST} estimates. As was expected, mean value of *F* frequencies were lower for the sampling sites included in the westernmost genetic cluster than in the other sites. These results are in agreement to the lower values of local F_{ST}s observed for these sampling locations, where the genetic differences between these sites and other sites within the study area were lower compared to the recorded for other locations.

Although PSR is located in the same sand dune diagonal than R154 and R1, this sampling site is partially isolated from them by a sequence of large salt flats and low flooded areas that break the continuity of the diagonal. Possibly, the flooding dynamics of the area is primarily affecting the current gene flow. In all analyses LM, SC, LE and PSR were the most differentiated and isolated sampling sites (with a significant interruption in the gene flow at recent times), denoting a higher effect of genetic drift relative to the other populations in the metapopulation.

The lack of an IBD pattern in the studied populations seems to indicate that their differentiation is not related to geographical distances, but with the landscape or habitat characteristics where the populations were sampled. Because of their isolation, populations in the East and in the Southwest are presumed to have been more influenced by the impact of genetic drift compared to the western ones. On the other hand, populations on the Northwest showed higher values of gene flow among them relative to the East or the Southwest. This pattern could explain the lack of equilibrium between gene flow and genetic drift across the entire distribution of the species, at least in recent times.

Although 2MOD supported an immigration-drift equilibrium model as the most probable scenario for explaining the recent demography in this species, gene flow has been asymmetric in the recent past. Mora et al. (2016) using mitochondrial DNA (mtDNA) in this species and considering the same arrangements of populations of this study suggested moderate population structure at regional level and a pattern of isolation by distance at historical times. In addition, these authors also pointed out higher levels of gene flow in the Northwest, possibly associated with population stability in the Southwest and the East, and a historical range expansion in the Northwest. In their study, historical dispersal patterns were highly asymmetric, mainly in the direction Southwest to Northwest, and with a significant degree of ancient isolation in the East.

Similarly to other species of the "*mendocinus* phylogenetic group", this species is characterized as specialist of sandy habitats. Furthermore, the habitat of this species is interrupted by several salt inland flats such as Salina Chica, by an important lagoon, Chasicó Lake, and by the Chasicó stream (Fig. 1). Thus, gene flow in our study area is probably restricted to the three sand dune diagonals, surrounded by fields where the environment is not optimal for the occupation of this species (highly altered landscape; see Mora et al. 2016). However, major differentiation at microsatellites was observed without clustering subpopulations into regions or major hierarchical units. These results agreed with those published by Mora et al. (2016) using mtDNA, where hierarchical subdivision of genetic variation was not observed when population groups were defined by the same criteria (e.g. sandy diagonals or by the geographic position of sampling sites relative to Chasicó Lake).

Moreover, the evolution of this sandy landscape in the Central region of Argentina during the Late Quaternary appears to have had a great impact on how the populations are currently connected by gene flow. Based on inferences of how the effective population size of the species has changed over time (Mora et al. 2016) and data on habitat during the Holocene (Quattrocchio et al. 2008; Zárate and Tripaldi

2012), current populations of *C.* "*chasiquensis*" seem to be relicts of a widespread historical distribution in the Argentinean Pampas, with an evident decline at the beginning of the Holocene, where the climatic conditions became gradually warmer and wetter (Tonni et al. 1999). Consequently, current dispersal seem to be related to the evolution of the landscape during the Late Pleistocene and Holocene (Mora et al. 2016) and to the dynamics of landscape modifications occurred in the past 200 years in this region.

Landscape genetics

Landscape features such as habitat fragmentation or variables related to the suitable habitat for the species strongly influence their dispersal patterns and, therefore, the population genetic structure (Mapelli et al. 2012a). In our study, some landscape characteristics explained better the genetic variation rather than the geographical distance between populations. Two variables, one associated with habitat quality (NDVI) and other denoting geographic position (longitude) were highly significant in order to explain the population genetic structure of the species in recent times. These variables have affected the way the individuals are moving today, showing the high specificity of this species to sandy soils with low plant cover. Interestingly, local F_{ST} values were significantly correlated with both geographical longitude and plant cover, denoting an important degree of population isolation from the West to the East, and showing a clear relationship of population structure with habitat quality; tuco-tucos prefers sandy soils with low plant cover (see also Mapelli et al. 2012a). Lower local F_{ST} values resulted from the most continuous habitat at the West, while the higher ones were found to the East, where the habitat quality was lower (site with higher vegetation cover) and where the habitat is more discontinuous (as a result of habitat fragmentation). In concordance with local F_{ST} values, LM and SC were two of the three sampling sites with the lowest allelic richness, suggesting smaller effective sizes for these populations.

Relative to the West, the eastern diagonal is characterized by a patchier configuration and to have smaller extension of suitable habitat (Mora et al. 2016). The habitat around this sand dune diagonal is strongly interrupted by flooded salt flats (e.g. Salina Chica), agricultural crops and other important human modifications like forest plantations and water channels (Zech et al. 2009). The presence of substantial non-optimal habitat in this diagonal would prevent the establishment of immigrant individuals from the West. As was expected, regional turnover of genes and individuals were higher in the West than in the eastern edge of our study area. These results are partially supported by a low proportion of migrants into the eastern side in current days. It should be noted, the Northwest and Southwest diagonals extend to the West of our study area (including a wide sandy region in La Pampa Province), where a significant increase in the availability of sandy landscape is observed. Although the distributional limits of this nominal species is currently not well-defined (see Mora et al. 2016), the extensive sandy habitat observed westwards might possibly explain the highest levels of gene flow amongst the western populations.

Another two potential explanations for this pattern are that western sampling sites have shared a common ancestor with each other more recently than they did with eastern sites, or that the eastern sites represent a recent expansion into that area, with rapid divergence due primarily to strong genetic drift. As was suggested previously, Mora et al. (2016) using mitochondrial control region DNA sequences showed both sudden and spatial demographic expansions in the northwestern diagonal; whereas populations from the eastern and southwestern diagonals seem to have maintained a demographic equilibrium at historical times. Due to this population expansion on the Northwest diagonal, results of dispersal patterns using microsatellite loci should be taken in caution. Incomplete lineage sorting could be a much more likely explanation for the observed similarity among some of the westernmost sampling sites (shared history) than ongoing gene flow, particularly given the large spatial scales separating these sites and the subjacent demographic pattern observed with the mtDNA.

On the other hand, there are few studies in subterranean rodents (and micromammals in general) showing the genetic consequences of dispersal in patchy landscapes, particularly at different spatial scales (Wlasiuk et al. 2003; Fernández-Stolz et al. 2007; Kittlein and Gaggiotti 2008; Mora et al. 2010, 2016; Gómez Fernández et al. 2012; Mapelli et al. 2012a). The species belonging to the "*mendocinus* phylogenetic group" are among the most impacted species within the genus *Ctenomys*, and like *C.* "*chasiquensis*", are strictly restricted to sandy soils. In this context, Wlasiuk et al. (2003), Fernández-Stolz et al. (2007), Mora et al. (2010) and Mapelli et al. (2012a) reported high genetic structure among different subpopulations of *C. rionegrensis, C. flamarioni, C. australis* and *C. porteousi*, respectively, associated to a surprisingly fine geographical scale (less than 20 km) on fragmented farm landscapes, suggesting that minor discontinuities have been of considerable importance in shaping the dispersal patterns of those species (additional data are given in Online Resource 5). The Rio Negro tuco–tuco (*C. rionegrensis*; Langguth and Abella 1970; considered endangered by IUCN) occurs on a highly fragmented landscape in a limited geographic range in Uruguay. Despite that populations of this species are isolated and separated by few kilometers among them (from 13 to 64 km) into a matrix of sand dune patches (Wlasiuk et al. 2003), Kittlein and Gaggiotti (2008) have suggested that they conform a typical metapopulation rearrangement along the coast of the

Uruguay River. Porteous's tuco–tuco (*C. porteousi*; Thomas 1916; considered vulnerable by IUCN) has an extremely narrow distributional range in Central Argentina (Mapelli et al. 2012a, b). Because of the unusual expansion of soybean crop growing in the area during the latter 20 years, the habitat fragmentation in this species has progressively increased and has limited their presence to small suitable patches of habitat (Mapelli and Kittlein 2009). At present, this species occupies only 175 from 509 km^2 of total suitable habitat in their entire distributional range (additional data are given in Online Resource 5). The sand dune tuco–tuco (*C. australis*; Rusconi 1934; considered endangered by IUCN) is currently restricted to the coastal, friable sand dunes present between Necochea and Bahía Blanca in the southeastern region of Buenos Aires Province, Argentina, occupying an extremely reduced area of suitable habitat of approximately 82 km^2 (Mora et al. 2006). Also, the coastal dune habitat of this species is gradually retreating because of progressive forestation and urbanization in the region, posing a threat to survival of the species (Mora et al. 2007). Flamarion's tuco–tuco (or the tuco–tuco das dunas, *C. flamarioni*; Travi 1981; considered endangered by IUCN) is endemic and limited to the coastal sand dunes along the southern coast of Brazil (State of Rio Grande do Sul), and occupies a linear and practically continuous habitat (Freitas 1995; Fernández-Stolz et al. 2007; Fernandes et al. 2007). Like *C. australis*, this species is mainly threatened by habitat loss due to urbanization and dune removal. At a smaller scale (<2.5 km), the habitat of this species is highly fragmented, and similar to *C. australis*, low inter-dune grasslands and flooded areas constitute significant barriers to dispersal and gene flow (Fernández-Stolz et al. 2007). As was suggested by these authors, only 500 m of non-suitable habitat was sufficient to detect high population subdivision, even more significant than *C. australis* at similar spatial scales. It should be noted, however, that other possible factors such as a polygynous breeding system and the environmental instability that characterizes the coastal dunes reported for *C. flamarioni* (Fernández-Stolz et al. 2007) and *C. australis* (Zenuto and Busch 1998) may have influenced the observed pattern.

Interestingly, analyses of microsatellite data suggest that *C. rionegrensis, C. flamarioni, C. australis* and *C. porteousi* are extremely structured geographically, with subpopulations constituting distinct genetic units and, like *C.* "*chasiquensis*", showing a strict association with some landscape variables like low vegetation cover and highly permeable sandy soils. In some species an absence of an isolation-by-distance pattern is also observed (Wlasiuk et al. 2003; Mora et al. 2010; see Online Resource 5). Other *Ctenomys* species belonging to different phylogenetic groups (additional data are given in Online Resource 5) also show an important population genetic structure in their particular geographical ranges as a result of habitat configuration and stochastic processes. In general, like *C.* "*chasiquensis*", these species show high genetic differentiation at very short geographical distances using microsatellite loci (e.g. Gómez Fernández et al. 2012; Roratto et al. 2015). At this scale of the analysis (between 3 and 70 km), all studies show that genetic drift (a purely stochastic process) might be more significant in promoting genetic differentiation in these species than linear distances among subpopulations. While the habitat configuration (fragmentation and suitable habitat) appears to be the main factor shaping the genetic population structure at shorter distances, other barriers like rivers seem to be responsible to disrupt the genetic connectivity at longer geographical scales (Cutrera et al. 2005; Fernandes et al. 2007; Fernández-Stolz et al. 2007; Mora et al. 2007, 2013, 2016; Mirol et al. 2010; Gómez Fernández et al. 2012; Lopes et al. 2013; Roratto et al. 2015; Online Resource 5).

History and habitat configuration appear to be two important clues in shaping current genetic diversity and gene flow patterns in these subterranean rodents. Beyond the differences in demographic histories amongst these species of *Ctenomys* (some of them strongly related to significant changes in the configuration of the sand dune habitats during the Late Pleistocene and Early Holocene), higher degree of habitat fragmentation seems to be associated to higher population structure. Overall, all the studies which consider similar spatial scales and comparable number of microsatellite loci have shown the importance of landscape discontinuities (e.g. terrain elevation or habitat quality) over the patterns of current gene flow, denoting a minor role of geographic distances.

Several studies carried out in *Ctenomys* using only a few number of microsatellite loci (e.g. from eight to nine polymorphic loci; Wlasiuk et al. 2003; Fernández-Stolz et al. 2007; Mora et al. 2010; Mapelli et al. 2012a) showed the same pattern of moderate to strong population structure, including sampling designs performed on minor spatial scales (<10 km; Cutrera et al. 2005; Mora et al. 2010). Here, at least four genetic clusters were detected using nine highly polymorphic microsatellite loci, and most individuals in these clusters were strongly assigned to the locality where they originally were sampled. At the same time, an updated appraisal using further hypervariable nuclear markers will be useful to comprehend how the current connectivity among different populations is affecting the genetic structure of these endangered species, assessing more rigorously the contribution of each environmental or landscape variable to population differentiation.

Final considerations

Low rates of dispersal and high specificity to inhabit sandy soils make the *Ctenomys* species an attractive model to study the effects of habitat fragmentation on the population

structure, particularly at lower spatial scales. Similar to the observed in other tuco-tucos at similar geographic scales (Wlasiuk et al. 2003; Mapelli et al. 2012a), results of this work shows moderate to high genetic structure in this species at regional level. Some habitat characteristics (such as habitat quality and geographic location) and landscape discontinuities (such as flooded salt flats, agricultural crops and other important human modifications like forest plantations) are important features that shaped the differentiation among populations, at least more intensely than geographic distance. In conjunction, all analyses show higher values of gene flow among populations on the Northwest sandy diagonal, and an important effect of genetic drift in populations from the East and Southwest, possibly breaking an isolation by distance pattern across the distribution of this nominal species in current days. Furthermore, estimation of dispersal patterns in the species show that the most isolated and differentiated populations are located in the East, where current human activity is causing a stronger impact on the environment.

Beyond the effect of landscape variables on the population genetic structure, the evolution of the sandy landscape in our study area during the Late Pleistocene/Early Holocene also appears to have had a great impact on how populations are currently connected by gene flow. Environmental and demographic parameters seem to show a progressive decline in the effective population size of this species at the beginning of the Holocene, where the climatic conditions became progressively warmer and wetter (Mora et al. 2016), which has affected the plant cover and precipitations in this region nowadays (Quattrocchio et al. 2008). The last 200 years of continuous human alterations on this landscape also have had a significant influence on this population decline and isolation (Mora et al. 2016). Therefore, both the degree of isolation and the genetic variability of populations in their entire geographical range should be considered in order to define the most important units concerning their conservation status. Preservation of the genetic diversity of this species requires management strategies that consider not only the major (salt flats and lagoons) but also the minor landscape discontinuities (such as low and waterlogged habitats, harvests and forest plantations, human alterations) affecting gene flow.

On the other hand, habitat characteristics such as differences in vegetation cover observed between the western and eastern sampling units in *C.* "*chasiquensis*" could also promote the population divergence in adaptative features, further than the population genetic structure observed in neutral markers (e.g. microsatellites). The extent of genetic adaptation among widely distributed populations will help us to reveal those candidate gene loci that may be important as genetic markers to assess adaptive potential and guide conservation efforts.

Acknowledgements We are grateful to all the members of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" for their invaluable support and advice. Financial support was provided by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP-0138), UNMdP (Project EXA598) and FONCYT (PICT-2010-1551). To these persons and institutions, we express our deep gratitude.

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