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Genetic structure of populations of the Pampean grassland mouse, *Akodon azarae*, in an agroecosystem under intensive management.

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Abstract

Agroecosystems in central Argentina are a good example of landscape modification by human activities. We used the Pampean grassland mouse (*Akodon azarae*) as a biological model to assess the effects of landscape fragmentation on the genetic structure of natural populations present in the region. The species is a habitat specialist that is numerically dominant in relatively stable environments, such as remnant areas of native vegetation, stream borders, roadsides and railway banks. We used seven microsatellite loci to analyze the genetic population structure and to explore if there is sex-biased dispersal during the reproductive season at a fine geographical scale. Rodents were captured seasonally in trap lines located on roadsides in an agroecosystem of central Argentina. Values of genetic differentiation among populations and temporal patterns of spatial autocorrelation revealed that the genetic populations occupy areas larger than the sampling area. Causal modeling analyses showed that unfavorable habitats (secondary roads and crop fields) were not barriers to dispersal of *Akodon azarae*. The high levels of gene flow and the short duration of the low population density phase, followed by a fast recovery, would contribute to the maintenance of highly polymorphic populations. As expected for *A. azarae*'s mating system, males were not genetically structured. However, females' spatial genetic structure varied greatly over the year, which would be related to availability and quality of habitat, and to intrasex interactions. Our work contributes to the understanding of dispersal strategies in small mammals in anthropogenically fragmented habitats like intensively managed agroecosystems.

Introduction:

Agroecosystems are a particular type of anthropogenically fragmented habitat characterized by the conversion of large natural areas into small plots cultivated with different crops crossed by rural roads (Banks et al., 2005; Clark et al., 2001). This alteration of the habitat configuration and the consequent loss of available resources can change the relationship of species with their habitats in a way that depends on several factors such as sex, age, dispersal ability, ecological specialization, and tolerance to predation; that relationship can be expressed through different space use and movement patterns. In particular, the presence of new elements of anthropogenic origin in the habitat can disrupt movement patterns; therefore, a key issue is to understand how habitats and their configuration affect the dispersal of individuals of a species.

A representative example of a habitat loss and fragmentation process driven by agricultural intensification is observed in the plains of central Argentina, where cultivated areas have increased steadily in the last decades, with a current total area of approximately 36 million hectares (Manuel-Navarrete et al., 2005; Volante et al., 2015). Other important changes include producers' preference for some types of crops at the expense of others and the general use of no-till practices (Bilenca et al., 2007; Paruelo et al., 2005; Viglizzo et al., 2001). Currently, the landscape consists almost exclusively of crops and livestock pastures, with remnants of natural habitats persisting mostly as narrow strips along railways, water streams, roads and fence lines (the so called 'border' habitats), which are variable in width and can be several kilometers long (Ghersa et al., 2002; Medan et al., 2011). These habitats play an important role because they are the most similar to the original ones and contain resources for survival and reproduction of animal species (Chiappero et al., 2016; Gomez et al., 2011; Gomez et al., 2015; Sommaro et al., 2010). In central Argentina, border habitats harbor a community of rodent species of the subfamily Sigmodontinae that differ in habitat use, competitive ability, space use, and mating system (Gomez et al., 2015; Simone et al., 2010). One of the most abundant species is *Akodon azarae,* commonly known as "the Pampean grassland mouse" (de Villafañe 1981; Priotto and Polop 1997; Priotto and Steinmann 1999). This species is a good indicator of habitat quality due to its preference for relatively undisturbed habitats with high cover such as natural pastures or border habitats (Bonaventura and Kravetz 1989; Busch et al., 2001; Coda et al., 2015), and is competitively dominant over most of the other species of the assemblage (Busch et al., 1997; Busch et al., 2005). Populations show seasonal fluctuations in abundance, reaching maximum values in late autumn and

early winter, and a drastic drop between late winter and early spring (Andreo et al., 2009; Gomez et al., 2011). The reproductive cycle spans 6-8 months, starting in spring and ending in autumn (Bonatto et al., 2013; Zuleta et al., 1988). The species has a polygynous mating system that operates through female defense (Bonatto et al., 2012; 2013). In the breeding season, males and females keep exclusive home range areas that are larger in males than in females (Bonatto et al., 2012; Priotto and Steinmann 1999; Zuleta, 1989). In a previous study involving the same sampling animals as in the present work and using capture–mark–recapture (CMR) methods, Gómez et al. (2011) found that sex and reproductive period influenced the movement pattern independently of population abundance in border habitats of agroecosystems. During the breeding period, males moved longer distances than females, whereas in the non-breeding period movement was similar in both sexes. The authors also detected that 10% of the marked individuals moved more than 300 m, in some cases crossing a 5-m wide secondary road. The maximum dispersal distance was recorded for a male that moved 920 m.

Species that show specific habitat requirements have been found to be more affected by fragmentation than those that can exploit multiple habitat types or food resources. Likewise, in species showing sex differences in space use and movement patterns, fragmentation will affect them differentially (Didham 2010; Doerr et al., 2014; Kierepka et al., 2016; Schweizer et al., 2007). The alteration of the dispersal ability of organisms can have important consequences on populations, such as reduced levels of gene flow, which leads to loss of genetic diversity, increased inbreeding and a lowered capacity to adapt to environmental changes (Coulon et al., 2004; Frankham et al., 2002).

Dispersal, however, is difficult to estimate. Direct estimates of dispersal by means of traditional CMR methods are time-consuming, often fail to detect dispersal over long distances and can only measure the mobility of individuals rather than the effectiveness of dispersal determined by successful reproduction after immigration. On the other hand, genetic-based methods aim to estimate gene flow based on the pattern of spatial distribution of alleles at marker loci. The combined use of highly polymorphic genetic markers like microsatellites and methods of analysis based on individuals rather than on predefined populations allows us to make inferences of contemporary patterns of gene flow resulting from the particular configuration of the available habitat in a landscape (Coulon et al., 2004; Lancaster et al., 2011; Slatkin, 1985; Schweizer et al., 2007; Taylor et al., 2007). In this work, we continued our studies on the mode in which the particular configuration of habitats in an agroecosystem affects the dispersal patterns of

Sigmodontinae rodents. For that purpose, we complemented our previous studies that applied CMR techniques (Gómez et al., 2011) using microsatellite markers to estimate the genetic structure of *A. azarae* populations from border habitats and infer their gene flow patterns in an intensively managed agroecosystem. We predict that the presence of anthropogenic features, such as rural roads and crop plots, will impede gene flow, which will occur mainly along border habitats. Gene flow along those habitats would result in genetic structuring among different border habitats within the study area. We also examined evidence for sex-biased dispersal. Considering that *A. azarae* is a polygynous species with female defense, we hypothesized that the species would exhibit male-biased dispersal, resulting in increased population genetic structure among females relative to males.

Materials and Methods

Study area and sample collection

The study was conducted in linear habitats of an agricultural ecosystem in Río Cuarto Department (Córdoba Province, Argentina) from November 2005 to July 2006. Phytogeographically, the area is within the "Espinal Province" (Cabrera, 1953), but the original vegetation has been markedly altered by agriculture and cattle raising activities. At present, the landscape consists mainly of crop fields surrounded by wire fences, and crossed by paved and dirt roads and water streams; "border" habitats develop along these landscape elements. Borders are habitats with more homogeneous plant cover throughout the year than their surrounding habitats in farm landscapes (Priotto et al., 2002).

   Two groups of transects were laid out on field borders on opposite sides of two 7-m wide secondary dirt roads (A opposite to B, C opposite to D, respectively. Fig.1). The A-B and C-D transect groups were separated by at least 4 km. Transects A and B consisted of five trap lines, and C and D consisted of three trap lines, separated by 500 m from each other. Lines consisted of 30 Sherman-like live traps placed every 10 m and baited with a mixture of peanut butter and cow fat.

      Four seasonal capture, mark and recapture (CMR) trapping sessions were conducted for 5 consecutive days in November 2005 (spring, beginning of the breeding period), March 2006 (end of summer, middle of the breeding period), May 2006 (autumn, end of the breeding period) and July 2006 (winter, non-breeding period), in order to study rodent dispersal in border habitats by means of direct (CMR) and indirect (genetic markers)

methods. Each trap was georeferenced with a GARMIN eTrex Legend Cx GPS. Trapped animals were marked with numerically coded ear tags, weighed, sexed and assigned to age classes (juveniles or adults) following the criteria proposed by Dalby (1975) and de Villafañe (1981). Results of the CMR study were published in Gomez et al. (2011). We also obtained tissue samples by removing a small piece of the animal's tail tip; pieces were preserved in ethanol (90%) for the population genetics studies (this paper). This sampling procedure does not affect the animal's survival. Trapped animals were treated in accordance with the Argentine National Act 14346.

DNA extraction and microsatellite analysis

DNA was extracted from tail tips using a standard phenol–chloroform procedure (Maniatis et al.,1982). Seven microsatellite loci (Aaz1, Aaz2, Aaz3, Aaz4, Aaz5, Aaz6, Aaz8) developed by Vera et al. (2011) for the species, were amplified via the polymerase chain reaction (PCR) using fluorescently labelled forward primers and following the authors' protocols. The molecular size of the amplification products was determined using an ABI3100 sequencer at Macrogen Inc (Seoul, Korea). Fragments were scored using the software PeakScanner (Applied Biosystems, 2006) and binned using MsatAllele (Alberto, 2009; R Development Core Team), which defines the bin limits based on the distribution properties of the observed fragment sizes.

Population genetic structure

The program Microchecker (van Oosterhout et al., 2004) was used to detect the presence of null alleles or genotyping errors (e.g. stuttering or large allele dropouts). Genetic variability was measured by season as mean expected heterozygosity (He), mean observed heterozygosity (Ho) and mean allelic richness (AR). The significance of differences in genetic variability among seasons was calculated using the Friedman test in Infostat (Balzarini et al., 2008).

The genetic structure of *A. azarae* populations was estimated via Wright's F-statistics, using the estimators f and θ (Weir and Cockerham 1984) for F_{IS} and F_{ST}, respectively. The index f was used to assess the conformance to Hardy–Weinberg equilibrium (HWE) for each locus and season; significance levels were assessed through 28000 randomizations of alleles among individuals within samples and adjusted using the Bonferroni correction for multiple tests (Rice, 1989). Theta was calculated between pairs

of transects for each season and significance was tested by permutation of alleles among transects and evaluated using the Bonferroni correction. Due to the low number of individuals obtained in transects C and D in spring, summer and winter, genetic differentiation was estimated considering them as a single subpopulation. In autumn (peak of population density) each transect was considered a different subpopulation. The program FSTAT version 2.9.3 (Goudet, 2001) was used for calculations.

The spatial pattern of genetic variation (spatial genetic structure, SGS) was analysed by means of spatial genetic autocorrelation. Analyses were performed for each season, for the total population and for each sex separately, using the program Genalex 6.5 (Peakall and Smouse 2006). This statistical tool estimates the geographical extension to which individuals are more related than expected by chance. The program uses pairwise geographical and genetic distance matrices to calculate a multivariate autocorrelation coefficient r, which measures the genetic similarity of pairs of individuals whose geographical separation falls within a series of consecutive distance classes. The coefficient r ranges between [-1, +1]; a significantly positive r indicates that individuals within that distance class are genetically more similar than expected by chance, whereas a significantly negative r indicates no dependence among genotypes at the spatial scale considered, which is expected under restricted gene flow at that scale. The distance at which the autocorrelation crosses the x-axis ($r = 0$) is considered the "spatial range" or the "patch size" of the spatial pattern of genetic relatedness (Peakall et al., 2003). Spatial autocorrelation analyses were performed using the option "Single Pop" in Genalex. Distance classes were chosen to include an intra-trap line component (100 m, 200 m and 300 m), and an inter-trap line component (starting from 1100 m, at 800 m intervals) and were set using the "variable distance classes" option. In each analysis we performed 1000 random permutations of genotypes among distance classes; permutated "r" values (rp) were sorted and the 2.5th and 97.5th values were taken to define the upper and lower bounds of the 95% confidence interval (CI) about the null hypothesis of no autocorrelation ($r = 0$) (Peakall et al., 2003). To detect specifically positive autocorrelation at short distances, a one-tailed test for positive spatial genetic structure was performed by comparing individual rp values with observed r values to estimate the probability of achieving a value greater than or equal to the observed r. If this probability is less than 0.05, the alternative hypothesis of positive spatial genetic structure is accepted. Finally, a bootstrap analysis with 1000 resamplings within each distance class was performed to obtain the 95% CI about each value of r. According to Peakall et al.

(2003), the latter procedure is more conservative than permutation tests and favors the null hypothesis more frequently.

The effect of different landscape elements (borders, crop fields, and secondary roads) and geographic distance on genetic differentiation between individuals was evaluated using a Causal Modeling analysis (Cushman et al., 2006; Cushman and Landguth 2010). Resistance maps of the study site, representing different hypotheses about the difficulty of *A. azarae* individuals to disperse through different landscape elements, were drawn at the 1:1250 scale from a Google Earth image using the Open Layer plugin within QGIS 2.6.1 (QGIS Development Team 2014). The elements of the landscape were classified into three classes (secondary roads, crop/pasture fields and borders) and were assigned a resistance value representing the cost of movement for *A. azarae*. Five hypotheses about connectivity in the agroecosystem were considered (Table 1): Fields and secondary roads were assigned alternatively high and low resistance values, to generate four isolation-by-resistance hypotheses, whereas borders had minimum resistance in all models due to the species' preference for tall vegetation. The null model considered only Euclidean distance as a factor that provides resistance to gene flow. Circuitscape 4.0 (McRae et al., 2014) was used 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 the program Genalex 6.5 (Peakall and Smouse 2006). First, simple Mantel tests were performed between genetic distance and each of the geographic distances or resistance matrices (Table 1). Second, partial Mantel tests were performed: 1) between genetic distance and each landscape resistance hypothesis, partialling out the effects of geographic distance and, 2) between genetic distance and geographic distance, discounting the effect of resistance hypotheses that gave a significant correlation in point 1). All Mantel tests were carried out using the program Zt (Bonnet and Van De Peer 2002). If a particular hypothesis of landscape resistance affects gene flow, a simple Mantel test between genetic distance and the particular hypothesis is expected to be significant. In addition, partial Mantel tests between genetic distance and the hypothesis, partialling out the effect of geographic distance, is also expected to be significant, but the partial Mantel test between genetic distance and geographic distance, partialling out the effect of the resistance hypothesis, is expected to be non-significant (Cushman et al., 2006).

Results

A total of 357 individuals were captured: 83 in spring, 76 in summer, 133 in autumn and 66 in winter. In spring we captured more males than females; in summer, autumn and winter, the number of captured males and females was similar (Table S.1, Supplementary Data). In spring and summer, most individuals were adults, whereas juveniles prevailed during autumn and winter (Table S.2, Supplementary Data).

The analysis using Microchecker did not reveal the presence of null alleles, stuttering or large allele-dropout at any locus. Genetic variability was uniformly high in all seasons throughout the year (Friedman test P = 0.387 for AR, P=0.863 for Ho and P=0.799 for He) (Table 2). Departures from HWE were observed in all seasons after Bonferroni correction. Seasonal f values ranged between 0.081 and 0.104, and showed a small but significant heterozygosity deficiency (Table S.3, Supplementary Data).

Rodent populations from different borders were genetically homogeneous during spring and summer (Table 3a). In autumn, genetic differentiation between transects A and B was also non-significant, whereas a significant differentiation was detected for the remaining pairwise comparisons (Table 3b). In winter, only transect A was genetically different from the other transects (Table 3a).

The SGS pattern was different among seasons and between sexes (Fig 2 and 3). In spring, i.e. beginning of breeding season, the population was mainly composed of adults born at the end of the previous breeding period. The SGS was positive and significant only for the first distance class, as shown by the one-tailed test (100 m; P < 0. 05), and positive autocorrelation extended up to 2182 m (Fig 2a). Females showed a significant SGS for the first distance class, as shown by the one-tailed test (P < 0.05), and the autocorrelation was positive up to 1088 m, whereas males showed a random pattern (Fig 3a).

In summer, there was a positive and significant autocorrelation coefficient for the first distance class (P < 0.05, one-tailed test) and positive r extended up to 259 m (Fig. 2b). However, males and females displayed a random pattern of genetic structure (Fig. 3b).

In autumn, when population density peaks and the breeding season is ending, the population consisted mostly of juveniles. The SGS analysis showed a positive autocorrelation up to 225 m and significant at the first distance class (100 m) for the three significance tests (Fig. 2c). In females, the one-tailed test yielded a positive and significant r value for the first distance class (P<0.05), and the extent of autocorrelation was 199 m. Males showed a random pattern (Fig. 3c). In winter, i.e. the non-breeding

season, the population consisted mainly of juveniles born in the late breeding season and showed a random pattern (Fig 2 and 3d).

Simple Mantel tests between genetic distance and geographic distance or resistance hypotheses were non-significant in spring (Table S4, Supplementary Data). On the contrary, a significant correlation of genetic distance with geographic distance was recorded in summer, autumn, and winter. Genetic distance was significantly correlated with hypotheses two and three in autumn (Table S4, Supplementary Data). All partial Mantel tests between genetic distance and each hypothesis, partialling out geographic distance, were non-significant (Table S4). Therefore, no significant effects of landscape elements on the genetic differentiation between individuals were found. In summer, autumn and winter, genetic differentiation among individuals was best explained by geographic distance alone. However, when we excluded the juveniles from the analysis, there is no significant correlation between genetic and geographic distance (Table S4, Supplementary Data).

Discussion

Genetic variability and structuring

The positive and significant f values at the geographical scale analyzed (<10 km) indicated a general departure from HWE in all seasons (Table S3). Given that the absence of null alleles was confirmed by the Microchecker analysis, and that f values were calculated by season across the whole study area, the most probable explanation is a spatio-temporal Wahlund effect. On the one hand, the significant value of θ between transects in autumn and winter (Table 3a and b) indicates spatial subdivision in these seasons that could result in a Wahlund effect. On the other hand, the overlap of age classes (juveniles and adults) within different seasons would contribute to a temporal subdivision.

Akodon azarae populations are characterized by seasonal density changes throughout the year. Andreo et al. (2009), Gómez et al. (2011) and Bonatto et al. (2012; 2013) reported that the maximum density values occur in autumn, during the end of the breeding season, with a significant drop in winter. Nevertheless, polymorphism levels remained uniformly high throughout the study period. Sommaro (2012) and Chiappero et al. (2016) found uniformly high genetic variation despite large density fluctuations in populations of two rodent species of the genus *Calomys*, *C. musculinus* and *C. venustus,*

studied in the same border habitats during the same study period as in the present work. *Oligoryzomys longicaudatus* populations from southern Argentina also showed similar results (González Ittig et al., 2015). All these species show a common pattern of short periods of low population density followed by a fast recovery, and of dispersal among neighboring demes; these processes, together, would help to maintain high levels of polymorphism. A similar mechanism would operate in *A. azarae*, since the spatial autocorrelation analysis showed a pattern of random genetic structure at a long distance, suggesting a high rate of dispersal within the study area.

Simple Mantel tests for the complete data set suggest a pattern of isolation by distance in summer, autumn and winter, which is absent when we excluded juveniles from analysis. During these seasons, juveniles constitute an important proportion of the population (Table S.2, Supplementary Data); these individuals, staying near their mothers, may bias the results toward a significant correlation between spatial and genetic distances (Frantz et al. 2008, Renner et al. 2016). Causal modeling analyses showed that rural roads and crop fields do not act as barriers to dispersal in the Pampean grassland mouse; these results reinforce the idea of high levels of gene flow at a small geographical scale. Gómez et al. (2011) estimated dispersal using CMR methods for the same sample analyzed in the present work. They found that 10% of marked *A. azarae* individuals moved beyond the limits of their trap line (300 m); one male moved 900 m between trap lines along the same border, and 19 individuals crossed the secondary road between transects A and B (Gómez et al., 2011). Although these results do not reflect the reproductive success of migrants in the new population, they support the idea that landscape elements in the agroecosystem do not impair gene flow, at least at the scale of this study (25 km^2), and explain the random SGS found consistently beyond the 100 m distance class. Similar SGS patterns were found in the syntopic rodent species *C. musculinus* (habitat generalist) and *C. venustus* (habitat specialist) (Sommaro 2012; Chiappero et al. 2016). In the three species, the geographical scale of the study was not sufficiently broad to determine the spatial limits of a single deme. A similar pattern of low genetic differentiation between local populations was found in the pygmy rabbit *Brachylagus idahoensis* in eight sampling locations in two study areas in Idaho-North America (Estes-Zumpf et al., 2010). Although the species is a habitat specialist and its populations were isolated, they did not show a marked genetic structure because the water streams and roads did not act as substantial barriers to gene flow. Centeno-Cuadros et al. (2011) demonstrated that, although considered a habitat specialist, the southern

water vole *Arvicola sapidus* uses a wide variety of landscape types during dispersal, thus increasing gene flow. Serafini et al. (2019) assessed how the different rodent species perceive and respond to landscape complexity in an agroecosystem of central Argentina and found that, although *A. azarae* shows a strong preference for undisturbed habitats, it was present all across the habitat complexity range, i.e., from very simple landscapes to the most heterogeneous ones. This finding reinforces our results, since it indicates that, despite showing a strong preference for a particular type of habitat, this species would be able to disperse across a variety of sub-optimal areas.

Spatial genetic structure by sex and season.

        A. azarae males showed no genetic structuring year-round (Fig 3 a-d), which is in line with the known behavior of males. This species presents a polygynous mating system, in which males typically gain access to multiple females by displaying aggressive behavior against other rival males in order to exclude them from the breeding area, and females typically mate with only one male (Bonatto et al., 2012; 2013). The intensity of aggressive behavior exhibited by males in the presence of females varies: residents display high levels of aggression to intruders, whereas intruders show submissive behavior. Longer residence times of males in their territories may lead to stronger aggressive responses against other males that try to trespass the boundaries. Therefore, expulsion by adults would trigger natal dispersal of juvenile males (Bonatto et al., 2013).

        Spatial genetic structure in *A. azarae* females varied throughout the year. CMR studies showed that females move smaller distances than males at the beginning of the breeding season (Gómez et al., 2011). In spring, they show a weak spatial structure at a short distance. Bilenca and Kravetz (1998) found that during the breeding season, sexually mature females occupy microhabitats with high vegetation cover, unlike those occupied by inactive females. Thus, reproductive females would have a territorial behavior to defend reproductive spaces (areas with high vegetation cover), and to optimize maternal care at the beginning of the breeding season.

        In summer, female SGS patterns showed no structuring. *A. azarae* breeding females are aggressive against female intruders as a strategy to protect their offspring from potentially infanticidal females and that intensity of aggressiveness increases with increasing residence time of the defending female (Bonatto et al., 2017). If we consider that in this season the population density begins to increase, that the proportion of juveniles increases as observed in this work and in other studies (Zuleta et al. 1988; Zuleta and

Kravetz 1992), and that enclosure experiments showed that females keep an exclusive home range independent of density, then the lower availability of free breeding territories would result in increased aggressive interactions between females (Ávila et al., 2016). In addition, several authors have shown that individuals of *A. azarae* can occasionally migrate to crop fields in summer, which coincides with increasing population density during the flowering period of crops (Bilenca et al., 1992; Bilenca and Kravetz 1998; Cavia et al., 2005). Therefore, the rising population density, aggressive behavior of resident females and the availability of new space offered by mature crops would explain the lack of structure observed in summer.

In autumn (population density peak), the death of overwintering females would allow adult females to colonize optimal habitats; most of these females were born between February and April and have their last opportunity of reproducing in the breeding season. Their female offspring will not reproduce until the following season. Therefore, staying near their mothers' territory would increase their probability of surviving the winter, since they would receive maternal care that would guarantee their survival under the unfavorable conditions of autumn and winter (Suárez, 1996; Zuleta, 1989); this phenomenon would explain the high positive autocorrelation observed in females in the first distance class (Fig. 3c). For the same reason, juvenile males would also stay near their mothers: autocorrelation in the first-distance class was also positive and significant when all juveniles were considered (Fig. S.1, Supplementary Data).

During winter, the quality of border habitats is markedly reduced and the plant cover is distributed in patches (Bush and Kravetz 1992). *Akodon azarae* winter SGS is random, which would be explained partially by random death of individuals and partially, by inter-patch movements towards those with high plant cover along borders (Bonaventura and Kravetz 1989, Cittadino et al., 1994).

Conclusions

Dispersal is a key factor for the persistence of natural populations in anthropogenically fragmented habitats such as agricultural landscapes. To continue the study of persistence in, and use of, border habitats by small mammals, in this work we estimated the spatial genetic structure of *A. azarae*. We hypothesized that, since this species shows a strong preference for relatively undisturbed habitats with good cover, rural roads and crop plots would impair gene flow and therefore, genetic structure would be present in different border habitats. Our results rejected this hypothesis, since *A. azarae*'s genetic

structure was characterized by a random dispersal pattern at the spatial scale analyzed in this study. No evidence of an effect of roads or crop fields on gene flow was found. This result is in line with a recent study by Serafini et al. (2019) showing that, although this species prefers complex landscapes, it is also present in simple ones. On the other hand, as expected for *A. azarae*'s mating system, males were not genetically structured. However, females' spatial genetic structure varied greatly throughout the year, which would be related to changes in habitat quality and availability, and to intrasex interactions.

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Figure

Figure. 1: Distribution and scheme of transects established across an agro-ecosystem in Río Cuarto Department (Córdoba province, Argentina).
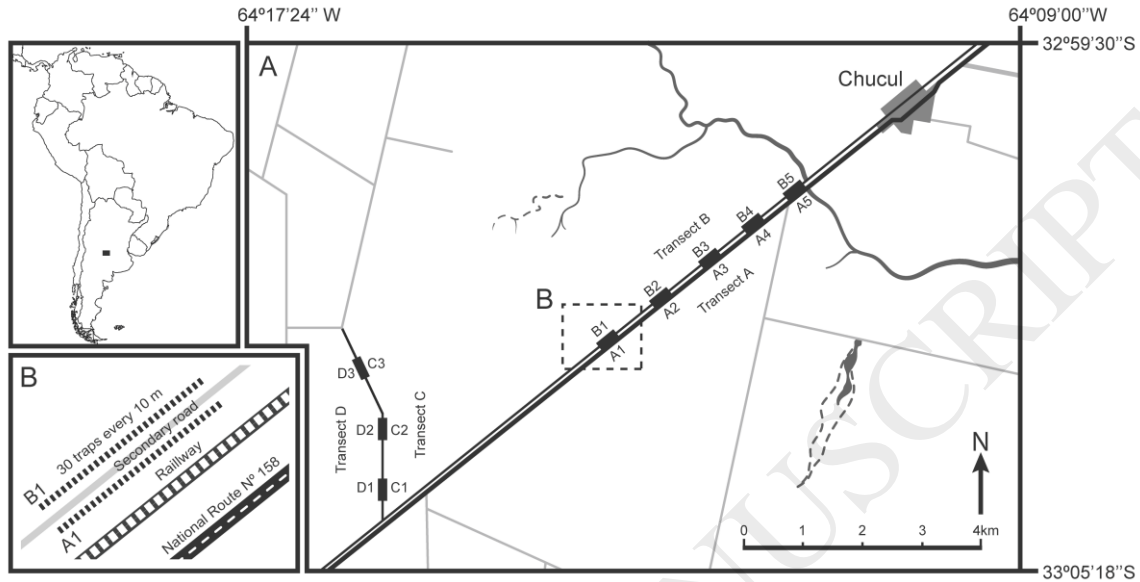

Figure 2: Spatial genetic autocorrelation analysis for *Akodon azarae* in spring (a), summer (b), autumn (c) and winter (d) performed with the "single pop" option in GENALEX. The diamonds connected by the solid line show the genetic autocorrelation coefficient *r*, and the dotted lines indicate the upper and lower bounds for the 95% confidence interval about the null hypothesis of no spatial structure ($r = 0$) obtained by 1000 random permutations of genotypes among distance classes. The vertical bars indicate the 95% confidence interval around "r" obtained by 1000 bootstrap resamplings within each distance class. The star indicates significantly positive "*r*" values with the one-tailed test ($P < 0.05$) and the arrows, the extent of positive spatial autocorrelation.
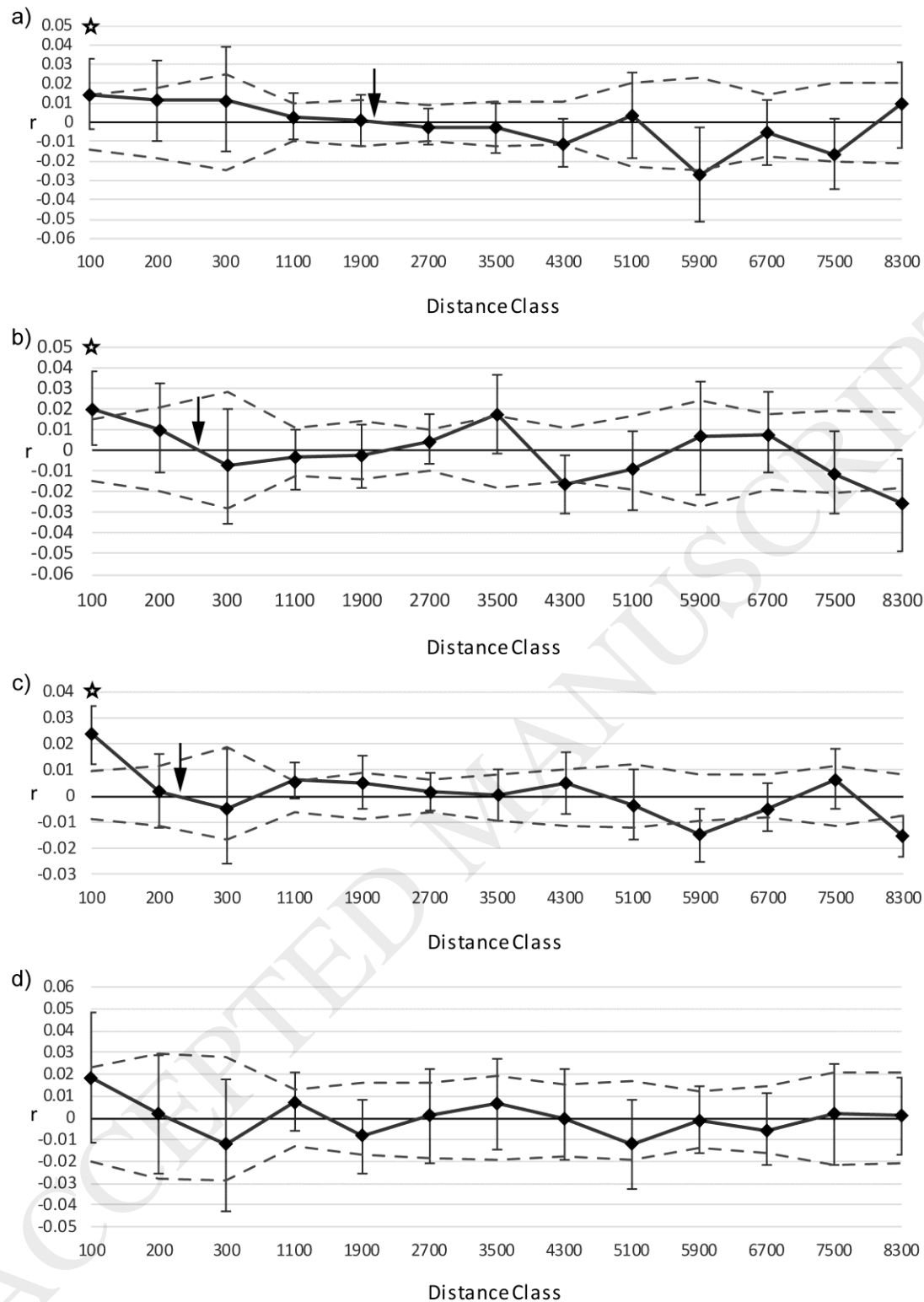
Figure 3: Spatial genetic structure by sex in spring (a), summer (b), autumn (c) and winter (d) performed using the "single-pop" option in GENALEX. The diamonds connected by the solid line show the genetic autocorrelation coefficient *r*, and the dotted lines indicate the upper and lower bounds for the 95% confidence interval about the null hypothesis of no spatial structure (r = 0) obtained by 1000 random permutations of genotypes among

distance classes. The vertical bars indicate the 95% confidence interval around "r" obtained by 1000 bootstrap resamplings within each distance class. The star indicates significantly positive "*r*" values with the one-tailed test (P < 0.05) and the arrows, the extent of positive spatial autocorrelation.
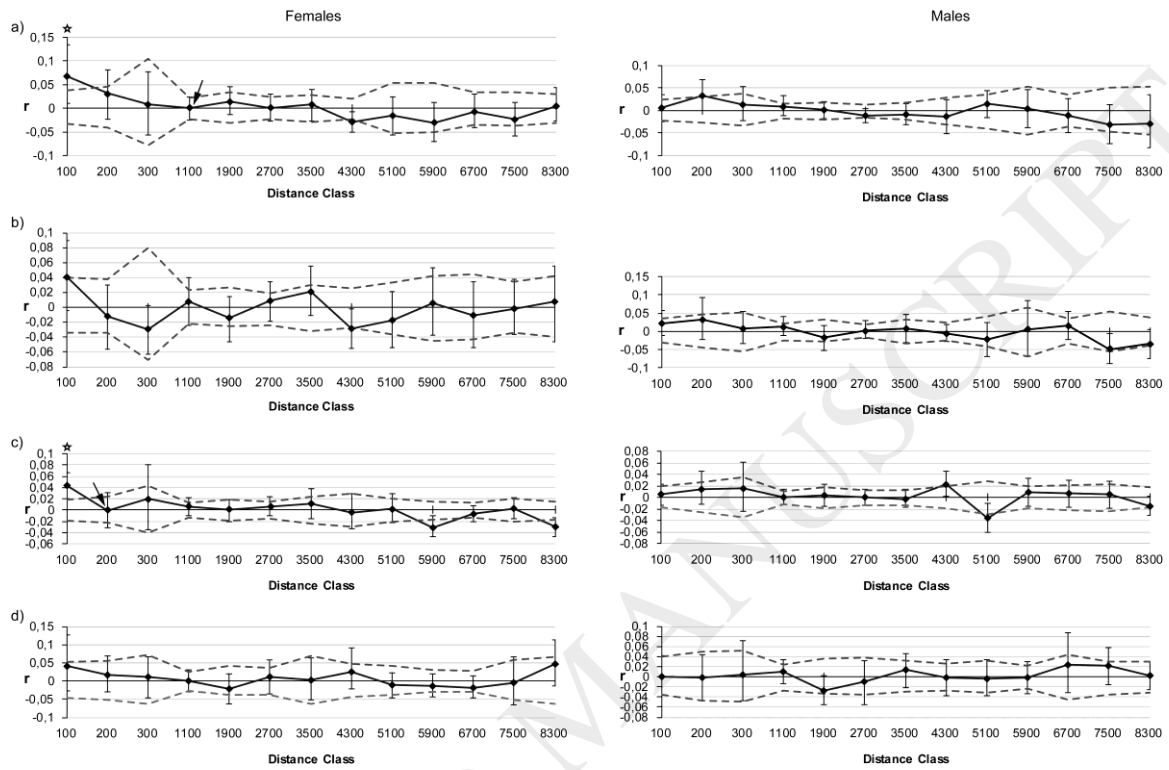
Table 1. Hypotheses about landscape resistance to gene flow in *A. azarae* and resistance values assigned to landscape elements.

Hypothesis	Borders	Crop/pasture field	Secondary roads
1- Fields show high resistance to gene flow; secondary roads show low resistance to gene flow	1	50	10
2- Fields and secondary roads show low resistance to gene flow	1	10	10
3- Fields show low resistance to gene flow; secondary roads show high resistance to gene flow.	1	10	50
4- Fields and secondary roads show high resistance to gene flow	1	50	50
Null- None of the habitat types show resistance to gene flow	1	1	1

Table 2. Levels of genetic variability in *Akodon azarae* by season. H_e: mean expected heterozygosity, H_o: mean observed heterozygosity and AR: mean allelic richness.

	Spring	Summer	Autumn	Winter	Mean value
H_o	0.811	0.815	0.812	0.812	0.81
H_e	0.885	0.885	0.875	0.879	0.88
AR	16.710	17.570	18.410	16.610	17.330

Table 3. Genetic differentiation (Θ) between transects. a) In spring, summer and winter, transects C and D were merged due to the small sample sizes. The significance of Θ values was obtained after the Bonferroni correction with 3000 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.017; b) In autumn, transects C and D were considered separately. The significance of Θ values was obtained after the Bonferroni correction with 6000 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.008. Statistically significant Θ values are indicated with an asterisk. The number of individuals analyzed per transect are in parenthesis.

a)

Season	Spring			Summer			Winter		
Transects	A (41)	B (30)	C+D (12)	A (37)	B (27)	C+D (14)	A (18)	B (25)	C+D (23)
A	0.0000			0.0000			0.0000		
B	- 0.0020	0.0000		0.0025	0.0000		0.0099*	0.0000	
C+D	0.0032	0.0076	0.0000	0.0088	0.0071	0.0000	0.0098*	0.0047	0.0000

b)

Season	Autumn			
Transects	A (40)	B (56)	C (15)	D (22)
A	0.0000			
B	0.0033	0.0000		
C	0.0281*	0.0167*	0.0000	
D	0.0173*	0.0113*	0.0189*	0.0000