SHORT COMMUNICATION

Genetic approach reveals a polygynous-polyandrous mating system and no social organization in a small and isolated population of the screaming hairy armadillo, *Chaetophractus vellerosus*

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Abstract

The development of agro-ecosystems in the pastures of the Pampean Region has substantially modified their structure and functioning. Many wild mammal populations in the Argentinean Pampas face habitat loss and/or fragmentation due to human activities, resulting in harmful genetic effects. The screaming hairy armadillo (*Chaetophractus vellerosus*) is a species considered an indicator of the state of preservation of the environments it inhabits. However, very little information is available about its mating system in the wild. In this sense, an isolated population of the screaming hairy armadillo in the northeast of Buenos Aires Province, which is separated from the main distribution area of the species by about 500 km, requires special attention. Genetic studies that analyzed social behavior and mating systems in Xenarthra are scarce but necessary to establish conservation actions for the isolated screaming hairy armadillo population under study. Thus, we analyzed the existence of a possible social organization in the species, together with its mating system, using a set of previously characterized microsatellites. Our results showed a complex scenario for the dispersal and mating system in this *C. vellerosus* population. Males disperse and females have a philopatric tendency with some degree of dispersal. This strategy, in combination with a polygynous-polyandrous mating system, could enhance genetic variability in this small and isolated population. In addition, no evidence of social organization was found.

Keywords Social organization · Mating system · Armadillos · Habitat fragmentation · Molecular ecology

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Introduction

Synergistic effects between habitat degradation, fragmentation and global climate warming can compound the effects of habitat loss on biodiversity (Hilty et al. 2012). The first consequences of this phenomenon are isolation and reduced population sizes. Small isolated populations can suffer the effects of inbreeding and loss of heterozygosity, leading to a decrease in reproductive success and an increase in extinction probability (Frankham et al. 2002). Early detection of potentially deleterious genetic load and loss of genetic variability maximizes our ability to implement a management approach aiming to limit or reverse these effects before they become substantial or irreversible (Hedrick 2001).

The development of agro-ecosystems in the pastures of the Pampean Region has substantially modified its structure and functioning (Viglizzo et al. 2011). Many wild mammal populations in the Argentinean Pampas face habitat loss and/or fragmentation due to human activities

related to cattle raising and farming, resulting in harmful genetic effects (Viglizzo et al. 2011; Bilenca et al. 2012). The screaming hairy armadillo (*Chaetophractus vellerosus*; Xenarthra, Cingulata, Chlamyphoridae) is a species considered as indicator of the state of preservation of the environments it inhabits (Pagnutti et al. 2014). In this sense, an isolated population of the screaming hairy armadillo in the northeast of Buenos Aires Province, Argentina, which is separated from the main distribution area of the species by about 500 km (Crespo 1974; Carlini and Vizcaíno 1987; Abba et al. 2011) (Fig. 1), requires special attention. This isolated population is associated with the shelly beach ridges on the coast of the Río de la Plata Estuary, covering an area of less than 900 km^2 (Abba and Superina 2010). These animals live in close proximity to humans and, in many cases, are harmed by their activities (Abba et al. 2007). Their main threats are the loss of habitat generated by agricultural-livestock activity and urbanization, mining activities, hunting for consumption and control of abundance, the attack of dogs and the run over by vehicles (Redford and Eisenberg 1992; Abba et al. 2007; Abba and Superina 2010). Because of these threats, this isolated population is currently at high risk of extinction and the species is regionally listed as Endangered

(Abba et al. 2019). Such disturbances are thought to affect both individual behavior and population dynamics.

The assessment of a species' mating system is a vital step for conservation. Reproductive behavior affects many biological processes that differentially impact the effective population size (*Ne*) (Darden and Croft 2008), including inbreeding (Meagher et al. 2000) and offspring viability (Newcomer et al. 1999). This highlights the importance of knowing the mating system, for example, in translocation programs (Sigg et al. 2005). In addition, mating systems may promote the evolution of populations through assortative mating and/or reproductive isolation, which would lead to a posterior speciation process (Panhuis et al. 2001), or increasing their extinction risk (Møller and Legendre 2001).

To our knowledge, only one paper analyzed the mating system of armadillos. McDonough (2000) investigated spatial and mating relationships in a population of individually marked nine-banded armadillos (*Dasypus novemcinctus*) in Texas, USA. The author suggested a polygynous mating system for the species because most breeding males paired with more than one female during the breeding season. In addition, Superina and Abba (2018) mentioned that a preliminary study on the screaming hairy armadillo found a polygynous-polyandrous pattern. This comment was based
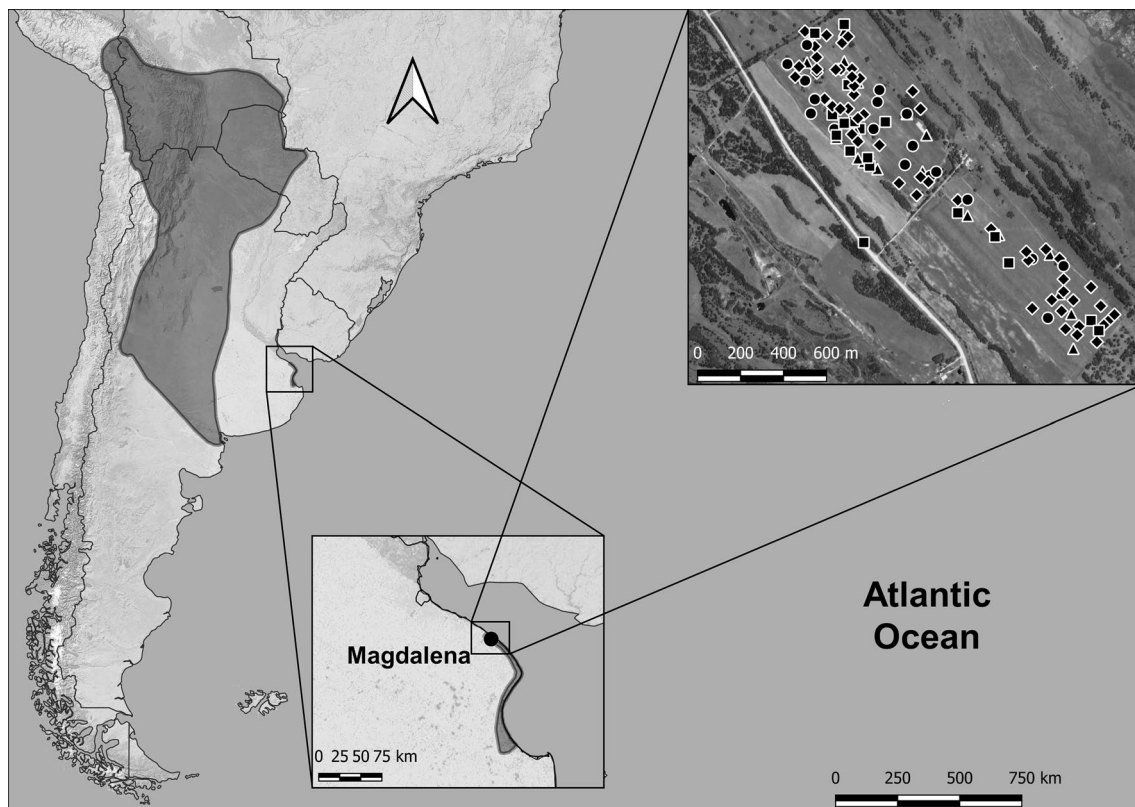
Fig. 1 Geographic distribution of *C. vellerosus* (in dark grey) and the 92 individuals assigned to each of the three genetic groups. Squares, diamonds and triangles indicate individuals that belong to each of the genetic groups. Circles indicate individuals not assigned to any group

on a genetic analysis with a small sample pool performed by some of the authors of the present work (Dobler et al. 2016).

Regarding the social organization, several field studies carried out by one of the authors of this work (Abba et al. 2007, 2011; Abba and Cassini 2010; Pagnutti et al. 2014) have confirmed most of the general characteristics postulated previously for Xenarthra (Vizcaíno and Loughry 2008). In general, armadillos are solitary and little aggressive. Outside the breeding season, they have flexible-sized home ranges that overlap considerably between individuals. The aggressions are sporadic, collaboration seems non-existent and no form of social bond has been recorded, not even between relatives. During the breeding season, temporary couples are formed. The females are slightly larger than males, so sexual selection is not expected to operate strongly on the males. Pagnutti et al. (2014) analyzed the home range of screaming hairy armadillos in the same area studied here, which is divided in two pastures with different use intensity, a more disturbed pasture and a less disturbed one (see Materials and Methods section for details). Their results showed that the home range was reduced by human disturbance and that individuals from the more disturbed pasture presented a more aggregated distribution when compared to the less disturbed pasture.

Given the scarce genetic studies related to the social behavior and mating system in Xenarthra, and the need to establish conservation actions for the isolated population of screaming hairy armadillo under study, our goal in this paper is to analyze the mating system and a possible social organization in the species. Taking into account the background information cited above, we hypothesized that individuals from the more disturbed pasture are more related between them than to individuals from the less disturbed pasture as a result of inbreeding. In addition, we expect a direct relationship between the spatial location of individuals and their kinship relationship and a polygynous-polyandrous mating system.

Materials and methods

Study area, sampling and DNA extraction

During 8 years (2006–2013) armadillos were sampled in a 100 hectare cattle farm located in Magdalena, Buenos Aires, Argentina (35° 10.45′ S, 57° 20.66′ W; Fig. 1). The field limits to the west by the Provincial Route #11, to the east by the Rio de la Plata Estuary and to the north and south by two artificial canals (built approximately 50 years ago) that flow into this estuary. These boundaries represent *a priori*, physical barriers to dispersal. Screaming hairy armadillos could cross the route, but the terrain on the other side is not suitable for dispersal. This study area is divided into two similarly-sized pastures (approximately 50 hectares each) with different use intensity. The northern one, characterized by a low intensity of use, is mainly used for cattle and sheep breeding, whereas the southern one, with high intensity of use, is covered by modified grassland used for livestock feeding.

The mean sampling effort was 15 days/year, capturing between 29 and 76 individuals each year (Abba et al. 2017). Population was estimated around 100–150 individuals, but it had a high replacement rate, evidenced by a low resampling. Individuals were captured by hand, sometimes helped by a net. Location of each captured individual was registered with a hand-held GPS. Small ear punches of tissues were collected from 110 armadillos, 74 from the northern less disturbed pasture and 36 from the southern more disturbed one. Three types of marks were used to identify captured individuals: (1) permanent mark: it consisted of one to three small cuts made with a notcher in the ear; tissue was collected for subsequent DNA extraction in the laboratory, (2) semi permanent mark: earrings or ear tags with identification numbers were affixed, and (3) temporary mark: stickers with different shapes and colors were used to identify armadillos during the same field campaign. The latter were intended to prevent the capture and handling of the same individual during the same season. Tissue samples were used for DNA extraction using a phenol:chloroform and DNA precipitation method (Sambrook et al. 1989). Precipitated DNA was resuspended in buffer TE, pH = 8.0, quantified in a spectrophotometer at 260/280 nm and stored at −20 °C.

Microsatellite amplification

Optimal PCR conditions were described, characterized and determined for a microsatellite set useful for this species in a previous study (Nardelli et al. 2016) in which 69 of the 110 individuals analyzed here were genotyped. Six microsatellite loci proved to be informative enough for individual identification with a probability greater than 0.99.

The PCR amplification protocol consisted of one step of denaturation at 95 °C for 3 min; followed by 35 cycles, each involving denaturation at 95 °C for 30 s, 45 s at annealing temperature (Table 1) and extension at 72 °C for 30 s; with a final extension step at 72 °C for 5 min. PCR amplifications were performed in 25 µl volumes containing 10 ng of DNA, 1 × PCR buffer (PB-L, Argentina), 3 mM MgCl$_2$, 0.2 mM of dNTPs mix (Genbiotech, Argentina), 0.4 µM of each primer (Genbiotech, Argentina), 0.5 U of *Taq* DNA polymerase (PB-L, Argentina), and sterile distilled water to reach final volume. One of the primers of each pair was dyed with FAM or HEX fluorochromes. Amplification products were visualized by migration on 2% agarose gel electrophoresis at 4 V/cm.

Table 1 General features of microsatellite loci and genetic diversity for the screaming hairy armadillo (*C. vellerosus*) in the study area

Locus name	Primer sequences	Repeat motif	T_a (°C)	n	Size range (bp)	N_A	PIC	H_o	H_e	P_{HWE}	F_{IS}
M1	GACCCG GTTCGA TTTAATA CACTGCTTG ACATTC TCATT	$(AG)_{13}$	56	108	95–111	10	0.718	0.648	0.751	***	0.137
M2	CTGGGT ATTCAC ACCAGAA GGGGTG ACGAAA GTTAAAG	$(AC)_{14}$	56	109	86–108	15	0.766	0.624	0.781	***	0.201
M4	CTAACC GTGCAT TTTATGG GGCCTA AGACGG TATTACA	$(TC)_8$	54	108	71–142	8	0.538	0.648	0.585	***	−0.089
M5	TCAAAG ACAATG TCCCCT A ATTTTC CAGCCT TGATCTG	$(AC)_{15}$	54	108	77–112	14	0.756	0.787	0.782	***	−0.006
M6	CAAGCA AGCAAG CAAG GCC ACGGTT TAGTTA ATCA	$(AAC)_8$	49	102	87–109	18	0.635	0.598	0.679	***	0.119
M7	ACCCTT CAAAAA CACTTATT TAAAAA CAAGCA AGCAAGC	$(TTG)_8$	48	108	77–168	26	0.834	0.602	0.848	***	0.290
					Mean	15.17	0.708	0.651	0.739		

T_a annealing temperature. n, individuals, N_A number of alleles, *PIC* polymorphic information content, H_o observed heterozygosity, H_e expected heterozygosity, P_{HWE} p value for exact test of *Hardy–Weinberg* equilibrium, F_{IS} inbreeding coefficient

*** $P < 0.0001$

Data analyses

Genotypes were determined using GeneMarker v. 2.2.0 (Softgenetics). Allelic richness and observed and expected heterozygosities were estimated with GenAlEx v. 6.5 (Peakall and Smouse 2012). Adjustment to Hardy–Weinberg Equilibrium (HWE) and F_{IS} values for all loci were calculated using GENEPOP v.4.2 (Raymond 1995). Polymorphic Information Content (PIC) was evaluated using Microsatellite Toolkit v. 3.3.1 (Park 2001). Finally, population structuring in our data set was tested using STRUCTURE 2.3.4 (Pritchard et al. 2000). This approach uses a Bayesian clustering analysis to assign individuals to clusters (K) without prior knowledge of their population affinities. STRUCTURE simulations were performed using an Admixture Model with the number of presumed clusters ranging from $K = 1$ to $K = 7$ and 20 runs per tested K value. For each run, the initial burn-in period was set to 100,000 followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations. The most probable number of clusters was determined by plotting Delta K as a function of K using Structure Harvester (Earl and vonHoldt 2012), an on-line application that applies the Evanno´s method (Evanno et al. 2005). We chose a proportion of membership threshold value of $q \geq 0.8$ to assign individuals to clusters. This value provides a statistical cutoff within the range of suggested values in the literature (Manel et al. 2002) and indicates that $\geq 80\%$ of ancestry can be attributed to the respective subpopulation.

To calculate genetic relatedness between pairs of individuals, we used the coefficient of relationship known as r, calculated by the method of Queller and Goodnight (1989). It was calculated using GeneAlEx v. 6.5.

In order to analyze kinship, we ran the Colony v 2.0.6.4 software (Jones and Wang 2010). Four runs were performed with the following settings: female and male polygamy with inbreeding for a monoecious species, no clones allowed, length of run was very long under the full-likelihood method with very high precision, allele frequencies estimated from the data set and updated as the analysis is run, sibship scaling set to yes with no sibship prior assigned. Allelic dropout rate was set to 0.001 and mutation/error rate to 0.001. Individuals were loaded as offspring when we could not determine possible parents and offspring in the pool of animals sampled. For final kinship assessment, we checked the results for concordance between sampling and genetic data, taking into account sex, age, weight and date of capture of each individual.

In order to test the existence of a possible social organization in the species, we analyzed if there was a correlation between the geographical distance among individuals and the parentage relatedness between them. This analysis was performed for the whole data set and for males and females separately. For this purpose, we plotted the geographic distribution of the individuals in a Geographical Information System implemented in the QGIS v3.6 software. We used the geographical location of the individuals at the time of their capture as an input to create their distribution layer. Subsequently, the geographic distance between all pairs of individuals was calculated using the Distance Matrix algorithm in QGIS. Finally, a correlation analysis between geographical and genetic distances between the individuals was made through a Mantel Test in GenAlex v. 6.5. The possible conformation of family groups was also evaluated. To address this issue, we tested if there were significant differences between the r values of those individuals that were less or more than 100 m apart at the time of capture. In this case, a Spearman correlation using RStudio package was done. The decision to take 100 m as a parameter was related to the fact that this species has a maximum home range of approximately 0.80 ha (Pagnutti et al. 2014).

Results and discussion

Genetic diversity and population structure

In a previous study (Nardelli et al. 2016) we analyzed genetic diversity and population structure of this isolated population and reported the results obtained by a set of six microsatellites specially designed for genetic studies in *C. vellerosus*. In the same study, genetic diversity parameters were calculated over 69 individuals. Here, we recalculated these parameters using a greater sample size (n = 110; Table 1).

The addition of 41 new samples to the 69 samples pool previously analyzed only contributed a new allele in the M5 locus. PIC value suffered a slight reduction (0.752 to 0.708), but all loci were still highly informative (Table 1). In addition, Probability of Identity (P_{ID}) and Probability of Identity among Siblings (P_{IDsibs}) for the whole set of loci were 5.4×10^{-7} and 4.3×10^{-3}, respectively, which confirmed that any individual in this population could be identified, and distinguished from the other individuals in the population, with a probability greater than 0.99.

Observed heterozygosities ranged from 0.598 to 0.787, averaging 0.651, which is higher than the value calculated before ($H_o = 0.583$). Expected heterozygosities varied from 0.585 to 0.848, with a mean value of 0.739. This value was slightly lower than the obtained in the previous study ($H_e = 0.776$, Nardelli et al. 2016). However, these differences between both works seem to be a random variation produced by sample sizes more than a significant difference. Therefore, none of the six polymorphic loci adjusted to HWE (p < 0.001; Table 1). Waples (2015) conducted an exhaustive study analyzing the possible causes of departures from HWE in natural populations. The possible causes include overlapping generations, population structure, endogamy

and small effective population size, among others. Departure from HWE in our data set could be due to the effect of over-lapping generations, taking into account that samples used in our study were taken from 2006 to 2013, and that captured individuals included offspring, juveniles and adults. Thus, 2 or 3 generations may have been sampled in our study period, taking into account some species characteristics such as life expectancy and reproductive strategy. Population genetic structure (Wahlund effect) would be another possible cause of the HWE deviations observed. This departure from HWE was previously attributed to the presence of three sympatric subpopulations in the area (Nardelli et al. 2016). We attempted to confirm this previous finding by performing a new STRUCTURE analysis that incorporates the 41 new individuals. The output of STRUCTURE also showed the presence of the three sympatric subpopulations previously described (Figs. 1 and 2). Ninety-two of the 110 individuals (84%) were assigned to one of the three groups. A larger subpopulation of 54 armadillos, and two small ones with 20 and 18 individuals were identified, while 18 animals could not be assigned to any genetic group (Fig. 1). The genome of these individuals not assigned to any genetic group is composed by a mixture of the alleles that defined each subpopulation, and evidenced the existence of gene flow between them. Compared to the previous study, a greater proportion of individuals was assigned to a subpopulation (71% in Nardelli et al. 2016). Field surveys did not detect evidence of physical barriers to dispersal in the study area that might explain this genetic structuring. The observed genetic structure might thus be due to the social behavior or the mating system of the species. To test the effect of sex on genetic structure, we also conducted a STRUCTURE
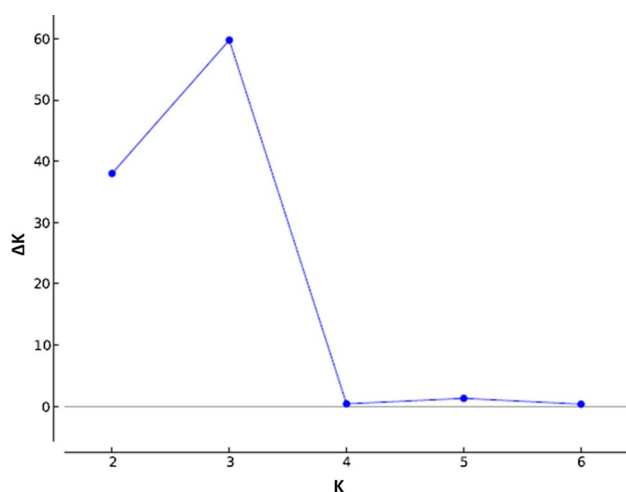
Fig. 2 Delta K ($\Delta K = mean|L''(K)|/std.\ dev.\ [L(K)]$) plot to determine the optimal number of clusters in STRUCTURE, determined using Structure Harvester (Earl and vonHoldt 2012). The highest delta K is observed at $K = 3$

analysis for males and females separately. We obtained three subpopulations for females and only two for males. Again, we found a sympatric distribution for these subpopulations.

Relatedness analyses

Relatedness coefficient (r) between all pairs of individuals was calculated. The mean r value was -0.011 (S.E. = 0.003), indicating low inbreeding levels in the population. We also calculated the mean r values between individuals sampled in the same pasture. We expected a higher and significantly different r value for the southern pasture, according to the results of Pagnutti et al. (2014). In that research, the authors described an aggregated distribution of screaming hairy armadillos in the southern pasture and a random arrangement in the northern one, suggesting that this arrangement was influenced by land use intensity. However, mean r values obtained were not significantly different (Northern pasture: -0.015, S.E. = 0.004; Southern pasture: -0.033, S.E. = 0.010; Mann-Whitney U-test; Z = -1.285, p = 0.20). This result suggests that inbreeding is not a consequence of the aggregated distribution described previously for the southern pasture. In a related work, Colle-vatti et al. (2007) performed a genetic relatedness analysis in a fragmented population of *Myrmecophaga tridactyla* in Emas National Park, Brazil. The authors found low genetic diversity, high levels of inbreeding and that animals with overlapping home ranges were highly related. Conversely, the *C. vellerosus* population under study presents a high genetic diversity comparable to that obtained for populations of *D. novemcinctus*, which are abundant and inter-connected (Nardelli et al. 2016). Results obtained in the present work confirmed these previous findings. In spite of being a small, isolated population affected by human activities, it presents high values of genetic diversity.

For the whole set of individuals analyzed together, Mantel test did not reveal significant correlation between geographical distance among individuals and their genetic related-ness ($R_{xy} = -0.009$, p = 0.530). This result is in agreement with the sympatric distribution of the three subpopulations (Fig. 1). Because Pagnutti et al. (2014) described a larger home range for males than females in the reproductive season, we also performed this analysis for each of the sexes separately, in order to test for differences between sexes. Mantel test did not show a significant correlation between geographic and genetic distances for males ($R_{xy} = 0.016$, p = 0.340). In the case of females, a significant but weak and negative correlation was observed ($R_{xy} = -0.096$, p = 0.040). This result suggests a male-biased dispersal tendency and a possible female philopatry phenomenon. In line with the latter, during field work carried out in November 2018, we recaptured a female individual 2 or 3 m away from its capture site in October 2012. Sex-biased dispersal studies

in the nine-banded armadillo, *D. novemcinctus*, reached different conclusions.Loughry and McDonough 1998, 2001) found no sex-biased dispersal in a population of individually marked armadillos in Florida, United States, while Binns (2013), using a set of 8 microsatellite loci, described a female-biased dispersal pattern in a population of the species located in western Mississippi. *D. novemcinctus* give birth to litters of monozygotic quadruplets, so dispersal could be a strategy to avoid competition for resources. Instead, litters of *C. vellerosus* consist of 1–2 offspring, hence remaining near the mother's home range could enhance the possibility of survival.

Taking into account the average home range calculated previously for the study population (Pagnutti et al. 2014; see Materials and Methods) section, we also evaluated the possible existence of social groups composed of related individuals. Our results showed a non-significant correlation between r and the condition of being more or less than 100 m away at the time of sampling ($\rho = 0.022$, $p = 0.20$). The same tendency was observed for males and females when they were analyzed separately ($\rho = 0.010$, $p = 0.73$; $\rho = -0.017$, $p = 0.48$; respectively). Although a female philopatry phenomenon is possible in this population, as suggested by Mantel test results, we could not detect indications of the existence of social groups composed by females. It is possible that the sympatric distribution of the three subpopulations may mask its existence.

Mating system

In order to correctly interpret our results about mating system, some biological characteristics of this species should be taken into account. Screaming hairy armadillos become sexually mature at about 11 months of age (males and females). Mating period initiates at the end of winter to the beginning of spring. Gestation length is about 60 days, and litters have 1–2 offspring. A single litter per year is born inside the burrow, in which the female sometimes builds a nest with plant material. Offspring remain inside the burrow during most of the lactation period and start foraging outside the burrow in summer. Average life expectancy has been estimated at approximately 3 years, and maximum longevity is estimated to be 6–10 years (Superina and Abba 2018).

Pedigrees were built taking into account the outputs of the Colony program. Surprisingly, we could determine only 3 mothers and 3 fathers. Other parents may not have been sampled, due to the high replacement rate of individuals or the number of microsatellite markers used may not be sufficient for reliable parentage assignment. On the other hand, pedigrees in Fig. 3 shows that these individuals present multiple litters. We also could identify 32 full-sibs and more than 40 half-sibs relationships. All of the kinships attributed had a probability value greater than 0.5 in the Colony output and
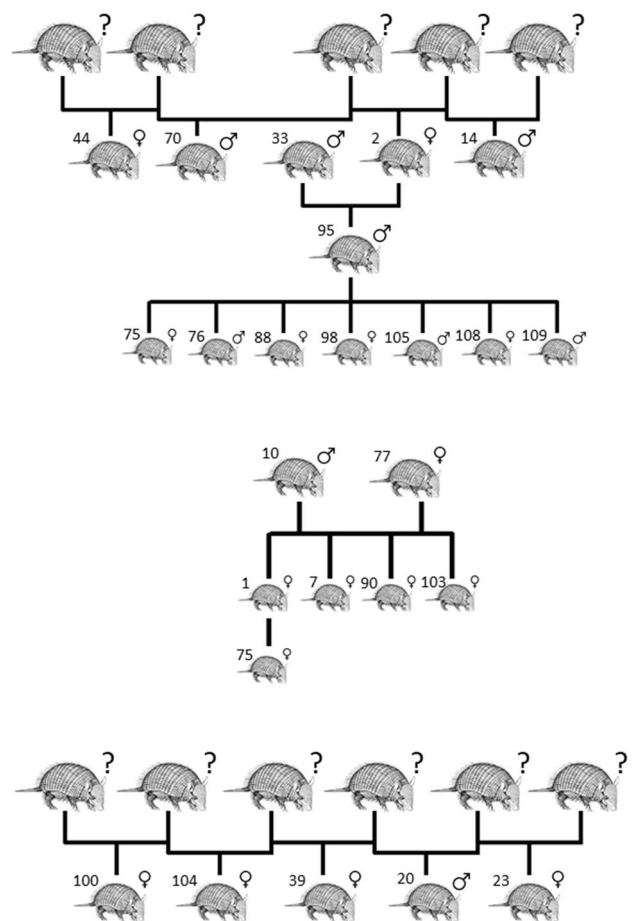
Fig. 3 Some pedigrees of *C. vellerosus* performed using Colony output. Upper right symbols indicate sex (♀ or ♂) or not sampled individuals (?). Upper left numbers represent individual's number

were corroborated using age and size data. Some of these results are shown in Fig. 3. The reconstructed pedigrees were consistent with the polygynous-polyandrous mating system previously inferred (Dobler et al. 2016). The keys to establishing the polygynous-polyandrous mating system were the half-sibhood relationships found. For example, the pedigree at the bottom of Fig. 3 shows that individual 104 has a half-sib relationship with individuals 100 and 39. However, individuals 100 and 39 are not related. Then, individual 39 has a half-sib relationship with individuals 104 and 20, but again, the latter two are not related. Although we have not identified the parents of these individuals, we could infer that at least one female mated with (at least) two males, and that at least one male mated with (at least) two females in order to obtain the half-sibhood relationships described.

Besides the studies mentioned above about mating systems in cingulates (McDonough 2000; Dobler et al. 2016), a few other studies address this phenomenon in other xenarthrans. Peery and Pauli (2012) described that the mating system of the Hoffmann's two-toed sloth, *Choloepus hoffmanni*,

Table 2 Geographic distances between full-sister and full-brother pairs

Sister pair		Distance (m)	Brother pair		Distance (m)
1	7	1694	6	22	68
1	90	1518	33	76	343
1	103	1608	33	105	1240
7	90	179	33	109	430
7	103	86	33	110	1415
75	88	1613	37	38	230
75	98	1597	71	92	933
75	108	960	76	105	153
88	98	209	76	109	701
88	108	719	76	110	1753
90	103	92	105	109	811
98	108	652	105	110	185
			109	110	986

involves a mixture of polygyny and promiscuity. The same authors proposed that the brown-throated three-toed sloth, *Bradypus variegatus*, exhibits a strongly polygynous system where males exclude competitors from their core ranges (Pauli and Peery 2012). Then, the same research group re-analyzed the mating system in both species using long-term genetic and space-use data (Garcés-Restrepo et al. 2017). They described that many female sloths mated with different males, recognizing a promiscuous pattern in both species. Also, they suggested that mate switching behavior employed by female sloths, enhances genetic diversity and the possibly to select higher-quality males. Our results suggest a similar strategy in *C. vellerosus* that might be the cause of the high genetic diversity in our study area.

In the case of *C. vellerosus*, the polygynous-polyandrous mating system observed impacts positively on the viability of this isolated population, increasing the number of individuals that mate in comparison to other mating systems as monogamy. In consequence, inbreeding and extinction risk would be reduced, through increasing genetic variability and heterozygosity.

In most polygynous and promiscuous populations of mammals, juvenile males are the predominant dispersers, whereas in monogamous species both male and female juveniles disperse equally often and equally far (Dobson and Jones 1985). The philopatric tendency observed for female individuals in this isolated population agrees with what was expected for polygynous and promiscuous mammal species. Nevertheless, full-sister dispersal distances revealed that some female dispersion occurs (Table 2). A possible explanation for this finding is that in a litter consisting of two females, one of them could remain near the mother and the other disperses, thus avoiding competence for resources. Full-brothers showed dispersal

distances similar to those of full-sisters (Table 2). As a consequence, non-significant differences between sexes were found (Mann-Whitney U-test; $Z = -0.598$, $p = 0.57$). Dispersal in random directions could mask the expected pattern for males.

Thus, our results evidence a complex scenario for the dispersal and mating system in this *C. vellerosus* population. Males disperse and females have a philopatric tendency with some degree of dispersal. This strategy, in combination with a polygynous-polyandrous mating system, could enhance genetic variability in this small and isolated population.

Results from genetic studies of mating patterns and social organization have often differed markedly from results of exclusively observational studies (Hughes 1998). There is increasing evidence that breeding systems may be substantially more complex and incorporate a greater variety of strategies than formerly documented (Kitchen et al. 2006). The structure and stability of breeding groups may also be more variable than previously thought, and long-term studies incorporating observational and genetic information are needed to fully describe social organization and mating systems.

References

Abba AM, Vizcaíno SF, Cassini MH (2007) Effects of land use on the distribution of three species of armadillos in the Argentinean pampas. J Mammal 88:502–507

Abba AM, Cassini MH (2010) Ecological differences between two sympatric species of armadillos (Xenarthra, Mammalia) in a temperate region of Argentina. Acta Theriol 55:35–44

Abba AM, Superina M (2010) The 2009/2010 Armadillo red list assessment. Edentata 11:135–184

Abba AM, Cassini GH, Cassini MH, Vizcaíno SF (2011) Historia natural del piche llorón *Chaetophractus vellerosus* (Mammalia: Xenarthra: Dasypodidae). Rev Chil Hist Nat 84:51–64

Abba AM, Benitez VV, Doyle SR (2017) Population ecology of *Chaetophractus vellerosus*: the first report for an armadillo in South America. Zoologia 34:e20785

Abba AM, Camino M, Torres RM, Ferreiro AM, Tamburini DM, Decarre J, Soibelzon E, Castro LB, Rogel TG, Agüero AJ, Albrecht CD, Superina M (2019) *Chaetophractus vellerosus. Categorización 2019 de los mamíferos de Argentina según su riesgo de extinción. Lista Roja de los mamíferos de Argentina.* http://cma.sarem.org.ar. Accessed 21 Dec 2019

Bilenca D, Codesido M, Fischer CG, Carusi LP, Zufiaurre E, Abba A (2012) Impactos de la transformación agropecuaria sobre la biodiversidad en la provincia de Buenos Aires. Rev Mus Argent Cienc Nat 14:189–198

Binns LA (2013) Spatial genetic structure of the nine-banded armadillo in western Mississippi. Doctoral Dissertation, Valdosta State University

Carlini AA, Vizcaino SF (1987) A new record of the armadillo *Chaetophractus vellerosus* (Gray, 1865) (Mammalia, Dasypodidae) in the Buenos Aires Province of Argentine: possible causes for the disjunct distribution. Stud Neotrop Fauna Environ 22:53–56

Collevatti RG, Leite KC, de Miranda GH, Rodrigues FH (2007) Evidence of high inbreeding in a population of the endangered giant anteater, *Myrmecophaga tridactyla* (Myrmecophagidae), from Emas National Park, Brazil. Genet Mol Biol 30:112–120

Crespo JA (1974) Comentarios sobre nuevas localidades para mamíferos de Argentina y de Bolivia. Rev Mus Argent Cienc Nat 11:1–31

Darden SK, Croft DP (2008) Male harassment drives females to alter habitat use and leads to segregation of the sexes. Biol Lett 4(5):449–451. https://doi.org/10.1098/rsbl.2008.0308

Dobler D, Ibañez EA, Abba AM, Túnez JI, Nardelli M (2016) Introducción al estudio del comportamiento social de *Chaetophractus vellerosus* mediante el uso de marcadores moleculares en una población aislada y afectada por el impacto antrópico. XXIX Jornadas Argentinas de Mastozoología, San Juan

Dobson FS, Jones WT (1985) Multiple causes of dispersal. Am Nat 126:855–858

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Res 4:359–361

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Frankham R, Briscoe DA, Ballou JD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Garcés-Restrepo MF, Peery MZ, Reid B, Pauli JN (2017) Individual reproductive strategies shape the mating system of tree sloths. J Mammal 98:1417–1425

Hedrick PW (2001) Conservation genetics: where are we now? Trends Ecol Evol 16:629–636

Hilty JA, Lidicker WZ Jr, Merenlender AM (2012) Corridor ecology: the science and practice of linking landscapes for biodiversity conservation. Island Press, Washington, DC

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. Ecology 79:383–399

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Res 10:551–555

Kitchen AM, Gese EM, Waits LP, Karki SM, Schauster ER (2006) Multiple breeding strategies in the swift fox, *Vulpes velox*. Anim Behav 71:1029–1038

Loughry WJ, McDonough CM (1998) Spatial patterns in a population of nine-banded armadillos (*Dasypus novemcinctus*). Am Mid Nat 140:161–170

Loughry WJ, McDonough CM (2001) Natal recruitment and adult retention in a population of nine-banded armadillos. Acta Theriol 46:393–406

Manel S, Berthier P, Luikart G (2002) Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conserv Biol 16:650–659

McDonough CM (2000) Social organization of nine-banded armadillos (*Dasypus novemcinctus*) in a riparian habitat. Am Mid Nat 144:139–152

Meagher S, Penn DJ, Potts WK (2000) Male-male competition magnifies inbreeding depression in wild house mice. Proc Natl Acad Sci USA 97:3324–3329

Møller AP, Legendre S (2001) Allee effect, sexual selection and demographic stochasticity. Oikos 92:27–34

Nardelli M, Ibañez EA, Dobler D, Justy F, Delsuc F, Abba AM, Cassini MH, Túnez JI (2016) Genetic structuring in a relictual population of screaming hairy armadillo (*Chaetophractus vellerosus*) in Argentina revealed by a set of novel microsatellite loci. Genetica 144:469–476

Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of polyandrous females. Proc Natl Acad Sci USA 96:10236–10241

Pagnutti N, Gallo J, Superina M, Vizcaíno SF, Abba AM (2014) Patrones estacionales de distribución espacial y área de acción del piche llorón, *Chaetophractus vellerosus* (Cingulata: Dasypodidae), en Magdalena, Buenos Aires, Argentina. Mastozool Neotrop 21:59–65

Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. Trends Ecol Evol 16:364–371

Park SDE (2001) Trypanotolerance in West African cattle and the population genetic effects of selection. Doctoral Dissertation, University of Dublin

Pauli JN, Peery MZ (2012) Unexpected strong polygyny in the brown-throated three-toed sloth. PLoS ONE 7:e51389

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research an update. Bioinformatics 28:2537–2539

Peery MZ, Pauli JN (2012) The mating system of a 'lazy' mammal, Hoffmann's two-toed sloth. Anim Behav 84:555–562

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Raymond M (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Redford KH, Eisenberg JF (1992) Mamm Neotr. Chicago University Press, Chicago

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. Cold Spring Harbor Laboratory Press, New York

Sigg DP, Goldizen AW, Pople AR (2005) The importance of mating system in translocation programs: reproductive success of released male bridled nailtail wallabies. Biol Conserv 123:289–300

Superina M, Abba AM (2018) Chlamyphoridae. In: Wilson DE, Mittermeier RA (eds) Handbook of the mammals of the world. Lynx Edicions, Barcelona, pp 48–73

Viglizzo EF, Frank FC, Carreño LV, Jobbagy EG, Pereyra H, Clatt J, Pincén D, Ricard MF (2011) Ecological and environmental footprint of 50 years of agricultural expansion in Argentina. Glob Change Biol 17:959–973

Vizcaino SF, Loughry WJ (2008) The biology of the Xenarthra. University Press of Florida, Gainesville

Waples RS (2015) Testing for Hardy–Weinberg proportions: have we lost the plot? J Hered 106(1):1–19