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Genetic structure of Greater Rhea (*Rhea americana*) populations in two regions with different land-uses in central Argentina

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Abstract. The distribution of populations of Greater Rhea (*Rhea americana*) are fragmented, probably owing to the conversion of grasslands to croplands. This study analyses the genetic structure of populations of Greater Rhea in areas with different degrees of anthropogenic alteration. Molecular variation was evaluated using Inter Simple Sequence Repeats (ISSR) markers in five wild populations that inhabit an agroecosystem and an area of semi-natural grassland. The populations were polymorphic for 23.33% of loci, with an average genetic diversity of 0.0822, and the populations of the two regions showed similar levels of variability. This low level of polymorphism may have be a result of historical bottlenecks and the long generation time of this species. The analysis of molecular variance showed highly significant differences among populations and non-significant differences between regions. The effects of habitat changes might not be the most important factor determining the genetic differentiation among populations currently. However, as modification of landscape structure of the Pampas grasslands could be more severe in the future, conservation of the Greater Rhea requires careful land-use planning.

Additional keywords: conservation, gene flow, grassland, habitat loss, ISSR.

Introduction

The intensification of agricultural practices to increase production has changed and restructured many landscapes, reducing the value of farmed lands for wildlife (Bennett *et al.* 2006). Several authors have observed that habitat loss or configuration changes, or both, may potentially reduce the persistence of populations in a landscape (Fahrig 2003; Gaggiotti and Hanski 2004) and may have consequences at the genetic level, which are directly related to stochastic processes associated with the small size of remaining populations (Frankham 2005).

Grassland ecosystems are one of the most widely and most profoundly transformed because they have been traditionally used for agriculture (Houghton 1994; Guerschman and Paruelo 2005). These transformations, involving loss and fragmentation of habitat, have resulted in the decline of many grassland birds (Vickery and Herkert 2001; Fernández *et al.* 2004; Stephens *et al.* 2004).

In Argentina, the original landscape structure of the Pampas grasslands has been changing rapidly owing to the application of intense and specialised agricultural practices (Díaz-Zorita *et al.* 2002; Bilenca and Miñarro 2004; Demaría *et al.* 2004; Brown *et al.* 2005). Since the beginning of this process in the early 1900s, there have been significant shifts in the ecoregional pattern of land-use (Viglizzo *et al.* 2001). In the last decade, there have been changes in the production practices in the Pampas region, with an increase in the crop production area and a decline in cattle grazing. This intensified grain production has been pronounced in some regions, such as the central and southern Córdoba Province (Díaz-Zorita *et al.* 2002; Bilenca and Miñarro 2004).

The Greater Rhea (*Rhea americana*) is endemic to South America and a typical inhabitant of the Pampas grassland of Argentina; it commonly lives in flocks in a loosely cohesive social system (Bruning 1974; Martella *et al.* 1995; Reboreda and Fernández 1997). This species has a peculiar mating system that combines simultaneous polygyny with sequential (and probably simultaneous) polyandry, in which several females lay their eggs in communal nests. Males alone incubate the eggs, and care for the chicks for several months (Handford and Mares 1985; Navarro and Martella 2002). Although Greater Rheas can use areas of mixed agriculture and patches of natural habitat (Martella *et al.* 1996), they do not use crops either as food or as nesting sites and often avoid them during their movements, so that Rheas tend

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not to occur where there have been large-scale conversions of grasslands (Bellis *et al.* 2004*a*). As remaining patches of suitable habitat in the agroecosystem are separated by distances larger than its average daily movements (<1.1 km, mean home range ± s.e., $5.04 \pm 1.41\,\text{km}^2$; Bellis *et al.* 2004*a*, 2004*b*; Juan *et al.* 2008), Rheas occur in a number of largely isolated populations, though with occasional exchange of individuals (Giordano *et al.* 2008*b*).

Additionally, subsistence hunting and egg harvesting of Rheas have been common practices among rural people, whereas the controlled egg harvest for incubation purposes at commercial farms of this species started in the 1990s. Thus, this emblematic bird of the Pampas has progressively become less abundant (Martella and Navarro 2006) and has subsequently been listed as Near Threatened (IUCN 2008). To guarantee the conservation and sustainable use of the Greater Rhea, the species has been included in Appendix 2 of The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, see http://www.cites.org/eng/app/appendices.shtml, accessed 8 May 2009). Although international protection measures, and provincial and national regulations on hunting, exportation, traffic, and trade of this species have been implemented since 1986, their populations have not increased significantly (Martella and Navarro 2006).

A previous study on the relationship between land-use and habitat suitability for the Greater Rhea in southern Córdoba Province (Giordano *et al.* 2008*a*) showed that the increase in area of agriculture leads to an increased fragmentation of suitable habitat. As a consequence, the spatial distribution of Greater Rhea populations is also increasingly fragmented and their densities within remaining habitats become lower. This situation could negatively affect the conservation status of the species, because it would lead to a loss of genetic variability and evolutionary potential, as has happened in other birds, such as the Taita Thrush (*Turdus helleri*) (Galbusera *et al.* 2000) and the New Zealand Kokako (*Callaeas cinereus wilsoni*) (Hudson *et al.* 2000). A previous study on the genetic structure of populations of Greater Rhea in agroecosystems of Buenos Aires Province (Argentina), based on RAPD markers (Bouzat 2001), reported similar levels of genetic variability in wild populations and captive ones that were used as an inbred ‘control’. Bouzat (2001) concluded that the isolation and small size of populations could affect the genetic variation in this species, based on the assumption that the lower variability exhibited by a captive population used as control was a consequence of high levels of inbreeding. However, recent work found no evidence of inbreeding in captive populations (Alonso Roldán 2006). Consequently, it is important to conduct further research on population genetics in Greater Rheas occurring in fragmented habitats to understand the relationship between the dynamics of genetic variability and habitat modification in the species.

Among the different molecular markers that can be used to study the genetic structure of natural populations, microsatellites (or simple sequence repeats, SSR) are the most informative, since codominant alleles can be scored at individual loci and they usually show a high degree of genetic variation. In species for which primers to amplify polymorphic microsatellite loci are not available (such as Greater Rhea), the use of arbitrary primers can be a successful alternative. In recent years, techniques based on polymerase chain reaction (PCR) amplifications of Inter Simple Sequence Repeats (ISSR) have provided new nuclear genetic markers in non-coding DNA. ISSR-PCR primers consist of repetitions in tandem of randomly designed basic motifs of 2–6 nucleotides that amplify the fragments of DNA between inverse-oriented microsatellite loci, the oligonucleotides being anchored in microsatellites themselves. This technique does not require prior knowledge of DNA sequences, generates highly reproducible band patterns, and reveals high levels of polymorphism (Zietkiewicz *et al.* 1994; Bornet and Branchard 2001).

Here we analyse the genetic structure of several wild populations of Greater Rheas in areas with two different land-uses in central Argentina, employing ISSR as genetic markers. These results provide a genetic basis for the management of natural populations of this species in Argentina and other regions in South America, as well as for wild populations accidentally established in other parts of the world, such as that occurring in north-eastern Germany (A. Korthals and F. Philipp, pers. comm.).

Materials and methods

Study area

We analysed the genetic variability of five wild populations of Greater Rhea on six ranches (Fig. 1) that ranged in size from 2000 to 8100 ha. The groups of Rheas on all but two of the ranches were considered different populations because they were separated by at least 7.8 km, and most interactions between individuals and groups occur over much smaller distances (Bellis *et al.* 2004*b*). The Rheas of the adjacent ranches of El Águila and La Colina were considered as belonging to one population because, although the ranches were fenced with six-wire fences, Greater Rheas can cross these easily (J. L. Navarro and M. B. Martella, pers. obs.). The average density of Rheas in each population was 3–4 individuals km^{-2} (calculated from G. Bazzano, unpubl. data).

The studied populations were located in two areas with differing land-uses: the populations of three ranches (El Toro, La Panchita, Campo Grande) were in an agroecosystem in south-western Córdoba Province where the original environment has been almost totally replaced by crops; the other two populations were on three ranches (La Colina–El Águila, and Los Guaicos) in central-southern San Luis Province, where a high proportion of the land cover is semi-natural grasses and gramineous steppe devoted to cattle grazing. Because no other grassland meets the same conditions as the areas surveyed here, there were no replicates available at the landscape-scale level. Similarly, the number of sampling sites was limited by the large size of the study areas.

The agroecosystems of south-western Córdoba have a low prairie climate, with mean daily maximum temperatures of 23°C in summer and mean daily minimum of 2°C in winter (annual average 16°C) (Servicio Meteorológico Nacional 2009). The area is characterised by flat to gently rolling dunes. Rainfall is concentrated in spring–summer (October–March) and the average annual rainfall is ~680 mm (Gorgas and Tassile 2002). The original landscape in this region was a forest and grassland mosaic, but since the beginning of the 20th century it has been transformed by the planting of crops of Corn (*Zea mays*) and Wheat (*Triticum aestivum*), and, in the last two decades, of

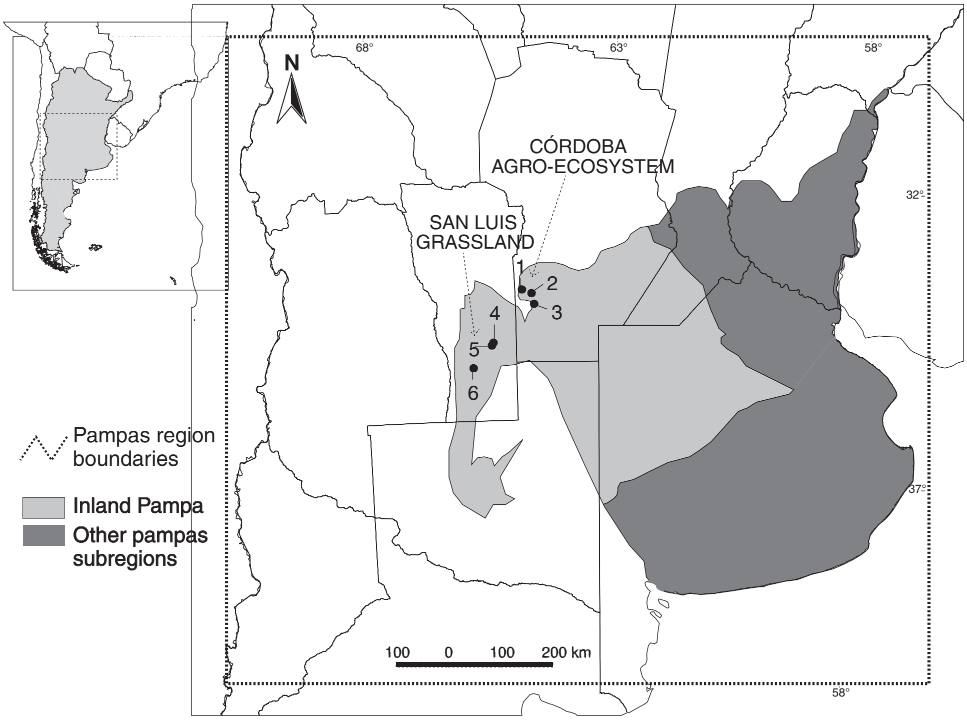

Fig. 1. Location of sampling sites. 1 = El Toro, 2 = La Panchita, 3 = Campo Grande, 4 = La Colina, 5 = El Águila, and 6 = Los Guaicos.

Sunflower (*Helianthus annus*), Soybean (*Glycine max*) and Peanuts (*Arachis hypogaea*) (Ghersa and León 1999; Díaz-Zorita *et al*. 2002).

The grassland of San Luis Province is characterised by sandy soils and rolling hills with fixed and live dunes (Anderson *et al*. 1970). Summer temperatures can reach 43°C with mean daily maximum temperatures of 25°C, while winter temperatures can be as low as −15°C with mean daily minimum of 1°C (average 16°C). Annual average rainfall is ~450 mm, concentrated between October and April (Servicio Meteorológico Nacional 2009). The main vegetation is composed of native grasses, including: *Sorghastrum pellitum*, *Elyomurus muticus*, *Bothriochloa springfieldii*, *Chloris retusa*, *Schizachyrium plumigerum*, *Eragrostis lugens*, *Sporobolus subinclusus*, *Aristida spegazzini*, *Poa ligularis*, and *Poa lanuginosa*, with small patches of trees, including *Geoffrea decorticans*, *Prosopis caldenia* and *Prosopis alpataco* (Anderson *et al*. 1970; Anderson 1973). Exotic grass species, such as *Eragrostis curvula* and *Digitaria eriantha*, were introduced to ranches to increase the carrying capacity for livestock (Covas and Cairnie 1985). Land is mostly used for cattle grazing and is only sporadically devoted to crop production because of the low annual rainfall (León *et al*. 1984), which combine to help maintain its natural state.

Sampling procedure

During the reproductive season (October–December), we conducted monthly ground surveys across all ranches, on foot or by vehicle, in search of carcasses, feathers, and abandoned nests (resulting from predation or desertion) of Greater Rheas. We collected feathers entangled in barbed-wire fences immediately after Rheas had crossed the six-wire fences separating paddocks. We did this at several sites at least 500 m apart, to ensure that samples were from different individuals. Where nests were found (three nests in Córdoba Province and nine in San Luis), samples of muscle tissue of up to three embryos inside unhatched eggs from each nest were obtained. In addition, two complete clutches from El Águila were collected and artificially incubated, and feathers of live chicks (up to three per clutch) were obtained after hatching. The sampling procedure employed and the mating system characteristic of Greater Rheas (polygynic–polyandric, with communal nesting) reduce the probability of sampling related individuals for genetic analysis. Furthermore, recent work in our laboratory that included inter-individual comparisons of ISSR data from within and among nests, grouped all the samples at random, suggesting a low rate of relatedness among chicks from the same nest (unpublished results).

DNA extraction and ISSR procedure

Individual total genomic DNA was isolated from embryonic tissue and the calamus of feathers of 101 individuals (which represents between 20 and 25% of the total number of Rheas inhabiting the entire study area; estimated from Giordano *et al*. 2008*a*), following an alkaline extraction method (Malagó *et al*. 2002). The quality of DNA obtained was determined by electrophoresis in a 1% agarose gel in 0.5 × TBE buffer. DNA concentration in each sample was measured in an Eppendorf BioPhotometer (Eppendorf AG, Hamburg, Germany).

We performed several pilot assays using different molecular markers with negative results: loci coding for allozymes proved to be highly monomorphic in the species; the four microsatellite primers specific for *Rhea americana* (GenBank accession numbers: AF230714–AF230717; Kimwele and Graves 2003) and those available for another ratite (Ostrich (*Struthio camelus*)) did not amplify or, if they did, were monomorphic in all individuals from several populations. For this reason, we decided to employ dominant arbitrarily primed markers, preferring ISSR to RAPDs given the high repeatability of the former.

After an initial screening using 30 primers, we selected eight anchored ones (those with the addition of one to five arbitrary nucleotides in order to increase the specificity of the annealing) because they originated clear, polymorphic, and 100% repeatable bands (Table 1). Amplification reactions were carried out in 25 μL of 20 ng of DNA, 1 mM $MgCL_2$, 200 μM of each dNTP, 6 pmol of each primer, 2.5 μL of PCR buffer (Amersham Biosciences Argentina S. A., Buenos Aires), and 0.75 U of Taq DNA Polymerase (Amersham Biosciences). A Biometra Uno II thermocycler (Whatman Co., Göttingen, Germany) was used, and cycling parameters were as follows: 94°C for 2 min, followed by 40 cycles of 94°C for 30 s, 40°C for 1 min, and 72°C for 1.5 min, with a final extension step of 72°C for 5 min. PCR products were separated by horizontal gel electrophoresis (1.2% agarose in 0.5 × TBE buffer), stained with ethidium bromide and photographed under UV light using a Kodak-DC290 digital camera. Fragment sizes were estimated by comparison with 100-bp ladder (Promega, Madison, WI, USA).

Table 1. Sequence of the primers used to amplify ISSR markers

Primer	Sequence
ISSR1	$5'$-$(AG)_8Y$-$3'$
ISSR3	$5'$-$(CA)_8RT$-$3'$
ISSR7	$5'$-$(AC)_8YT$-$3'$
ISSR10	$5'$-$(CAC)_4RC$-$3'$
ISSR11	$5'$-$(CA)_6RG$-$3'$
AEISSR1	$5'$-$(GA)_8C$-$3'$
Pa3	$5'$-$(CA)_7CTCTT$-$3'$
Boa4	$5'$-$(AC)_8C$-$3'$

Data analysis

Values of 1 and 0 were assigned to presence and absence of bands, respectively, in a matrix. Allelic frequencies, percentage of polymorphic loci and Nei‘s genetic diversity were calculated using the PopGen 1.31 program (Yeh and Boyle 1997). Levels of genetic variability among populations and between the two studied regions were compared using a *z* test (standard errors were obtained by a bootstrap procedure).

Genetic differentiation among populations and between pairs of populations were evaluated through an analysis of molecular variance (AMOVA) using Arlequin (Schneider *et al*. 2000). In order to compare levels of differentiation and to estimate levels of gene flow among the populations of the two regions, G_{ST} (equivalent to F_{ST}; Slatkin and Barton 1989) and the effective number of migrants (Nm, a measure of the relative strength of gene flow to genetic drift), were calculated using PopGen.

The possible existence of isolation by distance pattern was checked by a Mantel test, using TFPGA (Miller 1997).

Given that there are no previous data proving Hardy–Weinberg equilibrium available for the Greater Rhea, inbreeding coefficient in populations (F_{IS}) was calculated by a hierarchic Bayesian model (Holsinger *et al*. 2002), using the program Hickory (Holsinger and Lewis 2005). This program measures the goodness of fit of the genetic data to different models: a full model (with $F_{ST} \neq 0$ and $F_{IS} \neq 0$), one assuming $F_{ST} = 0$, and another one assuming $F_{IS} = 0$. The best model was selected on the basis of the Deviance Information Criterion (DIC), which is similar to Akaike’s Criterion. A difference greater than 5 or 6 units among models indicates that the most complex model should be preferred over the simplest one (Spiegelhalter *et al*. 2002).

Results

Eight to 14 bands were obtained with each primer (Fig. 2) and of a total of 90 bands scored, only 22 were polymorphic.

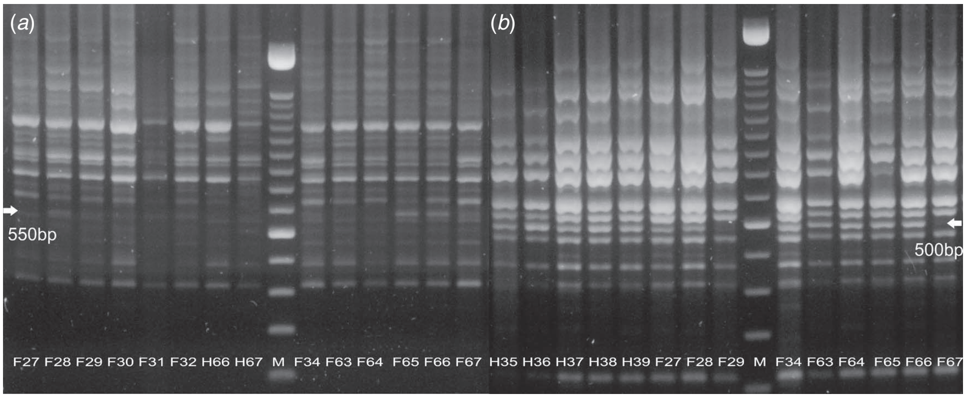

Fig. 2. Representative example of the ISSR patterns obtained (*a*) primer ISSR11 and (*b*) primer Pa3. All individuals are from the El Águila–La Colina population. The arrows indicate polymorphic bands. M: 100 kb molecular size marker.

Nei's genetic diversity (h) varied between 0.0637 and 0.0809, and the percentage of polymorphic loci ranged between 14.44% and 22.22% (Table 2). The *z* test showed no significant differences in genetic diversity values ($z = 0.24$, $P > 0.05$) between the different land-use regions (Córdoba and San Luis Provinces). G_{ST} and Nm values were 0.119 and 3.682 for the population from Córdoba and 0.078 and 5.917 for the population from San Luis, respectively.

The AMOVA test showed highly significant differences among all populations of the two land-use regions ($F_{ST} = 0.143$, $P < 0.001$). The fixation index was also significant when populations of each region were compared ($F_{SR} = 0.126$, $P < 0.001$), whereas no significant differences were found between regions ($F_{RT} = 0.019$) (Table 3). All pairwise comparisons between populations showed significant differences, with F_{ST} values ranging from 0.089 to 0.186 (Table 4). There was no correlation between Nei's genetic and geographical distances (Mantel test, $r = -0.289$, $P = 0.867$).

The difference in DIC between the complete model (DIC = 471.35) and the one assuming $F_{IS} = 0$ (DIC = 471.14) was lower than five units, indicating no evidence of inbreeding.

Discussion

The ISSR proved to be appropriate markers for analysing the genetic structure of wild populations of Greater Rhea. Despite the limitation of their lack of codominance and the rather low number of polymorphic bands obtained, patterns were 100% repeatable, allowing an accurate estimation of population parameters.

Table 2. Genetic variability indices of the studied populations of Greater Rhea

Campo Grande, El Toro and La Panchita populations from Córdoba Province; Los Guaicos and El Águila–La Colina populations from San Luis Province; h = Nei's genetic diversity. All populations from Córdoba were calculated with all individuals analysed

Population	*n*	h	Percentage of polymorphic loci
Córdoba			
Campo Grande	10	0.0643	14.44%
El Toro	12	0.0637	15.56%
La Panchita	20	0.0645	16.67%
San Luis			
Los Guaicos	19	0.0661	16.67%
El Águila–La Colina	40	0.0809	22.22%
Córdoba region	42	0.0725	21.11%
San Luis region	59	0.0816	22.22%
All populations	101	0.0822	23.33%

Levels of genetic variability obtained here are only loosely comparable with other studies in birds, since this is the first report using ISSR. Nevertheless, wild populations of Greater Rhea from central Argentina tend to show a lower percentage of polymorphic loci (23.3%) than those reported for other birds using RAPDs as genetic markers (e.g. 39.1% in Marsh Wren (*Cistothorus palustris*) and 45.7% in Tufted Titmouse (*Baeolophus bicolor*); Bowditch *et al*. 1993), but more similar to that reported for the threatened Clapper Rail (*Rallus longirostris levipes*) (15.9% of polymorphic RAPD loci; Nusser *et al*. 1996). Low levels of diversity were also reported for the Ostrich in a study based on DNA fingerprinting and microsatellites as genetic markers (Kawka *et al*. 2007). In a study based on RAPD loci, Bouzat (2001) reported a mean genetic diversity of 0.25 in Greater Rhea populations from Buenos Aires Province. However, this value is not comparable with the genetic diversity value we obtained, because the value obtained in the Buenos Aires populations was calculated using only the polymorphic loci.

Levels of polymorphism in populations from Córdoba Province were not significantly lower than those from San Luis Province, contrary to what would be expected considering that the former are affected by loss and fragmentation of habitat with intense agricultural land-use. The low variability found in the species, even in populations occurring in continuous habitats, suggests that habitat change would not be the determinant factor of the present levels of polymorphism, because the severe transformation of this agroecosystem is fairly recent (over the past 150 years; Díaz-Zorita *et al*. 2002; Brown *et al*. 2005). An alternative explanation for the poor level of polymorphism found is that this phenomenon was originated by historical processes, like the hunting pressure exerted on this species for centuries (Martella and Navarro 2006). Several studies suggest that hunting causes retractions in wild populations of Greater Rhea (Martella *et al*. 1996; Bellis *et al*. 2004*b*), which would have originated genetic bottlenecks. In addition, this is a long-lived species with a generation time greater than 1 year (age of first reproduction is 18–20 months; calculated from Bowthorpe and Voss 1968; Lábaque 2006), a long lifespan (up to 13 years; Navarro and Martella 2002), and in which only a small proportion (4.5 to 6.1%) of males contribute to the following generation (Fernández and Reboreda 1998). These characteristics have been correlated with low levels of average heterozygosity in a review that included 551 vertebrate species (Nevo *et al*. 1984).

Despite the low levels of polymorphism present in the Greater Rhea, the occurrence of polyandry, egg stealing (Fernández and Reboreda 1995), extra-pair copulations (Martella *et al*. 1998), and

Table 3. AMOVA analysis

Percentage of variation probabilities are significant at *, $P < 0.001$; **, $P < 0.001$; n.s., not significant

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation probabilities
Between regions	1	15.401	0.06471	$1.95^{n.s.}$
Among populations within regions	3	30.128	0.41180	12.39*
Within populations	96	273.203	2.84587	85.66**
Total	100	318.733	3.32238	

Table 4. Pairwise F_{ST} values between populations of Greater Rhea
Probabilities are significant at: *, $P < 0.05$; **, $P < 0.01$

	Campo Grande	El Toro	La Panchita	Los Guaicos	El Águila–La Colina
Campo Grande	0	**	*	**	**
El Toro	0.155	0	**	**	**
La Panchita	0.089	0.101	0	**	**
Los Guaicos	0.120	0.175	0.185	0	**
El Águila–La Colina	0.133	0.134	0.125	0.145	0

adoption of unrelated chicks (Codenotti and Alvarez 1998; Lábaque *et al*. 1999; Barri *et al*. 2005) may be preventing further decreases in the overall genetic variability, thus counterbalancing the negative effects that a depauperate gene pool may have on the viability of the populations.

The studied populations of Greater Rhea showed a high degree of genetic structuring ($F_{ST} = 0.143$) and significant differences in allelic frequencies in all pairwise comparisons, even in the region where the habitat can be considered continuous (San Luis Province). Levels of genetic structuring in Greater Rhea are even higher than those found in other bird species, such as the Western Capercallie (*Tetrao urogallus*) ($F_{ST} = 0.103$; Segelbacher *et al*. 2003) and the Spanish Imperial Eagle (*Aquila adalberti*) ($F_{ST} = 0.041$; Martínez-Cruz *et al*. 2004) using microsatellites as genetic markers.

The genetic structuring found in this work may be a result of both spacing and social behaviour of Greater Rheas, which may contribute to limit the gene flow among populations. In this flightless herbivorous species, movements and home-range depend on the distribution, abundance, and quality of food, rather than on human disturbance (Bellis *et al*. 2004*b*). In Rheas, movements seem restricted to those sites that provide regular forage production over time and good visibility for avoiding predation (Bellis *et al*. 2004*a*). Additionally, Codenotti and Alvarez (1997) reported that 23% of the harems of Rheas showed evidence of cooperative breeding between a dominant adult male and a subadult male of the same mixed pre-breeding group, suggesting a social reproductive structure that would also favour the genetic differentiation among populations.

Bouzat (2001) also reported significant levels of genetic divergence among populations of Greater Rheas in the Pampas region, which were interpreted as being a consequence of recent fragmentation in the area and isolation increasing local differentiation by genetic drift and inbreeding. Although our results agree with those of Bouzat, because of the experimental design we used we conclude that isolation among populations owing to habitat fragmentation would not be the most important factor causing population divergences in Greater Rheas. Different populations were used as control in the two works: while Bouzat used a captive population as a control, assuming that it represents the situation of poorest genetic variability, we compared the distribution of genetic variation across several wild populations from regions with different land-uses, taking the semi-natural grassland area as the control. As this is the habitat that best resembles the original condition in the region, we assumed that Greater Rhea populations from that area would exhibit the best genetic status. Consequently, the non-significant differences we found between regions ($F_{RT} = 0.019$, 1.96% of the total variance observed), the absence of an isolation-by-distance pattern, the equally low level of polymorphism, and the similarity in pairwise F_{ST} values in both regions suggest that genetic differentiation among populations is not essentially a result of the habitat fragmentation process.

Despite the lack of significance in the degree of genetic differentiation between regions, G_{ST} values were slightly higher for populations from Córdoba than from those of San Luis. The fairly short period since the beginning of large habitat changes in the south-west of Córdoba (<20 years; Bilenca and Miñarro 2004), in combination with the long generation time of Greater Rhea, may delay the appearance of effects of habitat modification at the genetic level. A similar pattern seems to occur in the Emu (*Dromaius novaehollandiae*), another ratite that shows low divergence in populations that were separated 100 years ago (Hammond *et al*. 2002).

An alternative explanation for the lack of significance in the degree of genetic structuring between regions could be that the high values of variance in allele frequencies among populations in a single region mask possible differences in the genetic structure between populations from the two areas with different land-use. Further studies involving a higher number of comparisons among populations from diverse habitats would help to clarify this.

The results presented here highlight the ability of Greater Rheas to use planted grasses and for surviving in a mosaic of patches of natural habitat and agricultural land, as suggested in a previous study (Martella *et al*. 1996). Nevertheless, the great expansion of intensive agriculture, which is projected to continue, could be a risk for the species in the future. A greater homogenisation of the landscape through intensive cropping for grain production could further reduce the area and connectivity of suitable habitats for Greater Rheas (grasslands and pastures). Therefore, the increasing disturbance driven by agricultural practices could further divide and isolate the existing populations, and threats that are not detectable today could become evident in the future. Promoting policies tending to diversify production practices and systematically monitoring wild populations will be the keys for the effective conservation of Greater Rheas.

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