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BRIEF REPORT

# May Captive Populations of Greater Rhea (*Rhea americana*) Act as Genetic Reservoirs in Argentina?

**V. Alonso Roldán,[1] J. L. Navarro,[1] C. N. Gardenal,[2] and M. B. Martella[1]***

*[1]Centro de Zoología Aplicada, Universidad Nacional de Córdoba, Córdoba, Argentina*
*[2]Cátedra de Genética de Poblaciones y Evolución, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina*

The Greater Rhea (*Rhea americana*) is a characteristic bird of the Argentine Pampas. Despite the increasing farming interest of this ratite, their natural populations are progressively decreasing in size and range. The object of this study was to evaluate the status of captive populations as potential genetic reservoirs. Using Inter-Simple Sequence Repeats as molecular markers, levels of genetic variability of F1 individuals from two captive populations were estimated and compared with those of wild populations in the same region. The captive populations were polymorphic for 12.22 and 13.33% of the loci, with a genetic diversity of 0.050. Differences with wild populations were not significant ($z = 1.79$; $P > 0.05$). Therefore, captive populations of rheas in Argentina should not be overlooked as genetic reservoir and source of individuals for reinforcement of natural populations, through reintroduction and translocation. Zoo Biol 29:1–6, 2010. © 2010 Wiley-Liss, Inc.

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The present address of V. Alonso Roldán: Centro Nacional Patagónico-CONICET. Bv. Brown 2825, U9120ACF Puerto Madryn, Argentina.

*Correspondence to: M. B. Martella, Centro de Zoología Aplicada, Universidad Nacional de Córdoba, CC 122, CP: 5000. Córdoba, Argentina. E-mail: martemo@com.uncor.edu

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## INTRODUCTION

Farming of Greater and Lesser Rheas (*Rhea americana* and *R. pennata*) has increased within the natural ranges of both species worldwide [Martella and Navarro, 2006]. Despite the economic importance of the Greater Rhea and of several protection measures undertaken, wild populations have remained low [Martella and Navarro, 2006; Giordano et al., 2008a], and the species is currently included in the Near Threatened category [IUCN, 2008]. A recent study in natural populations of Central Argentina using Inter-Simple Sequence Repeats (ISSR) as genetic markers showed low levels of genetic variability (mean percentage of polymorphic loci = 23.33% and mean Nei's genetic diversity = 0.0822) and an important degree of genetic structuring ($F_{ST} = 0.143$) [Alonso Roldán et al., 2009]. Thus, the conservation status of Greater Rhea is likely to worsen because the loss of genetic variability can diminish adaptability to environmental changes.

The translocation of captive-reared individuals into the wild has emerged as a management strategy that may contribute effectively to avoid local extinction of rhea populations. Several pilot experiences of this type have been successfully conducted in both rhea species in different regions of Argentina [for a review see Navarro and Martella, 2008]. Additionally, simulation models reinforce the idea that, if current trends of land use in Central Argentina are maintained, small wild populations will be at serious risk, unless translocations for reinforcement are implemented [Giordano et al., 2008b]. Thus, captive populations may play a role as demographic and genetic reservoirs, for repopulation or for increasing the size and the genetic variability of wild populations. Nevertheless, before considering translocation of individuals—a common management technique for the rescue of threatened species [Sinclair et al., 2006]—captive populations must be characterized in order to verify an acceptable degree of genetic similarity between the source and the possible target populations in the wild. Source populations must have variability levels that allow a rescue effect in the target ones while their allelic frequencies should be similar enough to avoid causing outbreeding depression.

In this article, levels of polymorphism are estimated in captive populations of Greater Rhea in Central Argentina and compared with those found in the wild in the same region, using ISSR as genetic markers. This technique usually provides a high number of polymorphic markers presenting high repeatability and does not require earlier knowledge of the sequences to be amplified [Haig et al., 2003], being particularly suitable when codominant markers, such as microsatellites, have not been described in the species.

## METHODS

The analysis was conducted on F1 individuals of two captive populations, which were found with rheas coming from different wild populations of Central Argentina [Córdoba and San Luis provinces]: one of them comprised a breeding stock of 4 males and 14 females, housed at the Córdoba Zoo (Córdoba), and the other was at the Estación Experimental Agropecuaria INTA, San Luis (Villa Mercedes, San Luis), with its breeding stock consisting of 9 males and 12 females. Results from both captive stocks were, in turn, compared with those of five geographically close wild populations [data from Alonso Roldán et al., 2009].

Individual total genomic DNA was isolated from the muscle tissue of embryos inside unhatched eggs and calamus of feathers of 17 individuals (8 from Córdoba Zoo and 9 from INTA), 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 *R. americana* [GenBank accession numbers: AF230714 to 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.

ISSR markers were analyzed using 8 primers (Table 1), which showed 100% of repeatability in earlier assays. Amplification reactions were carried out in 25 µl volumes consisting of 20 ng of DNA, 1 mM $MgCl_2$, 200 µM of each dNTP, 6 pM of each primer, 2.5 µl of PCR buffer (Amersham), and 0.75 U of Taq DNA Polymerase (Amersham Biosciences Argentina S. A., Buenos Aires). 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 sec, 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). Values of 1 and 0 were assigned in a matrix of presence–absence of bands Promega, Madison, WI, USA.

Allelic frequencies, percentage of polymorphic loci, and genetic diversity [Nei, 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70: 3321-3323] were calculated using the PopGen 1.31 program [Yeh and Boyle, 1997]. Levels of genetic variability between wild and captive populations were compared using a z test (standard errors were obtained by a bootstrap procedure). Captive and wild populations were also compared by means of a hierarchical analysis of molecular variance (AMOVA) using Arlequin [Schneider et al., 2000].

**TABLE 1. Name and base sequence of primers used**

| Primer | Sequence |
|---|---|
| ISSR1 | 5′-$(AG)_8$Y-3′ |
| ISSR3 | 5′-$(CA)_8$RT-3′ |
| ISSR7 | 5′-$(AC)_8$YT-3′ |
| ISSR10 | 5′-$(CAC)_4$RC-3′ |
| ISSR11 | 5′-$(CA)_6$RG-3 |
| AEISSR1 | 5′-$(GA)_8$C-3 |
| Pa3 | 5′-$(CA)_7$CTCTT-3 |
| Boa4 | 5′-$(AC)_8$C-3 |

The five wild populations were considered as one group and the two captive populations as a different group; the corresponding fixation index was calculated ($F_{CT}$).

## RESULTS

Analyses were performed on the basis of 22 polymorphic ISSR bands [see details in Alonso Roldán et al., 2009]. Although captive Greater Rhea populations exhibited diversity index values that were apparently lower than wild populations (Table 2), the differences were not significant ($z = 1.79$; $P > 0.05$). The hierarchical study of genetic structuring (Table 3) also indicated that captive populations are not significantly different from the group of wild populations analyzed ($F_{CT} = 0.079$, $P > 0.05$).

## DISCUSSION

This article shows that captive populations of Greater Rhea, founded with individuals from Central Argentina, preserve most of the genetic variation present in wild populations of the same region. Therefore, those captive populations may play a significant role as a source of individuals for translocation into the wild.

The similarity between wild and captive populations may be attributed to three main factors: (1) the relative short time elapsed since farms of this species were established compared with the long adult life expectancy and generation time of the Greater Rhea; (2) the breeding stocks may have comprised descendants of the same (or geographically close) wild populations, so the captive population could in fact represent a sample of the wild ones; and (3) deliberate artificial selection has not been performed up to the present, reducing the possibility of divergences in allelic frequencies owing to human intervention. Similar results were reported for captive populations of an Australian ratite species, the emu [Hammond et al., 2002], using microsatellites as genetic markers.

The release of captive stocks to increase wild populations poses risks of introducing some problems associated with captive breeding. Of particular concern is the possible loss of genetic variability owing to inbreeding and/or to the effect of domestication selection during captivity. Furthermore, descendants of matings

**TABLE 2. Genetic variability indices of captive and wild populations; *h* = Nei's genetic diversity**

| Population | *N* | *h* | % polymorphic loci |
|---|---|---|---|
| *Wild*[a] | | | |
| Campo Grande | 10 | 0.0643 | 14.44 |
| El Toro | 12 | 0.0637 | 15.56 |
| La Panchita | 20 | 0.0645 | 16.67 |
| Los Guaycos | 19 | 0.0661 | 16.67 |
| Águila-Colina | 40 | 0.0809 | 22.22 |
| *Captive* | | | |
| UNC-INTA | 9 | 0.0495 | 12.22 |
| Córdoba Zoo | 8 | 0.0495 | 13.33 |

[a]Data from Alonso Roldán et al. [2009].

**TABLE 3. Hierarchical analysis of the molecular variance (AMOVA) in populations of Greater Rhea**

| Source of variation | Degrees of freedom | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among groups | 1 | 14.213 | 0.25192 | 7.86[a] |
| Among populations within groups | 5 | 45.629 | 0.39232 | 12.24[b] |
| Within populations | 111 | 284.158 | 2.55998 | 79.89 |
| Total | 117 | 344 | 3.20422 | |

[a]$F_{CT} = 0.079$, n.s.
[b]$F_{SC} = 0.133$, $P < 0.001$.

between wild and captive individuals may lose adaptation to the local environment, generating outbreeding depression by the breakdown of coadapted gene complexes [Storfer, 1999].The similarities found here reveal that rhea farms should not be discarded as genetic reservoirs and sources of individuals for translocation within the region. However, similar studies to the one presented here should be necessary before implementing translocation programs in other areas within the geographic range of Greater Rhea, to avoid possible outbreeding depression. Management of captive populations of rheas should thus include measures toward enhancing or maintaining their conservation value, especially if natural populations of Greater Rhea continue to decline.

## CONCLUSIONS

1. Captive populations of rheas in Central Argentina should not be overlooked as genetic reservoir and source of individuals for reinforcement of natural populations in the same region.
2. Measures should be adopted to prevent reduction or loss of the current conservation value of captive populations.

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