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Use of molecular and environmental analyses for integrated in situ and ex situ conservation: The case of the Mexican prairie dog

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ABSTRACT

An important step in species conservation is to identify populations that significantly contribute to it. Considering both in situ and ex situ populations provides an integrated approach to the preservation of a species' evolutionary potential. The joint use of molecular and environmental analyses allows conservation schemes to be implemented when reintroducing captive populations, and wild populations to be prioritized for conservation purposes. We used genetic data and environmental analyses to select candidate areas for the reintroduction of a captive population of the Mexican prairie dog, *Cynomys mexicanus*, and prioritize wild populations for the conservation of this endangered endemic species. We estimated the levels of genetic diversity and differentiation of the captive population and compared them with those of six wild populations. We used species distribution modeling (SDM) to perform forecasts under future climate change scenarios and identify areas with suitable environmental conditions for the populations to persist in the medium to long term. The captive population showed high levels of genetic diversity ($H_d = 0.692$, $H_E = 0.52$), but was genetically differentiated from the wild populations. The genetic structure of wild populations should therefore be considered when reintroducing captive Mexican prairie dogs. In the wild populations, we found a correlation between colony area and nuclear genetic diversity, suggesting that genetic drift and/or inbreeding have been stronger in smaller colonies. The occupied climate space was well differentiated among wild colonies. The impact of agriculture and roads was stronger in the northeastern area of the species range, where SDM forecasts suggest that environmental conditions may remain suitable in the future. Finally, we identified three colonies as conservation priorities based on both genetic and ecological criteria.

1. Introduction

Conservation focused on the species level tends to overlook population-level diversity, which is important for a species' ability to respond to environmental change (May et al., 2011). As populations are the relevant units for evolutionary processes and ecological functioning (Luck et al., 2003; Ceballos et al., 2015), an important step in species conservation is to assess their levels of genetic variation and identify populations that are evolutionarily significant (Moritz, 1999).

Species distribution models (SDMs) have been used to determine the climatic factors that limit the distribution of lineages and species (Wiens et al., 2010). These approximations allow the climate space that defines the distribution of species to be projected into past climate or future global climate change scenarios (Elith et al., 2011). Such an approach has great utility for the conservation of many taxa, as a joint genetic and ecological approximation can provide important information to establish conservation priorities (May et al., 2011; Diniz-Filho et al., 2012) and help predict changes in species range and population extinctions as a response to climate change (Diniz-Filho et al., 2012; Wiens et al., 2013). The joint use of genetic data and SDM analysis has become common practice in phylogeography and conservation biology (Richards et al., 2007; Waltari et al., 2007; Diniz-Filho et al., 2009, 2012; Knowles and Alvarado-Serrano, 2010; May et al., 2011; Fordham et al., 2013; Moreno-Letelier et al., 2013; Ramírez-Barahona and Eguiarte, 2014; Bleyhl et al., 2015; Castellanos-Morales et al., 2016; Scheinvar et al., 2016).

Projections of species geographic range into future scenarios suggest that a large number of species will be threatened by climate and land-use changes (Pritchard et al., 2011; Diniz-Filho et al., 2012; Wiens et al., 2013). Some of the challenges that require research and management efforts are thus to assess the impact of global climate change on biodiversity, taking genetic data into consideration, and to develop management schemes that mitigate it (Frankham, 2010; Scoble and Lowe, 2010).

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Traditionally, conservation biology has been divided into in situ and ex situ conservation, seen as two distinct approaches to the protection of wild species (Pritchard et al., 2011). In situ conservation refers to the protection of species in their natural habitat, while ex situ conservation involves their protection outside of their natural surroundings, commonly in zoos, aquaria, botanical gardens, arboreta and seed banks (Pritchard et al., 2011). Current conservation needs, however, call for a change of strategy: whenever possible, an integrated approach that includes both in situ and ex situ conservation should become a common practice in conservation and management (Pritchard et al., 2011).

In this sense, captive populations of known origin can provide material for genetic research, with a view to better understand a species' diversity (Gippoliti and Amori, 2007). One of the main goals of ex situ conservation is also to breed in captivity highly threatened species in order to increase the number of individuals to re-establish wild populations. According to Fischer and Lindenmayer (2000), however, only about 23% of reintroduction programs are successful, and survival may be low even after careful pre-release preparation (Mathews et al., 2005).

Genetic variation is essential for species to respond to environmental change (Frankham et al., 2004; Laikre et al., 2010). Genetic management of captive populations has thus focused on maintaining genetic diversity and minimizing inbreeding (Frankham, 2010; Witzenberger and Hochkirch, 2011). Even so, the reintroduction of captive populations to the wild should be done with caution: the viability and fitness of the wild populations may be reduced, indeed, because of altered genetic composition, disrupted population structure, and broken local adaptation (Laikre et al., 2010).

A first step in reintroduction programs should be to assess the levels of genetic variation of both captive/founder and wild populations, with a view to maximize survival through their integrated management (Laikre et al., 2010; Pritchard et al., 2011; Maschinski et al., 2013). This strategy should be followed by the identification of populations that are geographically, ecologically and/or climatically distinct, hence securing conservation efforts in the medium to long term (Gippoliti and Amori, 2007). Considering genetic information during the development and implementation of management plans should thus ensure that unique and distinctive regional patterns are retained (Brandt et al., 2014).

According to Witzenberger and Hochkirch (2011), most publications in ex situ conservation genetics have focused only on captive populations. However, comparisons with wild populations are needed to assess whether the goals of breeding programs are really being met (Witzenberger and Hochkirch, 2011). In this study, we therefore combined genetic data from captive and wild populations and performed environmental analyses, with a view to provide an integrated in situ and ex situ conservation proposal that can be applied to a wide set of endangered taxa.

We used the endangered endemic Mexican prairie dog (*Cynomys mexicanus*) as a case study. The Mexican prairie dog is a sciurid endemic to an area of ca. 477 km^2 located in the intermontane valleys dominated by gypsum grasslands of the central Chihuahuan Desert in the states of Nuevo León, Coahuila and San Luis Potosí, Northwestern Mexico (Treviño-Villarreal and Grant, 1998; Scott-Morales et al., 2004, 2005; Castellanos-Morales et al., 2015, 2016). Along with the other species in the genus, the Mexican prairie dog is considered a key species and an ecosystem engineer (Slobodchikoff et al., 2009; Martínez-Estévez et al., 2013).

Mexican prairie dogs live in colonies consisting of several family groups or coteries. Each coterie in turn consists of several females, one or two unrelated males and their offspring. Females are philopatric and dispersal is male-biased (Ceballos and Wilson, 1985). Previous genetic analyses have shown that levels of genetic variation are high in wild populations, and that genetic structure is influenced by differentiation among family groups within each colony (McCullough and Chesser, 1987; Castellanos-Morales et al., 2015).

The Mexican prairie dog is listed as endangered by Mexican law (NOM-ECOL-2010–SEMARNAT, 2010) and the IUCN (Álvarez-Castañeda et al., 2008). It is also listed in the Appendix I of CITES (2016, www.cites.org). The historical distribution of this species was reduced up to 62% by 1998 due to changes in land use to agriculture as well as overgrazing and drought (Treviño-Villarreal and Grant, 1998). A captive population was established north of the species range at the Museo del Desierto in the city of Saltillo, state of Coahuila. This population was established ca. 10 years ago from five founders (sex and age unknown) captured in Rancho Los Ángeles (LA, 25.11°N −100.98°W; Fig. 1), Coahuila, managed by the Universidad Autónoma Agraria Antonio Narro (UAAAN; Fernando Toledo, Wildlife Director of the Museo del Desierto, personal communication). Captive breeding has been successful, and this population is being considered for reintroduction to the wild.

We thus combined genetic data from one captive and six wild populations of the Mexican prairie dog and performed environmental analyses, with the aim of assessing the captive population's reintroduction potential, selecting candidate areas for the reintroduction of captive-bred individuals to the wild, and prioritizing wild populations for conservation purposes. We used mitochondrial DNA (control region and cytochrome *b*) and 10 nuclear microsatellite loci to compare levels of genetic diversity between these populations. We also estimated the levels of genetic differentiation between captive and wild populations. We performed SDM to determine the climate space occupied by wild populations. Finally, we used SDM projections into future climate change scenarios to identify possible changes in the Mexican prairie dog distribution.

2. Materials and methods

2.1. Sample and data collection

In February 2012 we obtained 18 samples (10 adult females and eight adult males) from the ex situ population of *C. mexicanus* in the Museo del Desierto (MD), that is, 80% of the total population. We set Tomahawk traps in a 4 × 5 grid for three days. The traps were baited with a mixture of seeds and nuts provided by the Museum. Tissue samples were taken from the tip of the tail following the method described in Castellanos-Morales et al. (2015). Tissue was stored in 2-mL Eppendorf tubes and maintained at −80 °C until DNA extraction.

2.2. Genetic data

We performed total genomic DNA extraction with a DNeasy Blood and Tissue Kit (QIAGEN Sample and Assay Technologies, Hilden, Germany) following the protocol provided by the manufacturer. We amplified the control region and cytochrome *b* of the mtDNA following the conditions reported in Castellanos-Morales et al. (2014, 2015). We also amplified 10 of the nuclear microsatellite loci designed for *C. ludovicianus* by Jones et al. (2005), following the conditions reported in Castellanos-Morales et al. (2014) and Gutiérrez-Guerrero (2014).

To compare genetic variation between captive and wild populations (Fig. 1), we retrieved from Dryad (http://dx.doi.org/10.5061/dryad.pk944) data of 10 nuclear microsatellite loci and 78 individuals from the six wild populations of *C. mexicanus* (including the putative founding population, LA) reported in Castellanos-Morales et al. (2015). In addition, we retrieved from GenBank (PopSet 916354862) mtDNA sequences of the control region and cytochrome *b* for the same six wild populations reported in Castellanos-Morales et al. (2015).

We used Micro-Checker 2.2.3 (Van Oosterhout et al., 2004) to control for artifacts associated with the presence of null alleles. To corroborate our data, we also used FreeNA (Chapuis and Estoup, 2007) to estimate F_{ST} confidence intervals (CI) both with and without null alleles (ENA correction).

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