A note on the population structure of leopards (Panthera pardus) in South Africa

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INTRODUCTION

The leopard (Panthera pardus) occurs throughout Africa and Asia, although their range in Africa has declined by 37% in the last century (Ray et al. 2005). The main factors contributing to the decline in leopard distribution seem to be a combination of habitat destruction, human persecution, and prey depletion (Henschel et al. 2011). These activities have increased habitat fragmentation and can affect leopard behaviour through home range shifts and limited dispersal (Ngoprasert et al. 2007).

Limited dispersal, and hindered gene flow, can decrease genetic variation and increase genetic differentiation in fragmented subpopulations due to inbreeding, genetic drift and selection (Haag et al. 2010). Lowered genetic diversity may in turn cause reduced survival and reproduction success because of increased accumulation of deleterious mutations and increased probabilities in allele loss (Whitlock 2000; Reed & Frankham 2003; Frankham et al. 2004).

Recent work indicates that suitable leopard habitat in South Africa has become fragmented (Swanepoel et al. 2013), which could potentially lead to limited leopard dispersal and depleted genetic diversity. However, leopards are one of the most opportunistic large predators in Africa, which allows them to disperse through modified and unsuitable habitats (Marker & Dickman 2005). Nonetheless, a recent genetic study on Indian leopards (P. pardus fusca) showed increased genetic differentiation associated with habitat fragmentation, indicating restricted dispersal (Dutta et al. 2013a). These findings, combined with the suggested fragmentation of South African leopard habitat, highlights the importance of an understanding of leopard population structure in South Africa.

The main objective of this study was to identify genetic structuring and diversity patterns among leopard populations in South Africa to assess whether dispersal still occurs. We adapted the suitable leopard habitat map from Swanepoel et al. (2013) to delineate four core areas and we hypothesized that genetic differentiation between these leopard populations is likely to exist (Fig. 1).

METHODS

Specimens were retrieved from museums, taxidermists and biologists, and brought to the molecular laboratory of the Department of Animal Ecology at the VU University of Amsterdam. The samples were cut and ground, cooled with nitrogen and admixed with 100 µl PBS in order to pulverize the material. For DNA extraction of skin and skull tissue, the Promega SV protocol and DNA IQ™ System bone isolation protocol were used respectively (DNA IQ™ System Database Protocol Technical Bulletin, TB297, 2009).

Polymerase chain reaction (PCR) amplification followed, for which the protein-coding NADH-5 gene (311 bp; F:GGTCACTCCAAATAAAAAG; R:GGGTCTGAGTTTATATC) and non-coding central conserved region of the D-loop (349 bp; F:TCAACTGTCCGAAGAAGTGCCT; R:CCTGTTGAAGCAATAGGAATT) were used. PCR reactions (25 µl) were prepared for each sample with 13.3 µl H2O; 5 µl 5x GoTaq® Flexi Reaction Buffer; 1.5 µl MgCl2 Solution (25 mM); 2 µl dNTPs (2.5 mM each); 1 µl of each forward and reserve primer (5 µM each); 0.2 µl of GoTaq® DNA Polymerase (Promega, 2–3 U/µl); and 1 µl of genomic DNA.

Amplification reactions were run in a Biometra T3 Thermocycler, with an initial denaturation step of 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 45 s, extension at 72°C for 45 s, and a final elongation step at 72°C for 10 min. The DNA yield was verified on an agarose gel. The cycle-sequencing PCRs were performed with a total volume of 10 µl, of which 1.0 µl consisted of the DNA template, 1.5 µl 5x sequencing buffer (Applied Biosystems), 1.0 µl primer (5 µM), 1.0 µl Big Dye® ready reaction mix (Applied Biosystems) and 5.5 µl H2O. Cycle-sequencing PCRs were run with 96°C for 1 min,