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Original Investigation

Phenotypic differences, spatial distribution and diversity at the *Cytb* and *BMP4* genes in springbok (*Antidorcas marsupialis*)

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ABSTRACT

The springbok (*Antidorcas marsupialis*) is a significant contributor to the economically important game ranching sector in Southern Africa. Phenotypic variation between springbok from the Karoo and Kalahari regions has been reported by several sources, with springbok from the Kalahari regarded as the larger form. There is no consensus on whether the two variants are determined by heredity, environment or a combination of the two. We studied variation in 80 individuals from four springbok populations using both a gene widely used for population studies (*Cytb*) and a gene that effects growth (*BMP4*). Results from *Cytb* haplotypes and *BMP4* diploid gene sequences reveal moderate differentiation among springbok sampled from different regions. We also found a CA tandem repeat motive with high variability at the 3′ end of the *BMP4* gene region sequenced (the third exon). There is some support for a hypothesis that nominally short and long fragments at this *BMP4* repeat are associated with different populations, which may indicate either neutral genetic differentiation between spatially isolated forms, or a relationship between phenotype and *BMP4* genotype. We also present new primer sequences to amplify both a partial fragment of the *BMP4* gene region and the complete *BMP4* tandem repeat motive in springbok.

Introduction

Springbok occurs in the arid areas of Southern Africa (Fig. 1) where grasslands or savannas with short-growing grasses occur (Chain et al. 2004). The springbok is a significant contributor to the economically important game ranching sector and is widely utilized in the commercial wildlife ranching and hunting industry in South Africa (SA). It is the game species most extensively cropped in South Africa for the local and export venison market (Hoffman and Wiklund 2006; Hoffman et al. 2007).

Phenotypic variation in springbok is well known with several color variants that have been reported (Kruger et al. 1979; Hetem et al. 2009). Normal coloration consists of cinnamon-brown dorsal parts with white ventral areas, separated by a reddish-brown band (Estes 1991). Color aberrations that occur at low frequencies have been exploited by commercial game farmers, leading to a proliferation of black, white and copper colored springbok populations. Size differences between springbok from different regions of Southern Africa have also been reported. The size differences correspond broadly to two geographic regions, the Karoo and Kalahari. Estes (1991) reported that male springbok from the Karoo region have an average shoulder height of 73 cm and weigh 30.6 kg, compared to 77–87 cm/41 kg for males from the Kalahari region. There is currently no consensus on whether the size variants are determined by heredity, environment or a combination of both. Large body size has been used as a criterion for active selection by farmers resulting in translocation of Kalahari type animals to the Karoo region in South Africa, in an attempt to improve average body size in populations. Anecdotal evidence from some farmers suggested that gains are short-lived and the average size of individuals revert to pre-augmented standards within 2–3 generations; whereas others claim that augmentation followed by selection resulted in sustained improvements in the average body size of herds.

Early genetic and taxonomic studies of springbok focused on the status of sub-species in *A. marsupialis*. Three subspecies of springbok were formerly recognized, with *A. m. marsupialis* (Zimmermann, 1780) at the southern edge of the distribution range in South Africa; *A. m. hofmeyri* (Thomas, 1926) in the northern parts of South Africa, as well as in Botswana and Namibia; and with *A.m. angolensis* (Blaine, 1922) in Angola (Bigalke 1970; Ansell 1972). Robinson (1975) questioned the continued recognition of the three subspecies, based on karyological evidence, allozymes and skull morphology. Subsequently, Peters and Brink (1992) suggested that there may be significant size differences among springbok, corresponding to the ranges of *A. m. marsupialis* and *A. m. hofmeyri*. In later genetic studies of springbok, the focus shifted to population

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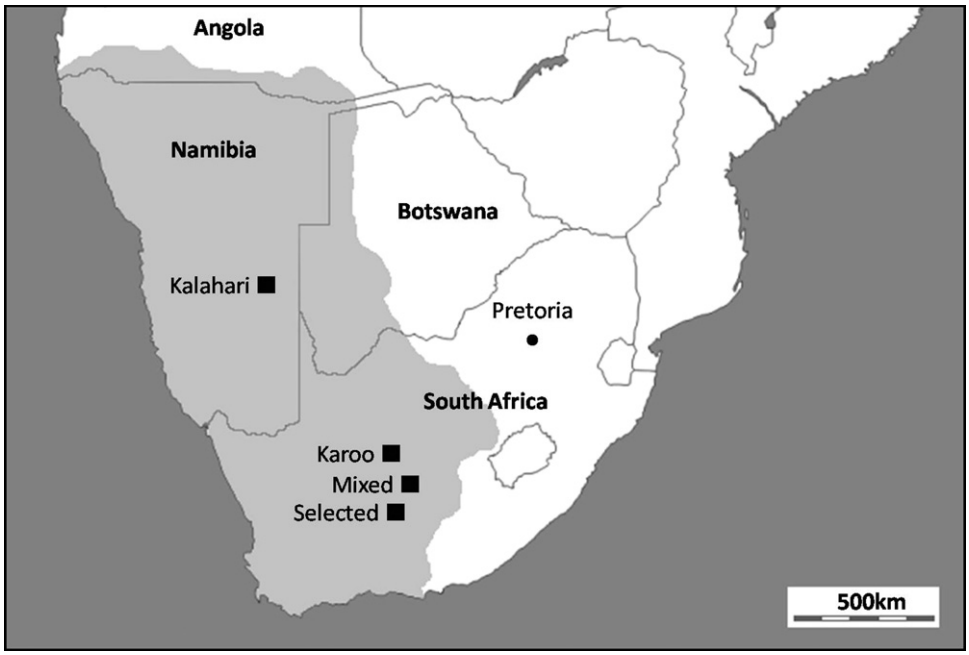

Fig. 1. The distribution of springbok in Southern Africa (shaded in light grey), with sampling localities (■).

genetics (Bigalke et al. 1993) and a possible link between inbreeding and fluctuating asymmetry in this species (Grobler et al. 1999).

Studies in conservation genetics routinely aim to use neutral markers, to ensure that the effects of processes such as drift and inbreeding are not masked by the signature of selection. For example, *Cytb* has often been used to elucidate taxonomic disputes in the Cetartiodactyla (Hassanin and Douzery 1999; Rodríguez et al. 2009) and also the Bovidae, to which the springbok resort (Matthee and Robinson 1999). However, with possible genetic differentiation in springbok potentially expressed as a detectable size difference, the use of a marker with possible adaptive significance relating to bone growth could add valuable insight into the evolutionary biology of this species, considering that the confusion on subspecies status was at least partially based on size differences. One candidate gene region for such analysis is the bone morphogenetic protein (BMP) genes. Among the 20 genes in this group, *BMP4* has been widely studied and postulated to play a role in skeletal development (Hogan 1996), bone density (Mangino et al. 1999) and fracture healing (Nakase et al., 1994). In domestic animals, an association between a tandem repeat motive within *BMP4* and phenotypic variation in cattle was suggested by Zhong et al. (2010). Since a potential link between *BMP4* and bone parameters exists, it was decided to use diversity at this gene as an added measure of differentiation between the two phenotypic forms in springbok.

In this paper, we describe a project where diversity at a neutral gene and a presumably more adaptive gene was correlated to geographic origin and phenotypic differences in springbok. Specifically, we aim to describe differences in *Cytb* and *BMP4* variability in springbok from four populations with diverse founding and management histories and investigate the possible link to the phenotype.

Material and methods

Sampling

Twenty springbok were sampled from each of four farms [with abbreviation used in brackets] (Fig. 1). Springbok described as pure Karoo type were collected from the farm Renostervlak in the Northern Cape (NC) Province of SA [Karoo]. Pure Kalahari type springbok were sampled from the farm Süs in the Mariental district of Namibia [Kalahari]. Two mixed populations were also sampled: (i) Wonderboom farm (NC, SA), which hosts a population based on former Karoo-type springbok, with intensive directional selection for increased body size and with augmentation using Kalahari type springbok [Selected]. (ii) Jakkalsfontein farm (NC, SA), which hosts a mixed population of both indigenous Karoo- and introduced Kalahari type springbok, but without the intense selection practiced at the Wonderboom locality [Mixed]. Sampling kits (with ethanol-filled tubes) and information pamphlets were distributed to the four farmers, who then collected samples when springbok were hunted or culled on their properties. Muscle tissue samples (approximately 2 cm^3) were stored in 10 ml collection tubes with 90% ethanol and sent to the Department of Genetics at the University of the Free State.

Genetic analysis

Genomic DNA was isolated using the Roche Diagnostics High Pure Template Preparation kit (Roche Diagnostics, Germany) following manufacturers specifications. The *Cytb* mtDNA gene region was amplified using the universal primers L14724 and H15149, as described by Irwin et al. (1991) and modified by Nagata et al. (1995) (L14724: 5′-GATATGAAAAACCATCGTTG-3′; H15149: 5′-CTCAGCCTGATATTTGTCCTCA-3′). Primers to amplify the *BMP4* gene region were designed using a selection of mammalian *BMP4* gene sequences available on GenBank, based on conserved areas across aligned sequences. The sequences of the designed primers were: BMP4-NEW-F: 5′-CCTCTTTAACCTCAGCAGCATCC-3′; BMP4-NEW-R: 5′-GCTATAAGGAAGCRGTCTGTGTAG-3′. Identical reaction mixtures were used for both gene regions. Mixtures contained 12.5 μl Econotaq plus green master mix (Lucigen, Middleton, WI), 1 μl of each primer (from a 10 μM solution), ±80 ng genomic DNA and 9.5 μl ddH_2O, for a final volume of 25 μl. Thermal cycling conditions for *Cytb* consisted of an initial denaturation step of 95 °C for 5 min, followed by 45 cycles each of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min; with a final extension step of 72 °C for 10 min and a hold at 4 °C. Thermal cycling conditions for *BMP4*