Single base-pair deletion in *ASIP* exon 3 associated with recessive black phenotype in impala (*Aepyceros melampus*).

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Impala (*Aepyceros melampus*) are common medium-sized antelope in Southern Africa[1]. Black impala have a black coat in comparison to the wildtype reddish-fawn animals (Fig. 1). They are currently intensively bred on many game ranches in South Africa. Carriers of the black mutation display a wildtype phenotype. They are routinely bought and sold on auction with no genetic evidence to support their status. A commercial genetic test for the "black gene" is essential to prevent fraud and improve breeding practices.

Mutations in the *ASIP* gene have been associated with a black phenotype in many domestic species[2-4]. To identify the molecular basis of the black phenotype in impala, we sequenced the *ASIP* exons (2, 3 and 4) in three black and five wildtype animals using Sanger sequencing (Table S1). Sequences were deposited in GenBank (KU564955, KU564954).

A c174delA was found in all black phenotype sequences (Fig. S1). Translation predicted a premature stop codon (239-241bp) (Fig. S1). Parentage with standardised microsatellite markers (S.M. Miller *et al*., in preparation) confirmed simple recessive inheritance of the mutation.

We designed a fragment test flanking the c.174delA (Table S1). Two hundred and fifteen black animals (131 blood and 84 hair samples) were homozygous (166 allele), 26 wildtype animals were homozygous (167 allele) and 231 carrier animals were heterozygous (166/167 alleles) (Fig. 2). A further 347 suspected carriers were homozygous (167 allele) and parentage testing of some of these animals excluded the proposed black phenotype parent. Sixty-one black-faced impala (*A. m. petersi*) from Etosha National Park, Namibia were homozygous (167 allele).

Fig. 1. Phenotypes of a) wildtype and b) black common impala (*Aepyceros melampus*).
63x68mm (300 x 300 DPI)

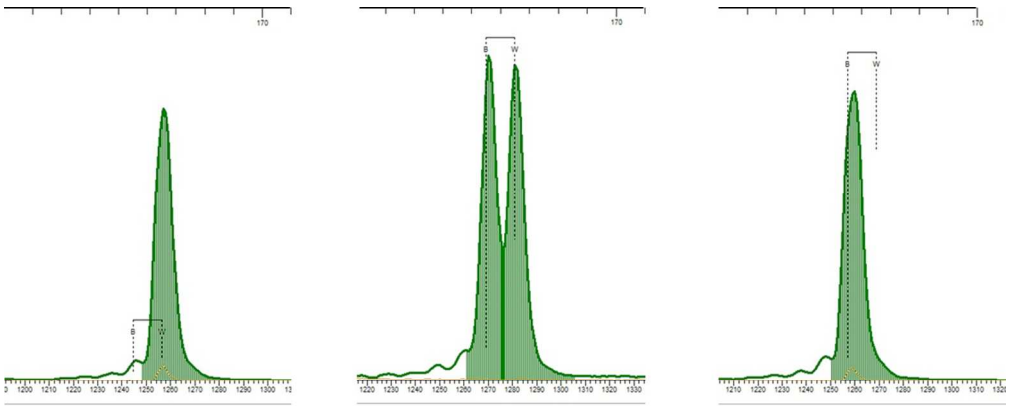

Fig. 2. Fragment analysis of the c.174delA in *ASIP* of the recessive black impala phenotype as compared to the wildtype phenotype and a carrier of the mutation.
99x46mm (300 x 300 DPI)

Conclusions: A single base-pair deletion in the *ASIP* gene has been associated with a black phenotype in impala. The fragment test described here allows for detection of carriers (wildtype phenotype) of the mutation.

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Conflict of interest: The authors have filed three patent applications: ZA2014/05233, ZA2015/03778 and PCT/IB/2015/055391. Funding was partially provided by the industry for which the test was developed (WRSA); however they were not involved in the experimental design or testing.

References

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Supporting Information - **Table S1.**

Table S1. Primer sequences used in Sanger sequencing (1, 2 and 3) of the *ASIP* exons 2,3 and 4 in impala (*Aepyceros melampus*) and for the fragment test (4) to detect a one base pair deletion in exon 3 (c.174delA) associated with the black phenotype. Underlining indicates universal Tail C and Tail D sequences from Blacket *et al.* (2012). Bold indicates Pig-tail designed by Brownstein *et al.* 1996.

Primers for *ASIP* exon 2 and 3 were designed using conserved sequences in horse (*Equus ferus caballus*; AF288358), pig (*Sus scrofa*; AY916525), cow (*Bos taurus* X99691) and sheep (*Ovis aries* EU420022); primers for *ASIP* exon 4 were designed using a full genome of the common impala (unpublished).

Sequencing Primers were supplied by Whitehead Scientific (Cape Town, South Africa). Primers for the fragment test were AB Prism Primers supplied by Applied Biosystems (Thermo Scientific, Johannesburg, South Africa).

Targeted Region	Forward Primer	Reverse Primer
Sequencing Primers		
1. *ASIP* exon 2	5'-CAGGACCAGGCTACCGTGAGCACCAGCCCAAAGRAWCA-3'	5'-CGGAGAGCCGAGAGGTGCTGCAGCCCCTTCTCATGG-3'
2. *ASIP* exon 3	5'-CAGGACCAGGCTACCGTGCAGAAGCTGCTGGCCTAAGT-3'	5'-CGGAGAGCCGAGAGGTGCTGACAATGWGAACTCCCAGGCCT-3'
3. *ASIP* exon 4	5'-CAGGACCAGGCTACCGTGTAGTCCGAGGAGTGTCCAGG-3'	5'-CGGAGAGCCGAGAGGTGCTGATTTTAGCCCAGCCCTAGCG-3'
Fragment Primers		
4. Deletion in Exon 3	5'-CTCTGCTCCTCCCACTTCAC-3' labelled with VIC	5'-**GTGTCTT**CAATGWGAACTCCCAGGCCT-3'

References

Blacket M.J., Robin C., Good R.T., Lee S.F., Miller A.D. (2012). Universal primers for fluorescent labelling of PCR fragments – an efficient and cost-effective approach to genotyping by fluorescence. Molecular Ecology Resources 12, 456-463.

Brownstein M.J., Carpten J.D., Smith J.R. (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. BioTechniques 20, 1004-1006.

Supporting Information - Fig. S1

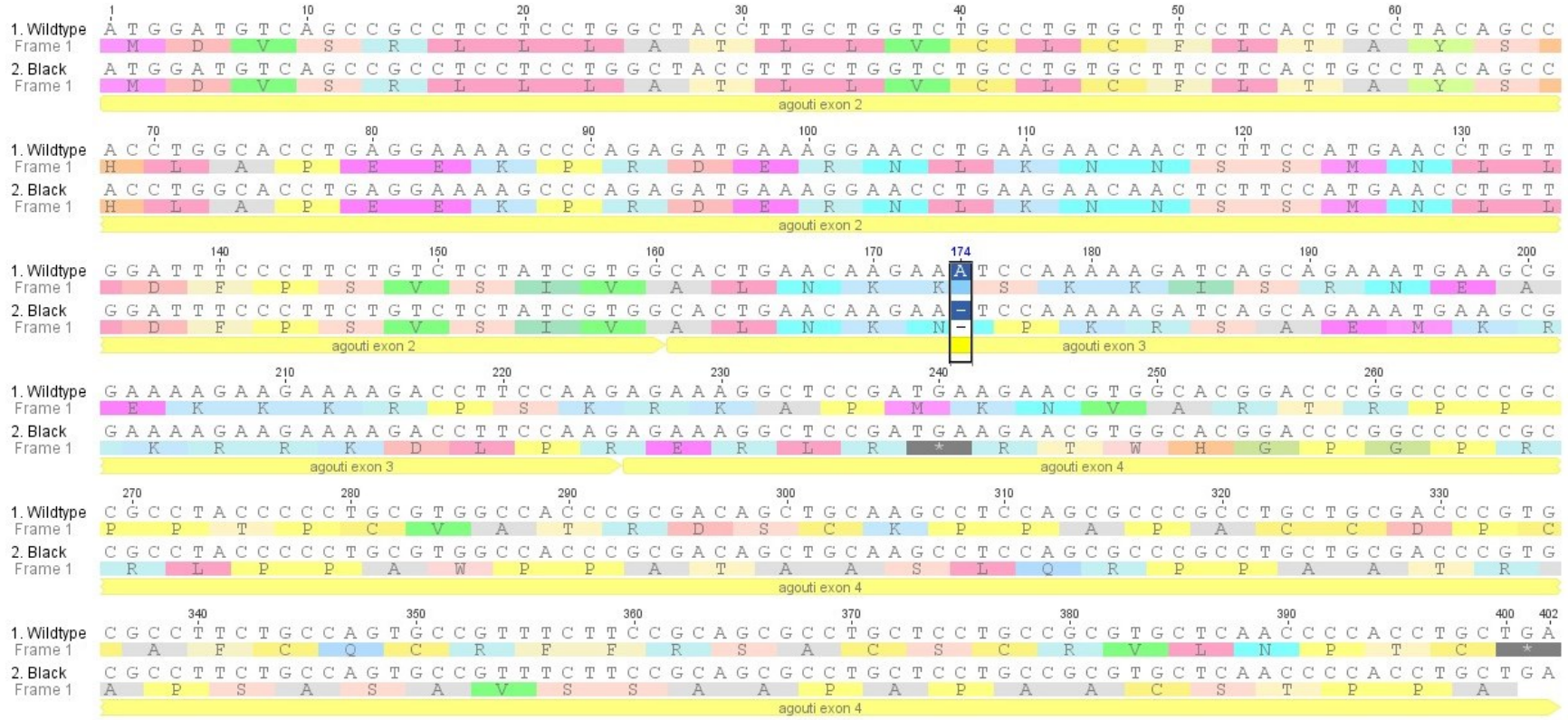

Fig. S1. Alignment of ASIP exons 2, 3 and 4 for (1) wildtype and (2) black impala. Single basepair deletion in exon 3 of the black impala is highlighted. A predicted premature stop codon caused by this frameshift mutation is indicated by the * at 239-241bp. Alignment and translation performed in Geneious v.8.1.7 (http://www.geneious.com, Kearse *et al.,* 2012).

Reference: Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647-1649.