Anthropogenic movement results in hybridisation in impala in southern Africa

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Abstract

Hybridisation caused by anthropogenic movements of animals is a conservation concern. Black-faced impala (*Aepyceros melampus petersi*) are endemic to north-western Namibia and south-western Angola and are geographically isolated from common impala (*A. m. melampus*). Common impala have been translocated into the black-faced impala range creating a hybridisation risk. We validated 13 microsatellite markers for the detection of recent hybridisation events. We used these markers to assess the genetic variation and differentiation among impala within Etosha National Park (NP), Southern Cross Private Game Reserve (SCPGR), Namibia, and private game ranches across South Africa. We confirmed that "black-nosed" impala in South Africa were *A. m. melampus*, thus providing more evidence that the black blaze on the face cannot be used to distinguish between the two subspecies. We detected four hybrids and one common impala on SCPGR. These five individuals were removed from SCPGR at time of sampling. We found two potential hybrids in southern Etosha NP. Further sampling of animals within Etosha NP is recommended to determine the extent of hybridisation within the park. The Namibian Ministry of the Environment & Tourism is developing a management plan for black-faced impala across Namibia that includes genetic testing for hybrids.

Keywords: *Aepyceros melampus melampus; Aepyceros melampus petersi;* allopatric; antelope

Introduction

Hybridisation due to anthropogenic disturbance has been recognised as a conservation problem for many years (Allendorf et al. 2001). Southern Africa has a history of translocating animals, mostly ungulates, around the region with minimal attention to genetic considerations (Spear and Chown 2009). This practice raises concerns and potentially threatens the viability of native species (or subspecies) and more research is needed into the evolutionary effects of wildlife ranching (Russo et al. 2019). The use of genetic techniques to detect hybridisation is often essential, as morphological

differences are not always diagnostic, especially between subspecies, and back-crosses are challenging to identify (e.g. Le Roux et al. 2015; van Wyk et al. 2017).

The impala (*Aepyceros melampus*), a common game ranch antelope species, is no exception. Two subspecies of impala are recognised: common impala (*A. m. melampus*) and black-faced impala (*A. m. petersi*). Black-faced impala are endemic to north-western Namibia and south-western Angola, with the Angolan population feared extinct (Green and Rothstein 1998; Fig. 1). They are listed as Vulnerable by the IUCN (IUCN SSC Antelope Specialist Group 2017). In Namibia, common impala only naturally occur in the Zambezi Region in the extreme northeast of the country (Skinner and Chimimba 2005; Fig. 1). Hybridisation between black-faced and common impala would have historically been prevented through geographical separation.

Fig 1. Map of sampling locations in Etosha National Park (NP) and Southern Cross Private Game Reserve (SCPGR). Inset indicates the historic ranges of common and black-faced impala (modified from Skinner and Chimimba 2005) as well as the location of Etosha NP in Namibia (NAM). Numbers indicate the number of samples per sampling area with each colour/shade representing a different region of Etosha NP. *Indicates sampling area of two suspected hybrids. Common impala sampling was from private ranches across South Africa (RSA)

Through a series of translocation events between 1968 and 1971, a population of black-faced impala was established in Etosha National Park (NP) to secure a population within a protected area close to their natural range (Green and Rothstein 1998). With the successful establishment and growth of this population, surplus animals from Etosha NP were translocated to multiple private farms across Namibia (Green and Rothstein 1998; Matson et al. 2003). The 2016 IUCN Red List assessment reported an estimated 3 250 – 3 300 black-faced impala on private ranches and conservancies in 2007, with approximately 1 000 in their original native range (north-west Namibia; IUCN SSC Antelope Specialist Group 2017). Common impala have also been introduced into private game farms across the country (Green and Rothstein 1998). These translocation events have eliminated the geographic isolation of the subspecies and hybridisation is a real threat (Green and Rothstein 1998). Using mitochondrial DNA (mtDNA), Nersting and Arctander (2001) confirmed the status of black-faced impala as subspecies and found minimal evidence of hybridisation with common impala in samples from Etosha NP. No hybridisation was detected by Lorenzen and Siegismund (2004) using microsatellite markers in samples collected in 2002.

Black-faced impala are named for the characteristic black blaze on the face, hence their common name (Fig. 2). While such markings are not traditionally evident in common impala (Skinner and Chimimba 2005), "black-nosed" impala are found on many South African game farms and are morphologically similar to black-faced impala (pers. obs. authors; Fig. 2). Grobler et al. (2017) used mtDNA to show that black-nosed impala from a farm in northern South Africa were consistent with the *A. m. melampus* haplotype, not *A. m. petersi*. The authors proposed that the black-nosed trait was not restricted to *A. m. petersi* and thus should not be used to distinguish between the two subspecies (Grobler et al. 2017). Genetic testing is therefore essential to determine the subspecies status of individuals and to prevent hybridisation and introgression (and potential negative impacts) within Namibia's black-faced impala population.

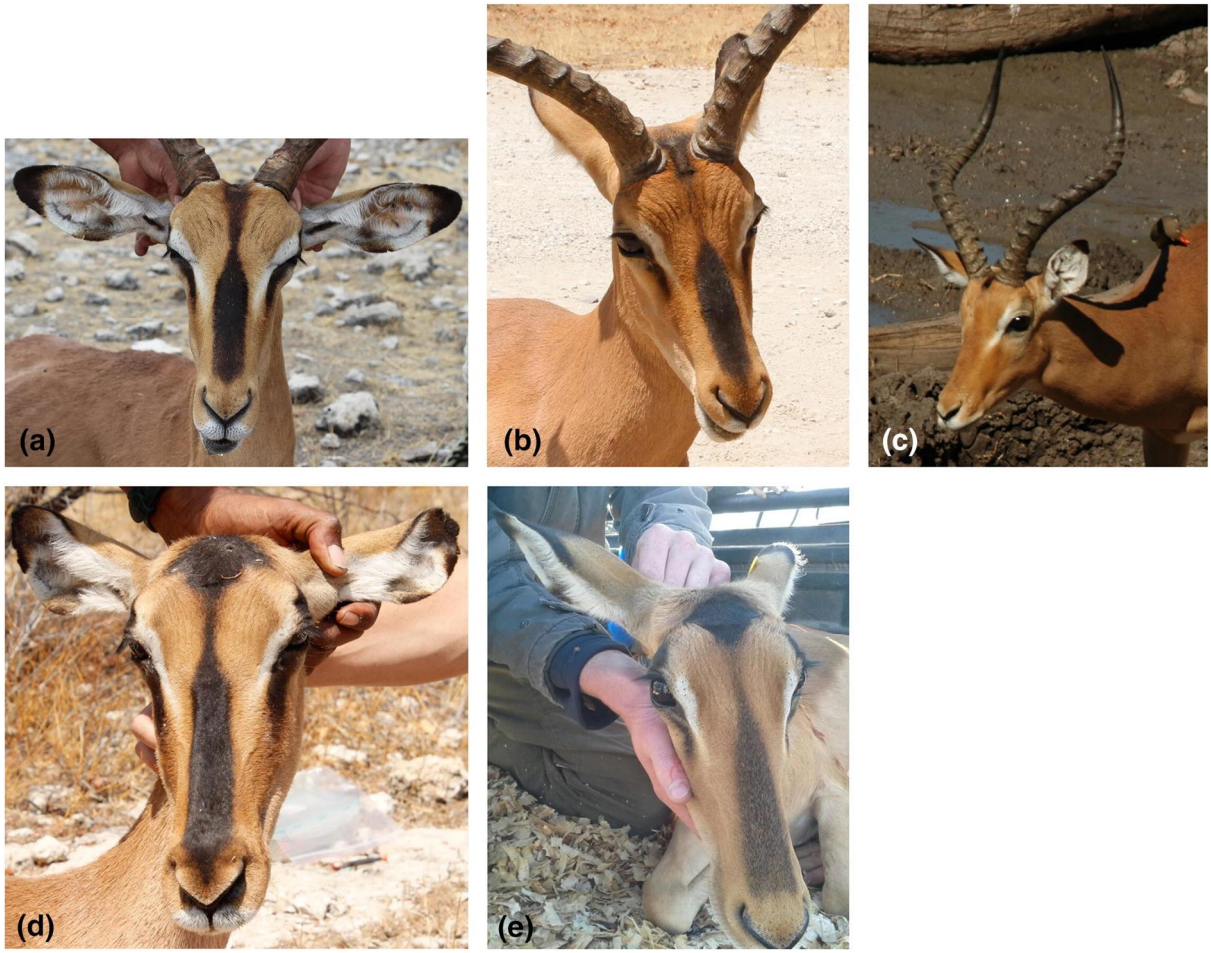

Fig 2. Variation in markings between black-faced impala (*Aepyceros melampus petersi*), common impala (*A. m. melampus*) and a possible hybrid. a Black-faced male, Etosha NP; b possible hybrid male, Etosha NP; c common impala male, Mapungubwe NP, South Africa; d black-faced female, Etosha NP; e "black-nosed" common impala, Kimberley, South Africa

The Ministry of Environment & Tourism (MET) in Namibia has a draft management plan based on Matson's proposed plan for protection of black-faced impala (Matson 2006). This plan includes a core area of approximately 200 000 km^2 reserved for black-faced impala only; certification to ensure only black-faced impala are present in this area and to prevent hybridisation between the two subspecies is being developed and will be based, in part, on the methods described in this publication (pers. comm. U. Muzuma, MET, Namibia). In this study we validated the use of 13 previously described microsatellite markers for common impala (Miller et al. 2016a) for identifying hybrids between

common and black-faced impala. We then tested impala across Etosha NP, Southern Cross Private Game Reserve (SCPGR), a privately owned reserve geographically close to Etosha NP, and South African game reserves for any evidence of hybridisation and introgression.

Methods

From September to December 2014, 62 black-faced impala in Etosha NP were darted from a vehicle and blood samples taken in EDTA vacutainer tubes (Fig. 1). Photographs of the face and whole animal were taken and animals were marked with 20 x 20cm cross of Terramycin spray (blue in colour) on the hip area to prevent resampling of the same animal. Different waterholes were visited most days to ensure a high level of unsampled individuals per session and coverage of the park. Twenty five samples from SCPGR in the Kamanjab region southwest of Etosha NP (Fig. 1), were collected in August 2015 and a further five samples in 2016, for a total of 30 samples. The five animals sampled in 2016 were all suspected hybrids.

All samples were extracted using the Prepfiler® Automated Forensic DNA extraction kit (Thermo Scientific, South Africa). Common impala individuals were routinely submitted to the Onderstepoort Veterinary Genetics Laboratory (VGL) for genotyping. Six hundred and thirty seven microsatellite profiles of common impala were used, including four "black-nosed" impala that were noted to have a similar black-faced marking to the black-faced impala from Etosha NP (please note we did not have phenotypic data for most samples so there could be more black-nosed individuals in our samples). Existing samples from game ranch animals were used as a proxy for natural populations of common impala as sampling animals from open systems was not possible due to budget constraints and they were native to the area. As previously noted, none of the black-faced impala samples from Etosha NP carried the gene associated with the black impala phenotype (Miller et al. 2016b). Only samples from common impala that did not carry the black gene mutation were used in this analysis. Genetic profiles

were generated using 13 microsatellites recommended for common impala in South Africa, excluding sex-linked I701, but including TGLA263 (Miller et al. 2016). Only samples with complete profiles were analysed: 59 Etosha NP, 29 SCPGR, 637 common impala.

This project was approved by the University of Pretoria Animal Ethics Committee (Project Number V039-15) and by the Namibian MET (Permit Number 1971/2014). Veterinary import permits were acquired for the transport of the samples from Namibia to the laboratory in South Africa (South African permit no: 13/1/1/14/2/1/11/201411000887; Namibia: through the Central Veterinary Laboratory Master Export Permit).

Validation of microsatellite markers for detecting hybrids

While the microsatellite markers used here have all been previously validated for use in common impala (Miller et al. 2016a), they have not been validated for assignment testing between common and black-faced impala. Probability of identity and sibling-identity were calculated in the Excel macro GenAlEx v6.5 (Peakall and Smouse 2012); Etosha NP and SCPGR populations were combined to represent Namibian impala. F_{ST} was calculated in GenePop v4.1.4 (Rousset 2008). A power analysis to determine the level of F_{ST} separation that can be reliably detected was carried out using the sample planning tool, SPTOG, on the ConGRESS website (Laval and Excoffier 2004; Excoffier and Lischer 2010; Hoban et al. 2013). The "Assignment Module" was used with the following parameters: number of genetic markers, 13; number of individual samples, 20 (to reflect the 20 controls as determined below); normal allele frequencies, 6, 9, 12, 15; population differentiation (F_{ST}), 0.05, 0.10, 0.20, 0.30, 0.40, 0.50; number of populations, 2 (South Africa and Namibia); and number of runs, 250.

Common vs black-faced impala

Two methods were used to determine the level of differentiation between common (South Africa) and black-faced impala (Etosha NP and SCPGR), prior to selection of control individuals for simulations and hybrid detection: Principal Component Analysis (PCA) in R v3.6.0 (R Core Team 2018) in RStudio v 1.1.456 (RStudio Team 2016) using the *adegenet* package (Jombart 2008) and STRUCTURE (Pritchard et al. 2000). All 725 genotypes were used for both analyses. In STRUCTURE *K*-values from one to four with 100 000 of burn-in and data collection of 100 000 chains were used and the "Admixture Model" with correlated allele frequencies was applied. This was replicated 10 times per value of *K*. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to aid in determining the most realistic *K* value. STRUCTURE runs were averaged using CLUMPAK (Kopelman et al. 2015).

Control populations

Based on the initial PCA and STRUCTURE analysis, there was a statistically supported split between common and black-faced impala (Supplementary Information Fig. S1, Fig. 3, see results for more details). Twenty control profiles for both black-faced and common impala were chosen for use in further analyses as follows: Relatedness values were calculated between the 637 common impala profiles using the Wang method in Coancestry (Wang 2011). The Friends and Family program (de Jager et al. 2017) was used to determine the least related individuals with a cut-off value of 0.25 relatedness level for "friends" or unrelated individuals. These friends were combined with the Etosha NP black-faced animals, excluding one animal that appeared to be admixed based on the initial STRUCTURE analysis, for a further STRUCTURE analysis using the same parameters are before. STRUCTURE Harvester was used to determine the most likely *K* value. This was determined to be *K*=2 (see results for details). The *Q* values for each individual were averaged over the 10 runs using CLUMPAK. These

Q values were used to determine the 20 animals from each population that were the "most pure". These were used as control animals for each population in subsequent analyses.

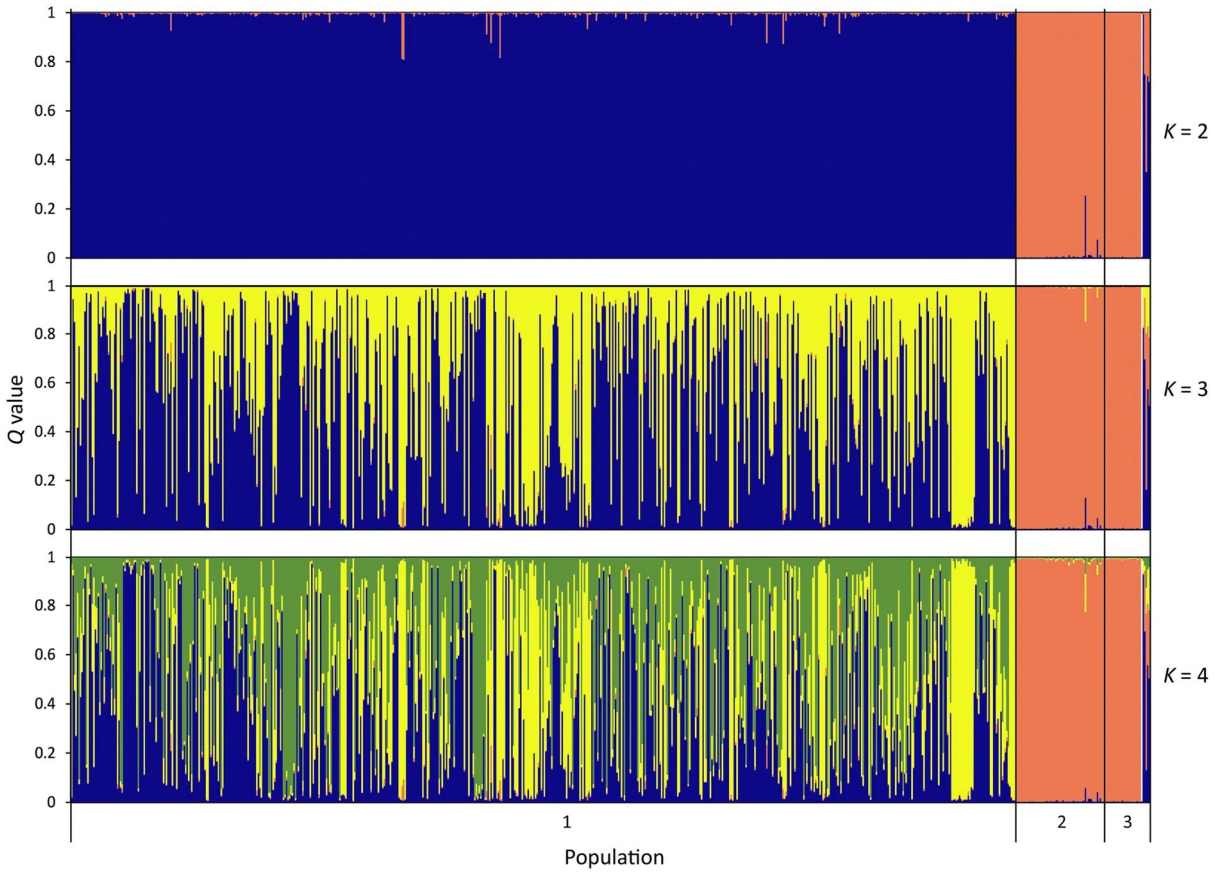

Fig 3. Differentiation between common and black-faced impala using STRUCTURE analysis (*K* = 2–4) based on 13 microsatellites. Population 1: Common impala, South Africa; Population 2: Black-faced impala, Etosha National Park, Namibia; Population 3: Impala, Southern Cross Private Game Reserve, Namibia

Simulation

One hundred simulated genotypes each were generated in HybridLab v1.0 (Nielsen et al. 2006) for: P1 (common), P2 (black-faced), F1, F2, back-crosses of F1 to both common (P1Bx) and black-faced impala (P2Bx) and a second back-cross of P1Bx to common controls (P1Bx2) and P2Bx to black-faced controls (P2Bx2). Controls determined above (*z* designation) and simulated genotypes were analysed with

NewHybrids v1.1 (Anderson and Thompson 2002) to validate the marker set for detecting hybrids. A burn-in period of 5 000 was followed by 10 000 sweeps based on the graphical version of NewHybrids (Supplementary Information Fig. S2). Ten replicates using Jeffrey's priors were tested and summarised using CLUMPAK. The graphical version of NewHybrids was also used to visualise the Kullback-Leibler divergence between populations for each locus.

These simulated data were also analysed using STRUCTURE with *K*-values from one to four with 100 000 of burn-in and data collection of 100 000 chains. The "Admixture Model" was applied. This was replicated 10 times per value of *K*. STRUCTURE HARVESTER was used to evaluate which *K* value was most likely. STRUCTURE runs were averaged using CLUMPAK (Kopelman et al. 2015).

Detection of hybrids

All 725 complete genotypes (simulations not included) were analysed using NewHybrids. The control animals were identified using the *z* designation. A burn-in period of 5 000 was followed by 10 000 sweeps. Ten replicates using Jeffrey's priors were tested. CLUMPAK was used to summarise the ten replicates for each prior. Ideally we would have classified individuals as P1, P2, F1, F2, P1Bx, etc. however, given the limitations of our microsatellite panel (see results), we have simply classified individuals with a value lower than 0.9 for either P1 or P2 as a hybrid. STRUCTURE was not used to evaluate the data for hybrids as it was not as effective in assigning the simulated genotypes to the appropriate clusters (see results for details).

Assignment Testing

Assignment testing was conducted to assign individuals to one of two populations (South African common impala or Namibian black-faced impala) using the same controls as for the NewHybrids testing. Rannala and Mountain (1997) criteria were used with Paetkau et al. (2004) simulation of

1 000 000 individuals with Type 1 error equal to 0.01 as implemented in GeneClass2 v 2.0.h (Piry et al. 2004). The assignment threshold of scores was set at 0.001.

Population summary statistics

Genotypes were grouped by populations: South African common, Etosha NP black-faced, SCPGR black-faced, black-faced (Etosha NP and SCPGR). The five impala on SCPGR that were culled after sampling were not included in these calculations. In order to account for sample size inequalities, a further 10 "populations" were included: 59 random common impala genotypes (out of the 637 South African common impala genotypes) selected 10 times. The average statistics from these 10 "populations" were used to compare to the other populations. For each population H_O, H_E and individual heterozygosity levels were calculated in GenAlEx and allelic richness (A_R), using rarefaction to account for unequal samples sizes was calculated, in FSTAT (Goudet, 1995). Inbreeding coefficients (F) were also calculated using the following equation (Frankham et al. 2010; equation 12.9):

$$1 - \frac{HInbred}{HOutbred}$$

where *HInbred* is the observed heterozygosity of the population for which the inbreeding coefficient is being calculated and *HOutbred* is the observed heterozygosity of 59 randomly sampled South African common impala genotypes. Unlike the traditional F_{IS} calculation, this equation estimates the accumulated inbreeding coefficient of a population resulting from the loss of genetic diversity over time (Frankham et al. 2010) or in our case, compared to the outbred common impala population.

Results

Full (13 markers) microsatellite profiles were generated for 59 Etosha NP, 29 SCPGR, and 637 common impala (Supplementary Information File S1; all supplementary material is available at

doi:10.25375/uct.8241632). Probability of identity for all markers combined for South African impala was $4.6x10^{-13}$ and for Namibian impala was $1.0x10^{-9}$. Probability of sibling-identity was $1.8x10^{-5}$ and $1.3x10^{-4}$ for South African and Namibian impala, respectively. The F_{ST} value generated by GenePop was 0.224. The number of alleles per microsatellite marker ranged from six to fifteen. The Assignment Module in the SPTOG revealed that 13 microsatellite markers would be able to assign individuals to populations with an F_{ST} of 0.2 at six, nine, 12 or 15 alleles (Supplementary Information Fig S3). The power of exclusion (with an F_{ST} of 0.2) was 0.99 for nine, 12 and 15 simulated alleles and 0.93 for six simulated alleles (Supplementary Information Fig. S3).

Common vs black-faced impala

The PCA analysis separated Etosha NP and SCPGR populations from the South African population. The first two axes from the PCA explained 16 percent of the variation in the data (12.1% for the first and 4.59% for the second axis; Supplementary Information Fig. S1). Four samples from SCPGR were closer to the South African population (Supplementary Information Fig. S1). These genotypes were the same genotypes that were found to be either common impala or hybrids in other analyses. The remaining suspected hybrid from SCPGR clustered more closely with the other black-faced impala samples.

STRUCTURE analysis revealed two populations: one predominantly consisting of common individuals and one of black-faced individuals (Fig. 3). The Evanno ΔK statistic indicated the strongest population split at $K = 2$ (Supplementary Information Fig S4). Larger values of K resulted in splits within the common impala population (Fig. 3) and had no support with STRUCTURE HARVESTER (Supplementary Information Fig. S4).

Choice of Control Individuals

Relatedness values between the 637 common impala as calculated in Coancestry are reported in Supplementary Information File S2. Friends and Family identified 146 unrelated individuals (Supplementary Information File S2). STRUCTURE analysis of these 146 unrelated common individuals and 58 black-faced individuals from Etosha NP are presented in Supplementary Information File S2; *K* = 2 *Q* values were averaged using CLUMPAK and sorted by Q value (Supplementary Information File S2). The highest 20 Q values for each population (common and black-faced) were chosen as controls for the rest of the analyses. All individuals chosen as controls had *Q* values > 0.997 averaged over the 10 replicates in STRUCTURE (Supplementary Information File S2).

Detection of Simulated Hybrids with NewHybrids

Control and simulated genotypes are available in Supplementary Information File S3. All of the controls were correctly identified by the NewHybrids software with posterior probabilities > 0.88 for all individuals (Fig. 4a). The cut-off for pure P1 and P2 was therefore set at a posterior probability equal to 0.90.

Ninety-seven percent of the P1 and 98 percent of P2 simulated genotypes were correctly identified (Fig. 4a). None of the F1 or F2 simulated genotypes were mistaken for P1 or P2 genotypes (Fig. 4a). Three percent of the P1Bx simulated genotypes were mistaken as pure P1 genotypes (posterior probabilities of 0.94, 0.96, 0.96; Fig. 4a) and one percent of the P2Bx simulated genotypes were mistaken as pure P2 genotypes (posterior probability of 0.92; Fig. 4a). Twenty one percent and 27 percent of double back-cross simulated genotypes (P1Bx2 and P2Bx2) were mistaken for pure P1 and P2 genotypes, respectively (Fig. 4a). As the classification of individuals in the P1Bx, P2Bx, P1Bx2 and

P2Bx2 categories was not well defined, we did not use these specific classifications for the assessment of our samples, but rather used the cut-off at *Q* value of 0.9 for pure versus hybrid as explained above.

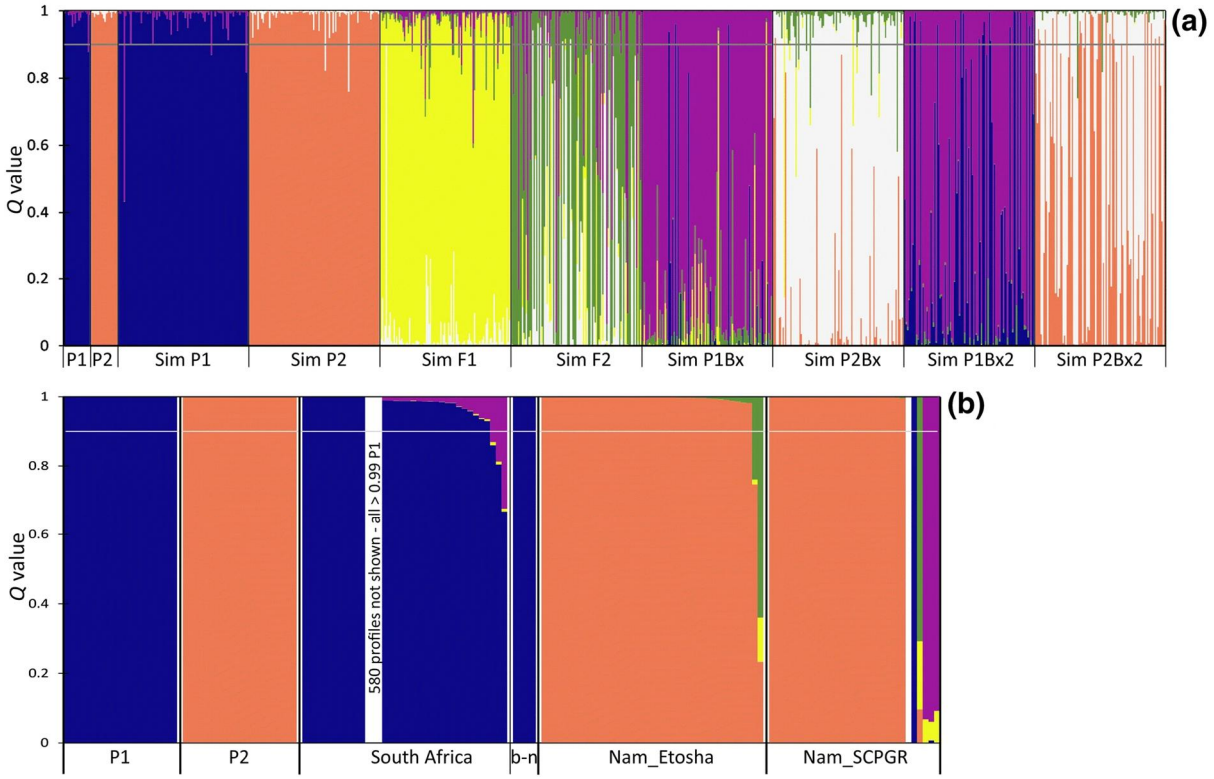

Fig 4. NewHybrids analysis of a Simulated genotypes with control samples from common (P1) and black-faced (P2) impala (20 genotypes each) and simulated F1, F2, F1 backcrosses to P1 (P1Bx), F1 backcrosses to P2 (P2Bx), P1Bx backcrosses to P1 (P1Bx2), and P2Bx backcrosses to P2 (P2Bx2) (100 simulated genotypes each); and b Actual genotypes with control samples of P1 and P2; South Africa, including five black-nosed individuals (b-n); Etosha NP (Nam_Etosha) and Southern Cross Private Game Reserve (Nam_SCPGR) in Namibia. A 0.9 cut-off was applied to all analyses as indicated by the solid grey line. Colours/shading reflect clustering based on NewHybrid settings, see text for details

Based on the Kullback-Leibler divergence between populations for each locus, TGLA263, TGLA122 and I4 were the three most informative microsatellite markers for distinguishing between common and black-faced impala while SPS113 was the least useful (Supplementary Information Fig. S5).

Detection of Simulated Hybrids with STRUCTURE

The Evanno Δ*K* statistic indicated the strongest population split at *K* = 2 (Supplementary Information Fig. S6). At *K* = 2 all of the control genotypes were correctly identified with a cut-off value of 0.9. Ninety-nine percent of simulated P1 genotypes and 100 percent of P2 genotypes were correctly identified. All of the F1 and F2 genotypes were correctly classified as hybrids. Five percent of the P1Bx and 11 percent of the P2Bx simulated genotypes were mistaken as pure P1 and pure P2 genotypes, respectively. Seventy nine percent of P1Bx2 simulated genotypes were mistaken as pure P1 genotypes and 65 percent of P2Bx2 simulated genotypes were mistaken as pure P2 genotypes (Supplementary Information Fig. S7).

Detection of hybrids with NewHybrids

Two out of 617 common impala samples had posterior probability values < 0.9 for the P1 population designation (P1; Fig. 4b). All four black-nosed individuals had posterior probability values > 0.99 for the P1 population designation (Fig. 4b). Two of the 39 individuals from Etosha NP were classified as hybrid animals (posterior probability < 0.8; Fig. 4b). Twenty four of the 29 individuals from SCPGR had posterior probabilities > 0.99 for the P2 population designation (Fig. 4b). Of the remaining five, which were all suspected hybrids, one had a posterior probability of 0.99 for the P1 designation and the remaining four were hybrids (Fig. 4b).

Assignment testing with GeneClass2

One of the 39 individuals from Etosha NP was below the threshold for assignment to the black-faced impala population (p = 0.0008; the western most individual identified by NewHybrids). Of the five suspected hybrids from SCPGR, one identified as a common impala (same individual as in NewHybrids), three were below the threshold for assignment to the common impala population and

one was below the threshold for assignment to either population. Nine of the South African individuals were below the threshold for assignment to the common impala population. Full results can be found in the Supplementary Information File S4.

Population summary statistics

Basic summary statistics are presented in Table 1. Allelic richness, observed and expected heterozygosity levels were highest in South African common impala. Black-faced impala on SCPGR had similar values to those on Etosha NP. Combining the two black-faced populations resulted in slightly higher values for A_R, H_O and H_E and a lower F value.

Table 1. Basic population summary statistics for South African (SA) common impala, Etosha NP black-faced impala, SCPGR black-faced impala, black-faced combined and a random sample of common impala. N = number of genotypes, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F = inbreeding coefficient

	SA common	Etosha NP black-faced	SCPGR black-faced	Black-faced (Etosha NP + SCPGR)	SA common59
N	637	59	24	83	59*
A_R	8.69	4.14	4.15	4.93	6.22
H_O	0.65	0.56	0.55	0.56	0.65
H_E	0.69	0.56	0.54	0.57	0.69
F	-	0.13	0.15	0.11	-

*59 genotypes randomly sampled from 637 common impala genotypes 10 times to create 10 "populations" of 59 individuals. Statistics calculated for each "population" and the average presented here.

Discussion

We found evidence of hybridisation between common and black-faced impala in both Etosha NP and one private farm (SCPGR) in Namibia. Of the five suspected hybrids on SCPRG, one clustered with the common impala and four tested as hybrids. Fortunately, all five of these animals were removed from the population at the time of sampling, due to suspected hybrid status. Two suspected hybrids were

identified in the western half of Etosha NP. Subsequent attempts to sample more animals from this region were unsuccessful as they had moved off due to reduced rainfall. Several farms bordering this part of the park may contain common impala. Hybridisation could, therefore, have been a result of movement between private farms and the national park. To prevent movement of common impala from private farms to Etosha NP, a buffer zone could be created in which common impala are prohibited. This conservation challenge is addressed in the MET's draft management plan for the species (pers. comm. U. Muzuma, MET, Namibia).

We confirmed that animals with the black-face marking found on a private reserve in South Africa clustered with common impala. This corroborates the results of Grobler et al.'s (2017) mtDNA test of black-nosed animals on a farm in the Limpopo Province. Although our results were based on a small sample size of animals on an isolated farm in the Northern Cape, we did not find any common impala individuals clustering with the Etosha NP individuals and only a few suspected hybrids, suggesting that most black-nosed individuals in South Africa are not black-faced impala.

Four software programs using differing algorithms (STRUCTURE, NewHybrids, PCA in R and GeneClass2) were able to distinguish between common and black-faced impala using genotypes generated from 13 microsatellite markers. We detected hybridisation between common and black-faced impala using NewHybrids software. This software was preferred as it was efficient at detecting hybrids with a very low error rate. While the classification of simulated genotypes by hybrid type (e.g. F1 vs F2 vs BxP1 vs BxP2) was not completely accurate, the number of simulated genotypes that were misidentified as a pure P1 or P2 individual was very low. A further level of back-crosses (P1Bx2 and P2Bx2) resulted in approximately 25% misidentification. This was not unexpected and similar results were reported for hybridisation within roan antelope (*Hippotragus equinus*) using 27 microsatellite markers (van Wyk et al. 2019). Therefore, while we are confident that the analysis presented here is

a useful tool for the detection of hybrid individuals from recent hybridisation events, it is not suitable for detecting multiple generations of backcrossing.

The microsatellite markers were developed and optimised for parentage assessment of common impala on South African game ranches (Miller et al. 2016a). If detection of hybrids after multiple generations of backcrossing hybridisation is required, the addition of more markers may improve the accuracy. The Kullback-Leibler divergence for the current loci indicates that they were not equally informative. Interestingly the two most informative markers were first developed for cattle (TGLA), outperforming the impala-specific markers. Any additional loci could be tested for Kullback-Leibler divergence and either replace or augment the microsatellites used here. Vähä and Primmer (2005) outlined Bayesian methods for detecting hybrids with varying numbers of loci. More recently, Randi et al. (2014) suggested some additional statistical testing for detecting dog/wolf hybrids with microsatellites which could be applied to impala.

The historical separation of black-faced and common impala is mirrored in many other savanna species as reviewed by Lorenzen, Heller, and Siegismund (2012) and includes giraffe (*Giraffa camelopardalis*) (Brown et al. 2007; Bock et al. 2014), greater kudu (*Tragelaphus strepsiceros*) (Nersting and Arctander 2001), sable antelope (*Hippotragus niger*) (Pitra et al. 2002), mountain zebra (*Equus zebra*) (Moodley and Harley 2005) and African lion (*Panthera leo*) (Bertola et al. 2016). Unique lineages within this “south west” region should be preserved to maintain local adaptive potential. Unfortunately, anthropogenic movement of impala, and other game species, is routine throughout southern Africa.

This study provides another example of the potential pitfalls associated with this practice and the potential for loss of local adaptations if mixing occurs. Hybridisation is not confined to impala: van Wyk et al. (2013, 2017) detected blesbok (*Damaliscus pygargus phillipsi*) and bontebok (*D. p.*

pygargus) hybridisation, Grobler et al. (2011, 2018) detected blue (*Connochaetes taurinus*) and black wildebeest (*C. gnou*) hybridisation and van Wyk et al. (2019) detected hybridisation between subspecies of roan antelope. In all of these cases, including our impala case, genetic evidence already exists for the separation as subspecies or, for wildebeest, species. Nersting and Arctander (2001) and Lorenzen and Siegismund (2004) both found evidence supporting differentiation between the impala subspecies (F_{ST} = 0.19) which is comparable to our findings (F_{ST} = 0.224). This is similar to other F_{ST} values between subspecies: roan antelope subspecies (F_{ST} = 0.165; van Wyk et al. 2019) and waterbuck subspecies (F_{ST} = 0.19; Lorenzen et al. 2006). In all cases hybridisation between subspecies/species was due to reintroduction efforts resulting in naturally allopatric subspecies or closely related species co-habiting. Integrating genetic data into conservation planning is highly recommended (Hoban et al. 2013) and should be encouraged in future conservation planning by southern African nations.

We have presented evidence that the genetic variation (allelic richness, heterozygosity) was lower in the black-faced impala compared to the common impala population of South Africa. The inbreeding coefficient for the black-faced impala was also lower relative to the presumed outbred common impala in South Africa. Sampling within South Africa was much more extensive and thus more sampling of the black-faced impala populations in Namibia is needed to confirm this. As many of the black-faced impala populations in Namibia are fenced and therefore isolated, management actions should include the periodic movement of genetically pure individuals between fenced populations and augmentation from any remaining free-roaming populations. This is an approach currently applied to carnivores within South Africa due to the movement restrictions imposed by fencing, as well as the general lack of dispersal corridors between fragmented conservation areas and between fenced game ranches (Davies-Mostert et al. 2009; Miller et al. 2015; Buk et al. 2018). A similar managed metapopulation plan could be developed for black-faced impala and management planners should consider this approach.

Conclusions

Hybridisation between common and black-faced impala following anthropogenic movement of common impala into the range of black-faced impala has occurred in Namibia. Phenotypes cannot be used to determine if an animal is a black-faced or a common impala as some common impala have extensive black markings on their faces. Current genetic testing can detect recent hybridisation events, however more robust testing would need to be developed to detect hybrids beyond one generation of back-crossing. Population genomics research could be used to further explore differentiation between the subspecies and to determine the evolutionary implications of admixture.

To prevent further admixture, it may be useful to limit anthropogenic movement of common impala by creating an impala-free buffer zone between black-faced and common impala populations. Any farms bordering Etosha NP should not be permitted to maintain common impala populations. Furthermore, any black-faced impala being introduced into an area, should be tested for "purity" before release. These concerns are addressed in the draft management plan being developed by the MET in Namibia (pers. comm. U. Muzuma, MET, Namibia).

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