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RESEARCH ARTICLE

Assessing introgressive hybridization between blue wildebeest (*Connochaetes taurinus*) and black wildebeest (*Connochaetes gnou*) from South Africa

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Abstract

Introgressive hybridization poses a threat to the genetic integrity of black wildebeest (*Connochaetes gnou*) and blue wildebeest (*Connochaetes taurinus*) populations in South Africa. Black wildebeest is endemic to South Africa and was driven to near extinction in the early 1900s due to habitat destruction, hunting pressure and disease outbreaks. Blue wildebeest on the other hand are widely distributed in southern and east Africa. In South Africa the natural distribution ranges of both species overlap, however, extensive translocation of black wildebeest outside of its normal distribution range in South Africa have led to potential hybridization between the two species. The molecular identification of pure and admixed populations is necessary to design viable and sustainable conservation strategies, since phenotypic evidence of hybridization is inconclusive after successive generations of backcrossing. The aim of this study was to assess levels of hybridization in wildebeest using both species-specific and cross-species microsatellite markers. Black wildebeest (157) and blue wildebeest (122) from provincial and national parks and private localities were included as reference material, with 180 putative hybrid animals also screened. A molecular marker panel consisting of 13 cross-species and 11 species-specific microsatellite markers was developed. We used a Bayesian clustering model to confirm the uniqueness of blue- and black wildebeest reference groups, assign individuals to each of the two clusters, and determine levels of admixture. Results indicated a clear partition between black wildebeest and blue wildebeest (the average proportions of membership to black wildebeest and blue wildebeest clusters were QI = 0.994 and QI = 0.955 respectively). From the putative hybrid samples, only five hybrid individuals were confirmed. However, high levels of linkage disequilibrium were observed in the putative hybrid populations which may indicate historical hybridization. Measures of genetic diversity in the black wildebeest populations were found to be lower than that of the blue wildebeest. The observed lower level of genetic diversity was expected due to the demographic history of the specie. This study will make a significant contribution to inform a national conservation strategy to conserve the genetic integrity of both species.

Keywords Black wildebeest · Blue wildebeest · Hybridization · STRUCTURE · HYBRIDLAB · Microsatellites

Introduction

Anthropogenic disturbances such as habitat modification and species translocation (Allendorf et al. 2001) are major causes driving loss of biodiversity, leading to introgressive hybridization and reverse speciation (Seehausen et al. 2006). Human induced hybridization may have detrimental effects on species survival including reduced fertility, genetic swamping or assimilation and may ultimately lead to extinction of populations and/or species (Rhymer and Simberloff 1996; Levin et al. 1996; Allendorf et al. 2001; Buerkle et al. 2003; Vuillaume et al. 2015). In South Africa, the blue wildebeest (*Connochaetes taurinus*) and the black

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wildebeest (*C. gnou*) display reproductive isolation under natural conditions, due to differences in breeding behaviour and habitat preferences (Brink 2005; Vrahimis et al. 2016). However, extra-limital translocations and the disruption of social structures through confinement on small areas have led to hybridization. Grobler et al. (2011) provide a detailed review of the proximate and ultimate causes of hybridization in wildebeest. In this paper, we investigate the challenging circumstances of detecting and managing hybridization between the two species.

Fossil records suggest that the blue- and black wildebeest diverged approximately one million years ago (Corbet and Robinson 1991; Brink 1993, 2005). Both species resort within the tribe Alcelaphini and have the same number of chromosomes, with 2n = 58 (Buckland and Evans 1978). Blue and black wildebeest share many morphological similarities but can be readily distinguished by a number of external phenotypic features. The first trait is horn conformation (Fig. 1): the black wildebeest have horns that start from an enlarged base, sweep downward, forward and then curve upwards while blue wildebeest horns curve outward and slightly downward from an extended swollen base and then rise upward with the tips pointing inward (Skinner and Chimimba 2005). In terms of coat colour, the blue wildebeest is generally a silvery-blue sheen colour, with black wildebeest generally buffy brown in colour (Skinner and Chimimba 2005). The latter species have also been known as the white tailed gnu because of their off-white tails that nearly reaches the ground (Skinner and Chimimba 2005). Finally, the blue wildebeest is also the larger of the two species (Skinner and Chimimba 2005) with males being approximately 130 cm tall at the shoulder and weighting between 210 and 260 kg (Attwell 1977; Fabricius et al. 1988). Male black wildebeest are approximately 120 cm tall at the shoulder with an average weight of 170 kg (Von Richter 1971, 1974; Skinner and Chimimba 2005).

Black wildebeest are endemic to South Africa with a historical distribution throughout the central region (Free State, North West, Gauteng, Northern Cape, Eastern Cape, Mpumalanga and KwaZulu-Natal) (Fig. 1). It was driven to near extinction in the early 1900s due to habitat destruction, hunting pressure and disease outbreaks (Von Richter 1974). In this regard, it is estimated that the population today is descendent from a founder population of approximately 300 animals (Kirkman 1938). According to the Vrahimis et al. (2016) the total extant population size is estimated at

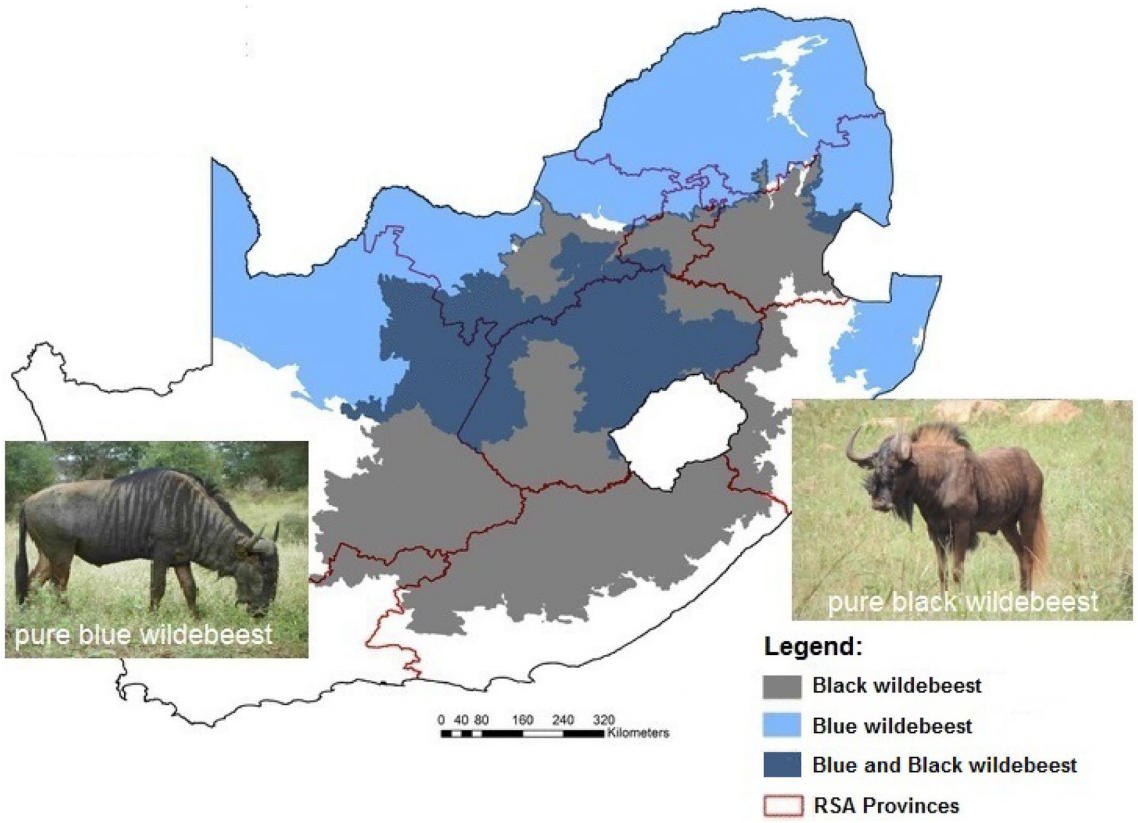

Fig. 1 Map indicating the inferred natural distribution ranges of the blue wildebeest (*Connochaetes taurinus)* and black wildebeest (*C. gnou)* in South Africa, based on historical accounts and recent archaeological records. (Reproduced with permission from (Birss et al. 2017; Russo et al. 2018). Photographs courtesy of Kobus Raath and Anri van Wyk). (Color figure online)

only 16,260 animals. In contrast, the blue wildebeest occurs throughout southern and east Africa and the International Union for Conservation of Nature (IUCN) estimates the total number of the blue wildebeest at 1,550,000 (Estes and East 2009). Threats for the blue wildebeest include habitat destruction, population fragmentation, poaching and disease outbreaks (East 1999; Estes 2013). Historically, the main threats to black wildebeest included hunting pressure, habitat loss and disease outbreaks (Vrahimis 2013). However, the only current significant threat to this species is hybridization with its congener (Benjamin-Fink and Reilly 2017; East 1999; Grobler et al. 2011).

In South Africa, the historical distribution ranges of these species are largely distinct with black wildebeest normally restricted to open grassland while blue wildebeest are associated with a wide variety of savannah habitat types including woodland, open-grassland and semi-desert environments (Skinner and Chimimba 2005). The geographical ranges of the two species did formally overlap in a number of areas (Fig. 1). Historical cases of natural hybridization were not documented and it was assumed that differences in habitat preferences and behavioural differences of the species would result in reproductive isolation (Brink 2005). Hybridization between these species was first reported in the 1960s. Later on, Fabricius et al. (1988) investigated a hybrid wildebeest population on a private farm in the Northern Cape Province of South Africa and observed that the hybrid offspring were fertile, with neonates that accompanied the females as well as some yearlings. As a precautionary principle, the Polokwane Game Reserve (Limpopo Province, South Africa) culled all the blue- and black wildebeest in 1982 due to possible hybridization (Grobler et al. 2005). The history of this game reserve indicated that in 1963, five pure black wildebeest individuals were translocated to the reserve with a blue wildebeest population. Between 1963 and 1982, 71 animals from this reserve were translocated to six new locations including a government protected area that acted as source population leading to potential rapid growth in putative hybrids. A similar situation occurred at Spioenkop Nature Reserve (KwaZulu-Natal Province, South Africa) where both species were introduced and the entire herd eventually culled due to suspected hybridization. Subsequent phenotypic and anomalous cranial morphological characteristics confirmed hybridization (De Klerk 2007; Ackermann et al. 2010), however, a number of animals had already been translocated to three government owned protected areas as well as a number of private properties (Grobler et al. 2011). From these localities, further translocations took place to numerous other properties possibly spreading the number of hybrid animals (Grobler et al. 2011). The actual extent of introgression in the national herd is not known, however, in 2011 it was estimated that more than 120 properties in South Africa harboured both species. It is also known that thousands of black wildebeest have been exported to Namibia, Swaziland and Botswana (Grobler et al. 2011). If hybrid animals were translocated from the 120 or more properties harbouring both species, a large proportion of the black wildebeest population in southern Africa may be admixed.

A clear definition of the genetic characteristics of each of the two species is essential to allow for hybrid detection. Early studies failed to detect significant genetic differences between blue- and black wildebeest. A study by Corbet and Robinson (1991) found no species specific G or C-banded chromosomal markers for mitotic chromosomes and mitochondrial DNA. Two different studies using allozymes also indicated very low genetic distance values between the two species (Corbet et al. 1994; Grobler and Van der Bank 1995). Grobler et al. (2005) conducted a study based on five bovine microsatellite markers and suggested fixed allele differences between the two wildebeest species at two loci (ETH 10 and BM1844). However, a larger sample size with better geographic coverage of pure reference populations indicated that the assumed fixed difference at ETH10 did not hold true (Kotze and Grobler, unpublished results). Initially, a total of four alleles were described at this locus, with two each unique to blue- and black wildebeest, but the subsequent sampling suggested that one of the potential black-specific alleles were in fact shared between the two species.

The success of hybrid detection in wildebeest will be influenced by backcrossing between hybrids and pure black wildebeest, since successive generations of backcrossing will lead to dilution with a biological, practical and monetary limit to hybrid detection ultimately reached. Boecklen and Howard (1997) confirmed that the detection of ultra-low levels of admixture can become unrealistic and impractical. In view of these restrictions, scientists and conservation managers may need to come up with pragmatic decision's, such as absolute "purity" versus "pure enough" and conserving pure individuals versus pure herds. The close evolutionary relationship that exists between the two wildebeest species suggest that it might be impractical to restrict conservation efforts to absolute purity, as was also the conclusion during efforts to conserve pure bontebok (van Wyk et al. 2017). The selection of a threshold Q-value (hybridization or admixture index from clustering algorithms like STRUCTURE) that allows for a measure of shared ancestry is thus recommended. Various Q-values have been suggested in the literature, with thresholds for purity ranging from 0.7 to 0.99 (Valbuena-Carabaña et al. 2007; Sanz et al. 2008; Lepais et al. 2009; Hoban et al. 2012). The optimal Q-value can be guided by simulations (Lepais et al. 2009; Cullingham et al. 2011; van Wyk et al. 2017) and depends on the application and the target species.

Hybridization has been successfully detected and managed between two endemic South African antelope species,

the bontebok (*Damaliscus pygargus pygargus*) and the blesbok (*Damaliscus pygargus phillipsi*) (Van Wyk et al. 2013; van Wyk et al. 2017), using the approach described above. The Western Cape Provincial Conservation Agency (CapeNature) used these studies to develop the Bontebok Conservation, Translocation and Utilization Policy (BCTUP, 2014). The BCTUP serves as a regulatory mechanism to inform permitting and translocation.

From a management and conservation perspective, it should be noted that backcrossing (either deliberate or accidental) may also act to dilute the effects of hybridization to a point where it is no longer considered important. In this regard (Hedrick 2009) proposed a course of action where cattle/bison hybrids are deliberately crossed with pure bison, to swamp the effects of hybridization. Such a course of action is however only feasible if sufficient pure animals exist.

In this study we aimed to (1) genetically characterise pure blue- and black wildebeest populations; (2) identify admixed individuals and suggest thresholds for purity; and (3) assess the impact of introgression over time using simulations based on real data.

Materials and methods

Sampling and molecular analysis

A total of 461 samples from black wildebeest, blue wildebeest and putative hybrids were collected over a period of 10 years (2006–2016) from various provinces in South Africa (Table 1). Black wildebeest populations from provincial Nature Reserves where the management history suggested low risk of contact with blue wildebeest were included in the study as representative of typical conserved herds of the species. Pure blue wildebeest samples were collected from the Kruger National Park where there is no known risk of hybridization. The blue wildebeest samples also included a large number of animals from a selection of commercial game ranches in South Africa. Putative hybrid samples were collected from localities that indicated phenotypic signs of hybrid animals and/or where the management history suggested risk of hybridization. Two known F1 hybrids were also included in the study. Collection and possession of black wildebeest samples were sanctioned under TOPS permit 036006 (University of the Free State)

Table 1 Provinces, sample sizes and classification of black wildebeest (*Connochaetes gnou*) and blue wildebeest (*Connochaetes taurinus*)

Province	Sample size	Classification
Reference black wildebeest population A, Free State	20	Pure black wildebeest
Reference black wildebeest population B, Western Cape	17	Pure black wildebeest
Reference black wildebeest population C, North West	8	Pure black wildebeest
Typical black wildebeest reserve population A, North West	9	Black wildebeest
Typical black wildebeest reserve population B, Free State	15	Black wildebeest
Typical black wildebeest reserve population C, Free State	25	Black wildebeest
Typical black wildebeest reserve population D, Free State	6	Black wildebeest
Typical black wildebeest reserve population E, Free State	20	Black wildebeest
Typical black wildebeest reserve population F, Gauteng	6	Black wildebeest
Typical black wildebeest reserve population G, Free State	11	Black wildebeest
Typical black wildebeest reserve population H, Eastern Cape	20	Black wildebeest
Reference blue wildebeest population, Mpumalanga	18	Pure blue wildebeest
Commercial blue wildebeest ranch population A, Limpopo	7	Blue wildebeest
Commercial blue wildebeest ranch population B, Limpopo	18	Blue wildebeest
Commercial blue wildebeest ranch population C, Limpopo	7	Blue wildebeest
Commercial blue wildebeest ranch population D, North West	14	Blue wildebeest
Commercial blue wildebeest ranch population E, Northern Cape	3	Blue wildebeest
Commercial blue wildebeest ranch population F, Unknown	2	Blue wildebeest
Commercial blue wildebeest ranch population G, Unknown	5	Blue wildebeest
Commercial blue wildebeest ranch population H, Unknown	41	Blue wildebeest
Commercial blue wildebeest ranch population I, Unknown	7	Blue wildebeest
Known hybrids, Free State	2	Known hybrids
Putative hybrid population A, Mpumalanga	19	Putative hybrids
Putative hybrid population B, Free State	20	Putative hybrids
Putative hybrid population C, Free State	80	Putative hybrids
Putative hybrid population D, KZN	61	Putative hybrids

and a standing permit 03309 (National Zoological Gardens of South Africa). Ethical clearance from the respective Institutional Research Ethics Committees was also obtained; UFS-AED2015/0067 (University of the Free State) and P7/12 (National Zoological Gardens of South Africa).

DNA was extracted from samples (blood, tissue or hair) using the Roche High Pure Template Preparation kit (Roche Diagnostics, GmbH) and the Qiagen DNeasy Blood and Tissue kit (GmbH, Germany) following the manufacturer's protocols. Genotyping was performed using three sets of microsatellite markers. First we used loci from the set developed by Røed et al. (2011) for wildebeest from East Africa (CT-02, CT-03, CT-10, CT-17, CT-18, CT-19, CT-25, CT-27, CT-30). We also used loci from the StockMarks® cattle genotyping kit (Thermo Fisher Scientific, Inc.) that were previously shown to amplify in wildebeest species (ETH10, TGLA53, TGLA122, BM1824, BM2113, OARCP26), Finally, we used species-specific markers described in van Wyk et al. (unpublished) (BLAWB6, BLAWB7, BLAWB9, BLAWB10, BLAWB13, BLAWB16, BLUW5, BLUW6, BLUW7, BLUW8, BLUW10, BLUW11, BLUW15). PCR reactions were prepared with a total volume of 12.5 µl, using KAPA2G Robust HotStart ReadyMix, and with primer concentrations of 0.5 µM forward and reverse primer and 50 ng genomic DNA template. The conditions for PCR amplification were as follows: 3 min (min) denaturation at 95 °C, 35 cycles each of 15 s (sec) at 95 °C, 15 s at 55–66 °C and 15 s at 72 °C, followed by extension at 72 °C for 10 min. PCR products were pooled together and run against GenescanTM 500 LIZ™ internal size standard on an ABI 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA). Samples were genotyped using GeneMapper v. 4.0 software (Applied Biosystems, Inc., Foster City, CA, USA).

Genetic diversity and differentiation

Genetic diversity was assessed in each species for individual populations including the putative hybrid populations. MICRO-CHECKER (van Oosterhout et al. 2004) was used to detect possible genotyping errors, allele dropout and non-amplified alleles (null alleles). The mean number of alleles per locus (A), allelic richness (AR), observed heterozygosity (H_o) and unbiased heterozygosity (H_z = expected heterozygosity adjusted for unequal sample size; Nei 1987) was calculated with GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Arlequin 3.5 (Excoffier et al. 2005; Excoffier and Lischer 2010) were used to test for deviations from Hardy–Weinberg equilibrium (HWE), with a Markov Chain length of 10^5 and 100,000 dememorization steps. Linkage disequilibrium between pairs of loci in all the populations, including the putative hybrid populations were tested for using Arlequin 3.5 (Excoffier et al. 2005; Excoffier and Lischer 2010) with 100 initial conditions followed by ten permutations, based on the exact test described by Guo and Thompson 1992. Sequential Bonferroni correction was used to adjust for multiple tests at a significance level of 0.05 (Rice 1989). F_{st}-based hierarchical analysis of molecular variance (AMOVA) was used to determine genetic differentiation among and within the black wildebeest and blue wildebeest populations (Arlequin 3.5; Excoffier et al. 2005; Excoffier and Lischer 2010).

Population structure and admixture analysis

Identification of genetic clusters and individual assignment of pure animals as well as putative hybrid individuals were performed using the Bayesian clustering approach implemented in STRUCTURE software version 2.3.4 (Pritchard et al. 2000; Falush et al. 2007; Hubisz et al. 2009). The evaluations were conducted with a model that assumes admixture, correlated allele frequencies and without prior population information. STRUCTURE was run for five replicates each with K = 1–10, with a run-length of 500,000 Markov chain Monte Carlo repetitions, following a burn-in period of 20,000 iterations. The five values for the estimated ln(Pr (X|K)) were averaged, from which the posterior probabilities were calculated. The K with the greatest increase in posterior probability (ΔK, Evanno et al. 2005) was identified as the optimum number of sub-populations using STRUCTURE HARVESTER (Earl and vonHoldt 2012). The membership coefficient matrices (Q—matrices) of replicate runs for the optimum number of sub-populations was combined using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) with the FullSearch algorithm and G′ pairwise matrix similarity statistics. The results were visualized using DISTRUCT version 1.1 (Rosenberg 2004). From the selected K value, we assessed the average proportion of membership (QI) of the sampled populations to the inferred clusters. Individuals (parental or admixed classes) were assigned to the inferred clusters using an initial threshold of $qi > 0.9$ (Barilani et al. 2007).

Assessing the persistence of introgression over time

HYBRIDLAB software (Nielsen et al. 2006) was used to investigate the effect of backcrossing and find limits to the detection of hybrids over time. We modelled a presumably typical scenario where hybridization occurred, blue wildebeest are removed from the locality (perhaps, but not necessarily, along with some F1 hybrids), and where remaining undetected F1 hybrids are left to backcross with pure black wildebeest. It will become increasingly more difficult to detect hybrid individuals with each backcross involving a hybrid animal with a pure black wildebeest and we aimed to determine the likelihood that hybrids will still be detected at a given generation following successive backcrosses.

Genotypes of pure black wildebeest and blue wildebeest (n = 100 for each species) with qi > 0.95 (from STRUCTURE-based analysis) were used as ancestral pure populations for the simulations. HYBRIDLAB software (Nielsen et al. 2006) was used to create 100 F1 hybrids. The 100 F1s were then "crossed" with pure black wildebeest to create backcross generations one (BC1). This cycle was then repeated a number of times, with each backcross generation created by mating with the original pure black wildebeest allele pool with the latest hybrid generation, to provide the next generation of backcross individuals. The range of admixture in the animals from each generation of back-crossing was then determined using STRUCTURE 2.3.4. The STRUCTURE software was run using the admixture model with correlated allele frequency for five replicates with K = 2, with a run-length of 100,000 repetitions of Markov chain Monte Carlo, following the burn-in period of 20,000 iterations.

Results

Genetic diversity and differentiation

A summary of the genetic diversity for reference black- and blue wildebeest populations, typical black wildebeest reserve populations, commercial blue wildebeest ranch populations, known hybrids and putative hybrid populations are presented in Table 2. From a total of 247 alleles detected, 90 alleles were shared between the two species, with 133 private alleles present in blue wildebeest and 24 in black wildebeest. Overall, the genetic diversity for the black wildebeest populations was lower compared to the blue wildebeest populations (Table 2). The mean number of alleles per locus (A) was 2.41, 4.74, 2.75 and 4.21 while the average allelic richness (AR) was 1.79, 2.6, 2.01 and 2.91 for reference black wildebeest, reference blue wildebeest, typical black wildebeest reserve populations and commercial blue wildebeest ranch populations respectively (Table 2). The observed heterozygosity (H_o) ranged from 0.35 to 0.47 in the 11 black wildebeest populations and from 0.42 to 0.63 in 10 the blue wildebeest populations while the unbiased heterozygosity (H_z) varied from 0.36 to 0.52 and 0.52 to 0.65 in all black- and blue wildebeest populations respectively (Table 2). H_o values were generally lower compared to H_z values, which may be an indication of the Walhund effect (considering that opportunistic sampling was used), or real loss of heterozygosity in smaller populations. The overall genetic diversity for the known hybrids and putative hybrid populations were mostly intermediate compared to the blue- and black wildebeest populations, however, the putative hybrid population D showed lower levels of diversity compared to the other putative hybrid populations (Table 2).

Significant deviations from expected proportions of genotypes under Hardy–Weinberg equilibrium was observed at five loci (BLUW6, BLUW8, BLUW10, BM184 and TGLA122) in five of the black wildebeest populations and five loci (TGLA53, CT-27, BLAWB13, BLUW6, BM1824) in five of the blue wildebeest populations, following Bonferroni correction. These markers indicated significant heterozygote deficit in the respective population with H_o values lower than H_e values (data not shown) which may be an indication of the presence of possible null alleles. Results from MICRO-CHECKER did indicate that the loci might contain possible null alleles in the respective populations. No general pattern was observed, the same markers did not deviate significantly from Hardy–Weinberg equilibrium in

Table 2 Summary of samples used, sample sizes and genetic diversity of black wildebeest (*Connochaetes gnou)*, blue wildebeest (*Connochaetes taurinus*) and putative hybrid populations: A = mean number of alleles; AR = Allelic Richness; H_o = observed heterozygosity; H_z = unbiased heterozygosity

Samples	Sample size	Mean number of alleles per locus (A)	Allelic richness (AR)	Unbiased heterozygosity (H_z)	Observed heterozygosity (H_o)
Reference black wildebeest[a]	45	2.41 (2.26–2.63)	1.79 (1.72–1.88)	0.38 (0.36–0.40)	0.38 (0.35–0.41)
Reference blue wildebeest	18	4.75	2.6	0.63	0.60
Typical black wildebeest reserve populations[b]	112	2.75 (2.35–3.46)	2.01 (1.61–2.44)	0.45 (0.39–0.52)	0.43 (0.37–0.47)
Commercial blue wildebeest ranch populations[c]	104	4.21 (2.13–6.92)	2.91 (1.83–3.84)	0.61 (0.52–0.65)	0.56 (0.42–0.63)
Known hybrids	2	2.52	2.08	0.67	0.69
Putative hybrid population A	19	4.08	2.95	0.62	0.40
Putative hybrid population B	20	4.63	3.17	0.66	0.46
Putative hybrid population C	80	4.46	2.48	0.55	0.52
Putative hybrid population D	61	3.50	2.24	0.46	0.42

[a]Reference black wildebeest consists of three populations listed in Table 1

[b]Consists of eight typical black wildebeest reserve populations listed in Table 1

[c]Comprises of nine commercial blue wildebeest ranch populations listed in Table 1

the same populations and all loci were included in further analysis.

To determine the hierarchical distribution of overall genetic diversity, we used a locus-by-locus Analysis of Molecular Variance (AMOVA), as implemented in ARLEQUIN 3.5 (Excoffier et al. 2005; Excoffier and Lischer 2010). The AMOVA analysis was performed in two different ways, with different parameters. Firstly, in order to determine genetic differentiation between blue- and black wildebeest, the reference black wildebeest (n = 45; three populations), typical black wildebeest reserve populations (n = 112; eight populations), reference blue wildebeest (n = 18; one population) and commercial blue wildebeest ranch populations (n = 104; nine populations) was used. An average Fst = 0.441 (P = 0.000) revealed a significant genetic differentiation between the two species indicating that they are genetically distinct as expected for separate species (Table 3). Secondly, the variation within both species was determined. AMOVA indicated non-significant differentiation between the 11 and 10 black- and blue wildebeest localities respectively (Table 3). The genetic differentiation within the black wildebeest population (84.11%) was slightly lower compared to the blue wildebeest populations (90.33%) (Table 3).

Bayesian assessment of admixture and hybridization

STRUCTURE analysis with the admixture model and K = 1–10 identified K = 2 as the most likely true K value with the largest increase in ΔK (Fig. 2), in line with the taxonomic status of these animals as separate species. Black- and blue wildebeest were assigned to two distinct clusters with individual coefficient of membership (QI) for pure black wildebeest QI > 0.994 and for pure blue wildebeest QI > 0.955. The two known F1 hybrids indicated mixed ancestry with $qi_{black} = 0.492$ and $qi_{black} = 0.475$ (Fig. 3). From the putative hybrid populations only five hybrid individuals were identified using the $qi \leq 0.90$ threshold and 156 individuals were identified as black wildebeest and 19 as blue wildebeest (Figs. 3, 4). Two black wildebeest individuals from provincial Nature Reserves were classified as hybrids with $qi_{black} = 0.777$ and $qi_{black} = 0.811$ (Fig. 3). High levels of

Table 3 Analysis of molecular variance (AMOVA) for black- and blue wildebeest

Source of variation	Variance	Percentage variation	Fst	P value
Among black- and blue wildebeest population	1.80715	35.45	0.44129	0.0000
Among con-specific populations	0.44237	8.68		
Within black- and blue wildebeest populations	2.84811	55.87		
Among black wildebeest populations	0.39673	15.89	0.15889	0.0000
Within black wildebeest populations	2.10007	84.11		
Among blue wildebeest populations	0.41929	9.67	0.09670	0.0000
Within blue wildebeest populations	3.91665	90.33		

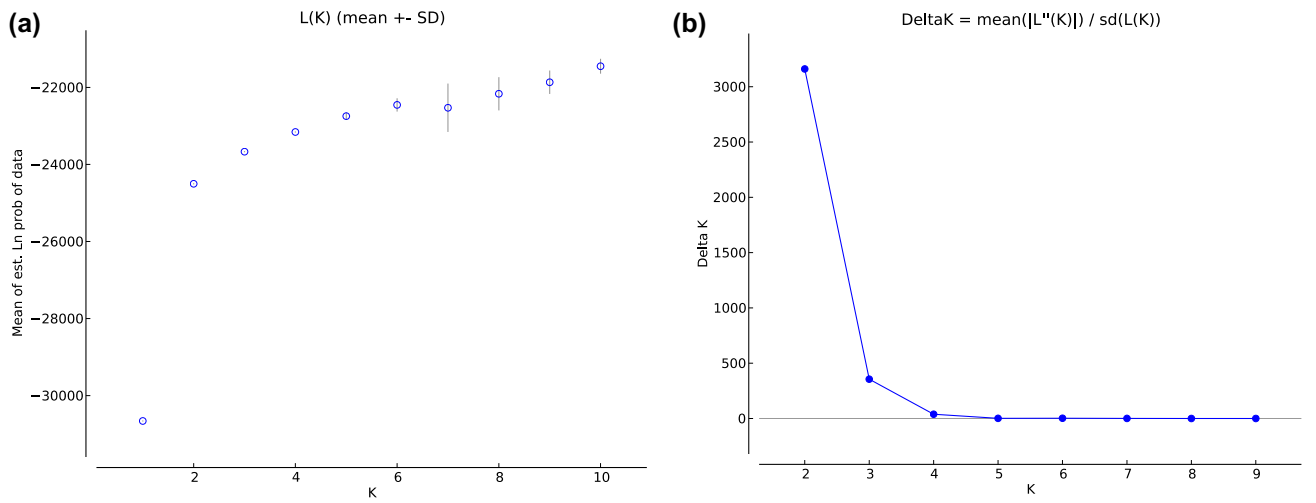

Fig. 2 **a** Probability (− LnPr) of K = 1–10 averaged over 5 runs. **b** Delta K values for real population structures of K = 1–10

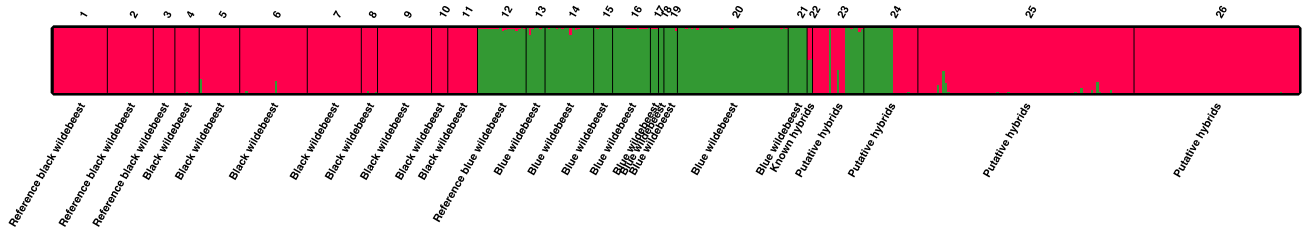

Fig. 3 STRUCTURE analysis (performed with K = 2) of microsatellite genotypes of reference black wildebeest, reference blue wildebeest, black wildebeest and blue wildebeest from reserves and game farms, known hybrids, and putative hybrid populations. Each individual is represented by a single vertical line, with lengths proportional to the estimated individual coefficient of membership in each cluster

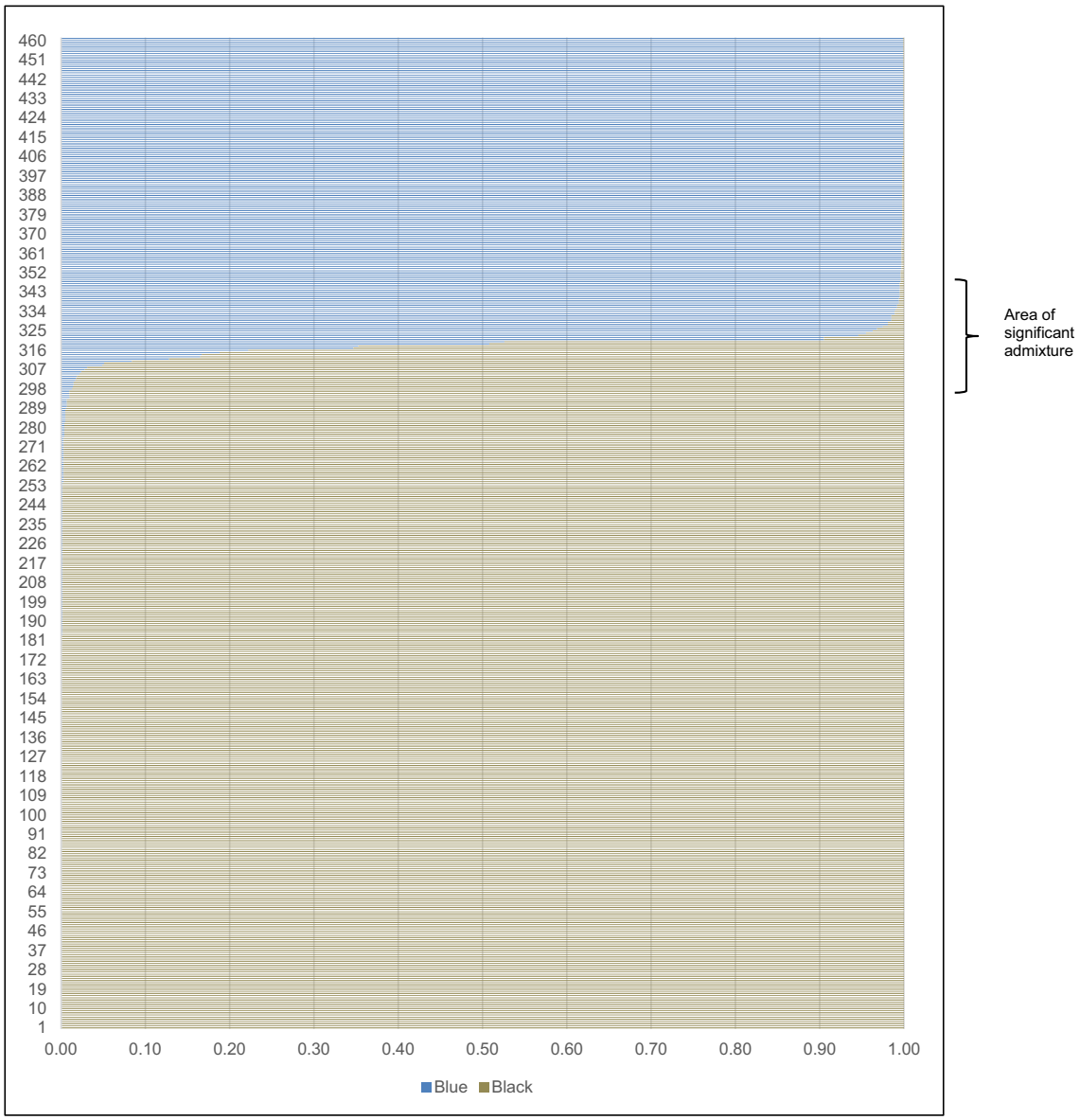

Fig. 4 A overall view of the genetic make-up of all 461 individual animals analysed. "Blue-like" and "Black-like" are represented by their corresponding colours. The proportion of animals that show admixture is surprisingly small. (Color figure online)

linkage disequilibrium were observed in the putative hybrid populations.

Assessing the persistence of introgression over time

In the absence of selection, each backcross generation should carry a proportion 1/4, 1/8, 1/16 etc. of introgressed genes (Goodman et al. 1999). In practise, a range of levels of admixture will be found in animals crossed under the same circumstances, since individuals will start with different components of shared and species-specific alleles that will either delay or advance the rate of admixture. The average levels of admixture in successive generations of hybrids closely follow theoretical expectations (Fig. 5) and based on these averages, a hybrid will on average not be detectable after BC2 or BC3, depending on the threshold used ($qi \leq 0.90$; $qi \leq 0.95$). However, there is a range of possible outcomes for each generation, with hybrids displaying more "blue-like" or "black-like" characteristics. Where random combinations of alleles result in a "black-like" profile, nominal hybrids will be classified as pure black as early as generation BC2 ($qi \leq 0.95$) or even BC1 ($qi \leq 0.90$).

Discussion

In this study, lower levels of genetic variation were observed in black wildebeest (H_o: 0.35–0.47) in comparison to blue wildebeest (H_o: 0.42–0.63) populations. This observation is in accordance with findings from previous studies. Corbet et al. (1994) reported low gene variation in black wildebeest (H: 0.018 ± 0.013) in comparison to blue wildebeest (H: 0.081 ± 0.030) based on 31 protein encoding loci. Grobler and Van der Bank (1995) additionally used allozymes and found lower average heterozygosity in the black wildebeest (3.25%) in contrast to blue wildebeest (4.71%). Lastly, a study using five cross-species microsatellite markers confirmed lower heterozygosity in black wildebeest (H_o: 0.397) in comparison to blue wildebeest (H_o: 0.646) (Grobler et al. 2005). Loss of genetic diversity in black wildebeest has been attributed to population bottlenecks in the late 1800s and in the early 1900s that resulted in a reduction in population size to approximately 300 animals (Kirkman 1938; Von Richter 1971). The bottleneck was reported to have impacted several generations, with surviving individuals divided into several smaller populations. Higher diversity in blue wildebeest could be attributed to the historical occurrence of very large migratory herds that consisted of tens of thousands of individuals (Corbet et al. 1994).

We also noted a significant number of alleles found in typical black wildebeest reserve populations and blue wildebeest populations that are sometimes excluded from the reference black wildebeest populations. Superficially, this may suggest large scale hybridization on provincial reserves but is almost certainly not the case, based on the results obtained from STRUCTURE, where there is no distinction between reference black wildebeest and typical black wildebeest reserve populations. This emphasizes the importance

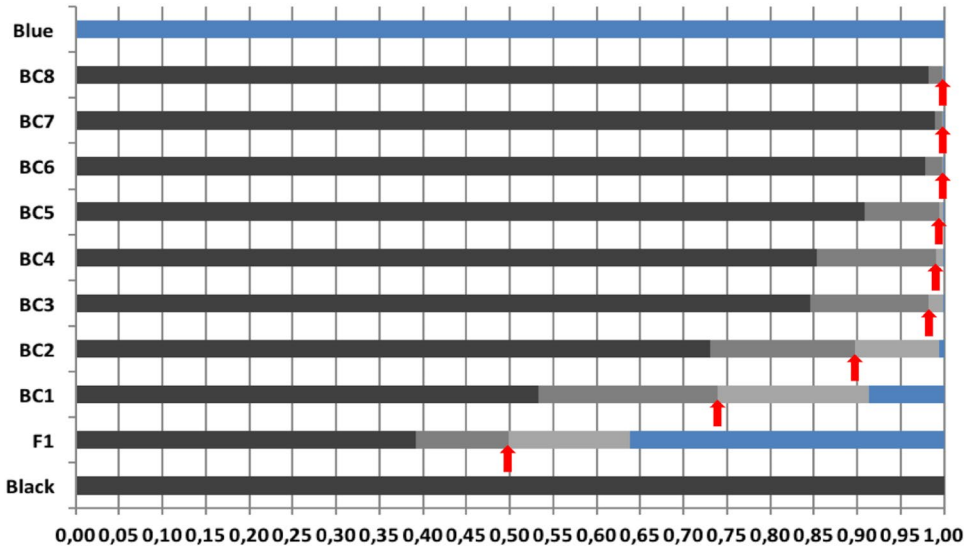

Fig. 5 Simulated levels of admixture of blue-like and black-like alleles in successive generations of hybridization and backcrossing in wildebeest. Grey areas of bars represent the spectrum of levels of admixture observed in 100 wildebeest for each generation. Red arrows indicate the average level of admixture in each generation. Our results [with expected values in brackets], were as follows: F1 = 49.9% [50], BC1 = 74.0% [75], BC2 = 89.8% [87.5], BC3 = 98.2% [93.8], BC4 = 99.1% [96.9], BC5 = 99.4% [98.4], BC6 = 99.8% [99.2], BC7 = 99.8% [99.6] and BC8 = 99.8% [99.8]. (Color figure online)

of large effective populations and the significance of translocation and meta-population management (Karsten et al. 2011). These alleles most likely confirm that the provincial reserves house an important part of the overall pool of diversity in *C. gnou*. These populations require special consideration and conservation efforts, in order to preserve unique alleles in *C. gnou*.

Our genetic survey using a large panel of diagnostic markers and more than 400 samples from various locations in South Africa, including both game farms and nature reserves allowed us to determine the extent of hybridization among blue and black wildebeest. Historically, blue and black wildebeest are reported to occur in overlapping geographic ranges (Du Plessis 1969). Past natural hybridization between the two species has not been previously reported which could be attributed to different habitat preferences and variations in social behaviour (Von Richter 1971; Hirst 1975). In unnatural circumstances, inadequate favourable habitat and disruption of social organization could result in hybridization. In recent times, hybridization between blue and black wildebeest has occurred mainly due to management (Grobler et al. 2005) whereby several provincial and private game farms in South Africa accommodated both species. Due to the translocation history of both species, high levels of introgression were expected in the study presented here. However, hybridization was observed to be negligible with only five out of 180 (2.78%) animals from putative hybrid populations being identified as hybrid, using a threshold of qi ≤ 0.90. Inadequate marker panels or control samples, small sample sizes and ancestral polymorphism between the species could result in misclassification of hybrid individuals as pure (Smith et al. 2013). Previous efforts to identify diagnostic markers to detect hybridization in wildebeest had limited success and were unable to quantify the extent of hybridization between the two species. The analysis based on allozymes reported frequency differences and some species-specific alleles at five polymorphic loci at low frequencies, but no fixed diagnostic species-specific alleles were found (Grobler and Van der Bank 1995). Cross-species markers originating from bovid identified species-specific alleles for numerous markers (Grobler et al. 2005); however analysis on a larger set of samples revealed that several of these were in fact shared by the two species (Grobler, unpublished results). Thus, our extensive genetic dataset allowed for the first time a detailed analysis of the hybridization pattern between blue- and black wildebeest. The resolution of the marker panel developed and the control samples used here is considered adequate as both known F1 hybrids ($qi = 0.48–0.49$) fulfilled the criteria of F1 hybrids with qi values of approximately 0.5 and a genotype heterozygous for blue- and black wildebeest at all loci. The number of markers used here is in line with several hybridization studies, for example Randi et al. (2014) conducted a study on hybridization between feral dogs (*Canus lupus familiaris*), Ethiopian wolf (*C. simensis*) and grey wolf (*C. lupus*) and reported that 24 divergent microsatellites were equally or more informative than a panel of 39 loci. However, the possibility exists that introgression between blue- and black wildebeest has extended beyond the detection of the current microsatellite marker panel. Boecklen and Howard (1997) indicated the number of markers used to detect backcrossed individuals increases with each generation that passes following the initial hybridization event. Although larger marker sets or whole genome approaches have been suggested in several hybridization studies, financial constraints currently limit their application in conservation genetics and as a means of genetic monitoring (Randi et al. 2014). Thus informative mitochondrial and Y-linked markers should be investigated in order to establish the potential existence of advanced backcrosses.

The observed low percentage of hybrid individuals observed in this study may be due to previous over-estimation of hybridization in wildebeest. Formerly, the identification of hybrid individuals has been based on direct observation of morphological characteristics due to differences between species with regards to horn conformation, body size and coat characteristics (Grobler et al. 2005; Ackermann et al. 2010). However, morphological identification of hybrids can become complex due to ancestral polymorphism or mutations that may give the appearance of hybrids (Mallet 2005). Thus, following the advancement of molecular techniques, genetic analysis has been used as a more rigorous test in order to detect hybridization or introgression. Lastly, the observation of lower than expected numbers of extant hybrids may be related to fitness, survival and infertility. It is unlikely that hybrid offspring are infertile as fecundity in hybrid individuals has been confirmed osteologically (Fabricius et al. 1988). However, hybridization may either lead to increased or reduced performance or fitness in the hybrid offspring compared to the parental taxa (hybrid vigor or heterosis versus outbreeding depression) (Lynch and Walsh 1998; Allendorf et al. 2013). The hybrid wildebeest offspring may have reduced fitness and performance compared to either parental taxa and this may be as a result of intrinsic outbreeding depression (chromosomal or genic) or extrinsic outbreeding depression (Allendorf et al. 2013). In the case of chromosomal intrinsic outbreeding depression, the differences in structure can result in aneuploidy gametes that reduces survival of progeny (Allendorf et al. 2013). Genic intrinsic outbreeding depression may cause reduced fitness because of genetic interactions between genes from the different taxa (Whitlock et al. 1995). Rawson and Burton (2002) presented examples of the functional interaction of a co-adapted gene complex that may lead to functional incompatibilities and may cause reduced fitness in hybrid offspring. Extrinsic outbreeding depression may lead

to reduced fitness in hybrid offspring due to loss of adaption by environmentally mediated selection. This includes increased disease susceptibility in the hybrid offspring (Sage et al. 1986; Currens et al. 1997; Goldberg et al. 2005) as well as ineffectiveness to evade predators which was observed in whitetail- and mule deer hybrid offspring (Lingle 1992).

The current dataset provides a reliable foundation for determining the status of hybridization in wildebeest. We recommend future research to include studies on the fertility and fitness of hybrid offspring as well as supplementing the existing microsatellites with SNPs, mitochondrial and Y-linked markers, which may improve the probability of detecting advanced backcrosses.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Black wildebeest samples were sanctioned under TOPS permit 036006 (University of the Free State) and a standing permit 03309 (National Zoological Gardens of South Africa). Samples from the Free State Provinces were collected under permit no. 01/30307 issued by DESTEA. Ethical clearance from the respective Institutional Research Ethics Committees was also obtained; UFS-AED2015/0067 (University of the Free State) and P7/12 (National Zoological Gardens of South Africa).

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