

ORIGINAL ARTICLE

Phylogeny and vicariant speciation of the Grey Rhebok, *Pelea capreolus*

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A South African endemic antelope, the Grey Rhebok (*Pelea capreolus*), has long been an evolutionary enigma in bovid systematics—its phylogenetic intractability attributed to its curious combination of derived and primitive morphological attributes and the consequences of a rapid radiation. By using a combination of DNA sequences, chromosomal characteristics and quantitative and qualitative morphological features we show that the species is a sister taxon to a clade that comprises the waterbuck, reedbuck and allies. Our finding of few unambiguous synapomorphies reinforces suggestions of a rapid radiation and highlights the effects of incomplete lineage sorting, including the hemiplasic nature of several chromosomal rearrangements. We investigate these data to address the general question of what may have led to *Pelea* being both genetically and ecologically distinct from the Reduncini. We argue that its adaptation to exposed habitats, free of standing water, arose by vicariance prompted by increasing aridity of the extreme south/southwestern region of the African continent in the Miocene. Ancestral lineages leading to the extant *Redunca* and *Kobus*, on the other hand, retreated to water-abundant refugia in the north during these mostly globally cool phases. The mosaic of water-rich environments provided by the Okavango and the drainage systems in the southwestern extension of the East African Rift system are considered to have facilitated speciation and chromosomal evolution within these antelope.

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INTRODUCTION

The Grey Rhebok (*Pelea capreolus*) is an endemic Southern African antelope noteworthy for its mix of derived and primitive morphological characters that have confounded its taxonomic affiliations and whose phylogenetic position has remained problematic even under intensive scrutiny. For example, although the monotypic *Pelea* is frequently placed with the reduncines (subfamily Reduncinae), especially in more recent DNA sequence investigations (Decker *et al.*, 2009; Bibi, 2013), it has also been assigned to the Antilopini (Oboussier, 1970), the Neotragini (Georgiadis *et al.*, 1990; Gentry, 1992), the Caprinae (Gentry, 1970), its own tribe, Peleini (Vrba, 1976; Vrba *et al.*, 1994) or tribe indeterminate (Gentry, 1992; Gatesy *et al.*, 1997). This taxonomic inconsistency reflects a reliance on morphology and the confounding effects of homoplasy on many of the characters (Gentry, 1992) used in morphologically-based investigations. It is further compounded by the inconsistent use of the tribe and subfamily as taxonomic categories for reduncine antelope. We follow Wilson and Reeder (2005) and use Reduncinae to accommodate the inclusion of *Pelea*. The tribe Reduncini is used to delimit *Redunca* + *Kobus* (i.e., excluding *Pelea*) and Peleini includes *Pelea*.

Morphology aside, even sequence-based investigations are at odds concerning the Grey Rhebok's phylogenetic affinities. Although a sister relationship to other reduncines is most usually indicated (Gatesy *et al.*, 1997; Hassanin and Douzery, 1999; Matthee and Robinson, 1999; Matthee and Davis, 2001; Decker

et al., 2009; Bibi, 2013), a close affiliation to either *Kobus* (the waterbuck and its close allies) or *Redunca* (the reedbuck and allies) is frequently detected. Examples of this phylogenetic ambiguity can be seen in the analysis of rDNA data where Gatesy *et al.* (1997) find *Pelea* most closely related to the Reduncini in 7/14 analyses. Moreover, based on the likelihood scores from the Kishino-Hasegawa tests their best tree was not statistically different from those in which *Pelea* was basal to either *Kobus* or to *Redunca*. This instability was mirrored in subsequent studies. These showed (i) *Pelea* in a sister relationship with *Redunca* in a strict consensus tree from a molecular supermatrix analysis—admittedly with very low bootstrap support (Marcot, 2007), (ii) the inability of cytochrome b sequences to resolve the relationship of *Pelea* to 11 Reduncini taxa (Birungi and Arctander, 2001) and (iii) that even a detailed analysis of complete mitochondrial genomes was unable to unequivocally assign this taxon (Hassanin *et al.*, 2012). In fact, the only exception to its apparent phylogenetic intractability resulted from high-throughput assays of genome-wide single nucleotide polymorphism genotyping (Decker *et al.*, 2009). A strict consensus cladogram of 40 843 single nucleotide polymorphism loci placed *Pelea* basal to the Reduncini and importantly, with 100% bootstrap support.

Chromosomal data (based on G-banding and/or Q-banding) are available for four of five *Kobus* species (*K. ellipsiprymnus* 2n = 50, *K. kob* 2n = 50, *K. leche* 2n = 48, *K. megaceros* 2n = 52 Kingswood *et al.*, 2000) and one of three *Redunca* species (*R. fulvorufa* (RFU)

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$2n = 56$, Rubes *et al.*, 2007). Since these data form the basis for our comparison with *Pelea*, a point of taxonomic clarification is required. We follow Wilson and Reeder (2005) in recognizing eight species in Reduncini (*K. ellipsiprymnus*, *K. kob*, *K. leche*, *K. megaceros*, *K. vadoni*, *R. fulvorufula*, *R. redunca* and *R. arundinum*) and not the more recent revision by Groves and Grubb (2011). Our position reflects the current debate that the latter treatment (which was subsequently incorporated by Wilson and Mittermeier, 2011) is biased by 'taxonomic inflation' as it nearly doubles the number of bovid species from 143 to 279. It has been argued that this new taxonomy has elevated ecotypes and subspecies to the level of full species based solely on a change in the species concept used (Heller *et al.*, 2013). Irrespective of the divergent taxonomic views, however, the published cytogenetic data for the six species listed above clearly show (i) that chromosomal evolution in the reduncines is driven predominantly by Robertsonian fusions (the joining of two non-homologous acrocentric chromosomes with a concomitant reduction in diploid number or $2n$), (ii) that this class of rearrangement is near selectively neutral (in Bovidae) and can occur convergently in different lineages and finally (iii) that the reduncines' rapid radiation permitted the persistence of chromosomal rearrangements across species nodes (i.e., hemiplasy—Avise and Robinson, 2008; Robinson *et al.*, 2008), thus displaying potential to obscure the actual history of species divergences (see Robinson and Ropiquet, 2011).

With this as background we set out to determine whether a supermatrix approach would provide additional insights to the evolutionary relationships of the enigmatic *Pelea*. We report on the molecular cytogenetic analysis of its karyotype using Fluorescence *in situ* hybridization (FISH) of microdissected whole chromosome painting probes derived from cattle and cloned Y sequences from *R. fulvorufula*. These data in conjunction with morphology (Vrba *et al.*, 1994) and sequences from four nuclear and two mtDNA genes/fragments representative of all eight Reduncini species and *P. capreolus* provide compelling evidence that the monotypic *P. capreolus* is sister taxon to the Reduncini. Our findings reinforce suggestions of a rapid radiation of the tribe, highlighting the effects of incomplete lineage sorting and the hemiplastic nature of several rearrangements, and permit a novel hypothesis for the origin of *Pelea* and the subsequent radiation of Reduncini.

MATERIAL AND METHODS

(i) Cell culture, genomic DNA extraction and chromosome banding
Chromosomal preparations of *P. capreolus* were derived from cryopreserved fibroblasts that were established in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 15% fetal bovine serum. In the case of *R. fulvorufula*, *K. leche*, *K. megaceros* and *K. ellipsiprymnus*, short-term cultures (Rubes *et al.*, 2007; Pagacova *et al.*, 2011) were obtained from peripheral blood of captive animals held at the Zoo Dvur Kralove, Dvur Kralove, Czech Republic. Biological material was obtained in accordance with conditions required by CapeNature (South Africa) and the Dvur Kralove Zoo (Czech Republic), respectively. The differential staining of chromosomes (G-banding and C-banding) followed conventional procedures with the G-banded *Pelea* chromosomes arranged to conform to those of the cattle standard (ISCNDB2000) (2001). Total genomic DNA was extracted from peripheral blood or cultured fibroblasts using the Wizard SV Genomic Purification System (Promega, Madison, WI, USA).

(ii) Microdissection and construction of sex chromosome-specific clones and whole chromosome painting probes

We used a subset of whole chromosome painting probes obtained from cattle (*Bos taurus*: BTA 1–29) for cross-species painting among the Reduncinae. The paints were produced by microdissection using a PALM Microlaser system

(P.A.L.M. GmbH, Bernried, Germany) and degenerate oligonucleotide-primed PCR (DOP-PCR) (Kubickova *et al.*, 2002). The probes were labeled with Orange-dUTP or Green-dUTP (Abbott, Illinois, USA) by secondary PCR. Microdissection was similarly used for the isolation of RFU Y chromosomal DNA. The Y-specific DNA was amplified by DOP-PCR and amplicons ligated into a pDrive vector (Qiagen, Hilden, Germany). A total of 20 clones were screened by DOT BLOT hybridization (Pauciullo *et al.*, 2006) of which seven were selected on intensity, fluorescently labeled with Orange-dUTP by Nick translation and subsequently tested for cross-species FISH. FISH-positive clones were sequenced using BigDye terminator chemistry on an automated sequencer.

(iii) Phylogenetic analyses

Our phylogenetic analysis included all eight species of the Reduncini as well as the problematic *P. capreolus*, the focus of our investigation. Sequence data were generated for three gene regions (mainly introns) using the primers and methods outlined in Mathee *et al.* (2001). These were Protein-Kinase C1, B-Spectrin nonerythrocytic 1 and Stem cell factor. To increase the data available for phylogenetic analyses, sequences from two mtDNA (Cyt-B; COI) and one additional nuclear marker (MC1R) were sourced from Genbank and included in the analyses (Supplementary Table 1). Sequences were aligned manually and trimmed to avoid missing data. Sequences from *Hippotragus equinus*, *Gazella/Nanger/Eudorcas* spp and *Oerotragus oerotragus* served as outgroups for the gene tree analyses.

Parsimony analyses of the DNA sequences were conducted using PAUP version *4.0b10 (Swofford, 2002). The Branch and Bound option was selected and nodal support determined by 1000 bootstrap iterations. All gaps and heterozygous sites were treated as missing data; heterozygous sites were coded using NC-IUB nomenclature (<0.5% of sites represented clear heterozygous positions and all of these changes were confined to transitional changes - Y or R).

Data were analysed separately for each DNA fragment (to observe potential incongruence among gene trees) and then combined in a single analysis (Wiens, 1998). Bayesian phylogenetic analyses used MrBayes v3.2 (Ronquist *et al.*, 2012). The AICc criteria (Burnham and Anderson, 2004) were applied in the selection of the best-fit models of sequence evolution using jModelTest v0.1.1 (Posada, 2008). Data analysis was performed in a partitioned fashion and parameters were 'unlinked' across partitions. Two parallel Markov Chain Monte Carlo simulations (4 chains each) were undertaken for 5 million generations with a sampling frequency of 100. Parameter convergence and ESS values were established in Tracer v1.5 (Rambaut and Drummond, 2007) and 20% of the sample was excluded as burn-in.

We next conducted a parsimony analysis of a supermatrix using the same methodology described for the gene tree analyses. The supermatrix initially included all six DNA fragments in combination with the *P. capreolus* chromosomal characters presented above, as well as those reported for *R. fulvorufula*, *K. kob*, *K. ellipsiprymnus*, *K. leche*, and *K. megaceros* (Kingswood *et al.*, 2000; Rubes *et al.*, 2007; Pagacova *et al.*, 2011—see Table 1) and confirmed by cross-species FISH in the present study. Species for which chromosomal information was not available were coded as missing data. The cattle (*Bos taurus*) karyotype was used to polarize the chromosomal data. We subsequently expanded the supermatrix to include the two classes of morphological characters presented by Vrba *et al.* (1994). The first entailed mostly quantitative osteological characters from horns and skulls that were allometrically scaled by the authors to remove the effects of body weight (the species vary considerably in body weight, and shape differences are commonly a consequence of differences in size) and coded based on tests for significant character differences between taxa. The second contains non-osteological, qualitatively coded phenotypic characters (colour, pelage length, glands, dentition and behaviour) (see Vrba *et al.*, 1994 Tables 6 and 7 for the two classes of data). Branch and bound parsimony analysis, with character substitutions assigned equal weights, was performed on the complete data set (molecular sequences, chromosomal and morphological characters i.e., total evidence). The morphological characters were polarized using the dibatag, *Ammodorcas clarkei*, as outgroup.

Table 1 Presence(+) / absence(–) matrix for the 12 Robertsonian chromosomal fusions identified in Reduncinae and confirmed by cross-species FISH using whole cattle chromosome painting probes

Species	1;11	6;18	7;11	5;17	3;11	3;10	6;10	6;29	5;13	1;19	4;7	2;25	X	Y
PCA	+	–	–	–	–	–	–	–	–	–	–	+	+	+
RFU	–	–	–	–	–	–	+	–	–	–	–	+	+	+
KKO	–	–	–	–	+	–	–	+	+	+	–	+	+	–
KEL	–	+	+	+	–	–	–	–	–	+	–	+	+	–
KLE	–	+	–	–	–	+	–	–	+	+	+	+	+	–
KME	–	+	–	–	–	–	–	–	–	+	+	+	+	–
Out	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: KEL, *K. ellipsiprymnus*; KKO, *K. kob*; KLE, *K. leche*; KME, *K. megaceros*; Out, outgroup; PCA, *P. capreolus*; RFU, *R. fulvorufa*. The last two columns show the patterns on X and Y chromosomes of the various species resulting from FISH of a microdissected *R. fulvorufa* Y-chromosome painting probe (clone sequence under accession number AM904697).

RESULTS

Identification of chromosomal orthologues in Reduncini and Pelea

The *P. capreolus* karyotype ($2n=56$) (Figure 1a) differs from the bovid ancestral karyotype ($2n=60$) through the presence of two Robertsonian (Rb) fusions that correspond to BTA 1;11 (PCA 1) and BTA 2;25 (PCA 2) on cross-species FISH using BTA painting probes (Figures 1b and c). The X chromosome is a large acrocentric with a prominent pericentromeric heterochromatic region, while the Y is intermediate in size (between pairs 15–19) appearing almost entirely heterochromatic on C-banding but with a more intensely staining region in the middle of Yq (Figure 2a). The corresponding C-band patterns of RFU and *K. ellipsiprymnus* (which are broadly typical to those of the other *Kobus* species examined) are shown in Figures 2b and c, respectively. Although there is some variation in extent of C-positive material within and among species, the location is invariably conserved.

Previous studies have identified a series of Rb fusions in *Redunca* and *Kobus* species (Kingswood et al., 2000; Rubes et al., 2007; Pagacova et al., 2011) all of which were confirmed by cross-species FISH in the present investigation. Based on these data, and the new information presented for *Pelea* (Table 1), we can conclude that Rb 2;25 is a synapomorphy that unites *Pelea* + *Kobus* + *Redunca*, thus firmly placing *Pelea* within Reduncinae. Its presence in *Antilope cervicapra* (tribe Antilopini) and the persistence time required to qualify as a hemiplasy (Robinson and Ropiquet, 2011) suggests that it arose convergently in *A. cervicapra*. The second fusion identified in *Pelea* (BTA 1;11) is not present in any of the other species for which data are available and, given the unfused state in outgroup species (and the presumed bovid ancestor), is considered an autapomorphy for *Pelea*.

FISH of Y-chromosome clones

Of the seven clones tested for efficacy on RFU chromosomes by FISH, five were positive. The sequence homology of the clones was high (95–100%) and, not surprisingly, resulted in identical FISH patterns. The most divergent sequences have been deposited in the National Center for Biotechnology Information database under accession numbers: AM 904696 and AM904697. One of them (AM904697) was used for the cross-species hybridization in *Kobus* and *Pelea*. The hybridization showed positive signals on X and Y chromosomes of *P. capreolus* (and of course *R. fulvorufa*) but importantly, only on the X chromosome of the *Kobus* species (Figure 3).

Closer inspection of the FISH data shows that signal is strongly localized to a region approximately two-thirds down the RFU Yq (with evidence of a weaker signal at the distal end of the chromosome). It also hybridized to the proximal one fifth of this species' acrocentric X chromosome, a region that is strongly C-positive on

conventional C-banding (Figure 2). Heterologous painting to *Pelea* results in the same patterns of hybridization (the distal signal faintly detected in RFU is sometimes detected), while the *Kobus* Y is completely devoid of signal (Figure 3). Cross-species painting to Caprini, Hippotragini and Alecelaphini suggests that the derived state (i.e., hybridization to the Y chromosome) unites *Redunca* + *Pelea* to the exclusion of *Kobus* (Table 1).

Phylogenetic analyses

(i) *DNA sequences*: The monophyly of *Redunca* + *Kobus* + *Pelea* clade is strongly supported by our analyses (Figure 4a) although individual gene trees based on parsimony revealed variable support for the placement of *P. capreolus* (Supplementary Figure 1). *Pelea capreolus* showed a single autapomorphic 2bp insertion in the Protein-Kinase C1 gene. The remaining gap characters were confined to single bp inserts or deletions in single species; in some instances, larger autapomorphic changes occurred when comparing the data to the outgroup. After bootstrapping, three of the gene trees (Stem cell factor, Cyt-B and COI) suggest an equidistant relationship between *Pelea*, *Redunca* and *Kobus*. In contrast, MC1R and B-Spectrin non-erythrocytic 1 indicate a sister taxon relationship between *Redunca* and *Kobus* (83 and 57% bootstrap support, respectively) with *Pelea* basal in the tree. Protein-Kinase C1 clustered *Pelea* within *Redunca* with 77% bootstrap support. The combined Parsimony (complete molecular data set) supported the equidistant relationship between the three genera included in the present study (Figure 4a); the basal position of *Pelea* is not supported by the Bayesian analysis. There is strong support for a sister species relationship between *K. megaceros* + *K. leche*, and between *K. kob* + *K. vardoni* as well as between *R. redunca* + *R. arundinum* with RFU basal in the genus. These phylogenetic associations (i.e., within Reduncini) similarly enjoyed strong statistical support in the only other study to benefit from full taxon representation, that of Birungi and Arctander (2001).

(ii) *Supermatrix analysis of DNA sequences and chromosomal characters*: Our analysis of a supermatrix that included the nuclear sequences described above, FISH mapping of the cloned *R. fulvorufa*-Y painting probe, and the 12 Rb fusions identified by cross-species FISH using cattle whole chromosome painting probes resulted in a bootstrap consensus tree that was partly unresolved (Figure 4b). This reflects the differences among individual gene trees coupled to homoplasies (both those resulting from convergent changes and hemiplasies) described in Robinson and Ropiquet (2011) as well as the conflicting *R. fulvorufa*-Y painting data that groups *P. capreolus* + *Redunca* to the exclusion of *Kobus*. Nonetheless, *P. capreolus* was basal in this analysis (with <50% bootstrap support), consistent with the presence of the Rb 2;25 fusion, which unites the Reduncinae.

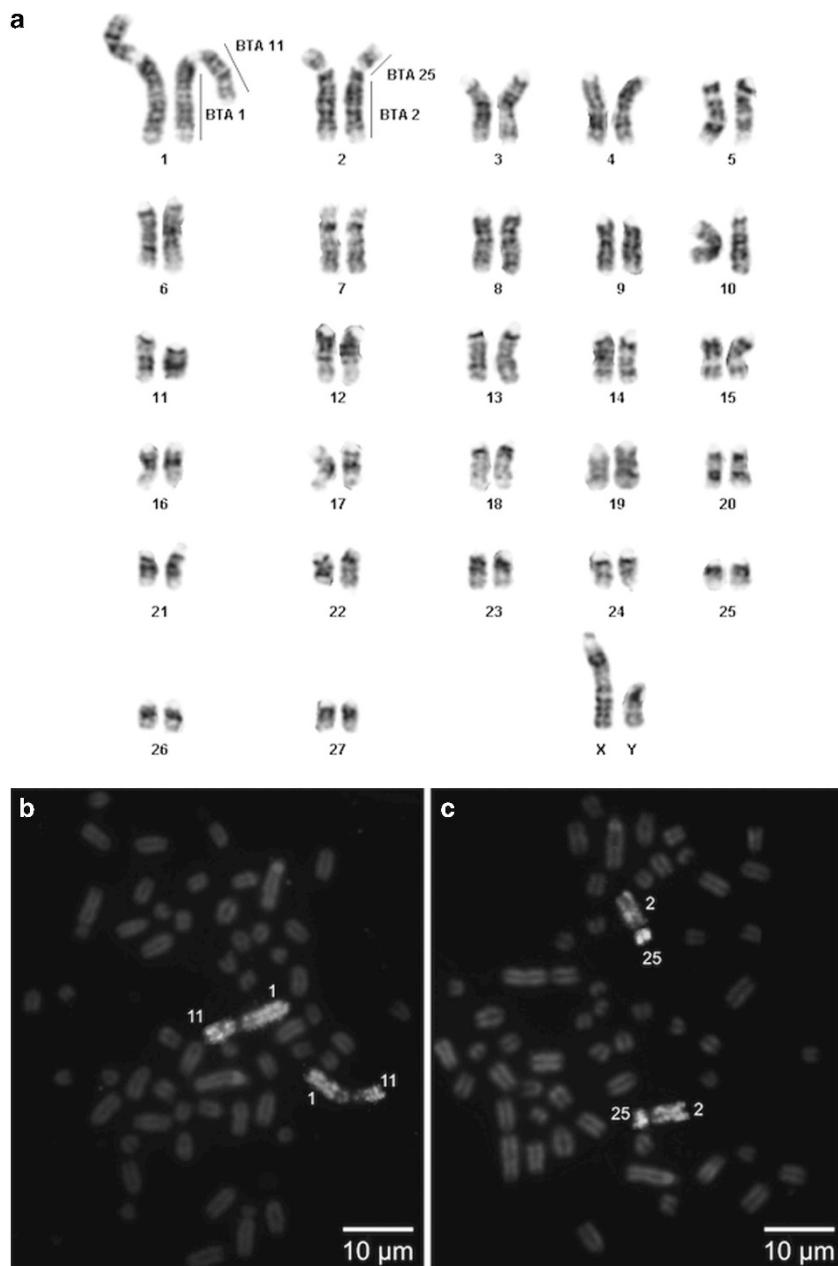


Figure 1 (a) G-banded karyotype of *Pelea capreolus* ($2n=56$). The Robertsonian fusion chromosomes (pairs 1 and 2) correspond to unfused acrocentric chromosomes in cattle (BTA). (b) Confirmation by cross-species FISH showing the cattle orthologues to pair 1 of the *Pelea* karyotype using cattle painting probes BTA1 and BTA11 and (c) BTA2 and BTA25 to pair 2 of the *Pelea* karyotype.

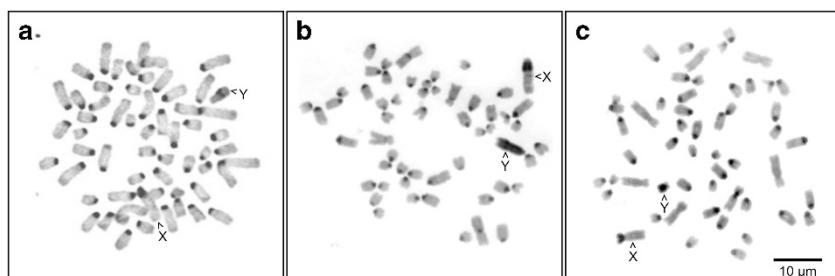


Figure 2 C-banded metaphase spreads of (a) *P. capreolus*, (b) *R. fulvorufa* and (c) *K. ellipsiprymnus*. The X and Y chromosomes are arrowed in each instance.

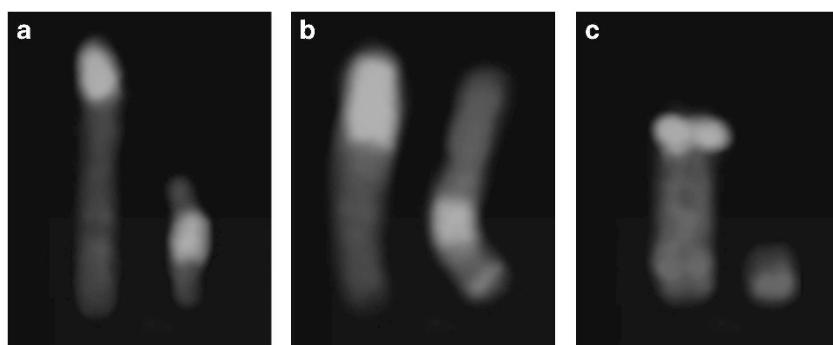


Figure 3 Cross-species FISH with a microdissected *R. fulvorufula* Y-chromosome painting probe showing hybridization patterns on the X and Y chromosomes for (a) *P. capreolus*, (b) *R. fulvorufula* and typically for the species within *Kobus*, (c) *K. ellipsiprymnus*.

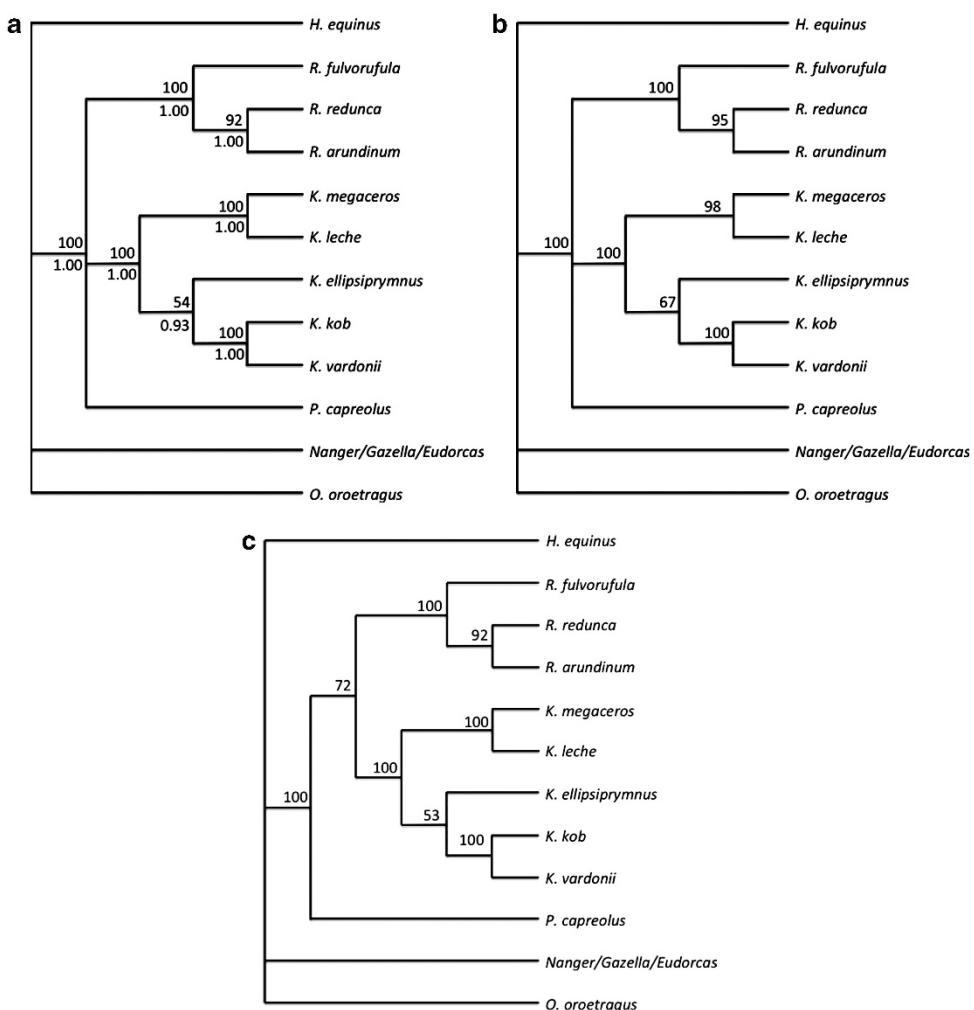


Figure 4 (a) Phylogenetic tree based on DNA sequences from two mitochondrial DNA (Cyt-B and COI) and four nuclear gene fragments (B-Spectrin nonerythrocytic 1, PRKC1, Stem cell factor and MC1R). Bootstrap values are presented above each node, while Bayesian posterior probability values are presented below. Prior model specification for the Bayesian analyses were as follows: B-Spectrin nonerythrocytic 1 and MC1R: nst = 2, rates = gamma; PRKC1: nst = 2, rates propinv; Stem cell factor: nst = 6, rates propinv; Cyt-B: nst = 6, rates = invgamma; COI: nst = 6, rates = gamma, (b) Supermatrix parsimony phylogeny based on the mtDNA and nuclear sequences presented in (a) above, together with Robertsonian fusion chromosomal data and FISH patterns from a microdissected *R. fulvorufula* Y-chromosome painting probe. Bootstrap values are presented above each node. (c) Supermatrix parsimony phylogeny based on the complete data set (i.e., sequences, chromosomes and morphological characters). Bootstrap values are presented above each node.

(iii) *Supermatrix analysis of DNA sequences, chromosomal characters and morphology*: An expanded data set comprising 5783 characters (658 parsimony informative) from mtDNA and nuclear sequences,

chromosomal data and osteological and non-osteological features (taken from Tables 5 and 6 of Vrba *et al.*, 1994) placed *P. capreolus* as sister to *Redunca* + *Kobus* (with 72% bootstrap support). The analysis

of the complete data set reaffirmed the monophyly of *Redunca* and *Kobus* (100% bootstrap support, respectively) although the association of *K. ellipsiprymnus* to *K. kob* and *K. vardonii* is weak (53%, Figure 4c). Interestingly, comparison of the cladograms presented in Vrba *et al.* (1994) show that a major difference between constructs based on quantitative osteology characters from horns and skulls versus qualitatively (discretely coded) characters such as colour and pelage length, subcutaneous glands, tooth morphology concerned the placement of *K. ellipsiprymnus*, a species thought to exhibit morphological paedomorphosis (the retention of juvenile physical characteristics in adults).

DISCUSSION

The evolutionary relationships of the endemic South African Grey Rhebok, *P. capreolus*, have long been a source of conjecture fueled by its ambiguous morphology and the rapid radiation of the Antelopinae in general (Matthee and Davis, 2001) and reduncines in particular (Bibi, 2013). This is reflected in past taxonomic treatments that have variously regarded *Pelea* as a member of the Reduncini, Antilopini and Neotragini (among others) or as warranting recognition as a separate tribe (Oboussier, 1970; Gentry, 1970, 1992; Vrba, 1976; Vrba *et al.*, 1994). More recently, comprehensive sequence-based investigations have tended to converge by finding *P. capreolus* as a part of Reduncinae (Gatesy *et al.*, 1997; Hassanin and Douzery, 1999; Matthee and Robinson, 1999; Matthee and Davis, 2001; Decker *et al.*, 2009; Bibi, 2013), although its relationship to species within Reduncini has been unclear. The outcome of the supermatrix analysis (sequences, chromosomes and morphology) presented herein, however, persuasively places the Grey Rhebok as the first lineage to diverge within this clade—a finding that is consistent with the primitive state of its cutaneous glands, dentition and horns (Vrba and Schaller, 2000). It also provides a robust evolutionary framework in which to examine the likely sources of conflict among data sets.

Certainly the earlier taxonomies, underpinned by morphological characters, were influenced by evolutionary convergence that resulted in the grouping of taxa on the basis of homoplasy, rather than their shared evolutionary history. A significant number of the morphological characters conventionally used in bovid phylogenetics (particularly those of dentition and limbs, i.e., Gentry, 1992 among others) are thought to have experienced selection for improved mastication and locomotion (Marcot, 2007) leading to rampant homoplasy. As the terrestrial Neogene environments in Africa became less forested, environmental changes are thought to have resulted in shared selection pressures causing most ungulates to respond with similar morphological adaptations. This probably accounts for the poor resolution of *Pelea* in the Gatesy *et al.* (1997) study where *Pelea*'s position in molecular-based phylogenies altered radically with the inclusion of Gentry's (1992) morphological characters in their analyses. This is further borne out by their investigation of the skeletal characters in isolation which showed limited taxonomic congruence with either their rDNA gene tree topologies or with the traditional classifications of bovids. In contrast, Vrba *et al.* (1994) relied on quantitative characters that were corrected for allometry, as well as qualitative coded, independent phenotypic characters that when used singularly, or in combination, reinforced the molecular findings of the present study. Our combined data set contains information from genes that may be influenced by a variety of selective regimes (a protein coding exon, three non-coding introns, two mtDNA gene fragments), chromosomal rearrangements—considered rare genomic changes that have lower probabilities of convergence, as well as morphology. The resulting supermatrix allows these disparate data sets to be analysed simultaneously, thus permitting

their convergence on the most likely hypothesis of reduncine evolutionary relationships. Moreover, it corroborates the identical associations suggested by possibly the most comprehensive molecular study to date—that based on 40 843 genome-wide single nucleotide polymorphism genotypes (Decker *et al.*, 2009)—and is confirmed by the recent multi-calibrated analysis of the pruned Hassanin *et al.* (2012) mitochondrial DNA dataset (Bibi, 2013).

A second reason for *Pelea*'s equivocal relationships is rapid cladogenesis which allows little time for evolutionary transitions to accumulate along the intervening branch between successive speciation events. The nearly contemporaneous divergences among the Reduncinae lineages resulted in few unambiguous synapomorphies supporting the basal nodes in the tree (those defining the relationships between *Pelea*, *Kobus* and *Redunca*). As expected, the limited signal is also reflected in low bootstrap and non-significant posterior probabilities for these nodes. For example, placing *Pelea* as a sister taxon to *Redunca* will increase the total tree length by only two steps and placing *Pelea* as a sister taxon to *Kobus* would require three additional steps. In comparison, there are 74 synapomorphies that support the monophyly of the expanded reduncine clade (i.e., *Pelea* included in the Reduncinae).

The effects of the rapid radiation on chromosomal characters has similarly been noted (Robinson and Ropiquet, 2011). Of the 12 Rb fusions identified in the six species for which data are available (Table 1), three are synapomorphies (Rb 2;25; Rb 1;19; Rb 4;7) that define natural clades (the Reduncinae, the genus *Kobus* and the sister relationship between *K. megaceros* + *K. leche*, respectively), two are hemiplasies (Rb 5;13, Rb 6;18)—that is, they are thought to have persisted across successive speciation nodes by virtue of short internodes—and the balance are true homoplasies having arisen independently in other non-reduncine lineages. Interestingly, the presence of the Rb 1;19 fusion at the base of *Kobus* and the Rb1;11 autapomorphy in *P. capreolus* suggests that monobrachial fusions (Baker and Bickham, 1986) may have facilitated *Pelea*'s reproductive isolation. This would not create a barrier with *Redunca*. That said, the Protein-Kinase C1 gene tree (Supplementary Figure 1) places *P. capreolus* within the *Redunca* clade (with 77% bootstrap support) and, given the similar Y chromosome FISH patterns (Figure 3), one may speculate that these shared characters reflect introgressive hybridization in their distant past, although there is no modern evidence to suggest this (in captive settings or in the wild). It is significant, however, that hybridization has been recorded among many species of bovids (Gray, 1972) and has been suggested as a reason for phylogenetic discordance between the mtDNA and nuclear genomes (see Hassanin and Ropiquet, 2007; Ropiquet *et al.*, 2008; Robinson and Ropiquet, 2011).

Molecular dating (Hassanin and Douzery, 1999) estimates the appearance of the Reduncini (including *Pelea*) at 9.0–10.8 mya although a new multicalibrated mitochondrial phylogeny suggests 7.5 mya (6.5–8.5 mya), an age that is considerably younger (Bibi, 2013). There is reasonable agreement with the earliest fossil evidence for the group reported from the Siwaliks formations of India and Pakistan, which may be 8 my or older (the oldest substantiated African reduncines are only known as far back as ~7 myr; Bibi *et al.*, 2009). If the Late Miocene divergences of *Pelea*, *Redunca* and *Kobus* occurred almost contemporaneously, different tempos of chromosomal evolution are apparent. One chromosomal change characterizes the *Pelea* lineage consistent with a low tempo of chromosomal evolution (~0.1 changes per million years), whereas there are 10 rearrangements leading to the terminal taxa subsequent to the *Redunca*/*Kobus* split (~1.4 changes per million years)—a value that

would be considered at the upper end of the range for many eutherian mammals (see Pardini *et al.*, 2007 and references therein). It is tempting, therefore, to suggest that the accelerated tempo of chromosomal evolution in Reduncini is, in some way, a reflection of climatic and vegetation shifts that have impacted on their evolutionary history.

Redunca and *Kobus* are antelope that tend to occupy specialized habitats with permanent water being a necessary requirement (Skinner and Chimimba, 2005). They are found predominantly in wetlands and tall or tussock-grassland, and it seems reasonable to conclude that they probably arose from 'presence-of-permanent-water-adapted' ancestry (Vrba, 2006). If this is correct, their evolutionarily-persistent water adaptation resulted in recurrent fragmentation of their distributions during arid (and mostly globally cool) phases. This mimics what has been proposed for grazers with a preference for open habitats where a general increase in the ratio of grass to wood cover during the African Miocene/Pliocene (Cerling *et al.*, 1997; Arctander *et al.*, 1999; Hassanin and Douzery, 1999; Vrba and Schaller, 2000 among others) is thought to have led to varying degrees of regional genetic differentiation in a variety of African bovids (Lorenzen *et al.*, 2006).

But what does a basal *Pelea* tell us of a species that is both genetically and ecologically distinct from the other Reduncini? It is possible that *Pelea* (an endemic, montane specialist considered to have retained many primitive phenotypic characters, Vrba and Schaller, 2000) arose by vicariance in the extreme southern/southwestern regions of the continent in response to the onset of arid biotypes in the Namib that continued deep into the Neogene. This would be consistent with Pickford's (2004) hypothesis that posits a long period of adaptation to arid and semi-arid conditions in relative isolation from similar biotypes elsewhere, and which has been invoked to account for the high levels of endemism encountered in numerous South African plant and vertebrate lineages. Should this hold, the increasingly dry conditions in the late Miocene led to *Pelea*'s adaptation and persistence in exposed habitats, free of standing water (Skinner and Chimimba, 2005), while the lineages giving rise to the extant 'presence-of-permanent-water-adapted' species survived in contracted, water-abundant refugia such as those offered by the Okavango delta and the drainage systems in the southwestern extension of the East African Rift system (Moore *et al.*, 2012). In this regard, it is particularly noteworthy that the vast majority of new reduncine 'species' presented in Groves and Grubb (2011), most likely regional ecotypes and subspecies (Heller *et al.*, 2013; Zachos *et al.*, 2013), are found in these areas. This gives additional credence to our view that the fragmented nature of these environs both promoted speciation in *Redunca* and *Kobus*, as well as provided conditions that permitted the fixation of chromosomal rearrangements through drift (if selectively neutral) or selection (if overdominant).

In summary, the phylogenetic relationships of the South African endemic Grey Rhebok, *P. capreolus*, have long been problematic. We show by constructing a supermatrix of DNA sequences, chromosomal characters and quantitative and qualitative morphological features, that a persuasive argument can be made for its basal placement in a clade that comprises the waterbuck, reedbuck and allies. The phylogenetic data support a rapid radiation with short internodes that have impacted on the accumulation of unambiguous synapomorphies and provide a rationale for the persistence of chromosomal fusions across successive speciation nodes. These data, in conjunction with molecular estimates of divergences with Reduncinae and their correspondence with the fossil record and paleoecological trends in the late Miocene/Pliocene, provide a novel hypothesis of the origin and subsequent cladogenesis of Africa's reduncine antelope.

DATA ARCHIVING

GenBank accession numbers can be found in Supplementary Table 1.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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