The biogeography of introgression in the critically endangered African monkey *Rungwecebus kipunji*

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In the four years since its original description, the taxonomy of the kipunji (*Rungwecebus kipunji*), a geographically restricted and critically endangered African monkey, has been the subject of much debate, and recent research suggesting that the first voucher specimen of *Rungwecebus* has baboon mitochondrial DNA has intensified the controversy. We show that *Rungwecebus* from a second region of Tanzania has a distinct mitochondrial haplotype that is basal to a clade containing all *Papio* species and the original *Rungwecebus* voucher, supporting the placement of *Rungwecebus* as the sister taxon of *Papio* and its status as a separate genus. We suggest that the *Rungwecebus* population in the Southern Highlands has experienced geographically localized mitochondrial DNA introgression from *Papio*, while the Ndundulu population retains the true *Rungwecebus* mitochondrial genome.

Keywords: baboons; hybridization; kipunji; *Papio*; *Rungwecebus*; Tanzania

1. INTRODUCTION

The significance of introgressive hybridization in the evolution of wild populations is poorly understood. In cercopithecine primates, hybridization and persistent introgression have been reported in macaques (*Macaca*; e.g. Kanthaswamy *et al.* 2008), guenons (*Cercopithecus*; e.g. Tutin 1999; Detwiler 2002) and baboons (*Papio*; e.g. Phillips-Conroy & Jolly 1986;

Samuels & Altmann 1986; Alberts & Altmann 2001; Zinner *et al.* 2009b), as well as between baboons and geladas (*Theropithecus*; Dunbar & Dunbar 1974; Jolly *et al.* 1997), suggesting that reproductive isolating mechanisms may be weak or absent. The long-term consequences of hybridization vary widely and depend largely on natural selection, population genetics, demography, anthropogenic influences and the complex dynamics of gene flow among populations. In this study, we report new information about the role of introgression in the evolutionary history of the kipunji (*Rungwecebus kipunji*), a unique, geographically restricted, and 'critically endangered' African monkey (Davenport & Jones 2008).

The kipunji was originally described as a new species of mangabey, *Lophocebus kipunji* (Jones *et al.* 2005), on the basis of external morphology and behaviour. When a dead subadult from the type locality, Mt Rungwe in Tanzania's Southern Highlands, became available, Davenport *et al.* (2006) used genetic data to show that this classification was incorrect. They erected a new genus, *Rungwecebus*, recognizing the kipunji's phylogenetic placement—sister to *Papio* in their analyses—and its lack of the diagnostic morphology of baboons. Olson *et al.* (2008) confirmed this phylogenetic result with additional molecular markers, and Singleton's (2009) geometric morphometric analysis showed that *Rungwecebus* is morphologically distinct from both *Lophocebus* and *Papio*. Continuing ecological and behavioural study has also improved our understanding of the kipunji's biology and distribution (e.g. Davenport & Jones 2008; Davenport *et al.* 2008).

Two recent studies with broader taxonomic and geographical representation for *Papio* (Burrell *et al.* 2009; Zinner *et al.* 2009a) have renewed debate about the evolutionary history of *Rungwecebus*. Both showed that the mitochondrial haplotype from the original *Rungwecebus* voucher specimen is nested within *Papio*, in which introgression and mitochondrial polyphyly seem to be common (Zinner *et al.* 2009b). Zinner *et al.* (2009a) suggested that *Rungwecebus* is sister to *Papio* with past introgressive hybridization, as previously proposed by Olson *et al.* (2008), while Burrell *et al.* (2009) suggested that *Rungwecebus* originated via hybridization between *Lophocebus* and *Papio*. However, the genetic information for *Rungwecebus* has never been geographically complete. *Rungwecebus* is known from two populations in Tanzania (figure 1)—one in the Southern Highlands (Mt Rungwe and the adjacent Livingstone Forest in Kitulo National Park), and the other 350 km to the northeast in Ndundulu Forest, within the Kilombero Nature Reserve in the Udzungwa Mountains (Davenport *et al.* 2008). The original voucher specimen, which was the source of all previous *Rungwecebus* sequences, came from Mt Rungwe; no genetic material from the Udzungwas has been available until now. Here, we add the first DNA sequences of *Rungwecebus* from Ndundulu, as well as two additional sequences from Mt Rungwe-Kitulo.

2. MATERIAL AND METHODS

We extracted and sequenced mtDNA from six kipunji stool samples from the Ndundulu population and two additional fresh tissue specimens from the Southern Highlands—one from Mt Rungwe and one from Livingstone Forest—using standard methods for PCR

Figure 1. Locations in Tanzania and across Africa (inset) for *Papio* and *Rungwecebus* samples. Filled circle, *Papio*; filled star, *Rungwecebus*.

amplification, sequencing, and fragment assembly (table 1; figure 1). Based on the collection locations, dates and conditions, we are very confident that the sequenced samples come from six different individuals. We sequenced four mtDNA fragments and aligned them to sequences from GenBank (see the electronic supplementary material, table S1): (i) 600 bp of the cytochrome oxidase I (COI) gene; (ii) 500 bp of the cytochrome oxidase II (COII) gene; (iii) 408 bp of the 12S rRNA gene; and (iv) 897 bp spanning part of NADH dehydrogenase subunit 4 (ND4), 3 tRNAs, and part of NADH dehydrogenase subunit 5 (ND5). We performed phylogenetic analyses of the individual fragments and a combined dataset of COI, COII, and 12S with reduced taxon sampling. Details of specimen collection and laboratory and analytical methods are in the electronic supplementary material.

3. RESULTS AND DISCUSSION

The six stool samples share a single haplotype for all sequenced fragments. Phylogenetic analyses of the individual fragments and the combined data are consistent with respect to the placement of *Rungwecebus* (figure 2 and see the electronic supplementary material, figures S1–S7). The original sample (FMNH 187122) and the two new samples from Mt Rungwe-Kitulo (SHCP 2458 and WTS 9308) cluster together, within the extant mitochondrial diversity in *Papio*. However, the Ndundulu kipunji haplotype is strongly supported as the sister lineage to this *Papio*–*Rungwecebus* clade. The mean uncorrected genetic distance between the Ndundulu and Mt Rungwe-Kitulo kipunji haplotypes is 4.81 per cent (table 2). The proximity between Mt Rungwe-Kitulo and *Papio* haplotypes is unlikely to be owing to incomplete lineage sorting, which results in shared haplotype lineages that are old relative to species divergences. The disparity in genetic diversity between the two kipunji populations suggests differing effective population sizes or demographic histories, although stronger inference from diversity statistics will require larger sample sizes.

Overall, our results, together with previous observations of morphological distinctiveness, indicate that *Rungwecebus* is the sister lineage of *Papio* but has

Table 1. *Rungwecebus* specimen and GenBank information. FMNH, Field Museum of Natural History; SHCP, Southern Highlands Conservation Programme; UAM, University of Alaska Museum; WTS, William T. Stanley field catalogue.

sample	COI	COII	12S	ND4/5	locality	collection date	coordinates (WGS84)
FMNH 187122	DQ381473	DQ381471	DQ375756	GU068078	Tanzania, Mbeya Region, Rungwe District, Mt Rungwe, 1769 m	3 Aug 2005	–9.16412, 33.632
SHCP 2458	GU068063	GU068070	GU068059	GU068079	Tanzania, Mbeya Region, Rungwe District, Livingstone Forest, approximately 2.5 km east of Kiguru village, 1872 m	15 Jul 2008	–9.20483, 33.89046
WTS 9308	GU068064	GU068071	GU068060	GU068080	Tanzania, Mbeya Region, Rungwe District, Mt Rungwe, Syukula village, 1770 m	1 May 2007	–9.16651, 33.63304
UAM 103004		GU068072		GU068081	Tanzania, Ndundulu Forest Reserve	16 Nov 2005	–7.7973, 36.51195
UAM 103006	GU068066	GU068074		GU068083	Tanzania, Ndundulu Forest Reserve	19 Feb 2006	–7.7973, 36.51195
UAM 103007	GU068065	GU068073		GU068082	Tanzania, Ndundulu Forest Reserve	16 Nov 2005	–7.7973, 36.51195
UAM 103008	GU068067	GU068075		GU068084	Tanzania, Ndundulu Forest Reserve	30 Jun 2007	–7.7973, 36.51195
UAM 103009	GU068068	GU068076	GU068061	GU068085	Tanzania, Ndundulu Forest Reserve	30 Jun 2007	–7.7973, 36.51195
UAM 103010	GU068069	GU068077	GU068062	GU068086	Tanzania, Ndundulu Forest Reserve	1 Jul 2007	–7.7973, 36.51195

Figure 2. Bayesian 50 per cent consensus tree for combined data. Shaded boxes show posterior probability (PP), parsimony bootstrap (PB), and likelihood bootstrap (LB) support. Outgroups not shown.

Table 2. Uncorrected pairwise genetic distance between populations or species. Above diagonal, minimum; below diagonal, mean; diagonal, nucleotide diversity.

	<i>Rungwecebus</i> Ndundulu	<i>Rungwecebus</i> Mt Rungwe- Kitulo	<i>Papio</i> <i>cynocephalus</i>	<i>Papio</i> <i>anubis</i>	<i>Papio</i> <i>papio</i>	<i>Papio</i> <i>ursinus</i>	<i>Papio</i> <i>hamadryas</i>	<i>Theropithecus</i>	<i>Lophocebus</i>
<i>Rungwecebus</i> Ndundulu	0.0000	0.0481	0.0426	0.0440	0.0474	0.0467	0.0440	0.0735	0.0639
<i>Rungwecebus</i> Mt Rungwe- Kitulo	0.0481	0.0009	0.0055	0.0302	0.0309	0.0130	0.0316	0.0680	0.0659
<i>Papio</i> <i>cynocephalus</i>	0.0457	0.0203	0.0247	0.0027	0.0254	0.0076	0.0041	0.0639	0.0639
<i>Papio anubis</i>	0.0468	0.0328	0.0282	0.0197	0.0185	0.0323	0.0007	0.0646	0.0625
<i>Papio papio</i>	0.0484	0.0322	0.0296	0.0242	0.0021	0.0330	0.0247	0.0646	0.0660
<i>Papio ursinus</i>	0.0488	0.0226	0.0286	0.0374	0.0348	0.0237	0.0350	0.0687	0.0639
<i>Papio</i> <i>hamadryas</i>	0.0453	0.0330	0.0258	0.0154	0.0267	0.0388	0.0037	0.0680	0.0666
<i>Theropithecus</i>	0.0735	0.0680	0.0668	0.0669	0.0656	0.0701	0.0696	XXXX	0.0762
<i>Lophocebus</i>	0.0656	0.0694	0.0706	0.0684	0.0684	0.0706	0.0704	0.0776	XXXX

experienced geographically localized introgressive hybridization in the Southern Highlands, introducing *Papio* DNA long after the divergence between these two genera. We base our conclusion on samples from two different populations across the known range of the kipunji in the Southern Highlands, both of which contain apparently introgressed haplotypes. We consider the Ndundulu haplotype to represent the true (non-introgressed) *Rungwecebus* mitochondrial genome, although rigorously testing this will require both additional genetic data and morphological corroboration using voucher specimens.

The presence of introgressed *Papio* haplotypes in one kipunji population implies past (and perhaps present) contact between *Rungwecebus* and baboons. Both *Rungwecebus* populations live in forest near *Papio cynocephalus*, which inhabits the dry bush adjacent to Ndundulu as well as open habitats and farmland adjacent to Mt Rungwe and Kitulo. The two species are separated primarily by habitat preference, but *P. cynocephalus* is known to enter forest and *Rungwecebus* has been observed on the ground in recently cultivated areas outside the forest (Davenport & Jones 2008). The frequency with which kipunji and baboons have

encountered each other may vary between the two areas, and may have changed over time as a result of local cycles of climate change, wildfires, and other disturbance. Anthropogenic factors may also be important in determining very recent interactions; being persistent crop-raiders and legally classified in Tanzania as 'vermin,' baboons are now increasingly rare in the areas of high human density that surround the Mt Rungwe forest.

It is unsurprising that the Ndundulu and Mt Rungwe-Kitulo kipunji populations may have different evolutionary histories. As well as having local differences in habitat and ecology, the two areas are separated by 350 km of non-forest habitat, preventing any gene flow between them. Indeed, the Udzungwas are considered the southernmost range of the Eastern Arc Mountains (Lovett & Wasser 1993), which until recently were thought to be biogeographically distinct from the Southern Highlands. The large number of endemic taxa in each region suggests long-term isolation, as does the fact that some appear to be of ancient origin and have geographically distant sister taxa (Dinesen *et al.* 1994; Stanley *et al.* 2005). In the past few years, however, the discovery in Mt Rungwe, Kitulo and other Southern Highlands sites of a wide range of vertebrates previously thought to be Eastern Arc endemics has cast doubt on the biogeographic distinction between these two areas (Davenport 2004; Carleton & Stanley 2005). It now seems probable that they were once linked by a forest corridor that has since disappeared, isolating populations of forest-dependent taxa such as the kipunji.

Further understanding of the evolutionary history of *Rungwecebus* and the differences between the two populations will require adult specimens from both Mt Rungwe-Kitulo and Ndundulu suitable for morphological and genetic research. The Livingstone Forest specimen (SHCP 2458) possesses all the diagnostic features of *Rungwecebus* (i.e. erect crest of hair on the crown, black eyelids, long tail with light tip, and lack of a long rostrum and mandibular fossae). Unfortunately, this specimen, though an older individual than the original voucher, is another subadult, and the osteomorphology of adult *Rungwecebus* will remain a matter of conjecture until adult specimens become available.

The original description of the kipunji in 2005 was trumpeted as an example of how little we still know about biodiversity and additional research has accentuated this point. The two existing *Rungwecebus* populations have experienced a complex, idiosyncratic history of independent divergence and subsequent hybridization with a closely related genus, probably dependent on an intricate and dynamic background of population connectivity and demography. Our ongoing research on additional aspects of the biology of this unique and critically endangered genus will enhance our understanding of hybridization, evolution, and biogeography in a region of Africa that remains surprisingly poorly known.

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