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Evolutionary biology

Inverted intergeneric introgression between critically endangered kipunjis and yellow baboons in two disjunct populations

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Intergeneric hybridization and introgression was reported from one of two populations of the recently discovered kipunji (*Rungwecebus kipunji*), a critically endangered African monkey species of southern Tanzania. Kipunjis of the introgressed population (from Mount Rungwe) carry a mitochondrial DNA (mtDNA) haplotype closely related to those of parapatric yellow baboons (*Papio cynocephalus*), whereas the second kipunji population, in the Udzungwa Mountains, carries the original kipunji mtDNA haplotypes, which diverged from the baboon lineage about 3 million years ago. Interestingly, in our study of yellow baboons in Tanzania, we found that baboons from the southeastern boundary of the Udzungwa Mountains carry mtDNA haplotypes closely related to the original kipunji haplotype, whereas baboons from the northern boundary, as expected, carry mtDNA haplotypes of the northern yellow baboon clade. These findings provide evidence for a case of inverted intergeneric admixture in primates: (i) a baboon mtDNA haplotype introgressed the Mount Rungwe kipunji population by mitochondrial capture and (ii) an Udzungwa Mountains kipunji mtDNA haplotype introgressed a small subpopulation of yellow baboons by either mitochondrial capture or nuclear swamping. The baboon–kipunji example therefore constitutes an interesting system for further studies of the effects of genetic admixture on fitness and speciation.

1. Introduction

Advances in molecular genetics have tremendously influenced our understanding of the impact of hybridization and introgression on the evolution of animal taxa [1,2]. Introgression is a long known phenomenon [3,4] and is defined as the infiltration of genetic material of one species into another through repeated backcrossing of hybrids to one or both parental species [5]. As a result of introgression certain genetic or phenotypical characters of species 'A' can be found in species 'B'. Strong uni-parental backcrossing can lead to mitochondrial or Y-chromosomal capture so that species 'A' might carry mitochondria or the Y-chromosome of species 'B' [1,6]. First indications for introgression are incongruent phylogenies derived from different genetic markers or from genetic and phenotypic characters [1,7].

A remarkable case of intergeneric introgression in primates was reported from the kipunji (*Rungwecebus kipunji*), a critically endangered African monkey, discovered in 2003 in southern Tanzania [8]. Kipunjis occur in two isolated populations: in the forests of Mount Rungwe and the adjacent Livingstone Forest and about 350 km to the northeast in the Ndundulu Forest of the

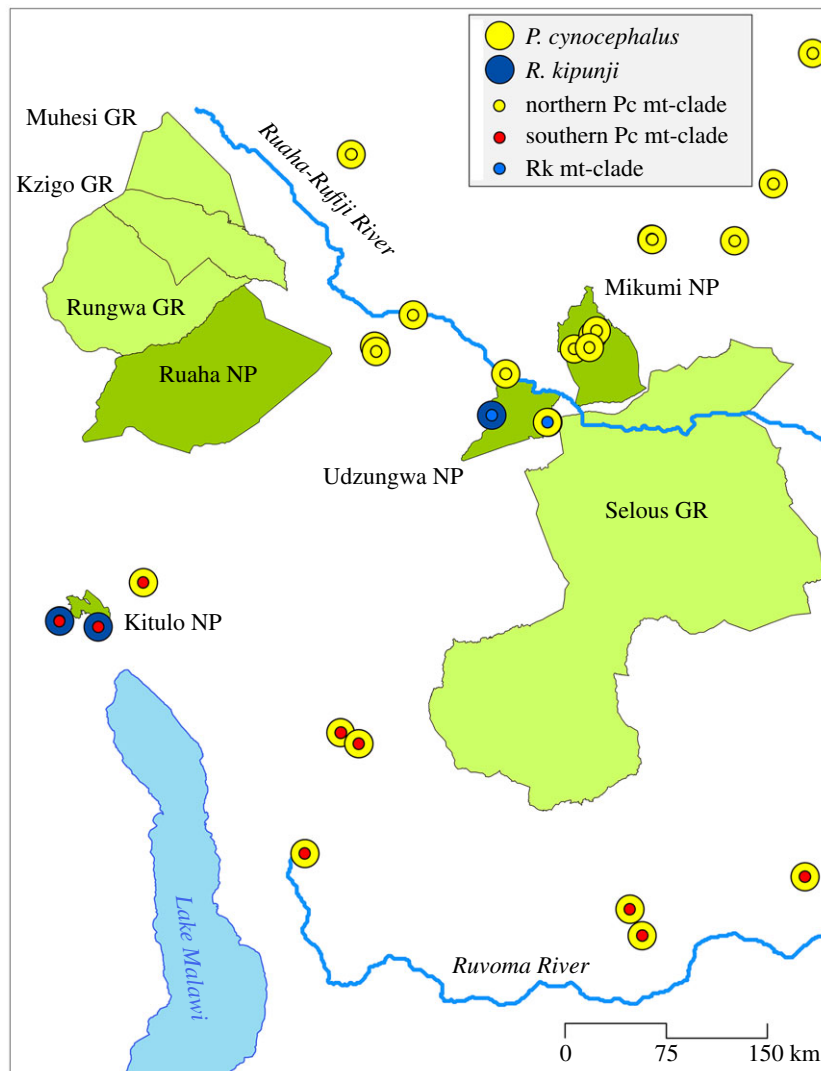


Figure 1. Map of southern Tanzania with yellow baboon (yellow circle) and kipunji (blue circle) sampling locations and geographical occurrence of mtDNA haplotypes of northern (small yellow circles) and southern (small red circles) yellow baboons and kipunjis (small blue circles). Protected areas: GR, game reserve; NP, national park, Pc, *Papio cynocephalus*; Rk, *Rungwecebus kipunji*. Details on samples can be found in the electronic supplementary material, table S1.

Udzungwa Mountains [9]. Analyses of mitochondrial DNA (mtDNA) of the Mount Rungwe population revealed that the kipunji mtDNA sequences are nested within the geographically proximate southern yellow baboon (*Papio cynocephalus*) clade [6,10,11]. In contrast, the population of the Udzungwa Mountains carry mitochondria concordant with a position as sister taxon to baboons [12]. This pattern was interpreted as a result of introgression with mitochondrial capture in the Mount Rungwe population. In contrast the Ndundulu haplotype from the Udzungwa Mountains was considered to represent the true, non-introgressed kipunji haplotype [12]. Here we report on the mtDNA phylogenetic relationships among the Udzungwa kipunjis and sym- and parapatric yellow baboons and a case of inverted intergeneric introgression.

2. Material and methods

During a Tanzania-wide screening for infection of non-human primates with the pathogenic bacterium *Treponema pallidum*, we collected tissue samples from 17 yellow baboons in the Udzungwa Mountain National Park (see electronic supplementary material) (figure 1). After DNA extraction, we amplified and sequenced the Brown region [13] of the mtDNA genome using methods outlined in Newman *et al.* [14] and Zinner *et al.*

[15]. Obtained haplotypes were aligned against orthologous sequences derived from GenBank of other baboons, kipunjis, and related taxa that were used as outgroups (electronic supplementary material, table S1). We reconstructed maximum-likelihood (ML) and Bayesian trees and estimated divergence ages. Full details of all analyses and an ethical note are provided in the electronic supplementary material.

3. Results and discussion

As in earlier studies on baboon phylogeny using mtDNA markers [14–19], baboons segregate into various strongly supported clades (ML bootstrap support (BS): greater than 90%, Bayesian posterior probabilities (PP): greater than 0.90). However, the branching pattern among them is not well resolved. Moreover, these clades follow a geographical pattern and their distribution is not always concordant with the boundaries of the six species now generally recognized. As in previous studies, yellow, olive (*Papio anubis*), and chacma baboons (*Papio ursinus*) show para- or polyphylies, which were regarded as results of ancient introgressive hybridization events [15,20].

In our tree reconstruction, as previously found [10–12], the mtDNA lineage of Mount Rungwe kipunjis clusters

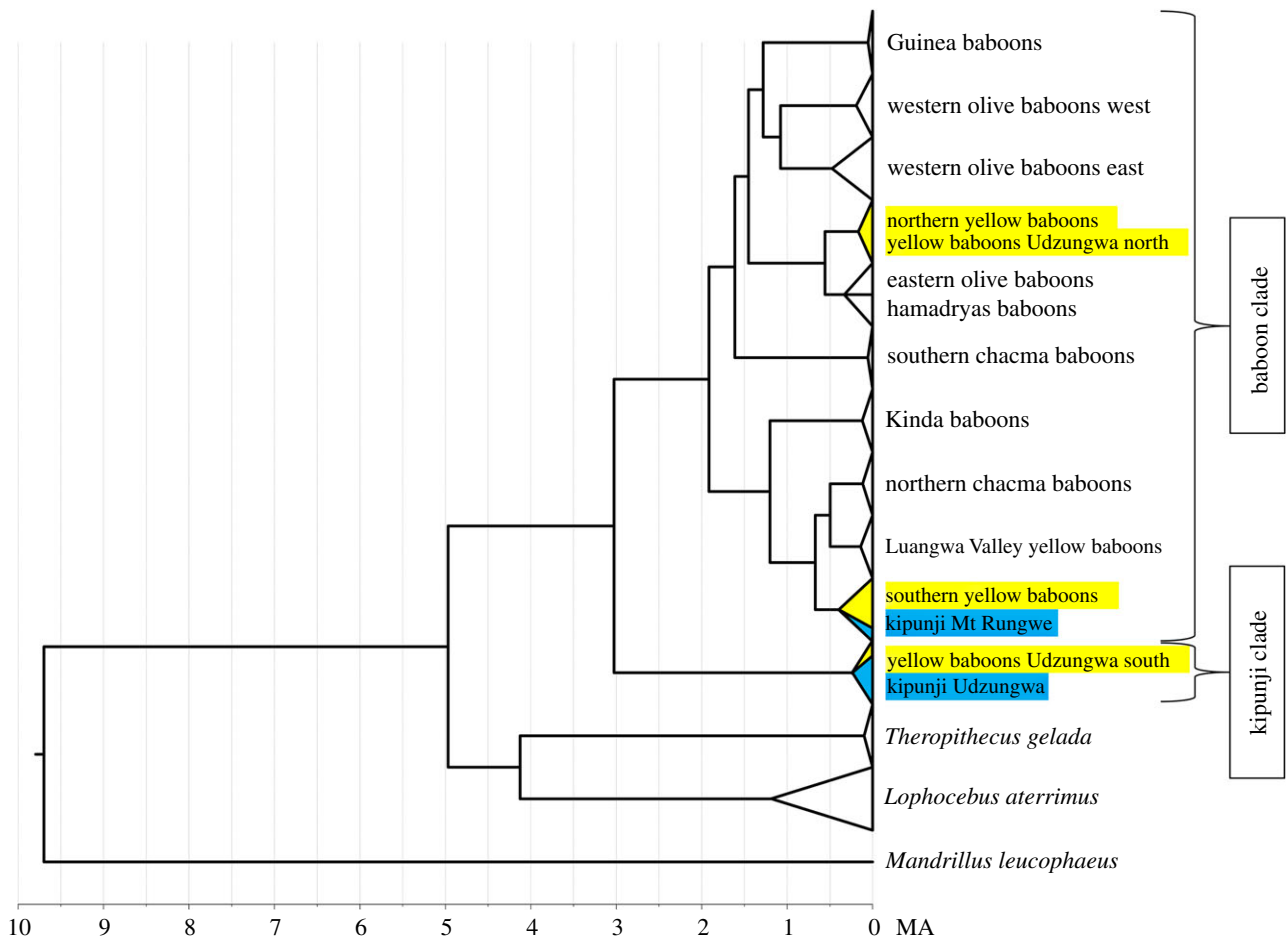


Figure 2. Chronogram showing phylogenetic relationships and divergence times among various baboon and kipunji mtDNA lineages. The mtDNA lineage of the Mount Rungwe kipunji clusters with southern yellow baboons, while yellow baboons from southern Udzungwa cluster with the Udzungwa kipunji. In contrast, yellow baboons from northern Udzungwa cluster with their conspecifics of the northern yellow baboon mtDNA clade (see also electronic supplementary material, figure S1 and table S2). (Online version in colour.)

with southern yellow baboons and the kipunji mtDNA lineage from Udzungwa constitutes a sister clade to the *Papio* clade, with a divergence time of 3.03 million years ago (Ma) (95% highest posterior density (HPD): 2.06–4.09 Ma) (figure 2; electronic supplementary material, table S2). Also expected is the grouping of yellow baboons from northern Udzungwa with conspecifics of the northern yellow baboon clade. However, yellow baboons from southern Udzungwa do not cluster either with southern or with northern yellow baboons. Instead, they represent a closely related sister clade to the mtDNA lineage of Udzungwa kipunjis. We estimated the split of the Mount Rungwe kipunji mtDNA lineage from its nearest southern yellow baboon lineage at 0.05 Ma (0.00–0.13 Ma), while the yellow baboon mtDNA clade from southern Udzungwa diverged from the Udzungwa kipunji lineage at 0.24 Ma (0.07–0.44 Ma) (electronic supplementary material, table S2).

The phylogenetic relationships of the two kipunji populations with respective para- or sympatric yellow baboons suggest three introgression scenarios. Alternative explanations for the observed pattern such as incomplete lineage sorting (ILS) are highly unlikely, because in small populations, as in kipunjis [21], lineage sorting should be relatively fast and the distribution pattern of haplotypes follows a geographical structure, while ILS should be random in respect to geography [15,22]. The first scenario assumes the mtDNA lineage found in Udzungwa kipunjis and baboons represents an

ancient *Papio* mtDNA lineage, which would mean that this is the oldest *Papio* mtDNA lineage discovered so far and also that the Udzungwa kipunjis captured baboon mitochondria, similar to the scenario envisioned for the Mount Rungwe kipunji population [11]. The two other scenarios assume that the Udzungwa kipunji mtDNA lineage indeed is the original kipunji mtDNA lineage [12], which would mean that the southern Udzungwa yellow baboons either captured mitochondria from female kipunjis (scenario 2) where theoretically a single event of a female kipunji breeding successfully with a male baboon is sufficient or that baboon males introgressed a subpopulation (e.g. a small isolated group) of the Udzungwa kipunji population over generations leading to nuclear swamping (scenario 3). In scenario 3, male-mediated gene flow from baboons into kipunjis would alter the nuclear gene pool of the kipunjis, up to completely converting the kipunji phenotype into the baboon phenotype, while leaving the ancestral kipunji mtDNA sequences in place. We believe the first scenario is unlikely, because if the Udzungwa kipunji mtDNA lineage represented an old *Papio* lineage, we would expect to find it more frequently in the baboon population of southern Tanzania, which, however, was not the case [19]. It would be interesting to investigate whether this lineage can be also found in baboons of the adjacent Selous Game Reserve. To test the hypothetical scenarios, we would need to analyse whole nuclear genomes inferring amount and frequency of gene flow between kipunjis and baboons. It

would be interesting to see which genes were exchanged, in which direction and whether similar mosaic genome patterns can be detected in both kipunji populations. Also, given that the baboon population carrying the kipunji mtDNA seems to be rather small, it might be interesting to examine whether these baboons show any indications of fitness loss related to genetic admixture, as compared with unadmixed conspecifics.

The kipunji–baboon case is, to our knowledge, the first reported case of inverted intergeneric introgression in primates, and maybe mammals overall. However, inverted introgression among species of the same genus has been reported e.g. in *Lepus*. Brown hares of northern Spain (*L. europaeus*) carry mtDNA lineages of mountain hares (*L. timidus*), whereas mountain hares in northern Russia carry mtDNA lineages of brown hares [23–26]. In the case of Spanish hares, ancient introgression is obvious, because the mountain hare went extinct in the region after the last glacial period, whereas introgressive hybridization is still ongoing in Sweden and Russia. Ongoing hybridization between *Rungwecebus* and *Papio*, however, is not reported.

Ethics. No animals were captured specifically for this study. The study was in line with the Veterinary Act of 2003 and Tanzania Wildlife Research Institute's (TAWIRI) Guideline for Conducting Wildlife Research (2001). Further information is available in the electronic supplementary material.

Data accessibility. Detailed methods are available as electronic supplementary material. Newly generated haplotypes have been deposited in GenBank (accession numbers MG569923–MG569944).

Authors' contributions. D.Z. and C.R. conceived the study. I.S.C. collected samples; S.K. did laboratory work; C.R. did the phylogenetic analyses. All authors discussed the results, collaborated in writing the paper, approved the final version of the manuscript and agreed to be held accountable for the content therein.

Competing interests. The authors declare no competing interests.

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