Discordance Between Spatial Distributions of Y-Chromosomal and Mitochondrial Haplotypes in African Green Monkeys (*Chlorocebus* spp.): A Result of Introgressive Hybridization or Cryptic Diversity?

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Abstract Introgressive hybridization may cause substantial discordances among phylogenies based on different genetic markers. Such discordances have been found in diverse mammal species including primates. A recent study of mitochondrial DNA (mtDNA) revealed several poly- and paraphyletic relationships in African green monkeys (*Chlorocebus*), suggesting contemporary and/or ancient introgressive hybridization among almost all parapatric species of the genus. However, mtDNA analyses alone do not allow us to draw conclusions concerning introgression events. In this study we analyzed two Y chromosomal (Y-chr) markers for 30 African green monkey samples and compared the resulting genetic relationships to those based on published mtDNA data. In line with the results for mtDNA, we found no Y-chr evidence of hypothesized hybridization among *Chlorocebus sabaeus* and *C. tantalus* in the northern part of the contact zone in West Africa, and we found two distinct and distantly related Y-chr haplotypes within the range of *C. tantalus*, suggesting possible cryptic genetic diversity rather than ancient introgressive hybridization in this species.

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In contrast, Y-chr data revealed monophyletic relationships within *Chlorocebus pygerythrus* from East Africa, suggesting that mtDNA paraphylies found in this species are most likely to be the result of ancient introgressive hybridization and subsequent cytonuclear extinction of an earlier taxon. Our results accentuate the importance of analyzing sex chromosomal data in addition to mtDNA to obtain more information on the potential outcomes of hybridization with respect to genetic and species diversity. Analysis of more diverse nuclear marker sets is needed to obtain a more complete picture of the African green monkey evolution.

Introduction

Molecular genetic studies, often based on mitochondrial DNA (mtDNA), have provided evidence of hybridization in diverse animal taxa in the last two decades (Abbott *et al*. 2013; Mallet 2005; Zinner *et al*. 2011). Introgressive hybridization, or the transmission of genetic material from one species into the genome of another, closely related species, accounts for a large proportion of reticulated phylogenies and apparent para- or polyphyletic relationships (Mallet 2005). As the heterogametic sex is often inviable or sterile in hybrids (Haldane 1922), one common result of introgressive hybridization in mammals is the transmission of the maternally inherited mitochondrial genome (Mallet 2005; Orr 1997; Schilthuizen *et al*. 2011). However, factors such as sex-biased dispersal or unequal population sizes may lead to backcrossing with only one of the parental species, which may cause nuclear swamping, mitochondrial or Y-chromosomal (Y-chr) capture (Zinner *et al*. 2011).

Evidence of frequent introgressive hybridization has been found in diverse African savannah mammals, and this has been attributed to recurrent changes in the extent and distribution of the savannah biome, and hence in range expansions and retractions of populations with the formation of secondary contact zones due to climatic changes in the last million years, e.g., hartebeests (Flagstad *et al*. 2001) and warthogs (Muwanika *et al*. 2003). Among the primates, baboons (*Papio*) are widely distributed in sub-Saharan African savannahs, and hybridization is ongoing in most places where the ranges of two species meet (Charpentier *et al*. 2012; Jolly *et al*. 2011; Keller *et al*. 2010; Tung *et al*. 2008). Moreover, discordant patterns between mtDNA and morphotypes also occur in regions where no current hybrid zone exists (Zinner *et al*. 2009). As in most mammalian taxa, males are predominantly the dispersing sex in baboons, suggesting that the discordant patterns are due to nuclear swamping through male-mediated introgressive hybridization (Zinner *et al*. 2009). In some of these cases introgression presumably led to the cytonuclear extinction of earlier baboon taxa or populations, whose mitochondrial genomes persist only as a trace in extant species (Keller *et al*. 2010; Zinner *et al*. 2009).

Like baboons, African green monkeys of the genus *Chlorocebus* constitute parapatric species that inhabit sub-Saharan savannahs (Hill 1966; Kingdon 1997; Lernould 1988). Hybridization has been assumed between most of the species in East Africa based on morphological data such as the occurrence of intermediate morphotypes (Kingdon

1997; Mekonnen *et al.* 2012; Napier 1981). Further, a recent study of mtDNA including samples from almost all contact zones throughout the genus' range found several discordant phylogenetic patterns, suggesting that introgressive hybridization might be common among all African green monkeys (Haus *et al.* 2013). Most of these discordances support previous observations of ongoing hybridization in contemporary contact zones between *Chlorocebus aethiops* and *C. djamdjamensis* and between *C. aethiops* and *C. pygerythrus* in Ethiopia as well as between *C. tantalus* and *C. pygerythrus* in Uganda and Kenya (Fig. 1a; Haus *et al.* 2013; Kingdon 1997; Napier 1981). In addition, Haus *et al.* (2013) found some inconsistencies between morphology and mtDNA beyond current contact zones. Their analysis revealed two distinct clades within the range of *Chlorocebus tantalus*: a western clade including samples from Ghana, Burkina Faso, and Togo (C 9, Fig. 1a) and an eastern clade with samples originating from Nigeria, Cameroon, Uganda, and northwest Kenya (C 1, Fig. 1a). Similarly, samples of *Chlorocebus pygerythrus* cause polyphyletic relationships in the mtDNA tree with distinct clades found in southeast Ethiopia and Somalia (C 6), in Kenya and Tanzania (C 8), and in Southern Africa (C 4, Fig. 1a; Haus *et al.* 2013). Because these mtDNA clades are geographically localized, they are unlikely to be explained by random sorting failures such as incomplete lineage sorting (Avise 2004; Funk and Omland 2003). A possible alternative reason for the discordant patterns might be nuclear swamping and
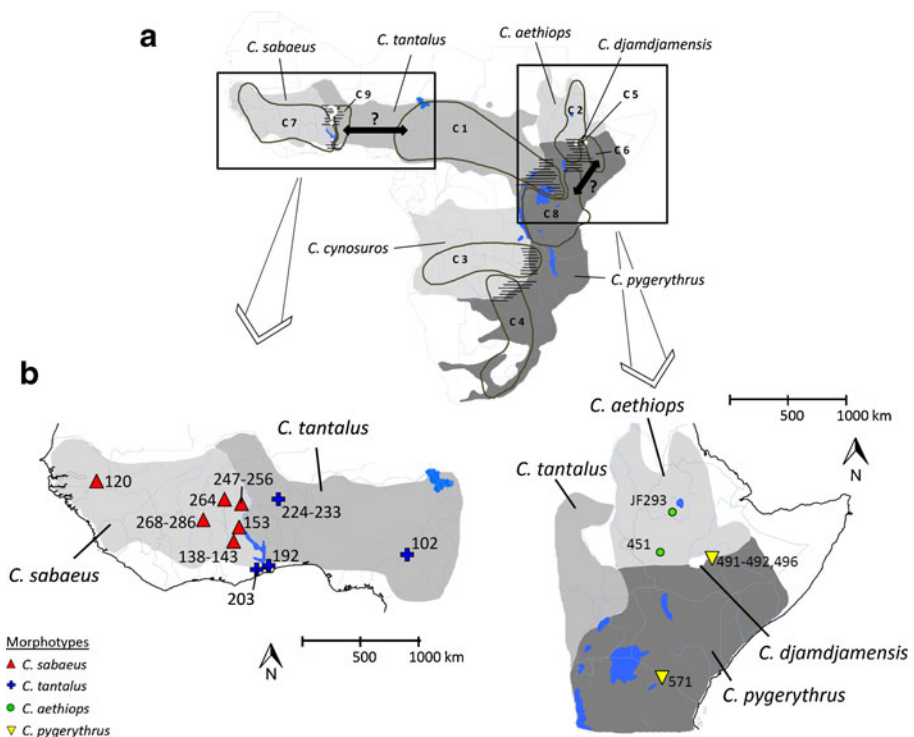
Fig. 1 Distribution map of African green monkeys (*Chlorocebus* spp.). **(a)** Distribution of mitochondrial DNA (mtDNA) clades and presumed hybridization in contemporary contact zones (hatched areas) according to Haus *et al.* (2013). Black arrows indicate possible ancient introgression events we test in this study. Possible hybridization in the West African contact zone is indicated with a white question mark. **(b)** Collection sites for samples of morphotypes from West and East Africa used in this study.

subsequent cytonuclear extinction through ancient introgression, as suggested for some baboon taxa (Zinner *et al.* 2009).

There is conflicting information on the occurrence of hybridization between *Chlorocebus sabaeus* and *C. tantalus*. These taxa seem to be separated by the White Volta and the Oti River in Ghana, with *Chlorocebus sabaeus* west and *C. tantalus* east of the rivers (Fig. 1; Booth 1956, 1958; Haus *et al.* 2013). However, hybridization has been assumed to occur in the northern part of the contact zone in Burkina Faso, where confluents of the Volta River are unlikely to represent geographical barriers for the monkeys (Fig. 1a; Lernould 1988). The analysis of samples collected east and west of the Volta River and its tributaries in northern Ghana and in Burkina Faso yielded concordant results between mt-haplotype variation and morphotype distribution, suggesting that no gene flow exists in the northern part of the contact zone (Haus *et al.* 2013). An exceptional population of *Chlorocebus tantalus* population occurs west of the Volta River in south Ghana (Booth 1956, 1958; Groves 2001). Despite its morphology, which clearly resembles that of *Chlorocebus tantalus*, mtDNA of this population falls within the *C. sabaeus* clade (C 7), suggesting introgressive hybridization in the south (Haus *et al.* 2013; Fig. 1). However, mtDNA analysis alone cannot detect hybridization processes such as Y-chr capture, and the possibility remains that hybridization also occurred in the northern range.

We here analyze two Y-chr markers for 30 African green monkey samples and compare genetic relationships based on Y-chr sequences to those based on previously published mtDNA sequences and morphology. We focus 1) on potential ancient introgression events within *Chlorocebus pygerythrus* from East Africa and within *C. tantalus*, which may have caused the observed polyphyletic relationships in the mtDNA phylogeny (Haus *et al.* 2013); and 2) on the hypothesized hybridization in the West African contact zone between *Chlorocebus sabaeus* and *C. tantalus* (Fig. 1b). If some mtDNA clades in *Chlorocebus pygerythrus* and *C. tantalus* represent relict mitochondrial genomes resulting from ancient introgression, then we predict a pattern of Y-chr DNA relationships that is concordant with the species morphology, but not with the native mtDNA. If male-mediated introgressive hybridization has occurred or is ongoing among African green monkey species in the West African contact zone, we predict that our analysis will reveal discordant phylogenetic relationships based on Y-chr and mtDNA data.

Methods

Data Sampling

We collected fecal samples of *Chlorocebus aethiops* and of the population of *C. pygerythrus* from southeast Ethiopia. We also collected samples at eight different sites on both banks of the Volta River and its confluents in Ghana and Burkina Faso covering the distribution of the western clade of *Chlorocebus tantalus* as well as the potential contact zone of *C. sabaeus* and *C. tantalus* (Fig. 1b, Table I). We preserved feces in >90% ethanol for ≥24 h, dried them, and transferred them into tubes with silica beads for further storage (Nsubuga *et al.* 2004). Whenever we observed a green

Table I Sample identities, collection sites, and GenBank accession numbers of the two Y-chr markers SRY and ZFY and the mitochondrial cytochrome *b* gene (cyt *b*) for *Chlorocebus* samples used in this study

ID	Species	Country	Location	Longitude latitude	GenBank accession no.		
					SRY	ZFY	Cyt *b*
JF293	aet	—	—	—	JF293182[b]	JF293267[b]	—
102	tan	Nigeria	Gashaka Gumti NP	11.19677 31.73892	KC843980	KC844009	JX983843[a]
120	sab	Senegal	Niokolo Koba NP	13.02577 13.23736	KC843991	KC844020	JX983828[a]
138	sab	Ghana	Bui NP	8.29083 −2.28465	KC844002	KC844031	JX983804[a]
139	sab	Ghana	Bui NP	8.29114 −2.28431	KC843997	KC844026	JX983805[a]
142	sab	Ghana	Bui NP	8.29114 −2.28431	KC843992	KC844021	JX983806[a]
143	sab	Ghana	Bui NP	8.29090 −2.28388	KC843990	KC844019	JX983807[a]
153	sab	Ghana	Mole NP	9.26008 −1.86058	KC843993	KC844022	JX983811[a]
192	tan	Ghana	Kalapka RR	6.45293 0.38055	KC843985	KC844014	JX983840[a]
203	tan	Ghana	Shai Hills RR	5.89777 0.06897	—	—	JX983823[a]
224	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	KC843984	KC844013	JX983831[a]
225	tan	Burkina Faso	Park D'Arly	11.60094 1.39187	KC843981	KC844010	JX983832[a]
226	tan	Burkina Faso	Park D'Arly	11.58281 1.46103	KC843982	KC844011	JX983833[a]
233	tan	Burkina Faso	Park D'Arly	11.58087 1.45781	KC843983	KC844012	JX983829[a]
247	sab	Burkina Faso	Ranch Nazinga	11.14870 −1.62334	KC843999	KC844028	JX983796[a]
255	sab	Burkina Faso	Ranch Nazinga	11.1554 −1.61030	KC843987	KC844016	JX983801[a]
256	sab	Burkina Faso	Ranch Nazinga	11.15547 −1.61030	KC843988	KC844017	JX983802[a]
264	sab	Burkina Faso	Deux Bale Foret	11.55079 −2.95757	KC843998	KC844027	JX983792[a]
268	sab	Burkina Faso	Comoe—Leraba NP	9.90260 −4.65454	KC844004	KC844033	JX983778[a]
269	sab	Burkina Faso	Comoe—Leraba NP	9.90260 −4.65454	KC843989	KC844018	JX983779[a]

Table I (continued)

ID	Species	Country	Location	Longitude latitude	GenBank accession no.		
					SRY	ZFY	Cyt b
271	sab	Burkina Faso	Comoe—Leraba NP	9.90260 −4.65454	KC844003	KC844032	JX983780[a]
275	sab	Burkina Faso	Comoe—Leraba NP	9.84896 −4.62302	KC843995	KC844024	JX983783[a]
277	sab	Burkina Faso	Comoe—Leraba NP	9.85291 −4.61415	KC844000	KC844029	JX983784[a]
284	sab	Burkina Faso	Comoe—Leraba NP	9.86207 −4.66974	KC843996	KC844025	JX983788[a]
285	sab	Burkina Faso	Comoe—Leraba NP	9.86207 −4.66974	KC844001	KC844030	JX983789[a]
286	sab	Burkina Faso	Comoe—Leraba NP	9.86207 −4.66974	KC843994	KC844023	JX983790[a]
451	aet	Ethiopia	Bonga	7.26645 36.23131	KC843976	KC844005	JX983743[a]
491	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	KC843977	KC844006	—
492	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	KC843978	KC844007	—
496	pyg	Ethiopia	Sof Omar Caves	6.91205 40.84583	—	—	JX983764[a]
569	sab	PEI, Barbados	—	—	KC843986	KC844015	—
571	pyg	PEI, TZ/KY	—	—	KC843979	KC844008	—

aet = *aethiops*; pyg = *pygerythrus*; tan = *tantalus*; sab = *sabaeus*; PEI = Paul Ehrlich Institute; TZ/KY = Tanzania/Kenya; NP = National Park; RR = Resource Reserve.

[a] Haus et al. (2013).

[b] Roos et al. (2011).

monkey we recorded information on external morphological characters based on Hill (1966), Napier (1981), and Groves (2001) to determine the species (Table II). We recorded all geographic localities using GPS (Table I). We also analyzed fecal samples from *Chlorocebus tantalus* (East Nigeria) and *C. sabaeus* (Senegal) (Haus *et al.* 2013), and blood samples from captive *C. sabaeus* and *C. pygerythrus* from the Paul Ehrlich Institute (PEI) in Langen, Germany. These individuals were offspring of green monkeys originating from Barbados and from East Africa (Kenya or Tanzania), respectively (Fig. 1b, Table I).

We conducted data collection in compliance with the animal care regulations and the principles of the American Society of Primatologists for the ethical treatment of nonhuman primates. We collected all fecal samples from wild, nonhabituated groups without threatening or harming the subjects. We obtained blood samples as part of regular diagnostic checks by veterinarians from the PEI. The research complied with

Table II External morphological characters used to identify different *Chlorocebus* species in West and East Africa in this study

Species	Diagnostic morphological features[a]			IDs of assigned groups/ samples
	Face	Tail	Extremities	
aethiops	Narrow white brow band, elongated white cheek whiskers, fine white moustache	Paracaudal white tuft, whitish tale tip	Gray limbs, hands and feet pale	451
pygerythrus	White brow band, very short whiskers form a complete face-ring	Red subcaudal patch, no tuft, black tail tip	Color of limbs like dorsum, black hands and feet	491, 492, 496, 571
sabaeus	Brow band poorly expressed or absent, yellow whiskers directed upward in front of ears, whorl in front of ears	No tuft, yellow golden tail tip	Grizzled golden limbs, hands and feet pale	120, 138, 139, 142, 143, 153, 255, 256, 264, 247, 268, 269, 271, 275, 277, 284, 285, 286, 569
tantalus	Long but tiff whiskers, yellowish, brushed up, tapered white brow band, black bar from whiskers	Paracaudal white tuft, pale or creamy tail tip	Gray limbs, pale feet	102, 192, 203, 224, 225, 226, 233

Sample IDs correspond to IDs in Fig. 1b and Table I.

[a] Hill (1966); Napier (1981) and Groves (2001).

protocols approved by the German Primate Center and the PEI in Germany, the Ethiopian Wildlife and Conservation Authority (EWCA) in Ethiopia, the Centre National de la Recherche Scientifique et Technologique (CNRST) in Burkina Faso, and the Forestry Commission (FC) of Ghana, and adhered to the legal requirements of the countries in which the research was conducted.

DNA Extraction, Amplification, and Sequencing

We extracted total genomic DNA from fecal and blood samples using the QIAamp DNA Stool Mini Kit and the DNeasy Blood & Tissue Kit from Qiagen (Qiagen, Germany), respectively. We applied standard protocols as provided by the company with only minor changes for the extraction of fecal samples (Haus *et al*. 2013). We stored blood sample extracts at 4°C and fecal sample extracts at –20°C in 50-µl aliquots for up to 24 mo before further processing.

Only samples from males can be used in the analyses of Y-chr markers. Because the collection of fecal samples from unhabituated wild animals does not allow the assignment of samples to individuals, we obtained information on the individuals' sex using a gonosomal polymerase chain reaction (PCR) –based sexing method (Roos *unpubl. data*).

We amplified and sequenced fragments of two Y-chr genes: 695 bp of the last intron of the zinc finger (ZFY) and a 783 bp long fragment of the sex determining region (SRY). We amplified both markers via two overlapping fragments, each measuring *ca.* 450–500 bp length, using the primer pairs ZFY-F1: CCTGATTCCAG GGAGTACC and ZFY-R1: AGTAAAGCTTAACTGCACCTAT, ZFY-F2: AGGACA GATTACTATCCTGTG and ZFY-R2: CAGTATGAGTGCTTAATCAAAC, SRY-F1: CTTGAGAATGAATACATTGTCAGGG and SRY-R1: GTATCCCAGCTGCTTGC TG, and SRY-F2: GATCAGAGGCGCAAGATGG and SRY-R2: AGGTCTTTGTAGC CAATGTTACCCG. We used 1 U BiothermTaq 5000 (Genecraft, Germany) in a 30-μl PCR mix (1× reaction buffer, 0.16 mM for each dNTP, 0.33 μM for each primer, and 0.6 mg/ml bovine serum albumin), with the following thermo cycler conditions: 94°C for 2 min, followed by 60 cycles of 94°C for 1 min, 60°C (SRY) and 58°C (ZFY) for 1 min, 72°C for 1 min, and 72°C for 5 min. We conducted all PCR reactions with at least one PCR blank (HPLC-purified water). We ran and checked PCR products on 1–2% agarose gels, excised DNA fragments of relevant lengths, and purified PCR products with the Qiagen Gel Extraction Kit (Qiagen, Germany). We used the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Germany) and the respective forward and reverse primers to run sequences on an ABI 3130 *xL* sequencer. We deposited all sequences in GenBank (Table I).

Analysis of Genetic Structures

To compare the results obtained from the Y-chr data with those for mtDNA in African green monkeys, we used published cytochrome *b* (cyt *b*) gene sequences from Haus *et al.* (2013). We selected cyt *b* sequences from the same samples for which we sequenced Y-chr markers. In cases where mitochondrial sequences were not available we used sequence information from samples that were collected at the same site, assuming that indivduals from the same social group carry similar mtDNA (Fig. 1, Table I).

We assembled and aligned all Y-chr sequences with the program Geneious Pro 5.0.4 (Drummond *et al.* 2011) and added an additional sequence from GenBank representing *Chlorocebus aethiops* (Roos *et al.* 2011; Table I). We also used Geneious Pro 5.0.4 to prepare two separate alignments, one concatenated alignment of the two Y-chr markers ZFY and SRY, and a second alignment including all cyt *b* sequences. To remove deletions in the Y-chr data set we used Gblocks (Talavera and Castresana 2007), leading to a final length of the Y-chr alignment of 1476 bp, for which we measured variable and parsimony-informative sites with Mega 5.0 (Tamura *et al.* 2011). To compare genetic patterns of Y-chr and mtDNA we calculated median-joining networks for both data sets separately, using Network 4.610 (Bandelt *et al.* 1999). Finally, we displayed and edited resulting networks with Network Publisher.

Results

The samples in our analysis represent four morphological species: *Chlorocebus aethiops*, *C. pygerythrus*, *C. sabaeus*, and *C. tantalus* (Fig. 1). PCR-based sexing indicated that our fecal sample data set includes 28 samples from male individuals

originating from all sampling sites except the population of *Chlorocebus tantalus* west of the Volta River in south Ghana, for which we were not able to sample a male. For this site we have only mtDNA information (ID 203 in Fig. 1b).

The analysis of Y-chr markers showed a low genetic variability among the 30 samples analysed, with nine variable and seven parsimony-informative sites within the concatenated 1476 bp data set of ZFY and SRY. Accordingly, we refrained from reconstructing a rooted phylogenetic tree, and instead used median-joining networks to depict haplotype diversity and genetic distance among haplotypes and clades, respectively. We found 6 Y-chr haplotypes, whereas the corresponding cyt *b* sequences represent 17 haplotypes forming 6 mtDNA clades. Y-chr sequences of *Chlorocebus pygerythrus* from southeast Ethiopia are identical to the *C. pygerythrus* sequence from Kenya/Tanzania and thus constitute a single haplotype, whereas mtDNA reveals two distinct clades, one for southeast Ethiopia and one for Kenya/Tanzania (Fig. 2). In contrast, the analysis of samples of *Chlorocebus tantalus* from east of the Volta River and its confluents in Ghana and Burkina Faso indicates a distinct Y-chr haplotype, which differs from the haplotype obtained from East Nigerian samples of *C. tantalus*. This is in agreement with the mtDNA results, where two distinct clades are also found. Y-chr sequences of *Chlorocebus sabaeus* and *C. tantalus* from Ghana and Burkina Faso represent two different haplotypes, which are clearly separated east and west of the Volta River, again reflecting the same pattern as the mtDNA data.

Discussion

East Africa

Based on mtDNA two distantly related *pygerythrus* clades occur in East Africa, one in southeast Ethiopia (C 6), which is closely related to *Chlorocebus djamdjamensis*, and a second in Kenya and Tanzania (C 8, Fig. 1; Haus 2013). Our results show that both clades possess the same Y-chr haplotype despite their large mtDNA divergence. Because the social system of African green monkeys is characterized by male-biased dispersal (Jaffe and Isbell 2011; Kingdon 1997), male-mediated introgressive hybridization is the most probable explanation for this discordant pattern between maternal (mtDNA) and paternal (Y-chr) inherited markers. Given the large mtDNA divergence (Haus 2013), it is unlikely that this discordance has been caused by geographic isolation and the higher rate of mtDNA evolution in comparison to Y-chr DNA, which can induce a similar discordance. Therefore, ancient introgressive hybridization and nuclear swamping might have led to a morphotype of *Chlorocebus pygerythrus* in southeast Ethiopia that carries Y-chr DNA of *C. pygerythrus* (and likely autosomal DNA), but the mtDNA of a possible now cytonuclear extinct taxon.

West Africa

We found a different picture in *Chlorocebus tantalus*. Both Y-chr and mtDNA data support the differentiation of *Chlorocebus tantalus* into a western and eastern clade despite their morphological similarity (Figs. 1 and 2). Here, the genetic concordance of maternal and paternal inherited markers is inconsistent with introgression patterns
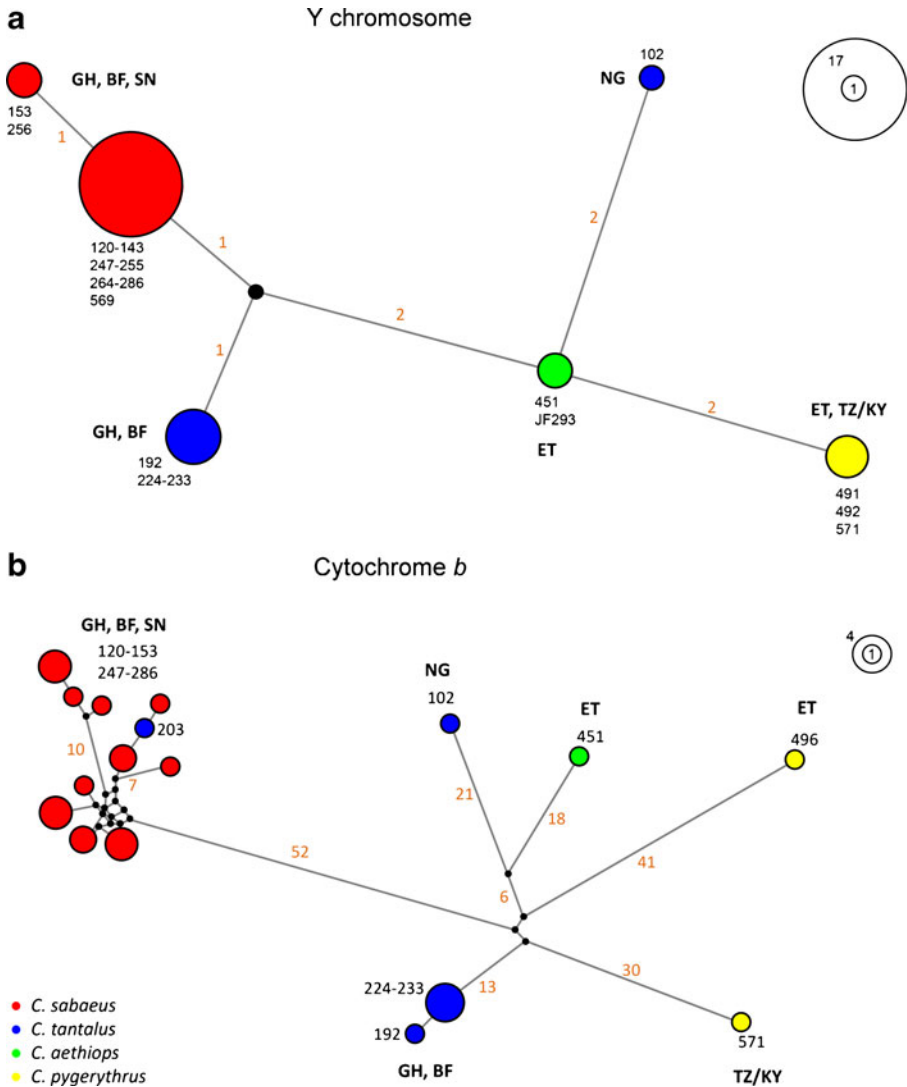
Fig. 2 Median-joining networks of **(a)** Y-chr and **(b)** cyt *b* haplotypes of samples of *Chlorocebus* from West and East Africa. Circle sizes specify haplotype frequencies as shown by the scales. Colors represent morphotypes, orange numbers indicate mutated positions of major branches, and black numbers correspond to sample IDs in Fig. 1b and Table I. GH = Ghana; BF = Burkina Faso; SN = Senegal; NG = Nigeria; ET = Ethiopia; TZ/KY = Tanzania/Kenya.

caused by male dispersal. However, a population genetic approach with more diverse nuclear markers and a larger data set from other parts of the range of *Chlorocebus tantalus* are needed to test for potential admixture in other regions of the genome. An alternative explanation for the discrepancy between genetic relationships and morphotype variation may be cryptic genetic, and thus, possibly taxonomic diversity. Although recent primate taxonomies recognize only the *Chlorocebus tantalus tantalus* subspecies for the region west of Cameroon, Schwarz reported a different morphotype from Togo in 1926 (Schwarz 1926), but this was not accepted by other authors (Booth

1956; Groves 2001, 2005; Hill 1966). Another *tantalus*-like morphotype (*chrysurus*) with unknown type locality was described by Blyth (1845), which is currently recognized as a synonym of *tantalus* (Hill 1966) or *sabaeus* (Groves 2001; Napier 1981). A detailed morphological analysis covering the whole range of *Chlorocebus tantalus* is required to determine whether the two detected genetic clades are similar or differ in morphology. Because there are no type localities for *tantalus* or *chrysurus*, further genetic and morphological analyses should include both type specimens to test whether they belong to one of the clades detected and whether further nuclear data also support two different genetic clades.

Hybridization between *Chlorocebus sabaeus* and *C. tantalus* was thought to occur only in the northern range of the Volta River and its tributaries in Ghana and Burkina Faso (Lernould 1988), but mtDNA results provided evidence for a contradicting picture with hybridization in the south but not in the north (Haus *et al.* 2013). Although our data set may not include all populations of *Chlorocebus sabaeus* and *C. tantalus* along the Volta River and its tributaries, the analysis of samples from the northern part of their potential contact zone showed no inconsistencies between the genetic patterns of Y-chr and mtDNA, nor between the Y-chr and morphological data. Accordingly, there is also no Y-chr evidence of interspecific gene flow across the Volta River in northern regions. In contrast, discordance between morphotype and mtDNA from a single population of *Chlorocebus tantalus* from the Accra plains west of the Volta River in Ghana suggests introgressive hybridization in the south (Fig. 1a; Haus *et al.* 2013). Unfortunately, our data set did not include male samples from this population, preventing us from analyzing Y-chr markers. However, there is no evidence of morphological individuals of *Chlorocebus sabaeus* in or close to this area and because this population of *C. tantalus* carries the mitochondrial genome of *C. sabaeus* (Booth 1956, 1958; Haus *et al.* 2013), introgressive hybridization remains the most plausible explanation here. The factors that led to this unexpected pattern remain unclear. One possible explanation is that limited hybridization across the Volta River occurred throughout the contact zone of *Chlorocebus sabaeus* and *C. tantalus* in the past, when African green monkey populations were distributed more commonly along the river banks than today. If this is the case, then the *C. tantalus* population west of the Volta River in South Ghana may represent the only remaining hybrid population. Alternatively, if the Volta River represents a geographic barrier to the distribution of both species, individuals of *Chlorocebus tantalus* from the eastern river bank may have been introduced into a population of *C. sabaeus* west of the Volta River by humans. Such anthropological impact is not unlikely, as African green monkeys are often kept as pets (Riley *et al.* 2011).

Conclusions

Our analysis of sex-linked markers indicates that two populations exist in East African *Chlorocebus pygerythrus* carrying distinct mtDNA haplotypes, but the same Y-chr haplotype, suggesting that male-mediated gene flow between these populations existed in the past or is still ongoing. In contrast, the two populations of *Chlorocebus tantalus* in West Africa, which were detected based on mtDNA, also carry distinct Y-chr haplotypes. Here it remains to be tested whether (cryptic) speciation is in progress

or if genomic admixture due to ancient introgression has occurred in a part of the range of *Chlorocebus tantalus*. The analysis of the potential West African contact zone showed that *Chlorocebus sabaeus* and *C. tantalus* differ in mtDNA as well as Y-chr DNA. These results suggest that ongoing interspecific gene flow between these species across the Volta River is strongly reduced or even absent, with one exception in South Ghana, where introgressive hybridization may have led to a phenotypic population of *C. tantalus* west of the Volta River carrying mtDNA of *C. sabaeus*. Our study highlights the importance of the analysis of Y-chr data in addition to mtDNA to obtain information on hybridization patterns, and especially, on ancient events, which might have influenced the genetic diversity of some taxa.

Our results indicate that the evolution of African green monkeys was influenced by introgressive hybridization, most probably with the cytonuclear extinction of some populations or taxa. This aspect may also play an important role in the survival of the endemic species *Chlorocebus djamdjamensis*. Continuing asymmetric introgression between *Chlorocebus aethiops* and *C. djamdjamensis* in Ethiopia may also lead to the cytonuclear extinction of the restricted species *C. djamdjamensis* (Haus *et al.* 2013; Kingdon 1997; Mekonnen *et al.* 2012). A comprehensive genetic analysis of samples including all remaining populations of *Chlorocebus djamdjamensis* is needed to explore the progress and pattern of introgression.

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