Mitochondrial DNA analyses show that Zambia’s South Luangwa Valley giraffe (Giraffa camelopardalis thornicrofti) are genetically isolated

Julian Fennessy1*, Friederike Bock2, Andy Tutchings1, Rick Brenneman1,3 and Axel Janke2,4

1Giraffe Conservation Foundation, 26 Grasmere Road, Parley, Surrey, CR8 1DU, England, U.K. 2Senckenberg Gesellschaft für Naturforschung, Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, Frankfurt am Main, 60325, Germany, 3Grewcock Center for Conservation and Research, Omaha’s Henry Doorly Zoo and Aquarium, 3701 South 10th Street, Omaha, NE 68107, U.S.A and 4Goethe University Frankfurt, Siemensstr. 70, 60323, Frankfurt am Main, Germany

Abstract

Thornicroft’s giraffe, Giraffa camelopardalis thornicrofti, is a geographically isolated subspecies of giraffe found only in north-east Zambia. The population only occurs in Zambia’s South Luangwa Valley, an area which interestingly places it between the current distribution of Masai (G. c. tippelskirchi) giraffe to the north, and the Angolan (G. c. angolensis) and South African (G. c. giraffa) giraffe in the south-west and south, respectively. Specific studies have been undertaken on the ecology of this subspecies, but their population genetics remains unknown. We studied 34 individuals from the South Luangwa National Park and adjacent Lupande Game Management Area and seven individuals from northern Botswana. The complete cytochrome b and control region sequences of the mitochondrial genome were sequenced and analysed together with database data by maximum likelihood tree reconstruction and maximum parsimony network analyses. The giraffe from Zambia’s South Luangwa Valley are most closely related to the subspecies G. c. tippelskirchi and part of their radiation. However, they form a unique population that would benefit from increased research and conservation management.

Key words: Giraffa, phylogeny, population genetics, Thornicroft’s giraffe, Zambia

Résumé

La girafe de Thornicroft, Giraffa camelopardalis thornicrofti, est une sous-espèce isolée géographiquement que l’on ne trouve qu’au nord-est de la Zambie. Cette population ne vit que dans le sud zambien de la Vallée de la Luangwa, une zone qui la situe entre la distribution actuelle de la girafe masai (G. c. tippelskirchi) au nord, celle de la girafe d’Angola (G. c. angolensis) et celle d’Afrique du Sud (G. c. giraffa), respectivement au sud-ouest et au sud. Des études spécifiques ont été entreprises sur l’écologie de cette sous-espèce, mais la génétique de cette population reste inconnue. Nous avons étudié 34 individus du Parc National de South Luangwa et de l’Aire de gestion de la faune de Lupande qui lui est proche et sept individus du nord du Botswana. Les séquences complètes du cytochrome b et d’une région de référence du génome mitochondrial ont été réalisées et analysées en regard des données de référence par des analyses par reconstruction de l’arbre selon le principe du maximum de vraisemblance et par la méthode de parcimonie maximale. Les girafes de la Vallée de South Luangwa en Zambie sont plus étroitement apparentées à la sous-espèce G. c. tippelskirchi et font partie de leur radiation. Cependant, elles constituent une population unique qui aurait tout intérêt à faire l’objet de nouvelles recherches et d’une meilleure gestion de conservation.

Introduction

Thornicroft’s giraffe, Giraffa camelopardalis thornicrofti Lydekker, 1911, is an isolated population restricted to Zambia’s South Luangwa Valley. The population has been loosely monitored since the mid 1960’s and appears to be
viable and stable. Although no formal or accurate census has been conducted, the size of the population is estimated to be between 750 and 1000 individuals over the 13,890 km² of the South Luangwa National Park and Lupande Game Management Area. Little is known about this giraffe race beyond a range movement study (Berry, 1978) and recently published reproductive and herd size studies (Bercovitch & Berry, 2009a,b). The regional habitat is characterized by riparian forest, mopane and mungo woodlands, thickets, scrub and open grassland (Astle, Webster & Lawrance, 1969; Fanshawe, 1969). The giraffe population in the South Luangwa Valley is geographically isolated from the nearest giraffe populations by a minimum of 400 km north to Tanzania (G. c. tippelskirchi) and 450 km south and south-west to western Zambia, Botswana and Zimbabwe (possibly G. c. angolensis and/or G. c. giraffa respectively).

Giraffe (Giraffa camelopardalis spp.) taxonomy has been confusing and even contradictory for nearly 250 years (Fennessy, 2004, 2008). Even today, many aspects remain unresolved, but recent efforts using molecular genetic techniques are providing insight into untangling the twisted status (Brown et al., 2007; Hassanin et al., 2007). It is widely accepted that there are nine Giraffa camelopardalis subspecies. In the most recent literature, Brown et al. (2007) provided phylogenetic trees for six of the currently recognized subspecies: G. c. angolensis, G. c. giraffa, G. c. peralta, G. c. reticulata, G. c. rothschildi and G. c. tippelskirchi and indicated that some of these may actually be distinct enough as stand-alone species.

We sequenced and analysed mitochondrial DNA fragments to study the population genetics of Thornicroft’s giraffe matriline from Zambia’s South Luangwa Valley. Previous studies on giraffe genetics utilized the complete mitochondrial cytochrome b and control region sequences for which 35 haplotypes have been described for G. c. angolensis, G. c. antiquorum, G. c. giraffa, G. c. peralta, G. c. reticulata, G. c. rothschildi and G. c. tippelskirchi (Brown et al., 2007) as well as G. c. antiquorum and G. c. peralta (Hassanin et al., 2007). With this approach, we determined the genetic variability of the matriline and closest giraffe subspecies to the Zambia’s South Luangwa Valley giraffe.

Material and methods

In July 2008, 34 samples from Thornicroft’s giraffe individuals from the free-ranging population of Zambia’s South Luangwa National Park and the adjacent Lupande Game Management Area were taken using remote delivery biopsy darting. Skin biopsies were stored at room temperature in a tissue preservative buffer (Seutin, White & Boag, 1991) with glutaraldehyde prior to DNA isolation. In addition, seven individuals from northern Botswana were sampled by the same technique. A blood sample from the okapi (Okapia johnstoni) was collected from a zoo individual (Basel, Switzerland) to give the phylogenetic analysis a direction as the closest outgroup to the giraffe. Whole genomic DNA was extracted from tissue and blood using standard procedures (Sambrook, Fritsch & Maniatis, 1989).

Giraffe specific PCR primers were constructed from database sequence (accession number AP003424) for PCR amplifying and sequencing the complete cytochrome b (forward: 5’TGAAAAACCATCGTTGCTGT-3’; reverse: 5’-GTGGAAGCGGAAGATC-3’) and complete control region sequences (forward: 5’TACACTGTCCTCTTGAAGC-3’; reverse: 5’TCCGTTTGTGTTTAAAGC-3’).

PCR amplification of mitochondrial genes was performed on 10 ng DNA using Ex-Taq (TAKARA BIO, Inc., Otsu, Shiga, Japan) according to the manufacturers’ recommendation. For the DNA sequencing, the purified PCR products were eluted in water using 5 ng of PCR product for one reaction. The cycle sequencing was accomplished with a BigDye terminator sequencing kit 3.1 (Applied Biosystems Inc. [ABI], Life Technologies, Carlsbad, CA, USA). The reactions were then analysed in house on an ABI 3730 DNA Analyzer (accession numbers HF571176 – HF571217).

The sequences were aligned by Clustal X (Thompson et al., 1997) and manually edited in Geneious version 5.6.4 (Biomatters – www.geneious.com). The corresponding sequences from the O. johnstoni were added to determine the root of the phylogenetic tree. The programme Treefinder (TF) version of October 2008 (Jobb, von Haeseler & Strimmer, 2004) was used for model testing. The individual mitochondrial sequences and a combination of both were analysed using maximum likelihood.

The statistical parsimony networks of the control region and cytochrome b sequences were generated using the TCS programme version 1.21, with a setting to a 95% connection limit (Clement, Posada & Crandall, 2000). Standard graphics programmes were used to improve trees and networks graphics.

Results

Most samples were amplified and sequenced from both ends of the cytochrome b gene. The control region amplified well.
but it could only be sequenced from the L-strand for 858 nt to the polycytidine region. All attempts to sequence across the repetitive region, with specific H- or L-strand primers, failed to yield clear sequences for all individuals. Except for the length variations in the poly-C region, the remainder of the control region sequences provided unambiguous sequence alignments. Therefore, the control region sequence was limited to 789 nt after the problematic 206 nt were discarded. The alignment was straightforward with no gaps or ambiguous sites in the 1140 nt long cytochrome b gene, and only few in the control region (789 nt alignment length). The position of the gaps was unambiguous, but is ignored by the TF programme in the ML analyses. The combined data set was 1929 nt long.

The sequences within the G. c. thornicrofti were identical for the cytochrome b gene and control region sequences. The mean sequence distance among all giraffes was 0.024 + /− 0.003, with a maximum distance of 0.048 for one G. c. tippelskirchi relative to a G. c. giraffa individual, to a G. c. tippelskirchi individual as well as to five individuals of the Botswana samples. TF model testing recommended the HKY model (Hasegawa, Kishino & Yano, 1985) of sequence evolution with five categories of gamma rate variation (5G) for the individual and combined data set analysis.

The ML analysis of the concatenated data set is shown in Fig. 1. Zambia’s South Luangwa Valley giraffes (LVNP), G. c. thornicrofti, are a monophyletic group that is supported by an 87% TF support value. The G. c. thornicrofti are part of the G. c. tippelskirchi radiation, which are relatively close to the G. c. antiquorum and G. c. reticulata lineage. The ML tree shows some G. c. tippelskirchi within G. c. antiquorum and G. c. reticulata. As expected from the geographical distance, the most distant group in this data set is G. c. angolensis. The G. c. giraffa individuals do not cluster together on a branch but appear in different places along the tree. The TF support values for these results are given in the figure indicating that the main findings are well supported with 80–100% support value.

The tree analyses of individual sequences generally confirm these findings (Fig. S1, S2). The individuals from zoos (marked by *) and the two from which the population but no geographical origin was given (marked by +) are grouping with the expected subspecies. The analyses of the mitochondrial loci separately confirm these findings.

MP network analysis of the concatenated data set supports the results from the ML analyses, but under the given strict settings, some populations are not connected to each other when the network method cannot find a MP

Discussion

While the taxonomic status of the Thornicroft’s giraffe (G. c. thornicrofti) remained without synonyms since first
described by Lydekker (1911), this has not been without dissenting discourse among scientists. As with the other giraffe subspecies, there has been controversy over the taxonomic status with some agreeing that this is a true subspecies, some suggesting that it may be identical with G. c. inflamata (synonym), others suggesting that it should be grouped with other southern African subspecies (Dagg & Foster, 1982), and most recently, that it is a separate species (Groves & Grubb, 2011).

Seymour (2002) studied museum specimens of six of the giraffe subspecies and characterized skull morphologies, horn morphologies and pelage pattern differences, as well as mtDNA sequences from the D-loop or control region. The pelage pattern of the Thornicroft’s giraffe is unique with the spots generally having sharper edges than those of other subspecies and being larger over the front quarters and much smaller on the back quarters (Seymour, 2002). Similarly, Seymour (2002) found the skull demonstrated a more robust morphology yet with smaller parietal horns than those found in most other subspecies. This trait although is not diagnostic as the phenotypic distribution does overlap the distributions of other southern African giraffe subspecies.

While these characteristics suggested the differentiation into subspecies, a single mitochondrial haplotype was sequenced by Seymour (2002) among all of the G. c. thornicrofti samples studied, and this haplotype was shared by some G. c. tippelskirchi museum specimens from southeastern Kenya and Tanzania. However, in this study, the single haplotype encountered was monophyletic to G. c. thornicrofti but found nested within the G. c. tippelskirchi clade representing the populations of the western Rift Valley (Brown et al., 2007).

From a molecular genetics perspective, G. c. thornicrofti might be a candidate for being subsumed into G. c. tippelskirchi. The apparent lack of genetic diversity in this highly variable mtDNA region is striking and suggests that this population likely grew out of a very small number of founders or survivors from a time when the last common ancestor of what we know today as G. c. tippelskirchi and G. c. thornicrofti may have inhabited the region of Africa extending from Tanzania and southern Kenya to, potentially, Botswana. Morphologically, however, there are skull and pelage differences that do separate it from G. c. tippelskirchi. It is entirely possible that subtle phenotypic differences in the purported founders could have been maintained or even enhanced through genetic drift or selection in subsequent generations of the small and isolated population in South Luangwa Valley. At this time, none of these considerations are understood well enough to make a sound taxonomic change from the current status.

From an evolutionary perspective, the population may be quite important to the genus as it fills a niche in a habitat isolated by perhaps 400 km from the nearest northerly giraffe populations in Tanzania and some 450 km from the giraffe populations to the south in Zimbabwe. It is very surprising to find such a genetically homogenous group that shows no variation amongst their mitochondrial genome. This genetically despauate state suggests either very recent colonization of the area by females that were identical for their mt genome, or a recent and severe bottle-neck. Furthermore, it suggests that no other maternal lineage from the G. c. tippelskirchi subspecies entered the South Luangwa Valley. Therefore, the founding population of the current day Thornicroft’s giraffe survive as an isolated population with a limited amount of genetic variability and a rather small effective
population size. The uniqueness of the Thornicroft’s giraffe, combined with the clustering of the population separately from all other subspecies, is fascinating from a conservation perspective.

Two recent studies indicate that giraffe have a fusion/fusion social system with kinship exerting a major impact on giraffe social associations (Bercovitch & Berry, 2013; Carter et al., 2013). Both studies were conducted at sites with relatively isolated giraffe populations – South Luangwa National Park, Zambia (Bercovitch & Berry, 2013) and Etosha National Park, Namibia (Carter et al., 2013). It is unlikely that the similar social structures found at these two different field sites reflect a pattern common to giraffe elsewhere, but whether the patterns might be a consequence of a limited amount of genetic variation within the population increasing the probability of kinship as a factor regulating herd structure. Only further studies linking genetics and sociality among giraffe will determine whether this is accurate and whether it has any conservation implications.

Therefore, we suggest that the G. c. thornicrofti is a valid and important evolutionary unit and that no changes in subspecific designation be made until the complete phylogenetic tree can be constructed with most of the major giraffe populations and additional genetic markers included. Evolutionary trajectories of isolated populations like this population cannot be predicted, and in a changing world, such populations should be recognized, protected and conserved.

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References

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Supporting information 
Additional Supporting Information may be found in the online version of this article: 
Figure S1 ML phylogram of the control region sequence. 
Figure S2 ML phylogram of cytochrome b sequence. 
Figure S3 Individual MP networks of control region (A) and cytochrome b (B) sequences.