Short Communication

Conservation genetics of an isolated giraffe population in Swaziland

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Introduction

Giraffe (*Giraffa camelopardalis*) are a critical component of the savannah's browsing guild (Birkett, 2002), although their distribution is discontinuous and the species has declined over most of the range (Fennessy, 2008). Often confined to protected areas, management of the giraffes has increasingly focused on maintaining small, closed populations including private lands or conservation areas (Brenneman *et al.*, 2009b). The mating system and social structure of giraffe evolved in large, continuous savannah habitats. Females may only be sexually receptive for a few days every 2 weeks of oestrous cycle (Bercovitch, Bashaw & del Castillo, 2006), and males will travel up to 20 km per day in search of oestrous females (Du Toit, 2001). The nature of their highly varied range sizes (van der Jeugd & Prins, 2000; Fennessy, 2009), and potential for long-distance seasonal movements (Le Pendu & Ciofolo, 1999; Berger, 2004), makes understanding the potential impacts of managing giraffe as closed, fragmented populations an important research goal.

Populations in closed, geographically restricted areas face potential hardships not limited to lowered nutritional benefits (Brenneman *et al.*, 2009b) and the potential for deleterious genetic consequences associated with small populations, including low heterozygosity and inbreeding. Contemporary giraffe populations display a substantial amount of genetic structuring, even within recognized subspecies, possibly reflecting recent fragmentation of the savannah due to human population expansion and increased aridity (Brown *et al.*, 2007).

Our goal was to quantify the neutral genetic diversity of South African giraffes (*G. c. giraffa*) in a wild and free-roaming, but fenced (i.e. closed) population in NE Swaziland. Giraffes in Swaziland are thought to be extralimital, with no European records of their presence south of the Komati River in South Africa (Goodman & Tomkinson, 1987), although giraffe bones have been identified in KwaZulu-Natal from the warm Atlantic period of the Holocene (Cramer & Mazel, 2007). This population exists in two fenced areas, and our specific objectives were to characterize the genetic variation, degree of differentiation and amount of inbreeding, in order to evaluate the genetic consequences of closed giraffe population management. Given the small population size, restricted number of founders and lack of migration, we predicted that genetic variation would be low relative to other published giraffe genetic studies. We discuss the potential consequences and implications of isolated small giraffe populations to generalize about the genetic impacts of isolation and small population size and make suggestions for future work towards more resilient giraffe populations.

Methods

Mbuluzi Game Reserve is in northeast Swaziland and encompasses an area of 30 km^2 of lowveld acacia savannah. Mbuluzi is bisected by a two-lane paved road, and both sides of the reserve are fenced or bordered at the north and east by steep topography and the Mbuluzi River, effectively isolating giraffe in the north and south from one another and the surrounding agricultural and protected areas that lack giraffe populations (Fig. 1).

In 1994, ten giraffe were introduced into the southern portion; four giraffe sourced from Mopane and six from South Hampton Pens near Hoedspruit, South Africa. In 1997, eight giraffe were introduced into the northern

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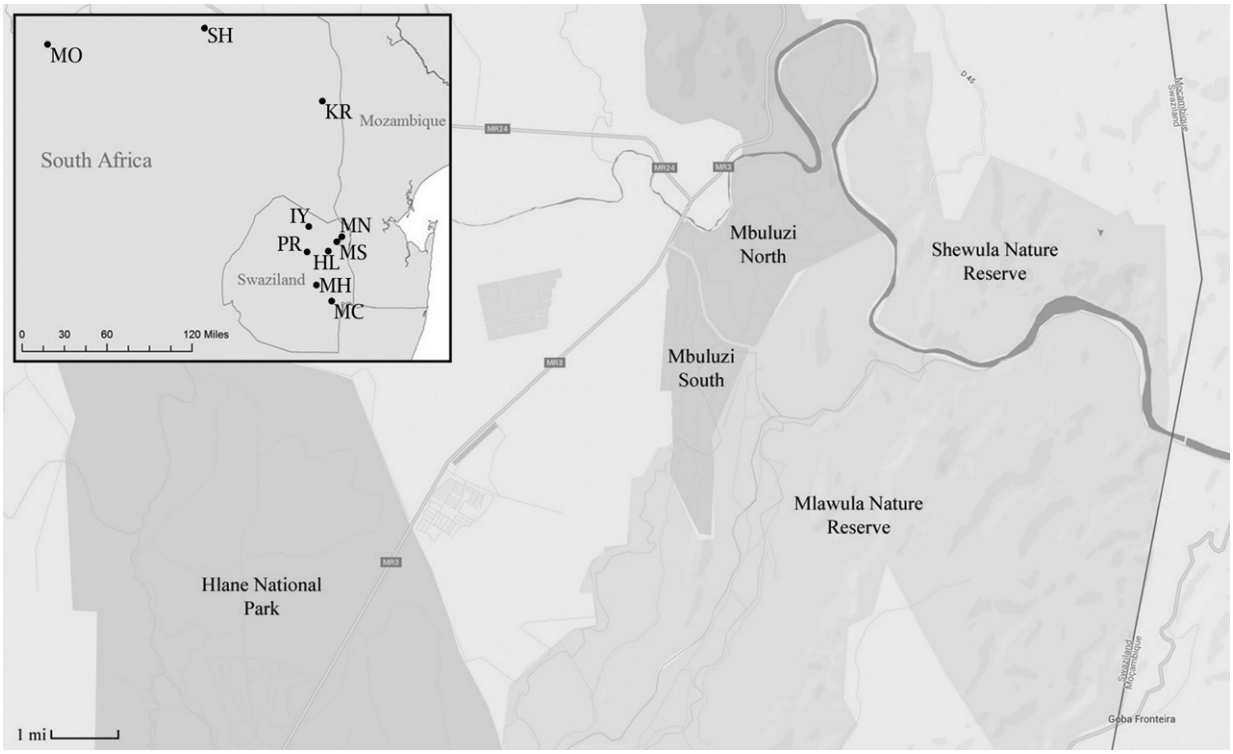

Fig 1 Location of the Mbuluzi Game Reserve within the Lubombo Conservancy lands in northeast Swaziland. Inset: Distribution of introduced giraffe populations in Swaziland (Mbuluzi North, MN, Mbulusi South, MS; Hlane Royal National Park, HL; Inyoni Yami Sugar Irrigation Scheme, IY; Mkhaya Game Reserve, MH the Mhlosinga Conservancy, MC; Panata Ranch, PR) and original source for Mbuluzi giraffe (Mopane, MO and South Hampton Pens, SH). Approximate location of Kruger Park (KR) is also indicated

portion of the reserve from Mhlosinga Nature Reserve, Swaziland. The stock origin of these giraffe is not known. Although no formal population estimates had been conducted at the time of sampling (2013), Mbuluzi staff estimated 20–25 giraffe on each of the north and south.

We collected giraffe faecal pellets over a 3-week period in June 2013 and June 2014. Giraffe were located after sunrise (06:00–10:00) and late afternoon (15:00–17:00) drives. We photographed individual giraffe, recorded gender, assigned age class (juvenile, adult) and noted any distinguishing scars. Photo IDs were subsequently used to identify animals in the field. At each sighting 'spotters' noted the location of defecation. We placed the fresh pellets in 95% ethanol or into envelopes for desiccation at ambient temperature. Over the 2 years, we obtained 48 faecal samples (putatively unique individuals), consisting of 25 from the south and 23 from the north. Two included individuals from the north were classified as juveniles.

We processed individual stool samples one at a time, under clean room conditions. We removed the outer layer of three pellets from each giraffe sample using a razor and placed into a common 25-ml Sarstedt tube, and then DNA was isolated using Qiagen QIAamp Stool Extraction kit (Qiagen Inc, Valencia, CA, U.S.A.). A final blank extraction was included for each day. We PCR amplified fourteen microsatellite loci (Gica9905, Gica9976, Gica13905, Gica16120, Gica10894 and Gica16160, Crowhurst *et al.*, 2013; 11HDZ562 and 11HDZ1004, Huebinger *et al.*, 2002; Gca_01, Gca_12, Gca_21, Gca_22 and Gca_25, Gca_26, Carter *et al.*, 2012) and combined individual loci into pools of 2–3 loci for electrophoresis. PCR amplifications consisted of 25 µl volume reactions containing 5.9 µl H_20, 7.5 µl Qiagen multiplex PCR Mastermix (Qiagen), 0.1 µM M13 labelled forward primer, 10 µM reverse primer, 10 µM M13 labelled dye primer (hexachlorofluorescein or 6-carboxyfluorescein)

and 20 ng DNA. Thermocycling conditions consisted of: 95°C for 15 min, 35 cycles at 94°C for 0.5 min, 58°C for 1.5 min, 72°C for 1.5 min and a final extension at 72°C for 10 min. Products were electrophoresed on an ABI 3130xl and scored alleles using GeneMarker® software (SoftGenetics, LLC, State College, PA, U.S.A.) with manually confirmation.

We assessed PCR error after two independent amplifications, followed by a third amplification for approximately 50% of loci to confirm homozygosity or to obtain consensus genotypes. We evaluated potential errors using MicroChecker (van Oosterhout, Hutchinson & Wills, 2004). All PCRs included a negative control. We checked for matching genotypes using Genalex 6.5 (Peakall & Smouse, 2006). We used GENEPOP 4.0 (Rousset, 2008) correcting for significance of multiple tests using sequential Bonferroni correction (Rice, 1989) to confirm Hardy–Weinberg proportions (HWP) and linkage disequilibrium (LD).

We calculated the numbers of alleles, effective number of alleles, per cent polymorphic loci and private alleles at each site and the observed and expected heterozygosity at each site and for each locus across the entire sample. We also calculated individual multilocus heterozygosity among giraffe. Pairwise genetic differentiation (F_{ST}) and inbreeding (F_{IS}) were measured using 1000 permutations to assess significance using Spagedi 1.5 (Hardy & Vekemans, 2002).

We evaluated the power of the data set to differentiate among individuals using the probability of identity (PI) test, and the sib-correction (PI_{sibs}). We tested the ability of assignment tests (Paetkau *et al.*, 1995) to assign giraffe to their sample location. We then calculated the frequency distribution of the relatedness coefficient r_{qg} (Queller & Goodnight, 1989) between all giraffe pairs within each location. We estimated confidence intervals for mean within-population relatedness using 1000 bootstrap replicates, and a null distribution of relatedness among unrelated individuals was generated using 999 permutations of the data set. We conducted all diversity and differentiation measures, assignment tests and permutation analyses using Genalex. Finally, we searched Web of Science for other giraffe populations that have been characterized using microsatellites to examine general patterns of microsatellite diversity across the continent and among free-range and fenced populations. We included only population samples ≥10 individuals genotyped.

Results

We collected 48 faecal samples from which we were able to identify 33 unique giraffe; sixteen from the north and seventeen from the south of the reserve. Three DNA extractions failed to amplify for more than two or three loci, reducing our final genotypes to fourteen in the north and sixteen in the south. One genotype was removed from the north sample due to it being an identical match (see below) with another. For the remaining genotypes, PCR error was detected at two loci (Gca25, n = 2; Gica9905, n = 1) or 0.7% (3/381). In each case, the initial genotype was mistakenly scored as a homozygote due to weak amplification at larger allele sizes. Adjusting primer concentrations between PCR replicates confirmed heterozygote genotypes for these three examples. Additional error was manifested as PCR failure at individual loci. Our final data set contained 26 gaps (~7% missing loci from entire data set), ranging from zero missing (Gca_12 and 11HDZ1004) to as many as eight (Gica13905) (mean number of missing loci per giraffe = 2.3, SD = 1.4).

Linkage disequilibrium between loci was detected at nine comparisons ($\alpha = 0.05$) in the north and seventeen pairwise comparisons in the south. Between the north and south, only three significant pairwise comparisons were in common. Significance of LD tests decreased to 0 and 1 (north and south respectively) following stepwise Bonferroni correction. Overall, these results suggest that LD is related to sampling error (i.e. small population size) not physical linkage. Deviation from HWP was significant at Gca_12 in both north ($P = 0.008$) and south ($P = 0.003$), but was nonsignificant following Bonferroni adjustment and at Gica16160 ($P = 0.03$, ns following Bonferroni adjustment) in the south.

Two loci (Gica10894, Gca_26) were monomorphic, and the number of alleles detected in the twelve polymorphic loci ranged from 2 to 6. (see Table 1 for summary). Private alleles were present in low frequencies (range 0.036–0.143) at four loci (six alleles) in the north and four loci (four alleles) in the south. Mean H_o was 0.351 and 0.296 in the north and south respectively (Table 1), with the range of individual giraffe H_o from 0.1 to 0.58 in both the north and south (not shown). Differentiation in allele frequencies was moderate ($F_{ST} = 0.133$, $P = 0.0001$).

The estimated inbreeding coefficient was high overall and significantly greater than 0 ($F_{IS} = 0.190$, 0.05 SE, $P < 0.001$). Examined separately, the south was significantly more inbred ($F_{IS} = 0.164$, 0.07 SE, $P = 0.007$)

Table 1 Genetic diversity of giraffe populations reported in the literature

Location	Taxon	N	No. loci	Na	H_e	H_o	F_{IS}	Ref.
Mbuluzi (combined)	*G. c. giraffa*	29	14	2.6 (2.9)	0.371 (0.504)	0.324 (0.447)	0.139	This study[a]
Mbuluzi North		13	14	2.5 (2.9)	0.379 (0.505)	0.351 (0.447)	0.062	
Mbuluzi South		16	14	2.6 (2.9)	0.363 (0.438)	0.296 (0.345)	0.196	
Kruger N.P., South Africa	*G. c. giraffa*	27	14	–	0.437	0.266	–	Brown *et al.*, 2007
Etosha N.P., Namibia	*G. c. angolensis*	70	11	2.9	0.459	0.476	–	Carter *et al.*, 2012
Etosha N.P., Namibia	*G. c. angolensis*	15	12	3.3	0.466	0.408	0.140	Brenneman, Louis & Fennessy, 2009a
Etosha N.P., Namibia	*G. c. angolensis*	15	14	–	0.350	0.352	–	Brown *et al.*, 2007
Namib Desert, Namibia	*G. c. angolensis*	18	12	3.6	0.424	0.406	0.043	Brenneman, Louis & Fennessy, 2009a
Hoanib River Namibia	*G. c. angolensis*	15	14	–	0.350	0.352	–	Brown *et al.*, 2007
Katavi N.P., Tanzania	*G. c. tippelskirchi*	40	8	6.2	0.527	0.513	–	Crowhurst *et al.*, 2013
Athi River Ranch, Kenya	*G. c. tippelskirchi*	25	16	4.3	0.535	0.355	–	Huebinger *et al.*, 2002
Niger	*G. c. peralta*	28	14	–	0.523	0.450	–	Brown *et al.*, 2007
Lake Nakuru N.P., Kenya	*G. c. rothschildi*	18	14	4	0.541	0.527	0.028	Brenneman *et al.*,2009b[b]
Ruma N.P., Kenya	*G. c. rothschildi*	17	14	3.1	0.488	0.471	0.035	Brenneman *et al.*, 2009b[b]
Yoder Flowers Farm, Kenya	*G. c. rothschildi*	12	14	4.9	0.648	0.714	−0.106	Brenneman *et al.*, 2009b[b]
Murchison Falls N.P., Uganda	*G. c. rothschildi*	51	14	4.7	0.541	0.518	0.044	Brenneman *et al.*, 2009b[b]
Murchison Falls N.P., Uganda	*G. c. rothschildi*	51	14	–	0.479	0.402	–	Brown *et al.*, 2007
Nakuru N.P., Kenya	*G. c. rothschildi*	18	14	–	0.412	0.390	–	Brown *et al.*, 2007
Meru N.P., Kenya	*G. c. reticulata*	10		–	0.536	0.490	–	Brown *et al.*, 2007
Samburu N.R., Kenya	*G. c. reticulata*	18		–	0.647	0.546	–	Brown *et al.*, 2007
Laikipia	*G. c. reticulata*	52[c]		–	0.639	0.624	–	Brown *et al.*, 2007

[a]North and south Mbuluzi combined. Values for polymorphic loci only in parentheses are presented for comparative purposes.
[b]Brenneman *et al.* (2009b) reported summary statistics for polymorphic loci only.
[c]Laikipia listed as representing three distinct sites in same vicinity.
N sample size; n_a, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; F, fixation index.

than the north (F_{IS} = 0.107, 0.08 SE, ns). Mean relatedness in the north was negative, although not significantly different from 0 (i.e. unrelated; mean r_{qg} = −0.078, 95% CI: −0.193 to 0.025, ns). In contrast, giraffe in the south were significantly more related than random (mean r_{qg} = 0.088, 95% CI: 0.016–0.157, P = 0.03).

The probability of any two individuals (PI) having the same genotype was low in the north and south (3.7×10^{-6} and 5.17×10^{-6} respectively) increasing to 2.8×10^{-3} and 3.6×10^{-3} when accounting for the possibility of siblings (PI_{sibs}). Despite the moderate genetic differentiation between the two locations, assignment tests failed to correctly assign three southern giraffe to their correct subpopulation. Two of the misassigned giraffe had missing loci (two and three loci respectively of the twelve total).

We identified five studies quantifying microsatellite diversity in a total of twelve locations (Table 1). In general, heterozygosity (He) and mean number of alleles were lower in *G. c. giraffe* than in other subspecies.

Discussion

Levels of microsatellite diversity in Mbuluzi giraffe are among the lowest reported from either fenced or free-ranging giraffe population (Brown *et al.*, 2007; Brenneman, Louis & Fennessy, 2009a; Brenneman *et al.*, 2009b; Carter *et al.*, 2012; Crowhurst *et al.*, 2013). The lower diversity in *G. c. giraffa* relative to other subspecies (Table 1) should be interpreted cautiously as representative studies are few, the markers used are not the same across studies, and in some cases, only polymorphic loci were reported. However, low diversity in *G. c. giraffe* is of interest because it is one of the only subspecies considered to be stable or increasing (Fennessy, 2008).

The observed heterozygosity of south Mbuluzi is particularly low; 0.296 relative to 0.351 in the north, despite having the similar numbers of alleles (Table 1). This, despite the fact that the south population was sourced from two different locations (Mopane and Hoedspruit, Fig. 1) and, thus, potentially could have had greater initial

diversity. The inferred level of inbreeding is substantially greater in the south than the north. Both inbreeding and low heterozygosity have been implicated in reducing individual survival and population fitness of wildlife (Chapman *et al.*, 2009). Continuous inbreeding can lead to reduced survival and fertility of individuals (Charlesworth & Willis, 2009) particularly under stressful environmental conditions and in populations with low reproductive rates (Wang *et al.*, 1999).

This limited genetic diversity is not surprising given the small number of founders and lack of gene flow into the population since its establishment, which would reduce allelic diversity directly through genetic drift (Allendorf, 1986). The distinction between the north and south is indirectly influenced by drift; reduced heterozygosity is also a direct result of mating patterns (Crow & Kimura, 1970). The sex ratio of founders, for example, is unknown, but if highly skewed may explain differences in zygosity. Assuming most giraffe sampled are third generation (F_2), the current level of inbreeding likely reflects inbreeding effects from previous generations. Records of founder sex ratios, mortalities, translocations between the north and south and changes in population size are nonexistent, and therefore understanding the genetic patterns in terms of the demographic history remains speculative.

The north and south Mbuluzi populations are moderately differentiated, and one short-term management objective would be to exchange giraffe between the north and south to encourage outbreeding and increase heterozygosity. However, ultimately maintaining genetically diverse populations in Mbuluzi will require facilitating gene flow with outside populations of South African giraffe. Allowing population admixture, while simultaneously encouraging population growth, would benefit from the removal of fences within Mbuluzi and between Mbuluzi and neighbouring protected areas in Swaziland (Mlawula Nature Preserve and Hlane Royal National Park, Fig. 1).

As giraffe are attractive for ecotourism and trophy hunting, their numbers will continue to be bolstered by the private sector. This is a controversial idea as stocking and closed populations carry risks associated with overgrazing, inbreeding, disease and increased parasite loads (Daszak, Cunningham & Hyatt, 2000; Hoenerhoff *et al.*, 2006; Brenneman *et al.*, 2009b), problems associated with reduced genetic diversity. Therefore, we argue that while private game farms have increased giraffe numbers in places like South Africa and Swaziland, increased vigilance on the consequences of unregulated trade in giraffe would go a long way towards improving the long-term viability of giraffe populations.

Increasingly, both public and private protected areas need to balance the goal of conserving biodiversity in increasingly fragmented ecosystems (Margules & Pressey, 2000) with financial security (Dharmaratne, Sang & Walling, 2000). Achieving tourist or private shareholder satisfaction is therefore recognized as a goal in the management of many game preserves and protected areas (Langholz & Kerley, 2006). In Mbuluzi, the removal of fences may be considered as antagonistic to these goals (e.g. reducing the density and visibility of animals) and could have management implications that are counter to the objective of conserving healthy giraffe populations in the near term (e.g. increased poaching). Since the founding of the giraffe population at Mbuluzi, additional giraffe populations have been established (or attempted) at additional locations within Swaziland, including Hlane Royal National Park, Inyoni Yami Sugar Irrigation Scheme, Mkhaya Game Reserve, the Mhlosinga Conservancy and Panata Ranch. Although the provenance of these giraffe is not publically available, some of these locations were sourced from the current stock at Mbuluzi. It is likely that the genetic status of giraffe populations throughout Swaziland is questionable. As a management objective, planning for giraffe exchanges would be of potential benefit. Genetic assessments of existing populations would be a valuable tool in informing translocations and should be implemented.

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