Original Research Article

Relatedness and genetic variation in wild and captive populations of Mountain Bongo in Kenya obtained from genome-wide single-nucleotide polymorphism (SNP) data

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Abstract

To assess the relatedness and amount of genetic variation of wild and captive Mountain Bongo Tragelaphus eurycerus ssp. isaaci, both non-invasive and invasive samples were efficiently analyzed using SNP's. Mountain Bongo is estimated to remain in Kenyan forest with less than 96 individuals, possibly as low as 73 individuals, split in five subpopulations whereof four populations are isolated from each other. The genetic diversity of wild animals was studied using fecal samples, and using tissue samples from the 62 animals presently held captive at the Mount Kenya Wildlife Conservancy. In strategic conservation of the wild Mountain Bongo, the captive animals constitute a potential genetic input to wild populations. Our study shows there is still genetic variation in the wild population and that the subpopulations are to some extent genetically differentiated. This leads to an overall effective population size of around 14 in the wild population, which is good relative to the small population, but dangerously small for long-term, or even short-term, survival. Most individuals in the wild population were unrelated, while in the captive population most individuals were related at the level of half-sibs. The captive population still host genetic variation and is differentiated slightly to the wild population. Careful restocking from the captive populations could be an effective means to enhance the genetic variation in the wild, but most importantly make the dwindling population less vulnerable to stochastic events.

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1. Introduction

Global decrease in biodiversity strikes hard on numerous wild mammals and in several cases dwindling wild populations are smaller than captive populations. Conservation strategies may then imply re-stocking of protected wild populations with...
captive animals, however, the genetic and evolutionary impact from such anthropogenic conservation will consequently be in focus. Ideally, relatedness or genetic health should be investigated before action is taken, but practical problems may come into account. To evaluate the relatedness in populations targeted for applied strategic conservation we have chosen a modern genomic methodology to obtain genetic variation data, using both non-invasive and invasive sampling techniques, suitable for undersized wild and captive populations of a critically endangered mammal.

The Mountain Bongo Tragelaphus eurycerus ssp. isaaci, also referred to as the Eastern Bongo, is endemic to Kenya and classified as critically endangered (CR C2a(i) ver 3.1.). In an assessment made 2008 by IUCN was the wild population estimated to a total of 75–140 individual animals composed of four different subpopulations (IUCN, 2008). The mountain Bongo is a large size forest ungulate (♀ ≤ 276 kg; ♂ ≤ 405 kg) with a complex social behavior. At a sexual maturity of 2 years of age, one offspring may be born after a gestation time of 38 weeks (Kingdon, 2015). Disturbance from human activities may have significant impact on the breeding success of this species (Prettejohn, 2017).

At present, there are five known wild subpopulations with an uneven population distribution, accounting for an estimated total population of 96 animals based on information from camera traps in combination with experience from several years of surveillance in the forests. However, evidence based solely on camera trap identification results in 73 individuals only (see Fig. 1; Prettejohn, 2017; Shears, 2015). The smaller population size would qualify for classification as critically endangered with criteria D as it is likely that less than 50 individuals are mature (IUCN, 2001).

The five wild subpopulations are not managed actively, thus natural genetic exchange between the present subpopulations may only occur within Mau forest, see Fig. 1. Consequently, inbreeding is an imminent problem and conservation strategies improving genetic diversity have been raised (Mallon, 2013; CBSG, 2010).

Historically, the Kenyan subpopulations of mountain Bongo have been described in Cherengani Hills, Londiani forest and crater, Mau highland forest, Aberdare mountain range, the mountain area of Ol Donyo Eburr and Mt Kenya commonly habituated in mountainous forest between 2100 m–3000 m altitude (Kingdon, 2015; Ralls, 1978; Price, 1969). Before human exploitation of the land, animals could possibly be able to pass between these locations and thus there were possibilities of gene flow. The habitat at these highland forest areas in Kenya, and likely some few more highlands in the region, is in total a relatively small area thus could not host a large total population of animals. This becomes further obvious in relation to the species relative, the Low Land Bongo Tragelaphus eurycerus ssp. eurycerus, also called the Western Bongo, inhabiting a vast area stretching from Sierra Leone in the west, to Southern Sudan in the east, the Congo Basin and to northern Angola in the south (Kingdon, 2015; Ralls, 1978). Therefore, it can be expected that the genetic diversity of the mountain Bongo has been severely reduced, and thus it is of interest with regard to the present status of the species and the conservation strategy for sustainable population growth.

The Mount Kenya Wildlife Conservancy (MKWC) is located on the slopes of Mt. Kenya bordering the Mt. Kenya Forest, a UNESCO world heritage site (Fig. 1). At present, 63 Mountain Bongo (25 bulls, 38 cows) are ranging in an enclosure of 60 ha mostly comprised of natural forest and grassland. The land has further been subdivided into 4 breeding groups, 3 bachelor groups and 2 nursery groups. A majority of these animals would not survive in the wild because they have to some extent lost their natural instincts, for example to fend or evade predators, or they may not be able to meet their nutritional

Fig. 1. Map showing the present Bongo populations and sizes in Kenya. Estimates are based on information from camera traps in combination with experience from several years of surveillance in the forests. Population size solely by evidence from camera trap identification is presented within parenthesis. The captive population is found at Mt. Kenya Wildlife Conservancy (A) and the wild populations are Ragati at Mt. Kenya (B), Honi-Salient in the Aberdares (C), South West Mau (D) and Maasai Mau (E) in the Mau Forest and Eburr (F) in Mau Eburr.
requirements because they get >80% of supplements in captivity. Some animals have lost immunity to diseases and would suffer from disrupted veterinary care. Aiming towards future restocking of captive animals to wild habitats, some animals have deliberately been kept to maintain a feral behavior. This population constitutes a possibility of genetic exchange with wild populations. The captive Mountain Bongo pedigree dates back to the early 1970s and started from five wild individuals, two bulls and three cows, captured in the Aberdares. In the 2004 when the MKWC population had grown to 18 individuals, additional 18 Mountain Bongo were repatriated from fourteen zoos and institutions in the US to MKWC. However, 11 animals were lost to endemic diseases, within the first two years. Most succumbed to the tickborn disease Theleria, some had a combination of Anaplasmosis and Babesiosis which are also borne by ticks. Offspring from the US sourced animals are considered as good as locals. Relatedness within the MKWC population at present is evident, but to different degrees between the individuals because breeding has been both natural and through selection. In the past, good looking males were made to breed more by placing them in a group of females, however the recent approach has been a careful selection of individual males and females through the Population Management 2000 (PM2000) software package providing a suite of tools for genetic and demographic analysis for management of pedigreed animal populations with additional recommendations from the Association of Zoos and Aquariums (AZA) cooperatively managed Species Survival Plan® (SSP) Program for Mountain Bongo coordinator. Animals have so far always been let to mate naturally in enclosed areas, thus no artificial insemination has been done.

A previous study by Faria et al. (2011) investigating genetic diversity in wild mountain Bongo based on the mtDNA control regions from sampled fecal DNA concluded that the diversity among the four subpopulations Mount Kenya National Park, Aberdare National Park, Eburu and Mau forests, was low with only two mtDNA haplotypes found. However, mtDNA is often not variable enough to provide in-depth population studies of individuals. Therefore, in order to get a much better resolution of the genetic variation in the populations we used single nucleotide polymorphisms (SNP) spread over the whole genome.

Our study has to have two main aims, first, to test whether this technique can be used for samples of low quality as feaces collected in the wild, and secondly, to assess the patterns of genetic diversity and genetic effective population size among the wild individuals in two wild subpopulations in the Aberdares and Mt. Kenya (fecal samples) and in the captive Bongo population in Mount Kenya Wildlife Conservancy (biopsy samples).

2. Materials and method

2.1. The population localities

The population locations are given in Fig. 1; A) The MKWC comprise an enclosure of 60 ha bordering the Mt. Kenya Forest (approx. 0°02′16.00″S 37°07′43.50″E, alt. 2137 m). B) In Mt. Kenya wild animals have during recent years mainly been traced to the south-west part of the forest called Ragati (approx. 0°19′31.70″S, 37°13′47.90″E, alt. 2630 m) and this area is presently within a fencing programme. C) In the Aberdare National Park the wild Bongo population in recent history has been split on several groups, however lately observations have only identified two remaining groups commonly present at the Salient and Honi areas (approx. 0°20′10.50″S, 36°44′50.00″E, alt. 2640 m). It may be expected that these relatively large groups are flexible and may split occasionally. The entire national park is fenced. D) The south west of Mau forest reserve habituates one group of wild Bongo (approx. 0°25′50.00″S, 35°22′00.00″E alt. 2190 m), this forest area is not fenced. E) The south east part of the Mau forest reserve referred to as the Maasai Mau area have the most recently discovered group of wild Bongo (approx. 0°47′10.00″S, 35°47′30.00″E, alt. 2370 m). The forest area is not fenced. The two groups in Mau forest are not separated by any fence. F) The mountain Ol Doniyo Eburru situated in the Mau Eburru area comprises a fenced forest inhabited by one group of wild Bongo (approx. 0°39′31.00″S, 36°10′30.00″E, alt. 2573 m).

2.2. DNA sampling procedure

The MKWC population was identified by individuals and habituated to human presence (in particular the author P. Fundi) thus approaching the individuals within 1 m for sample collection was less problematic, especially when feeding on commercial foods. A small tissue sample from the ear lobe edge was cut off using an ear notching tool. The biopsy samples were put in 10 ml test tubes and added 99% ethanol before storage at −20 °C. The ear notching tool was flame sterilized using a 1350 °C butane torch during 3 min before sampling a different individual. The fresh wound after biopsy was treated by broad spectrum oxytetracycline spray. The MKWC animals were sampled within a period of three weeks. In the wild, animals were not sampled by knowledge of individuals, thus sampled with a opportunistic method. After identification of Mountain Bongo dung in the wild by the specialized Bongo Surveillance Team (Rhino Ark Trust, 2017; Shears, 2015), plastic test tubes of 50 ml were filled approximately to half the volume with feces and half the volume with ethanol, then stored in a fridge at −18 °C. The samples were not frozen during a period of three days due to air transport, but were frozen again at −18 °C until DNA extraction. The wild samples used in the study were collected within a period of one year. Because of the spatial and temporal intervals between the dung samples collected it is not likely that the same individual have been sampled twice. The study comprises a total of 97 samples whereof 60 were from captive animals at MKWC and 37 from the wild (Aberdares 22, Mt Kenya 9, Mau Forest 4 and Eburu 2). Please refer to a detailed list of samples in the Supplementary Materials.
2.3. DNA extraction method

Tissues samples were cut in a 1—2 mm² piece and extracted with DNase QI Tissue Kit (Qiagen) according to the manufacturer protocol. Fecal samples were dried overnight, weighed to 200 mg and extracted with QIAamp Fast DNA Stool Mini Kit (Qiagen) according to the manufacturer protocol and added 10 µl RNaseA to the 100 µl elute. The DNA quality was controlled with both Qubit and agarose gel.

2.4. RADseq

We did a genome-wide scan for single-nucleotide polymorphisms (SNP) using RADSeq (e.g. Baird et al., 2008; Davey and Blaxter, 2010), which has been shown to be useful for population genetic studies of natural populations of non-model species, using an Illumina platform (made by SNPSaurus, CA). Since this was done de novo we used blood and skin from three captive individuals to obtain a good starting point. Only the SNP’s that were of good quality (see below) in the skin samples were used on the fecal samples to avoid contamination from other sources of DNA. In total 1052 SNP’s were found in the blood samples. The ratio of transitions versus transversions (ts/tv) was 1.57 across all SNP’s. C-T/T-C transitions were more common (53.6%) than G-A/A-G transitions (46.3%; X² = 8.19, P = 0.0042). C-T transitions were more common than expected (42.7% of total X²) and A-G transitions less common than expected (56.2% of total X²). The pattern of transversions were slightly significant (X² = 14.4, P = 0.044), with T-G transversions more common than expected (47.3% of X²), and T-A/A-T transversions less common than expected (5.9% of X²).

The RADseq analysis gives likelihoods for each genotype and we only choose loci for each individual where the difference in scaled likelihoods (Q-score) was 13 which corresponds to p = 0.05, i.e. the probability that the alternative genotype(s) are the correct one given the data. This is more relaxed compared to other studies where p = 0.01 (Q = 20) is used, but given the quality of the wild samples we found that necessary to be able to use the data efficiently in terms of power. We will also give data from using the stricter cut-off.

2.5. Statistics

Basic estimates of genetic variation (Hₑ and Hₒ = expected and observed heterozygosity and Fᵢₛ) were estimated for each group using Arlequin 3.5.1.2 (Excoffier and Lischer, 2010), and the test for genetic differentiation was made using a locus-by-locus AMOVA in Arlequin.

We estimated pairwise relatedness using the method of Lynch and Ritland (1999). Since we access to the studbook (Shears, 2015), we could calibrate the molecular estimates of relatedness with the known ones. The relatedness among the captive individuals was calculated using the R-program pedantics (Morrisey and Wilson, 2009). The mean relatedness in the captive group using this software was 0.074. The molecular estimates, on the other hand returned values with a mean of 0.77, i.e. the estimates were seriously biased upwards. Thus, the estimates obtained using real data was corrected correspondingly. The method of Lynch and Ritland (1999) uses allele frequencies in the estimation of relatedness. In our case these can be estimated in two ways; by using the whole data set, or estimating allele frequencies in each group (wild, captive) independently. Using all data has the advantage that as much data as possible is used in the estimation, which will result in a good estimate, but has the disadvantage that it pools two groups that are slightly genetically different (see below). Using allele frequencies for each group separately has the advantage that potential problems with pooling is avoided, but has the disadvantage that estimation of allele frequencies is less robust given the small group of wild individuals. Here we only present the result from the pooled group using this software was 0.074. The molecular estimates, on the other hand returned values with a mean of 0.77, i.e. the alternative genotype(s) are the correct one given the data. This is more relaxed compared to other studies where p = 0.01 (Q = 20) is used, but given the quality of the wild samples we found that necessary to be able to use the data efficiently in terms of power. We will also give data from using the stricter cut-off.

Based on resampling we found that the standard error of the estimates was 0.06. Relatedness is normally not a uniform distribution but with discrete values, such as 0.5 for parent-offspring and full-sibs, and 0.25 for half-sibs. We estimated the relative frequencies of different relationships of dyads (unrelated, half-sibs, full-sibs and parent-offspring, inbred (r = 0.75), and identical) by calculating the likelihood of a relationship given the relatedness estimate (r) and the standard error. This is the probability that a given estimate belongs to a normal distribution with a mean of, say 0.5 for full-sibs, and a variance being the same as the sampling variance. These likelihoods were then transformed into Akaike Information Criteria (AIC) = 2-2Log(Likelihood). The relatedness with the lowest AIC was then scored as the relatedness within the particular dyad.

Effective population size (for review see Luikart et al., 2010) was estimated using the software NetEstimator 2.01 (Do et al., 2013) using the molecular coancestry method (Nomura, 2008) and the heterozygote excess method (Pudovkin et al., 1996; Luikart and Cornuet, 1999). We did not use the test using linkage equilibrium (LD) since this method returned an infinite effective population size. This happens when the expected and estimated LD are very close and then the denominator in the equation determining Ne becomes close to zero. This means that when there is no, or little, LD in the population, Ne becomes hard to estimate with precision. One interpretation favoured by Do et al. (2013) is that is indicates a very large Ne. However, small sampling errors in estimating LD will give large differences in output, hence we refrain from this estimator. It is worth noting that in an iteroparous species like the bongo and with a sample of individuals from different cohorts this does not give the true effective population size but rather the number of breeders, Nₑ (Wang et al., 2016), which is likely to be higher than Nₑ (Waples et al., 2013) and it is a measure of inbreeding effective population size, which relates to the loss of heterozygosity.
If there is substantial number of matings between close relatives then this value be lower. In accordance to the suggestions in Nomura (2008), we also calculated the harmonic mean of the estimates from the two methods.

We also used the data from the captive population where the mating patterns are known, and thus the sibships. We used two methods; first, by using the variance in mating success by Nomura (2002; eq 4), which takes into account the variance in mating success between males and females, and the variance in offspring number. Secondly, we used the sibship method by Wang (2009), where $1/N_e = (Q_{HS} + 2Q_{FS})/4$, if there is no inbreeding, and where $Q_{HS}$ and $Q_{FS}$ is the proportion of half- and full-sibs among all possible dyads, respectively. This is an estimate of the variance effective population size, which relates to the variance of allele frequencies. Variance effective size is more affected by recent population reductions, while inbreeding effective size relates to the probability of inbreeding (Luikart et al., 2010).

3. Results

3.1. Quality of samples

The number of reads different between the skin and the fecal samples (Fig. 2a and b), with a mean number of reads for the skin data being 165 ($sd = 81.3$, range: 1–335), and for the fecal data (mean = 5.0, $sd 26.6$, range 1–328). However, the importance issue is not the number of reads per se but the ability to correctly infer genotypes. This is usually measured as $Q$, where $Q = 13$ corresponds to an error rate of 5%, and $Q = 20$ corresponds to an error rate of 1%. In Fig. 2c and d we plot the probability of a $Q$ larger than 13, or 20, as a function of the number of reads over all individuals and loci for the two groups. Clearly, the probability of a high confidence in genotype scoring (high $Q$) increases with the number of reads, but it levels off at a fairly low number of reads. The difference between the two curves can in part be explained by the fact that there were only a few data points for the lower number of reads for the skin data. It is worth noticing that even when the number of reads were small, the probability of getting a high $Q$-score was above 80%.

For the skin data and for $Q = 13$, we found that 86.4% of the loci had a sample size of 50 individuals or more, and 29.6% of the loci a sample size of 60 individuals or more (Fig. 3a). Using the stricter criterion ($Q = 20$) we found that 70.5% of the loci had a sample size of 50 or more individuals, and 24.5% a sample size of 60 or more individuals (Fig. 3a). We then used the loci

![Fig. 2.](image-url)
where we had 50 or more individuals and tested for Hardy-Weinberg equilibrium (HWE) and used the loci that did not differ significantly from HWE. Using the less strict criterion we found 197 suitable loci that was used in the following. The corresponding data for the feces data is shown in Fig. 3b.

3.2. Population genetics

Since we have access to the breeding data of the captive population we can compare the estimates effective population size from the molecular analyses to the estimates using the known data of variance in mating and breeding success and sibship in the captive population. There were 10 males and 23 females in the captive population. The mean mating success in males was 4.3 (SD = 4.49), and in females 1.8 (SD = 0.83). Thus, the variance in mating success was almost 30 times larger among males than among females (20.2 vs 0.7). The mean number of offspring from each mating was 1.3 (SD = 0.62). The pedigrees for males and females are shown in Fig. 4, where the larger variance in male reproductive success is evident. Using these number results in a variance effective population size of 14 using Nomura’s method (Nomura, 2002). If we, on the other hand, use the sibship method (Wang, 2009) we obtain a variance effective population size of 17.

The levels of expected heterozygosity did not differ between the two groups (captive: 0.40, SD = 0.14, wild: 0.45, SD = 0.10), and neither did the observed heterozygosity (captive: 0.70, SD = 0.36, wild: 0.72, SD = 0.26). Mean $F_{IS}$ over loci was $-0.65$ (sd = 0.38) for the wild population, and $-0.82$ (sd = 0.50) for the captive population. This difference is not significant ($P = 0.070$, Mann-Whitney U-test). The inbreeding effective population size ($N_e$) was 14 (95% CI: 8–21) in the wild population using the molecular ancestry method, and in the captive population $N_e = 3$ (95%: 2–4). The heterozygote excess method ($HE$) gave an estimate of $N_e = 1$ in both cases. The mean over all loci for $HE$ was 1.85 (sd = 1.70), and 2.96 (sd = 6.78) in the captive population. This difference was not significant ($P = 0.68$, Mann-Whitney U-test). The harmonic mean of the two estimates was 3.3 for the wild population, and 1.5 in the captive population.

![Fig. 3. Quality of the SNP in a) the captive population and b) the wild population. The graph shows the number of individuals with a certain number of loci of high quality (see text for details).](image)
We were able to correct for the bias in the relatedness using the molecular data but the variance was too low compared to the real data. In particular, the dyads with a relationship of 0.5 (full sibs and parent–offspring) was not picked up using the molecular markers. The distribution of relatedness values in categories of 0.1 (truncated to zero for negative numbers) is given in Fig. 5a and it is clear that the long right-hand tail in the real data is not recovered in the molecular data, but is truncated to lower numbers. When discretized into three categories it is clear that the full-sibs are now in the half-sib category (Fig. 5b). This means that inference from the data on the wild population needs caution, but the results does not show any sign of drastic inbreeding in terms of high degree of relatedness.

The wild and the captive population differed genetically with a $F_{ST} = 0.028$ (95%: 0.016–0.042; $P < 0.0001$). This was in particular due to nine loci with an $F_{ST}$ larger than 0.2 (Fig. 6).
4. Discussion

4.1. Population genetics

The results show that we can use RADseq to obtain a set of usable SNP's even for samples of lower quality such as feces. The results with regard to effective population size differed depending on the method used. In the captive population variance effective population size was larger than the inbreeding effective population size estimated by molecular markers. This is typical for populations that have recently gone through a bottleneck with only few founders but where the population has started to increase. This is what has happened in the captive population and hence this difference is as expected. However, it needs to be clear that the inbreeding effective is an estimate of the probability of inbreeding and given the structure of the captive population this can happen if mating is not controlled. Unfortunately, we could not do the same comparison for the wild population due to the lack of demographic information. However, the molecular data hints that the inbreeding population size might be larger in the wild population, but even if there is a difference, the actual size is very small and thus the risk of inbreeding is high. This does not seem to be the case as the inbreeding coefficient ($F_{IS}$) is negative overall, with only a handful of loci showing otherwise. This suggests that even if the population is small and in danger of inbreeding, this is not the case to any measurable degree at the time the samples were taken.

The estimation of relatedness using the molecular markers differed from the known data insofar that high levels of relatedness was not found, when the original bias was removed. We tried different way of calculating allele frequencies and

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**Fig. 5.** Relatedness in the captive population using known relationships (black bars), estimated from the SNP’s (grey bars) and in wild population (white bars). A) distribution of relatedness values. B) relatedness values transformed into most likely relatedness category.
different number of loci, but the results did not differ in any qualitative sense (results not shown). This means that the results are only suggestive as some close relatives might have gone unnoticed in the wild population, as was evident in the captive population. Nevertheless, the results from the wild population did not differ strongly from the captive population; most individuals are not related to each other, but with some relatives around. This is not surprising if the sampling in the wild was from parent and offspring, or siblings. Hence, taking into account the uncertainty of the method of calculating relatedness using the molecular markers, we have no reason to assume that the wild population is inbred.

The samples of the wild are taken from a meta-population, and it is well known that the effective size of a meta-population is larger than for a single population of the same number of individuals (Hedrick and Gilpin, 1996). This is due to genetic drift in the different parts of the meta-population that when taken as a whole result in a larger genetic variation. Thus, the genetic variability can be larger overall, but small within each subpopulation. However, if the different subpopulations had widely different allele frequencies a pooling into one would lead to an observed deficit of heterozygotes due to the Wahlund effect. This is the case here, where 78.9% of the loci had a heterozygote deficiency. However, the median deficit was 0.077, indicating a modest differentiation among the subpopulations. Since the observed frequency of heterozygotes equals $H_{exp}(1-F_{ST})$, we can estimate the differentiation among the wild populations ($F_{ST}$). The median $F_{ST}$ is 0.17 (for frequency distribution see Fig. 5). In the case of a di-allelic system like this an $F_{ST}$ of 0.17 means that, on average, the probability that the populations are fixed for alternative alleles is 0.17. Thus, even if there were two (out of 197) loci where this was the case, most loci are fairly similar in terms of allele frequencies among subpopulations (Fig. 7). Since the effective size of a metapopulation is increased relative to a single population by a factor $1/(1-F_{ST})$ (Barton and Whitlock, 1997), the effective population size is about 20% larger than in a undivided population. The scarcity of samples from the wild populations makes a deeper analysis of the differentiation unfeasible. The result suggests that the wild population host a larger genetic diversity. However, the effective population size is by all standards very low due to the small population size, thus protecting the fragile wild populations and their habitats is of utmost importance. Naturally, at any point of introducing genetic variation into the wild populations from the captive population at MKWC, siblings or half siblings according to the pedigree should not be introduced in the same population. In fact, the wild population is so small that even if the genetic variability is still at a very low, but not catastrophic, level, the main threat might be demographic stochasticity such as failed reproduction or skewed sex ratios. This can in turn rapidly lead to a reduction in numbers and thus also in genetic variability.

4.2. Conservation

Fencing of the wild habitats is a recent event and made solely for protective reasons. No translocation of animals have so far taken place, but translocations between wild subpopulations may well be necessary in future to achieve genetic sustainability in conservation management. Given that there is still genetic variation in the wild population, albeit low (see also Faria et al., 2011), and that the genetic composition of the captive group slightly differs from the wild population, a restocking can be an important means by which genetic variation can be enhanced in the wild population. The more practical question then arises, which population is best suited for restocking? The habitats have been investigated systematically (Estes et al., 2011, 2010, 2008). The typical habitats have declined drastically due to deforestation, cutting firewood, charcoal burning, clearing land for agriculture, cattle grazing and human settlements (Shears, 2015; Lambrechts et al., 2003). These human activities cause disintegrated populations with loose breeding cycles. Scattered individuals are also easier killed off by hyena or leopard. Poaching with dogs and trap snares constitutes the main reason for rapid population decrease, however diseases like rinderpest also constitute a potential threat (Kock et al., 1999). Fencing a conservation area is a method that has been
used; the entire Aberdare National Park and the Eburru forest have been fenced. It has been shown that the primary effect of electrical fencing is positive with a decrease in human introgression and increase in animal population size, however, over a longer period of time the effect of the fence is as good as the management that comes with it (Massey et al., 2014). Some areas are more difficult to monitor for rangers because of challenging topology, for example Mt Kenya and Mau Forest. From this perspective, the Londiani Crater forest and adjacent highland offer suitable future conservation area for Bongo as well as other rare species. The Londiani area has historically held Mountain Bongo populations (Price, 1969) and after expeditions made by Bongo Surveillance Team and one of the authors (Svengren, H.) it stands clear that pristine highland forest between 2100 and 3000 m altitude has an abundance of favoured food plants. In addition, the rim around the crater provides excellent patrolling conditions with visibility to the surrounded forest below. Presently, the fragile wild populations of Mountain Bongo still have a chance to recover given that protection of individual animals and their habitats succeeds with an executive action.

5. Conclusion

At present there are five remaining subpopulations of wild Mountain Bongo in Kenya that are spatially separated, some are even to large extent fenced, and the total number of individuals are estimated to 73–96 individuals. Evidently there is need of active conservation management to sustain genetic variability as well as provide security for the habitat and animals. Translocation of selected animals between subpopulations is one possibility. Another possibility is offered by the sixth subpopulation that is captive in Mount Kenya Wildlife Conservancy that comprises 63 animals, whereof some few are suitable for restocking to wild populations. Management of interchanging genetics between subpopulations should be done with care and after a genetic survey have been done. This study provides the first investigation regarding genetic variation within and in between both wild and captive populations. Restocking could help to keep genetic variation at a reasonable level, but most importantly it helps to reduce the risk of extinction due to stochastic events that invariably hits q small population more than a large one. Assuming that genetic interchange between wild subpopulations can be made, that restocking from captive animals to wild can be made and that stochastic events are reduced, there are good chances that the Mountain Bongo can grow to a future sustainable population size in Kenya.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.gecco.2017.07.001.

References
