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Short Communication

Evaluating ex situ conservation projects: Genetic structure of the captive population of the Arabian sand cat

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ABSTRACT

Ex situ conservation plays an increasingly important role in the conservation of endangered species. Molecular genetic markers can be helpful to assess the status of captive breeding programmes. We present the first molecular genetic analysis of the captive population of the Arabian sand cat (*Felis margarita harrisoni*) using microsatellites. Our data indicates that the captive population of *F. m. harrisoni* comprises three genetic clusters, which are based on different founder lineages. Genetic diversity was relatively high, the effective population size even exceeded the number of founders. This was presumably caused by subsequently integrating unrelated, genetically diverse founders into the captive population and a careful management based on minimizing kinship. However, we detected an error in the studbook records, which might have led to incestuous matings and underlines the usefulness of molecular evaluations in captive breeding programmes for endangered species.

Ex situ conservation and reintroduction projects play an increasingly important role in the conservation of threatened species (Fischer and Lindenmayer 2000; Frankham et al. 2010). However, the success of such breeding programmes is strongly determined by genetic processes, such as inbreeding depression, loss of genetic diversity (reviewed in Witzenberger and Hochkirch 2011), outbreeding depression (e.g. Marshall and Spalton 2000) and adaptation to captivity (e.g. Williams and Hoffman 2009). In order to avoid such negative effects, studbooks have been established for many endangered species. Data on the genetic diversity and the relationships within a captive population can provide valuable information for the improvement of ex situ conservation programmes for endangered species (O'Brien 2006).

The sand cat (*Felis margarita*) is a small felid species with a disjunct distribution in northern Africa and western Asia, where it occurs at low densities. It is classified as near threatened on the IUCN red list (Mallon et al. 2008). Four subspecies have been described, only two of which (*F. m. harrisoni* and *F. m. scheffeli*) have ever been represented in captivity (Akers 2009). The first individuals bred in captivity mostly belonged to the subspecies *F. m. scheffeli*, a subspecies which is endemic to Pakistan. Nowadays, the captive population consists almost exclusively of the Arabian sand cat (*F. m. harrisoni*), which occurs on the Arabian Peninsula and in Jordan (Akers 2009). Based upon the international studbook, coordinated ex situ breeding of this subspecies started in the 1970s and the current population can be traced back to 18 founders of *F. m. harrisoni* (Akers 2009). Further 10 potential founders have been integrated but have yet to provide offspring. Since 1988 the captive population of the Arabian sand cat has been coordinated globally by an international breeding programme, which is however split into two more or less independent regional subsections: The North American Species Survival Plan (SSP) which provided the basis for this breeding population and the European endangered species programme (EEP),which was established in 1998 (Magiera 2011). Until the end of 2009 the complete captive population has reached 200 individuals (Akers 2009), 102 of which are registered in the EEP (Magiera 2011).

Here, we present the first genetic analysis of the captive population of the Arabian sand cat using nine microsatellite loci. Our aim was to analyze whether the relatively small breeding stock is affected by inbreeding and the loss of genetic diversity and if the pedigree information is correct.

The European studbook for *F. m. harrisoni* currently records 23 holders (Magiera 2011). All holders registered in the EEP studbook were contacted and asked to contribute samples to this analysis. We obtained 44 samples from nine zoos. DNA was extracted from tissue and blood using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's instructions (with a special protocol for blood). For hair samples we extracted the DNA with a modified Chelex 100 protocol, using 400 μl of a 10% Chelex solution with addition of 7 μl Proteinase K (18 mg/ml) per sample and an overnight lysis step (Estoup et al. 1996; Walsh et al. 1991).

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All samples were genotyped at nine di-nucleotide repeat microsatellite loci (FCA08, FCA23, FCA45, FCA58, FCA77, FCA90, FCA126, FCA132 and FCA149). These primers were originally characterized in the domestic cat (Menotti-Raymond et al. 1999; Menotti-Raymond and O'Brien 1995). Amplification was performed in a Mastercycler (Eppendorf) using the 2.5× 5PRIME HotMasterMix (5PRIME). For each PCR we used 5 μl reaction mix containing: 1.2 μl genomic DNA, 2.2 μl HotMasterMix, 2.2 μl water and 0.1 μl of the forward and reverse primers. The PCR conditions were as recommended by the manufacturer, with an annealing temperature of 55 °C for most of the primers (exceptions: FCA45: 62 °C, FCA77: 58 °C, FCA90: 52 °C). The 5′-end of each forward primer set was labelled with a fluorescent marker (5-FAM, TMR or JOE). The products were genotyped on a MegaBACE 1000 automated DNA sequencer (GE Healthcare). Fragment lengths were determined using MegaBACE Fragment Profiler (Amersham Biosciences). To minimize genotyping errors such as allelic dropout due to low DNA concentrations we applied a multiple tube approach, as recommended in Taberlet et al. (1996). Samples were only included into further analyses if they yielded unambiguous results in three independent replications.

We tested our data for the occurrence of null alleles with Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) and for linkage disequilibrium with Fstat 2.9.3.2 (Goudet 1995). The genetic structure of our sample was analyzed using structure 2.3.3 (Pritchard et al. 2000). For this we assumed admixture to be possible and chose the correlated allele frequency model with a burn-in of 100,000 simulations followed by one million Markov chain Monte Carlo simulations. Tests were run for $K = 1–10$ with ten iterations per K. As the method for inferring the optimal K value suggested by Evanno et al. (2005) tends to result in low K values (Campana et al. 2010; Hausdorf and Hennig 2010) and generally works better for scenarios with strong genetic differentiation (Waples and Gaggiotti 2006), we used both the method described by Pritchard et al. (2000) and the method suggested by Evanno et al. (2005) to infer K. In our case, both methods revealed an identical value for K. The individuals were assigned to genetic clusters using the highest assignment probability. A F_{ST} based AMOVA with 9999 iterations was performed in GenAlEx 6.4 (updated from Peakall and Smouse 2006) with the three structure based genetic clusters as populations.

We used Fstat to calculate the number of alleles (n_a) and the inbreeding coefficient (F_{IS}). Expected and observed heterozygosities (H_e and H_o) for each locus and population were determined in GenAlEx. The accuracy of linkage disequilibrium methods to assess the effective population size has been criticized by several authors (e.g., Beebee 2009; England et al. 2006), while the Bayesian approach was rated to be more accurate (Beebee 2009). Therefore, the effective population size (N_e) of the captive populations was determined with ONeSAMP (Tallmon et al. 2008), which uses a Bayesian approach. The upper and lower bounds of the prior distribution for N_e were 2 and 500, respectively. Additionally we performed a Wilcoxon signed rank statistic implemented in the software package Bottleneck 1.2.02 (Cornuet and Luikart 1996) to test for genetic signatures of population bottlenecks. The two-phase mutational model (TPM) is the most likely mutation model for microsatellites (Piry et al. 1999). We therefore used the TPM and set the proportion of stepwise mutations to 0.3 (setting the proportion to 0.7 did not change the results).

The mean relatedness between individuals was calculated using Coancestry (Wang 2010). This programme calculates seven different relatedness estimators. We tested all of them and assessed their performance by comparing the results with known relationships. Based upon these comparisons, we chose the estimator based on Queller and Goodnight (1989), as it showed the smallest deviation from our known relationships and the smallest variance. Additionally, we tested the relatedness between the breeding pairs and the four sampled founders with the same estimator. To measure individual F, four estimators are available in Coancestry. We used the TrioML estimator based on Wang (2007) as it fitted our data best based on the relatedness data for the whole captive population (see above).

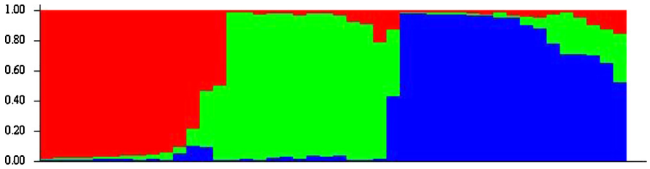

Fig. 1. Genetic clusters obtained from the Structure analysis ($K = 3$) for all samples ($n = 44$). For definition of clusters see text. Each individual is represented by a single vertical line, divided into K colours. The coloured segment shows the individual's estimated proportion of membership to that genetic cluster.

There was no evidence for null alleles, large allele drop-out or scoring errors and all pairwise tests for linkage disequilibrium were not significant ($P > 0.05$). However, two juveniles did not match their parents and siblings at six of the nine analyzed loci but perfectly matched a second breeding pair of the concerning zoo.

The most likely number of genetic clusters (K) revealed by structure was three (Fig. 1), independent of the method used to determine the optimal K. According to studbook data, these three clusters can be explained by their ancestry. Our sample includes genetic information from twelve of the original 18 founders (four via direct sampling and further eight by indirect sampling of their descendants). Individuals assigned to cluster 1 (red in Fig. 1) show a direct descent from three founders captured at Ad Dawhah, Qatar (studbook # 403, 409 and 504). Nearly all individuals from this cluster are currently kept in the Al-Wabra Wildlife Preservation (Qatar). Individuals assigned to cluster 2 (green) can be traced back to eight founders. These include individuals which are descendants from four founders from Al-Wabra (studbook # 403, 409, 504, 510), two from Saudi Arabia (studbook # 231 and 340) and two from Riyadh Zoo (Saudi Arabia, studbook # 261 and 228). Cluster 3 (blue) includes individuals with a complex ancestry, which can be traced back to eleven founders. It includes all already mentioned founder lineages (except for studbook # 510), with the addition of one Saudi Arabian founder from Riyadh Zoo (studbook # 235) and another Saudi Arabian breeding line which originated in the Society of Scientific Care Inc (USA, studbook # 143, 149 and 150). The individuals assigned to cluster 2 and 3 are now living in several zoos without any obvious geographic correlation to the genetic clusters. Individuals from Al-Wabra Wildlife Preservation are found in all clusters except cluster 3, which comprises only sand cats from European zoos. Cluster 2 is a mixture of sand cats from Al-Wabra Wildlife Preservation and Ebeltoft Zoo. The AMOVA revealed, that most of the genetic variation occurred within individuals (85%), the difference among individuals was not significant (0%), but a significant portion was explained by the difference between the three genetic clusters (15%).

H_e and H_o were rather high in the ex situ population of the Arabian sand cat and mean F_{IS} was not significant (Tables 1 and 2). The estimated N_e was 22.16 (median, 95% CI: 18.7–37.6). We detected a significant sign for a recent genetic bottleneck (Table 1). The mean relatedness between dyads was $r = -0.024 \pm 0.009$. The mean

Table 1
Genetic parameters of the captive populations of *Felis m. harrisoni* (with n = number of samples, n_a = mean number of alleles per locus, H_0 and H_e = observed and expected heterozygosity) and two-tailed P-values of the Wilcoxon test for heterozygosity excess or deficiency under the two-phase mutational model (TPM).

n	n_a	H_0		H_e		TPM
44	4.67	0.649	95% CI: 0.58–0.72	0.655	95% CI: 0.60–0.71	0.00391*

* Significant values ($P < 0.05$).