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Novel mtDNA haplotypes represented in the European captive population of the Endangered François' langur (*Trachypithecus francoisi*)

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Abstract

Assessing the genetic diversity of captive populations of endangered species is key to the successful management of conservation-breeding programs. In this study, we sequenced a 393-bp fragment of the mitochondrial DNA (mtDNA) control region of 23 captive individuals of the Endangered François' langur (*Trachypithecus francoisi*) to assess the mtDNA diversity of the European captive population and to identify the possible geographical origins of the population founders. Combined with 42 sequences previously published from 29 wild François' langurs, we identified a total of 40 haplotypes in *T. francoisi*, including 12 haplotypes in the 23 samples from the European captive population. Only one of the haplotypes from captive animals has previously been reported from wild populations; the remaining 11 haplotypes are newly reported here. Our results suggest that the captive *T. francoisi* population currently holds a relatively good genetic diversity compared with many other captive populations, that this diversity originates from a fairly broad range across the species' distribution in the wild, and that the captive population could play a significant role in increasing genetic diversity of isolated wild populations. However, the European captive population is currently quite small, and genetic diversity could be lost rapidly, which has been demonstrated in other captive populations. We recommend further investigation of the genetic diversity of captive and wild *T. francoisi* populations, as well as the effective conservation of this diversity.

Keywords François' langurs · mtDNA · Conservation genetics · Captive populations

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Assessing the genetic diversity of captive populations of endangered species is important for management of conservation breeding programmes. Whilst the genetic status of some captive populations suggests they are unlikely to have any direct conservation value (Ogden et al., 2018), other captive populations may have the genetic potential to provide animals for reintroduction or reinforcement projects that aim to help species recovery (Svengren et al., 2017).

François' langur (*Trachypithecus francoisi*) is classified as Endangered on the IUCN Red List (Nadler et al., 2020), with wild populations occurring in fragmented habitats in southern China and northeastern Vietnam. Hunting and habitat loss have caused severe population declines in both countries (Nadler et al., 2020), whilst live-trapping for captive-breeding and exhibition in zoos has also been identified as a threat to the species (Li et al., 2007). Approximately 140 *T. francoisi* are reported in captivity, 40 of them in European zoos. The size of the global captive population provides the prospect of reinforcing current wild populations or reintroducing *T. francoisi* to its former range, if sufficient habitat survives. The European captive population, managed as an EAZA Ex situ Programme (EEP), is fairly newly established, with the first founders of the current EEP population being imported in 1992 and the European studbook being established in 2005. Pedigree records of EEP individuals, available within zoos records, suggest that retention of original founder diversity is likely to be high, but no genetic assessment of the EEP has previously been conducted.

In this study, we sequenced a 393 bp fragment of the mtDNA D-loop from 23 captive *T. francoisi* from European zoos, using samples previously collected and stored by zoos and museums (see Supplementary Material for details). Combined with 42 sequences previously published by Liu et al. (2013) from 29 wild *T. francoisi*, 12 *T. leucocephalus*, and 3 *T. poliocephalus*, we produced a multiple alignment showing 96 polymorphic sites. Pair-wise sequence difference ranged from 0% to 6.8%, with a mean of 3.4%. We designed the sampling of captive individuals of *T. francoisi* to maximise the number of matrilines sequenced and included almost all known matrilines in European zoos, this is reflected in a high haplotype diversity (h) (0.925 ± 0.001), similar to that in wild populations of this species, while nucleotide diversity (π) was slightly lower (0.026 ± 0.0002) than in wild animals (Liu et al., 2013).

We identified a total of 40 haplotypes in *T. francoisi*, including 12 haplotypes in the 23 samples from the captive EEP population. Only one of the haplotypes from EEP animals has previously been reported from wild populations, while the remaining 11 haplotypes are newly reported. This suggests that either not all extant wild haplotypes were sampled by Liu et al. (2013) or that some wild haplotypes became extinct prior to the sampling of the wild populations.

We constructed a minimum-spanning haplotype network (Fig. 1), which illustrates that haplotypes from wild populations of *T. francoisi* show strong geographical specificity and local homogeneity, consistent with the previous analysis (Liu et al., 2013). One captive EEP langur (studbook #27) presents the same haplotype as wild *T. francoisi* sampled in Guizhou Province, China. Nine new haplotypes identified in the EEP population cluster with haplotypes from Guangxi Province, China, and two (haplotypes Tf10 and Tf11) link haplotypes from Vietnam and

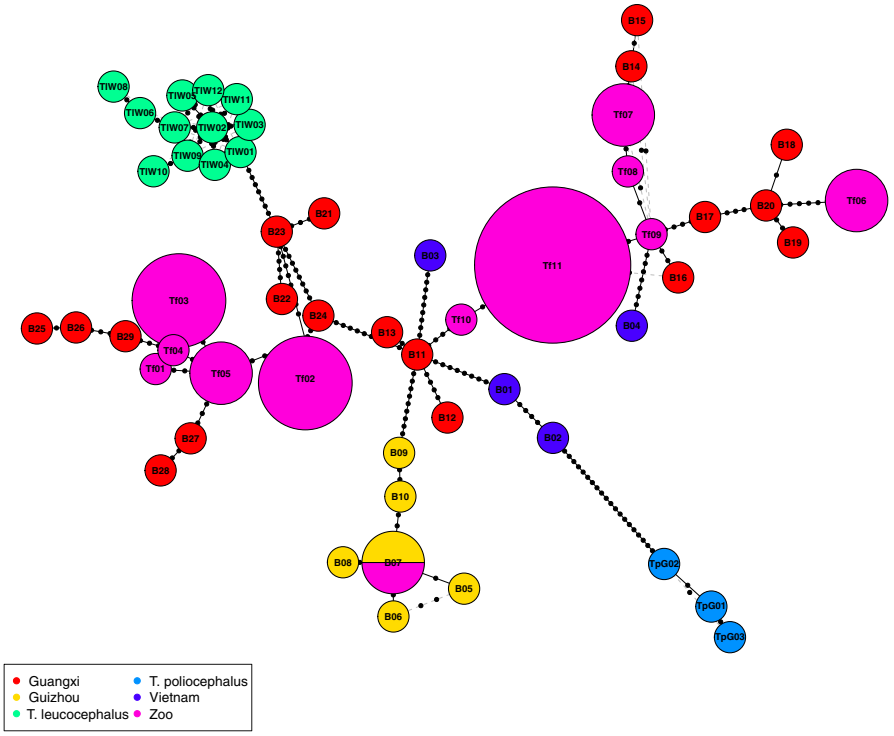

Fig. 1 Minimum-spanning haplotype network of *Trachypithecus* species included in the study. Each circle represents a haplotype and the diameter scales to haplotype frequency. Samples obtained from wild populations by Liu et al. (2013) or from the European captive population are colour-coded. The number of mutational steps is shown as dots on the lines connecting haplotypes

Guangxi (Fig. 1). This network shows that the EEP population appears to represent genetic diversity from a relatively large portion of the species' range. As previously reported, *T. leucocephalus* haplotypes form a unique haplogroup, separated by nine mutational steps from Guangxi haplotypes of *T. francoisi*, whereas *T. poliocephalus* haplotypes, forming another unique haplogroup, are separated by 20 steps from Vietnamese haplotypes of *T. francoisi* (Liu et al., 2013).

Our results suggest that the *T. francoisi* EEP population currently holds relatively good genetic diversity and that this diversity originates from a broad range across the species' current distribution in the wild. This suggests that the EEP population could play a significant role in increasing genetic diversity of isolated wild populations. The 12 mtDNA haplotypes identified show that the *T. francoisi* EEP population is relatively diverse compared with many other captive populations. For example, Ogden et al. (2018) identified only two to four mtDNA haplotypes within each of five different European captive populations of ungulate taxa. However, the EEP population is currently quite small, and genetic diversity could be lost rapidly as has been demonstrated in other captive populations. At least two of the haplotypes in the *T. francoisi* EEP will not persist, because they are represented by only male founders

(Tf01 and Tf09). Other haplotypes are also likely to be lost if matrilines representing rare haplotypes do not produce female descendants. This illustrates the potential for rapid loss of genetic diversity in small populations, a serious issue impacting the viability of many captive populations. We recommend further analysis, including nuclear DNA markers, to better assess genetic diversity within the EEP and wild populations and the implications for the long-term viability of the populations.

This study adds to the growing body of work attempting to provide relevant genetic information to help improve the management of captive populations for direct conservation benefits (Ogden et al., 2018; Svengren et al., 2017). It has illustrated the benefit of sampling mtDNA diversity in a captive population prior to this diversity being lost to help ascertain the geographical origins of the founders of the captive population. In this respect, this study reflects the importance of storing genetic material of captive individuals, especially of founder individuals and their offspring, preferably as museum specimens. In this study, we were fortunate to be able to sample five founder individuals that had been preserved in the CryoArks biobank at National Museums Scotland. We recommend increased efforts to use genetic and other tools to assess captive populations, their direct conservation value, and the potential for integrating captive populations into effective conservation planning.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10764-022-00295-x.

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Data availability All data generated or analysed during this study are included in this published article. Fasta sequences are submitted to GenBank with IDs: ON159335-ON159357.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics statement Our work was conducted according to international guidelines. Samples from living animals were obtained during routine veterinary procedures.

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