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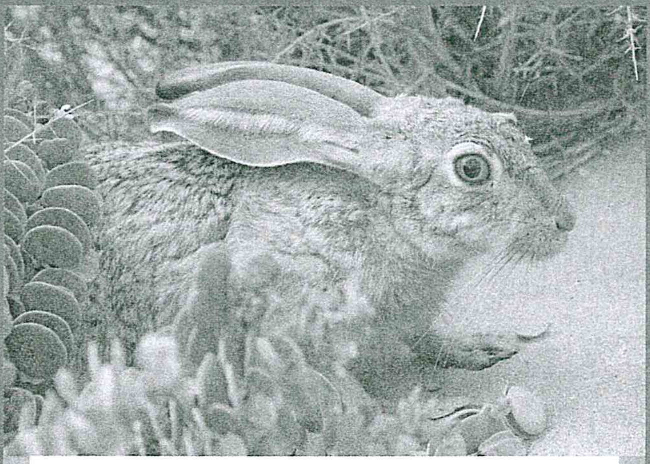

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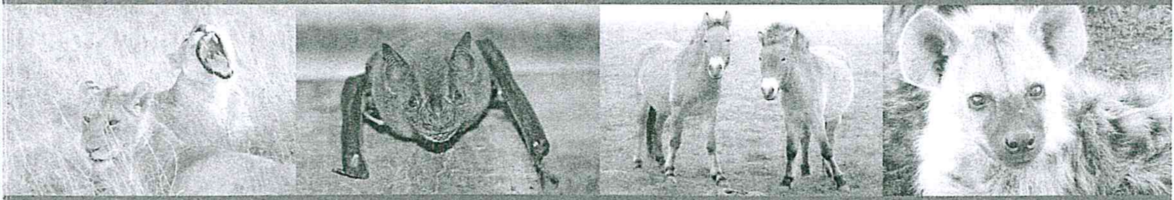

Parentage analysis on a colony of African penguin (*Spheniscus demersus*) hosted in an Italian biopark

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The knowledge of genealogical relationships and of the inbreeding status can assist zoo managers in decision making on captive animals relocation. Aim of the present study was to carry out parentage analysis on a colony of African penguins in order to confirm the pedigree derived from field observations and certificates of birth and to assess extra-pair mating. The colony has been hosted in an Italian biopark since 2009. It originated by the union of three *ex situ* groups: two from different parks in the Netherlands and one coming from a German facility. Blood samples of 56 penguins were collected in EDTA tubes and a spin column-based nucleic acid purification method was used to extract DNA. Sex of each animal was determined through the amplification of the chromo-helicase-DNA-binding1 (CHD1), as described in literature. A panel of 11 STRs was analysed using 5' labelled primers: eight STRs were already characterised in *Spheniscus demersus* and three were described in *Spheniscus humboldti*. Genotype data were analysed with COLONY (version 2.0.5.9) that implements full-pedigree likelihood methods to simultaneously infer sibship and parentage among individuals using multilocus genotype data. Runs was carried out several times, assuming different mating systems (polygamy or monogamy of male and female separately or simultaneously), using different random number seeds, and repeating analyses with different lengths of the run. Paternity and maternity exclusion data were supplied based on animal age considering three years as breeding age. Results on full-sib, maternity and paternity were considered. The previous family tree was mostly confirmed, even though the probabilities of assignment varied widely (0.67-1 for full likelihood methods; 0.55 - 0.95 % with pure pairwise likelihood approach). Four discordances with assigned kinships were discovered and unknown genetic relationships among two couples of individuals were found. The most uncertain attributions were observed between clusters originating from the two Dutch zoos: in two cases penguins belonging to the different groups resulted to be relatives.

Evaluation of the inbreeding status of the population gave a nonsignificant F*is* value of -0.005 (p-value = 0.57). We recommend additional investigations to understand if previous exchanges between the two Dutch zoos could have occurred or a documental issue could be taken into account. Overall, our results showed that documented evidence of parentage within a colony alone is not a reliable mean to trace relationships among members and that both certificates and genetic methods should be applied.