Conservation Genet Resour
DOI 10.1007/s12686-011-9529-x

TECHNICAL NOTE

Molecular sexing of tigers, *Panthera tigris*

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Received: 27 June 2011 / Accepted: 6 September 2011

Abstract We report the development of a fast and reliable PCR-based method for sex identification of tiger DNA designed to be incorporated into fluorescent short tandem repeat (STR) profiling. A single primer pair, consisting of a fluorescently-labelled forward primer and an unlabelled reverse primer, is used to co-amplify homologous fragments of a zinc finger (ZF) protein intron which exhibits size polymorphism between the X and Y chromosomes. The ZFX and ZFY amplicons differ in size by 12 bp and can thus be differentiated by capillary electrophoresis.

Keywords Molecular sex identification · Tiger · Zinc finger protein · Forensics · Felids

Introduction

Tiger parts are highly sought after, primarily as an ingredient in traditional Asian medicine and as ornamental skins. The demand for tiger parts along with habitat destruction was the principle cause for the decline in tiger numbers during the twentieth century, and despite international legislation and domestic bans on tiger part trade, poaching and illegal activity continue to threaten the world's tigers today (Linkie et al. 2003). Six extant subspecies of tiger are currently recognized, *Panthera tigris tigris, Panthera tigris corbetti I, Panthera tigris corbetti II (jacksoni/malayensis), Panthera tigris altaica, Panthera tigris sumatrae* and *Panthera tigris amoyensis*, and all are listed as 'endangered' or 'critically endangered' on the IUCN Red List of Threatened Species (IUCN 2010). Population monitoring of these species, including sex ratio data, is therefore vital to provide information for their conservation and management. Although the sex of adult tigers can be determined by an experienced observer, field observations may be hampered by distance, position relative to the animal or by vegetation. Furthermore, juvenile tigers are difficult to sex, and illegally traded parts and derivatives of tigers have no sexual characters.

Here, we report the development of a non-invasive molecular sexing technique for tiger DNA. In this study, we exploit a size difference (~12 bp) between ZFX and ZFY homologs, elucidated by capillary electrophoresis, to determine the sex of tiger samples. The current research forms part of a larger project to develop genetic markers for linking tiger parts to their individual source animal, in order to help investigate illegal poaching and trade of tigers, and their parts.

Methods

Samples

Samples of blood or hair were collected from zoo tigers of known sex (male = 15, female = 17) from four of the six

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Published online: 01 October 2011