ARTICLE IN PRESS

Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Multiple paternity: A compensation mechanism of the Chinese alligator for inbreeding

Huan Wang[a], Peng Yan[a], Shengzhou Zhang[a], Long Sun[a], Min Ren[a], Hui Xue[a], Fang Zhang[a], Rong Wu[b], Xiaobing Wu[a,*]

[a] Key Laboratory for Conservation and Use of Important Biological Resources of Anhui Province, College of Life Sciences, Anhui Normal University, Wuhu, Anhui 241000, China
[b] Alligator Research Center of Anhui Province, Xuanzhou 242000, China

ARTICLE INFO

Keywords:
Multiple paternity
Chinese alligator
Mating
Genetic diversity hypothesis
Inbreeding

ABSTRACT

The Chinese alligator *Alligator sinensis* is a critically endangered species endemic to China. Knowledge about reproductive strategies of a species contributes to their conservation. Little is, however, known about the reproductive strategies and its impact on the population. In the present study, an easy and non-invasive genetic method was used to improve the understanding of mating system of Chinese alligators and its effect on the population genetic diversity by nine polymorphic microsatellite loci. There was a high incidence of multiple paternity among 50 clutches, with a total 60% of the clutches having multiple paternity and up to three males contributing to single clutches. In addition, polyandry females choose to mate with males that are more distant in relatedness compared with monogamy females. Multiple paternity can decrease the inbreeding coefficient, while there is no significant difference between single and multiple paternity ($P > 0.05$). Furthermore, there was an increased allelic diversity (though not heterozygosity) in multiple paternity sired offspring compared with the single paternity sired offspring in F2 generations ($P < 0.05$), as predicted by the genetic diversity hypothesis. Multiple paternity may function as an important inbreeding avoidance compensation mechanism leading to the potential of the species to avoid extinction. These findings will not only enhance the understanding of the mating system and the biological traits of the Chinese alligator, but also improve the captive breeding program management and conservation strategies of the endangered species.

1. Introduction

Polyandry is a mating system in which individual females mate with multiple males, often leads to multiple paternity, and is very common in many species of both invertebrate and vertebrate animals (Pizzari and Wedell, 2013; Croshaw et al., 2017). While multiple mating in female animals can be a species liability, it can be a costly behavior in terms of time and energy expenditure, elevated risks of predation, injuries, and sexually transmitted diseases (Thonhauser et al., 2013). These energetic and endangerment costs suggest that there are compensating benefits for mating with multiple males, which provides benefits to the female directly or indirectly (Tregenza and Wedell, 2002). Unlike many bird and mammal species, parental care of hatchlings in reptiles does not occur or is rudimentary and males do not provide direct resources to the female before, during or after mating (Uller and Olsson, 2008). Polyandry females may, therefore, mainly obtain indirect genetic benefits derived from the co-occurrence of sperm from two or more

* Corresponding author at: College of Life Sciences, Anhui Normal University, 1 Beijing East Road, Wuhu, Anhui Province 241000, China.
E-mail address: wuxb@mail.ahnu.edu.cn (X. Wu).

http://dx.doi.org/10.1016/j.anireprosci.2017.10.016
Received 15 August 2017; Received in revised form 13 October 2017; Accepted 25 October 2017
0378-4320/

males, which may promote sperm competition and determine which sperm are most viable, and additionally, polyandry can also increase offspring viability (Blouindemers et al., 2005; Eizaguirre et al., 2007; Whittingham and Dunn, 2010). For female reptiles, the advantage of multiple mating is not well understood (Bocedi and Reid, 2015).

Based on observations of crocodilians in captivity, males have behaviors of establishing breeding territories, excluding other adult males, and mating with multiple females, and as a consequence, the mating system was long believed to be polygynous and monandrous (Grigg and Kirshner, 2015; Rossi Lafferriere et al., 2016). Molecular genetics can be used to genotype individuals, determine parentage, and facilitate the determination of patterns of mating systems and gain information on the reproductive biology that would otherwise be extremely difficult to document (Stewart and Dutton, 2011; Clark et al., 2014; Freda et al., 2016). Due to characteristics such as the high variability, neutrality, co-dominance, and Mendelian inheritance of these types of markers, genetic markers such as DNA microsatellites are beneficial for studying mating systems and for determining paternity (Bórquez and Brante, 2017). Evidence of multiple paternity has been detected in several crocodilian species by using such methods and data (Rossi Lafferriere et al., 2016).

The mating system has an important role in determining amount of genetic diversity in captive and wild populations (Anthony and Blumstein, 2000). The maintenance of genetic diversity in populations is also strongly influenced by mating systems, which ultimately determine how variation is transmitted across generations (Pérez-González et al., 2014). Genetic variation within and among populations is, however, influenced by the genetic content of the founders and the migrants following establishment, especially if the populations are small, and composed of multiple mated females. This can lead to more genetic variation in founder groups than single mated females, and the mating system can affect the effective population size and evolution of the species (Rafajlović et al., 2013; Sari et al., 2017).

The Chinese alligator, *Alligator sinensis* is a freshwater crocodilian species which is endemic in China and one of the most endangered species of the world. The wild population is now estimated to be less than 150 individuals. In 1972, the Chinese government listed this species as a first-level state-protected species, while the IUCN Red List of Threatened Species listed as "Critically Endangered". Captive breeding is an integral part of many species recovery plans. In response, nature reserves and farms for the Chinese alligator were established in the Anhui and Zhejiang Provinces (Wu et al., 2002). Meanwhile, artificial breeding and the related basic research with Chinese alligators were conducted since 1980s (Chen et al., 2003). Though the issues with artificial incubation and breeding of the Chinese alligators have been successfully resolved, a lack of genetic diversity and genetic variation existed in this small population (Yan et al., 2006). Knowledge of the genetic mating system is essential for effective management and can be important knowledge with the endangered species for its recovery and development (Ojeda et al., 2017).

It is difficult to observe the mating behavior of Chinese alligators, however, as they mate underwater. The previous study using molecular genetic methods indicated that multiple paternity existed in *A. sinensis*. There were three of ten clutches that were sired by at least two different males, however, the sample size was small. The mating system of Chinese alligators could, therefore, not be definitively resolved when considering results from the previous study (Hu and Wu, 2010). Nonetheless, results from the previous study provided for evidence for further studying the mating system of this ancient reptile species and impact on the genetic diversity.

In the present study, a molecular genetics method was used for studying greater numbers of individuals so as to enhance understanding of the mating system of the Chinese alligator and why individuals of this species have the mating system that is beneficial for species with small populations such as the Chinese alligator. To investigate this, male-female relationship, the inbreeding coefficient, genetic variation and differentiation, the genetic diversity was analyzed. The results will enhance the understanding of reproductive system and sexual selection of the Chinese alligator, and also improve the captive breeding program management and conservation strategies of this endangered species.

2. Materials and methods

2.1. Study site and sampling

Samples of captive Chinese alligators were collected in Wuhu (WH), Xuanzhou (XZ) and Gaojingniao (GJM; Anhui Province, China; 118°30′–119°35′E, 30°18′–31°18′N; Fig. 1).

It should be noted that the Gaojingmiao forestry farm has an alligator re-wilding training area and wild-release area. In re-wilding training area those alligators that resided in semi-natural state were chosen from the Xuancheng Alligator Breeding Center. After training for a lengthy period, those alligators that were well-adapted to their environment are discharged to the wild-release area. Eleven clutches of the alligators that had transitioned to the wildness training area were collected at Gaojingmiao re-wilding training area. There were 11 clutches of the captive *A. sinensis* from which samples were collected at Xuancheng Alligator Breeding Center, and another 28 clutches of the captive alligators were collected at the Wuhu Alligator Farm. In total, samples were collected from 50 clutches (total number of eggs, $n = 755$) during the breeding season of 2015 with all of the offspring being from the third filial generation (F3). Eggs from each clutch were incubated separately in wooden boxes containing the nests material and hay. Upon hatching, the umbilical cord tissue samples were taken from all alligators. Samples from two or three eggshell and eggshell membranes were chosen from each clutch at random. Samples were cleaned with 0.70% physiological saline, and stored at −20 °C in 95% ethanol. In addition, 50 blood samples of the second generation (F2) individuals hatched in 2003 and 2004 were collected and stored at −80 °C in the laboratory.

All procedures were approved by the Forestry Authorities of China and the Animal Care and Welfare Committee of the College of Life Sciences, Anhui Normal University.