Multiple Paternity: A Compensation Mechanism of the Chinese Alligator for Inbreeding

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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. 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. Polyandry females may, therefore, mainly obtain indirect genetic benefits derived from the co-occurrence of sperm from two or more males, which may promote sperm competition and determine which sperm are most viable, and additionally, polyandry can also increase offspring viability. 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.

Methods

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. The DNA of offspring was extracted from umbilical cords. The maternal DNA was extracted from the eggshell and eggshell membranes. To assess for multiple paternity in the 50 clutches, the GERUD2.0 program. The maternal and reconstructed paternal genotypes that were determined utilizing COANCESTRY were used to measure relatedness estimates (R) between the maternal and paternal genotypes using previously described procedures of Wang and the paternal inbreeding coefficient (F) using previously described procedures of Ritland.

Results

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 (Table 1). 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 (Table 2).

Table 1 Presence of multiple paternity in 50 clutches of Chinese alligators

Family	Brood size	No. of fathers	No. of offspring sired by father 1	No. of offspring sired by father 2	No. of offspring sired by father 3	Family	Brood size	No. of fathers	No. of offspring sired by father 1	No. of offspring sired by father 2
GJM1	13	1	13			WH2	17	1	17	
GJM2	12	1	12			WH4	16	2	13	3
GJM3	17	3	7	5	5	WH5	18	2	15	3
GJM4	24	2	22	2		WH6	8	2	7	1
GJM5	11	1	11			WH7	13	1	13	
GJM6	32	1	32			WH8	6	1	6	
GJM7	16	1	16			WH9	6	1	6	
GJM8	20	2	17	3		WH10	9	2	6	3
GJM9	16	1	16			WH11	7	2	5	2
GJM10	21	2	19	2		WH12	13	2	11	2
GJM11	23	2	21	2		WH13	7	1	7	
XZ1	19	1	19			WH14	14	2	13	1
XZ2	20	2	10	10		WH15	20	2	15	5
XZ3	10	1	10			WH16	13	1	13	
XZ4	34	1	34			WH17	12	1	12	
XZ5	13	2	7	6		WH18	12	2	9	3
XZ6	14	2	11	3		WH19	9	1	9	
XZ7	10	2	9	1		WH20	16	2	15	1
XZ8	11	2	10	1		WH21	6	1	6	
XZ9	22	3	12	8	2	WH23	15	2	8	7
XZ10	18	1	18			WH24	10	2	7	3
XZ11	11	2	10	1		WH25	12	2	6	6
WH1	14	2	10	4		WH26	15	1	15	
WH3	29	3	24	3	2	WH27	9	2	8	1
WH22	13	3	7	5	1	WH28	18	2	14	4

Table 2 Genetic diversity comparison between the F2 and two types of F3 generations

Locus	F2	F3 (M)	F3 (P)	F2	F3 (M)	F3 (P)
	Na	*Na*	*Na*	*Ho/He*	*Ho/He*	*Ho/He*
Alsu7	3	2	6	0.3/0.2648	0.3263/0.2736	0.2773/0.2476
Alsu8	3	3	3	0.38/0.3533	0.4246/0.4014	0.4585/0.4408
Alsu10	3	3	3	0.58/0.5190	0.5825/0.6618	0.5677/0.5507
Alsu11	5	5	7	0.8/0.5972	0.7193/0.5831	0.5186/0.5632
Alsu12	3	3	3	0.62/0.4988	0.5228/0.4815	0.5611/0.5518
Amiu18	5	6	9	0.62/0.5661	0.4561/0.6072	0.5262/0.5683
Asiu1	3	4	7	0.48/0.4063	0.4877/0.4570	0.4672/0.4703
Asiu20	3	3	4	0.6122/0.5115	0.6211/0.5274	0.548/0.5199
Alsu6	2	2	5	0.24/0.2432	0.1965/0.2263	0.2751/0.2771
Mean±SE	3.33 ± 0.33^{b}	3.44 ± 0.44^{b}	5.22 ± 0.72^{a}	0.51 ± 0.06^{m}/ 0.44 ± 0.04^{n}	0.48 ± 0.05^{m}/ 0.47 ± 0.05^{n}	0.47 ± 0.04^{m}/ 0.47 ± 0.04^{n}

Note: F2: F2 generation group; F3: F3 generation group; M: monogamy group; P: polyandry group. Na: number of alleles per locus; Ho: observed heterozygosity; He: expected heterozygosity. Different superscripts a, b indicate differences among groups ($P < 0.05$); Superscript “m” and “n” of Ho and He means no difference in the three groups ($P > 0.05$).

Conclusions

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.