Parentage determination of an isolated Yangtze finless porpoise population *Neophocaena phocaenoides asiaeorientalis* in the Yangtze Tian-e-Zhou Baiji National Natural Reserve based on molecular data*

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Received May 31, 2004; revised August 30, 2004

Abstract Reproductive behaviors are poorly known for the Yangtze finless porpoise *Neophocaena phocaenoides asiaeorientalis*. In this study, the parentage of an isolated Yangtze finless porpoise population inhabiting the Yangtze Tian-e-Zhou Baiji National Natural Reserve is determined by analysis of microsatellite loci and mitochondrial DNA (mtDNA) control region sequences, and the porpoise's reproductive behaviors are studied. Overall 4 full parentage assignments and additional 3 single parentage assignments were determined for the population of 23 individuals. The analysis shows that their estimated reproductive cycle is shorter than that reported previously and there probably exists an overlapping between gestation and lactation period. The study also shows that the female does not show fidelity to a particular male for breeding and vice versa, the oldest males did not monopolize mating and the dominance rank could not be so strict for the porpoise society. Moreover, the porpoise's mating pattern and relatedness among candidate parents are discussed here. These results provide important information for making guidelines of management and conservation for this protected population.

Keywords: Yangtze finless porpoise, isolated population, reproductive behavior, parentage.

It is usually difficult to observe cetacean reproductive behaviors and patterns of parentage, yet such knowledge is important for their conservation and management. In recent years, the use of genetic markers to identify parent-offspring relationships is becoming an important tool in molecular ecology[1]. The assessment of precise parental relationships within populations through parentage allocation allows researchers to define social structure, mating patterns, kinship and quantify reproductive success[2]. Such studies in cetaceans have been performed only in several species[3-7], and no data are available for those of the Yangtze finless porpoise *Neophocaena phocaenoides asiaeorientalis* up to date.

The Yangtze finless porpoise, an endemic and endangered small cetacean population, is only distributed in the middle and lower reaches of the Yangtze River. Previous studies have shown that their minimal age at maturity is 4 years old for female and 4.5 for male[8]. Regardless of the reproductive rest, the female reproductive cycle is estimated to be about one and a half years[8], in which gestation period is 11 months[9] and lactation period is estimated to be about 6 months[8]. Mating appears to be seasonal with a peak from late February to middle June and mainly from March to May[10]. Additionally, the peak season for birth is from February to May, although it could be observed almost year around[8,11]. Generally, the Yangtze finless porpoise's mating system is described as polygyny, but there is no direct evidence for this description[8,12]. Because of the complexity of reproductive behaviors and tactics, the results only based on the behavior observation and/or limited collected specimens are not enough to confirm their reproductive behaviors.

Ex situ conservation is one of the important measures to save the Yangtze finless porpoise. The establishment of a breeding Yangtze finless porpoise population in the Yangtze Tian-e-Zhou Baiji National Natural Reserve represents the first attempt at *ex situ* conservation efforts for a cetacean species in the world[11,13], which is very important to genetic conservation and management of the Yangtze finless

* Supported by the National Natural Science Foundation of China (Grant No. 30170142), Chinese Academy of Sciences (Grant No. KSCX2-SW-118), the Institute of Hydrobiology, CAS (Grant No. 220103), and Ocean Park Conservation Foundation in Hong Kong (2002—2003)

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porpoise, baiji *Lipotes vexillifer* and other rare species. In this study, we try to determine the maternity and paternity by analysis of microsatellite loci and mtDNA control region sequence and explore some reproductive behaviors of the reserve population. These results provide important guidance for management and conservation of this endangered species.

1 Materials and methods

1.1 Study area and population

In 1992, the Tian-e-Zhou Oxbow, located in Shishou, Hubei, China, was approved by the central government as a natural reserve for baiji, which is a critically endangered cetacean species occurring in the Yangtze River. This oxbow was formed naturally when it was cut off from the main stream of the Yangtze River in 1972. It is 21 km long, 1—1.5 km wide, and has an average bottom depth of 4.5 m (Fig.1)[11]. Instead of the baiji, the Yangtze finless porpoises were first introduced into the reserve as early as in 1990 to test the feasibility of the establishment of the reserve during a base-line study. A long-term observation had shown that there were 1—3 individuals born in the reserve each year (Table 1). Since the animals were moved in and out, or died, only four individuals were left in the reserve in early 1997[11, 13]. Thereafter, another three and six individuals were introduced into the reserve from the Yangtze River in 1998 and 1999, respectively[11]. The population size of the reserve was 22 when the present study started[11, 13].

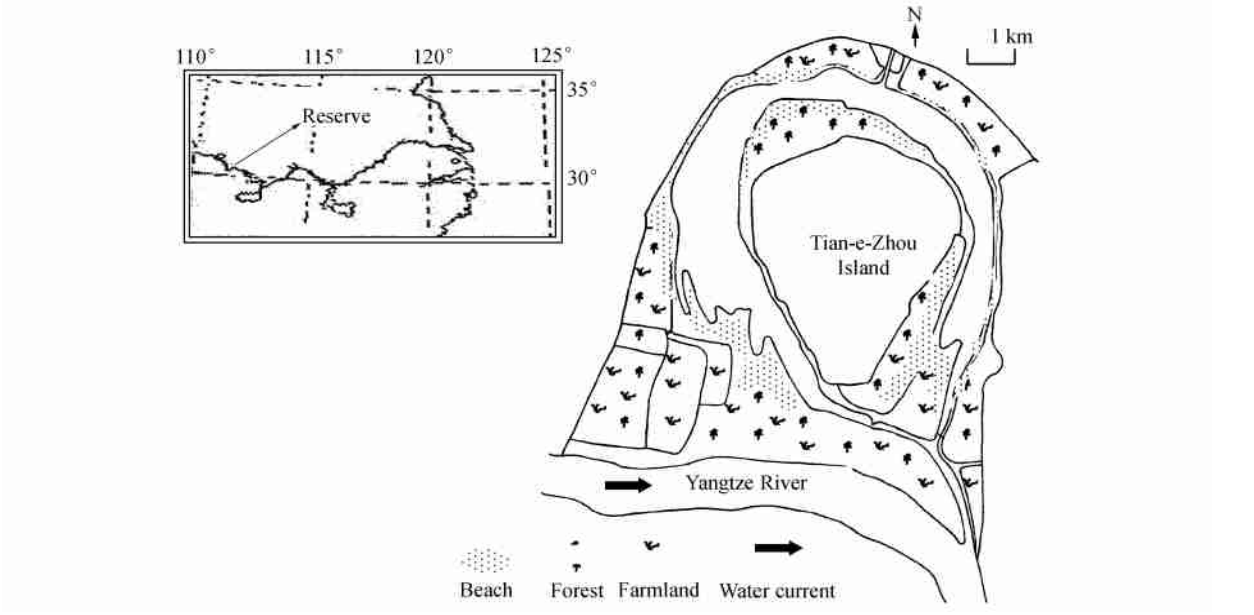

Fig. 1. The geographical location and basic shape of the Yangtze Tian-e-Zhou Baiji National Natural Reserve which was periodically connected to the Yangtze River; the location of the reserve on the middle reach of the Yangtze River, China, is indicated.

Table 1. Historical records and observation results for the reserve population

Date	Animals introduced[a]		New born	Population size
	Females	Males		
Early spring 97				4
Apr-97			1++	5
Apr-98			2++	7
Dec-98	2	1		10
Apr-99			1+1++	12
Dec-99	2	4		17[b]
Apr-00			1+ 1++	19
Apr-01			3++	21[c]
Apr-02			1++	22
Total	4	5	11	22

a) the number of animals introduced from the Yangtze River; b) the population size after one was removed to captivity; c) the population size after one died during this period; '+' conceived in the Yangtze River; '++' conceived in the reserve (Refs.[11, 13])

1.2 Sample collection

During early June 2002, we captured all porpoises in the reserve by nets with the permission of the Department of Fisheries Management, Hubei Province. A whole blood sample of 10—20 mL was drawn from each porpoise for DNA extraction. Another female porpoise, captured from the reserve in 1999 and raised in the Institute of Hydrobiology, CAS, was also involved in the study. Totally, 23 individuals were included for analyzing the parental relationship of the population (Table 2).

Table 2. Samples of the Yangtze finless porpoises in analysis

Animal code	Sex	Length (cm)	Age (Year)	Grouping
02TF0101	F	74	0.01	−
02TF0202	F	147	11.9	+
02TM0103	M	107	0.6	−
02TM0204	M	138	3.8	−
02TM0305	M	136	3.4	−
02TF0306	F	129	3.0	−
02TM0407	M	143	4.9	−
02TF0408	F	149	13.7	+
02TM0509	M	114	1.0	−
02TM0610	M	134	3.1	−
02TM0711	M	162	11.8	+
02TM0812	M	149	6.5	+
02TF0513	F	132	3.8	−
02TM0914	M	151	7.1	+
02TM1015	M	144	5.1	+
02TM1116	M	144	5.1	+
02TF0617	F	139	6.6	+
02TM1218	M	161	11.3	+
02TM1319	M	140	4.2	−
02TF0720	F	127	2.5	−
02TM1421	M	155	8.6	+
02TM1522	M	174	⩾13.0	+
02TF0823	F	130	3.2	−

Individuals at the age below 5 years are indicated by "−", whereas individuals at the age above 5 years are indicated by "+".

1.3 DNA extraction

Total genomic DNA was extracted using the protocols included in E.Z.N.A® Blood DNA Kit (Omega Bio-tek, Inc.) and used as the template in PCR.

1.4 Sequencing of mtDNA control region and microsatellite genotyping

A 1.1kb fragment of the mtDNA control region was amplified by PCR with primers L: 5′-GAA TTC CCC GGT CTT GTA AAC C-3′ and H: 5′-TCT CGA GAT TTT CAG TGT CTT GCT TT-3′[14]. Amplification reaction was performed in a 50μL volume containing about 200ng DNA template, 0.8μmol/L of each primer, 2.5mmol/L of Mg^{2+}, 0.24mmol/L of each dNTPs, 3U pfu DNA polymerase (Sangon) and 1× PCR buffer on a PTC-100TM Programmable Thermal Controller (MJ Research Inc.). PCR cycling profile consisted of an initial denaturation of 3min at 95 ℃, followed by 30 cycles of 40s at 95 ℃, 1min annealing at 63 ℃, and 2min at 72 ℃, with a final extension of 7min at 72 ℃. Amplified fragments were purified with QIAquick® PCR purification kit, and sequenced from both ends with primers L and H, respectively, on an ABI377 automated DNA sequencer. The mtDNA control region sequences were aligned using the program Clustal X[15] with all parameters set to default values. After correction by hand, we combined L and H sequences from the same sample into a consensus sequence and then analyzed and compared the haplotypes that defined.

Thirty-seven microsatellite loci were amplified for each individual in reactions as described (Table 3)[5,16-21]. PCR products were run on 6.0% denaturing polyacrylamide gels, and then each locus was re-amplified and run at least twice to ensure accuracy of scoring. For microsatellite data, likelihood ratio test for departures from Hardy-Weinberg equilibrium at each locus and linkage disequilibrium between pairs of loci were investigated by the program POPGENE (ver. 1.3.1)[22].

Table 3. Microsatellite primers used in this study

Primer	Reference
PPHO110, PPHO137, PPHO133, PPHO142, PPHO130, PPH0104	Rosel et al.[16]
Texvet3, Texvet2, Texvet7	Rooney et al.[17]
GT575, GT023, GT271, GT101, GT195, GT211, GT307, GT310, GT509	Berube et al.[18]
rw34, rw26, rw2-17, rw31, rw48, rw25, rw2-19, rw4-10	Waldick et al.[19]
Ev10PmA, EV1Pm, EV94Mn, EV104Mn	Valsecchi[20]
JB651A, JB69, JB64	Bond and Amos[21]
409/470, 415/416, 464/465, 468/469	Amos et al.[5]

1.5 Age estimating

Gender was identified morphologically and body length was also measured for each individual. The age for each porpoise was estimated from its body length using the following formula: $Y_{♂} = 114.4458X^{0.1410}$ ($♂ \leqslant 13.0$ Year); $Y_{♀} = 116.2519X^{0.0947}$ ($♀ \leqslant 16.5$ Year), where Y is body length and X is age[8].

Based on historical records[11,13], some of the individuals in the reserve should be more than 5 years old, and all born in the reserve after 1998 should be younger than 5 years when they were sampled in this study. To assign parentage to all the offspring born in the reserve, the population was divided into two age groups: the founder group (age⩾5; 11 individuals) and the offspring group (age<5; 12 individuals). A summary of the information about gender, body length and age of all the samples is given in Table 2.

1.6 Parentage analysis

Parentage analysis was conducted using the program Cervus (ver 2.0), by which the parentage was determined by analyzing the genotypic data using a likelihood based approach[23]. For accurate assignment of the parentage, the analysis consisted of a series of steps. (1) The observed and expected heterozygosity (Ho and He), standard exclusion probabilities, null allele frequency for each locus and for all loci combined were calculated, and then loci with high null allele frequencies were excluded from further parentage analysis. (2) Assigning maternity to a female first. Once a female was assigned, then to assign paternity to a sexually matured male assuming the first parent was assigned correctly. We calculated 0.99 for the proportion of loci typed, and assumed 0.01 for rate of typing error, 80% and 95% for the relax and strict confidence level, respectively. And the proportion of sampled candidate parents was set at 0.9 for the possibly existing missing observation and capturing, accidental death and/or some candidate fathers were not introduced into the Reserve. (3) Because mtDNA is inherited and transmitted from mother to offspring, in order to avoid the incorrect assignment, we examined the difference of the mtDNA haplotypes between the paired candidate mother and offspring based on the mtDNA sequence analysis. If there existed true pairs of mother and offspring, then both should possess the same haplotype. (4) Because sexual maturity age is at least 4 years old for female and 4.5 for male[8], and gestation period is 11 months[9], the age differences therefore should surpass 5 years between true pairs of mother and offspring or 5.5 years between true pairs of father and offspring. (5) If the first parent was not assigned to a special offspring, then the second parent would also not be assigned by the program Cervus. The offspring who had not found mother (the first parent) yet therefore were used to assign paternity to a male first and maternity second, then discriminated true paternity using the age difference criterion as step (4).

Eventually, based on the data of the polymorphic microsatellite loci we evaluated the relatedness for each candidate parent pair and parent-offspring pair with the program NEWPAT (ver 5) as the program can analyze parentage using loci with a high-frequency of null alleles[24].

2 Results

2.1 mtDNA sequence analysis

The 930 bp sequences of mtDNA control region from the 23 individual samples were aligned for analysis. Two polymorphic sites were detected, and both were from transitions. Three unique haplotypes (H1, H2 and H3; Table 4) were defined.

Table 4. Polymorphic sites in mtDNA sequence and distribution of haplotypes in the population

Haplotype	Variable sites[a]		Individuals
	143	415	
H1	C	T	02TM0103, 02TM0305, 02TF0306, 02TF0408, 02TM0812, 02TM1015, 02TM1116, 02TM1218, 02TM1319, 02TM1522, 02TF0823
H2	T	.	02TF0101, 02TF0202, 02TM0204, 02TM0407, 02TM0509, 02TM0610, 02TM0711, 02TF0513, 02TF0617, 02TF0720
H3	.	C	02TM0914, 02TM1421

a) Identified with the 930bp sequence where a '.' indicates identity with haplotype H1. Haplotypic sequences were deposited into GenBank(Accession No. AY334099-AY334101)

2.2 Test for Hardy-Weinberg equilibrium and null allele frequency

Totally 37 microsatellite loci isolated from several cetacean species were used for examination of parentage of the Yangtze finless porpoises in the reserve in this study. Of which, 14 loci were stable and polymorphic for amplification (Table 5). The number of alleles detected per locus ranged from 2 to 7, the number of alleles for the 14 loci sum to 55, average number per locus was 3.93. There were high frequencies of null alleles at locus PPHO104, rw410 and GT575, and locus GT575 deviated significantly from Hardy-Weinberg expectations ($P = 0.000007$). As the high frequency of null alleles would impact the reliability of results from the program Cervus (ver 2.0), these three loci were excluded from further parentage analyses. Additionally, the proportion of the locus EV10pm typed was relatively low, this locus therefore was also excluded. No loci left significantly deviated from Hardy-Weinberg equilibrium and no linkage disequilibrium was detected between loci. The total exclusionary power was 0.9533 for the first parent and 0.9954 for the second parent (assuming the first parent was assigned correctly) at the 10 loci, which means the loci could provide sufficient power to detect parentage.

2.3 Parentage analysis

There were only three mature females (02TF0202, 02TF0408, 02TF0617; > 5 years) considered to be candidate mothers within the population. We assigned 7 of 12 offspring to those with > 95% confidence. There were 6 mature males (02TM0711, 02TM0812, 02TM0914, 02TM1218, 02TM1421, 02TM1522; > 5.5years) considered to be candidate fathers in the population. We assigned 5 of the 7 offspring to the candidate fathers with > 95% confidence. Then, three pairs of offspring and candidate mother with one loci mismatching (02TM0407 and 02TF0202) and/or age difference of < 5 years (02TM0610 and 02TF0617, 02TF0513 and 02TF0617) were excluded. In addition, one pair of offspring and candidate father (02TM0610 and 02TM1218) were excluded because the first parent (02TF0617) was assigned incorrectly.

Then, the 8 offspring having not found father or mother yet were used to assign paternity to a male first and maternity to a female secondly. Five pairs of father-offspring were assigned at > 80% confidence level, in which 2 pairs were assigned at > 95% confidence level. And then, two pairs with the age difference less than 5.5 years and one loci mismatching were also excluded.

Table 5. Summary of descriptive statistics for the population in the reserve at 14 microsatellite loci

Locus	k	Ho	He	Excl(1)	Excl(2)	HW	Null freq
GT271	3	0.435	0.518	0.128	0.228	NS	+0.0713
PPHO104	3	0.435	0.595	0.169	0.312	NS	+0.1511
rw410	2	0.217	0.322	0.050	0.133	NS	+0.1829
PPHO142	4	0.652	0.573	0.162	0.314	NS	−0.0658
PPHO130	7	0.870	0.824	0.439	0.616	NS	−0.0415
rw34	6	0.435	0.500	0.132	0.295	NS	+0.0716
EV10pm	3	0.300	0.309	0.045	0.151	NS	+0.0425
PPHO137	6	0.944	0.837	0.449	0.626	NS	−0.0759
PPHO110	7	0.957	0.854	0.494	0.666	NS	−0.0676
GT575	2	0.043	0.414	0.082	0.162	*	+0.8052
GT509	3	0.565	0.479	0.110	0.247	NS	−0.0991
TV2	2	0.391	0.496	0.118	0.184	NS	+0.1068
GT104	5	0.870	0.643	0.224	0.392	NS	−0.1981
GT310	2	0.435	0.502	0.121	0.185	NS	+0.0612

For each locus in the population the observed number of alleles (k), the observed (Ho) and expected (He) heterozygosity, test for Hardy-Weinberg equilibrium (HW) and null allele frequency (Null freq) are listed. Excl (1), exclusion probability of the locus for the first parent; Excl (2), exclusion probability of the locus for the second parent (with first parent assigned). Likelihood ratio test for HW was investigated by the program POPGENE (ver. 1.3.1)[22]. The departure from Hardy-Weinberg equilibrium was indicated by '*' ($P < 0.05$); NS, not significant.

Finally, overall 4 full parentage assignments were determined, and additionally 3 pairs of single parentage assignment were obtained. The results of paternity analyses are shown in Table 6.

Table 6. The summary of parentage assignment

OS	FP	SP	LM	H(O)	H(M)	Age(O) (Year)	AD(M-O) (Year)	AD(F-O) (Year)	F
02TF0101	02TF0202**	02TM0812**	0	H2	H2	0.0	11.9	6.5	+
02TM0103	02TF0408**	02TM1522**	0	H1	H1	0.6	13.1	> 12.4	+
02TM0407	02TF0202**	—	1	H2	H2	4.9	7.0	—	?
02TM0509	02TF0202**	02TM1421**	0	H2	H2	1.0	10.9	7.6	+
02TM0610	02TF0617**	02TM1218**	1	H2	H2	3.0	3.6	8.3	?
02TF0513	02TF0617**	—	0	H2	H2	3.8	2.8	—	?
02TF0720	02TF0202**	02TM1218**	0	H2	H2	2.5	9.4	8.8	+
#02TM0204	02TM1218*	—	0	—	—	3.8	—	7.5	+
02TF0306	02TM1218*	—	0	—	—	3.0	—	8.3	+
02TM0407	02TM0914*	—	1	—	—	4.9	—	2.2	?
02TM1319	02TM0812**	—	1	—	—	4.2	—	2.3	?
02TF0823	02TM1218**	—	0	—	—	3.2	—	8.1	+

Offspring identity (OS), first parent identity (FP), and second parent identity (SP), OS-FP-SP or OS-FP loci mismatching (LM), haplotype for offspring (H(O)), mother H(M), offspring age (Age(O)), difference in age between mother and offspring (AD(M-O)) or father and offspring (AD(F-O)) are reported respectively. '—', lack of datum; '+', the probable family, and '?' represents the excluded family. '**' indicates at > 95% confidence level, '*' at > 80%. '#' indicates the analysis was done to assign paternity to a male firstly and maternity to a female secondly.

2.4 Relatedness for each candidate parent pair and parent-offspring pair

Based on the data of the 13 polymorphic microsatellite loci conforming to the Hardy-Weinberg expectations we evaluated the relatedness for each candidate parent pair and each parent-offspring pair. The analysis indicated that there might be no close relationship between candidate parents (Table 7). Except the mother and offspring pair of 02TM0204 and 02TM1218, the relatedness for other pairs was much higher than 0.25 (Table 8), showing the

result of parentage was very accurate.

Table 7. The relatedness among the candidate mother and father

Individual	02TM0711	02TM0812	02TM0914	02TM1218	02TM1421	02TM1522
02TF0202	0.10[a)]	0.11	0.04	−0.15	0.04	0.19
02TF0408	−0.05	0.40	0.05	−0.24	−0.27	0.20
02TF0617	−0.23	0.08	0.00	0.13	0.17	0.16

a) Relatedness

Table 8. Relatedness between parents and between parent and offspring pair

OS	FP	SP	R(M-F)	R(M-O)	R(F-O)
02TF0101	02TF0202	02TM0812	0.11	0.59	0.58
02TM0103	02TF0408	02TM1522	0.20	0.43	0.67
02TM0509	02TF0202	02TM1421	0.04	0.60	0.39
02TF0720	02TF0202	02TM1218	−0.15	0.34	0.44
02TM0204	02TM1218	—	—	—	0.09
02TF0306	02TM1218	—	—	—	0.44
02TF0823	02TM1218	—	—	—	0.56

Offspring identity (OS), first parent identity (FP), second parent identity (SP) and relatedness between mother and father (R(M-F)), mother-offspring (R(M-O)) and father-offspring (R(F-O)) pair are reported respectively. '—', lack of datum.

3 Discussion

There were extremely high frequencies of null alleles at locus PPHO104, rw410 and GT575. One of the reasons might be that the population had been mixed and genetically subdivided, for two small populations had been introduced into the reserve in December 1998 and December 1999. Another is that, at the microsatellite loci, a null allele most often occurs because of mutations in one or both primer binding sites, sufficient to prevent effective amplification of the microsatellite allele[23]. As the microsatellite loci used in this study were isolated from other whale species, thereby relatively high null allele frequencies could result from sequence differences. The null allele frequencies would be lower if the analysis performed using microsatellite loci isolated from the Yangtze finless porpoise itself.

Many statistical approaches to paternity analysis are available[23, 25-27]. One of the key questions relating to these methods is how to assess the confidence of a particular paternity assignment[11]. Due to the effects of typing error, un-sampled candidate females or males, and missing genotypes, maternity or paternity was often assigned at a statistical confidence level without complete certainty. In addition, most paternity studies performed were just based on the nuclear data regardless of the mtDNA and other related data such as age difference between animals[3, 28]. However, the age difference of some presumed parent-offspring pairs (e.g. 02TM0610 and 02TF0617, 02TF0513 and 02TF0617) at high confidence level assigned only by the nuclear data in our study were not satisfied with the age criterion, indicating that some of the presumed parentage were falsely assigned and suggesting that the reliability of paternity only based on some nuclear loci needed to be treated with caution.

Female mammalian reproductive behavior is highly constrained by the demands of gestation and lactation[4]. The maternity analysis showed that the mature female 02TF0202 had three progenies (02TF0101, 02TM0509 and 02TF0720) in the reserve. The age difference was 0.99 year between 02TF0101 and 02TM0509, 1.50 between 02TM0509 and 02TF0720, and 2.49 between 02TF0101 and 02TF0720. That is, the female 02TF0202 had borne three offspring within two and a half years that consisted of two gestation periods and two lactation periods. Regardless of the reproductive rest, the female reproductive cycle is estimated to be one and a half years, in which gestation period is 11 months and lactation period estimated to be 6 months[8,9]. But our data showed that the reproductive cycle for the female porpoise is about one year to one and a half years, which means that there probably exists an overlapping between gestation and lactation period. In fact, such an overlapping was reported by Kasuya et al.[9], though it is not common, for the female finless porpoise in the Inland Sea of Japan. Thereby the estimated reproductive cycle was shorter than that reported previously.

The paternity testing by DNA fingerprinting in this study revealed that four males fathered seven offspring, so the males that owned progenies occupied 67% (4/6) of the candidate fathers in the population. According to the age difference between father and offspring and the gestation period (11 months; near 0.92 year)[8,9], it was estimated to be 5.6 years old for 02TM0812, 6.7 for 02TM1421, 6.6, 7.2, 7.4 and 7.9 for 02TM1218, >11.5 for 02TM1522 when they mated for these offspring,

respectively (Table 6). The analysis showed that the oldest males did not monopolize mating, it was possible that males from all age classes above 5.6 years could father offspring and the dominance rank could not be so strict for the porpoise society in the reserve. Meanwhile, since the estimated ages for the offspring 02TF0306 and 02TF0823 were 3.0 and 3.2 years, respectively (Table 2 and Table 6), it was possible that their father 02TM1218 mated with their mothers at one single season, which confirms that a male might mate with several females within a mating season[8, 12].

The analysis also revealed three males fathered the female 02TF0202's offspring (Table 6 and Table 8). According to the ages estimated for the offspring 02TF0101 and 02TM0509, which was 0.01 and 1.00, respectively, the two offspring therefore were conceived within nearest two years. However, the nearest addition of new males into the reserve was in December 1999, so both fathers (02TM0812 and 02TM1421) were in the reserve at both conceptions showing that the female did not show fidelity to a particular male. The mating pattern for the Yangtze finless porpoise used to be considered to be polygynous[8, 12]. However, Parson and Wang reviewed that the finless porpoise that inhabits the South China Sea could be promiscuous[29]. Our data combined with the results of the previous studies[8, 12] suggested that even if a male can mate with several females, a female can also mate with several males, implying female choice for partner diversity. This provides additional evidence to Parson and Wang's conclusion[29].

Amos et al. reviewed that "recent data from harbour seals, *Phoca vitulina*, red deer, *Cervus elaphus* and sheep, *Ovis aries*, showed that the primary requisite for selection against polygyny does exist in large mammals"[30]. As for this reserve population, one of the possibilities is that since activity of this population coming from different segments of the Yangtze River was limited in the isolated natural reserve, the animals had more opportunities to access the different opposite sexes and mate with them than those inhabiting the Yangtze River. Another possible explanation is that there existed female choice for avoidance of inbreeding and/or increase of genetic diversity. Many authors have already addressed this problem. For example, Blouin et al. reported that there was abundant evidence that many species avoided mating with kin, and Amos et al. also reported that examination of three long-lived vertebrates, the long-finned pilot whales, *Globicephala melas*, the grey seal, *Halichoerus grypus*, and wandering albatross, *Diomedea exulans*, revealed significant negative relationships between parental similarity and genetic estimates of reproductive success[31, 32]. The analysis showed that there is no close relationship between each candidate parent pair in our study. As the female could make no selection for less related males during mating, we could not compare and evaluate whether there possibly exists avoidance mechanism of inbreeding for the porpoise or not at present. Meanwhile, because just several parent pairs were determined, some of the results of this study must be treated with caution. Further researches based on a larger population size would be expected.

Ex situ conservation is one of the important measures to save rare or endangered species. The results acquired in this study provide important information for making guidelines of management and conservation for the finless porpoise population in the reserve and this endangered species as a whole. At the same time, since the reserve is isolated and the population is small, isolated from the population in the Yangtze River and consists primarily of males, whereas most of them are female in wild population according to Chen et al.[10], it therefore would be very interesting theoretically and academically to take these special chances in a long term to monitor and study what the influences will be on the mating system, reproductive behaviors and tactics of the Yangtze finless porpoise because of the differences of sex ratios, population size and environments, and explore the genetic structure, its changes of the genetic diversity among different generations, the impact of the genetic management on the genetic structure, the process of losing of the genetic diversity, and the population viability of the small and isolated population as well as the endangered mechanism of endangered species in general.

Acknowledgements We wish to thank X. Zhang, Q. Zhao, Z. Wei, K. Wang, X. Wang and B. Yu in our department and staff of the Yangtze Tian-e-Zhou Baiji National Natural Reserve for their help in capturing and taking care of the animals.

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