RESEARCH NOTE

The loss of genetic diversity in the Chinese paddlefish (*Psephurus gladius* Martens) as revealed by DNA fingerprinting

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Introduction

Examining patterns of genetic diversity has become an integral component of many management plans concerning endangered species. Chinese paddlefish (*Psephurus gladius* Martens), a large-sized freshwater fish, is the only member of the family Polyodontidae present in Asia and in the main stream of the Yangtze river. Since the Gezhouba Dam, which was built in 1981, blocked the channel and prevented sturgeon from swimming into the lower-middle section of the Yangtze river, currently the Chinese paddlefish is found only in the upper main stem of the Yangtze river. Furthermore, in the last two decades, the over-catching, habitat destruction and pollution have caused a rapid reduction of the wild population of Chinese paddlefishes, such that the species is now listed as endangered (Category I of the State Key Protected Wildlife List, in the CR Category of the IUCN and on Appendix II of the CITES; Yue *et al.* 1998). Multiple factors are implicated in species extinction, including habitat loss, and fragmentation, demographic instability, inbreeding and other genetic factors (Lande 1988). If one of these factors, namely recent changes in genetic diversity, can be tested and perhaps rejected, then research and management can focus on the other extinction mechanisms. Moreover, species endangerment often derives from the erosion of genetic diversity (Chen 1993). The loss of genetic diversity predicts lower individual fitness and lower population adaptability (Lande 1998). Consequently, information on genetic diversity may contribute to a better understanding of the causes of species endangerment and thereby help in the development of management strategies for species conservation.

Genetic diversity surveys in endangered populations typically determine the variation currently maintained in the population rather than the magnitude or rate of loss of genetic diversity over time (Matocq *et al.* 2001). Finding evidence of temporal changes in genetic diversity is challenging. Difficulties include finding appropriate molecular markers, methods of analysis (Luikart *et al.* 1998) and establishing a baseline or reference point from previous specimens against which to measure change (Taylor *et al.* 1994).

DNA fingerprinting is a good marker system for small populations (Wan *et al.* 2004) and has revealed the loss of genetic diversity (Wan and Fang 2002, 2003; Wan *et al.* 2003a), genetic variation between subpopulations (Fang *et al.* 2002a, 2003), and genetic differentiation of populations (Wan *et al.* 2003b) of endangered animals. Besides a reliable genetic marker, detecting the loss of genetic diversity also requires archival specimens from which DNA can be obtained. A considerable number of historic samples were preserved by formalin-fixation. The formalin-induced cross-linking between DNA and proteins resulted in the extraction of only fragmented DNA from fixed samples, limiting the use of these archival specimens in conservation genetics (Wan *et al.* 2004). However, a recent study reported the isolation of high molecular weight DNA from formalin-fixed samples and the successful use of such DNA for fingerprinting studies (Fang *et al.* 2002b).

In this study, we compared genetic diversity of historical collections of Chinese paddlefishes with recent collections, to see whether there has been a loss of genetic diversity in this species as its range and population size

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have shrunk. We assayed genetic diversity of using DNA fingerprinting on two sets of formalin-fixed samples collected in 1957–1959 and 1995–1999, respectively.

Materials and methods

Materials

All samples are formalin-fixed and collected from Wanxian, Peiling and Yibin. The first set of paddlefish samples consisted of 17 individuals (body length ranging from 480–721 mm), collected between 1957 and 1959. The second set of paddlefishes consisted of 18 random individuals (body length ranging from 678–1063 mm), collected between 1995 and 1999.

Isolation of DNA

The formalin was completely removed from formalin-fixed samples according to the protocol of Fang *et al.* (2002b). High molecular weight DNA was then extracted from the processed tissues by conventional phenol/chloroform methods (Sambrook *et al.* 1989).

DNA digestion and electrophoresis of DNA fragments

Six μg DNA per sample was digested with 20 U *Hinf* I and then subjected to ethanol precipitation at –20°C. The resulting DNA fragments were dissolved in 6 μl TE buffer, then loaded into a 20 cm long 0.8% agarose gel. Gels were electrophoresed in TAE buffer (40 mM Tris-acetate, 20 mM Sodium acetate, 1 mM EDTA, pH 8.3) for 48–60 h. In the electrophoresis, *Lambda* DNA/*Eco*RI + *Hin*dIII marker was used as the molecular weight standard.

Probe, hybridization and autoradiography

The gels were dried on a vaccum gel dryer (Biorad, Germany) and hybridized to [g-^{32}P] ATP-end (5′) labelled oligonucleotide probe (5′-AGA GGT GGG CAG GAG AGG TGG GCA GGT-3′). Oligouncleotide end-labelling was done in T4 kinase reaction and purified in Sephadex G-50. Hybridization was preformed at 45°C with the probe for 1–2 h in 5× SSPE (1× SSPE : 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 8.0), 5× Denhardt's solution (50× Denhardt's solution : 1% bovine serum albumine, 1% polyvinylpyrrolidone, 1% Ficoll in double distilled H_2O), 0.1% sodium dodecylsulfate, 10 μg/ml sonicated and denatured *E. coli* DNA and 1–2 × 10^6 cpm/ml of the labelled probe. After hybridization, the membrane was washed once in 2× SSC, 0.1% SDS at room temperature for 10 min and then twice in 1× SSC, 0.1% SDS at 45°C for 10 min. The membrane was kept moist and exposed to X-ray films (Kodak XAR-5) for 1–5 days at –80°C with intensifying screen.

Statistical analysis

Genetic diversity was assessed by computation of band-sharing coefficient (x) and allelic frequency (q) according to Blanchetot (1993). The genetic variability (V) was calculated using the equation of Kuhnlein *et al.* (1989). Heterozygosity (H_{ub} and H_{bc}) and the number of loci (L_{ub} and L_{bc}) were estimated according to the descriptive equations of Stephens *et al.* (1992), Jin and Chakraborty (1993) and Gilbert *et al.* (1990). The subscripts *ub* and *bc* are abbreviations for unbiasedly and biasedly corrected, respectively, indicating two different methods for the same measurement.

Results and discussion

The scored bands on the DNA fingerprint ranged from 2.3 to 12.1 kb (figure 1a and 1b). The historical samples of paddlefishes presented 16 bands per individual (figure 1a) whereas the modern samples of paddlefishes had 15 bands per individual (figure 1b). Differences between archival and current groups were statistically significant ($P < 0.001$). These two DNA fingerprints show that all individuals possessed the two bands close to 12.1 kb, suggesting that the two bands may be typical bands for the Chinese paddlefish.

The band sharing coefficient and allelic frequency increased from 0.1758 to 0.2033 and from 0.0921 to 0.1074, respectively, over the time between the two samples being collected from the wild (table 1). Thus, the modern sample of paddlefishes shared more bands than the archival sample, suggesting that genetic diversity was lost in the intervening 35–40 years. Genetic heterogeneity was studied with genetic variability, hypervariable loci and heterozygosity, and all three parameters showed a decrease from 1957 to 1999 (table 2), again suggesting the loss of genetic diversity.

When judging solely from existing populations, low genetic diversity cannot be used as the evidence for loss of genetic heterogeneity. Several recent studies report that some rare and endangered species, even those with an extremely narrow geographical distribution, may actually contain high levels of genetic variation (Soltis and Soltis 1991; Lewis and Crawford 1995; Ge *et al.* 1997, 1999). Conversely, some large populations often have low genetic diversity due to small effective population size (Hamrick and Godt 1996). As a result, conservation biologists have drawn attention to comparisons of widespread and restricted congeneric species (He *et al.* 2000; Ge *et al.* 1999), or have used archival specimens as reference points to distinguish the observation of low genetic diversity from the process of losing genetic diversity (Matocq *et al.* 2001).

Here we detected the loss of genetic diversity using DNA fingerprinting and two sets of samples collected in

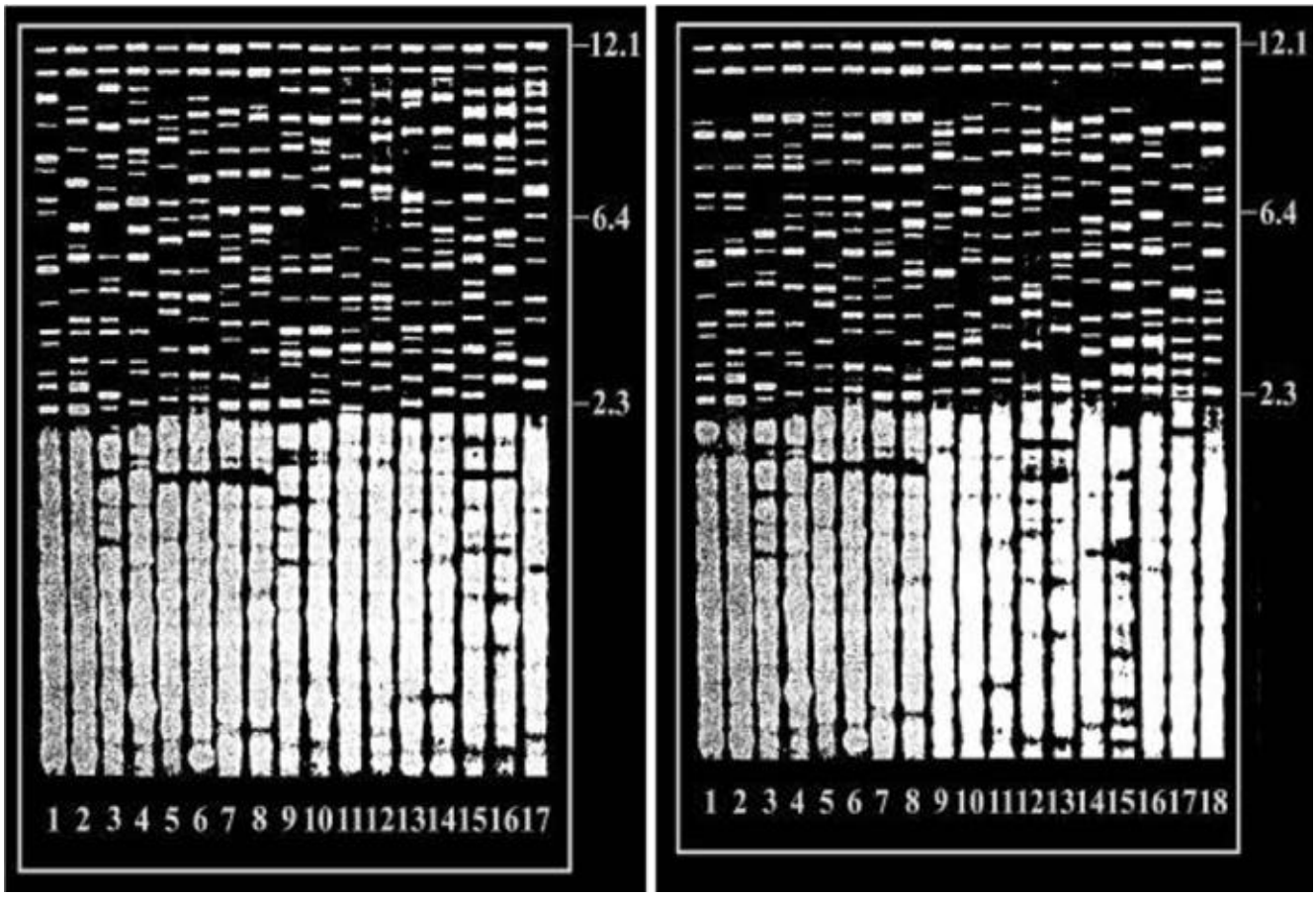

Figure 1. DNA banding patterns of Chinese paddlefishes collected in different periods: (a) Archival samples collected between 1957 and 1959. (b) Present-day collections between 1995 and 1999.

Table 1. The genetic diversity parameters for two sets of Chinese paddlefishes.

Detected group	Collection time	Number of bands (n)	Allelic frequency (q)	Band sharing (x)
I	1957–1959	16	0.0921	0.1758
II	1995–1999	15	0.1074	0.2033

1957–1959 and 1995–1999. We analysed the direct parameters (genetic variability, hypervariable loci and heterozygosity) and indirect parameters (band-sharing coefficient and allelic frequency). All parameters showed less genetic variation in the current population than in the historic population. Not only did the modern population possess a lower level of genetic diversity, but also the average allelic frequency was elevated to 0.1074 and the heterozygous ratio (0.8926) was lower than 0.9. Conservation biologists argue that the average heterozygous ratio must be higher than 0.9 so as to make sure the long-term viability of a species (Ballou *et al.* 1990), suggesting that the species of Chinese paddlefish may be at significant risk of extinction.

Because there are many differences in the two populations, we analysed the divergence between the current population and the historic population by calculating the average band sharing (S) according to Lynch (1991). If two populations are homogeneous, S is equal to 1.0. We calculated an S value close to 1.0 (1.024). Thus, the two populations have not diverged yet, even though the heterozygous ratio of current population has decreased to 0.8926.

Because loss of genetic variation may decrease the potential to persist in the face of abiotic and biotic environmental change, as well as the ability of a population to cope with short-term challenges such as pathogens (Lande 1988), modern populations of Chinese paddlefish

Table 2. Frequencies of unique bands, number of different bands and hypervariable loci, genetic variability (*V*), and heterozygosity in two sets of Chinese paddlefishes.

Detected group	Unique bands (%)	Different bands (*n*)	*V*	Locus number		Heterozygosity	
				L_{ub}	L_{bc}	H_{ub}	H_{bc}
I	9.52%	42	0.6409	7.930	7.787	0.779	0.772
II	6.25%	32	0.5588	7.642	7.422	0.608	0.617

may have lower adaptability than in the past. The present results lead to the recommendation that artificial breeding plans and subsequent reintroduction be implemented as soon as possible in order to maximize chances for persistence of this species.

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