Experimental reintroduction revealed novel reproductive variation in Crested Ibis *Nipponia nippon*

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The Crested Ibis *Nipponia Nippon* formerly occurred throughout East Asia, but since 1981 its unique population has been confined to a narrow area in Central China. During 2004-2005, 23 Crested Ibises were experimentally reintroduced to an isolated basin in Qinling Mountains, where they and their offspring exhibited variation in reproduction never observed in wild population. Crested Ibis has been considered to be a monogamous species, and breeding pair is solitary and territorial in breeding season. However, 3.4% of breeding females exhibited polyandry and 43.1% of nests were observed in colony in the reintroduced population during 2006-2014. The mating system flexibility is likely related to male-biased sex ratio in the small isolated reintroduced population, due to greater dispersal capacity by female. The colonial nesting is attributed to the availability of large nest trees and abundant food supply. First nest failure in reintroduced population occurred much earlier than that in wild, which resulted in significantly higher probability of renesting. The phenotypic plasticity in Crested Ibis may play important role in the restoration of this critical endangered species under a changing environment, and future reintroductions provide opportunity to further understand the degree and cost of adaptive phenotypic plasticity in Crested Ibis.

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INTRODUCTION

The Crested Ibis (*Nipponia nippon*), listed as endangered in the IUCN Red List, is a formerly widespread east Asia species (BirdLife International, 2014). Habitat degradation and over-hunting of the species led to rapid decline and subsequent extinction of the population in Russia, Korea Peninsula and Japan during the twentieth century (BirdLife International, 2001; Shi & Cao, 2001). In May 1981, a remnant population of seven birds was discovered in the Qinling Mountains of Central China (Liu, 1981). Since then great conservation efforts have been made to restore the population. As a result, the wild population of Crested Ibis increased to over 1000 individuals (Wang *et al*., 2014), with another 670 in captivity.

The reproductive biology of the Crested Ibis has been well studied and documented repeatedly since 1980s (Shi *et al*., 1989; Zhai *et al*., 2001; Yu *et al*., 2006, 2007). Crested Ibis is socially monogamous, with quite high mate fidelity and nest site fidelity between years (Lu *et al*., 1997; Shi & Cao, 2001). The birds build solitary nest on large trees near rice paddies and human settlements, showing territorial in the breeding season (Shi *et al*., 1989; Shi & Cao, 2001). Nesting altitudes range from 500 to 1200 m, but no significant variation is found in clutch size and breeding success across altitudinal zones (Zhai *et al*., 2001; Yu *et al*., 2006). Both male and female contribute to nesting, hatching and feeding chicks (Shi *et al*., 1989; Ding, 2004), and the breeding success is quite high in nidicolous birds due to stability of pair maintenance and human protection (Yu *et al*., 2006).

To reduce the risk of extinction inherent to a species confined to limited mountain area vulnerable to catastrophes such as epidemic diseases, climate disasters and feeding ground pollution, reintroduction of Crested Ibis has been planed to establish a second population in its historical range since 2000 (Zheng, 2000). To make technical preparation, an experimental reintroduction was conducted during 2004-2005, and 23 captive-bred Crested Ibises were released after acclimation at Huayang, 30 km from the main distribution area of wild population (Liu, Lu & Chu, 2005; Liu *et al.*, 2008). During 2006-2014, the breeding of the reintroduced and wild Crested Ibis were monitored, and unexpected reproductive traits of the reintroduced population were revealed and hereby described in this paper.

MATERIALS AND METHODS

Study site

The study was conducted in the transborder area of Yangxian, Chenggu and Xixiang

County (32°30′-33°40′N, 107°05′-107°50′E) of Shaanxi Province, Central China, on the south slope of the Qinling Mountains (Fig. 1). The breeding areas for wild Crested Ibis, ranging from 500 to 1200 m in altitude, are dominated by deciduous broadleaf forests and mixed broadleaf-conifer forests, with rice paddies, reservoirs, rivers, and farmlands in valleys. The experimental reintroduction of Crested Ibis was performed at Huayang town (33°34′ N, 107°31′ E; elevation averages 1100 m), which is an isolated basin on slope of Qinling Mountains, 30 km from the main distribution area of wild population and supporting a few wild Crested Ibises.

Breeding surveys

All of the 23 released birds were given unique numbered plastic bands, and 11 of them were collared with 11-g radio transmitters (type RI-2D; Holohil Systems Ltd., Canada; Liu, Lu & Chu, 2005; Liu *et al*., 2008). During 2006-2014, nests of released Crested Ibis and their offsprings were located either by homing on transmitter signals using receivers and handheld antennas (Wildlife Materials International, Inc., USA; Mech & Barber, 2002), or by investigation of breeding pairs' distinct territorial displays including copulation, vocalizing and flying around nest sites (Shi *et al*., 1989; Shi & Cao, 2001). Regular observation was conducted in an elevated hide or on high ground near nest, to determine the clutch size, brood size and number of chicks fledging from each nest. Nestlings, if accessible, were color banded at about 25 days of age. The capture and banding of Crested Ibis were granted by State Forestry Administration of China (No. 33 Forest Protection [2002]).

For wild population, most of nestlings have been color marked since 1987 (Lu *et al*., 1997), which ensures us to identify individuals. Comparisons to reintroduced population are based on data of wild population collected through the same methods and during the same period.

Data analysis

We used Independent-sample T test to compare differences in Clutch sizes, breeding success and productivity between reintroduced and wild population, and differences in diameter at breast height (DBH) between colonial and solitary nesting trees. We used Binomial test to examine differences in probability of first nest failure, probability of renesting after nest failure, percentage of nests in colony, and percentage of females exhibiting polyandry, between reintroduced and wild population. Statistics were reported as mean ± SD.

RESULTS

During 2006-2014, totally 65 and 1280 nesting attempts of the reintroduced and wild Crested Ibis were recorded, respectively. No significant differences of Clutch sizes (t = 1.660, P = 0.098), breeding success (t = 0.106, P = 0.916) and productivity (t = 0.645, P = 0.519) were observed between reintroduced and wild population (Table 1).

43.1% of nests were observed in colony in reintroduced population (Table 1), while this occurrence was not recorded in wild population. The colonial nesting trees had significantly larger DBH than solitary nesting trees (t = 8.229, P < 0.001; Fig. 2). The colonial nests averaged 3.1 ± 0.9 (n = 9, range 2-5) per tree, with average distance of 3.5 ± 1.3 m (n = 26, range 2-6 m).

The probability of failure for the first nest was similar in reintroduced and wild population (Binomial test, P = 0.421, Table 1). 72.8% of nest failures in reintroduced population occurred during March and April, while 45.2% in wild (Fig. 3). The probability of renesting after nest failure in reintroduced population was 0.36, significantly higher than that in wild population (Binomial test, P = 0.034, Table 1).

Wild Crested Ibis showed monogamous and both male and female contributed to nesting, hatching and feeding chicks. By contrast, two females (3.4%, n = 59) were observed to exhibit polyandry in reintroduced population (Table 1). In both cases, the female deserted her first clutch immediately after egg laying, and formed pair bond with another male to produce a new clutch. The male-only care for the first clutch or brood caused nesting failure in both cases.

DISCUSSION

The reintroduced Crested Ibis exhibited widespread colonial nesting never observed in the wild population. Archibald & Lantis (1979) once speculated that Crested Ibis probably nested in colony in Central China before 1950s when large trees were available, whereas it was solitary in Siberia and Japan due to scarcity of food. The nationwide logging of mature trees in China during late 1950s resulted in dramatic population decline (BirdLife International, 2001; Shi & Cao, 2001), and the last remnant population survived in remote Qinling Mountains where small amounts of nest trees and paddy field were available. The Crested Ibis exhibited territorial in the breeding season, and each pair occupied a territory of about 1-2 km^2 (Shi & Cao, 2001). As the population increasing, the breeding region of Crested Ibis gradually expanded to lower mountain, where the nests were getting concentrated and were as close as 10 m due to high quality of food supply (Liu, Ding & Chu, 2003). Moreover,

the species was recorded to nest together with Little Egret (*Egretta garzetta*) and Chinese Pond Heron (*Ardeola bacchus*) in *Quercus variabilis* (Liu, unpublished data), a relative large nest tree species available (Yu *et al*., 2006). Based on this information, our result indicated that a reasonable proximate explanation of the colonial nesting is the availability of large nest trees in Huayang basin with abundant food.

Renesting is an important breeding strategy used by birds to compensate for nest loss, and renesting propensity is affected by many factors including seasonal timing (Arnold, Devries & Howerter, 2010; Gates, Lanctot & Powell, 2013). In our study, 23% of unsuccessful pairs renested if their first nests failed before April but 5% renested after May, showing remarkable temporal correlation. Thus, the significantly different timing in first nest failure contributed to the different possibility of renesting between wild and reintroduced population. In wild population, death of altricial chicks due to shortage of food, predation, parasites and adverse weather conditions in early stage is the primary factor affecting breeding success (Ding, 2004; Yu *et al*., 2006). As a result, more than half of nest failure occurred in nestling stage after May. In reintroduced population, however, nest failure was mainly attributed to egg loss caused by the pre-captive parent treading, and occurred much earlier, which raised the possibility of initiating a renest.

Adult sex ratio plays a central role in mating systems, and females are more polygamous when males outnumber females (Liker, Freckleton & Szekely, 2013, 2014). Our observation of polyandry in reintroduced Crested Ibis population supports this theory. Female Crested Ibis shows significantly greater dispersal distance than male both in wild and reintroduced population (Yu *et al*., 2010; Ministry of Environment of Japan, 2014), which may has significant impact on adult sex ratio of small isolated population. In our reintroduction, 13 of 23 released birds inhabited in Huayang basin of the Qinling Mountain in spring of 2006, of which five were female (Liu, unpublished data). As a result, mating system flexibility occurred in this isolated population with male-biased sex ratio, which acted as a buffer mechanism to reduce the impact of environmental and demographic noise in small population (Rossmanith *et al.*, 2006). The nesting failure in the polyandrous females indicated biparental care of nestling stages is probably necessary for successful breeding in Crested Ibis.

Thus, the Crested Ibis showed phenotypic plasticity which is the ability of one genotype to produce more than one phenotype when exposed to different environments (Price, Qvarnstrom & Irwin, 2003). The phenotypic plasticity may play

149 important role in the restoration of this endangered species, especially under a
150 changing environment in China. Currently, reintroductions of Crested Ibis are being
151 conducted in several sites in China and Japan, which provide opportunities to
152 understand the degree and cost of adaptive phenotypic plasticity.

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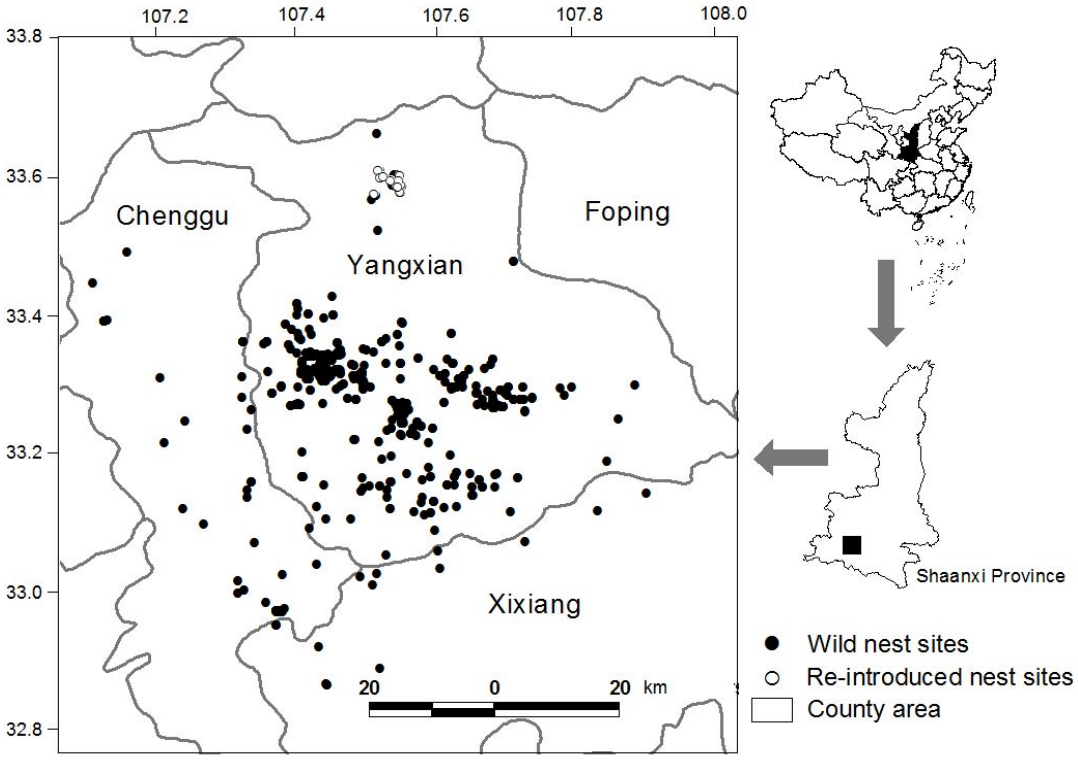

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220 **Figure 1** Map of the study area. The locations of nest sites of wild and reintroduced

221 Crested Ibis during 2006-2014 are marked.

222 **Table 1** Reproductive traits of reintroduced and wild Crested Ibis. "Failed first nest"
223 is the probability of failure for the first nest, "renesting" is the probability of renesting
224 after nest failure, "colonial nesting" is the percentage of nests observed in colony, and
225 "polyandry" is the percentage of breeding females exhibited polyandry. Sample size is
226 presented in parentheses.

Trait	Reintroduced	Wild
Clutch size	2.56 ± 0.67 (41)	2.73 ± 0.61 (323)
Breeding success	69.4 ± 23.7% (37)	69.0 ± 22.1% (291)
Productivity	1.81 ± 0.61 (48)	1.87 ± 0.64 (1031)
Failed first nest	0.19 (59)	0.17 (1248)
Renesting*	0.36 (11)	0.12 (217)
Colonial nesting*	43.1% (65)	0 (1280)
Polyandry*	3.4% (59)	0 (1248)

227 * Significant difference between reintroduced and wild population.

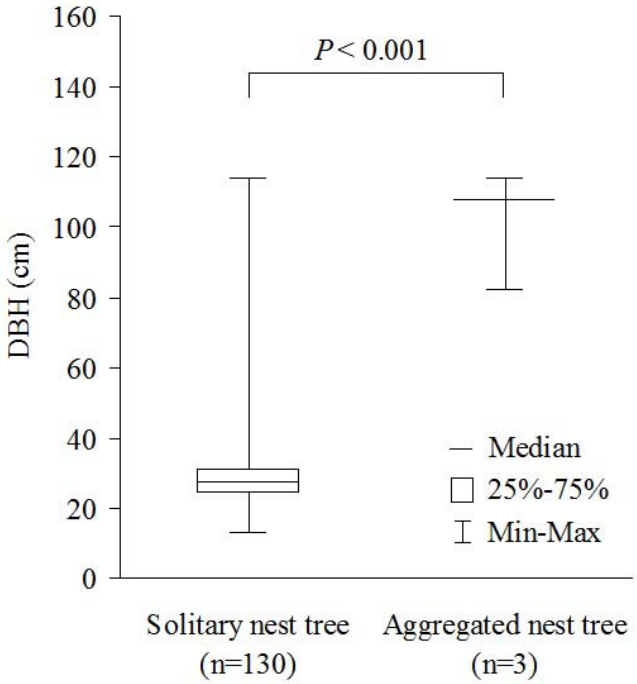

Figure 2 DBH of Solitary and Colonial nest trees in Crested Ibis. DBH of solitary nest trees varied in a wide range but concentrated extremely. Aggregated nest trees had significantly larger DBH.

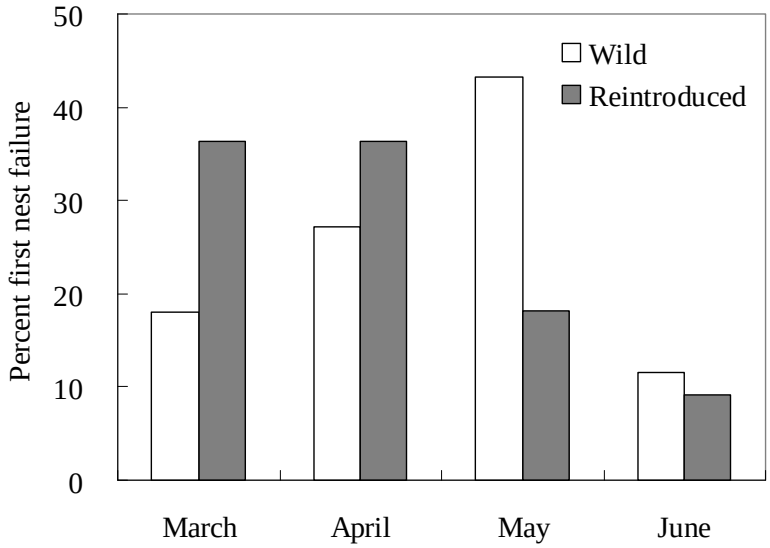

Figure 3 Percentage of first nest failure in wild and reintroduced Crested Ibis population by month during 2006-2014. In reintroduced population, 72.8% of nest failure occurred during March and April. By contrast, nest failure was significantly postponed in wild population.