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Genetic structure among wild populations of Elliot's Pheasant *Syrmaticus ellioti* in China from mitochondrial DNA analyses

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Summary

Genetic structure among five populations of Elliot's Pheasant sampled from five different provinces – Anhui (AH), Zhejiang (ZJ), Fujian (FJ), Hunan (HN) and Guizhou (GZ) – was assayed using mitochondrial control region sequences from 33 individuals. Using AMOVA, we found a high level of haplotype variation within populations, and a degree of genetic structure among groups (GZ population relative to all others pooled). However, this difference was not statistically significant and little geographical structure was indicated among the remaining populations. Furthermore, using a rooted maximum parsimony tree, we found the sequences of the GZ population were largely grouped in their own branch, while sequences of the other four populations were interspersed among branches. We identified a lower level of gene flow between the GZ population and all others, a finding supported by significant F_{ST} values. Conversely, we identified a larger amount of gene flow between the remaining four populations, particularly among the three easternmost populations (AH, ZJ and FJ). Given our results, further study should be focused on the GZ population and on management units for the purpose of maintaining the genetic structure of the species in the west of China.

Introduction

It has been documented that genetic structure among animal populations is fundamentally influenced by gene flow and historical demographic processes, as well as by natural selection and speciation (Laurent *et al.* 2003, Kvist *et al.* 1999). The genetic structure in species with a high potential for long-distance dispersal is expected to be homogeneous throughout a large geographical area. In contrast, low mobility causes significant genetic differences among populations (Newton 2003). The dispersal ability in birds seems to be good. The efficiency of gene flow is determined by dispersal ability together with geographic barriers. In addition to gene flow, historical demographic events are another important element in determining population structure, and should also be considered when an effective and sustainable management plan is developed.

Elliot's Pheasant (*Syrmaticus ellioti*), regarded as Vulnerable (http://www.redlist.org), is endemic to areas south of the Yangtze River in China. It is found in broad-leaved forest and mixed coniferous/broad-leaved forest habitats. In 1872 Swinhoe first found this species in southern Zhejiang and Anhui provinces. In the following year, Père David obtained specimens from Fujian province and instigated captive breeding of the species in Paris (Knoder 1983). Much research has been carried out on its conservation, morphology, breeding and ecology, especially since the 1980s (Delacour 1977, Long 1985, Ding and Zhuge 1988, Ding *et al.* 1990, Shi and Zheng 1997, Ding 1998). Its population size is believed to be declining because of continuing habitat loss, habitat fragmentation and hunting (Ding and Jiang 2000, BirdLife International

2006). It has been concluded that both male and female Elliot's Pheasants disperse in spring, over distances of 1.5–2.1 km (Peng and Ding 2005). The relatively low mobility of the species highlights the need for research into possible variation in the genetic structure among populations of Elliot's Pheasant, a topic that has not previously been investigated.

Mitochondrial DNA (mtDNA) possesses several properties that make it uniquely suitable for the purpose of intraspecific phylogeographic analysis, including relatively high mutation rates, maternal inheritance and no recombination (Avise 2000). The control region (D-loop region) is the most variable part of the mtDNA molecule, which has been chosen to study population structure and diversity in most major taxa of organisms (Wu *et al.* 2004, 2006, Zhang *et al.* 2004, Ruan *et al.* 2005, Wu and Fang 2005, Hu *et al.* 2006, Xu and Fang 2006). This study aimed to examine for the first time the genetic structure and gene flow among five wild populations of Elliot's Pheasant by detailed analysis of the mtDNA control region sequences. There was an expectation that genetic analysis would prove useful in forming management strategies.

Materials and methods

DNA extraction, amplification and sequencing

In total, 33 samples were obtained from five different localities shown in Figure 1: western China site, Leigongshan Nature Reserve in Guizhou (GZ) province; central China site, in Hunan (HN) province; eastern China (three sites), Gutianshan Nature Reserve in Zhejiang (ZJ) province, Ningguo country in Anhui (AH) province, and Fujian (FJ) province. Samples were collected as blood material or pads from feet of dried specimens (Table 1).

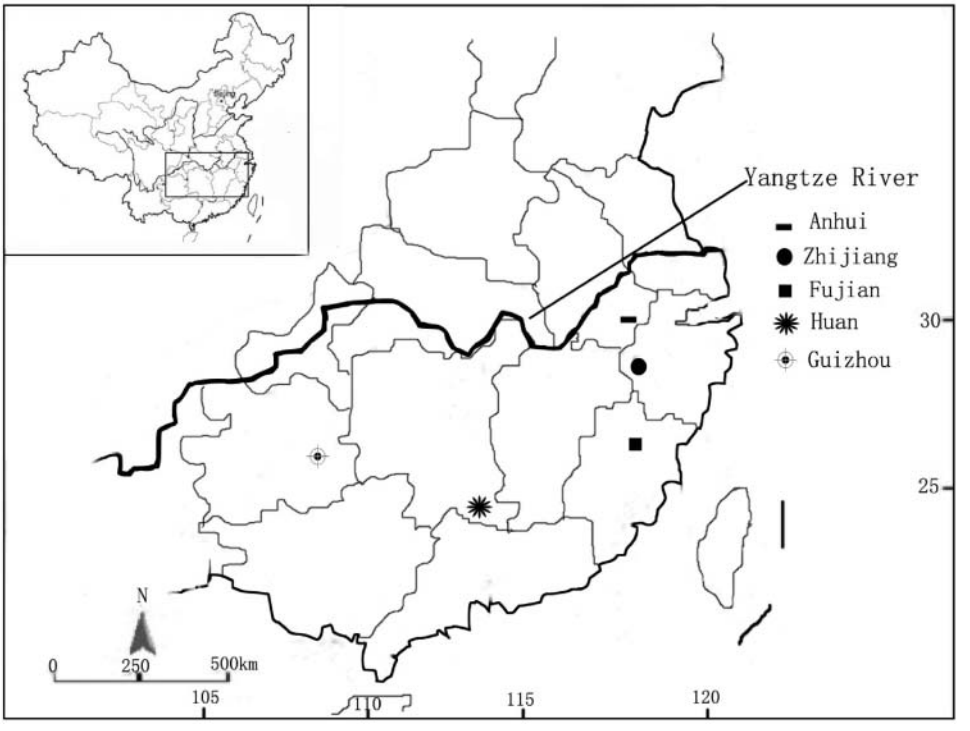

Figure 1. Sampling localities of Elliot's Pheasant.

Table 1. Measures of mitochondrial DNA diversity observed in the five populations of Elliot's Pheasant.

Population	*N*	Individual code	N_h	$h \pm$ SD	$\pi \pm$ SD
AH	6	AH01-04, AH07-08	5	0.933 ± 0.122	0.00272 ± 0.00055
ZJ	5	ZJ01, ZJ06-08, ZJ10	6	1.000 ± 0.126	0.00504 ± 0.00130
FJ	6	FJ01–04, FJ07, FJ13	6	1.000 ± 0.096	0.00510 ± 0.00108
HN	7	HN01-07	6	0.952 ± 0.096	0.00480 ± 0.00125
GZ	9	GZ01, GZ03–06, GZ08-11	9	1.000 ± 0.052	0.00575 ± 0.00100
Total	33		31	0.992 ± 0.010	0.00616 ± 0.00055

N, number of individuals; N_h, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity.

Genomic DNA was isolated using standard proteinase K digestion and phenol/chloroform extraction procedures (Sambrook *et al.* 1989). Polymerase chain reaction (PCR) amplification was performed in a 50 μl reaction volume on a PTC-0220 Peltier thermal cycler using a pair of universal primers: PHDL and PHDH (Randi and Lucchini 1998). The thermal cycling profile, and the DNA fragment cloning and sequencing settings, have been described in detail in Jiang *et al.* (2005).

Data analyses

Control region sequences were aligned using the program CLUSTAL X (Thompson *et al.* 1997) and were checked visually. Two standard measures of genetic diversity – nucleotide diversity (π) and haplotype diversity (*h*) – were calculated using the program DnaSP version 3.51 (Rozas and Rozas 1999). Initial sequence comparisons and measures of variability were performed using MEGA3 (Kumar *et al.*, 2004).

A hierarchical analysis was carried out among groups (a group of pooled AH, ZJ, FJ and HN populations versus a group of the GZ population) and populations by analysis of molecular variance (AMOVA) analyses (where Φ_{CT} is the genetic variation attributable to genetic differentiation among groups, Φ_{SC} that among populations within groups, and Φ_{ST} that among populations relative to the total sample). AMOVA analyses (Excoffier *et al.* 1992), values of migrating individuals (*Nm*) calculated from *F*-statistics (F_{ST}), were performed in the software package ARLEQUIN version 2.0 (Schneider *et al.* 2000). *Nm* was the historical average number of individual migrants contributing to a population's gene pool each generation, where *N* was the female effective population size and *m* was the female migration rate. It was calculated from the equation $F_{ST} = 1/(2M + 1)$ (Slatkin 1987, Baker *et al.*, 1994), where $M = Nm$ for haploid populations. To assess the relationship among mtDNA sequences, a phylogenetic tree was constructed with MEGA3 using the maximum parsimony (MP) method, rooted with one homologous sequence from Hume's Pheasant (*Syrmaticus humia*) as outgroup (GenBank accession number AY368069). Bootstrapping of 1,000 replicates supported branches.

Historical demography analyses – a size stationary model or a range expansion model – were tested for the four populations. Fu's F_S test, its corresponding *P* values and parameters related to a population growth expansion (where τ is expansion time, and θ_0 and θ_1 are expansion range) were performed in the software package ARLEQUIN version 2.0 (Schneider *et al.* 2000). Fu's F_S test, which was initially designed as a test of selective neutrality, was very sensitive to population demographic expansion with high negative values (Fu 1997). A null model of population range expansion was assumed if $\tau > 0$ and $\theta_1 > \theta_0$, or a null model of population stability if $\tau = 0$ or $\theta_1 = \theta_0$. Then the validity of the estimated demographic model was tested by the distribution of a *SSD* test (the sum of squared differences) between the observed and an estimated mismatch distribution, which was obtained by a bootstrap approach. A significant *SSD* value ($P < 0.05$) was taken as evidence for departure from a model of population range

expansion (when $\tau > 0$ and $\theta_1 > \theta_0$), or from a model of population stability (when $\tau = 0$ or $\theta_1 = \theta_0$) (Schneider and Excoffier 1999).

Results

Sequence diversity

Nucleotide sequence data comprising 1,152–1,154 base pairs (bp) from the mtDNA control region was collected from 33 individuals of Elliot's Pheasant. The nucleotide composition included 14% G, 26.7% A, 32.6% T and 26.6% C, showing a paucity of guanine. This is in agreement with the characteristics of other avian control region sequences (Baker and Marshall 1997), which confirmed that control region sequences and haplotypes obtained in this study were successfully amplified from a true mitochondrial origin rather than from a nuclear pseudogene.

Genetic variability is shown in Table 1. The mean haplotype diversity (h) of all individuals was 0.992, ranging from 0.933 to 1.000, while the mean nucleotide diversity (π) was 0.00616, ranging from 0.00272 to 0.00575. Thirty-one unique haplotypes were identified from 33 individuals by 55 variable nucleotide positions in the control region sequences. Haplotype AH03 was shared by both AH03 and AH07 individuals. Haplotype HN03 was shared by HN03 and HN07 individuals. All identified haplotypes are shown in Table 2.

Genetic structure

Since the GZ population appeared to have a somewhat different genetic structure from other populations in our preliminary analyses, the five populations were pooled into two groups: a group of pooled AH, ZJ, FJ and HN populations versus a group of the GZ population. The hierarchical analyses enabled a better understanding of the layer (groups, populations or individuals) to which the genetic difference was attributable (Table 3). Differences between two groups (Φ_{CT}) explained a larger proportion of the total genetic variance (33.7%), but were not significant. Differences among populations within each group (Φ_{SC}) explained only 4.42% of the total genetic variance. On the other hand, differences among populations relative to the total sample (Φ_{ST}) explained most of the genetic variance (61.9%), which was significant. Shown as the rooted MP tree (Figure 2), most individual sequences were mixed, except sequences from the GZ population which was largely grouped in its own branch.

The number of migrants Nm among AH, ZJ and FJ, as shown in Table 4, was above 10 individuals per generation, indicating a large amount of gene flow. The smallest amount of gene flow appeared between GZ and the other populations, which ranged from 0.73 to 1.39.

Demographic analyses

Demographic analyses (Table 5) showed evidence of range expansions of the group of pooled AH, ZJ, FJ and HN populations. Mismatch tests were consistent with a range expansion model in which significant P values for Fu's F_S test were obtained. Demographic parameters estimated by mismatch analyses corresponded to a null model of population range expansion ($\tau > 0$ and $\theta_1 > \theta_0$) that could not be rejected (the sum of squared differences' P values > 0.05).

Discussion

Genetic structure

These results suggested a high level of haplotype variation in wild populations of Elliot's Pheasant. A quite substantial degree of genetic structure was revealed, since 33.7% of the variation was distributed among groups (GZ population relative to all others pooled), though it

Table 2. Thirty-one mitochondrial haplotypes resolved from 33 individuals of Elliot's Pheasant.

Haplotype	Variation position					
						11
	11111122	2222222222	2222233333	4445555566	6666677899	99901
	7823447812	2223344446	8999911778	2590345912	4578967612	36665
	3752122320	2481402692	2367815252	4316292427	8588479571	81520
AH01	CTTAAACTCA	CTATCACCCT	TCTCTTCAAC	TCCATTCTCC	ACCTCACTTT	TCCTC
AH02	.C....T...	..G.....	....	....	......T...	.....
AH03	.C....T.T.	....	....	....	......T...	.....
AH04	......T.T.	....	....C.....	........T.	......T...	.....
AH08	......T.T.	....T.....	....	....	......T...	.....
ZJ01	T.....T...	....	....	....	......T...	.....
ZJ06	......T...	....	.......G..	...G..T...	......T..C	.....
ZJ07	......T...	....	....	....	......T...	.....
ZJ08	......T.T.	TC.C....T.	...T.C....	....	......T...	.....
ZJ10	......T.T.	....	....	C.........	......T...	...C.
FJ01	......T.T.	....	....	....	......T...	CT...
FJ02	...G..T.T.	........T.	....	....	....T.T...	.....
FJ03	......T...	....	........G.	.....A....	......TC..	.....
FJ04	.C....T.T.	....	....	....	......T...	.....
FJ07	......T.T.	....	....	....	T.....T...	.....
FJ13	......T.T.	TC.C....T.	...T.C....	....C.....	......T...	.....
HN01	.C...GT.T.	........TC	......T..T	....	.T..T.T...	.....
HN02	......T.TG	....	....C.....	....	...C..T...	.....
HN03	......T.TG	....	....C.....	....	......T...	.....
HN04	.CC...T.TG	C	....	....	.T..T.T...	.....
HN05	....T.T.TG	....	....C.....	....	......T...	.....
HN06	......T.T.	....TG....	....	....	......T...	.....
GZ01	.C....T.TG	........TC	....	....	.T....T...	.....
GZ03	.C...GT...	......T.TC	..C.......	....	.T..T.T...	....T
GZ04	.C....T.T.	........TC	..C.C.....	.T........	.T..T.T...	.....
GZ05	.C....TGT.	........TC	..C.C.....	....	.T....T.C.	..T..
GZ06	.C...GT.T.	........TC	.TC...T..T	.......G.G	.T..T.T...	.....
GZ08	.C....T.TG	.......T.C	..C.......	....	.TT...T...	.....
GZ09	.C....T.TG	........TC	..C.......	....	......T...	.....
GZ10	.C....T.T.	........TC	C.C.C.....	....	.T..T.T...	.....
GZ11	.C....T.TG	........TC	....	..T.......	.T...GT...	.....

Note. Position numbers (read vertically) refer to the location of each variable site in the sequence. Dots indicate similarity with haplotype AH01, and letters indicate base substitutions. Here, samples codes are used as haplotypes codes.

Table 3. Analysis of molecular variance (AMOVA) analyses for Elliot's Pheasant grouped into a group of AH, ZJ, FJ and HN versus the GZ group.

Source of variation	Elliot's Pheasant
Among groups	33.7%
Among populations within groups	4.42%
Within populations	61.88%
Φ_{CT} =	0.337 (P = 0.20)
Φ_{SC} =	0.067 (P = 0.01)
Φ_{ST} =	**0.381 ($P < 0.001$)**

Φ_{CT} represents the amount of genetic variation attributable to genetic differentiation among group; Φ_{SC} that among populations within groups; and Φ_{ST} that among populations relative to the total sample. Values in bold indicate significance at P < 0.001(10,100 permutations).

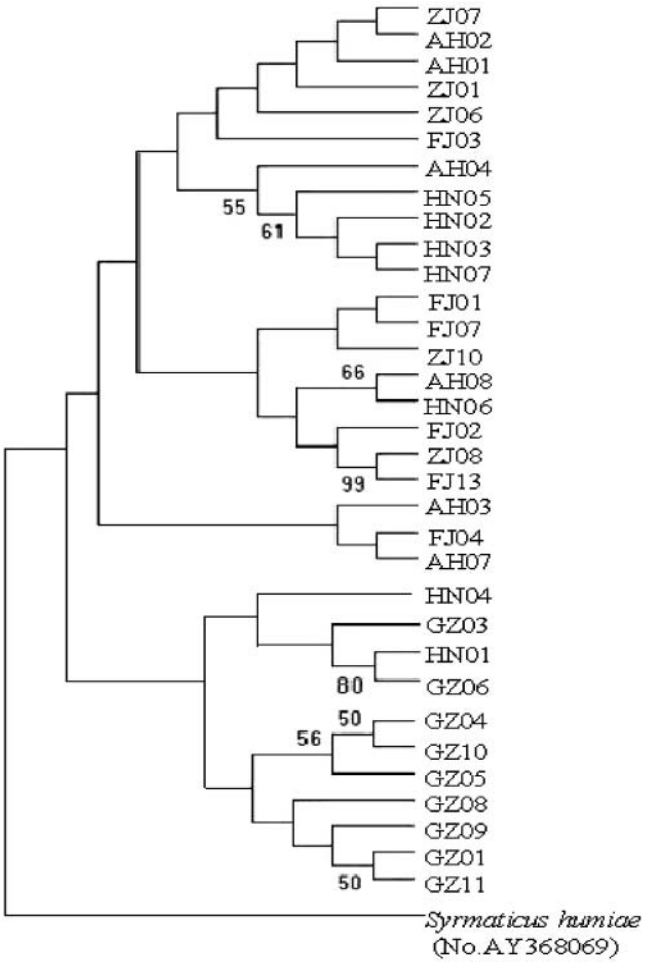

Figure 2. Phylogenetic relationships among the 33 mtDNA control region sequences constructed using maximum parsimony, rooted with a control region sequence of *Syrmaticus humiae* (GenBank accession number AY368069).

was not statistically significant in the AMOVA analysis. Furthermore, sequences of the GZ population were largely grouped in their own branch, while sequences of the remaining four populations were interspersed among branches. So what accounted for the different genetic

Table 4. Population pairwise F_{ST} values (lower left matrix) and the number of migrants (Nm, upper right matrix).

	AH	ZJ	FJ	HN	GZ
AH		10.00	40.5	3.34	0.74
ZJ	0.048		infinite	2.34	0.73
FJ	0.012	−0.054		3.85	0.90
HN	0.130	0.176	0.115		1.39
GZ	**0.403**	**0.407**	**0.357**	**0.265**	

Significant F_{ST} values are indicated in bold type ($P < 0.001$, 10,000 permutations).

Table 5. Demographic test to detect historical range expansion.

Group	F_S*	τ	θ_o	θ_1	*SSD*#
AH, ZJ, FJ, HN pooled	**−17.7**	3.44	2.10	7101	**0.004**

Both F_s, and *SSD* values in bold are statistically significant (*$P < 0.001$, #$P = 0.60$) and consistent with a model of range expansion.

structure of the GZ population? Lower level of gene flow serves as a factor. The average estimate of gene flow between the GZ population and the remaining four populations suggested limited exchange among populations. Ding (1998) deduced that the ancestor of *Syrmaticus* in China originated from the Wuling Mountainous Area, which extended from north-east Guizhou province to the south-west. The Wuling Mountainous Area (mean elevation 1,000 m) serves as a natural barrier between Guizhou and Hunan provinces. Elliot's Pheasant, with its limited dispersal ability, seems unable to cross it, leading to a lower number of migrants from the GZ population to the remaining four populations, since it mainly inhabits an elevation of 300–800 m in broad-leaved or mixed forest. Moreover, Johnsgard (1986) commented that Elliot's Pheasant was separated from Bar-tailed Pheasant (*Syrmaticus humia*) in the eastern Himalayas. Was the western habitat a refuge for ancient Elliot's Pheasant? It would require a complete phylogeny of the genus with appropriate outgroups to support this.

In contrast, little population structure was indicated among the remaining four populations. In this study, the *Nm* values among these four populations detected an extent of gene flow with a large number of migrants between connected sites, which are all located in the middle and lower reaches of the Yangtze River plain. Therefore, it is suggested that gene flow is sufficient to homogenize gene pools among the four populations. Secondly, population expansion, a historic demographic pattern, was another plausible explanation for the lack of genetic structure in the four populations. The demographic parameters estimated by mismatch analyses corresponded to a model of population range expansion. The idea of population expansion was further supported by the significantly negative Fu's F_S value.

Conservation implications

A good understanding of population genetic structure is critical to the design of an effective conservation programme for this species. Based on the substantial genetic differentiation among populations, the GZ population would be considered as one Management Unit (Moritz 1994). If a conservation programme aims to preserve the genetic distinctiveness of the species in the west of China, artificial introductions of Elliot's Pheasant from AH, ZJ, FJ and HN populations to the GZ population should be discouraged. Additional study should be focused on the GZ population regarding its origin, evolution, etc. Since gene flow and other genetic parameters are indicators of the cumulative evolutionary process, the ''current'' genetic structure is the result of past evolution. Recent habitat loss, fragmentation and degeneration would therefore have more negative effects on the future population genetic structure. The much reduced habitat has been further fragmented into smaller patches in recent decades (BirdLife International 2006) and, given the declining population, habitat management is seen as vital because demography and genetics are not independent.

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