Biochemical Systematics and Ecology 57 (2014) 69–77

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Population structure of the endangered Hume's pheasant (*Syrmaticus humiae*) inferred from a partial sequence of the mitochondrial DNA control region

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ARTICLE INFO

Article history:
Received 10 March 2014
Accepted 5 July 2014
Available online

Keywords:
Syrmaticus humiae
Population structure
Genetic diversity
Management unit

ABSTRACT

Genetic diversity plays a vital role in biological conservation, especially for those species under the threat of habitat loss and fragmentation. In this study, 73 samples of Hume's pheasant (*Syrmaticus humiae*), collected from 6 localities in Guangxi and Guizhou provinces of China, were used to examine the influences of habitat fragmentation on genetic diversity and structure based on 1143 bp sequence of mitochondrial DNA (D-Loop). The results showed that all populations expressed high genetic diversity while lacking genetic differentiation. Phylogenetic analyses suggested that the haplotypes identified from the four putative populations (TXL, LX, PJ and LD) did not cluster into separate geographic branches. Despite habitat fragmentation, we failed to find evidence of genetic depletion or impediment of gene flow within the 6 localities. Therefore, the four geographical populations should be regarded as an identical management unit (MU). Based on our results, we suggest that habitat restoration and hunting prohibition for protection should be highlighted for the increased conservation of the Hume's pheasant.

1. Introduction

One of the most important issues in conservation biology is the decrease of genetic variability and the increased likelihood of extinction caused by habitat fragmentation, defined as the fragmentation of one contiguous habitat (Andren, 1994; Frankham, 1996; Saccheri et al. 1998; Westemeier et al. 1998). Habitat fragmentation will directly result in population fragmentation, a major threat to a species' survivability (Brooks et al. 2002; Reed, 2004). The small population sizes that result from population fragmentation increase the chance of inbreeding and random genetic drift within the fragmented habitats (Wade, 2001; Fickel et al. 2012; Frantz et al. 2012). Therefore, it is very important to study the genetic diversity of fragmented populations in order to prevent population extinction (Hedrick and Kalinowski, 2000; Höglund, 2009).

The Hume's pheasant (*Syrmaticus humiae*) is a species that is distributed among the hill tracts of north-east India, north and west Myanmar, south-west China and north Thailand (Han, 1997; Fuller et al., 2000). The wild population size of the

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http://dx.doi.org/10.1016/j.bse.2014.07.006
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Hume's pheasant has decreased significantly over the past years (IUCN, 2014). It is now listed as a globally near-threatened (NT) species by BirdLife/IUCN (BirdLife International, 2014; IUCN, 2014) and ranked Grade I National Protected Wildlife in China (Zheng and Wang, 1998). In Guangxi Province of south-west China, *S. humiae* is distributed mainly along the Nanpan River and Hongshui River in the counties of Tianlin, Leye, Longlin, Xilin, Lingyun and Tian'e (Li et al. 1998). Hume's pheasant was initially found in Luodian, Guizhou Province in the winter of 2010 (unpublished data). In our previous field surveys, we found that the habitats of *S. humiae* are under pressure from intense logging and ongoing poaching, partially due to the local peoples' ignorance of wildlife protection laws (unpublished data).

Mitochondrial DNA (mtDNA) is commonly used for examining and exploring genetic diversity, population structure, as well as phylogeographic analyses of intraspecific populations (Avise, 2000). For example, the mtDNA provides sufficient variation for studying population structure and diversity (Brown et al. 1979; Moritz et al. 1987; Cao et al. 2013; Song et al. 2014). Hence, mtDNA data is a particularly useful marker for investigating the evolutionary subdivision of contemporary geographical patterns within species (Arbogast and Kenagy, 2001; Zhang et al. 2013). The aim of this study was to examine genetic diversity and population structure of *S. humiae* in the fragment habitats of the Guangxi and Guizhou provinces using mtDNA displacement loop (D-Loop) sequences.

2. Materials and methods

2.1. Sample collection

A total of 73 samples of *S. humiae* were obtained from 6 localities shown in Fig. 1. Longlin County, Guangxi Province ($N = 3$), Xilin County, Guangxi Province ($N = 2$), Tianlin County, Guangxi Province ($N = 22$), Leye County, Guangxi Province ($N = 13$), Tian'e County, Guangxi Province ($N = 24$). The samples from Xialao and Pojie towns were collected from two sides of the Hongshui River and Luodian County of Guizhou Province ($N = 9$). Three blood specimens were obtained form living trapping in the field and seventy specimens were came form muscle stored in faunal collections of nature reserves or local residents' home for the recent years. Four geographic populations were discriminated using artificial and natural landscapes, such as highways and rivers, and sampling intervals (Develey and Stouffer, 2001; Thinh et al. 2012; Dong et al. 2013): TXL (Tianlin, Longlin, Xilin), LX (Leye County and Xialao town), PJ (Pojie town in Tian'e) and LD (Luodian).

2.2. DNA extraction and sequencing

Genomic DNA was isolated using standard proteinase K digestion followed by phenol/chloroform extraction methods (Sambrook et al. 2001). Two oligonucleotide primers, PHDL (5'-AGG ACT ACG GCT TGA AAA GC-3') and PHDH (5'-CAT CTT GGC

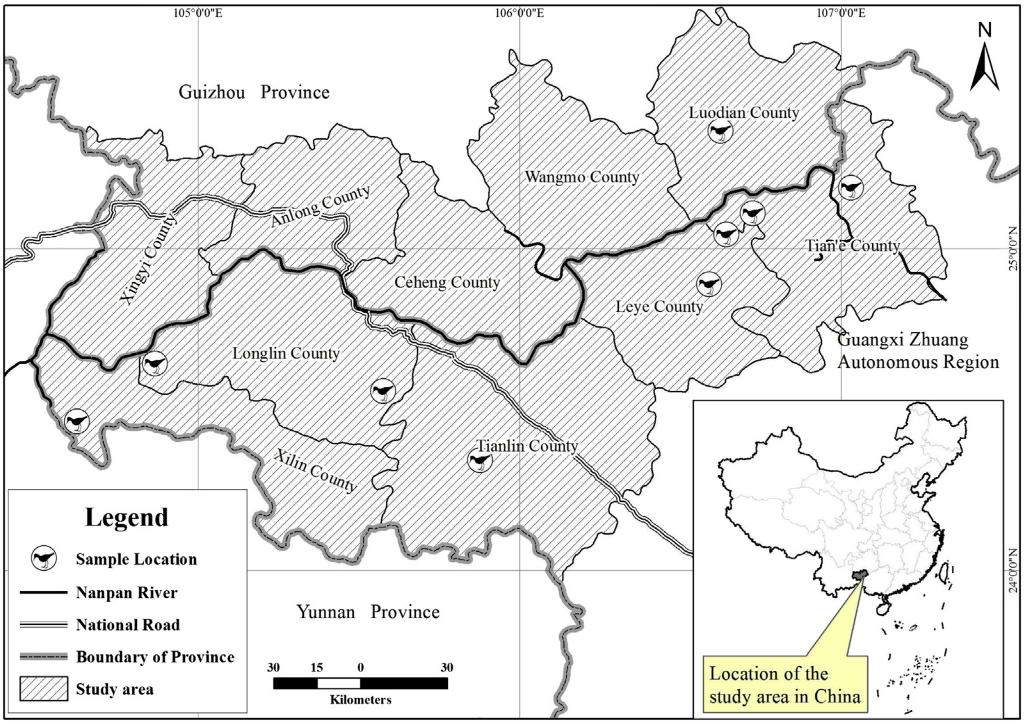

Fig. 1. Study area and sampling locations of the Hume's pheasant.