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Full Length Research Paper

Effects of environmental factors on the population genetic diversity of Chinese bamboo partridge (*Bambusicola thoracica*)

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Knowledge of factors that affect population demographic and genetic diversity is of importance to understand the evolutionary ecology. The environment plays an important role in the evolutionary process. This study used mtDNA control region sequences and nuclear microsatellites to infer the relationship between population genetic diversity and environmental factors of Chinese bamboo partridge (*Bambusicola thoracica*). A total of 1140 nucleotides of mitochondrial DNA control region were sequenced from 180 bamboo partridge sampled from thirteen populations in southeast China. Thirteen-nine polymorphic positions defined 50 haplotypes. The ten microsatellite loci were polymorphic in all populations of Chinese bamboo partridge. Bamboo partridge population genetic diversity (π-, h-, K-, H_E- and H_O- values) decreased with decreasing variation coefficients of temperature and rainfall. The more stable the climate, the higher the genetic diversity observed.

Key words: *Bambusicola thoracica*, mitochondrial DNA, microsatellite, genetic diversity, environmental factors.

INTRODUCTION

The quantification of intra- and inter-population genetic diversity is of importance in many areas of evolutionary biology and ecology (Angers et al., 1999). The intraspecific polymorphism of mitchondrial DNA (mtDNA) and microsatellites has abundant geographic variation in most species (Hu et al., 2011; Tsukagoshi et al., 2011). The extent of geographic variation results from a balance of forces tending to produce local genetic differentiation and forces standing to produce genetic homogeneity (Slatkin, 1987).

The environment plays an important role in the evolutionary process (Scheiner, 1993), which may influence genome size (Nardon et al., 2005) or genetic variation (Huang et al., 2005; Jin and Liu, 2008; Ruan et al., 2012). Environment is the direct force that influences the populations in nature (Vanhala et al., 2004). The interplay between a genetically variable population and its ever-changing environment is the focus of attention in ecological genetics (Merrell, 1981). There have been a lot of studies on gene (or genotype) and environmental interaction, and we all know that environmental factors are very important to maintain genetic variability. Some studies of the effects of environmental factors on population genetic diversity were carried out in arid region (Huang et al., 2005; Jin and Liu, 2008). They showed higher genetic diversity occurrence in stable condition. However, these studies did not involve humid area.

Chinese bamboo partridge (*Bambusicola thoracica*, Aves, Galliformes) is a popular game bird and a

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Abbreviations: AAT, Annual average temperature; **AAR,** annual average rainfall; **VCT,** variation coefficient of annual average temperature; **VCR,** variation coefficient of annual average rainfall.

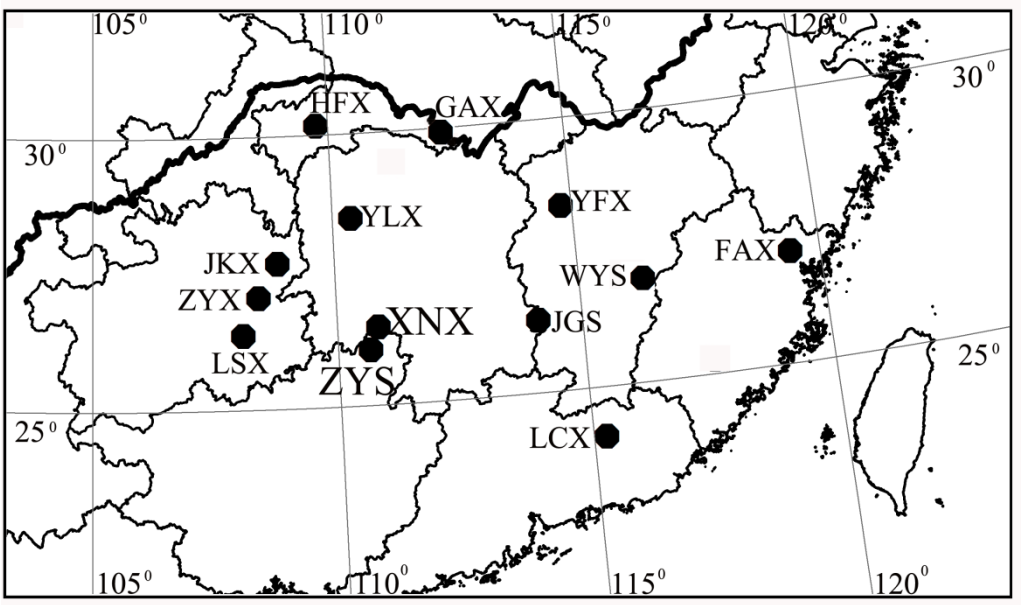

Figure 1. Sampling localities in this study.

nonmigratory endemic partridge in south China (Cheng, 1978). The partridge occurs mainly in hilly and flat terrain having shrubbery, bamboo forests and grassy areas below 1000 m in south China, a mosaic of mountains lower than 2000 m (Johnsgard, 1999). In a recent study, Huang et al. (2010) documented the phylogeograpical strucutre of Chinese bamboo partridge.

Genetic data have increasingly been used to provide an alternative means of resolving questions of ecology or genetics. The most commonly used molecular marker for the genetic diversity studies has been animal mtDNA. Microsatellites has emerged as molecuar markers with the finest resolution for population-level studies, due to their high variability, abundance, neutrality, co-dominance and the unambiguous scoring of alleles (Tautz, 1989). We selected latitude, longitude, altitude, annual average temperature, annual average rainfall, variation coefficient of annual average temperature and variation coefficient of annual average rainfall as envirnomental factors. The objective of this study was to investigate the effect of environmental factors on genetic variation among bamboo partridge populations using mtDNA control region sequences and nuclear microsatellites.

MATERIALS AND METHODS

Sample collection and laboratory methods

A total of 180 samples of 13 populations in bamboo partridge were collected from the following localities: FAX, GAX, HFX, JGS, JKX, LCX, LSX, WSY, YFX, YLX, ZYS, ZYX (Figure 1). Liver/muscle samples were dissected from birds and stored in 100% ethanol immediately after removal. All the samples were from the natural populations.

DNA was extracted from liver/muscle tissue with the ethanol sedimentation procedure. Two oligonucleotide primers, PHDL (5′-AGG ACT ACG GCT TGA AAA GC-3′) and PHDH (5′- CAT CTT GGC ATC TTC AGT GCC -3′) (Randi and Lucchini, 1998), were used to amplify PCR and sequence of about 1100 bp mitochondrial DNA control-region segment. There was 1 unit of *Taq* DNA polymerase in 35 µl reactions. The final concentrations were 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 150 µmol/L dNTP, 10 pmol/L primers and about 100 ng DNA templates. PCR conditions were as follows: 95°C 4 min; 35 cycles of 95°C 40 s, 55 to 58°C 40 s, 72°C 60 s; followed by 72°C 10 min in PE9600 thermocycler. After examination by agarose electrophoresis, PCR products were purified with Wizard™ PCR Preps DNA purification box (Promega Inc. USA). Sequences were obtained by the double-stranded DNA cycle sequencing with each of the primers used in the PCR amplification on an ABI 377 automated sequencer. The BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) was used to sequence, with conditions set in accordance with the provided protocol. All individuals were sequenced in both directions.

All samples were genotyped by PCR amplifications of ten microsatellites that were isolated originally from the relative species *Gallus gallus* genome: ADL268 (annealing temperature = 60°C), ADL136 (56°C), MCW0016 (62°C), MCW067 (56°C), MCW069 (58°C), MCW0111 (57°C), MCW0216 (60°C), MCW222 (62°C), MCW0295 (60°C) and LEI0192 (56°C). Primer sequences and information on markers can be retrieved from http://www.ncbi.nlm.nih.gov/sites/entrez. Amplified products, which ran on 8% polyacrylamide gel by electrophoresis, could be visualized by silver staining. PUC19 DNA/MspI (HpaII), as a size standard, was ran on each gel to determine fragment size using Bandscan 4.30 software (http://moleco.sjtu.edu.cn).

Data analysis

Sequences were aligned by clustal X procedure (Thompson et al., 1997) and refined manually. DnaSP5.10.01 (Librado and Rozas, 2009) was used to estimate average and population haplotype diversity (h), nucleotide diversity (π) and mean number of pairwise

Table 1. Environmental factors for 13 populations of *Bambusicola thoracica*.

Population	Latitude	Longitude	AAR (mm)	AAT(℃)	VCR	VCT
FAX	27°10'	119°21'	1539.9	19.3	0.181	0.022
GAX	30°02'	112°09'	1125.2	18.3	0.228	0.035
HFX	29°31'	110°01'	1500.0	17.5	0.180	0.020
JGS	26°22'	114°05'	1865.5	14.2	0.179	0.021
JKX	26°01'	110°23'	1369.0	16.2	0.127	0.023
LCX	24°04'	115°09'	1693.3	21.0	0.225	0.023
LSX	26°14'	108°03'	1375.0	15.3	0.171	0.018
WYS	27°10'	116°32'	2583.0	17.9	0.180	0.020
XNX	26°15'	110°31'	1331.1	17.0	0.141	0.023
YFX	28°14'	114°28'	1800.0	17.2	0.161	0.021
YLX	28°16'	110°14'	1440.0	16.6	0.164	0.024
ZYS	26°01'	110°23'	1773.0	16.4	0.178	0.019
ZYX	27°02'	108°15'	1093.3	16.4	0.151	0.025

AAR, Annual average rainfall; AAT, annual average temperature; VCR, variation coefficient of annual average rainfall; VCT, variation coefficient of annual average temperature.

differences (*K*). The software GENEPOP Version 3.2a (ftp://ftp.cefe.cnrs-mop-fr/pub/msdos/genepop) (Raymond and Rousset, 1995) was used to calculate allele frequencies, allelic richness, observed (H_O) and expected (H_E) heterozygosities. Tests of differences in haplotype frequencies among all populations were performed using Fisher's exact test. T-test was used to test the differences between H_O and H_E among all populations. A Mantel test (Mantel, 1967) was performed to estimate the relationship between two independent dissimilarity matrices: the genetic diversity and environmental factors. SPSS11.0 was used for the materials.

Environmental factors

Latitude, longitude, altitude, annual average temperature (AAT), annual average rainfall (AAR), variation coefficient of annual average temperature (VCT) and variation coefficient of annual average rainfall (VCR) were selected as environmental factors (Table 1). Climatic data over 30 to 50 years of sampling localities were collected from China Meteorological Data Sharing Service System (http://cdc.cma.gov.cn).

RESULTS

Mitochondrial DNA haplotype and variability

A total of 1140 nucleotides of the mtDNA control region genes were sequenced for all the samples. The mtDNA control-region sequence alignment showed 50 different haplotypes (27.78% of the all samples), defined by 39 polymorphic sites. The number of observed haplotypes within populations ranged from four in ZYX, HFX and GAX to ten in JGS (Table 2). The percentages of unique haplotypes were calculated by dividing the number of unique haplotypes by the total number of samples. Within each population, this percentage varied from 0 in XNX to 50.0% in LCX (Table 2). Statistically significant differences in haplotype frequencies were observed among all populations (χ^2 = 25.112, p<0.01) based on Fisher's exact test.

The mtDNA data revealed high level of haplotype and nucleotide diversity of the partridge (π = 0.0052, h = 0.94) (Table 2). Nucleotide diversity among the thirteen populations varied from 0.00123 (WYS) to 0.00395 (ZYX); at the same time, nucleotide diversity ranged from 0.644 (YFX) to 0.890 (JGS) (Table 2). The pairwise divergence between haplotypes (average K = 2.638) was lowest (K = 1.400) in partriges from WYS population and highest (K = 4.500) in partridges from ZYX population (Table 3).

Microsatellite genetic diversity

The ten microsatellite loci were polymorphic in all populations of Chinese bamboo partridge (Table 3). The number of alleles ranged from seven to ten. Mean number of alleles per locus varied from 2.70 in HFX to 5.00 in JGS. Allelic richness ranged from a minimum of 1.90 in LCX to a maximum of 4.06 in ZYX. Observed heterozygosity ranged from 0.39 in LCX to 0.54 in YLX, while expected heterozygosity varied from 0.39 in LCX to 0.65 in HFX. The average value of H_O = 0.48 was lower than H_E = 0.50 (t = 1.19, p>0.05).

The relationship between genetic diversity and environmental factors

Haplotype diversity (h), nucleotide diversity (π) and pairwise divergence between haplotypes (K), expected heterozygosity (H_E) and observed heterozygosity (H_O) showed negative correlation with latitude, longitude, AAT,

Table 2. Number of total haplotypes and unique haplotypes found within each population, percentages of number of unique haplotypes/total number of individuals (*S*) and number of the unique haplotypes/total number of haplotypes (*R*), ratio of number of individuals/total number of haplotypes (*T*), nucleotide diversity (π), haplotype diversity (*h*) and mean pairwise differences (*K*) of the eight populations in *B. thoracica.*

Population	Sample size	Total Haplotype	Unique haplotype	*S*	*R*	*T*	π*	*h**	*K**
FAX	16	7	7	0.438	1.000	2.286	0.00165	0.867	1.883
GAX	10	4	1	0.100	0.250	2.500	0.00174	0.778	1.978
HFX	10	4	1	0.100	0.250	2.500	0.00175	0.822	2.000
JGS	30	10	8	0.267	0.800	3.000	0.00304	0.890	3.462
JKX	11	5	3	0.273	0.600	2.200	0.00268	0.854	3.054
LCX	10	5	5	0.500	1.000	2.000	0.00168	0.667	1.911
LSX	15	6	3	0.200	0.500	2.500	0.00393	0.762	4.476
WYS	10	5	4	0.400	0.800	2.000	0.00123	0.756	1.400
XNX	13	5	0	0.000	0.000	2.600	0.00193	0.782	2.205
YFX	10	5	3	0.300	0.600	2.000	0.00160	0.644	1.822
YLX	22	6	1	0.045	0.167	3.667	0.00203	0.844	2.312
ZYS	14	6	2	0.143	0.333	2.333	0.00288	0.835	3.286
ZYX	9	4	1	0.111	0.250	2.250	0.00395	0.806	4.500

* From Huang et al. (2010).

Table 3. Summary of genetic diversity in bamboo partridge at ten microsatellite loci, mean number of alleles per locus (*A*), allelic richness (*Ac*), number of polymorphic loci (*P*) among the populations, expected heterozygosity (H_E) and observed heterozygosity (H_O).

Population	*A*	*Ac*	*P*	H_E	H_O
FAX	3.00	2.90	9.00	0.44	0.42
GAX	3.20	2.07	10.00	0.50	0.52
HFX	2.70	1.98	10.00	0.44	0.65
JGS	5.00	3.96	10.00	0.53	0.54
JKX	3.10	3.00	10.00	0.49	0.45
LCX	2.80	1.90	7.00	0.39	0.39
LSX	3.60	3.32	10.00	0.46	0.42
WYS	3.40	3.34	10.00	0.48	0.48
XNX	4.10	2.20	10.00	0.50	0.60
YFX	3.50	3.50	10.00	0.48	0.41
YLX	4.40	2.24	10.00	0.54	0.58
ZYS	3.70	2.08	10.00	0.47	0.57
ZYX	4.20	4.06	10.00	0.53	0.54

AAR, VCT and VCR, except H_E and latitude, H_O and latitude, H_E and VCR, H_O and VCR (Table 4). Significant negative relationship was observed between π and VCT ($r = -0.656$, $p<0.05$), π and AAT ($r = -0.670$, $p<0.05$), *K* and longitude ($r = -0.657$, $p<0.05$), *K* and AAT ($r = -0.671$, $p<0.05$), H_E and AAT($r = -0.697$, $p<0.05$) (Table 4).

DISCUSSION

The importance of preserving levels of genetic variation within populations has long been recognized for influencing short-term population viability as well as for maintaining a population's adaptability to environmental changes (Frankham, 1995). Populations throughout a range are adapted to local climates or are genetically distinct across latitude in a way that is related to climate. Many studies show that climate factors differs in importance to different types of organism and/or different region (Huang et al., 2005; Jin and Liu, 2008). The aim of this study is to reveal the correlations between environmental factors and population genetic diversity of

Table 4. Coefficient relationship between genetic diversity and environmental factors.

Parameter	Latitude	Longitude	AAT	AAR	VCT	VCR
π	-0.656*	-0.297	-0.670*	-0.415	-0.190	-0.347
h	-0.138	-0.110	-0.474	-0.187	-0.017	-0.294
K	-0.297	-0.657*	-0.671*	-0.416	-0.349	-0.191
H_E	0.325	-0.372	-0.697*	-0.193	-0.460	0.282
H_O	0.421	-0.483	-0.351	-0.190	-0.179	0.080

*Indicate significant correlation, $p<0.05$.

Chinese bamboo partridge. We found that the genetic diversity of the partridge correlated negatively with AAR and AAT, which were different from the results of *Alectoris chukar*, which is distributed in arid area (Huang et al., 2005). In arid and semi-arid region, rainfall was relevant to the vegetation (Pianka, 1970). Better plant growth in higher rainfall regions, will provide more food for phytophagous partridge. Thus, the population size would be larger with higher genetic diversity, due to reduction of bottleneck effects in genetics (Huang et al., 2005). However, in humid region, rainfall was not the determinant factor influencing the vegetation growth. Rainfall occurred mainly in the bird breeding season in Southern China. Excessive rainfall may affect the success rate of bird breeding in these areas. Therefore, the genetic diversity of the partridge correlated negatively with AAR.

There was a significant genetic differentiation among the population of Chinese bamboo partridge (Huang et al., 2010). Genetic differentiation within a species's range is determined by natural selection, genetic drift and gene flow (Zakharov and Hellmann, 2008). Natural selection acts directly on the phenotypes of individuals (Huang et al., 2005). To the extent that these phenotypes are controlled or influenced by genes or genotypes, natural selection will act indirectly on genes and genotypes. If the genotypes of individuals with different phenotypes differ, those genes and genotypes of the individuals favored by natural selection will tend to increase in relative frequency (Merrell, 1981). Chinese bamboo partridge genetic diversities decreased with increase in variation coefficients of temperature and rainfall. The more stable the climate, the higher the genetic diversity observed, which were same as the results of other partridges (Huang et al., 2005). Two general scenarios of natural selection effects on genetic population structure are stabilizing and diversifying selection types. Under uniform stabilizing selection, the same phenotype is selected across the range, and the degree of population subdivision is reduced relative to neutral expectation. Under diversifying selection caused by environmental heterogeneity, population subdivision is fostered and exceeds what is expected under neutral expectation (Volis et al., 2004). Vanhala et al. (2004) showed that a stable environment will not put much pressure on the plant for it to maintain much genetic variation as the plant grow in an unstable environment. Almost all kinds of genotypes can exist due to the environmental pressure relaxation in stable climate areas (Huang et al., 2005), thus genetic diversities are higher. However, in unstable climate areas, only the individuals with genotypes adapted to changeable conditions can survive, therefore lower genetic diversity occurred.

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