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GENETIC DIVERSITY IN PERIPHERAL AND CENTRAL POPULATIONS OF RUSTY-NECKLACED PARTRIDGE (*ALECTORIS MAGNA*) BASED ON MITOCHONDRIAL AND MICROSATELLITE DNA

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Though it has been long presumed that peripheral populations tend to exhibit low levels of genetic diversity due to isolation and genetic drift, results of empirical investigation remain ambiguous. Some rusty-necklaced partridge (*Alectoris magna*) populations have expanded their present ranges, resulting in several peripheral populations, due to recent deforestation by human beings in Northwestern China. On the basis of mitochondrial DNA control-region and microsatellite DNA data, we compare the genetic diversity (π-, *H*-, H_0-, and H_E-values) between three peripheral populations and five central populations. Maternal and biparental DNA markers indicated accordantly genetic diversity. Compared to central populations, the peripheral populations exhibited lower genetic diversity. The low genetic variability of the three peripheral populations appeared to result partly from isolation and natural selection.

Key words: *Alectoris magna*, peripheral population, genetic diversity, mitochondrial DNA, microsatellite

INTRODUCTION

Geographically peripheral populations are more likely to be imperiled than central populations. They tend to occur in less suitable environments and are often isolated from more central and continuous populations (LESICA & ALLENDORF 1995). Many theoretical works have revealed that genetic mechanisms such as inbreeding or genetic drift in small population caused by genetic bottlenecks and founder effects are important factors in reducing genetic variability (BARRETT & KOHN 1991). Genetic diversity is expected to be lower in peripheral populations than in central populations (CASSEL & TAMMARU 2003, ECKERT *et al*. 2008), due to genetic drift (NEI *et al*. 1975, HARTL & CARK 1997). Central populations are usually large, continuous and occupy favorable habitats. Peripheral populations, by contrast, can be more or less isolated, fragmented, and be subject to a more variable physical environment (LESICA & ALLENDORF 1995). Accordingly, peripheral populations will often experience different selection pressures than central popula-

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tions, which may lead to lower genetic divergence (KIRPATRICK & BARTON 1997). Genetic differences are most likely to occur in populations that become isolated at the periphery of the range (LESICA & ALLENDORF 1995, SAFRIEL *et al*. 1994). Empirical evidence supporting this hypothesis remains ambiguous (GARNER *et al*. 2004, HUANG *et al*. 2005). Some data support the hypothesis that peripheral populations exhibit lower genetic diversity (LAMMI *et a*l. 1999, HOU *et al*. 2002, WANG *et al*. 2001), while others show no such relationship (TIGERSTEDT 1973, WENDEL & PARKS 1985, PETITET *et al*. 1998). There is thus practical need for descriptive studies.

Rusty-necklaced partridge (*Alectoris magna*, Gallifromes, Phasianidae) is found in Qinghai, Ningxia and Gansu provinces, patchily distributed in dry and open rocky mountains (LIU 1992), with two described subspecies: *A. magna magna* (the Chaidamu Basin) and *A. magna lanzhouensis* (the Lanzhou Basin and the Liupanshan Mountain) (LIU *et al*. 2004). The partridge is representative species of arid and semiarid environments in northwestern China (HUANG *et al*. 2007*a*). Forest and farmland are generally avoided. Most forest has disappeared in the Gansu Province because of deforestation and cultivation by human beings. Rusty-necklaced partridge has expanded to Lixian, Beidao and Haiyuan, which result in peripheral populations, paralleling with *A. chukar* along the Liupan Mountains (Fig. 1). Introgressive hybridization between the two species was detected in the contact zone (CHEN *et al*. 1999, LIU *et al*. 2006). These populations provided the opportunity to investigate the genetic diversity of peripheral populations, compared to that of central populations.

Mitochondrial DNA (mtDNA), particularly focusing on fast-evolving segments of the noncoding control region, has been extensively employed to assess evolutionary questions (STANLEY *et al*. 1996, BONATTO & SALZANO 1997, VILA *et al*. 1997). Recently, the development of hypervariable genomic markers, microsatellites (GOLDSTEIN & SCHLOTTERER 1999) allowed the inferring of additional details on evolutionary processes and population structure (BALDING *et al* 2001). Here we examine the difference of genetic diversity in relation to geographic position (peripheral or central), using both mtDNA control-region sequences and nuclear microsatellites. To eliminate the effects of genetic variation between subspecies, we only analyze one subspecies, *A. magna lanzhouensis*. There were two aims: (1) assess whether maternal mtDNA and biparental microsatellite markers described concordant population genetic diversity; and (2) compare the genetic diversity between peripheral and central populations.

MATERIALS AND METHODS

Sample collection and laboratory methods

A total of 82 samples of eight populations in rusty-necklaced partridge are collected from the following localities: Lanzhou, Dingxi, Jingyuan, Haiyuan, Huining, Beidao, Lixian and Wushan (Fig. 1). Wild samples were collected during consecutive hunting seasons. Liver samples were dissected from birds and stored in 95% ethanol immediately after removal. The methods of DNA extraction, PCR amplification and sequence of mtDNA control region genes referred to HUANG *et al* (2007*a*). The sequences were deposited in GenBank and the accession numbers are from DQ157593 to DQ157619. These are just from HUANG *et al* (2007*a*).

All samples were genotyped by PCR amplifications of eight microsatellites: MCW135 (5'-ATA TGC TGC AGA GGG CAG TA–3', 5'-CAT GTT CTG CAT TAT TGC TCC–3', annealing temperature = 45 °C), MCW207 (5'-GAT CCT TAC AGC CTG CAA TGC–3', 5'-ATA CTG TTG GAA GAT GTA TGC G–3', 60 °C), MCW295 (5'-ATC ACT ACA GAA CAC CCC TCT C–3', 5'-TAT GTA TGC ACG CAG ATA TC–3', 50 °C), MCW323 (5'-GAA ATG GTA CAG TGC AGT TGG–3', 5'-TGA ATT CTC TCG GCT TCC ATC–3', 60 °C), that were isolated originally from the chicken (*Gallus gallus*), and AB121114 (5'-GAC TAG TAG TGA AGA CTG TT–3', 5'-AGA TTT CTG GCT TCT GCA–3', 52 °C), AB063167 (5'-GTC ACA CAC TGT ATC ATA CT–3', 5'-GTG ATC TCA GTG TTT ATC TT–3', 55 °C), AB035840 (5'-TGC ACC AAT CCC AGC TGT TT–3', 5'-ACA ATG GAA AGT GGG GTT C–3', 55 °C), AB063153 (5'-CAT AAC TGG GAT ATT GTT TA–3', 5'-ACA ACC ACT TCT CCA GCT A–3', 52 °C) that from common quail (*Coturnix coturnix*), which were obtained from GenBank. The PCR products were denatured at 94 °C 5 min using Dextran blue formamide solution. After polyacrylamide gel electrophoresis, the migration rate fragment size was determined using Bandscan 4.30 software (http//moleco.sjtu.edu.cn), with the marker pUC19 DNA/Msp I (Hpa II).

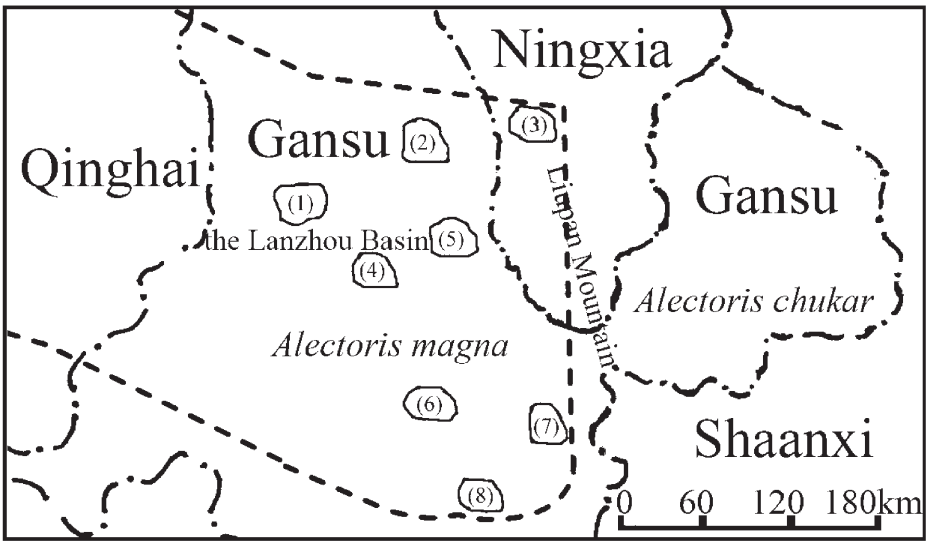

Fig. 1. Rusty-necklaced partridge sampling sites: 1 = Lanzhou, 2 = Jingyuan, 3 = Haiyuan, 4 = Dingxi, 5 = Huining, 6 = Wushan, 7 = Beidao, 8 = Lixian

Sequence analysis

All sequences were aligned using Clustal X (THOMPSON *et al*. 1997). Arlequin2.0 (SCHNEIDER *et al*. 2002) was used to compute the number of haplotypes in populations, number of polymorphic sites. DnaSP4.0 (ROZAS *et al*. 2003) was used to calculate population haplotype diversity (*H*), nucleotide diversity (π) and mean number of pairwise differences (*K*). Arlequin2.0 (SCHNEIDER *et al*. 2002) was used to compute pairwise population differentiation and to perform analysis of molecular variance (AMOVA, EXCOFFIER *et al*. 1992).

The software GENEPOP Version 3.2a (ftp://ftp.cefe.cnrs-mop-fr/pub/msdos/genepop) (RAYMOND & ROUSSET 1995) was used to calculate allele frequencies, observed (H_O) and expected (H_E) heterozygosities. Deviations from Hardy–Weinberg equilibrium for each locus and each population were assessed using the Markov chain method, as implemented in GENEPOP 3.2a. Genetic differentiations were tested among all pairs of populations for all loci (GENEPOP 3.2a). F_{ST} values for population subdivision were also calculated using GENEPOP 3.2a according to WEIR and COCKERHAM (1984). Tests of genotypic differentiation, based on the G-based exact tests of GOUDET *et al* (1996), were also performed using this program.

RESULTS

Mitochondrial DNA haplotype and variability

A total of 458 nucleotides of the mtDNA control region were sequenced of all the samples. The mtDNA control-region sequence alignment showed 25 different haplotypes, defined by 27 polymorphic sites (Table 1). The number of observed haplotypes within populations ranged from three in Beidao to seven in Lanzhou (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 17.64% in Lanzhou to 37.50% in Wushan (Table 2). The most common haplotypes were M2 with 29 individuals from all the sampling sites (Table 1). Many allied haplotypes, however, were localized. Results of AMOVA showed that 12.25% of the total mtDNA genetic variability was distributed within, and 87.75% among populations (Φ_{ST} = 0.63, P<0.01). Pairwise F_{ST} values test showed peripheral populations were significantly differentiated from central populations except Haiyuan and Huining (Table 3).

Nucleotide diversity among the eight populations varied from 0.0028 (Haiyuan) to 0.0069 (Dingxi, Table 2); and haplotype diversity ranged from 0.52 (Beidao) to 0.86 (Wushan, Table 2). The pairwise divergence between haplotypes (average k = 2.33) was lowest (k = 0.85) in partridges from Haiyuan population and highest (k = 3.18) in partridges from Dingxi population. Three peripheral populations (Lixian, Beidao and Haiyuan), possessed lower haplotype diversity (average 0.67) and nucleotide diversity (average 0.0030), compared to central geographic populations (average H = 0.80, π = 0.0057).

Table 1. Sampling locations, numbers and frequency in the total population of the 25 mtDNA haplotypes found in rusty-necklace partridge (HUANG *et al.* 2007*a*). Haplotype positions are aligned with the complete mtDNA D-loop sequence of *Alectoris* (RANDI & LUCCHINI 1998).

Haplo-type	Number (frequency, %)	Variable positions in sequences	Sampling location (sample size)
		00011222222222222222233333333344	
		11209111122233444457890113489994 5	
		79730017834856245638804045834796 4	
M1	2 (2.4)	AAAACAGCAGCTTTCTTTCTATTCTGATTTCCC	Lanzhou (1), Lixian (1)
M2	29 (35.4)	.....-.....	Huining (5), Beidao (1), Haiyuan (7), Lanzhou (6), Dingxi (2), Wushan (1), Lixian (3), Jingyuan (4)
M3	3 (3.6)	.....T.G.....	Lixian (3)
M4	1 (1.2)	.....A.....T.....	Lixian (1)
M5	5 (6.1)	.....A.....T..C.....C....	Beidao (5)
M6	1 (1.2)	.....T.....C....	Beidao (1)
M7	2 (2.4)	.....-.....A.....	Wushan (2)
M8	1 (1.2)	.G.....A.....T.....A.....	Wushan (1)
M9	1 (1.2)	.....C..T..C.....C....	Wushan (1)
M10	8 (9.7)	.....C....	Lanzhou (3), Jingyuan (1), Haiyuan (1), Wushan (3)
M11	5 (6.1)	.....C...C-.....	Dingxi (5)
M12	1 (1.2)	.....C...-.....	Dingxi (1)
M13	2 (2.4)	.....-.....GC.....	Dingxi (1), Huining (1)
M14	1 (1.2)	...G.....T..-.....	Huining (1)
M15	1 (1.2)	G.....C....-.....	Huining (1)
M16	2 (2.4)	.....T..-.....	Huining (1), Haiyuan (1)
M17	1 (1.2)	.....-.....C...	Haiyuan (1)
M18	1 (1.2)	.....-.....C.GG	Haiyuan (1)

Table 1 (continued)

Haplo-type	Number (frequency, %)	Variable positions in sequences	Sampling location (sample size)
M19	2 (2.4)	.....-.....G	Haiyuan (2)
M20	3 (3.6)	.....C..C.....G	Jingyuan (3)
M21	2 (2.4)	.....-.....C.G.	Jingyuan (2)
M22	2 (2.4)	.....-...C.....A..	Lanzhou (2)
M23	3 (3.6)	.....A....C.....T.....	Lanzhou (2), Dingxi (1)
M24	2 (2.4)	.G.T.....C.....	Lanzhou (2)
M25	1 (1.2)	.....T-.....	Lanzhou (1)

Microsatellites genetic diversity

The results of our PCR amplifications of the eight microsatellite loci in 82 rusty-necklaced partridge samples revealed a total of 54 alleles. The eight microsatellites were polymorphic in the partridge samples, with the exception of locus MCW207, which was monomorphic in the all samples. Allele frequencies at microsatellites were calculated for all individuals. Values of observed heterozygosity (H_O) ranged from 0.20 (Lixian) to 0.75 (Jingyuan), and values of expected heterozygosity (H_E) varied from 0.31 (Lixian) to 0.59 (Wushan) (Table 2). The averages of the eight geographic populations of rusty-necklaced partridge are 0.45 for H_O and H_e. Significant allele frequency differences were detected among all pairwise comparisons for the eight ps over all loci ($P < 0.001$). Probability tests for departure from Hardy–Weinberg performed in each population and cross each locus show that five loci (AB063153, MCW295, CW323, AB121114, AB035840) in each population were in equilibrium $P > 0.05$), and the MCW135 locus in the Lanzhou and Beidao populations was not equilibrium ($P < 0.05$). The multilocus test performed for Beidao population showed a heterozygote deficit, but the difference was not significant ($P > 0.05$). Other populations showed heterozygote redundancy, which was significant in populations Haiyuan and Jingyuan ($P<0.05$). Microsatellite genetic diversity was also significantly partitioned among the eight population (average multilocus $F_{ST} = 0.309$, $P < 0.01$). Pairwise F_{ST} values were significant between peripheral and central populations except Lixian and Wushan (Table 3).

Compared to central geographic populations, Lixian, Beidao and Haiyuan exhibited

Table 2. Haplotypes and genetic diversity of the eight populations.

Population	Sample size	Total haplotypes	Unique haplotypes	K*	π*	H*	H_O	H_E
Huining	9	5	2	2.17	0.0047	0.73	0.57	0.55
Wushan	8	5	3	3.14	0.0057	0.86	0.50	0.58
Jingyuan	10	4	2	2.47	0.0054	0.78	0.75	0.46
Lanzhou	17	7	3	2.60	0.0057	0.85	0.52	0.59
Dingxi	10	5	2	3.18	0.0069	0.76	0.48	0.41
Beidao	7	3	2	1.81	0.0039	0.52	0.22	0.32
Lixian	8	4	2	1.03	0.0023	0.78	0.34	0.38
Haiyuan	13	6	3	0.85	0.0028	0.72	0.20	0.31

*From HUANG *et al* 2007*a*.

low H_O (average 0.25) and H_E (average 0.34). A significant difference was found in H_O ($t = 2.2443$, $p = 0.044$) and in H_E ($t = 4.15$, $P = 0.007$) between peripheral and the central populations. Lixian population has the lowest observed heterozygosity ($H_O = 0.20$) and the lowest expected heterozygosity ($H_E = 0.31$), significantly different from heterozygosity values of all the other populations ($P < 0.05$; Wilcoxon's signed-rank test).

DISCUSSION

Though the mtDNA genome of animals is typically inherited in a uniparental (matrilineal) fashion and only has an effective population size one-fourth that of the nuclear genome (AVISE *et al*. 1987), the genetic diversities exhibited by

Table 3. Pairwise values of F_{ST} (microsatellites DNA, above the diagonal; mitochondrial DNA, below the diagonal) among populations of rusty-necklaced partridges.

Population	Huining	Beidao	Haiyuan	Jingyuan	Lanzhou	Dingxi	Wushan	Lixian
Huining		0.396*	0.264*	0.358*	0.281*	0.367*	0.198*	0.413**
Beidao	0.654**		0.194*	0.264*	0.287*	0.771**	0.180*	0.273*
Haiyuan	0.031	0.640**		0.147*	0.337*	0.284*	0.110*	0.373*
Jingyuan	0.150*	0.545**	0.082		0.392*	0.324*	0.103*	0.392*
Lanzhou	0.126*	0.504**	0.102*	0.041		0.343*	0.302*	0.485**
Dingxi	0.176*	0.629**	0.249*	0.081	0.138*		0.254*	0.464**
Wushan	0.245*	0.310*	0.192*	0.154*	0.080	0.299*		0.088
Lixian	0.294*	0.499**	0.285*	0.205*	0.146*	0.347*	0.127*	

*$P < 0.05$, **$P < 0.01$

mtDNA haplotypes and observed at microsatellites loci were accordant. The three peripheral populations, Lixian, Beidao and Haiyuan possessed lower nucleotide diversity (average $\pi = 0.0030$), haplotype diversity (average $H = 0.67$), and values of observed heterozygosity (average $H_O = 0.25$) and expected heterozygosity (average $H_E = 0.34$), while central populations owned higher genetic diversity (average $\pi = 0.0057$, $H = 0.80$, $H_E = 0.52$, $H_O = 0.58$).

Many authors believe that the peripheral populations often have reduced levels of genetic variability relative to central populations (LESICA & ALLERDORF 1995, GARCIA-RAMOS & KIRKPATRICK 1997, WANG *et al*. 2001). Our results support this hypothesis, since compared to central populations, the three peripheral populations exhibited lower genetic diversity. Populations located at range margins are more isolated from sources of immigrants and are thus more prone to genetic bottlenecks (KARRON 1987, ROWE & BEEBEE 2003), a situation that should deplete neutral genetic variation (GARNER *et al.* 2004). The genetic diversity of a population is related to the degree of isolation. Low levels of genetic diversity can be expected in populations at range limits as a result of low levels of immigration and high levels of genetic drift (e.g. SOULÉ 1973, HOFFMANN & BLOWS 1994). Rusty-necklaced partridge is a species indicative of arid and semiarid environments in northwestern China, while forest and farmland are generally avoided. This could explain the lower genetic diversity of the Haiyuan population, because it is isolated from other populations by farmlands, preventing gene flow. HUANG *et al.* (2007*b*) observed that the population genetic diversity of rusty-necklaced partridge was negatively correlated with the rainfall. Based on this environmental factor, natural selection could lead to a lower genetic diversity. Indeed, the Lixian and Beidao populations belong to wet areas with average annual rainfall of 510.0±126.2 mm (n = 40) and 547.8±130.5 mm (n = 40), a habitat little favorable for rusty-necklaced partridges, and possessed the lowest genetic diversities (Table 2).

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