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Introgressive hybridization and population genetic diversity between rusty-necklaced partridge and chukar partridge in northwestern China

Zuhao Huang[1,2], Zhisong Yang[2], Jiao Zhang[2] & Naifa Liu[2]

[1] **School of Life Sciences, Jinggangshan University, Ji'an, Jiangxi Province, 343009, China.**
[2] **School of Life Sciences, Lanzhou University, Lanzhou, Gansu Province, 730000, China.**

Corresponding author : Naifa Liu, School of Life Sciences, Lanzhou University, Lanzhou, Gansu Province, 730000, China. E-mail: naifaliu@sohu.com.

ABSTRACT. Introgressive hybridization is a common feature of the contact zone between divergent partridges of the genus *Alectoris*. The rusty-necklaced partridge (*Alectoris magna*) is paralleled with the chukar partridge (*A. chukar*) along the Liupan Mountain in northwestern China, and hybridization between the two species has been detected in the contact zone within this region. We examined nine populations of rusty-necklaced partridge and eight populations of chukar partridge to determine the extent and nature of the hybridization between them. A total of 458 nucleotides of mitochondrial DNA control-region were sequenced, revealing a strong asymmetry in introgression between the two taxa. The hybrids, morphologically identified as *A. magna*, were partly introgressed with genetic material from *A. chukar*. The haplotype diversity and nucleotide diversity decreased with increasing hybrid ratio among hybrid populations. The genetic integrity of the rusty-necklaced partridge is shown to be at risk from the introgressive hybridization.

KEY WORDS : *Alectoris magna*, *Alectoris chukar*, introgressive hybridization, genetic diversity, genetic assimilation

INTRODUCTION

Hybridization and introgression are increasingly recognized as important factors in the diversification of plants, and provide an excellent opportunity to study evolutionary processes (ARNOLD, 1997; KLINGENBERG et al., 2000). Speciation caused by introgressive hybridization occurs frequently in plants (ELLSTRAND & SCHIERENBECK, 2000), but its importance in animal evolution remains controversial. The importance of introgressive hybridization is increasingly supported by recent molecular and ecological studies (ROQUES et al., 2001). Hybridization is relatively common in birds (GRANT & GRANT, 1992), and many avian parapatric distributions have been described (e.g. RISING, 1983) that apparently represent stable zones of overlap and hybridization.

In some cases, hybrids may constitute a large part of the natural populations (CRESPIN et al., 1999). Also, serious problems for the conservation of rare native species can occur because of hybridization (LEARY et al., 1995). Natural hybridization is expected to increase genetic diversity and fitness (ROELKE et al., 1993), such as in plant species of hybrid origin, and in genetic exchange among microorganisms. Empirical evidence supporting this hypothesis remains ambiguous. Hybridization can result in genetic assimilation and hybrid depression (RISING, 1983; RYMAN et al., 1995). Hybridization can also compromise the genetic integrity of existing species to the point of causing genetic extinctions (GILL, 1994; ABERNETHY, 1994). Hybridizing avian populations often show low geographic variation and absence of diagnostic alleles at both nuclear and mitochondrial loci (RANDI & BERNARD-LAURENT, 1999). We are not yet able to make a priori judgments about when the "positive" or "negative" effects of hybridization will dominate. There is thus a practical need for advanced studies.

Two closely related species of *Alectoris* partridge, namely, rusty-necklaced partridge (*Alectoris magna*) and chukar partridge (*A. chukar*), are distributed in northern China. The former is limited to small areas in Ningxia, Gansu and Qinghai, while the latter is found in the broad Palearctic region. They live parapatrically along the Liupan Mountain (LIU, 1984; Fig. 1). The Liupan Mountain represents a barrier to dispersal and an area of secondary contact among many western and eastern taxa (WANG, 1988). Hybridization between the two species was detected in the contact zone (CHEN et al., 1999; LIU et al., 2006), and provided an interesting case to illustrate the consequences of asymmetrical introgression on genetic diversity. We thus undertook to clarify the genetic status of rusty-necklaced partridge in the parapatric region based on mitochondrial DNA (mtDNA) control-region. Our general aims were: (1) to infer the extent of introgressive hybridization between the two species, (2) to discuss the causes of the hybridization, and (3) to assess the effects of the introgressive hybridization on rusty-necklaced partridge.

MATERIALS AND METHODS

Sample collection and DNA extractions

A total of 106 birds from nine populations of rusty-necklaced partridge were collected along the Liupan Mountain from the following localities: Lanzhou (LZ, n=17), Dingxi (DX, n=10), Jingyuan (JY, n=10), Haiyuan (HY, n=22), Huining (HN, n=10), Beidao (BD, n=9),

Zhuanglang (ZL, n=10), Lixian (LX, n=10) and Wushan (WS, n=8) (Table 1, Fig. 1). Eighty-four birds from eight populations of chukar partridge were collected from the following localities: Tianshui (TS, n=10), Xichuan (XC, n=10), Quzi (QZ, n=10), Tongchuan (TC, n=10), Panke (PK, n=10), Gaoping (GP, n=14), Honghui (HH, n=10) and Wangxia (WX, n=10) (Fig.1). Wild birds were collected during three consecutive hunting seasons (2001, 2002 and 2003). Liver samples were dissected from birds and stored in 95% ethanol immediately after removal. Total DNA was extracted from liver by the ethanol sedimentation procedure as described by RANDI & LUCCHINI (1998).

TABLE 1

Number of hybrids and hybrid ratio in the nine populations of rusty-necklaced partridge

Population	N	Numbers of hybrid	Hybrid ration (%)
Huining	10	1	10.00
Wushan	8	0	0
Beidao	9	2	22.22
Haiyuan	22	9	40.91
Jingyuan	10	0	0
Lanzhou	17	0	0
Dingxi	10	0	0
Lixian	10	2	20.00
Zhuanglang	10	8	80.00

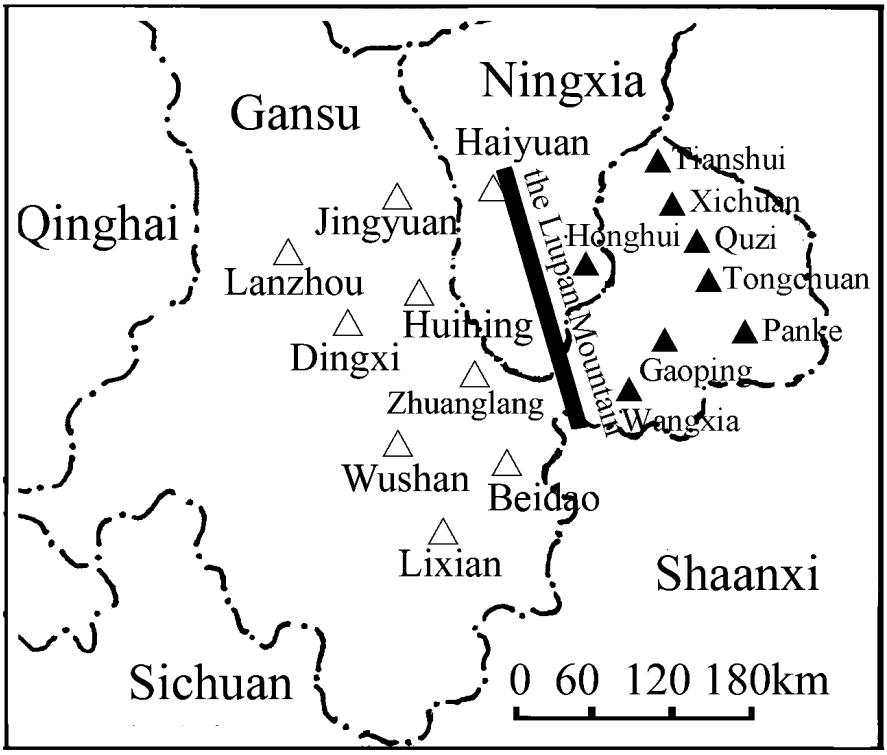

Fig. 1. – Map showing the sampling sites. △: *Alectoris magna*, ▲: *A. chukar*.

Laboratory methods

Two oligonucleotide primers, PHDL (5-AGGACTACGGCTTGAAAAGC-3) and PH1H (5-TTATGTGCTTGACCGAGGAACCAG-3) (RANDI & LUCCHINI, 1998), were used to amplify and sequence 458bp mitochondrial DNA control-region segment. There was 1 unit of Taq DNA polymerase in 35μL reactions. The final concentrations were 10mmol/L Tris-HCl (pH 8.3), 50mmol/L KCl, 1.5mmol/L $MgCl_2$, 150μmol/L dNTP, 10pmol/L primers and about 100ng DNA templates. PCR conditions were as follows: 95°C 4min; 35 cycles of 95°C 40sec, 55–58°C 40sec, 72°C 60sec; followed by 72°C 10min in PE2400 thermocycler. After examination by 1% agarose gel 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 amplifications on an ABI 373 automated sequencer. All individuals were sequenced in both directions. The sequences were deposited in GenBank and the accession numbers are from DQ157593 to DQ157619 (*A. magna*), and from AY190634 to AY190659 (*A. chukar*).

Sequence analysis

Sequences were aligned by Clustal X Procedure (THOMPSON et al., 1997) and refined manually. Arlequin 2.0 (SCHNEIDER et al., 2002) was used to define the haplotypes. DnaSP4.0 (ROZAS et al., 2003) was used to estimate population haplotype diversity (h), mean number of pairwise differences (k), nucleotide diversity (π). The difference of haplotype and nucleotide diversity between the hybridized populations and the pure ones was compared

statistically using a randomization test. The individuals were pooled and then randomly assigned to the two groups. The difference between these random values was stored and this procedure was repeated 1000 times. The observed differences were compared with this random distribution: the P value (p*)* is given by the proportion of random values greater than the observed one (using absolute values because the test is two-tailed).

The population differentiation was determined using the Arlequin 2.0 analysis of molecular variance (AMOVA) (SCHNEIDER et al., 2002). Unrooted haplotype networks were obtained using TCS1.21 Procedure (TEMPLETON et al., 1992). A Mantel test (MANTEL, 1967) was performed to estimate the relationship between two independent dissimilarity matrices: the genetic diversity and hybrid ratio.

RESULTS

Introgressive hybridization and hybrid ratio

Of all the samples, a total of 458 nucleotides of the mtDNA control region genes were sequenced. Twenty-eight samples of rusty-necklaced partridge from five populations (Zhuanglang, Lixian, Haiyuan, Beidao and Huining) had chukar partridge mtDNA genotype. The hybrids were morphologically similar to *A. magna.* However, there were no samples of chukar partridge with rusty-necklaced partridge genotype. We thus confirmed that the hybridization between the two species was asymmetrical introgression, gene flow being only from chukar partridge into rusty-necklaced partridge. The hybrid ratio ranged from 10.00% (Huining) to 80.00% (Zhuanglang) in the five hybrid populations (Table 1). The total hybrid ratio was 20.75% in rusty-necklaced partridge.

Mitochondrial DNA haplotype

The mtDNA control-region sequence alignment showed 50 different haplotypes, defined by 67 polymorphic sites including 65 substitutions (40 transitions and 15 transversions) and two deletes (187, *chukar*; 246, *magna*) (Table 2). There were 17 haplotypes (C1-C17) belonging to chukar partridge, among which C1 was the most common haplotype, with 41 individuals from all the sampling sites of chukar partridge (Table 2). The number of observed haplotypes within populations of chukar partridge ranged from three in Honghui to six in Gaoping. Twenty-five haplotypes (M1-M25) were found in rusty-necklaced partridge. The number of observed haplotypes within populations varied from one in Zhuanglang to seven in Lanzhou (Table 3). The most common haplotype M2 was geographically widespread, in 32 individuals of rusty-necklaced partridge. The hybrids had 12 haplotypes, among which four haplotypes (C1, C2, C3 and C12) were shared with chukar partridge and the others (H1-H8) were unique haplotypes. As in the chukar partridge, haplotype C1 was the most common haplotype with 16 hybrids.

The haplotype network showed that haplotypes of chukar partridge and hybrids grouped together, stemming from C1, which was at the centre of a star-like topology (Fig. 2). The haplotypes of rusty-necklaced partridge constituted the other group, stemming from M2, which was at the other centre of a star-like toplogy (Fig. 2). The pairwise percentage sequence divergence between chukar partridge and rusty-necklaced partridge was 0.0599.

TABLE 2

Haplotyes and variable sites of chukar partridge (C1~C15), rusty-necklaced partridge (M1~M25) and hybrids (C1~C3, C12, H1~H8)

Haplotype	Variable positions in sequences	Sampling location (sample size)
	00000000000000011111111222222222222222222222222222333333333333444	
	00011111222556700056899011111222233334444455788999000111347899944 5	
	13505789789054934821706020126834585678124563888901945601458134790 64	
C1	ACCTCAAAACCCGCTATACA-CCTTGGTAAGCTCTCACTCTCTCGCACTCTTACACCGACTCTACCC	*
C2	.....-.....A.....T..	**
C3	.....T.-.....	TS(1),HH(1)
C4	.....-..C.....	TS(1), HY(1)
C5	.....-.....T.....	TS(1),QZ(2)
C6	.....-.....A..T.....	XC(1),QZ(1)
C7	.....-T.....A..T.....	XC(1)
C8	.....-.....T.....	QZ(1)
C9	.....-.....T.....A.....T..	TC(3)
C10	.....-.....G.....T..	TC(1)
C11	.....-.....C.....A.....	PK(5)
C12	.....-.....T..	PK(2),WX(1),GP(1), LX(1)
C13	.....G.....-.....T..	WX(1)
C14	.....C.....-.T.....A.....	WX(1)
C15	..T.....-.....A.....T..	GP(1)
C16	C.....-.....A.....T..	GP(1)
C17	.....-.G.....T..	GP(1)
M1	.....T..CG.GT...AA.AG...T.TG.C..T...T...TC.GTC.T..G.T.CT..	LZ(1),LX (1)
M2	.....T..CG.GT...AA.AG...T.TG.C..T-..T...TC.GTC.T..G.T.CT..	***
M3	.....T..CG.GT...AA.AG...T.TG.C..T-..T...TC.GTC.T..G.T.CT..	LX(3)
M4	.....T..CG.GT...AA.AG..A.T.TG.C..T.T.T...TC.GTC.T..G.T.CT..	LX(1)
M5	.....T..CG.GT...AA.AG.A..T.TG.C..T.T.T..CTC.GTC.T..G...CT..	BD(5)

TABLE 2

Haplotyes and variable sites of chukar partridge (C1~C15), rusty-necklaced partridge (M1~M25) and hybrids (C1~C3, C12, H1~H8)

Haplotype	Variable positions in sequences	Sampling location (sample size)
M6	.....T..CG.GT...AA.AG....T.TG.C..T.T.T...TC.GTC.T..G...CT..	BD(1)
M7	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.TA.G.T.CT..	WS(2)
M8	.....G.....T..CG.GT...AA.AG.A..T.TG.C..T.T.T...TC.GTC.TA.G.T.CT..	WS(1)
M9	.....T..CG.GT...AA.AG....T.TG.C.CT.T.T..CTC.GTC.T..G...CT..	WS(1)
M10	.....T..CG.GT...AA.AG....T.TG.C..T...T...TC.GTC.T..G...CT..	LZ(3),JY(1),HY(1), WS(3)
M11	.....T..CG.GT...AA.AG....TCTG.C....-..T...TC.GTC.T..G.T.CT..	DX(5)
M12	.....T..CG.GT...AA.AG....T..G.C..T-..T...TC.GTC.T..G.T.CT..	DX(1)
M13	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.T.GGCT.CT..	DX(1),HN(1)
M14	.....T.GCG.GT...AA.AG....T.TG.CT.T-..T...TC.GTC.T..G.T.CT..	HN(1)
M15	.....G.....T..CG.GT...AA.AG....TCTG.C..T-..T...TC.GTC.T..G.T.CT..	HN(1)
M16	.....T..CG.GT...AA.AG....T.TG.CT.T-..T...TC.GTC.T..G.T.CT..	HN(1),HY(1)
M17	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.T..G.TCCT..	HY(1)
M18	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.T..G.T.CTGG	HY(1)
M19	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.T..G.T.CT.G	HY(2)
M20	.....T..CG.GT...AA.AG....TCTG.C.....T...TC.GTC.T..G.T.CT.G	JY(3)
M21	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TC.GTC.T..G.T.CTG.	JY(2)
M22	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TCCGTC.T..G.T..T..	LZ(2)
M23	.....T..CG.GT...AA.AG....T.TG.C..T-..T...TCCGTC.T..G.T..T..	LZ(2),DX(1)
M24	.....G....T..CG.GTT..AA.AG....T.TG.C.....T...TC.GTC.T..G.T.CT..	LZ(2)
M25	.....T..CG.GT...AA.AG....T.TG.C...-..T...TC.GTC.T..G.T.CT..	LZ(1)
H1	.....-.....T.....	BD(1)
H2	...C.....-.....A.....T..	ZL(1)
H3	C.....-.....	ZL(1)
H4	....A.....T.-.....	HY(1)
H5	.A.....-.....	LX(1)
H6	.....G..A.....T.-.....	HY(1)
H7	.....-.....A.....	HY(1)
H8	.....-.....T.....	HY(1)

*: TS(5),XC(7),QZ(5),TC(5),PK(3),WX(5),GP(4),HH(7), BD(1),HY(3),ZL(6)
**: TS(2),XC(1),QZ(1),TC(1),WX(2),GP(6),HH(2), HN(1)
***: HN(5),BD(1),HY(7),LZ(6),DX(2),WS(1),LX(3),JY(4), ZL(2).

TABLE 3

Total number of haplotypes and number of unique haplotypes found within each population, mean pairwise differences (K) and nucleotide diversity (π) and haplotype diversity (h) of *Alectoris chukar*, *A. magna* and hybrids.

Population	Sample size	Total haplotypes	Unique haplotypes	K	π (x±SD)	h (x±SD)
Alectoris magna						
Huining	9	5	2	2.17	0.0047±0.0018	0.73±0.16
Wushan	8	5	3	3.14	0.0057±0.0011	0.86±0.11
Beidao	7	3	2	1.81	0.0039±0.0018	0.52±0.04
Haiyuan	13	6	3	0.85	0.0028±0.0008	0.72±0.13
Jingyuan	10	4	2	2.47	0.0054±0.0009	0.78±0.09
Lanzhou	17	7	3	2.60	0.0057±0.0009	0.85±0.06
Dingxi	10	5	2	3.18	0.0069±0.0012	0.76±0.13
Lixian	8	4	2	1.03	0.0023±0.0005	0.78±0.12
Zhuanglang	2	1	0	0.33	0.0000	0.00
Total	88	25	19	2.33	0.0051±0.0005	0.83±0.04
Alectoris chukar						
Tianshui	10	5	1	1.31	0.0029±0.0007	0.76±0.13
Xichuan	10	4	1	0.96	0.0027±0.0009	0.53±0.18
Quzi	10	5	1	1.11	0.0027±0.0007	0.76±0.13
Tongchuan	10	4	2	1.93	0.0042±0.0008	0.71±0.12
Panke	10	3	1	1.47	0.0032±0.0005	0.69±0.10
Gaoping	14	6	3	1.62	0.0036±0.0006	0.77±0.09
Wangxia	10	5	2	1.53	0.0035±0.0009	0.76±0.13
Honghui	10	3	0	0.91	0.0020±0.0007	0.51±0.16
Total	84	17	11	1.49	0.0033±0.0003	0.73±0.05
hybrids	22	12	8	1.28	0.0035±0.0007	0.80±0.09

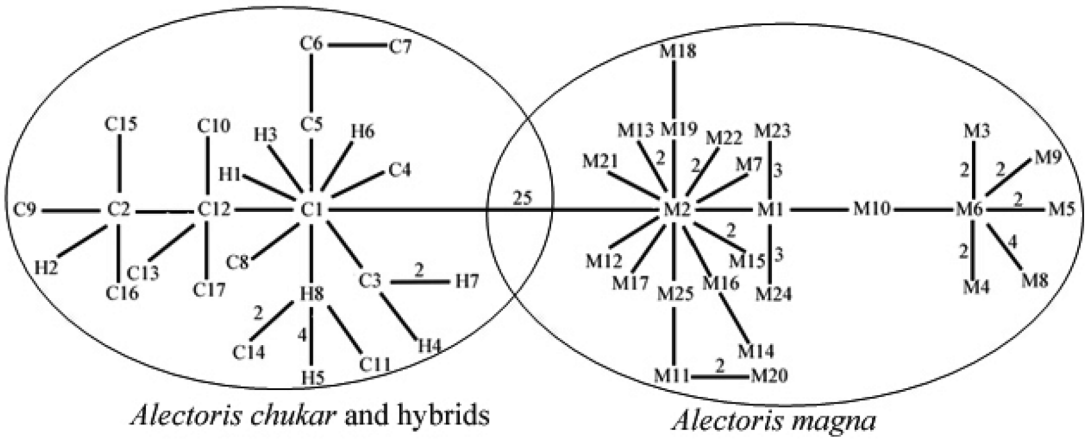

Fig. 2. – Haplotype network using the number of different mutations among 50 mtDNA haplotpyes of *Alectoris chukar*, *A. magna* and hybrids. Distances between linked haplotypes correspond to one mutation, except when shown by numbers.

Genetic diversity

Nucleotide diversity among the nine populations varied from 0.0000 (Zhuanglang) to 0.0069 (Dingxi); at the same time, haplotype diversity ranged from 0.00 (Zhuanglang) to 0.86 (Wushan) in rusty-necklaced partridge (Table 3). The pairwise divergence between haplotypes of rusty-necklaced partridge was lowest in Zhuanglang (k=0.00) and highest in Dingxi (k=3.18). Nucleotide diversity ranged from 0.0020 (Honghui) to 0.0042 (Tongchuan), and haplotype diversity varied from 0.51 (Honghui) to 0.76 (Tianshui, Quzi and Wangxia) among the eight populations of chukar partridge (Table 3). Nucleotide diversity and haplotype diversity of hybrids were 0.0035 and 0.80, respectively (Table 3).

The average nucleotide diversity and haplotype diversity in the five hybrid populations of rusty-necklaced partridge (Huining, Baidao, Haiyuan, Zhuanglang and Lixian) were 0.0027 and 0.55, respectively, while those of the other populations (Lanzhou, Dingxi, Jingyuan and Wushan) were 0.0059 and 0.81. Statistically insignificant differences in haplotype diversity and nucleotide diversity were observed between five hybridized populations and the pure ones ($p>0.05$) based on randomization tests. The Mantel test indicated that the haplotype diversity and nucleotide diversity showed negative correlation with hybrid ratio ($r=-0.847$, $p>0.05$, $n=5$; $r=-0.905$, $p<0.05$, $n=5$) among the five hybrid populations (Fig. 3). The results of AMOVA showed significant genetic differentiation ($\chi 2=32.06$, $p<0.01$, $n=9$) between the five hybrid populations and the other four non-hybrid population of rusty-necklaced partridge.

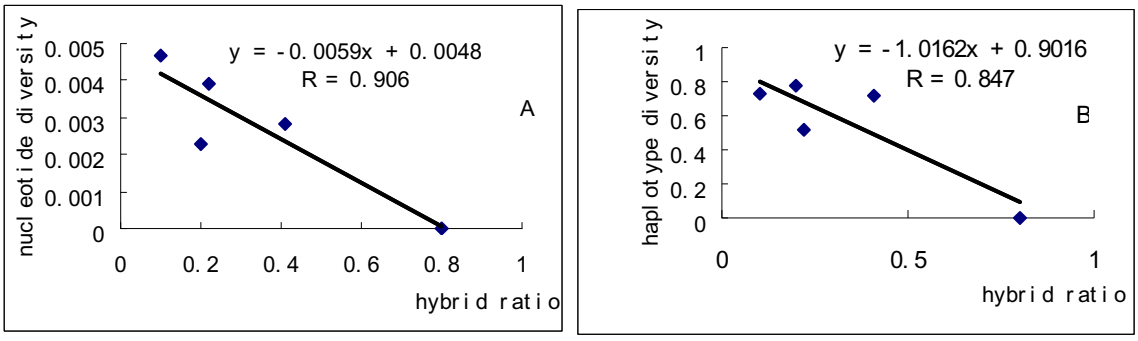

Fig. 3. – The relationship between genetic diversity and hybrid rate. A: nucleotide diversity, B: haplotype diversity. Regression equation and correlation coefficient (r) are given. Each dot represents one population.

DISCUSSION

A general difficulty in studies of hybridization in the wild is to assess the degree of hybridization of individuals (Crespin et al., 1999). Historically, morphological markers such as counts, measurements and colour patterns were first used to describe hybridization. More recently, molecular tools have provided very informative genetic markers. Mitochondrial DNA data showed a strong asymmetry in introgression between rusty-necklaced partridge and chukar partridge. Some partridges from the contact zone, morphologically identified as *A. magna*, are actu-

ally partly introgressed with *A. chukar*. The introgression demonstrates that the natural hybridization does not affect both taxa in the same way (CRESPIN et al., 1999). Behavioural, ecological or genetic factors must act in the hybrid zone, either favouring the advance of chukar partridge alleles into the rusty-necklaced partridge genome or impeding that of the latter alleles into the former genome. From a theoretical viewpoint, this asymmetry may be the result of various factors (ENDLER, 1977). One possibility is asymmetrical selection caused by an asymmetrical hybrid breakdown, as demonstrated by MORAN (1979). An alternative possibility is asymmetrical gene flow (MAY et al., 1975), resulting from differences in generation time (BARTON, 1986), in mating behaviour (LAMP & AVISE, 1986; KONKLE & PHILIPP, 1992) or some other component of fitness (ABERNETHY, 1994) between the two parental taxa. Among all these hypotheses, differences in mating behaviour and generation time do not appear to be relevant because laboratory experiments have revealed no such evidence. By contrast, an alternative hypothesis is that gene flow from chukar partridge to rusty-necklaced partridge is more important than gene flow in the reverse direction, because of the different habitat distributions of the two taxa. Chukar partridge has wide ecological amplitude and niche variation. In terms of its widespread Palearctic distribution and varied habitat affinities, chukar partridge may be considered the most successful of the seven species of *Alectoris* (JOHNSGARD, 1988). Because of its considerable adaptability and tolerance of conditions, hybrids involving chukar partridge have been recorded in other locations also: *A. chukar* × *A. rufa* in Italy (BARATTI et al., 2004) and *A. chukar* × *A. graeca* in Greece (DRAGOEV, 1974).

The introgressive hybridization cloud takes place through secondary contact between *A. chukar* and *A. magna* in the Liupan Mountain region. Fossils of *Alectoris* were recorded in early Pleistocene deposits in China (WETMORE, 1934), which demonstrated that *Alectoris* partridges were widespread in northwestern China during the early Pleistocene. Biochemical and molecular data (RANDI & LUCCHINI, 1998) suggested the divergence time between the two species was 1.90 million years ago, corresponding to Donau glaciation. Because of its lower altitude and precipitation, the Chaidamu Basin experienced no glacier effect during the Pleistocene (LI & LI, 1991), and thus it acted as a refuge for rusty-necklaced partridge. The ancestors of *A. magna* could have evolved in the Basin (HUANG et al., 2007). After the last glaciation, natural changes (such as desertification), and, more recently, anthropogenic habitat alterations such as deforestation and agriculture, produced a rapid extension of the ecological conditions suitable for rusty-necklaced partridge, which, in turn, resulted in their increased hybridization with chukar partridge along the Liupan Mountain.

Some authors have found that introgressive hybridization results in local genetic extinction of birds, such as *Anas platyrhynchos* × *A. rubripes* (ANKNEY et al., 1987), *Icterus galbula*× *I. bullocki* (RISING, 1983), *Passerina cyanea*× *P. amoena* (RISING, 1983) and *Vermivora pinus*× *V. chrysoptera* (GILL, 1994). ABERNETHY (1994) observed that the genetic integrity of the Scottish mainland red deer (*Cervus elaphus*) was shown to be at risk from sika (*Cervus nippon*). Rusty-necklaced partridge was strongly introgressed with chukar partridge, which raises questions about its genetic integrity. The haplotype diversity and nucleotide diversity decreased with increasing hybrid ratio among hybrid population. The Zhuanglang population exhibited the lowest nucleotide diversity and haplotype diversity, with the highest hybrid ratio (80.00%) and the least haplotypes (M2). Asymmetrical introgression between the two species may eventually result in local genetic assimilation of rusty-necklaced partridge populations.

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