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Authors: Larsson, Jobs Karl, Sun, Yue-Hua, Fang, Yun, Segelbacher, Gernot, and Höglund, Jacob

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Microsatellite variation in a Chinese grouse *Bonasa sewerzowi* population: signs of genetic impoverishment?

Jobs Karl Larsson, Yue-Hua Sun, Yun Fang, Gernot Segelbacher & Jacob Höglund

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Genetic variation in a Chinese grouse *Bonasa sewerzowi* population was assessed using five microsatellite markers. The mean number of alleles and allelic richness were comparable with what has been observed in other grouse, e.g. black grouse *Tetrao tetrix* and capercaillie *T. urogallus*, populations studied with the same markers regardless of whether these populations were isolated or from within the continuous range of the respective species. However, the proportion of heterozygotes and F_{IS} observed in Chinese grouse were more similar to isolated than to continuous grouse populations suggesting that the Chinese grouse population may show genetic signs of habitat fragmentation and relative isolation. Furthermore, demographic analyses indicated that the studied Chinese grouse population would not persist without immigration. We suggest that excess heterozygosity is a sign of a sink deme within a metapopulation system.

Key words: Chinese grouse, fragmentation, metapopulation, microsatellite variation, sink population

Jobs Karl Larsson & Jacob Höglund, Population Biology/EBC, Norbyvägen 18D, SE-752 36, Uppsala, Sweden - e-mail addresses: karl.larsson@ebc.uu.se (Jobs Karl Larsson); jacob.hoglund@ebc.uu.se (Jacob Höglund)

Yue-Hua Sun & Yun Fang, Department of Zoology, Chinese Academy of Sciences, Beijing 100080, PR China - e-mail addresses: sunyuehua@sina.com (Yue-Hua Sun); fangyun@vip.sina.com (Yun Fang)

Gernot Segelbacher, Max Planck Research Centre for Ornithology, Vogelwarte Radolfzell, Schloss Möggingen, Schlossallee 2, D-78315 Radolfzell, Germany - e-mail: segelbac@vowa.ornithol.mpg.de

Corresponding author: Jobs Karl Larsson

In order to survive in a changing environment, natural populations need to exhibit genetic variation. When populations become fragmented due to human-induced or natural causes, subpopulations of a previously continuous population become isolated and local populations grow small. As there is a direct link between the strength of genetic drift, the stochastic loss of genetic variants, and reduction in population size, fragmentation leads to loss of genetic variation (Gilpin & Soulé 1986, Lande 1988, Vrijenhoek 1994, Frankham 1996, Srikwan & Woodruff 2000, Hedrick & Kalinowski 2000). While populations may quickly recover from population size bottlenecks in terms of numbers, the regain of genetic variation takes longer being directly dependent on the mutation rate and gene flow between sub-

populations. In order to be able to survive in the long run, individuals in populations need to be able to adapt to new circumstances, and adaptation to changing environments is impossible without genetic variation (Frankel 1974, Lande & Shannon 1996, Frankham, Lees, Montgomery, England, Lowe & Briscoe 1999).

While both theoretical (Wright 1931, 1978) and empirical (e.g. Srikwan & Woodruff 2000) evidence support the notion that isolation and reduction in numbers as such lead to genetic erosion, it is less clear what is to be expected in metapopulation systems that are characterised by frequent extinction, colonisation and movement between already occupied patches (Hedrick & Gilpin 1997, Barton & Whitlock 1997). If some subpopulations are sinks in the sense that persistence in such patches

depends on immigration from larger ones that produce an excess of individuals beyond local carrying capacity (sources), it is reasonable to assume that the patterns of genetic variation among demes is different from what is predicted from models without metapopulation dynamics (Gaggiotti 1996).

In this paper, we present data on microsatellite variation in a population of the red-listed and threatened Chinese grouse *Bonasa sewerzowi*. This species is endemic to coniferous forests in the mountains east of the Tibetan high plateau in the Peoples Republic of China (PRC; Sun 2000). Little is known about the conservation status (Sun, Fang, Jia, Wang, Klaus & Scherzinger 1999, Sun, Swenson, Fang, Klaus & Scherzinger 2003) and even less about genetic variation in Chinese grouse. Thus, there is a need for diagnostic methods to screen natural populations of the species and to study whether Chinese grouse populations show any sign of genetic impoverishment. We have thus studied genetic variation in a population at the Lianhuashan Nature Reserve, in the province of Gansu, PRC. We compared different measures of genetic variation with what has been found in isolated and fragmented populations of the capercaillie *Tetrao urogallus* in Germany outside the Alps, which are remnants of a formerly connected metapopulation system and in more continuous populations of capercaillie in the Alps (metapopulation defined as in Segelbacher & Storch 2002), and two populations in the continuous distribution range of the black grouse *T. tetrix* in Scandinavia. If the Chinese grouse population were more similar in genetic architecture compared to isolated than to continuous grouse populations, this would indicate that the Chinese grouse population might suffer from habitat fragmentation. Because it was relevant in this study to ask whether the studied population is a source or a sink population, we also used previously published data on demographic parameters (Sun et al. 2003) to simulate whether the population would persist or go extinct in the absence of migration.

Methods

We extracted DNA from 22 Chinese grouse collected at the Lianhuashan Nature Reserve, PRC (34°31'-35°34'N, 103°44'-103°48'E), using a DNeasy® Tissue kit (QIAGEN, <http://www.qiagen.com/>) according to the manufacturers recommendations. The source of DNA varied from blood samples (N = 2), feathers (N = 6) and eggshells (N = 8) to remains after predation events (N = 6). Blood samples, eggshells and predation remains were stored in 96% ethanol and feathers were stored dry.

Transportation times from the field to the laboratory varied substantially, and some samples may have remained undetected in the field for long periods. Thus, DNA-degradation may have been substantial. However, the fragment lengths analysed here are so short (< 300 base pairs) that degradation did not pose a serious problem. Some samples were found at close geographical proximity, suggesting that they may have come from related individuals. However, the allelic distributions at the various loci did not reveal any obvious patterns (see below). Therefore, we treated all samples as unrelated in the subsequent analyses.

We screened the Chinese grouse population for genetic diversity using allele length variation at five microsatellite loci (TUT1, TUT2, TUT3, BG4 and BG20) as described in Segelbacher, Paxton, Steinbrueck, Trontelj & Storch (2000) and Piertney & Höglund (2001). PCR reaction conditions and temperature profiles followed the original publications. In all cases, PCR fragments were separated by electrophoresis on 6% denaturing polyacrylamid gels and visualised using silver staining.

We compared genetic variation in the investigated Chinese grouse population with data from seven capercaillie populations (Segelbacher, Höglund & Storch 2003) and two black grouse populations. Of the capercaillie populations, four were isolated (Fichtelgebirge, Black Forest, Thuringia and Vosges) and three were from within the continuous distributional area of the species (Varaldskogen in Østfold, Norway, and Jaroslaw and Archangelsk in Russia; Segelbacher 2002). The black grouse samples came from Petäjävesi, Finland (see Höglund, Alatalo, Lundberg & Lindell 1999 and Höglund, Piertney, Alatalo, Lindell, Lundberg & Rintamäki 2002) and from Østfold, Norway. Both of these populations were connected to other nearby populations. The isolated capercaillie populations have become fragmented both due to natural (deglaciation events affecting the distribution of capercaillie habitat) and human-induced causes (deforestation and other activities). Some may have been restocked, but the success of these attempts is unknown (Segelbacher 2002).

We report the following estimates of genetic diversity: mean number of alleles per locus, allelic richness (number of alleles per locus corrected for sample size; Petit, El Mousadik & Pons 1998), observed, H_o , and expected heterozygosity, H_e , and F_{IS} . We used the computer programs GENETIX 4.1 (Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France) and FSTAT 2.93 (Goudet 2001) in all calculations.

We performed a population viability analysis using RAMAS/age (Ferson & Akçakaya 1991) to simulate

Table 1. Estimates of genetic diversity in a Chinese grouse population, and in various populations of capercaillie (Segelbacher 2002, Segelbacher et al. 2003) and black grouse (Höglund et al. 2002). Isolated populations had a significantly lower number of alleles, allelic richness, observed heterozygosity and F_{IS} when compared to continuous capercaillie and black grouse populations (see text).

	N	Mean # alleles	Allelic richness	H_o	H_e	F_{IS}
Chinese grouse; 5 loci						
Lianhuashan	22	4.2	3.58	0.57	0.52	-0.04
Isolated capercaillie; 10 loci						
Fichtelgebirge	9	3.5	2.23	0.71	0.53	-0.26
Black Forest	62	5.1	2.22	0.53	0.54	0.02
Thuringia	11	4.2	2.39	0.57	0.57	0.05
Vosges	52	4.6	2.15	0.56	0.53	-0.05
Continuous capercaillie; 10 loci						
Østfold, Norway	18	4.9	2.53	0.62	0.65	0.05
Archangelsk, Russia	43	5.3	2.39	0.60	0.61	0.01
Jaroslavl, Russia	16	4.5	2.41	0.58	0.61	0.05
Scandinavian black grouse; 13 loci						
Østfold, Norway	25	7.5	7.33	0.68	0.72	0.08
Petäjävesi, Finland	141	8.8	8.11	0.70	0.74	0.06

population persistence of Chinese grouse using data from the same study population/site (Sun et al. 2003). We used five age classes (including a juvenile age class with no fecundity and 17% survival) and defined the following parameters: fecundity in older age classes was six eggs, adult survival was 61%, and sex ratio was 64% males. Coefficients of variation for these parameters were assumed to be a tenth of the above estimates, and we used a density independent model of population growth. Simulations were replicated 100 times. We estimated population persistence as the proportion of simulations that had not gone extinct after the simulated period of 30 years.

Results

We found, on average, 4.2 alleles per locus in the Chinese grouse population at Lianhuashan, which corresponds to an allelic richness of 3.58. The latter number is higher than any number observed in capercaillie, but lower than any number observed in black grouse (Table 1). The Chinese grouse population had an excess of heterozygotes yielding a negative F_{IS} , although it was not significantly different from zero (see Table 1).

When comparing isolated with continuous (excluding Chinese grouse) populations we found that isolated populations were signified by having fewer alleles, lower allelic richness, lower observed heterozygosity and lower F_{IS} (Mann-Whitney U-tests: # alleles, $U = 2$, $P = 0.032$; allelic richness, $U = 0$, $P = 0.008$; H_o , $U = 1$, $P = 0.016$; F_{IS} , $U = 1.5$, $P = 0.012$).

Since the number of loci screened differed among the populations and since this may affect the above-mentioned indices, we also restricted the analyses to only include the five loci for which all populations were screened. However, this restriction only lead to a slight

change in a few of the parameter estimates and did not affect the main conclusions or the relative size of any estimate. We therefore report the full data.

Using RAMAS/age we invariably found that the population would become extinct within 30 years (P of extinction = 1.000), regardless of slight variations in the estimated demographic parameters and their corresponding variation.

Discussion

When asking questions about genetic variation, one of the problems connected with the use of any genetic marker is to resolve what the estimates mean (Moss, Pierntry & Palmer 2003). Most microsatellite markers used in contemporary studies of conservation genetics are probably neutral or nearly so, i.e. selection is a negligible force affecting allele frequencies within and among populations, and mutation, migration and random genetic drift are more important (McDonald & Potts 1997, Scribner & Pearce 2000). Thus markers with a high and variable mutation rate, such as microsatellites, may show some variation while at other markers with slower mutation rates genetic variation is low. Therefore, it is difficult to compare different kinds of markers, such as allozymes versus microsatellites, and among different microsatellite loci. In our study, five of the markers were identical for all populations compared, thus reducing these sorts of errors.

Another potential bias is that the same microsatellite marker often shows variation in both amplification probability and variability among species (Primmer, Møller & Ellegren 1996). It is often observed that the marker is more variable in the species in which it was originally cloned, and that amplification probability and variability drops with the phylogenetic distance to

the species with which it is compared (Primmer et al. 1996, Primmer 1997). Applied to our study, we would thus predict that since the markers were either cloned in capercaillie or black grouse, the Chinese grouse would show less variation since capercaillie and black grouse are phylogenetically closer to each other than either is to Chinese grouse (Ellsworth, Honeycutt & Silvy 1996, Lucchini, Höglund, Klaus, Swenson & Randi 2001). However, as the allelic richness in Chinese grouse was higher than in any capercaillie population, the data did not show any obvious signs of any such effects.

Assuming that all our samples come from subpopulations within larger populations, comparing isolated capercaillie populations with capercaillie and black grouse populations from within the continuous range of the respective species allowed us to detect a few differences between isolated/fragmented populations as compared to continuous ones. First, the mean number of alleles detected in isolated populations was lower. Because this parameter obviously depends on sample size, it is less useful for diagnosing genetic variation. Therefore allelic richness, which is the number of alleles corrected for sample size, has been developed (Petit et al. 1998). In our case, isolated populations had lower allelic richness as compared to continuous populations indicating that isolated populations have suffered from genetic deterioration. Furthermore, isolated populations contained a lower proportion of heterozygote individuals than continuous populations. However, isolated populations contained more heterozygotes than would be expected from Hardy-Weinberg expectations yielding a low and sometimes negative F_{IS} . An excess of heterozygotes was also reported in Alpine capercaillie populations, was found to be most distinct in populations at the edge of the range and might be an effect of a very recent population decline (Segelbacher & Storch 2002). Excess heterozygosity was also observed in some, but not all, red grouse *Lagopus lagopus scoticus* populations in eastern Scotland (Piirtney, MacColl, Bacon & Dallas 1998).

Excess heterozygosity may have to do with the metapopulation dynamics. In disturbed metapopulation systems, it is often the small and most isolated subpopulations that go extinct, possibly due to inbreeding effects (Saccheri, Kuusari, Kankare, Vikman, Fortelius & Hanski 1998). These patches are then recolonised from larger and more heterozygous subpopulations. In undisturbed metapopulation systems the balance between extinction and recolonisation may be different. Because males are philopatric and females do not disperse after their first year, matings between close relatives may occur and cause an excess of homozygotes (Höglund et al.

2002). An alternative explanation can be that immigrations to largely isolated populations are extremely rare events. If individuals in such populations show signs of inbreeding depression, an immigrant may have unproportionally high reproductive success and hence contribute new and unique alleles to the population. Furthermore, small and isolated populations of charismatic and/or harvested species are sometimes restocked with individuals from captive breeding programmes or larger populations, and such individuals may reproduce at an unproportional rate in their new environment (Hansen, Nielsen, Ruzzante, Bouza & Mensberg 2000). We can, at present, not distinguish between any of these hypotheses. At any rate, and regardless of the explanation, we are left with the observation that continuous populations tend to have positive and isolated populations tend to have negative F_{IS} .

In this context, it is relevant to ask whether the studied Chinese grouse population is a source or a sink within a presumed metapopulation. According to published data, the population is stable around 28 individuals annually (Sun et al. 2003). Using the observed demographic parameters from Sun et al.'s (2003) study, our simulations showed that the population would become extinct within 30 years if not supported by immigration. It is important to note that the purpose of this exercise was not to model population growth and dynamics in the studied population. Instead, we wanted to see whether the studied population was dependent on immigration or not. It seems clear that it is, which has important implications for its management, but also for the interpretation of the genetic variation observed in the population. As the population is stable in size, it is clear that immigrants are needed to explain this. Thus, the observed excess heterozygosity is most likely an effect of immigrants from other populations.

What do these kinds of comparisons lead to when it comes to saying something about the genetic status of the studied Chinese grouse population? The allelic richness observed was higher than in any capercaillie population, but lower than in any black grouse population regardless of the compared population being isolated or not. Thus, the Lianhuashan population of Chinese grouse do not show any direct evidence of excessive loss of alleles. We did, however, observe non-significant excess of heterozygotes and a corresponding negative F_{IS} . This may be a sign of a sink population and, as such, a warning that the Lianhuashan population is endangered or at least dependent on immigration for persistence. The habitat surrounding the Nature Reserve that contains the Lianhuashan population is heavily affected by man-made habitat changes, and the forest habitat in which the Chi-

nese grouse lives is heavily fragmented. It should come as no surprise that such habitat alterations could have had genetic and demographic consequences.

In summary, we suggest that genetic variation at microsatellite marker loci could be used to detect signs of isolation, fragmentation and metapopulation dynamics in grouse. It is less certain if such signs are detectable within the studied Chinese grouse population at Lianhuashan. The studied population did not show any obvious signs of having lost alleles, but may contain more heterozygotes than an ecologically healthy population would.

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