Genetic Differentiation among Migrant and Resident Populations of the Threatened Asian Houbara Bustard

SAMUEL RIOU, OLIVIER COMBREAU, JACKY JUDAS, MARK LAWRENCE, MOHAMED SALEH AL BAIDANI, AND CHRISTIAN PITRA

Abstract

The Asian houbara bustard *Chlamydotis macqueenii* is a partial migrant of conservation concern found in deserts of central Asia and the Middle East. In the southern part of the species range, resident populations have been greatly fragmented and reduced by sustained human pressure. In the north, birds migrate from breeding grounds between West Kazakhstan and Mongolia to wintering areas in the Middle East and south central Asia. Extensive satellite tracking has shown substantial partitioning in migration routes and wintering grounds, suggesting a longitudinal barrier to present-day gene flow among migrants. In this context, we explored genetic population structure using 17 microsatellite loci and sampling 108 individuals across the range. We identified limited but significant overall differentiation (*F_{CT} = 0.045*), which was overwhelmingly due to the differentiation of resident Arabian populations, particularly the one from Yemen, relative to the central Asian populations. Population structure within the central Asian group was not detectable with the exception of subtle differentiation of West Kazakh birds on the western flyway, relative to eastern populations. We interpret these patterns as evidence of recent common ancestry in Asia, coupled with a longitudinal barrier to present-day gene flow along the migratory divide, which has yet to translate into genetic divergence. These results provide key parameters for a coherent conservation strategy aimed at preserving genetic diversity and migration routes.

Key words: *Chlamydotis macqueenii*, conservation, microsatellites, migration, population structure

The identification of management units in migratory species of conservation concern can be held back by lack of information on dispersal patterns and migratory behavior (Esler 2000; Martin et al. 2007). On one hand, the dispersal ability of long-distance migrants can limit genetic differentiation by maintaining gene flow throughout the range of the species (Barrowclough 1980; Arguedas and Parker 2000). On the other, the presence of partitioning in migratory routes coupled to philopatry can limit gene flow between populations and thus promote significant levels of genetic differentiation (Baker et al. 1990; Webster et al. 2002; Clegg et al. 2003). Obtaining estimates of population genetic structure is thus essential in this context (Fraser and Bernatchez 2001; Palsboll et al. 2007).

In several long-distance migrant birds, genetic differentiation has been shown to closely match the partitioning of migratory routes (Ruegg and Smith 2002; Ruokonen et al. 2004; Jones et al. 2005). Indeed, avian migration routes are often genetically determined, at least in part (Helbig 1996; Berthold 2001). But this is not a consistent pattern. Cryptic natal dispersal or the recent colonization of a species’ present range can result in a pattern of genetic homogeneity among populations. For example, highly philopatric populations of snow goose *Chen caerulescens* in North America and populations of red-billed quelea *Quelea quelea* in southern Africa split by a migratory divide have both failed to show genetic differentiation associated with migratory partitioning (Avise et al. 1992; Dallimer et al. 2003).

The Asian houbara bustard *Chlamydotis macqueenii* (Knox et al. 2002; Broders et al. 2003) is a shy bird distributed widely, but at low densities, in the deserts and steppes of the Arabian Peninsula (referred to here as Arabia) and South and central Asia (from Iraq to Mongolia). In spring and summer, migrant birds are mostly observed from north of Iran and West Kazakhstan to the Mongolian and Chinese Gobi (Figure 1). Due to hunting and habitat loss, these
populations have been on the decline since the end of the 20th century (Riou et al. 2011). These migrants spend the winter in Pakistan, Iran, Iraq, and Arabia, where they encounter residents. Fifteen years of satellite tracking have shown substantial longitudinal partitioning and fidelity in the migratory pathways and wintering areas of these birds: individuals from western Kazakhstan and northern Iran winter in Iraq and western Iran, birds from central and eastern Kazakhstan spend the winter in central and eastern Iran, and far eastern birds (Mongolia, China) winter in southern Iran and Pakistan (Combreau et al. 1999, 2001, 2006, 2011; Tourenq et al. 2004; Judas et al. 2006). Dispersal movements of young birds remain to be uncovered, but juveniles tracked to date have followed migratory paths similar to those shown in Figure 1 (Combreau et al. 2011).

Little is known regarding the extent of gene flow between migrants and residents in this species. Migrants could possibly contribute to the gene pool of Persian and Arabian residents, although migrants wintering in Arabia are thought to form a minority. Small to medium distance movements of resident birds within the peninsula may occur (Van Heezik and Seddon 2002) but it is unlikely, for example, that birds from eastern populations in Yemen and Oman could cross the vast expanses of the Rub’ Al Khali desert to join populations in northwestern Arabia (Osborne 1996). Also, residents in the Arabian Peninsula have been heavily impacted on by human pressure, eradicated from most of their former range they now face a high extinction risk in a few remnant patches in Yemen, Oman, northwestern Arabia, and the Levant (Seddon and Van Heezik 1996). Examining genetic differentiation among these threatened populations is thus clearly required.

Here, using 17 microsatellite loci among sampling locations spanning most of the species range, we examine the extent of genetic differentiation between and among migrant and resident populations of the Asian houbara bustard. We discuss the findings in light of known information on the partitioning of migratory routes and their conservation implications.

**Materials and Methods**

**Sample Collection and Microsatellite Amplification**

We sampled 108 individuals from 9 sites covering most of the species distribution range (Figure 1; Table 1). All migrant populations were sampled in spring. Fieldwork in

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**Figure 1.** Sampling locations (see Table 1) and the distribution range of the Asian houbara bustard. This map was created based on data from the literature and information gathered by National Avian Research Center. The dotted arrows symbolize migratory pathways known from satellite tracking (Combreau et al. 2006). Northern Iran hosts 2 types of migrants: In the spring, it hosts birds that spend the winter further southwest, and in the winter, it hosts birds that breed further north in Kazakhstan/China. We know from satellite tracking that the ones sampled in spring for this study all migrated southwest in autumn.
Yemen and Egypt was carried out in winter, but local knowledge and satellite tracking (National Avian Research Center, unpublished data) confirmed that the birds concerned were resident. Blood samples (<300 µl) were taken from the brachial vein and stored in 95% ethanol. DNA was extracted using the QIAamp (DNAeasy) extraction kit (QIAGEN). All individuals were genotyped at 17 microsatellite loci previously identified as being polymorphic in houbara bustards: A2, A10, A21, A22, A29, A106, A120, A204, A205, A210, D12, D110, D118, D119 (Chbel et al. 2002) and O26, O27, O38 (Pitra et al. 2004). All loci were amplified by PCR following standard conditions described in Lieckfeldt et al. (2001). Resulting products were resolved in POP-7 polymer on a 36-cm capillary array using a 3130xl Genetic Analyzer and sized using GS500 ROX internal size standard in GENEMAPPER (Applied Biosystems).

Genetic Diversity within Populations

The data set was checked for genotyping errors (such as null alleles) with MICROCHECKER 2.2 (Van Oosterhout et al. 2004). Allelic richness \( R \) (Petit, et al. 1998), observed heterozygosity \( H_o \), and expected heterozygosity \( H_e \) were calculated for each population and at each locus. Allelic richness controls for variation in sample size by a rarefaction method and was calculated using FSTAT 2.9.3 (Goudet 2001). GENEPOL 4.0 (Rousset 2008) was used to calculate \( H_o \), \( H_e \), and Wright’s inbreeding coefficient \( F_{IS} \). It was also used to perform exact tests for departure from Hardy–Weinberg proportions within populations and assessments of linkage disequilibria among all possible pairs of loci using the Markov chain procedure.

Heterozygosity tests implemented in BOTTLENECK (Cornuet and Luikart 1996) were used to detect departure from mutation–drift equilibrium. These tests compare 2 estimates of expected heterozygosity, one based on allele frequencies \( H_e \), assuming Hardy–Weinberg equilibrium (HWE) and another based on the number of alleles and sample size \( H_{eq} \), assuming mutation-drift equilibrium. At equilibrium, both estimates should be similar. If a population experiences a bottleneck, rare alleles will be rapidly lost, and therefore, \( H_{eq} \) will decrease faster than \( H_e \) (i.e., \( H_e > H_{eq} \)). The reverse (i.e., \( H_e < H_{eq} \)) could suggest population expansion. Estimates of expected heterozygosity at mutation–drift equilibrium were calculated using the stepwise mutation model and two-phase model with 10% of multistep changes, as suggested for microsatellites (Piry et al. 1999).

Population Structure

Population differentiation was assessed by calculating global and pairwise \( F_{ST} \) values following Weir and Cockerman (1984), and the G-based exact test for genotypic differentiation implemented in GENEPOP 4.0. We used hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN 3.1 (Excoffier et al. 2005) to test for genetic partitioning between migrants and residents and between Arabian (Yemen and Egypt) and central Asian populations. We calculated pairwise genetic distances in GENETIX 4.0 (Belkhir et al. 1996–2004), and built dendrograms based on these distances using the neighbor joining in MEGA 4.1 (Tamura et al. 2007). Mantel tests of the correlation between genetic and geographic distances were run in GENETIX 4.0.

We further investigated population structure using the Bayesian genotype clustering technique in STRUCTURE 2.3 (Pritchard et al. 2000). This method uses individual genotype information to cluster sampled individuals into a number of groups (\( K \)), minimizing departure from HWE and linkage equilibrium. Likelihood of the data for each value of \( K \) is calculated using a Markov chain: We made 10 runs for each \( K \) between 1 and 10, using the admixture model and a burn-in period of 100 000 steps followed by 100 000 iterations. We also calculated \( \Delta K \) following Evanno et al. (2005), as an alternative method to identify the most likely \( K \).

We used the individual-based assignment method of Rannala and Mountain (1997) implemented in GENCLASS 2.0 (Piry et al. 2004) to assign each individual to its most likely population of origin. This further assesses the strength of population subdivision and enables the identification of possible recent migrants.

Results

Estimates of Genetic Diversity

The number of alleles ranged from 4 to 12 across loci, and average \( H_o \) was 0.47 (range 0.16–0.73 across loci). There

<table>
<thead>
<tr>
<th>Population</th>
<th>Sampling location</th>
<th>Status</th>
<th>( N )</th>
<th>( R )</th>
<th>( H_o )</th>
<th>( H_e )</th>
<th>( F_{IS} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yemen (YEM)</td>
<td>1 (16.8N, 52.0E)</td>
<td>Resident</td>
<td>5</td>
<td>2.62</td>
<td>0.55</td>
<td>0.44</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>3 (29.3N, 33.8E)</td>
<td>Resident</td>
<td>13</td>
<td>2.91</td>
<td>0.52</td>
<td>0.51</td>
<td>0.20</td>
</tr>
<tr>
<td>Pakistan (PAK)</td>
<td>4 (27.2N, 64.5E)</td>
<td>Resident</td>
<td>13</td>
<td>2.94</td>
<td>0.45</td>
<td>0.47</td>
<td>0.01</td>
</tr>
<tr>
<td>Iran (IR)</td>
<td>5 (35.4N, 125.0E)</td>
<td>Migrant</td>
<td>17</td>
<td>2.98</td>
<td>0.37</td>
<td>0.45</td>
<td>0.12</td>
</tr>
<tr>
<td>West Kazakhstan (WK)</td>
<td>6 (42.9N, 75.0E)</td>
<td>Migrant</td>
<td>36</td>
<td>3.15</td>
<td>0.49</td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td>East Kazakhstan (EK)</td>
<td>7 (45.5N, 120.0E)</td>
<td>Migrant</td>
<td>10</td>
<td>2.95</td>
<td>0.47</td>
<td>0.46</td>
<td>0.22</td>
</tr>
<tr>
<td>Mongolian plateau (MON)</td>
<td>8 (39.0N, 102.7E)</td>
<td>Migrant</td>
<td>14</td>
<td>2.79</td>
<td>0.42</td>
<td>0.44</td>
<td>0.08</td>
</tr>
</tbody>
</table>

N, individuals sampled; \( R \), allelic richness; \( H_o \), mean observed heterozygosity; \( H_e \), mean expected heterozygosity; \( F_{IS} \), Wright’s inbreeding coefficient; associated standard deviations are shown in parentheses; see Figure 1 for a correspondence of sampling locations on the map.
was no evidence of genotyping errors due to large allele dropout or the presence of null alleles. Tests for linkage disequilibrium were significant for one pair of loci (D110 and D118; P < 0.0001). We therefore removed one of these loci (D118) from all subsequent analyses. Only one locus within one population (A204, Mongolian plateau) showed significant departure from HWE after Bonferroni correction.

Gene diversities were similar among populations (Table 1) as indicated by the low standard deviations of the mean allelic richness (2.91 ± 0.16), mean He, (0.47 ± 0.06), and mean Hc (0.50 ± 0.03). The inbreeding coefficient FIS was very low in residents from Yemen and Egypt and higher in some of the central Asian populations (Table 1). Heterozygosity tests revealed a significant heterozygosity deficit in all migrant populations under both mutation models (Table 2); in contrast, gene diversity in residents from Yemen, Egypt, and Pakistan was at mutation–drift equilibrium.

### Population Genetic Structure

We found highly significant genetic differentiation of the Yemen population (mean FST = 0.115, P < 0.001). Samples from Egypt were also significantly different, although this was less marked (mean FST = 0.042, P < 0.05). Among the central Asian samples, pairwise FST values were very low, and genetic differentiation was not significant with the exception of the group from West Kazakhstan (Table 3).

Differentiation was highest between West Kazakhstan and the Mongolian plateau (P < 0.001). AMOVA supported partitioning into 3 groups (Yemen, Egypt, and central Asia: FCT = 0.045, P = 0.05, respectively, 1.1% and 94.3% of the variation among populations within groups and within populations) but also a separation into 4 groups, treating West Kazakhstan separately from the rest of central Asia (FCT = 0.036, P = 0.03, respectively, 0.05% and 96.3% of the variation among populations within groups and within populations). AMOVA did not support a migrant–resident dichotomy (FCT = 0.002, P > 0.15).

Dendrograms built using either Nei (1978) or Cavalli-Sforza and Edwards (1967) genetic distances resulted in the same topology: Egypt and Yemen clustered together, and Yemen was a clear outlier, Egypt less so, to the otherwise closely knit group of populations; populations from the Mongolian plateau and East Kazakhstan clustered together with good bootstrap support within the central Asian group (Figure 2).

The correlation between genetic and geographic distances was not significant (Mantel test: R = 0.34, P = 0.2). It improved when using wintering locations of migrant birds but isolation by distance remained nonsignificant (R = 0.48, P = 0.13).

### Individual-Based Clustering

Providing no prior knowledge on sampling location, STRUCTURE assigned the highest likelihood to a model with K = 1 population. However, using clustering assisted by information on sampling locations, as is suggested for data sets with relatively weak signal of structure (Hubisz et al. 2009), the model with the highest Ln P(D) and ΔK was obtained for K = 4 (Ln P(D) values obtained for K = 1: −3655, K = 2: −3644, K = 3: −3619, K = 4: −3592, and K = 5: −3637). Only one inferred cluster, corresponding to the samples from Yemen, had a clear geographical basis, although the distribution of clusters was also markedly different in the Egyptian samples and, to a lesser extent, in the West Kazakh ones (Figure 3).

### Discussion

Genetic differentiation was mainly apparent between Arabian populations, particularly the one from Yemen, and all other central Asian populations of the Asian houbara.

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**Table 2** Heterozygosity tests for each population (performed in BOTTLENECK)

<table>
<thead>
<tr>
<th>Population</th>
<th>SMM</th>
<th>TPM, 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n_{def}</td>
<td>n_{exc}</td>
</tr>
<tr>
<td>EK</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>IR</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>PAK</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>MON</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>WK</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>EGY</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>YEM</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

n_{def} number of loci with heterozygosity deficiency, n_{exc} number of loci with heterozygosity excess under stepwise mutation model (SMM) and two-phased model of mutation (TPM). P is the probability of departure from mutation–drift equilibrium using a sign test. Boldface indicates populations where P ≤ 0.05 and population abbreviations are the same as ones defined in Table 1.

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**Table 3** Pairwise FST estimates (below diagonal) and Nei’s (1978) genetic distances (above diagonal)

<table>
<thead>
<tr>
<th></th>
<th>EK</th>
<th>IR</th>
<th>PAK</th>
<th>MON</th>
<th>WK</th>
<th>EGY</th>
<th>YEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EK</td>
<td></td>
<td>0.002</td>
<td>0.014</td>
<td>0</td>
<td>0.015</td>
<td>0.052</td>
<td>0.147</td>
</tr>
<tr>
<td>IR</td>
<td>0.002</td>
<td></td>
<td>0.023</td>
<td>0.005</td>
<td>0.019</td>
<td>0.030</td>
<td>0.172</td>
</tr>
<tr>
<td>PAK</td>
<td>0.007</td>
<td>0.000</td>
<td></td>
<td>0.015</td>
<td>0.021</td>
<td>0.05</td>
<td>0.146</td>
</tr>
<tr>
<td>MON</td>
<td>0.013</td>
<td>0.007</td>
<td>0.013</td>
<td></td>
<td>0.014</td>
<td>0.033</td>
<td>0.133</td>
</tr>
<tr>
<td>WK</td>
<td>0.009</td>
<td>0.011</td>
<td>0.014</td>
<td>0.017</td>
<td></td>
<td>0.033</td>
<td>0.133</td>
</tr>
<tr>
<td>EGY</td>
<td>0.047</td>
<td>0.019</td>
<td>0.026</td>
<td>0.049</td>
<td>0.023</td>
<td></td>
<td>0.127</td>
</tr>
<tr>
<td>YEM</td>
<td>0.131</td>
<td>0.094</td>
<td>0.139</td>
<td>0.135</td>
<td>0.103</td>
<td>0.090</td>
<td></td>
</tr>
</tbody>
</table>

P values of the exact tests of genotypic differentiation: ****<0.0001, ***<0.001, **<0.01, *<0.05, and NS>0.05. NS, not significant.
Population structure within the central Asian group was barely detectable with this set of markers. Nevertheless, $F_{ST}$-based statistics (Table 3; AMOVA), genetic distances (Figure 2), and the STRUCTURE analysis all tended to suggest a weak signal of divergence of birds from West Kazakhstan relative to those from further east.

Such a level of homogeneity in central Asia suggests either recent common ancestry or high levels of gene flow. Given that heterozygosity tests (Table 2) showed clear heterozygosity deficiency in migrants, a pattern that could be interpreted as recent population expansion (Donnelly et al. 2001), and that information derived from satellite tracking suggests philopatry and the presence of a migratory divide (Figure 1), and thus a longitudinal fault in present-day gene flow, the genetic similarity of western versus eastern populations is probably due to recent common ancestry (Avise et al. 1992). The weak signal of divergence seen in West Kazakh birds would thus be evidence of this lack of present-day longitudinal gene flow. In contrast, within eastern populations (Mongolian plateau, East Kazakhstan, and Pakistan), gene flow along the migratory pathway could help maintain genetic homogeneity. For example, satellite tracking has shown that a juvenile that was born and equipped in China and was wintered in Pakistan, spent its first summer in eastern Uzbekistan, and it is not impossible that it may have bred there. It is not impossible either that some males may occasionally breed during migration on their stopover sites (Rohwer et al. 2009), although we have no evidence for this at present.

The most obvious pattern of divergence was observed for residents in Arabia, where northwestern (Egyptian) and eastern Arabian birds were significantly differentiated from each other and from the central Asian group. This suggests that gene flow between migrants and resident Arabian populations is restricted. A previous study, using mitochondrial DNA, found strong genetic divergence of the African houbara $C.$ undulata and Asian houbara $C.$ macqueenii but failed to show obvious structure among populations within the Asian species (Pitra et al. 2004). Only birds from the Sinai showed signs of relative isolation, mirroring our findings here with microsatellites and a wider sample of Egyptian birds. That study did not sample residents from eastern Arabia (i.e., authors used migrants from Asia wintering in the United Arab Emirates), which explains why the pattern described here of relatively strong divergence of the eastern Arabian population was not uncovered earlier. Genetic distances were several fold higher between eastern and northwestern Arabia than they were between the latter and central Asia. This...
supports the suggestion that the Rub’ Al Khali desert acts as an important barrier to gene flow (Osborne 1996). This genetic structure among residents of the Asian houbara contrasts with genetic homogeneity described in the resident African species (Lesobre et al. 2010). This could possibly be due to the greater range size and presence of geographic barriers (deserts, mountains, and seas) in Asia and the Middle East than in North Africa. We acknowledge that the sample of birds from Yemen is small in our study, and one may argue that a larger sample of birds could alter our interpretation of population differentiation to some extent. However, by resampling our data set (1000 times) and drawing 5 individuals at random each time, not once did the random $F_{ST}$ value come close to our biological $F_{ST}$. Moreover, the individual-based approach carried out in STRUCTURE also supported the clustering of samples from Yemen. Finally and as further discussed below, the low number of sampled birds is beyond our control and is a direct consequence of the extreme rarity of houbara in Yemen.

Pitra et al. (2004) concluded that the African species was most probably ancestral and that Asia was secondarily colonized. Based on this and rooting the network in Figure 2 on an ancestral Egyptian houbara, the tree suggests an initial expansion into Arabia (Yemen), followed by central Asia (West Kazakhstan, Iran, and Pakistan) and lastly a colonization of the northeast (East Kazakhstan and Mongolian plateau). As mentioned above, heterozygosity tests support such a scenario of recent population expansion into northern and northeastern central Asia from Middle Eastern birds. Houbara may have colonized the advancing steppes and deserts of central Asia following the end of the last glacial period, a time at which many species colonized newly available habitats in northern latitudes (Avise et al. 1992; Milá et al. 2000; Zink et al. 2002).

Finally, whereas birds caught wintering in the United Arab Emirates have then been found to subsequently breed in Asia (Osborne et al. 1997), the reverse has been documented very rarely. Minimal gene flow between Yemen and central Asia also supports the notion that only a small proportion of birds breeding in Asia spend the winter in Arabia. Birds from eastern Arabia could thus be viewed as a separate evolutionary significant unit (Crandon et al. 2000). Sadly, this area encompasses only a few remnant populations in Yemen and Oman, poaching continues within these and likely to disappear in the near future. Based on the results of this study, Northwest Arabian populations and, arguably, the West Kazakh population could also be viewed as distinct genetic units. Larger resident populations are thought to be found in Iraq and Iran, and further genetic analyses should be carried out to determine their relatedness to Arabian and Asian populations.

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**References**


