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Phylogeography of the Common Pheasant *Phasianus colchicus*

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The Common Pheasant *Phasianus colchicus* is widely distributed in temperate to subtropical regions of the Palaearctic realm. Populations of Common Pheasant have been classified into five subspecies groups based on morphological variations in male plumage. Previous phylogeographical studies have focused on limited sets of subspecies groups in the eastern Palaearctic and knowledge on subspecies in the western Palaearctic region is still poor. In this study, we undertake the first comprehensive analysis of subspecies from all five defined subspecies groups across the entire Palaearctic region. Two mitochondrial (CYTB and CR) and two nuclear (HMG and SPI) loci were used to investigate genetic relationships of these subspecies groups and to infer their dispersal routes. Our results revealed that the subspecies *elegans*, with its range in northwestern Yunnan, China, was in the basal position among 17 studied subspecies, supporting a previous hypothesis that the Common Pheasant most probably originated in forests in southeastern China. Subspecies in the western Palaearctic region nested within the most subspecies-rich *torquatus* group (‘Grey-rumped Pheasants’), indicating that the *torquatus* group is not a clade but instead forms a gradation with other subspecies and subspecies groups. Our dating analysis suggested that the initial divergence among populations of Common Pheasant originated around 3.4 Mya with subsequent dispersal into the Western Palaearctic region during the Late Pliocene–Lower Pleistocene approximately 2.5–1.8 Mya. We propose two possible east-to-west colonization routes for the Common Pheasant and suggest conservation implications for some regional subspecies. Overall, this study demonstrates the lack of concordance between morphology-based subspecies delimitation and their genetic relationships. This is likely to be a consequence of initial isolation due to historical vicariance followed by population admixture due to recent range expansion of Common Pheasant in the western Palaearctic region.

Keywords: mitochondrial DNA, morphology, nuclear DNA, subspecies, vicariance.

Natural populations with large geographical ranges usually exhibit intra-specific variation, with subpopulations explicitly differing from one another in aspects of morphology, genetics and ecology (Mayr 1963). Increased population structure through population subdivision may be a historical consequence of isolation due to geographical barriers (Avise 2000) or evolutionary processes such as natural selection and genetic drift (Slatkin 1987). Phylogeography aims to infer the historical biogeographical processes that shape contemporary patterns of genetic variation within and between species (Avise 2000). For species with large
geographical ranges in particular, range-wide phylogeography can provide deep insights into how geological, environmental and evolutionary factors contribute to population structuring or species divergence. Conducting comprehensive range-wide studies is a challenge, however, as it requires the collection of field data on a very large scale, over hundreds to thousands of kilometres and across many countries (Liu et al. 2011, 2012a,b).

One example of a species with a large geographical range and pronounced intra-specific divergence is the Common Pheasant Phasianus colchicus. This species is the world’s most widespread pheasant, with a natural distribution in the Palaearctic region. Its range extends from eastern–southeastern Europe (east of the Black Sea) to far eastern Siberia and southwards to Indochina and Afghanistan (Hill & Robertson 1988). Thirty recognized subspecies categorized into five subspecies groups have been defined, with some subspecies occasionally afforded specific status (Madge & McGowan 2002). The validity of some subspecies, however, has been questioned because of the clinal variation existing among them (Cramp & Simmons 1980, Johnsgard 1999, Madge & McGowan 2002). The five subspecies groups are as follows: (1) the colchicus group (‘Black-necked Pheasants’ west and south of the Caspian Sea) including P. c. persicus, P. c. talschensis, P. c. colchicus and P. c. septentrionalis; (2) the principalis-chrysomelas group (‘White-winged Pheasants’ in Central Asia) including P. c. principalis, P. c. zarudnyi, P. c. chrysomelas, P. c. bianchii, P. c. zerafchanicus and P. c. shawii; (3) the tarimensis group (population in Tarim Basin in southeastern Xinjiang, China) including P. c. tarimensis; (4) the mongolicus group (Kirghiz Pheasants in northern Xinjiang, China and eastern Kazakhstan) comprising P. c. mongolicus and P. c. turestanicus; and (5) the most subspecies-rich group, the torquatus group (‘Grey-rumped Pheasants’, mostly distributed in East Asia) containing P. c. satscheuensis, P. c. pallassi, P. c. suhshanensis, P. c. torquatus, P. c. kiansuensis, P. c. rothschildi, P. c. karpowi, P. c. strauchi, P. c. elegans, P. c. vlangalii, P. c. hagenbecki, P. c. edzlinensis, P. c. alaschanicus, P. c. sohokhotensis, P. c. decollatus, P. c. takatsukasae and P. c. formosanus (Madge & McGowan 2002) (Fig. 1).

The designations of the five groups and thus their constituent subspecies have been based on biogeography and morphological characters in males, such as variation in body size, plumage patterns and the presence or absence of a white neck ring (Solokha 1994, Johnsgard 1999, Liu et al. 2010). Because plumage variation may vary considerably with diet and habitat (Cramp & Brooks 1988), a dependence on morphological traits may be unreliable for phylogenetic assessment (Alström et al. 2013). In contrast, phylogeographical analysis of spatial and temporal genetic variation can uncover population or subspecies affinities and associated evolutionary processes (Gu et al. 2013). For example, in a previous study, Qu et al. (2009) uncovered the existence of a major genetic gap among principalis-chrysomelas, mongolicus and torquatus groups and could not fully validate some subspecies. Although previous phylogeographical studies have revealed some patterns of populations/subspecies divisions within Common Pheasant (Qu et al. 2009, Liu et al. 2010), an analysis involving complete geographical coverage of all subspecies as well as the application of multilocus DNA data with both mtDNA and nuclear DNA has not been conducted.

In this study, we undertake the most comprehensive molecular phylogeographical analyses conducted on this species to date, with continent-wide sampling of subspecies (17 of 30 recognized subspecies). The analyses, which were based on a multilocus dataset including both fast-evolving mtDNA genes and more moderate- to slow-evolving nuclear DNA loci, allowed us to clarify whether the molecular data reflect the traditional taxonomy of all five morphological groups of Common Pheasant subspecies in the Palaearctic region, and to estimate the timing of divergence within Common Pheasant subspecies or groups. In addition, we attempted to infer possible east-to-west dispersal routes of Common Pheasant subspecies based on our phylogeographical results and testing proposed routes that have been based on morphology (Solokha 1994).

**METHODS**

**Taxonomic sampling**

We collected 85 fresh tissue, blood and feather samples from 17 subspecies (57% of defined subspecies) of Common Pheasant from all groups in the Palaearctic region (Fig. 1). Voucher specimens were deposited at the Zoology Museum of Ferdowsi University of Mashhad, Iran (ZMFUM; colchicus, talischensis, persicus and principalis), Beijing Normal University, China (satscheuensis, strauchi, rothschildi, vlangalii, kiansuensis, karpowi,
pallasi, torquatus, elegans and suehschanensis) and Sun Yat-sen University, China (mongolicus, shawii and tarimensis). A list of studied taxa and outgroups along with voucher numbers, localities and GenBank accession numbers are provided in Supporting Information Table S1.

**DNA sequencing and alignment**

Total genomic DNA was extracted from tissue, blood and feather samples. Muscle samples were preserved in 96% ethanol and stored at −20 °C. Blood samples were mixed immediately in blood storage buffer (0.1 M Tris-HCl, 0.04 M EDTA-Na2, 1.0 M NaCl, and 0.5% sodium dodecyl sulfate (SDS)). Feathers were kept in dry envelopes. DNA was extracted using a QIA quick DNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and the standard salt extraction method (Bruford et al. 1992). Before DNA extraction, samples were incubated overnight at 55 °C in extraction buffer (2% SDS and 0.5 mg/mL proteinase K).

Primer pairs L14731/H16065 (Kimball et al. 1999), L16757/H1259 (Randi & Lucchini 1998), SerpinCF/SerpinCR and HMG172F/HMG174R (Kimball et al. 1999) were used to amplify mitochondrial cytochrome b (CYTB) and the control region (CR), and autosomal intronic regions of nuclear genes High mobility group b (HMG) and Serpin peptidase inhibitor clade C (SPI), respectively. For the two mitochondrial loci, PCR amplifications followed the protocols described in Liu et al. (2010) except that an annealing temperature of 60 °C was used. For the two nuclear introns, PCR protocols followed Kimball et al. (2009). After visualization on 1% agarose gels stained with SYBR Gold (Invitrogen), PCR products were sequenced in both directions on an ABI 3730 XL automated sequencer by Macrogen (Seoul, Korea). The samples of Iranian and Chinese Common Pheasants were amplified separately in two different laboratories, one at Ferdowsi University of Mashhad and one at Beijing Normal University.

**Phylogenetic analysis**

The sequences of the mitochondrial (CYTB and CR) and nuclear (HMG and SPI) loci were aligned using MAFit v6.847b (Katoh & Toh 2008), with
subsequent adjustments in \texttt{BioEdit v7.0.5} (Hall 1999). We constructed phylogenetic trees using maximum likelihood (ML) and Bayesian inference (BI) in \texttt{PAUP v4.0b10} (Swofford 2003) and \texttt{MrBayes v3.2.1} (Ronquist & Huelsenbeck 2003), respectively. The phylogenetic trees were rooted using homologous sequences of a Green Pheasant \textit{P. versicolor} individual, the closest sister species of the Common Pheasant. The amino acid translations of gene coding regions were examined for stop codons to ensure all were functional proteins. To reveal different possible topologies and identify sources of conflict between genes, the three datasets (mtDNA, nuclear DNA and the concatenated data) were analysed for both ML and BI analyses, separately. All analyses were run under best-fit nucleotide substitution models estimated in \texttt{ModelTest v3.7} (Posada & Crandall 1998) using the Akaike information criterion (Akaike 1974). Using \texttt{ModelTest}, we identified TVM+I+G and GTR+I+G as the best-fitting substitution models for the mtDNA and combined mtDNA–nuclear datasets, respectively (Table S2).

For ML analyses, the best-fit substitution models were used in a subsequent ML heuristic tree search with 10 random addition sequence replicates and tree-bisection-reconnection branch swapping. To assess nodal support, 100 bootstrap replicates were run under ML with the parameters estimated automatically. For BI analyses, we performed four independent runs of Metropolis-coupled Markov chain Monte Carlo (MCMC) analyses on the above three datasets starting from random trees. The MCMC chains were run for 10 million generations with sampling every 10 000 generations. The first 10% of generations were discarded as burn-in, well after stationarity of the chains had been established, and posterior probabilities were calculated from the remaining samples (pooled from the two simultaneous runs). We determined convergence among the independent runs observing the ESS values of the posterior distribution of all resulting parameters in \texttt{Tracer v1.6} (Rambaut et al. 2014). The resulting tree was visualized using \texttt{FigTree v1.3.1} (Rambaut & Drummond 2009).

We visualized phylogenetic relationships in Common Pheasants by incorporating the polymorphism of the combined mtDNA–nuclear DNA dataset. Based on this combined dataset, we generated Neighbor-Net networks (Bryant & Moulton 2004) of \textit{P. colchicus} samples using uncorrected \textit{p}-distances in \texttt{SplitsTree v4.10} (Huson & Bryant 2006).

We further combined the mtDNA–nuclear DNA dataset to estimate divergence times between major evolutionary clades of Common Pheasants in \texttt{BEAST v1.7} (Drummond et al. 2012). XML files for the BEAST analyses were generated in \texttt{BEAUTi v1.7} (Drummond et al. 2012). Analyses were run under the Yule speciation model. We used separate nucleotide substitution models for each marker. For the mtDNA data, we implemented a normal prior for the substitution rate (ucld. mean parameter) using a robust overall divergence rate of 2.1 ± 0.1% per million years (Myr) (0.0105 ± 0.0005 substitutions/site/Myr; Weir & Schluter 2008). For the nuclear intronic data, we used a mean rate of 0.00135 ± 0.00045 substitutions/site/Myr (Ellegren 2007), also implemented as a normal prior. In preliminary runs, a relaxed uncorrelated lognormal rate distribution was used to check whether a strict molecular clock was appropriate for any of the datasets. Other priors were set with default values. For these analyses, \(5 \times 10^7\) generations were performed, with sampling every 1000 generations. Each analysis was run twice. The MCMC output was analysed in \texttt{Tracer v1.6}. (Rambaut et al. 2014) to evaluate whether valid estimates of the posterior distribution of the parameters had been obtained. The first 25% of generations were discarded as burn-in, well after stationarity of chain likelihood values had been established. Trees were summarized using \texttt{TreeAnnotator v1.7.4} (Rambaut & Drummond 2012) with options ‘Maximum clade credibility tree’ and ‘Mean heights’ and were visualized in \texttt{FigTree v1.3.1}.

To test for historical rapid population expansion, a mismatch distribution analysis among individuals was carried out using mtDNA loci (\textit{CYTB} and \textit{CR}) only under a population growth–decline model in \texttt{DnaSP v5.0} (Rogers & Harpending 1992). In such an analysis, demographic stability is illustrated by multimodal distribution, and a unimodal pattern is consistent with sudden expansion (Slatkin & Hudson 1991). Using the same dataset, we also performed the neutrality test of Fu’s \(F_S\) and Tajima’s \(D\).

**RESULTS**

**Sequence characteristics**

Sequencing of 85 \textit{P. colchicus} individuals from 17 subspecies of six groups generated 2435 base pairs...
(bp), including 1280 bp of mtDNA and 1155 bp of nuclear DNA. The aligned CR sequence dataset comprised 483 bp, of which 37 sites (7.66%) were parsimony-informative, and the CYTB dataset contained 797 bp including 24 (3.01%) parsimony-informative sites. The alignments of HMG with 641 bp and SPI with 514 bp contained no parsimony-informative sites. The total concatenated dataset comprised 61 parsimony-informative sites (4.8%).

**Multilocus phylogeny**

Separate phylogenetic analyses were performed on the mitochondrial and nuclear datasets (Supporting Information Figs S1 and S2). As our nuclear data contained much lower levels of polymorphism, the concatenated analysis relied on the information from the mtDNA loci (Fig. 2). Overall, the topology of the trees generated by BI was generally congruent with those recovered by ML and also between the mtDNA and combined dataset (Supporting Information Fig. S3). We thus focus mainly on the results obtained from Bayesian analysis of the combined mtDNA–nuclear DNA dataset.

In the trees generated from the combined dataset using MrBayes (Fig. 2) and BEAST (Fig. 3), individuals of the subspecies elegans formed a distinct clade with relatively low support, 0.80 for BI.
(Fig. 2) and 62% for ML (Fig. S3), which was sister to a clade that contained all other subspecies. Within this clade, pre-defined morphological groups did not correspond to monophyletic genetic groups. The genetic relationships of subspecies within the most subspecies-rich group *torquatus* were unresolved, with low support values in both analyses. However, among subspecies in the western Palaearctic, we identified six branches with high posterior probability values in the analyses. Clade A encompassed all sampled groups except for *torquatus* and *elegans* groups, consisting of *P. c. shawii*, *P. c. tarimensis* and *torquatus*. Clade B in turn was divided into C (*principalis*) and D, with the latter further divided into E (*mongolicus* group) and F (*colchicus* group) (Figs 2 and 3). Finally, Clade G, consisting of ‘Caucasus Pheasant’ *P. c. colchicus* individuals, was a distinct, strongly supported group nested within F.

The haplotype network analysis based on the concatenated dataset largely showed close genetic relationships among subspecies in the western Palaearctic (*principalis-chrysomelas*, *mongolicus*, *colchicus*). These taxa had closer relationships with the *tarimensis* subspecies in the southern Xinjiang Uygur region than the *torquatus* subspecies group in eastern Asia and *elegans* group in southwestern China (Fig. 4).

**Estimated divergence times**

The dating analysis (Fig. 3) suggested that the basal divergence separating the Green Pheasant from Common Pheasant occurred approximately 4.3 million years ago (Mya). The separation of the *elegans* group from the remaining taxa is estimated to have taken place approximately 3.4 Mya, with subsequent divergence of the *torquatus* group from other subspecies in the western groups around 2.5 Mya. The split between the other western groups and the *tarimensis* group took place later, beginning approximately 1.8 Mya. The *principalis* and the *colchicus* groups diverged from their most recent common ancestor approximately 1.6 Mya and further divergences within these subspecies occurred approximately 1.4 Mya (Fig. 3).

**Demographic analysis**

Demographic histories were inferred by a pairwise mismatch distribution analysis based on mitochondrial CYTB and CR haplotypes. The mismatch distribution for the entire set of samples (Fig. 5A) and four pheasant subspecies groups (Fig. 5B–E) was bell-shaped, which would be expected under a sudden expansion model. Two groups (*colchicus* and *torquatus*) fit both sudden and spatial expansion models (Fig. 5B,E). The inferred population
size expansion was further supported by significantly negative values of Fu’s $F_S$ and Tajima’s $D$ (Table 1). The mismatch distribution suggested the *colchicus* and *torquatus* groups of Common Pheasant underwent a rapid, recent population and range expansion. The *principalis-chrysomelas* group showed neutral variation according to Tajima’s $D$ value and recent population expansion based on Fu’s $F_S$ value. The *tarimensis* group may have decreased population sizes or has experienced over-dominant selection. No mismatch analysis was conducted on the *mongolicus* and *elegans* groups because of their small sample sizes (Table 1).

### Table 1. Statistics from mismatch analyses of *Phasianus colchicus* subspecies.

<table>
<thead>
<tr>
<th>Dataset (n)</th>
<th>Tajima’s $D$</th>
<th>Fu’s $F_S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total samples (46)</td>
<td>$-0.2134^{**}$</td>
<td>$-17.948^{**}$</td>
</tr>
<tr>
<td><em>colchicus</em> (12)</td>
<td>$-0.283^{**}$</td>
<td>$-0.622^{*}$</td>
</tr>
<tr>
<td><em>principalis-chrysomelas</em> (5)</td>
<td>0.000$^{**}$</td>
<td>$-0.701^{*}$</td>
</tr>
<tr>
<td><em>tarimensis</em> (29)</td>
<td>0.087$^{**}$</td>
<td>2.680$^{*}$</td>
</tr>
<tr>
<td><em>torquatus</em> (50)</td>
<td>$-0.512^{*}$</td>
<td>$-9.837^{*}$</td>
</tr>
</tbody>
</table>

Parameters were calculated under sudden and spatial expansion models. $n$ = number of polymorphic sites. The significance of Tajima’s $D$ and Fu’s $F_S$-values is indicated as follows: $^{*}P < 0.010$; $^{**}P < 0.001$.

### DISCUSSION

#### Phylogeography and taxonomic implications

The Common Pheasant is widely distributed in the Palaearctic region, mainly in forest grasslands, semi-arid forests, arable lands and along hillsides ranging in elevation between −10 m (along the Caspian Sea and Xinjiang) and 3000 m (Chang *et al.* 2008, Kayvanfar & Aliabadian 2013). This species has a long history as a captive gamebird, with inter-subspecies hybrid populations having been intentionally introduced beyond its natural range, for example to Western Europe and North America. Common Pheasant plumage patterns, especially the presence of a white collar and varied coloration on rump and wings in males, have long been considered important diagnostic characters for intra-specific taxonomy and have even been used to reconstruct evolutionary relationships among subspecies (Johnsgard 1999).

Although subspecies groupings were previously defined using morphological discontinuities among subspecies, the present study demonstrates some incongruence between morphology-based subspecies divisions and genetic relationships. In particular, the *elegans* subspecies, previously within the *torquatus* group because of morphological similarity, probably forms the earliest subspecies (although support was low). In addition to being genetically distinct, individuals in the *elegans* subspecies are distinguished from those of subspecies within the *torquatus* group by the black bars on the basal part of the much wider central tail feathers. Mantle and scapular feathers are darker, the upper feathers of the mantle are more spotted at their extremities with dark green, and lower back and rump feathers have rather wide sub-terminal dark-green bands.

Morphologically there is a major split between western ( *tarimensis*, *principalis-chrysomelas*, *mongolicus* and *colchicus*) and eastern groups ( *torquatus* and *elegans*) of Common Pheasant. In general, subspecies of the western groups have darker plumage and possess brownish-red or blackish-brown rumps, whereas most members of the eastern groups have lighter plumage and bluish to darkish-grey rumps (Fig. 2). Geographically, the eastern groups are allopatric with the four western groups, with the former distributed in humid and sub-humid zones and the latter occupying arid regions. However, our findings revealed that subspecies in the western Palaearctic are nested within the most subspecies-rich *torquatus* group (‘Grey-rumped Pheasants’), making the latter a grade of subspecies and subspecies groups (Figs 2–4), probably indicating rapid morphological evolution. In addition, no obvious clinal geographical variation in rump
coloration or the presence of a white collar is evident. Where subspecies ranges are contiguous, however, some degree of gradual changes in plumage characters (Liu & Sun 1992), and shared genetic polymorphism has been documented previously (e.g. Qu et al. 2009, Liu et al. 2010, Zhang et al. 2014) and in the present study (Figs S1 and S2). This probably suggests frequent admixture among adjacent subspecies and violates the status of some Common Pheasant subspecies (Zhang et al. 2014). In contrast, differences in morphology (Madge & McGowan 2002) and perhaps genetic variation can be more abrupt between slightly isolated subspecies. To analyse further the boundaries and genetic admixture among subspecies, efforts to quantify morphology and to genotype populations using high-polymorphic genetic markers will be necessary (Wang et al. 2017).

**Biogeographical conclusions**

Cyclical Pleistocene climatic oscillations are presumed to have played important roles in shaping the geographical distribution, demographic history and ultimately patterns of genetic diversification of
many plant and animal species in the Palearctic region (Avise 2000, Hewitt 2000). According to our study, the Green Pheasant and the Common Pheasant are estimated to have diverged from each other approximately 2.5–6.5 Mya (Fig. 3). This timeframe may be due to vicariance speciation that was associated with the isolation of the Japan archipelago from the East Asian mainland in the late Early Miocene (Millien-Parra & Jaeger 1999). The divergence between the *elegans* group and other *P. colchicus* groups happened approximately 3.4 Mya, with the *torquatus* group diverging from the latter around 2.8 Mya (Fig. 3). These events immediately pre-date the shift to a cooler and fluctuating climate that characterized the Pleistocene.

The placement of subspecies *elegans* at the most basal position within the species implies that Common Pheasant might have its origin in the subtropical forests of Yunnan and Sichuan in southwestern China, as postulated by Delacour (1977). These regions are hypothesized to have been refugia for several avian species during the last glaciations (Qu *et al.* 2011). Yunnan and Sichuan are adjacent to the Himalayan and Hengduan mountains and are surrounded by massive forests (Liu 1983). It is likely that populations of *elegans* were isolated and evolved independently in these regions.

Based on our phylogenetic relationships within the western group (Fig. 2), we tentatively propose the existence of two dispersal routes into the western Palearctic region. Under our colonization hypothesis, Common Pheasant may have dispersed from east to west during the Late Pliocene-Early Pleistocene, approximately 1.8 Mya; such a scenario is consistent with Solokha’s suggestion (Solokha 1994) that pheasants migrated from the Tarim Basin to the South Tajik Depression when populations dispersed ‘out of China’. This is a long route involving the division of *tarimensis* into the ranges of *mongolicus* and *colchicus*. This route was open until the Middle Pleistocene, as the Pamir-Alay mountain system might not yet have been a major barrier to species dispersal (Bidos 1985). At the same time, the already extant mountain ranges of Kunlun could have been a barrier to pheasant dispersal from the Tarim River drainage toward India in the southwest (Solokha 1994).

The other short route is associated with the expansion of *tarimensis* to *principalis*. After its colonization to the west, the range of Common Pheasant may have been continuous from the northern foothills of the East Tian Shan Depression to the Black Sea. Such a colonization wave would have continued along the southern shore of the Caspian Sea, the foothills of the Elburz Mountains and the Caucasus. In central and southern Afghanistan and Iran, the distribution range of Common Pheasant was probably restricted to the Parapamisus and Kopet Dagh mountains during the Early to Middle Pleistocene because of the cool and dry climate (Potapov 1978, Kehl 2009). The dispersal of western groups in an easterly direction was probably limited by the ancient Dzungar Desert (Solokha 1994). The aridification of Central Asia, which began at the end of the Neogene, took place when the Amu Darya, the major river in Central Asia, began flowing into the Aral-Sary Kamysh Depression, with consequent formation of the Garagum (Karakum) Desert. With increasing desertification, Pheasant populations were gradually confined to river valleys and humid foothills, resulting in disruption of the species’ range (Fed & Atamuradov 1994). Subsequent disruption of their western range and evolution of isolated populations led to the genetic differentiation of members in the *colchicus* group (*P. c. persicus*, *P. c. talischensis* and *P. c. colchicus*).

The shorter dispersal route was associated with the formation of the *principalis* group out of China. These populations could have penetrated valleys of the Murghab and Tedzhen mountains and the East Kopet Dagh foothills, leading to the colonization of the *principalis* subspecies in accordance with the findings of Solokha (1994) (Fig. 6). As the *mongolicus* and *colchicus* groups appeared more closely related to each other than to the *principalis* group, these two groups might have descended from a common ancestor from the Tarim basin with one ancestral group colonizing the upper Tian Shan Mountains to give rise to the *mongolicus* group (Figs 2–4, 6). The haplotype network analysis also confirmed that the *mongolicus* group was represented by a distinct haplotype. This finding suggests that populations of Common Pheasant from these regions probably remained isolated throughout the Pleistocene irrespective of climatic oscillations and desertification of northern China (Qu *et al.* 2009).

In conclusion, our proposed scenario based on genetic analysis is partially in agreement with the scenario by Solokha (1994), who proposed three dispersal waves of the Common Pheasants in central Asia and the Caucasus based on phenotypic variation and palaeo-geographical data (Fig. 6).
Our analysis also suggests different patterns of demographic histories among subspecies groups. The results of the mismatch distribution analysis suggest that some of the subspecies groups (colchicus, torquatus and principalis-chrysomelas) have experienced a rapid, recent population and range expansion. Whereas the recent expansion hypothesis is supported by the non-significant mismatch distribution, no evidence for changes in population size can be inferred for the principalis-chrysomelas group based on Tajima’s $D$ (Table 1). Because Fu’s $F_S$ is a more sensitive indicator of population expansion and genetic hitchhiking compared with Tajima’s $D$ (Fu 1997), the former may provide better evidence for expansion of the principalis-chrysomelas group. In contrast, the tarimensis group may have suffered a recent bottleneck, possibly due to desertification (Fig. 5).

**Conservation applications**

Dispersal and shifts in the ranges of various subspecies of Common Pheasant are still occurring (Braasch et al. 2011), primarily because of anthropogenic activities. In addition, the destruction of natural habitats has led to the reduction of the geographical range of some subspecies. For example, the destruction of *Tamarix pentandra* and *Populus euphratica* forests in the foothills of the Kopet Dagh range and the introduction of irrigation in the Tedzhen Oasis have caused a two-fold increase in the distribution range of *P. c. principalis* on former farm lands in the last 40 years without any artificial breeding attempts; this situation is similar to the southeastern range extension of *P. c. persicus* (Fet & Atamuradov 1994, Solokha 1994). The distribution of the ‘Northern Caucasus Pheasant’ *P. c. septentrionalis* is highly fragmented and only remains genetically pure in the lower reaches of the Samur River. Currently, many conservation programmes are introducing pheasants into their historical ranges in most North Caucasian countries; however, the genetic purity of those introduced pheasants is not clear (Koblik et al. 2006). The *in situ* population of *P. c. turcestanicus* is now extinct as a result of the aridification of the Aral

![Figure 6](image_url). Proposed dispersal routes of *Phasianus colchicus* from the eastern to western side of the Palaearctic region: the long route itself divides into two sub-routes, from the Tarim Basin to the range of subspecies mongolicus and from the Tarim Basin to the range of subspecies colchicus (yellow arrows), separately; the short one expanded from the Tarim Basin to the range of subspecies principalis (red arrow). Dashed lines refer to potential historical colonization routes that are most likely geographical barriers at present. The colours within each pie chart reflect the haplotypes.

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Sea area but this subspecies is still present in the region of the Syr Darya river in northeast Uzbekistan and southern Kazakhstan (Scott et al. 1987, Abdusalyamov 1988, Clements 2000, Lepage 2007). Although our study has provided the first multi-locus phylogeographical picture of Common Pheasant at a subspecies-group level, interesting questions still remain. First, the torquatus group and some subspecies in the torquatus group do not represent monophyletic groups (see also Qu et al. 2009) and relationships among these remain unresolved in the present study. On the other hand, the overlap of P. c. persicus and P. c. talischensis in the phylogenetic tree challenges the validity of these subspecies. This pattern is probably caused by shallow divergence and frequent recent population admixture (Liu et al. 2011). Genomic-scale markers derived from next-generation sequencing can provide higher resolution (Ekblom & Galindo 2011), and may be able to answer these remaining questions associated with speciation and local adaptation of Common Pheasant (Seehausen et al. 2014).

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Condensed Bayesian inference tree of subspecies of *Phasianus colchicus* based on mitochondrial cytochrome b (CYTB) and control region (CR) dataset. PP (posterior probability) values from the Bayesian analysis (10 million replicates) are indicated at the > 0.90 (**) and > 0.75 (*) significance level. Letters on branches represent similar branch splits on the phylogenetic tree in Figure 2.

**Figure S2.** Condensed Bayesian inference tree of subspecies of *Phasianus colchicus* based on concatenate nuclear High mobility group 17 (HMG) and Serpin peptidase inhibitor clade C (SPI) dataset. PP (posterior probability) values from the Bayesian analysis (10 million replicates) are indicated at the > 0.90 (**) and > 0.75 (*) significance level. Letters on branches represent similar branch splits on the phylogenetic tree in Figure 2.

**Figure S3.** Maximum likelihood inference tree of subspecies of *Phasianus colchicus* based on the concatenated mtDNA and nuclear DNA dataset. Numbers on nodes represent ML bootstrap values (given only if > 70%). The splits of the branches A, B, C, D, E, F and G are discussed in the text. Taxa names are represented in abbreviated forms.

**Table S1.** List of samples of subspecies of *Phasianus colchicus* included in this study along with museum voucher numbers, GenBank accession numbers and collection localities.

**Table S2.** Data partitions, estimated models of sequence evolution and total number of characters in each partition used in *Phasianus colchicus* phylogenetic analyses.

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