Evidence for Genetic Hybridization between Released and Wild Game Birds: Phylogeography and Genetic Structure of Chukar Partridge, *Alectoris chukar*, in Turkey

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Abstract: The Chukar Partridge (*Alectoris chukar*, Galliformes) is one of the most important game birds in its native range, spanning from the Balkans to eastern Asia, and the regions of Europe, North America and New Zealand where it was introduced. Previous studies found two main genetic lineages of the species forming an eastern and a western clade. Chukar Partridges are raised in game farms and released to supplement natural populations for shooting in the USA, Canada, Greece, and Turkey. To explore intraspecific genetic structure, phylogeography, and possible genetic admixture events of *A. chukar* in Turkey, we genotyped individuals from fourteen wild and five captive populations at two mitochondrial and ten microsatellite DNA loci in. Wild and farmed Chukar Partridge samples were analyzed together to investigate possible influences of intraspecific hybridizations. We found that the farmed chukars, which mainly (85%) cluster into the eastern clade, and wild ones were genetically distinct. The latter could be separated into six management units (MUs), with partridges from Gökçeada Island in the Aegean Sea forming the most divergent population. Intraspecific hybridization was detected between wild and captive populations. This phenomenon causes rampant introgression and homogenization. The phylogeographic analysis revealed admixture among wild populations; nevertheless, this did not impair pointing to Anatolia as likely having a “refugia-within-refugia” structure. We recommend that the genetic structure of Chukar Partridge and its MUs be taken into account when developing the policy of hunting, production, and release to preserve the genetic integrity of this species.

Keywords: population genetics; evolution; Anatolia; refugium; breeding station; admixture

1. Introduction

Genetic diversity plays a crucial role in the adaptation and survival of species [1], while their phylogeographic structure reflects the complex relationships between historical and ongoing evolutionary processes in a spatial framework. Phylogeographic studies helped elucidate gene flow patterns, hybridization, range expansion, and speciation among many bird species [2].

Climatic fluctuations have been occurring in the last three million years, alternating warm and cold periods in the Northern Hemisphere, especially in mountainous regions,
influencing the current phylogeographic structure of resident species. During the Quaternary ice ages, substantial areas of northern Europe and the mountain ranges of southern Europe and Asia were covered by ice sheets. In this period, Iberia, Italy, the Balkans, and Anatolia subsequently acted as glacial refugia for different species [3]. Southern Anatolia was one of the most important unglaciated areas in the western Palearctic region during the Pleistocene [3,4]. During that period, several animal and plant populations remained isolated in different refugia, which changed the genetic structure of the species to which they belonged. High altitudinal differentiation and a wide range of climates and microclimates may have promoted three possible refugia within Anatolia located, respectively, along the coastline of the Mediterranean region from Antalya to Hatay, along the coastline of the Aegean region from İzmir to Çanakkale, and in mid-northern Anatolia [4]. Moreover, various phylogeographic studies provided evidence that local populations of mammals [5], birds [4,6,7], amphibians [8], and insects [9] display a high level of genetic differentiation as the likely outcome of different ecological and climatic conditions. Widespread resident species such as *Alectoris* spp. may have different genetic structure in Anatolia.

Seven *Alectoris* (Galliformes) species occur in the Palearctic [10]. Although they are mainly allopatric, natural hybridization in their contact zones has been described [11]. The Chukar Partridge, *Alectoris chukar*, which is represented in the ancient Roman and Hellenistic mosaics, is one of the world’s most important game birds. The native distribution range of this species extends from the Balkans to eastern Asia [12], with 14 morphological subspecies [13] clustering into two well-distinct genetic lineages forming an eastern and a western clade separated by mountain ranges from Altay to Himalayas [14,15]. Eastern clade chukars are also raised in farms for hunting and meat production [16,17]. Moreover, European game farms breed *A. rufa* × *A. chukar* [18–20] and *A. graeca* × *A. chukar* producing hybrids [11,21] that are released into the wild for shooting purposes with the aim of supplementing natural populations. This practice, however, is now illegal in most European countries. Almost 70,000 farm-reared Chukar Partridges are produced and released in Turkey yearly since 2001 (www.milliparklar.gov.tr/resmiistatistikler, accessed on 1 August 2021).

Similar to what happens with the common quail (*Coturnix coturnix*) [22] and the mallard (*Anas platyrhynchos*) [23] across Europe, the anthropogenic introgressive hybridization turns into the rampant genetic homogenization [11,19,24] of *Alectoris* partidges at both inter- and intraspecific level. We genotyped partridges of fourteen wild and five farm (captive) *A.chukar* populations from Turkey at two mitochondrial and ten microsatellites DNA loci to determine (i) the phylogeographic structure, (ii) whether wild and farmed chukars are genetically different, and (iii) whether signs of admixture between them occur.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

Muscle tissue samples were collected from wild and captive individuals (*n* = 362) sampled during the 2018–2019 hunting seasons in fourteen localities throughout Turkey and six breeding stations (Table 1). Captive adult individuals were randomly selected in each breeding station. The MAKU-HADYEK-169 protocol controlled all the experiments on Chukar Partridges by MAKU, Local Ethical Committee on Animal Experiments regulations. All samples were preserved at room temperature in absolute ethanol. Total DNA was extracted using GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) or Dneasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions.
Table 1. Chukar Partridge sampling locations and sample size. Abbreviations for each wild and captive population surveyed in this study are spelled out.

| No. | Source       | Locality         | Mt DNA (n) | Microsatellites (n) | Reference       |
|-----|--------------|------------------|------------|--------------------|-----------------|
| 1   | Wild population | Çanakkale (CAN) | 24         | 21                 | This study      |
| 2   |              | Muğla (MUG)      | 19         | 16                 | This study      |
| 3   |              | Burdur (BUR)     | 19         | 18                 | This study      |
| 4   |              | Eskişehir (ESK)  | 21         | 20                 | This study      |
| 5   |              | Çankırı (CKR)    | 18         | 18                 | This study      |
| 6   |              | Niğde (NIG)      | 22         | 21                 | This study      |
| 7   |              | Sivas (SIV)      | 20         | 13                 | This study      |
| 8   |              | Kayseri (KAYS)   | 29         | 28                 | This study      |
| 9   |              | Bayburt (BAY)    | 14         | 14                 | This study      |
| 10  |              | Erzurum (ERZ)    | 19         | 19                 | This study      |
| 11  |              | Kars (KAR)       | 18         | 18                 | This study      |
| 12  |              | Bitlis (BIT)     | 13         | 13                 | This study      |
| 13  |              | Van (VAN)        | 14         | 14                 | This study      |
| 14  |              | Hakkari (HAK)    | 8          | 8                  | This study      |
| BS1 | Breeding station | Afyon (BSA)     | 19         | 15                 | This study      |
| BS2 |              | Gaziantep (BSG)  | 24         | 23                 | This study      |
| BS3 |              | Kahramanmaras (BSK) | 28         | 27                 | This study      |
| BS4 |              | Malatya (BSM)    | 18         | 17                 | This study      |
| BS5 |              | Uşak (BSU)       | 14         | 14                 | This study      |
| BS6 |              | Yozgat (BSY)     | 26         | 25                 | This study      |
| GenBank | 12 Countries  | 86                | -          | Barbanera et al. [13] |
|      | Alectoris greaca | 2                | -          | Barbanera et al. [13] |

Total 475 362

2.2. DNA Amplification and Sequencing

The partial cytochrome-b (Cyt-b, 1092 bp) and the entire Control region (CR, about 1155 bp) of the mitochondrial DNA (mtDNA) were amplified for all samples following Barbanera et al. [14]. PCR products were purified and sequenced on both strands at Macrogen (Seoul, Korea). Sequences were aligned in GENEIOUS PRIME 2021.2.2 with the MUSCLE plugin and further proofed manually [25]. The sequences were deposited in GenBank with accession numbers MZ706294 to MZ706461. We added 86 partial Cyt-b and CR GenBank sequences of Chukar Partridge from Europe and Asia. The accession numbers of the outgroup Rock Partridge (Alectoris greaca) and GenBank sequences were given in the tree of Supplementary Material S1 (Table 1).

As far as the microsatellites are concerned, we selected the ten most polymorphic loci in A. chukar among 130 from an A. rufa genomic library: Aru1A, Aru1B3, Aru1E7, Aru1E93, Aru1E97, AruF25, AruF114, Aru1G4, Aru1G49 [26], plus one Aru2D020, used for the first time in this study (forward primer: CACACACTAACCTTTTCTCTCG; reverse primer: CACATCAGTACGAAACATGG). The PCR conditions were as indicated in [27].

2.3. Phylogeographic Analysis

The Cyt-b and CR sequences were concatenated and aligned. The phylogenetic relationships were reconstructed in MEGA X [28] and BEAST 2 [29] using the Maximum Likelihood (ML) method. The TN93 + G + I algorithm was selected using MEGA X and following the Akaike Information Criterion (AIC). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with a superior log-likelihood value. There were a total of 2247 positions in the final dataset. Genetic differentiation among populations ($F_{ST}$) was evaluated by analyzing molecular variance (AMOVA) in ARLEQUIN 3.5 with significances assessed by 10,100 permutations [30] and a spatial analysis of molecular variance (SAMOVA) was
performed using SAMOVA 1.0 [31]. This method is based on a simulated annealing procedure aimed at identifying geographically homogeneous populations and maximally differentiated in terms of among-group components ($F_{CT}$) of the overall genetic variance without the prior assumption of group composition AMOVA relies on. The program was run for 10,000 iterations from each of 100 random initial conditions and tested all the grouping options (predefined number of groups $K$ ranging from 2 to 18). The optimal number of groups ($K$): $F_{CT}$ values (proportion of genetic variation among groups) reached a maximum, or a plateau was selected. A median-joining network was created to visualize haplotype relationships using Network 10 [32]. Haplotypes and mismatch distributions of demographic/population expansion were defined by DnaSP 6 [33] and the number of polymorphic sites ($S$), haplotype diversity ($Hd$), nucleotide diversity ($\pi$), average number of nucleotide differences ($K$), and number of haplotypes ($h$) were calculated using DnaSP or ARLEQUIN for the mtDNA dataset.

Microsatellites: Departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated for each microsatellite locus and population with an exact test using GENEPOP 4.7.5 [34]. The mean number of alleles ($A$), observed ($H_o$), expected ($H_e$) heterozygosity, and $F_{ST}$ distances were calculated using ARLEQUIN [30].

We used the Bayesian clustering method implemented in STRUCTURE 2.3.4 [35] to infer the population structure. Ten independent runs with $K=1$–10, where $K$ is the different number of subpopulations, were used with an admixture model taking sampling locations as priors and correlated allele frequencies between populations. Throughout the analysis, the burn-in period was fixed at 50,000, and the number of MCMC runs at 20,000. Besides, SAMOVA was performed to identify groups using SAMOVA [31]. The most likely number of groups was determined by 100 repeatedly running with two to 10 groups and choosing those partitions with a maximum $F_{CT}$ value and STRUCTURE HARVESTER [36] according to the method of Evanno et al. [37].

3. Results
3.1. Mitochondrial Nucleotide Sequences

The alignment of concatenated mt DNA loci for 354 individuals had a length of 2247 nucleotides, indels included. A total of 169 haplotypes were found, 146 belonging to the wild populations and 30 to the captive populations. Unique haplotypes ($n=148$) were mostly found in the wild populations ($n=139$), whereas captive populations yielded only seven haplotypes (Table 2). The most frequent haplotypes were Hap20 ($n=48$) and Hap74 ($n=25$) found only in captive partridges except for one wild individual (NIG) at Hap74 and Hap 26 ($n=14$) in only wild, respectively. The ML tree with the highest log likelihood ($−6412.72$) and posterior probability is shown in Figure 1. All haplotypes fell into one of the two main clades, i.e., either the western or the eastern one (Figure 1 and Supplementary Material). Besides, the median-joining haplotype network showed two main groups where captive and wild populations clearly clustered apart, even if evidences of admixture were also flagged (Figure 2. When star contraction of 169 haplotypes was applied, 96 haplotypes remained, with partridges from breeding stations and wild populations well separated from each other (Figure 2). The largest haplogroup included H19, H20, H74, and H88 haplotypes. These were mostly held by partridges from breeding stations (and in 60.5% of all captive individuals as opposed to only 0.9% of wild ones).

Basic summary statistics, including sample sizes, haplotype and nucleotide diversities, are provided in Table 2. We found the highest $Hd$ in KAH and KAR, followed by BAY and central populations, while the lowest was recorded in BSG, BSY, and BSA. Eastern haplotypes were found in the wild CAN, KAH, NIG populations, and all breeding stations. Nearly all samples from breeding stations (85.3%) and some from wild populations (2.1%) belonged to the eastern clade (Table 3).
Table 2. Summary statistics (±SD) of genetic diversity in Chukar Partridge populations. Haplotype diversity ($H_d$) and nucleotide diversity ($\pi$) for each sampling area. $N =$ number of individuals; $eN =$ number of east clade individuals; $S =$ polymorphic sites; $h =$ number of haplotypes; $eh =$ number of east haplotypes; $uh =$ number of unique haplotypes. *SAMOVA groups of breeding station.

| SAMOVA Groups | Population | $N$ | $eN$ (%) | $S$ | $h$ | $eh$ (%) | $uh$ | $H_d$ | $\pi \times 10^{-3}$ (±SD) |
|---------------|------------|-----|----------|-----|-----|---------|-----|-------|-----------------------------|
|              |            |     |          |     |     |         |     |       |                             |
| 1            | CAN        | 22  | 1 (4.5)  | 25  | 13  | 1 (7.7) | 8   | 0.91  | 1.63 ± 0.46                 |
| 2            | KAH        | 29  | 3 (10.3) | 36  | 28  | 3 (10.7)| 21  | 0.99  | 2.98 ± 0.30                 |
| 3            | BAY        | 14  | no       | 23  | 12  | no      | 8   | 0.98  | 1.91 ± 0.31                 |
| 4            | ESK        | 20  | no       | 10  | 8   | no      | 4   | 0.87  | 1.69 ± 0.13                 |
| Center       |            |     |          |     |     |         |     |       |                             |
| 5            | BUR        | 18  | no       | 28  | 15  | no      | 11  | 0.98  | 2.45 ± 0.36                 |
| 5            | MUG        | 18  | no       | 22  | 15  | no      | 13  | 0.98  | 2.04 ± 0.28                 |
| 5            | CRK        | 16  | no       | 16  | 13  | no      | 7   | 0.98  | 1.62 ± 0.24                 |
| 5            | NIG        | 19  | 1 (5.3)  | 28  | 16  | 1 (6.3)| 9   | 0.98  | 2.07 ± 0.43                 |
| 5            | SIV        | 12  | no       | 11  | 10  | no      | 3   | 0.97  | 1.25 ± 0.19                 |
| 5            | ERZ        | 19  | no       | 34  | 15  | no      | 11  | 0.97  | 2.84 ± 0.23                 |
| 6            | total center | 102 | 1 (1)    | 74  | 72  | 1 (1.4)| 54  | 0.99  | 2.17 ± 0.15                 |
| 6            | KAR        | 18  | no       | 23  | 16  | no      | 10  | 0.99  | 2.69 ± 0.24                 |
| 6            | BIT        | 12  | no       | 21  | 10  | no      | 7   | 0.97  | 2.32 ± 0.29                 |
| 6            | VAN        | 13  | no       | 20  | 10  | no      | 5   | 0.95  | 2.47 ± 0.37                 |
| 6            | HAK        | 8   | no       | 12  | 7   | no      | 1   | 0.96  | 2.35 ± 0.25                 |
| 6            | total east | 51  | no       | 34  | 35  | no      | 23  | 0.98  | 2.49 ± 0.13                 |
|              | Total wild | 238 | 5 (2.1)  | 103 | 146 | 5 (3.4)| 139 | 0.99  | 2.47 ± 0.10                 |
| 1*           | BSA        | 14  | 12 (85.7)| 14  | 4   | 3 (75.0)| no  | 0.71  | 1.77 ± 0.75                 |
| 2*           | BSC        | 22  | 21 (95.5)| 18  | 5   | 4 (80.0)| no  | 0.52  | 1.02 ± 0.56                 |
| 3*           | BSK        | 26  | 17 (65.4)| 22  | 18  | 12 (66.7)| 4   | 0.97  | 3.26 ± 0.32                 |
| 4*           | BSM        | 15  | 14 (93.3)| 11  | 6   | 5 (83.3)| 1   | 0.76  | 1.08 ± 0.35                 |
| 4*           | BSY        | 25  | 23 (92.0)| 16  | 8   | 6 (75.0)| no  | 0.66  | 1.29 ± 0.51                 |
|              | Total captive | 116 | 99 (85.3)| 26  | 30  | 20 (66.7)| 7   | 0.79  | 2.23 ± 0.27                 |
|              | TOTAL      | 354 | 104 (29.4)| 105 | 169 | 21 (12.4)| 148 | 0.97  | 3.81 ± 0.08                 |

Figure 1. ML tree built in MEGA X for the aligned haplotypes using the TN93 + G + I model. The posterior probability of trees in which the associated taxa clustered together is shown next to the branches. Haplotype details are given as Supplementary Documents S1 and S2. Admixed individuals were excluded from this analysis.
differences among and within wild populations accounted for 13.66% and 86.34%, while in the case of captive populations these figures were 24.71% and 75.29%.

Pairwise $F_{ST}$ estimates (Figure 3) revealed several well-distinct groups. Breeding station differing by high levels of divergence from wild populations but not from each other except for BSK ($F_{ST} = 0.29—0.40, p < 0.001$). Some wild populations were not distinguished from the other wild populations ($p < 0.001$). However, CAN, an island population, KAH, BAY, and ESK were differentiated from the other wild populations ($p < 0.001$; Figure 3).

![Figure 2](image1)

Figure 2. Heat map of mitochondrial (a) and microsatellite (b) pairwise $F_{ST}$ values across *A. chukar* populations in Turkey. Darker shades of blue rectangles indicate higher values of $F_{ST}$ (as displayed on the bar down of the heat map). Crosses indicate non-significant $F_{ST} p$-values ($p > 0.05$).

![Figure 3](image2)

Figure 3. Population group defined in SAMOVA (six wild and four captive) based on mtDNA data. Circles represent geographic locations of wild populations and red angles represent the breeding stations on the map. Median-joining haplotype network constructed from the combined mt Cyt b and CR sequence data in Network 10. Haplotypes are represented by circles with size proportional to individual number. Mutational steps are shown by the number of hatch marks.

Mismatch distribution was unimodal, and population expansion was accepted for wild populations, while for captive populations were multimodal and demographic expansion was not supported (Figure 4).

### Table 3. Spatial analysis of molecular variance (SAMOVA) of *Alectoris chukar* for the mtDNA.

| Mt DNA | Microsatellite |
|--------|----------------|
| $K$    | $F_{CT}$       | $K$    | $F_{CT}$       |
| 5      | 0.56*          | 3      | 0.10*          |
| 4      | 0.10*          | 5      | 0.10*          |
| 6      | 0.11*          | 7      | 0.12*          |
| 8      | 0.12*          | 9      | 0.11*          |
| 3      | 0.08*          | 4      | 0.07*          |
| 5      | 0.06*          | 6      | 0.05*          |
| 7      | 0.05*          |
| 8      | 0.05*          |

Some population differentiation was observed in the wild and captive Chukar Partridges in the SAMOVA for mtDNA. The highest $F_{CT}$ value was found at $K = 5$ ($F_{CT} = 0.56; p < 0.0001$). One group included all wild populations, and the breeding stations were divided into four groups (Tables 2 and 3). When only wild populations were analyzed, we did not observe an $F_{CT}$ plateau, rather it increased with the number of $K$. We selected $K = 6$ due to the highest $F_{CT}$ differentiation between $K = 6$ and $K = 7$ ($F_{CT} = 0.11; p < 0.001$; Figure 3, Table 3). The first population to split apart was CAN ($K = 2$), followed by KAH ($K = 3$).
The AMOVA revealed that differences among populations accounted for 48.37% of the overall genetic variance observed and differences within populations for 51.63%. The differentiation between wild and captive populations was moderate yet statistically significant ($F_{ST} = 0.48$, $p < 0.01$). The differentiation among individuals from only wild or captive populations was low statistically significant ($F_{ST} = 0.14$ and $F_{ST} = 0.24$, $p < 0.01$). The differences among and within wild populations accounted for 13.66% and 86.34%, while in the case of captive populations these figures were 24.71% and 75.29%.

Pairwise $F_{ST}$ estimates (Figure 3) revealed several well-distinct groups. Breeding station differing by high levels of divergence from wild populations but not from each other except for BSK ($F_{ST} = 0.29$—$0.40$, $p < 0.001$). Some wild populations were not distinguished from the other wild populations ($p < 0.001$). However, CAN, an island population, KAH, BAY, and ESK were differentiated from the other wild populations ($p < 0.001$; Figure 3).

Mismatch distribution was unimodal, and population expansion was accepted for wild populations, while for captive populations were multimodal and demographic expansion was not supported (Figure 4).
3.2. Microsatellite Analysis

The mean number of alleles per locus varied from 6.6 to 13.2 across wild populations and from 6.9 to 8.3 in captive ones (Table 4). A total of 203 alleles were found of which 43 at Aru1E97, followed by 35 at AruF25 and 24 at Aru1B3 (Table 5). The mean expected heterozygosity ranged from 0.69 to 0.87 in wild populations and from 0.71 to 0.79 in captive ones; observed heterozygosity ranged from 0.62 to 0.79 in the former and from 0.64 to 0.73 in the latter, respectively (Table 4).

Table 4. Summary statistics (±SD) of genetic diversity in Chukar partridge. Haplotype diversity (Hd) and nucleotide diversity (p) expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (FIS) for each sampling area. p values for heterozygote deficiency.

| Location | N  | A   | Ho  | He  | p       | FIS |
|----------|----|-----|-----|-----|---------|-----|
| CAN      | 21 | 7.5 | 0.63| 0.69| 0.0002  | 0.0991 |
| MUG      | 16 | 9.0 | 0.62| 0.82| 0.0000  | 0.2523 |
| BUR      | 18 | 10.4| 0.66| 0.86| 0.0000  | 0.2325 |
| ESK      | 20 | 10.0| 0.74| 0.81| 0.0000  | 0.2786 |
| CKR      | 18 | 10.1| 0.69| 0.81| 0.0000  | 0.1404 |
| SIV      | 13 | 9.7 | 0.74| 0.86| 0.0000  | 0.1482 |
| NIG      | 21 | 11.4| 0.74| 0.85| 0.0000  | 0.1344 |
| KAH      | 28 | 13.2| 0.71| 0.86| 0.0000  | 0.1749 |
| BAY      | 14 | 9.5 | 0.76| 0.84| 0.0000  | 0.1125 |
| ERZ      | 19 | 10.2| 0.79| 0.84| 0.0055  | 0.0500 |
| KAR      | 18 | 11.3| 0.78| 0.87| 0.0000  | 0.0995 |
| BIT      | 13 | 10.1| 0.79| 0.86| 0.0000  | 0.0790 |
| VAN      | 14 | 9.3 | 0.77| 0.86| 0.0000  | 0.1080 |
| HAK      | 8  | 6.6 | 0.71| 0.84| 0.0004  | 0.1644 |
| BSA      | 15 | 7.0 | 0.72| 0.75| 0.1004  | 0.0473 |
| BSG      | 23 | 8.2 | 0.73| 0.73| 0.1194  | −0.0062 |
| BSF      | 27 | 7.8 | 0.66| 0.74| 0.0000  | 0.0974 |
| BSY      | 25 | 8.3 | 0.70| 0.72| 0.0152  | 0.0368 |
| BSM      | 17 | 8.2 | 0.71| 0.79| 0.0000  | 0.1109 |
| BSU      | 14 | 6.9 | 0.64| 0.71| 0.0329  | 0.0913 |

Table 5. Microsatellite polymorphism. T: total number of alleles (range), Np: mean the number of alleles per population ± SD, HWE: Hardy–Weinberg equilibrium, HD: Heterozygote deficiency, HE: Heterozygote excess, values: the number of specific alleles in each population.

| Location | Aru1B3 | Aru1E7 | Aru1E97 | Aru1G4 | Aru1G9 | Aru2D020 | Aru1A | Aru1E93 | AruF114 | AruF25 |
|----------|--------|--------|---------|--------|--------|----------|-------|---------|---------|-------|
| T        | 24 (9–16) | 10 (5–10) | 43 (7–25) | 13 (4–9) | 23 (6–15) | 20 (4–13) | 13 (4–9) | 9 (2–9) | 23 (6–15) | 20 (4–13) |
| Np       | 12.6/1.9 | 6.4/1.3 | 14.7/4.6 | 6.9/1.3 | 11.5/2.1 | 8.5/2.7 | 7.1/1.4 | 4.4/1.8 | 8.7/1.2 | 11.7/4.0 |

| Location | Aru1B3 | Aru1E7 | Aru1E97 | Aru1G4 | Aru1G9 | Aru2D020 | Aru1A | Aru1E93 | AruF114 | AruF25 |
|----------|--------|--------|---------|--------|--------|----------|-------|---------|---------|-------|
| CAN      | -/HWE | -/HWE | -/HWE | -/HWE | -/HD | -/HD | -/HWE | -/HWE | -/HWE | -/HWE |
| MUG      | -/HD | -/HWE | 1/HWE | -/HWE | -/HD | -/HD | -/HWE | -/HWE | -/HWE | -/HWE |
| BUR      | -/HWE | -/HWE | 1/HD | -/HWE | -/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| ESK      | -/HD | -/HWE | 2/1HWE | -/HWE | -/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| CKR      | -/HD | -/HWE | -/HWE | -/HWE | -/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| SIV      | -/HWE | -/HWE | -/HWE | 1/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| NIG      | -/HWE | 1/HD | 2/HWE | -/HWE | -/HD | -/HE | -/HWE | -/HWE | -/HWE | -/HWE |
| KAH      | -/HD | -/HWE | -/HWE | 1/HWE | 1/HWE | 1/HD | 1/HD | -/HWE | -/HWE | -/HWE |
| BAY      | -/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| ERZ      | -/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| KAR      | 1/HWE | 1/HWE | 1/HWE | 1/HWE | 1/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| BIT      | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| VAN      | -/HWE | -/HWE | 1/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |
| HAK      | 1/HD | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE | -/HWE |

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The linkage equilibrium was rejected for only 22 out of 900 pairs of alleles. However, after sequential Bonferroni correction, exact tests for genotypic linkage disequilibrium was non-significant. These results indicated that the loci used segregate independently. Hardy–Weinberg Equilibrium (HWE) was not accepted for 54 out of 200 the loci in all localities (Table 5). Deviation from HWE was found in all populations except BSA and BSG (Table 4), which might be indicative of inbreeding, assortative mating or null alleles. Heterozygote deficiency appeared in one to 14 loci (Table 5). Heterozygote excess (HE) occurred in NIG at Aru2D020.

STRUCTURE HARVESTER indicated that the most likely number of clusters was $K = 2$ using the log-likelihood ($L(K)$) concept (Figure 5). Wild and captive populations separated at $K = 2$; inferences of $K = 3$ to $K = 6$ were similar, revealing three main groups with CAN always well differentiated. Captive samples showed evidence of admixture (Figure 6). Birds from the CAN island population are separated from the other wild populations at $K = 3$ to $K = 6$. By arbitrarily defining an individual as belonging to a specific cluster when assignment probability ($q$) was above 0.8, 346 of 362 individuals clustered together at $K = 3$ in Structure (Table 6). The highest percentages of admixed (less than 80% of the individuals assigned to the cluster) individuals between wild and captive clusters were observed in SIV, VAN, KAR, ESK, ERZ, and KAH (Table 4).

![Figure 5](image1.png)

**Figure 5.** (a) Log-likelihood ($L(K)$) means for each number of cluster ($K$) from 10 independent runs in Structure Harvester (error bars represent SD). (b) graph shows $\Delta K$ for each $K$ based on the first and second-order rates of change.

![Figure 6](image2.png)

**Figure 6.** STRUCTURE assignment of 362 individual microsatellites for $K = 2$ to $K = 6$. Each individual genotype is represented by a vertical bar. Black lines separate the 20 different populations, 14 wild and six captive. The most likely number of clusters is $K = 3$. Besides, CAN chukars are clustered separately from other wild populations ($K = 3–K = 6$). The sample size is shown above each respective pie chart.
Table 6. Sample size (n) and percentages of samples collected in different localities and assigned to the wild (two clusters) or captive cluster at K = 3 in STRUCTURE. Proportion of membership of each pre-defined population in each of the tree clusters ≤ 0.8 are considered admixed.

| Location | n   | Wild (%) | Captive (%) | Main Admixture (%) |
|----------|-----|----------|-------------|-------------------|
|          |     | Cluster I | Cluster II  | Cluster III       |
|          |     |           |             | Cluster I and II  |
|          |     |           |             | Cluster I and III |
|          |     |           |             | Cluster II and III|
| Wild     |     |           |             |                   |
| CAN      | 21  | 0         | 90          | 10                |
| MUG      | 16  | 100       | 0           | 0                 |
| BUR      | 18  | 95        | 0           | 6                 |
| ESK      | 20  | 95        | 0           | 0                 |
| CKR      | 18  | 89        | 0           | 6                 |
| NIG      | 21  | 95        | 0           | 5                 |
| SIV      | 13  | 85        | 0           | 8                 |
| KAH      | 28  | 75        | 0           | 18                |
| BAY      | 14  | 100       | 0           | 0                 |
| ERZ      | 19  | 95        | 0           | 0                 |
| KAR      | 18  | 94        | 0           | 0                 |
| BIT      | 13  | 92        | 0           | 6                 |
| VAN      | 14  | 86        | 0           | 8                 |
| HAK      | 8   | 100       | 0           | 0                 |
| Total    | 241 | 83        | 8           | 4                 |
| Breeding stations |     |           |             |                   |
| BSA      | 15  | 0         | 93          | 0                 |
| BSG      | 23  | 0         | 96          | 0                 |
| BSK      | 27  | 4         | 93          | 0                 |
| BSM      | 17  | 0         | 82          | 0                 |
| BSU      | 14  | 0         | 100         | 0                 |
| BSY      | 25  | 0         | 100         | 0                 |
| Total    | 121 | 1         | 94          | 0                 |
| Total    | 362 | 56        | 5           | 34                |

Comparable population differentiation was observed in wild and captive Chukar Partridges in the SAMOVA at ten microsatellite loci, and K = 4 distinguished among CAN, the other wild populations, BSU, and the other breeding stations ($F_{CT} = 0.12; p < 0.01$; Table 3). When only wild populations were analyzed, we did not observe an $F_{CT}$ plateau; rather it decreased with the number of K (Table 3). The first population to emerge as distinct was CAN ($K = 2$), followed by HAK ($K = 3$), MUG ($K = 4$), and BIT ($K = 5$), respectively. Pairwise $F_{ST}$ values based on microsatellites showed significant genetic differentiation among most localities (Figure 2). Non-significant values were obtained between neighboring east Anatolian localities.

4. Discussion

The goal of this study was threefold. First, we aimed to determine population structure and phylogeography of Chukar Partridges in Turkey; second, to investigate whether there is any difference between wild and farmed individuals; and third, to search for possible signatures of admixture between them.

4.1. Population Genetic Structure

Our mtDNA analyses of Chukar Partridges from Anatolia showed that farmed Chukars are genetically different from wild ones as well as that the two clusters they belong to fall within the western and eastern clade, respectively, emerged in previous studies [23,38] (Figure 1). Concordantly, microsatellites structure showed wild and captive birds to group in two distinct clusters ($K = 2$). CAN, an island population (Gökçeada Island), emerged as the most genetically differentiated one on the basis of mtDNA and microsatellites ($K = 3–6$) among the wild populations, and the other follow on the same order they are listed in Table 3. Noteworthy, this genetic picture emerged from $F_{ST}$ and SAMOVA of both genetic systems used (Figures 2, 3 and 5). Even if the wild populations clustered together, it is still possible to detect some internal differentiation among eastern Anatolian, central Anatolian and ESK, KAH, plus BAY populations as well as that evidences of admixture between them occur with the exception of CAN. The differentiation between
captive and wild populations was in line with the dissimilar shape and size of bill of their individuals [39]. When wild and captive populations were analyzed separately, it was found that the captive populations were highly differentiated from each other (captive $F_{ST} = 0.24$, wild $F_{ST} = 0.14$). This may be due to the fact that bloodlines used at breeding stations are sometimes reinforced with confiscated Chukar Partridges from illegal hunters.

Global populations of Chukar Partridges fall in an eastern and western clade; the farmed populations from Europe and the USA belong to the eastern clade [14]. Concordantly, we found that Turkish farmed Chukar Partridges mainly (85.3%) belong to the eastern clade. These captive partridges threaten the genetic integrity of wild populations. A number of studies unveiled the anthropogenic hybridization involving $A. rufa \times A. chukar$ [14,15,18–21,24,40–45], $A. graeca \times A. chukar$ [21] as well as intraspecific hybridization in Chukar Partridge [46,47].

We found some wild individuals falling in the eastern clade, CAN, KAH, and NIG (Table 2). Also, we have determined some genetic admixture between farmed (of eastern origin) and wild individuals in six wild populations and four farms. While 2% of hybrid individuals were found in the wild population, a higher hybridization rate (5%) occurred in farms (Table 6). If this process continues, these admixtures might significantly alter the gene pool of wild populations, possibly impair their fitness and affect female reproduction due to low carotenoid levels in blood plasma (as observed in the Red-Legged Partridges ($Alectoris rufa$) [48]). A similar genetic homogenization was found in the Mallard, another popular game bird in Europe, with captive-bred individuals changing the gene pool of wild populations [23]. Casas et al. [24] showed that extinction risk of wild and genetically preserved Red-Legged Partridge populations through releases of farmed hybrids is a possibility. Our results show that high haplotype and nucleotide diversity exists in wild Chukar Partridge populations in Turkey as opposed to farm populations (Table 2). Nevertheless, introgressive hybridization might reduce the distinctiveness and diversity of wild populations, impacting their fitness in the near future.

The unimodal mismatch distribution results indicated that all wild populations together experienced recent demographic expansion. However, when taken separately their multimodal mismatch distribution suggests that admixture of haplotypes from three previously isolated lineages (one of them was captive) might have occurred. Also, multimodal mismatch distribution of population separated by SAMOVA at $K = 6$ indicated previously isolated lineages. Although Anatolia is not covering all the range of Chukar Partridge, these lineages may be considered as potential refugia within Anatolia, one of the most important unglaciated areas in Western Palearctic during the Pleistocene. The phylogeographic analysis showed that possibly Anatolia might have been a refugium with “refugia-within-refugia” structure. This model was supported by previous studies indicating range shifts within this region, as in case of Kurper’s Nuthatch, $Sitta krueperi$ [49]. A dense forest cover existed in the northern Anatolia and its coastal belts [50], which, according to Albayrak et al. [4], might have hosted three refugia for Kruper’s Nuthatch in Last Glacial Maximum (LGM), with the late Quaternary glacial-interglacial cycles shaping subsequent demographic expansion. Overall, it is assumed that many Anatolian species underwent population expansion before the Last Interglacial (LIG) [51,52], or between LGM and LIG [49].

### 4.2. Heterozygosity and Inbreeding

Estimates of observed heterozygosity are significantly lower than expected, except in captive populations, BSG and BSA (Table 4). Widespread heterozygote deficiency (Table 5) might be indicative of a genetic diversity loss in wild and farmed Chukar Partridges. Inbreeding is confirmed, especially in MUG and BUR (indicated by $F_{IS}$ value higher than 0.2). The positive $F_{IS}$ is an indication of decreasing heterozygosity due to null alleles. Similarly, positive $F_{IS}$ was observed in the historical wild group of Mallards in Europe [23].
4.3. Taxonomic and Conservation Implications

Fourteen Chukar Partridge subspecies are recognized worldwide, and two of them, A. c. cypriotes and A. c. kurdestanica, occur in southwestern/south-central Turkey, respectively [13]. Our finding supports the two described subspecies (see Figure 3; depicted in blue and green, respectively). Moreover, (SAMOVA and FST results) might possibly support a new subspecies distributed in Gökçeada Island (CAN).

To preserve the genetic diversity of the Chukar Partridge in Turkey, a country where the release of captive individuals is a common practice, six management units (MUs) should be taken into account: CAN, KAH, BAY, ESK, south-eastern Turkey, and central Anatolia separately (Figure 3). Specific conservation efforts should be made for the population of Gökçeada Island (CAN), where partridge releases should be banned due to the occurrence of a genetically distinct and well-preserved A.chukar population. We advocate for the genetic identity of Chukar Partridges and their six MUs to be considered when developing hunting, production, and releasing policies to preserve the integrity and internal diversity in perpetuity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14070571/s1, File S1 and S2: The detailed haplotypes tree.

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