

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 in Europe, North America and New Zealand where it was introduced. Previous studies found two large genetic lineage 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 the intraspecific population structure, phylogeography, and possible hybridizations of the species, we here used ten microsatellite and two mitochondrial DNA loci on 14 wild and five farmland populations sampled in Turkey. 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%) derive from the eastern clade, and wild Chukar Partridges were genetically different. Wild populations can be separated into six management units, with Gökçeada Island in the Aegean Sea as the genetically most distinct population. Intraspecific hybridization was detected between wild and captive populations. The anthropogenic hybridization of Chukar Partridges causes introgression and homogenization. The phylogeographic analysis revealed an admixture among wild populations; possibly, Anatolia may figure as a refuge area with “refugia-within-refugia”. We recommend that the genetic diversity of Chukar Partridges and their MUs’ populations be taken into account when developing the policy of hunting, production and release of Chukar Partridges to conserve 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 3 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 mountains range of southern

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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, populations remained isolated in different refugia, which changed the genetic structure of the species. High altitudinal differentiation and a wide range of climates and microclimates may have promoted three possible refugia within Anatolia located, respectively, in the coastline of the Mediterranean region from Antalya to Hatay, along the coastline of Aegean region from İzmir to Çanakkale, and in mid-northern of Anatolia [4]. Moreover, various phylogeographic studies provided evidence that Anatolian populations of mammals [5], birds [4,6,7], amphibians [8], and insects [9], display a high level of genetic differentiation. This may have outcome resulted from the different ecological and climatic conditions.

Seven *Alectoris* (Galliformes) species occur in the Palearctic [10]. Although they are mainly allopatric, natural hybridization in *Alectoris* contact zones has been described [11]. 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 distinct genetic clades: an eastern and a western clade divided with mountainous barriers throughout 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 interspecific *A. rufa* × *A. chukar* [18–20] and *A. graeca* × *A. chukar* hybrids [11,21] are used to be released into natural populations for shooting. However, it is now illegal in most European countries. Almost 70000 farm-reared Chukar Partridges are produced and released in Turkey yearly since 2001 (www.milliparklar.gov.tr/resmi-statistikler).

Reintroduction studies have pros and cons. For example, a successful reintroduction projects on Peregrine Falcon (*Falco peregrinus*) helped to maintain the level of genetic diversity and inbreeding depression [22,23] and reintroduced two parrot species from captive population having similar genetic diversity with wild ones [24]. In contrast, a study about the Common Quail (*Coturnix coturnix*) [25] and the Mallard (*Anas platyrhynchos*) across Europe [26] showed that the anthropogenic introgressive hybridization turns into the rampant genetic homogenization of *Alectoris* partridges at both inter- and intraspecific level [11,19,27]. Therefore, we genotyped two mtDNA genes and ten microsatellites loci of Chukar Partridge samples from 14 wild and five farm (captive) populations from Turkey to determine (i) the population structure and phylogeography, (ii) whether wild and farmed chukars are genetically different (iii) the signs of admixture between wild and farmed chukars 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 14 localities throughout Turkey and six breeding stations (Table 1). Captive adult individuals were randomly selected in each breeding station. The MAKU-HADYK-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, USA) or Dneasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

2.2. DNA amplification and sequencing

The partial cytochrome-b (Cyt-b, 1,092 bp) and the entire Control region (CR, about 1,155 bp) of the mitochondrial DNA (mtDNA) were amplified for all samples following Barbanera et al. [14]. PCR products were purified and sequenced on both strand at

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Macrogen (Seoul, Korea). Sequence were aligned in GENEIOUS PRIME with the MUSCLE plugin and further proofed manually. The sequences were deposited in GenBank with accession numbers MZ706294 to MZ706461. We added 86 partial Cyt-b and CR haplotype sequences of Chukar Partridge from Europe and Asia deposited in GenBank. The Rock Partridge (*Alectoris graeca*) was used as outgroup (Table 1).

As far as the microsatellites are concerned, we selected the ten most polymorphic STR loci in *A. chukar* among 130 from an *A. rufa* genomic library: Aru1A, Aru1B3, Aru1E7, Aru1E93, Aru1E97, AruF25, AruF114, Aru1G4, Aru1G49 [28], plus one, Aru2D020, used for the first time in this study (forward primer: CAACTACTTAACCTTTCTCCTG, reverse primer: CACTTCATAGTACAGAAACATGG). The PCR conditions were as indicated in [29].

Table 1. Chukar Partridge sampling locations and sample size. An abbreviation is spelled for each wild and captive populations surveyed in this study.

no	Source	Locality	Mt DNA	Microsatellites	Reference
			(n)	(n)	
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		Kayramanmaraş (KAH)	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		Kahramanmaraş (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
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Total		475	362	

2.3. Phylogeographic analysis

The Cyt-b and CR sequences were concatenated and aligned. The phylogenetic relationships were reconstructed in MEGA X [30] and BEAST 2 [31] 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 [32] and a spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA 1.0 [33]. This method is based on a simulated annealing procedure aimed at identifying geographically homogeneous populations and maximally differentiated in terms of among-group component (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 [34]. Haplotypes and mismatch distributions of demographic/population expansion were defined by DnaSP 6 [35] and the number of polymorphic sites (S), haplotype diversity (H_d), nucleotide diversity (π), average number of nucleotide differences (K), and number of haplotypes (h) were calculated using DnaSP 6 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 [36]. The mean number of alleles (A), observed (H_o), expected (H_e) heterozygosity, and F_{ST} distances were calculated using ARLEQUIN [32].

We used the Bayesian clustering method implemented in STRUCTURE 2.3.4 [37] 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 [33]. 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.

3. Results

3.1. Mitochondrial nucleotide sequences

The alignment of concatenated mtDNA loci for 354 individuals had a length of 2,247 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) and Hap 26 ($n = 14$) in wild, respectively. The ML tree with the highest log likelihood (-6412.72) and posterior probability is shown in Figure 1. All haplotypes are divided mainly into two clades, i.e., the western and eastern clade (Figure 1, S1, and S2). Besides, the Median-joining haplotype network showed two main groups where captive and wild populations

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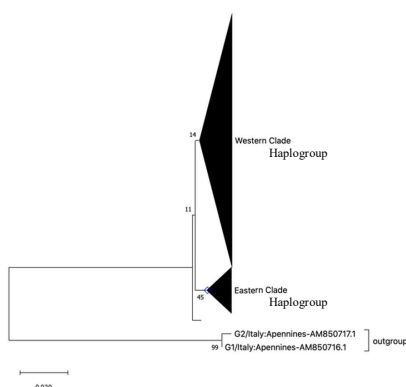
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Basic summary statistics, including sample sizes, haplotype and nucleotide diversities, are provided in Table 2. We found the highest Hd in KAH and KAR, followed by BAY and central populations, while the lowest was recorded in BSG, and BSY and BSA. 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).



Some population differentiation was observed in the wild and captive Chukar Partridges in the SAMOVA for mt DNA. 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 (Table 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 . $K=6$ was selected 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 ($K=3$).

Table 2. Summary statistics ($\pm$ SD) of genetic diversity in Chukar Partridge populations. Haplotype diversity (H_d) and nucleotide diversity (π) for each sampling area. N = number of individuals; nE = 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	<i>Population</i> <i>n</i>	<i>N</i>	<i>eN</i> (%)	<i>S</i>	Haplotypes			<i>Hd</i>	$\pi (\times 10^{-3})$
					<i>h</i>	<i>eh</i> (%)	<i>uh</i>		
1	CAN	22	1 (4.5)	25	13	1 (7.7)	8	0.91 $\pm$ 0.05	1.63 $\pm$ 0.46
2	KAH	29	3 (10.3)	36	28	3 (10.7)	21	0.99 $\pm$ 0.02	2.98 $\pm$ 0.30
3	BAY	14	no	23	12	no	8	0.98 $\pm$ 0.04	1.91 $\pm$ 0.31
4	ESK	20	no	10	8	no	4	0.87 $\pm$ 0.05	1.69 $\pm$ 0.13
5	BUR	18	no	28	15	no	11	0.98 $\pm$ 0.02	2.45 $\pm$ 0.36
5	MUG	18	no	22	15	no	13	0.98 $\pm$ 0.02	2.04 $\pm$ 0.28

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5	CKR	16	no	16	13	no	7	0.98 ± 0.03	1.62 ± 0.24
5	NIG	19	1 (5.3)	28	16	1 (6.3)	9	0.98 ± 0.02	2.07 ± 0.43
5	SIV	12	no	11	10	no	3	0.97 ± 0.04	1.25 ± 0.19
5	ERZ	19	no	34	15	no	11	0.97 ± 0.02	2.84 ± 0.23
5 - total center		102	1 (1)	74	72	1 (1.4)	54	0.99 ± 0.00	2.17 ± 0.15
6	KAR	18	no	23	16	no	10	0.99 ± 0.02	2.69 ± 0.24
6	BIT	12	no	21	10	no	7	0.97 ± 0.04	2.32 ± 0.29
6	VAN	13	no	20	10	no	5	0.95 ± 0.05	2.47 ± 0.37
6	HAK	8	no	12	7	no	1	0.96 ± 0.08	2.35 ± 0.25
6 - total east		51	no	34	35	no	23	0.98 ± 0.01	2.49 ± 0.13
Total wild		238	5 (2.1)	103	146	5 (3.4)	139	0.99 ± 0.00	2.47 ± 0.10
1*	BSA	14	12 (85.7)	14	4	3 (75.0)	no	0.71 ± 0.10	1.77 ± 0.75
2*	BSG	22	21 (95.5)	18	5	4 (80.0)	no	0.52 ± 0.11	1.02 ± 0.56
3*	BSK	26	17 (65.4)	22	18	12 (66.7)	4	0.97 ± 0.02	3.26 ± 0.32
4*	BSM	15	14 (93.3)	11	6	5 (83.3)	1	0.76 ± 0.08	1.08 ± 0.35
4*	BSU	14	12 (85.7)	20	5	3 (60.0)	no	0.72 ± 0.09	1.97 ± 0.85
4*	BSY	25	23 (92.0)	16	8	6 (75.0)	no	0.66 ± 0.09	1.29 ± 0.51
Total captive		116	99 (85.3)	26	30	20 (66.7)	7	0.79 ± 0.03	2.23 ± 0.27
TOTAL		354	104 (29.4)	105	169	21 (12.4)	148	0.97 ± 0.00	3.81 ± 0.08

Table 3. Spatial analysis of molecular variance (SAMOVA) of *Alectoris chukar* for the mtDNA. K, number of groups. F_{CT}, the proportion of total genetic variance due to the differences between groups. Population abbreviations as in Table 1. K of captive and wild populations was given in the first column.

	Mt DNA										Microsatellite									
	K	5	3	4	5	6	7	8	9		4	3	4	5	6	7	8	9		
	F _{CT}	0.56*	0.10*	0.10*	0.10*	0.11*	0.11*	0.12*	0.12*		0.12*	0.11*	0.08*	0.07*	0.06*	0.05*	0.05*	0.05*		
Wild populations	Group composition										Group composition									
	BUR	1	3	4	5	6	7	7	7		4	3	4	5	6	7	8	9		
	CAN	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1		
	MUG	1	3	4	5	6	5	5	5		4	3	3	3	3	3	3	3		
	ESK	1	3	4	5	4	4	4	4		4	3	4	5	6	7	8	2		
	CKR	1	3	4	5	6	7	7	7		4	3	4	5	6	6	6	6		
	SIV	1	3	4	5	6	7	7	7		4	3	4	5	6	7	8	9		
	NIG	1	3	4	5	6	7	7	7		4	3	4	5	6	7	8	9		
	KAH	1	2	2	2	2	2	2	2		4	3	4	5	6	7	8	8		
	BAY	1	3	4	3	3	3	3	3		4	3	4	5	6	7	5	5		
Breeding stations	ERZ	1	3	4	5	6	7	6	6		4	3	4	5	6	7	7	7		
	KAR	1	3	3	4	5	6	8	9		4	3	4	5	6	7	8	9		
	BIT	1	3	4	4	5	6	6	8		4	3	4	4	4	4	4	4		
	VAN	1	3	4	4	5	6	6	8		4	3	4	5	5	5	8	4		
	HAK	1	3	3	4	5	6	8	9		4	2	2	2	2	2	2	9		
	BSA	2									2									
	BSG	3									2									
	BSK	4									2									
	BSU	5									2									
	BSY	5									2									
	BSM	5									2									

* p < 0.01

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 2) 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 2).

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).

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	Mitochondria									
	K	5	3	4	5	6	7			
	F _{CT}	0.56*	0.10*	0.10*	0.10*	0.11*	0.11			
Wild populations	Group composition									
	BUR	1	3	4	5	6	7			
	CAN	1	1	1	1	1	1			
	MUG	1	3	4	5	6	5			
	ESK	1	3	4	5	4	4			
	CKR	1	3	4	5	6	7			
	SIV	1	3	4	5	6	7			
	NIG	1	3	4	5	6	7			
	KAH	1	2	2	2	2	2			
	BAY	1	3	4	3	3	3			
Breeding stations	ERZ	1	3	4	5	6	7			
	KAR	1	3	3	4	5	6			
	BIT	1	3	4	4	5	6			
	VAN	1	3	4	4	5	6			
	HAK	1	3	3	4	5	6			
	BSA	2								
	BSG	3								
	BSK	4								
	BSU	5								
	BSY	5								
	BSM	5								

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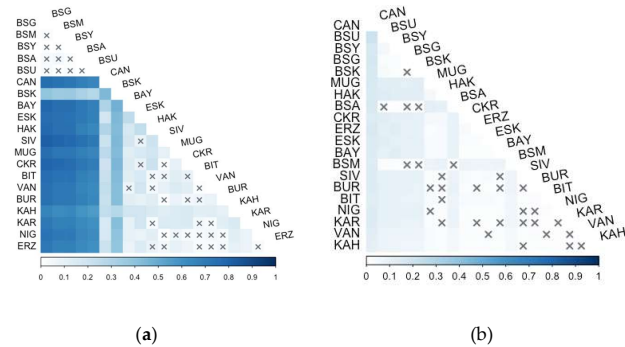


Figure 2. Heat map of pairwise F_{ST} values across populations, (a) mitochondrial, (b) microsatellite) of Chukar Partridges in Turkey. Darker shades of blue rectangles indicate higher values of F_{ST} (as displayed on the bar down of the heat map). Cross indicate F_{ST} p-values not significant ($p > 0.05$).

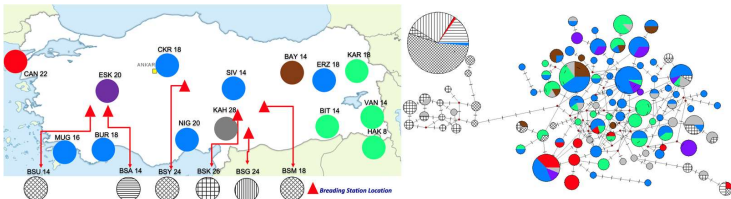


Figure 3. Population group defined in SAMOVA (six wild and four captive) based on mtDNA data. Circles represent geographic locations of wild populations and red angles represent the breeding stations on the map. Median-joining haplotype network constructed from the combined mt Cyt b and CR sequence data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

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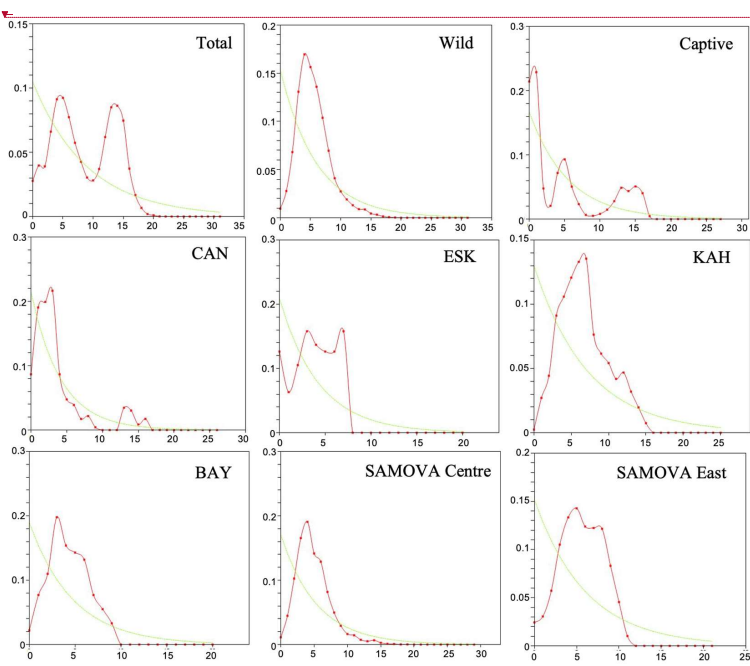


Figure 4. Mismatch distribution of Chukar Partridges using the SAMOVA groups. Pairwise differences of observed (red) and expected (green) values under the demographic expansion model.

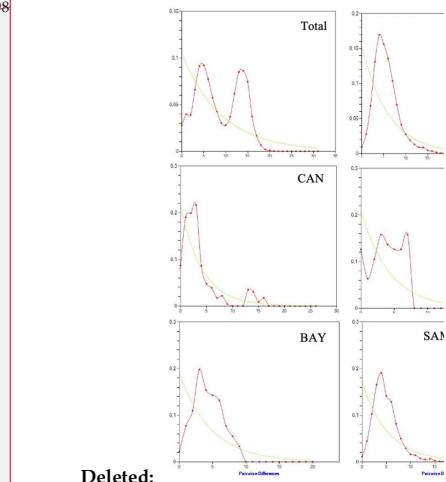
3.2. Microsatellite analysis

The mean number of alleles per locus varied from 6.6 to 13.2 across wild populations and 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). While private alleles were found in 11 populations, KAH was the one yielding the highest number (5 alleles; Figure 6). 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).

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.

Table 4. Summary statistics ($\pm$ SD) of genetic diversity in Chukar partridge. Haplotype diversity (H_d) and nucleotide diversity (p) expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{is}) for each sampling area. P values for heterozygote deficiency.

Location	N	A	H_o	H_e	p	F_{is}
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CAN	21	7.5±3.7	0.63±0.23	0.69±0.22	0.0002	0.0991
MUG	16	9.0±3.7	0.62±0.13	0.82±0.11	0.0000	0.2523
BUR	18	10.4±4.1	0.66±0.18	0.86±0.06	0.0000	0.2325
ESK	20	10.0±3.4	0.74±0.14	0.84±0.06	0.0000	0.1286
CKR	18	10.1±4.9	0.69±0.18	0.81±0.15	0.0000	0.1404
SIV	13	9.7±3.5	0.74±0.16	0.86±0.08	0.0000	0.1482
NIG	21	11.4±4.4	0.74±0.13	0.85±0.09	0.0000	0.1344
KAH	28	13.2±5.7	0.71±0.12	0.86±0.08	0.0000	0.1749
BAY	14	9.5±3.4	0.76±0.18	0.84±0.06	0.0000	0.1125
ERZ	19	10.2±3.6	0.79±0.09	0.84±0.08	0.0155	0.0506
KAR	18	11.3±4.4	0.78±0.15	0.87±0.06	0.0000	0.0995
BIT	13	10.1±3.2	0.79±0.17	0.86±0.08	0.0000	0.0790
VAN	14	9.3±3.5	0.77±0.18	0.86±0.07	0.0000	0.1080
HAK	8	6.6±2.4	0.71±0.26	0.84±0.08	0.0004	0.1644
BSA	15	7.0±2.8	0.72±0.14	0.75±0.15	0.1004	0.0473
BSG	23	8.2±3.3	0.73±0.15	0.73±0.18	0.1194	-0.0062
BSK	27	7.8±3.3	0.66±0.18	0.74±0.16	0.0000	0.0974
BSY	25	8.3±3.8	0.70±0.17	0.72±0.18	0.0152	0.0368
BSM	17	8.2±3.1	0.71±0.17	0.79±0.12	0.0000	0.1109
BSU	14	6.9±3.6	0.64±0.16	0.71±0.19	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, v: values: the number of specific alleles in each population.

Location	Aru1B3	Aru1E7	Aru1E97	Aru1G4	Aru1G49	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)	13 (6–10)	35 (6–20)
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
CAN	-/HWE	-/HWE	-/HWE	-/HD	-/HD	-/HD	-/HWE	-/HWE	-/HD	-/HWE
MUG	-/HD	-/HWE	1/HWE	-/HD	-/HD	-/HD	-/HWE	-/HWE	-/HD	-/HD
BUR	-/HD	-/HD	1/HD	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
ESK	-/HD	-/HWE	2/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
CKR	-/HD	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
SIV	-/HWE	-/HWE	-/HWE	1/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HWE	1/HWE
NIG	-/HWE	1/HD	2/HWE	-/HWE	-/HD	-/HE	-/HWE	-/HWE	-/HD	-/HWE
KAH	-/HD	-/HWE	-/HD	1/HWE	1/HD	1/HD	-/HWE	2/HWE	-/HD	-/HWE
BAY	-/HD	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE
ERZ	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	1/HWE
KAR	1/HWE	-/HWE	-/HWE	1/HWE	1/HD	-/HD	-/HWE	-/HWE	-/HWE	-/HWE
BIT	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HWE	2/HWE
VAN	-/HD	-/HWE	1/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE
HAK	1/HD	-/HWE	-/HD	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	2/HWE
BSA	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
BSG	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
BSK	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
BSY	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
BSM	-/HWE	-/HWE	-/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE
BSU	-/HWE	-/HWE	1/HWE	-/HWE	-/HD	-/HWE	-/HWE	-/HWE	-/HD	-/HWE

Structure Harvester indicated that the most likely number of clusters was K=2 (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

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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).

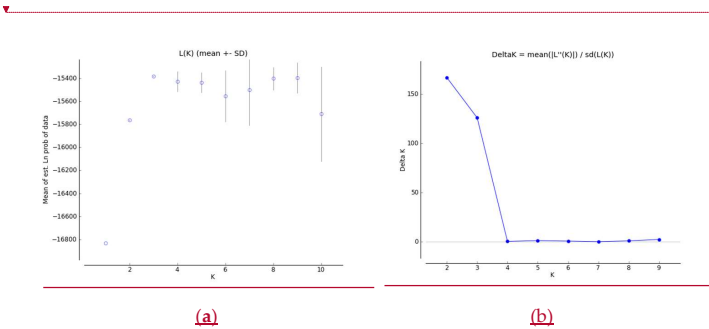


Figure 5. **a.** graph shows likelihood ($L(K)$) means for each number of cluster (K) from 10 independent runs in Structure Harvester (error bars represent SD). **b.** graph shows ΔK for each K based on the first and second-order rates of change.

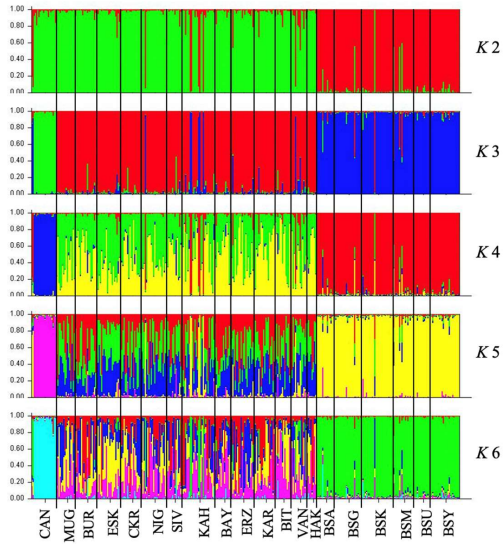
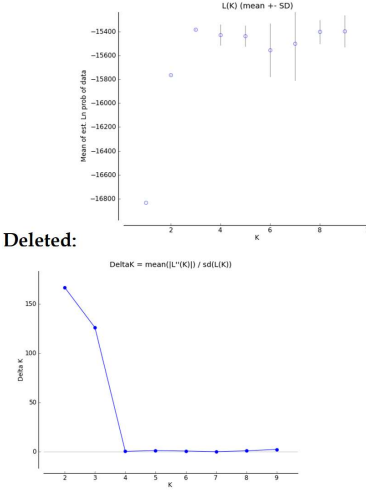


Figure 6. STRUCTURE assignment of 362 individual microsatellites for $K = 2$ to $K = 6$. Each individual genotype is represented by a vertical bar. Black lines separate the 20 different populations, 14 wild and six captive. The most likely number of clusters is $K = 3$. Besides, CAN chukars are clustered separately from other wild populations ($K = 3$ - $K = 6$).

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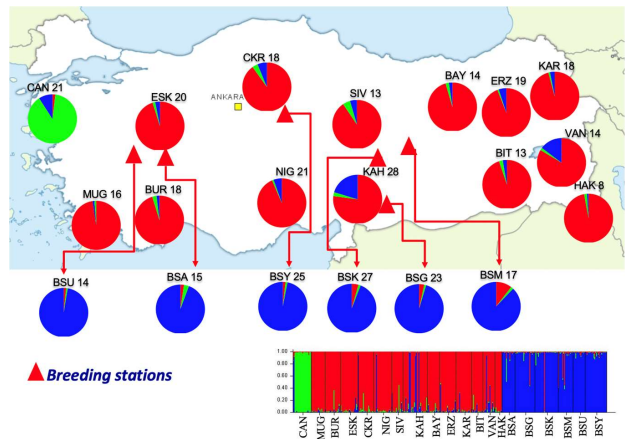


Figure 7. Proportion of wild (red) and farmed (blue) Chukar Partridges from 14 wild and six captive populations using a panel of ten microsatellites ($K = 3$). The sample size is shown above each respective pie chart.

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 using microsatellites. 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
CAN	21	0	90	10	0	0	0
MUG	16	100	0	0	0	0	0
BUR	18	94	0	0	6	0	0
ESK	20	95	0	0	0	5	0
CKR	18	89	0	6	6	0	0
NIG	21	95	0	5	0	0	0
SIV	13	85	0	0	8	8	0
KAH	28	75	0	18	4	4	0
BAY	14	100	0	0	0	0	0
ERZ	19	95	0	0	0	5	0
KAR	18	94	0	0	0	6	0
BIT	13	92	0	0	8	0	0
VAN	14	86	0	7	0	7	0
HAK	8	100	0	0	0	0	0
Total	241	83	8	4	2	2	0
BSA	15	0	0	93	0	0	7
BSG	23	0	0	96	0	4	0
BSK	27	4	0	93	0	0	4

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BSM	17	0	0	82	0	18	0
BSU	14	0	0	100	0	0	0
BSY	25	0	0	100	0	0	0
Total	121	1	0	94	0	3	2
Total	362	56	5	34	1	3	1

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 neighbouring east Anatolian localities.

4. Discussion

This study was threefold. First, we aimed to determine the population structure and phylogeography of the Chukar Partridges; second, to investigate whether there is any difference between wild and farmed chukars; and third to search for signatures of admixture between wild and captive.

4.1. Population genetic structure

Our mtDNA analyses of Chukar Partridges from Anatolia show that farmed chukars are genetically different from wild populations. The two genetic cluster emerged for the wild and captive chukar in Turkey fall within the western and eastern clade, respectively, found in other studies [26,38] (Figure 1). Moreover, microsatellites structure of Chukar Partridges showed that all the populations were divided into two clusters as wild and captive ($K = 2$). CAN, an island population (Gökçeada Island), is the most genetically differentiated 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 (Table 3). In particular, this genetic picture emerged from F_{ST} and SAMOVA of both genetic system used (Figure 2, 3, and 5). Even if the wild populations cluster together, still it is possible to detect some internal differentiation among eastern Anatolian, central Anatolian and ESK, KAH, and BAY populations and 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 Chukar Partridge [39]. When only wild or captive populations were evaluated themselves, it was found that the captive populations were more different from each breeding stations (captive $F_{ST} = 0.24$, wild $F_{ST} = 0.14$). This may be due to the fact that bloodlines used at breeding stations are 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* X *A. chukar* [14,15,18–21,27,40–45], *A. graeca* X *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

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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 (e.g. Red-Legged Partridges (*Alectoris rufa*) [48]). A similar problem was found in Mallard populations, another popular game bird in Europe with captive-bred individuals changing the gene pool of wild populations [26]. Casas et al. [27] showed that extinction risk of wild genetically intact 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 lower diversities in 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. Their multimodal mismatch distribution suggested that admixture of haplotypes from three previously isolated lineages (one of them was captive) might have occurred. Also, multimodal mismatch distribution of the separated population by SAMOVA $K=6$ 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” model. This model was supported because range shifts took place within Anatolia for Kruper's Nut-hatch, *Sitta krueperi* [49]. A dense forest cover existed in the northern Anatolia and its coastal belts [50]. Albayrak et al. [4] suggested that Anatolia might have hosted tree refugia for Kruper's Nut-hatch in Last Glacial Maximum (LGM). Through the Late Quaternary glacial-interglacial cycles affect demographic expansion of populations through the Late Quaternary glacial-interglacial cycles. The demographic population expansion experienced by many Anatolian species may be before 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 [26].

4.3. Taxonomic and conservation implications

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

To preserve the genetic diversity of *A. chukar*, the Chukar Partridge should be preserved in six management units, CAN, KAH, BAY, ESK, south-eastern Turkey, and central Turkey separately (Figure 3). Since the release of Chukar Partridges from farms is a common practice in Turkey, the individuals to be released into each area should belong to the local MU to preserve the Chukar Partridges' genetic structure. Specific conservation efforts should be applied to the population of Gökçeada Island (CAN) where Chukar Partridge releases should be banned due to potentially the new subspecies. We emphasize that the genetic diversity of Chukar Partridges and their six MUs should be considered when developing hunting, production and releasing policies to maintain the species'

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genetic integrity. The consequences of continued releases might be detrimental for long term conservation of wild Chukar Partridges.

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

Author Contributions: T.A. designed and directed the study. T.A., Ö.Ö., D.A. provided material. J.A.G.D. conducted the microsatellite analysis. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by The MAKU-HADYK-169 protocol controlled all the experiments on Chukar Partridges by MAKU, Local Ethical Committee on Animal Experiments regulations.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in GenBank with accession numbers MZ706294 to MZ706461.

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