

Passer hispaniolensis subpopulations in Turkey: allozyme variations and brief ecobiological notes

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Abstract: In this study, Spanish Sparrows were recorded in numerous locations and specimens were collected from Samsun, Çorum, and Denizli provinces in Turkey. The breeding subpopulations preferred various nesting sites, including wooded areas and White Stork nests. These sparrows were primarily observed in flocks of variable size and were rarely solitary. The largest flock recorded was found in juniper and pine woods in Denizli. We collected data on 23 allozyme loci to compare the genetic variation among the 3 Spanish Sparrow subpopulations. The low F_{ST} (0.1363) and high Nm (1.5842) values calculated from these data indicated that gene flow among the 3 subpopulations was high and that these subpopulations did not show substantial genetic isolation. The genetic distance (D) between Çorum and Denizli was particularly low (0.001), and these subpopulations showed the highest level of genetic similarity found in the study. Conversely, a high genetic distance (0.028) was found between Denizli and Samsun. The genetic structure of these subpopulations also demonstrated a low level of heterozygosity in the total population.

Key words: Allozyme, *Passer hispaniolensis*, Spanish Sparrow, Turkey

1. Introduction

The Spanish Sparrow, *Passer hispaniolensis*, is a small passerine bird. The females are usually not distinguishable from House Sparrow females, but the males of the 2 species differ: male Spanish Sparrows show broad black bibs, whitish cheeks, and black spots resembling drops on their flanks. This species is migratory in the western part and sedentary in the eastern part of the western Palearctic region. This species is not known to occur on the coasts of the Black Sea (but is common in the Kızılırmak Delta) or in the mountain areas of eastern and southeastern Turkey (Snow and Perrins, 1998; Mullarney et al., 2004); they migrate to Turkey to breed (Snow and Perrins, 1998). The Spanish Sparrow is the most colonial sparrow species in the Palearctic region (Summers-Smith, 1988). The birds typically nest in lowland areas, hot and humid areas, the edges of rivers or canals, olive groves, palm trees, acacia and eucalyptus trees, bushes, and reedbeds. The sparrows have also been reported to nest in forested or wooded areas where storks and eagles also nest (Snow and Perrins, 1998; Mullarney et al., 2004), and in human-made structures (Sacarrão and Soares, 1975; Alonso, 1984; Metzmacher, 1990; Marques et al., 2003). This species feeds primarily on seeds and can become a problem for cereal cultivation in certain regions (Marques et al., 2003).

Two subspecies of the Spanish Sparrow occur in Turkey (Roselaar 1995): *Passer hispaniolensis hispaniolensis* and *P. h. transcaspicus*. *P. h. hispaniolensis* is known to be distributed in western Turkey and *P. h. transcaspicus* in eastern Turkey, but the distribution of these subspecies in central Turkey is unknown.

P. hispaniolensis individuals have been observed between January and November in different localities in Turkey (Barış et al., 1984; Husband and Kasperek, 1984; Dijkzen and Kasperek, 1985; Kasperek, 1987; Kılıç and Kasperek, 1987; Dijkzen and Kasperek, 1988; Kılıç and Kasperek, 1989; Kırac, 1993; Kirwan, 1993; Jetz, 1995; Ertan, 1996; Kaya et al., 1999; Kirwan, 1999; Erdoğan, 2001; Aslan and Kızıroğlu, 2003; Kaya and Kurtonur, 2003; Karakaş and Kılıç, 2004; Perktaş and Ayaş, 2005; Nergiz and Tabur, 2007). Although avifaunal data on this species have therefore been recorded, no detailed studies of the population structure, abundance of colonies, or genetic and morphometric peculiarities are available for this species. Therefore, the aim of this study was to investigate the distribution of Turkish subpopulations of this species and to determine the morphological and genetic characteristics of the subpopulations.

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2. Materials and methods

This study was performed to determine the distribution and habitat preferences of Spanish Sparrows in various locations within the range of the species in Turkey, and then samples were collected in the provinces of Samsun (Çarşamba), Çorum (Alaca), and Denizli (Pamukkale) (Figure) to determine the genetic variation and genetic similarity among these subpopulations. The Denizli (Pamukkale) subpopulation within the distribution of *P. h. hispaniolensis* and the other subpopulations sampled in this study were in the central part of Turkey (Roselaar, 1995), where the distribution of the subspecies is unknown.

Spanish Sparrows were caught by mist netting; first, their coloration was evaluated to determine any differences between subpopulations, and then they were killed with ether to obtain breast muscle tissue samples, with permission from the Ankara University Local Ethics Committee for Animal Experiments. The homogenates obtained from breast muscle tissue were used, and all of the tissue was preserved at -80°C until the allozyme study was started. Nine specimens from Samsun, 15 from Çorum, and 8 from Denizli were examined by allozyme electrophoresis. The electrophoresis and staining protocols were modified from Shaw and Prasad (1970), Harris and Hopkinson (1976), Aebersold et al. (1987), Hillis et al. (1996), May (1998), Verimli et al. (2000), and Manchenko (2003). The allozyme variability was studied for the following 18 enzymes at 23 loci: ACON (4.2.1.3 aconitase hydratase, *Acon-m*); ALD (4.1.2.13 aldolase, *Ald*); CA (4.2.1.1 carbonic anhydrase, *Ca*); CK (2.7.3.2 creatine kinase, *Ck*); EST (3.1.1.1 esterase, *Est*); FUM (4.2.1.2 fumarase, *Fum*); G3PDH (1.1.1.8 glycerol-3-phosphate

dehydrogenase, *G3pdh-1*, *G3pdh-2*); GPI (5.3.1.9 glucose-6-phosphate isomerase, *Gpi*); IDH (1.1.1.42 isocitrate dehydrogenase, *Idh-s*, *Idh-m*); LDH (1.1.1.27 lactate dehydrogenase, *Ldh*); MDH (1.1.1.37 malate dehydrogenase, *Mdh-s*, *Mdh-m*); ME (1.1.1.40 malic enzyme, *Me-s*, *Me-m*); PGD (1.1.1.44 phosphogluconate dehydrogenase, *Pgd*); PGM (5.4.2.2 phosphoglucomutase, *Pgm*); PK (2.7.1.40 pyruvate kinase, *Pk*); PNP (2.4.2.1 purine nucleoside phosphorylase, *Pnp*); SOD (1.15.1.1 superoxide dismutase, *Sod-s*, *Sod-m*); and XDH (1.1.1.204 xanthine dehydrogenase, *Xdh*). The electrophoretic band patterns obtained were analyzed according to Harris and Hopkinson (1976). The presumptive alleles were designated alphabetically according to their relative mobility, and the electrophoretic data were evaluated with BIOSYS-II (Swofford and Selander, 1989). We calculated the following values: the allele frequencies (f), the mean number of alleles per locus (A), the proportion of polymorphic loci (P , 95% criterion; a locus was considered polymorphic if the frequency of the most common allele was ≤ 0.95) and the mean heterozygosity (H , H_o = observed and H_e = expected frequencies of heterozygotes under Hardy-Weinberg equilibrium). The amount of genetic divergence among the subpopulations was estimated with the indices of standard genetic identity (I) and distance (D). The genetic identity (I , the unbiased genetic identity) and distance (D , the unbiased genetic distance) values were calculated according to Nei (1978). F -statistics (F_{IS} , F_{IT} , and F_{ST}) were used to summarize the distribution of the genetic variation among and within the subpopulations. F_{IS} was used to represent the deficiency in heterozygosity due to inbreeding in the subpopulations. F_{IT} was used to



Figure. *P. hispaniolensis* sampling (DEN, ÇOR, and SAM) and observation localities in Turkey.

represent the total deficiency in heterozygosity due to inbreeding. F_{ST} was used to represent the total deficiency in heterozygosity due to the subpopulations. The impact of migration on F_{ST} was determined by the Nm value, given by the following formula according to Wright (1951, 1965): $Nm = (1 - F_{ST}) / 4(F_{ST})$, where N is the population size and m is the migration rate.

3. Results

3.1. Record locations, ecobiological notes, and morphological peculiarities

Spanish Sparrows were observed in many different localities but were only sampled in 3 locations in Turkey. Although Spanish Sparrows were observed less frequently than House Sparrows and Tree Sparrows, they were found to nest around small settlements (e.g., villages) along with the other 2 types of sparrow. Spanish Sparrows were found primarily on reeds, crops, shrubs, and trees during the field studies and were observed in dense flocks in these habitats. One of these flocks was observed on the reeds at the edge of a small watercourse (a canal) near a crop field in Çorum (Alaca), and a small flock consisting of several individuals was observed on crops in Hatay (Suvatlı). One of the small flocks was observed at the edge of a human-made water canal near Eber Lake (Afyonkarahisar) while they were drinking water and eating seeds of reeds. Spanish Sparrows were also observed to nest in White Stork nests in Mersin (Silifke), Çorum (Çatak village), and Samsun (Çarşamba). However, only one breeding pair was observed in a White Stork nest located on a power pole in Mersin (Silifke). In Çorum (Çatak village), Spanish Sparrows were found to occupy White Stork nests along with House Sparrows. In contrast to these sporadic sightings, Spanish Sparrows in Denizli (Pamukkale) were observed in large flocks in juniper and pine woods, which also contained many nests. These observations show that Spanish Sparrows were recorded in numerous areas during the field studies. Samples of the birds were collected in 3 different localities, Samsun (Çarşamba), Çorum (Alaca), and Denizli (Pamukkale) (Figure), and the nesting areas and abundance of these 3 subpopulations differed.

1. Samsun subpopulation (SAM): A dense flock nested in a White Stork nest on a tall tree in the garden of a village house in Samsun (Çarşamba) near the Yeşilirmak River. The birds were observed feeding in this garden. This flock was the most dense flock in a White Stork nest recorded in this study. Approximately 15 birds were observed in this nest. The breeding period of these birds occurred in June.

2. Çorum subpopulation (ÇOR): Individuals were observed in a flock consisting of approximately 20 birds, in reeds along the edge of a small canal near a wheat field in Çorum (Alaca). They were observed while they were feeding on the reed seeds during their migration in September. No nests were observed in the area.

3. Denizli subpopulation (DEN): Individuals were observed to nest in a dense flock consisting of approximately 100 birds in juniper and pine trees in Denizli (Pamukkale). Many nestlings and nests that had fallen from these trees were observed. Foxes were observed to eat these nestlings at night. The breeding period of these birds occurred in May. The birds were observed to leave this nesting site in July.

No within-sex color differences were observed in the individuals examined among the subpopulations, but the sexual dimorphism was very distinctive. During the breeding season, the males showed dark black areas on their neck and flank, and this coloration changed to resemble black drops after the breeding season in September. Moreover, the feathers on the dorsal side of their head and wings were dark chestnut during the breeding season.

3.2. Allozyme variations

A total of 5 of the 23 loci examined exhibited genetic variation among the 3 Spanish Sparrow subpopulations. The frequencies of the alleles detected at these loci by the allozyme variability analysis are shown in Table 1. All 5 of the loci were polymorphic in the ÇOR subpopulation. *Ck*, *Est*, and *Idh-s* were polymorphic for all of the subpopulations, although *Ca* was only polymorphic for the SAM and ÇOR subpopulations, and *Idh-m* was only polymorphic for the ÇOR and DEN subpopulations. The percentage of polymorphic loci (P) and the observed (H_o) and expected (H_e) heterozygosity are shown in Table 2. ÇOR had the highest percentage of polymorphic loci at 21.7%, whereas the other localities had identical values of 17.4%.

The SAM subpopulation at the *Ca* locus ($\chi^2 = 8.000$, $P = 0.005$); the ÇOR subpopulation at the *Est* ($\chi^2 = 8.337$,

Table 1. Allele frequencies of polymorphic loci in 3 *P. hispaniolensis* subpopulations.

Locus	Allele	Subpopulation		
		SAM	ÇOR	DEN
<i>Ca</i>	A	0.500	0.900	1.000
	B	0.500	0.100	0.000
<i>Ck</i>	A	0.667	0.233	0.250
	B	0.333	0.767	0.750
<i>Est</i>	A	0.125	0.208	0.250
	B	0.875	0.792	0.750
<i>Idh-s</i>	A	0.944	0.800	0.500
	B	0.056	0.200	0.500
<i>Idh-m</i>	A	1.000	0.733	0.750
	B	0.000	0.267	0.250

Table 2. Genetic variability at 23 loci in 3 *P. hispaniolensis* subpopulations (standard errors in parentheses).

Subpopulation	Mean sample size per locus	Mean number of alleles per locus (A)	Percentage of polymorphic loci* (P)	Mean heterozygosity (H)	
				Direct-count (Ho)	HdyWbg expected** (He)
SAM	8.8 (0.2)	1.2 (0.1)	17.4	0.069 (0.045)	0.059 (0.031)
ÇOR	14.9 (0.1)	1.2 (0.1)	21.7	0.027 (0.017)	0.071 (0.030)
DEN	7.9 (0.1)	1.2 (0.1)	17.4	0.033 (0.024)	0.076 (0.036)

*: A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

** : Unbiased estimate (see Nei, 1978).

$P = 0.004$), *Idh-s* ($\chi^2 = 17.530$, $P = 0.000$), and *Idh-m* ($\chi^2 = 16.762$, $P = 0.000$) loci; and the DEN subpopulation at the *Idh-s* ($\chi^2 = 9.143$, $P = 0.002$) and *Idh-m* ($\chi^2 = 10.182$, $P = 0.001$) loci showed significant deviations from Hardy–Weinberg equilibrium.

Although the mean F_{IS} and F_{IT} were 0.3962 and 0.4785, respectively, the *Ca* locus values of $F_{IS} = -0.6667$ and $F_{IT} = -0.2308$ indicated higher heterozygosity than the other loci. The mean F_{ST} was 0.1363 for all loci, and the *Nm* value was 1.584 (Table 3). Because $Nm > 1$ and, conversely, F_{ST} was relatively low, the total deficiency in heterozygosity due to the subpopulations was low. There was not high variation among the subpopulations. Among the 3 Spanish Sparrow subpopulations, the highest genetic distance (D) was 0.028 between DEN and SAM (Table 4).

4. Discussion

The Spanish Sparrow has not been previously found or recorded along the coast of the Black Sea, except in the Kızılırmak Delta (Bafra/Samsun). Therefore, this is the first report of Spanish Sparrows in Samsun (Çarşamba), near the Yeşilirmak River along the coast of the Black Sea.

Spanish Sparrows have been reported to use nest cavities in buildings, a breeding pattern similar to that of House Sparrows (Mullarney et al., 2004). We never observed nesting behavior of this type in the Turkish

population. In the present study, these sparrows were found primarily around houses in villages and were observed in dense flocks or as solitary individuals during the breeding season. These data are consistent with the findings of previous studies (Heinzel et al., 1995; Snow and Perrins, 1998; Mullarney et al., 2004). Although some researchers suggest that this species breeds in large flocks that may contain thousands of birds (Sacarrão and Soares, 1975; Summers-Smith, 1988; Metzmacher, 1990), such a dense flock was not found at our study locations in Turkey. We observed smaller flocks that consisted of approximately 4 or 5 birds, and the maximum, 100 birds, was observed only in the Denizli subpopulation.

We observed this species feeding primarily on the seeds of reeds and cereals. Although this species has been reported to be a problem for cereal cultivation in certain regions (Marques et al., 2003), we did not find any damage to crops during our study period and farmers did not mention it. There is no information about this problem in Turkey.

Certain passerine birds, including sparrows, frequently nest in the nests of White Storks (Bocheński, 2005; Tryjanowski et al., 2006; Kosicki et al., 2007), and *P. domesticus* and *P. montanus* were recorded more frequently in White Stork nests than *P. hispaniolensis* (Tobolka, 2011). In the Spanish Sparrow subpopulations

Table 3. F-statistics in loci in 3 *P. hispaniolensis* subpopulations.

Locus	F_{IS}	F_{ST}	F_{IT}	<i>Nm</i>
<i>Ca</i>	-0.6667	0.2615	-0.2308	0.7060
<i>Ck</i>	0.2722	0.1607	0.3892	1.3057
<i>Est</i>	0.2941	0.0106	0.3016	23.3349
<i>Idh-s</i>	0.8974	0.1515	0.9129	1.4002
<i>Idh-m</i>	1.0000	0.0906	1.0000	2.5094
Mean	0.3962	0.1363	0.4785	1.5842

Table 4. Matrix of coefficients of genetic similarity and distance (below the diagonal, genetic identity [I] [Nei, 1978]; above the diagonal, genetic distance [D] [Nei, 1978]) in 3 *P. hispaniolensis* subpopulations.

No.	Subpopulation	SAM	ÇOR	DEN
1	SAM	*****	0.017	0.028
2	ÇOR	0.983	*****	0.001
3	DEN	0.972	0.999	*****

that used the White Stork nests for breeding, we found that either flocks or a single breeding pair used these nests. This species was also occasionally observed to share these nests with *P. domesticus*, but not with *P. montanus*.

Morphological differences were not found among the subpopulations, but differences in coloration were found between the breeding and nonbreeding males. During the breeding season, the males were darker, primarily on their head and wing feathers and on their bibs and flank spots. These findings are consistent with previous results (Heinzel et al., 1995; Snow and Perrins, 1998).

The first *Passer* allozyme studies were conducted by Bush (1967) and Bush and Fraser (1969) using *P. domesticus*, and such studies are relatively common for *P. domesticus*. *P. hispaniolensis* was analyzed in an allozyme study that included only one sample (Parkin, 1988; Anderson, 2006); no other allozyme studies of this species were found in the literature. Therefore, the present study is the first to investigate allozyme variation in this species.

The ratios for the 5 polymorphic loci among the 23 loci evaluated in these 3 subpopulations are as follows: *Ca* (2/3), *Ck* (3/3), *Est* (3/3), *Idh-s* (3/3), and *Idh-m* (2/3). *Ca* showed a deviation from Hardy–Weinberg equilibrium only for SAM, and *Idh-s* and *Idh-m* showed a deviation from Hardy–Weinberg equilibrium for ÇOR and DEN. The average heterozygosity in the birds was determined as 0.045–0.065 (Barrowclough, 1983; Evans, 1987; Bates, 2000), and the average rate of polymorphic loci was between 0.222 and 0.240 (Corbin, 1983; Evans, 1987; Baker and Johnson, 1998). The estimated genetic distance value (D), based on allozymes and calculated according to Nei or Rogers, is generally lower in birds (Van Wyk et al., 2001; Saag et al., 2007) than in mammals. The genetic distance between bird species was estimated to be approximately 0.044, and the genetic distance between bird subspecies was estimated to be approximately 0.005 (Barrowclough, 1980). The average genetic distance at

the species level in Passeriformes was 0.148 (Ohta et al., 2000). In allozyme studies of the Spanish Sparrow's close relative *P. domesticus*, the average heterozygosity in natural populations was 0.074–0.157 and the rate of polymorphic loci was 27.8%–50% (Anderson, 2006). In the present study, the average heterozygosity was 0.072 among the *P. hispaniolensis* subpopulations, and the rate of polymorphic loci was 17.4%–21.7%. The highest D value found was 0.028 between the SAM and DEN subpopulations. These values are comparable to those found in previous studies of passerine birds. The genetic distance between ÇOR and DEN was 0.001. This value, the lowest found in the study, showed that the genetic similarity between these subpopulations was the highest among the subpopulations studied. According to the data from the allozyme studies, the genetic structure of these subpopulations showed a heterozygosity deficiency in the direction of $H_o < H_e$. The genetic variation within the species was 13.63%, and the calculated gene migration value of 1.5842 (>1) indicated that these subpopulations do not show significant genetic differentiation. The subpopulations' influences on the genetic variation of the total population were also low.

Accordingly, the level of gene migration was found to be relatively high among the 3 subpopulations sampled and might have produced less specialization in the subpopulations. DEN was found to be relatively genetically similar to the ÇOR subpopulation, but the genetic distance between DEN and SAM was high. Within this context, the high genetic distance between the DEN and SAM subpopulations may support the findings of Roselaar (1995), who explained that there were subspecies of this species in Turkey. The SAM subpopulation might be assigned to a new subspecies based on future research.

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