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Population structure and loss of genetic diversity in the endangered white-headed duck, *Oxyura leucocephala*

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Abstract

The white-headed duck is a globally threatened species native to the Palaearctic with a range extending from Spain in the west to the western edge of China in the east. Its populations have become fragmented and undergone major declines in recent decades. To study genetic differences between populations across the range and change in genetic diversity over time, we sequenced a portion of the mitochondrial DNA control region from 67 museum specimens (years 1861–1976) as well as 39 contemporary samples from Spain and seven from Greece (years 1992–2003). In the historical sample, we found a lack of significant genetic structure between populations in different areas. We found evidence that the species experienced a rapid expansion in the past, perhaps from glacial refugia centred around the Mediterranean following the last ice age. In Spain, the population went through a dramatic bottleneck in the 1970s and early 1980s, when only a few dozens individuals remained in the wild. Although population size has since recovered to a few thousand individuals, we found a highly significant loss of mitochondrial haplotype diversity between the historical and contemporary samples. Given ongoing declines in other areas, losses in genetic diversity that may reduce the adaptive potential of white-headed ducks in the future are a continuing concern throughout the geographic range of this species.

Introduction

Molecular genetic data obtained from museum specimens can provide information on the historical distribution and population structure of a species, as well as recent changes in its genetic diversity. Historical data may therefore improve our understanding of current patterns of diversity and contribute information critical to the development of management plans for endangered species (Cooper et al. 1996; Groombridge et al. 2000; Godoy et al. 2004; Leonard et al. 2005). Populations that are genetically distinct may require separate management (Moritz 1994), whereas source populations for reintroduction projects should be as genetically similar as possible to the historical population in an area (IUCN 1998). Mottled duck populations in Florida and Louisiana/Texas, for example, exhibit significant genetic differentiation, a result consistent with slight morphological differences and a lack of documented movements between these areas, thus providing increased impetus for managing these populations as evolutionarily significant units (McCracken et al. 2001; Williams et al. 2005).

The white-headed duck, *Oxyura leucocephala*, is the only stifftail (Oxyurini) native to the Palaearctic and is a globally threatened species

(classified as Endangered by the IUCN, BirdLife International 2000). Historically, it had an extensive range from Spain in the west to westernmost China in the east (Figure 1). At present, four populations of white-headed ducks are recognised, with two sedentary populations in the west and two largely migratory populations in the east (Scott and Rose 1996; Wetlands International 2002). The largest is a migratory population estimated at 5000–10,000 birds found in the Eastern Mediterranean, Middle East and Western Asia, with most birds breeding in Kazakhstan, Russia, the Caspian region and Turkey, and wintering in the Caspian region, eastern Mediterranean, and Black Sea countries (especially Turkey, Greece, Bulgaria and Israel). A second migratory population breeding in eastern Russia, western Mongolia, western China and the easternmost part of Kazakhstan, and wintering in Pakistan and Afghanistan, appears to be on the verge of extinction (Li and Mundkur 2003). A sedentary population estimated at 400–600 birds occurs in Tunisia and Algeria and a second sedentary population of around 2500 birds occurs in Spain, with occasional records from Morocco.

Since 1900, the species has suffered extensive population declines and fragmentation of its distribution, with extinctions of small populations in Egypt, Italy, Hungary and other European countries (Figure 1; Green and Hughes 2001). Birds in Egypt formed part of the main eastern migratory population, whereas those in Sardinia and other parts of Italy were probably associated with the population in Algeria and Tunisia. It is estimated that the global population exceeded 100,000 birds in 1900, but has since decreased to 8,000–13,000 owing to destruction of wetland habitats and hunting (Green and Anstey 1992; Wetlands International 2002). In Spain the population was severely impacted by hunting and population counts reached a low of only 22 individuals in 1977. An effective conservation programme, however, enabled a major recovery from this bottleneck leading to a maximum count of 4500 birds in 2000 (Torres and Moreno-Arroyo 2000; Almaraz and Amat 2004). Declines are continuing, however, in the eastern part of the range (Li and Mundkur 2003). In particular, numbers in the main Eastern population have crashed since 1991, including the loss of up to 11,000 birds that

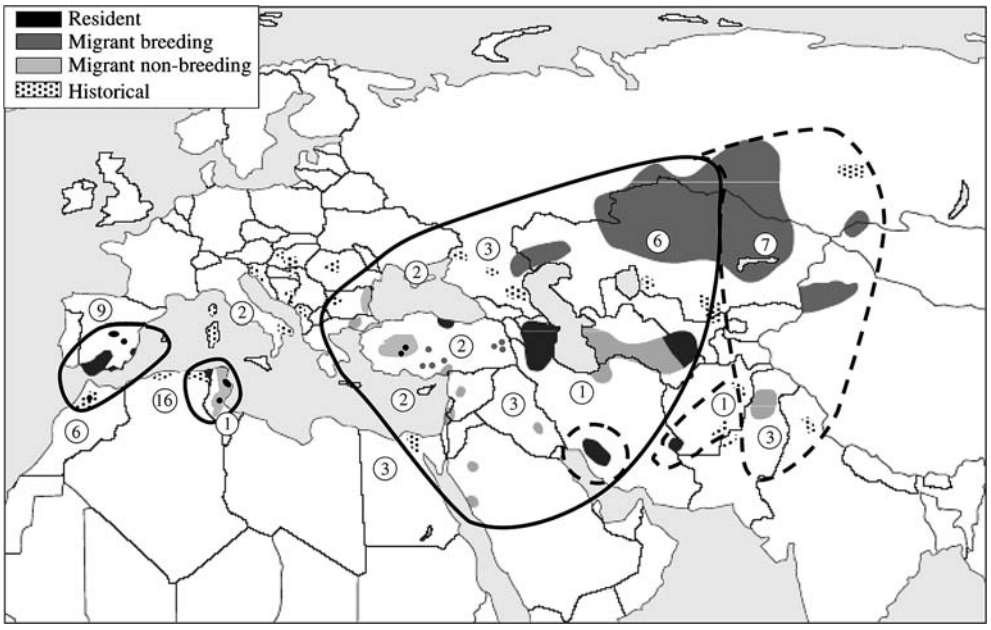

Figure 1. Past and present distribution of white-headed ducks. Information on distribution is based on: localities of the samples used in this study and on information in Green and Anstey (1992), Green and Hughes (2001) and Hughes and Green (2005). Numbers within circles indicate number of historical samples (years 1861–1976) from each country. Population boundaries are based on Scott and Rose (1996): dashed line = uncertain.

wintered at Burdur Lake in Turkey (Green et al. 1996).

In the present study, we assess the genetic diversity of historical and contemporary white-headed duck populations. Using museum specimens, we quantify mitochondrial DNA (mtDNA) variation in individuals collected between 1860 and 1976 to determine whether significant geographic structure existed prior to recent declines. In particular, we test whether eastern and western populations are genetically differentiated, as previously suggested by morphometric analyses (Amat and Sánchez 1982). We also test the prediction that the dramatic bottleneck suffered by the species would be reflected by a lower level of mtDNA diversity in the contemporary Spanish population. Finally, we consider the implications of our results for conservation programs.

Methods

Samples

A total of 113 white-headed duck individuals was analysed in this study (Appendix A.1). Of these, 46 were from contemporary specimens (years 1992–2003), including seven white-headed ducks from Greece and 39 from Spain. From these birds, fresh tissue was obtained, including blood, brain, muscle or feathers. Contemporary samples of white-headed ducks were from animals either injured and placed in recovery centres or found dead in the field. The other 67 samples were from museum specimens (years 1861–1976). From these, feathers or footpads were obtained, depending on the preference of museum curators. Museum specimens were selected from across the range, but with a special emphasis on the population in Spain and neighbouring Morocco prior to the recent bottleneck. We considered 1976 as the cut off point for historical samples because significant population declines in the east have occurred since then (Green et al. 1996; Li and Mundkur 2003). In Spain, the population began to pass through the bottleneck in the 1970s, but all of the historical samples from Spain were collected prior to 1968.

We also analysed 29 white-headed duck×ruddy duck (*Oxyura jamaicensis*) hybrids as a source of supplementary information on mtDNA diversity in the contemporary Spanish population. The North American ruddy duck was introduced to Europe about 50 years ago and hybridisation with an expanding ruddy duck population is now considered a major threat to the survival of white-headed ducks (Green and Hughes 2001). Previous studies have shown that the two species have divergent mtDNA sequences (McCracken et al. 2000) and we confirmed this for the control region sequences we used in this study (Muñoz-Fuentes et al., in prep.). From our sample of Spanish hybrids, 19 individuals had white-headed duck mtDNA, providing an opportunity to increase the total number of white-headed duck haplotypes sampled and therefore increased our chance of detecting low frequency haplotypes in the contemporary population. We recognise that hybrid individuals might yield a biased sample of mtDNA haplotypes due to possible cytonuclear interactions between mtDNA and a hybrid nuclear background (Arnold 1997) and therefore we completed analyses both with and without the data collected from hybrid individuals.

Molecular techniques

DNA extraction was performed using the protocol outlined in Gemmell and Akiyama (1996) or, in the case of feathers and occasionally for other tissues, using the DNeasy Tissue Kit (Qiagen). In the case of feathers, 30 μl of 100 mg/ml dithiothreitol (DTT) was added to the digestion buffer to achieve complete digestion of feather quills (Cooper 1994).

A portion of the mtDNA control region was amplified and sequenced. In the case of extracts from fresh tissue, we sequenced a region corresponding to nucleotide positions 82–767 in the chicken mitochondrial sequence (Desjardins and Morais 1990), which includes most of domain I and part of domain II. We used the primers L81 (5′-TATTTGGYTATGYAYRTCGTGCAT-3′) and H768 (5′-TATACGCMAACCGTCT-CATYGAG-3′) to amplify and sequence a product of 574 base pairs (bp). Given the lower quality of DNA extracts from museum specimens, we sequenced a smaller portion of the same region for the museum samples. For recently collected museum specimens (collection dates 1992 and 1993), we used primers L81 (see above) and H493 (Sorenson and Fleischer 1996) to amplify a region

of 300 bp. For the older historical samples (1861–1976), we used primers OxCF1a (5′-CCAGTACATATATTGATAGCCC AAC-3′) and OxCR1a (5′-GCTAGTCATAACG GACATTACGTG-3′) to amplify a region of 192 bp. Both of the shorter fragments included all of the polymorphic sites present in the 574 bp sequences for contemporary samples.

DNA was amplified using the polymerase chain reaction (PCR) carried out in 50-μl reactions containing 1× Buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 1 mM dNTPs (0.25 mM each), 0.5 μM forward primer, 0.5 μM reverse primer, 25–100 ng of genomic DNA and 1 U of AmpliTaq DNA polymerase (Applied Biosystems). PCRs were performed in a GeneAmp PCR System 9700 (Applied Biosystems) or PTC-100 Programmable Thermal Controller (MJ Research) using the following conditions: one segment of 94 °C for 1 min; 35 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 1 min; and a final segment of 72 °C for 7 min. We used AmpliTaq Gold DNA polymerase and its associated buffer (Applied Biosystems) when working with extracts from museum specimens. Thermal conditions were: one segment of 95 °C for 6 min; 45 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 1 min; and a final segment of 72 °C for 7 min.

PCR products were run in 1% agarose gels, excised, and purified using the QIAquick Gel Extraction Kit (QIAGEN). Both strands of each product were sequenced using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) in 11-μl reactions. We used Sephadex (G-50 Fine) spin columns to remove unincorporated dNTPs, and then electrophoresed reaction products in an automated sequencer (ABI 377 or ABI 3100, Applied Biosystems). Sequences from opposite strands were reconciled and edited using Sequence Navigator (Applied Biosystems), and were then aligned by eye using Se-Al v1.0a1 (Rambaut 1996). Sequences have been submitted to GenBank (Accession Numbers: AY836375-AY836506).

We found no evidence of nuclear copies (or Numts; see Sorenson and Quinn 1998) in our sequences. No double peaks were found when examining the electropherograms and DNA extracts from tissues that differ in the relative number of mtDNA and nuclear copies (e.g., blood and muscle tissue) provided equally clean and identical sequences.

Analysis of data

To measure mtDNA diversity, both haplotype diversity, Hd, and nucleotide diversity, π, and their standard deviations were estimated using DnaSP v4.0 (Rozas et al. 2003). To illustrate relationships among haplotypes, we constructed an unrooted parsimony network using TCS, version 1.13 (Clement et al. 2000). We tested for differences in haplotype diversity and nucleotide diversity between the historical sample from Spain and the contemporary sample from Spain and between the western historical samples (Spain, Morocco, Algeria, Tunisia) and the eastern historical samples (including all of the remaining sampling localities). To test whether the observed differences between populations were statistically significant, we performed permutation tests by randomizing haplotypes between populations and recalculating both indices (Hd and π) 1000 times. Significance was measured as the proportion of permuted data sets yielding greater differences in diversity measures between populations than in the observed data. To test for a decline in genetic diversity in Greece, we compared the contemporary Greek sample with the combined historical samples from the eastern half of the range, also doing permutation tests. In comparing historical versus contemporary diversity (Spain contemporary versus Spain historical, and Greek contemporary versus east historical), we used a 1-tailed test because our *a priori* prediction was lower diversity in the post-bottleneck populations (see Introduction). We used a 2-tailed test for the comparison of western versus eastern populations because we had no *a priori* prediction of which geographic region would have greater diversity.

To assess population genetic structure, we used analysis of molecular variance (AMOVA) (Excoffier et al. 1992) as implemented in ARLEQUIN 2.001 (Schneider et al. 2000). The analysis was based on historical samples only. We calculated Φ-statistics, analogues of *F*-statistics that incorporate information about genetic distance between haplotypes, and molecular variance components for the effects of individuals, populations and groups. Significance of both the Φ-statistics and the variance components was assessed using a permutation approach, which requires few assumptions and overcomes the problem of non-normality found in molecular data (Excoffier et al.

1992). We defined the following populations based on geographical proximity and knowledge of migratory movements (Scott and Rose 1996; Green and Hughes 2001; see Introduction): (i) eastern Kazakhstan, Pakistan and Afghanistan (EKaPaAf; $n = 11$); (ii) Turkey, Cyprus, Egypt, Iraq, Iran, Ukraine, Russia and western Kazakhstan (Tk-WKa; $n = 22$); (iii) Algeria, Tunisia and Italy (AlTuIt; $n = 19$); (iv) Morocco and Spain (MoSp; $n = 15$) (Figure 1). These populations were then grouped into east (i) and (ii) and west (iii) and (iv). Given the lack of band return data for white-headed ducks, there is uncertainty about possible movements among populations and we therefore repeated AMOVA analyses with alternative groupings along an east–west axis (see Results). Ideally, an analysis of population structure for a migratory waterfowl species should also consider possible differences between the sexes as well as location of sampling (i.e., breeding, migration, or wintering areas). Given relatively small sample sizes and reliance on museum collections, this was not possible in our analysis, but our examination of population structure on an east to west axis should be relatively unaffected by such factors given predominantly north to south migration (see Figure 1).

To test for evidence of recent population expansion we constructed a mismatch distribution and calculated Fu's *F*s (Fu 1997) and Fu and Li's (1993) *D** and *F** statistics to compare with Fu's *F*s. Thus, if *F*s is significant and *F** and *D** are not, it is an indication of population expansion, while the opposite indicates selection (Fu 1997). We also calculated the expansion coefficient (S/d), where S is the number of variable sequence positions and d is the mean number of pairwise nucleotide differences. A large value indicates recent population expansion and a small value constant population size (von Haeseler et al. 1996). We did not calculate Tajima's *D* (Tajima 1989) because *F*s is more powerful in detecting population expansion (Fu 1997; Ramos-Onsins and Rozas 2002). We used ARLEQUIN 2.001 (Schneider et al. 2000) and DnaSP v4.0 (Rozas et al. 2003) to perform these calculations. We also used a coalescent-based method as implemented in FLUCTUATE v.1.4 (Kuhner et al. 1998) to test for evidence of population expansion: FLUCTUATE should be more robust than simple metrics because it uses more of the information present in the data. The programme was run several times to ensure convergence of the estimates.

Results

Mitochondrial DNA variation

Among the contemporary samples of white-headed ducks from Spain and Greece, we found three different haplotypes for the 574 bp control region fragment (Table 1), and one among hybrids (Oleu_03) that was found in four individuals from Spain but not in any white-headed ducks. Given that all of the variable nucleotide sites defining these haplotypes occurred within a 148 bp portion of the control region, we amplified and sequenced for museum specimens a shorter, 192 bp region encompassing all of these variable sites. Among museum specimens we found 10 variable sites that defined 10 different haplotypes, among which Oleu_01, 02 and 03 were also found. The 11 variable sites found in both historical and contemporary samples included two with transversions (A–C) and nine with transitions (G–A, T–C).

We reconstructed the relationships among haplotypes in the pre-1976 historical sample using an unrooted parsimony network (Figure 2). There was no evidence of homoplasy among these closely related sequences (i.e., a single mutation at each variable site is sufficient to explain the data). Two common, central haplotypes, Oleu_01 and Oleu_02, differed from each other by one mutational step (Table 1, Figure 2), with Oleu_01 present in 45% and Oleu_02 in 30% of individuals. All other haplotypes were one or two steps divergent from one of the two central haplotypes and were less frequent, being found in only one to six individuals.

Past population diversity and structure

The genetic diversity of the historical sample was relatively high, with 10 haplotypes (Table 1). All haplotypes found in more than one individual were present in more than one population and were often geographically widespread. Thus, in addition to the two common haplotypes (Oleu_01 and 02), Oleu_07 was found in Algeria and Kazakhstan and haplotype Oleu_11 was present in

Table 1. Haplotypes observed in white-headed ducks defined by variable sites in the 192 bp portion of the control region sequenced along with number of individuals with that particular haplotype and their geographical location. Numbers within parentheses indicate white-headed duck×ruddy duck hybrids from Spain (only those with white-headed duck mtDNA)

	Nucleotide position											Geographical origin																	Total	
	1	2	3	3	5	7	8	12	12	13	15	Contemporary		Historical																
Haplotype	2	3	3	5	2	0	8	7	9	9	9	SpC	Gr	Sp	Mo	It	Al	Tu	Tk	Eg	Cy	Uk	Ru	Ka	Iq	Ir	Af	Pa	Cont	Hist
Oleu_01	G	G	T	C	C	C	G	A	C	C	A	26 (11)	6	3	5		3	1	1	2		2		9	1	1	1	1	43	30
Oleu_02	.	A	.	.	.	.	.	.	.	.	.	13 (4)		3			8		1	1	1		1	2	1			2	17	20
Oleu_03	.	A	.	.	.	.	.	.	.	.	G	(4)			1		2												4	3
Oleu_04	.	.	.	.	.	A	.	.	.	.	.		1																1	
Oleu_05	.	A	.	.	.	.	.	.	.	T	G			1																1
Oleu_06	.	A	.	.	T	.	.	.	.	.	.													1						1
Oleu_07	.	A	.	.	.	.	.	C	.	.	.						1							1						2
Oleu_08	.	.	.	.	.	.	.	.	T	.	.										1									1
Oleu_09	A	.	.	.	.	.	A	.	.	.	.					1														1
Oleu_10	.	.	C	.	.	.	.	.	.	.	.			1			1													2
Oleu_11	.	.	.	T	.	.	.	.	.	.	.			1		1	1						2		1					6
Total												39 (19)	7	9	6	2	16	1	2	3	2	2	3	13	3	1	1	3	65	67

Contemporary samples correspond only to Spain and Greece, the rest are historical samples. Sp, Spain; Gr, Greece; Mo, Morocco; It, Italy; Al, Algeria; Tu, Tunisia; Tk, Turkey; Eg, Egypt; Cy, Cyprus; Uk, Ukraine; Ru, Russia; Ka, Kazakhstan; Iq, Iraq; Ir, Iran; Af, Afghanistan; Pa, Pakistan; Cont, Contemporary; Hist, Historical.

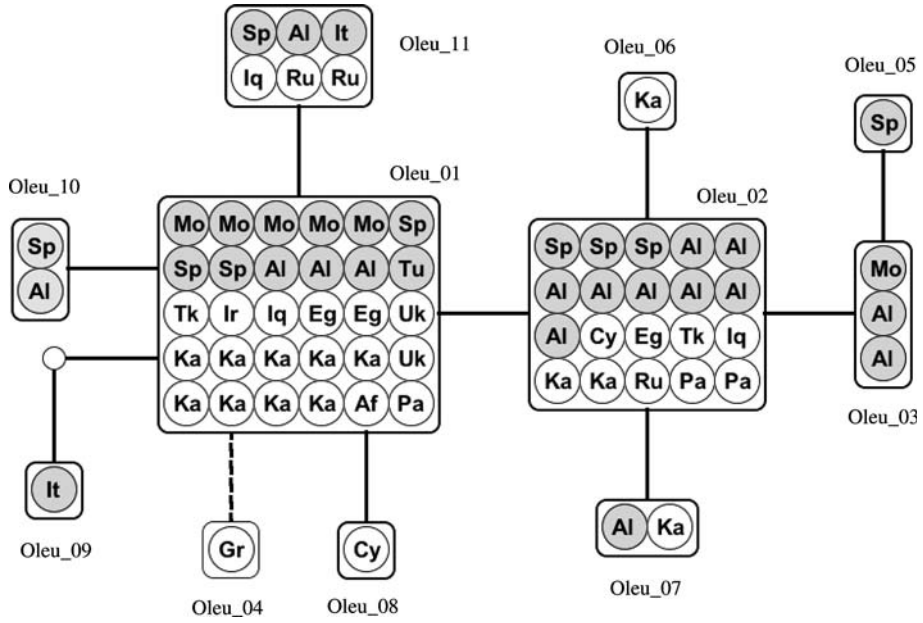

Figure 2. Haplotype network of white-headed ducks. Only historical samples are represented here, except for haplotype Oleu_04, which was found only in one contemporary sample (dotted line). Each circle corresponds to one individual and each box to one haplotype. Lines indicate one mutation. An empty circle corresponds to a haplotype not found. The letters inside the circle indicate country of origin (as defined in Table 1). Grey circles correspond to western populations and white circles to eastern populations (see text).

Spain, Italy, Algeria, Russia and Iraq (Table 1). Also, a diversity of haplotypes was found within a given location (Figure 2), such as in Spain (5 haplotypes among 9 individuals) or Algeria (6 haplotypes among 16 individuals). Thus, no obvious geographic structure was evident in the distribution of haplotypes.

Pairwise comparisons of Φ_{ST} between populations indicate that populations were not significantly differentiated regardless of the geographical distance between them ($P > 0.05$ in all cases). A pairwise comparison of sedentary populations in the western Mediterranean and migratory ones in the eastern Mediterranean and Asia was also non-significant ($\Phi_{ST} = 0.00946$; $P = 0.24$). Likewise, hierarchical AMOVAs based on alternative groupings of populations along an east to west axis suggest a complete lack of geographic structure (Table 2).

Despite the lack of significant population structure, the level of genetic diversity appeared to vary across the species range, with a trend towards

Table 2. Hierarchical AMOVAs for different groupings of white-headed ducks along a west to east axis. All Φ_{CT} and Φ_{SC} values were non-significant ($P > 0.05$). In the three analyses, either the two eastern populations (1), the two western populations (2), or both (3) are grouped at a higher hierarchical level. For country abbreviations see Table 1.

Group number	Groupings	Mitochondrial haplotype variation		
		Among groups, Φ_{CT}	Among populations within groups, Φ_{SC}	Within populations (%)
1	[SpMo] [AlTuIt] [Tk_WKaz, EKaAfPa]	0.013	0.000	98.68
2	[SpMo, AlTuIt] [Tk_WKa] [EKaAfPa]	−0.003	0.014	98.92
3	[SpMo, AlTuIt] [Tk_WKa, EKaAfPa]	0.006	0.008	98.69

higher diversity in the west. Two haplotypes observed in the east of the range (each found in one individual) were not observed in the west (Table 1), whereas four haplotypes from the west (found in one, one, two, and three individuals, respectively) were not observed in the east. Dividing the historical sample into west (Spain, Morocco, Algeria, Italy and Tunisia) versus east (all other locations), we found haplotype diversity estimates of 0.772 and 0.636, and nucleotide diversity estimates of 0.0065 and 0.0042, respectively (Table 3). Using the permutation approach (see Methods), the differences in haplotype diversity ($P = 0.103$) and nucleotide diversity ($P = 0.077$) approached statistical significance (Table 4).

Population size changes: past demography

The mismatch distribution was unimodal (Figure 3), a pattern that is compatible with recent population expansion (Rogers and Harpending 1992). This distribution is also strongly skewed to the left, reflecting the small number of nucleotide differences between haplotypes and suggesting that population expansion may have been recent. This view is also supported by the haplotype network (Figure 2), with two common haplotypes in the centre of the network and several rare ones that radiate from them (Slatkin and Hudson 1991). Fu's *F*s was negative and significant ($Fs = -4.56$; $P = 0.01$), whereas Fu and Li's F^* and D^* were not significant ($F^* = -2.02$ and $D^* = -1.88$; $P > 0.05$ in both cases). These results reject a neutral model with constant population size and are compatible with population expansion (Fu 1997). The expansion coefficient ($S/d = 9.66$) and exponential growth parameter obtained with FLUCTUATE ($g \approx 10^3$) also were consistent with recent population expansion.

Historical versus contemporary samples: recent loss of genetic variability

In Spain, there were fewer haplotypes in the contemporary sample than in the historical sample, with a bias towards the common haplotypes Oleu_01 and Oleu_02 (Table 1). Haplotype and nucleotide diversity were also lower at present than in the past (Table 3). Interestingly, haplotype frequencies in the contemporary samples of white-headed ducks and hybrids from Spain differed significantly ($\chi^2 = 9.07, P = 0.011$), with hybrids showing somewhat greater diversity (note that hybrids with ruddy duck haplotypes are excluded here). Although this result suggests a possible bias in the haplotype composition of hybrids, it unambiguously indicates that haplotype Oleu_03 must be extant in the Spanish white-headed duck population. Given greater diversity among hybrids, including them in our comparison of present versus past mtDNA variation is conservative with respect to our prediction of lower genetic diversity at present. Using the permutation approach (see Methods), we find significantly lower haplotype and nucleotide diversity at present than in the past, whether the hybrid sample is included or not (Table 4). Similarly, only two haplotypes were found in the Greek contemporary sample ($n = 7$

Table 3. Mitochondrial diversity in the white-headed ducks analysed in this study

Individuals	*n*	No. of haplotypes	Haplotype diversity, Hd*	Nucleotide diversity, π*
Overall historical sample	67	10	0.708 ± 0.040	0.00539 ± 0.00061
West historical sample	34	8	0.772 ± 0.047	0.00647 ± 0.00093
Spain historical, Morocco	15	6	0.705 ± 0.114	0.00585 ± 0.00151
Algeria, Tunisia, Italy	19	7	0.789 ± 0.076	0.00694 ± 0.00131
East historical sample	33	6	0.636 ± 0.069	0.00422 ± 0.00069
Eastern Kazakhstan, Pakistan, Afghanistan	11	4	0.673 ± 0.123	0.00473 ± 0.00115
Turkey, Cyprus, Egypt, Russia, Ukraine, Iraq, Iran, western Kazakhstan	22	4	0.636 ± 0.080	0.00392 ± 0.00071
Spain contemporary	39	2	0.456 ± 0.053	0.00238 ± 0.00028
Spain contemporary, including hybrids	58	3	0.511 ± 0.052	0.00313 ± 0.00041
Greek contemporary sample	7	2	0.286 ± 0.196	0.00149 ± 0.00102

In all cases, nucleotide and haplotype diversities were calculated for the 192-bp fragment that was sequenced in all samples and where all variable sites were found. Sample size. * Values ± standard deviation.

Table 4. Observed haplotype (Hd) and nucleotide (π) diversity in different groups of white-headed ducks, and *P*-values obtained from comparing observed differences between groups with the distribution of values obtained after randomizing haplotypes between populations 1000 times (see text)

	Hd	π
Regional		
West ($n = 34$)	0.772	0.00648
East ($n = 33$)	0.636	0.00422
Difference	0.135	0.00226
P	0.103	0.077
Spain		
Historical ($n = 9$)	0.833	0.00752
Recent ($n = 39$)	0.456	0.00237
Difference	0.377	0.00515
P	0.002	0.002
Spain, incl. hybrids		
Historical ($n = 9$)	0.833	0.00752
Recent ($n = 58$)	0.511	0.00313
Difference	0.323	0.00439
P	0.003	0.003
Greece		
Historical* ($n = 33$)	0.636	0.00422
Recent ($n = 7$)	0.286	0.00149
Difference	0.351	0.00273
P	0.136	0.120

*The historical sample used for the Greece comparison was the combined sample for all eastern populations.

individuals). Because no historical samples were available from Greece, we compared the contemporary Greek samples with the combined historical samples from the migratory populations in the eastern half of the range, assuming that this would reasonably reflect the potential diversity of the Greek population prior to population declines. While both haplotype and nucleotide diversity were lower in the contemporary Greek sample, neither difference reached statistical significance (Table 4), perhaps due to the low power provided by the small sample size for the contemporary population.

Discussion

Genetic diversity and population structure

Our analyses of historical samples indicate that the white-headed duck lacked strong genetic differentiation across its extensive range. Given the limited diversity in mtDNA haplotypes, however, and the relatively recent ancestry of all white-headed duck mtDNA lineages, it is difficult to separate recent migration among populations and recent coancestry as the explanation for the lack of genetic structure. Our analyses provide evidence for population expansion as seen from the haplotype network (Figure 2), the mismatch distribution (Figure3), the negative and significant value of Fu's Fs, and the non-significant values of Fu and Li's F^* and D^*. The expansion coefficient (S/d) and the maximum likelihood estimate for the parameter g also reached values interpreted in other studies as indicative of population expansions (von Haeseler et al. 1996; Lessa et al. 2003; Peck and Congdon 2004). Although both population expansion and a recent selective sweep can lead to rejection of a neutral model with constant population size (Fu 1997; Ramos-Onsins 2002), consistent results from the variety of tests applied above suggest population expansion as the most likely explanation. Thus, all white-headed duck populations may derive from a single ancestral population that was recent on an evolutionary time scale. Given the absence of fossil evidence and the fact that our data from historical samples comprise a short segment of the highly variable control region, it is difficult to establish a molecular clock. Portions of the control region evolve up to ten times as fast as the rest of the mtDNA in some waterfowl (Quinn 1992), so the small number of mutations separating white-headed duck haplotypes might have accumulated in the last few thousand years. Expansion from a single refugium following the most recent retreat of glaciers from Europe c. 10,000 years ago (Birks and Ammann 2000) seems consistent with the data.

The lack of significant genetic structure between east and west, coupled with slightly higher haplotype and nucleotide diversity in the west (Table 1, Table 3), suggest that glacial refugia for white-headed ducks may have been centred around the Mediterranean region during the last ice age. However, given relatively small sample sizes, differences in haplotype and nucleotide diversity between east and west were not statistically significant (Table 4).

The extinction of the white-headed duck in Egypt and several parts of central and eastern Europe between 1900 and 1960 (Green and Anstey 1992; Green and Hughes 2001) probably reduced

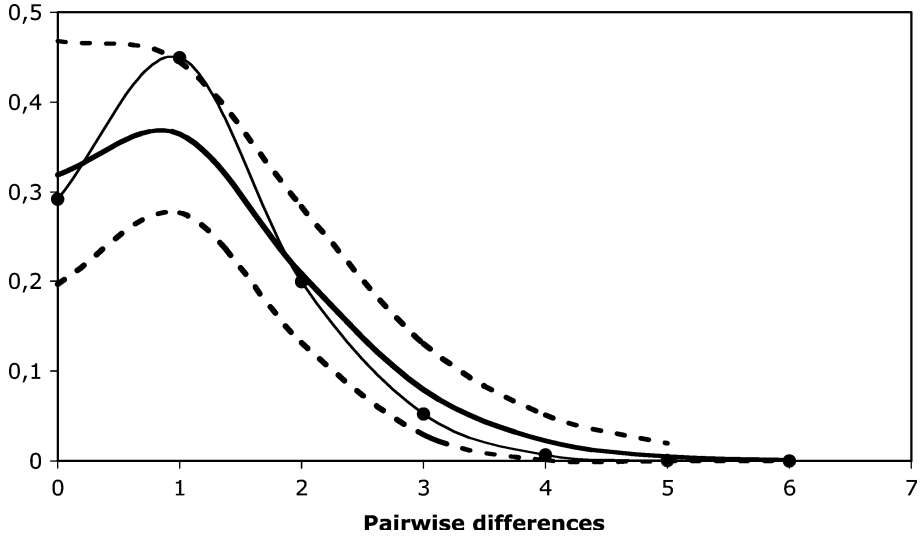

Figure 3. Observed mismatch distribution (thin line) based on mtDNA control region sequences for the historical sample of white-headed ducks, and distribution fitted to the data (thick line) assuming population expansion. The dashed lines indicate the 97.5 and 2.5 percentile values based on 1000 permutations. The observed distribution is compatible with recent population expansion.

the level of interchange between populations. While remaining populations may currently be isolated from each other due to range reduction and fragmentation over the last 100 years, we observed no genetic structure using mtDNA. In contrast, Amat and Sánchez (1982) found morphological differences between eastern and western populations. In a study of museum skins, western birds had significantly greater bill length and height and western males had more yellowish tertiary wing feathers (Amat and Sánchez 1982). However, these characters are affected by the methods used to preserve skins, and a separate study with a larger sample size found no significant differences (Violani and Grandi 1991; Brichetti and Violani 1992). Although selection due to varying environmental conditions could produce ecologically relevant differences that are not reflected in neutral genetic markers, slight morphological differences might also reflect purely phenotypic responses to varying environments.

While the power of our analyses to detect small differences in haplotype frequencies was limited by small sample size, we can conclude that all white-headed duck populations share recent common ancestry and show no evidence of the relatively deep historical divisions that molecular data have revealed in some other waterfowl species (Avise et al. 1990; Quinn 1992; McCracken et al. 2001; Paxinos et al. 2002; McCracken and Sorenson 2005), nor any evidence of significant structuring of mitochondrial variation produced by strong female philopatry (Scribner et al. 2001; Tiedemann et al. 2004). Lack of genetic structure coupled with recent expansion, apparently following the retreat of ice sheets after the last glacial maximum, has been observed in other avian species (Milá et al. 2000; Zink et al. 2002), including eiders (Pearce et al. 2004). In other migratory waterfowl breeding at high latitudes, slight but significant mitochondrial structure has apparently developed since the last glacial maximum (Ruokonen et al. 2004, 2005). Although little is known about philopatry and dispersal in white-headed ducks, their breeding chronology and social system differ markedly from other northern hemisphere ducks (Green and Hughes 2001), perhaps contributing to the observed lack of structure in maternally inherited mtDNA.

Loss of genetic variability

Our analyses indicate a substantial loss of genetic diversity in Spain and possibly other areas, in marked contrast to the genetic signature of an expanding population and a considerable level of haplotype diversity in the historical samples. Despite the small number of historical samples for

Spain, our analyses show significantly lower haplotype and nucleotide diversity in the contemporary population and an overall reduction in genetic diversity by about half (Table 4).

The additional sample of mtDNA haplotypes from hybrids, although apparently biased, was informative in revealing an additional haplotype (Oleu_03) that must be extant in the contemporary white-headed duck population in Spain. The different frequencies of mtDNA haplotypes in hybrids could be due to cytonuclear interactions (Arnold 1997), although strongly different interactions between these closely related mtDNA haplotypes and a hybrid nuclear background seem somewhat unlikely. Other possible explanations include somewhat different spatial distribution of the white-headed duck and hybrid individuals we sampled, non-independence of some hybrid samples (three of four hybrid samples with haplotype Oleu_03 were from the same locality and could have been related), or simply chance.

Implications for conservation

Low genetic variability at a given genetic locus may reflect one or more bottlenecks in a species' history, a recent selective sweep, inbreeding effects and/or low mutation rates (Amos and Harwood 1998; Charlesworth et al. 2003; Jiggins 2003). In the case of the contemporary white-headed duck population in Spain, the most likely explanation for low genetic variability is the severe bottleneck suffered by the population in the 1970s. Although the population is now recovering in size (Torres and Moreno-Arroyo 2000; Almaraz and Amat 2004), it appears that a considerable amount of genetic variability has been lost.

Because the population in Spain has expanded to several thousand birds (Torres and Moreno-Arroyo 2000), both genetic drift and loss of diversity are likely to have slowed. However, if the loss of genetic diversity detected in the control region of the mtDNA is representative of variation in ecologically important traits (Reed and Frankham 2001), the adaptive and evolutionary potential of the Spanish white-headed duck population may have been reduced by the bottleneck (e.g., Keller et al. 1994; Nieminen et al. 2001; Frankham et al. 2002; Reed et al. 2003). Although we did not collect data for contemporary populations in the east, a loss of genetic variation may also have occurred in other regions where populations have undergone a reduction in size (e.g., the population wintering in Pakistan; Li and Mundkur 2003). Therefore it is necessary and urgent to develop international management programmes to conserve remaining populations.

Despite the lack of mtDNA differentiation, the study of nuclear markers is required before concluding that all white-headed duck populations are part of the same management unit (MU) in the sense proposed by Moritz (1994). Furthermore, as recently discussed by Crandall et al. (2000), it is important to take into account not only the concept of "genetic exchangeability" but also the concept of "ecological exchangeability". In the case of the white-headed duck, eastern populations are migratory whereas western populations are generally sedentary, although this may simply reflect a flexible behavioural response to winter freezing of aquatic habitats in areas used for breeding by the eastern populations. Even if further study suggests there is no difference between these populations in nuclear markers, additional comparison of populations based on behavioural and morphological data is ideally required to make a full assessment of whether white-headed duck populations are differentiated in any meaningful way.

Reintroduction projects have taken place or are planned in Corsica, Hungary, Italy and Mallorca (Green and Hughes 1996; Hughes et al. 2004), and there has been considerable debate over the past 15 years about the merits of using birds from the captive population in Spain (which was derived from Spanish birds) or the UK (derived from birds captured in Pakistan) for such reintroductions. Birds bred in the UK were released in Hungary between 1986 and 1988 (Green and Hughes 1996). Based on our results, there is no evidence from mitochondrial markers that different lineages would be mixed or that genetic diversity would be lost by using these captive sources of birds for release. Nevertheless, if birds used for translocations or reintroductions originate from neighbouring populations, as recommended by the IUCN (IUCN 1998), they are more likely to be ecologically compatible. Given the observed lack of genetic variation in the Spanish population, we

Appendix A.1

Samples used in this study

Sample code	Organism[1]	Sex[2]	Locality and date	Country	Source[3]	Tissue type[4]	Haplotype
KAZ-1	*Oleu*	f	Ili River Delta; 1934	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-2	*Oleu*	?	Ili River Delta; 1948	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-3	*Oleu*	m	Ili River Delta 75 E 45 N; 22 Apr. 1948	Kazakhstan	AIZ	Footpad	Oleu_07
KAZ-4	*Oleu*	m	East Coast of Aral Sea 61 E 46 N; 1960–1970	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-5	*Oleu*	f	84 E 48 N; 1950	Kazakhstan	AIZ	Footpad	Oleu_02
KAZ-6	*Oleu*	m	Ili River Delta; 1954	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-7	*Oleu*	m	Kostanay Region/Qostanay (N); 1957	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-8	*Oleu*	m	Kostanay Region 64.30 E 54 N; 1958	Kazakhstan	AIZ	Footpad	Oleu_02
KAZ-9	*Oleu*	m	69 E 51 N; 1958	Kazakhstan	AIZ	Footpad	Oleu_01
KAZ-10	*Oleu*	f	Naurzum (southern Kostanay region); 1971–1976	Kazakhstan	VCNZ	Footpad	Oleu_01
KAZ-11	*Oleu*	m	Kostanay Region/Qostanay (N); older than 1970	Kazakhstan	KNSM	Footpad	Oleu_01
AMNH 424784	*Oleu*	m	Mellaha, Lake Marius; 24 Nov. 1920	Egypt	AMNH	Footpad	Oleu_01
AMNH 424785	*Oleu*	m	Mellaha, Lake Marius; Jan. 1918	Egypt	AMNH	Footpad	Oleu_02
AMNH 734074	*Oleu*	m	Crimea; Jun. 1910	Ukraine	AMNH	Footpad	Oleu_01
AMNH 734075	*Oleu*	f	Crimea; Jun. 1910	Ukraine	AMNH	Footpad	Oleu_01
AMNH 734077	*Oleu*	m	Petrowsk; 10 Apr. 1894	Russia	AMNH	Footpad	Oleu_11
AMNH 734083	*Oleu*	m	3 Apr. 1894	Morocco	AMNH	Footpad	Oleu_01
AMNH 734084	*Oleu*	?	8 Apr. 1894	Morocco	AMNH	Footpad	Oleu_01
AMNH 734085	*Oleu*	?	7 Apr. 1894	Morocco	AMNH	Footpad	Oleu_01
EBD 22194A	*Oleu*	f	Cádiz; 1968	Spain	EBD	Feathers	Oleu_01
EBD 22195A	*Oleu*	m	Cádiz; 1966	Spain	EBD	Feathers	Oleu_01
EBD 22196A	*Oleu*	?	Cádiz; 1968?	Spain	EBD	Feathers	Oleu_10
INFS 2244	*Oleu*	m	Laguna di Mistras, Cabras, Sardinia; 8 Nov. 1911	Italy	INFS	Feathers	Oleu_11
INFS 2291	*Oleu*	f	Unknown; 1900–1946	Italy	INFS	Feathers	Oleu_09
MAK 6387	*Oleu*	f	Lake Fetzara, Annaba; 9 Jul. 1917	Algeria	MAK	Feathers	Oleu_02
MAK 6388	*Oleu*	m	Lake Fetzara, Annaba; 13 Jul.1917	Algeria	MAK	Feathers	Oleu_01
MAK 6389	*Oleu*	m	Lake Fetzara, Annaba; 13 Jul. 1917	Algeria	MAK	Feathers	Oleu_10
MAK 6390	*Oleu*	f	Lake Fetzara, Annaba; 13 Jul. 1917	Algeria	MAK	Feathers	Oleu_02
MAK 6391	*Oleu*	m	Lake Fetzara, Annaba; 6 Jun. 1917	Algeria	MAK	Feathers	Oleu_03
MCZ 149634	*Oleu*	f?	SW Siberia, Semipalatinsk; May. 1922	Kazakhstan	MCZ	Feathers	Oleu_01
MCZ 158863	*Oleu*	m	Siberia, Semipalatinsk; 1922	Kazakhstan	MCZ	Feathers	Oleu_06
MCZ 158864	*Oleu*	f	Rostov-on-Don, vicinity Koisug; 28 Sept. 1910	Russia	MCZ	Feathers	Oleu_02
MCZ 58233	*Oleu*	m	South Russia (Volga, Sarper); 20 May. 1911	Russia	MCZ	Feathers	Oleu_11
MNHN 1861–451	*Oleu*	?	Uncertain location (Diff?); 1861 or older	Tunisia	MNHN	Footpad	Oleu_01
MNHN 1963–333	*Oleu*	f	Uncertain location (Oued Betb?); 11 Nov. 1939	Morocco	MNHN	Footpad	Oleu_03
MNHN 1973–153	*Oleu*	m	Doïet Roumi; 2 May. 1952	Morocco	MNHN	Feathers	Oleu_01
NHM 1893.5.3.1	*Oleu*	m	Peshawar; 30 Mar. 1893	Pakistan	NHM	Footpad	Oleu_01
NHM 1894.6.1.688	*Oleu*	m	Ghilzai, Khandahar; 20 Oct. 1897	Afghanistan	NHM	Footpad	Oleu_01
NHM 1915.7.28.1	*Oleu*	m	Laguna Modina [?Medina], N.E. Cadiz; 27 June 1915	Spain	NHM	Footpad	Oleu_01
NHM 1915.7.28.2	*Oleu*	f	Laguna Modina [?Medina], N.E. Cadiz; 27 June 1915	Spain	NHM	Footpad	Oleu_11
NHM 1921.4.15.2	*Oleu*	f?	Qalta; 20 Jan. 1921	Egypt	NHM	Footpad	Oleu_01

Appendix A.1 (Continued)

Sample code	Organism[1]	Sex[2]	Locality and date	Country	Source[3]	Tissue type[4]	Haplotype
NHM 1924.5.30.1	*Oleu*	m	60 miles N of Baghdad; 16 Mar. 1924	Irak	NHM	Footpad	Oleu_01
NHM 1924.5.30.2	*Oleu*	m	60 miles N of Baghdad; 16 Mar. 1924	Irak	NHM	Footpad	Oleu_11
NHM 1924.5.30.3	*Oleu*	m	60 miles N of Baghdad; 16 Mar. 1924	Irak	NHM	Footpad	Oleu_02
NHM 1941.5.30.9237	*Oleu*	f	Laguna de Sautololla (?), Doñana; 8 May 1883	Spain	NHM	Footpad	Oleu_05
NHM 1955.3.47	*Oleu*	m	4 May. 1910	Cyprus	NHM	Footpad	Oleu_08
NHM 1955.3.48	*Oleu*	f	Dec. 1910	Cyprus	NHM	Footpad	Oleu_02
NHM 1965.M.962	*Oleu*	m	Coto Doñana; 18 May. 1922	Spain	NHM	Footpad	Oleu_02
NHM 1965.M.963	*Oleu*	m	Quetta, Baluchistan; 24 Mar. 1914	Pakistan	NHM	Footpad	Oleu_02
NHM 1969.43.33	*Oleu*	f	Guadalquivir near Nuevas; 11 Feb. 1914	Spain	NHM	Footpad	Oleu_02
NHM 1985.2.3	*Oleu*	f	Khabbaki (?) Lake, Punjab Salt Range; 21 Feb. 1965	Pakistan	NHM	Footpad	Oleu_02
NRS 5853	*Oleu*	m	Lake Fetzara, Annaba; 25 May. 1913	Algeria	NRS	Footpad	Oleu_02
NRS 5854	*Oleu*	m	Lake Fetzara, Annaba; 2 Jun. 1913	Algeria	NRS	Footpad	Oleu_03
NRS 5855	*Oleu*	m	Lake Fetzara, Annaba; 2 Jun. 1913	Algeria	NRS	Footpad	Oleu_02
NRS 5856	*Oleu*	f	Lake Fetzara, Annaba; 6 Jul. 1913	Algeria	NRS	Footpad	Oleu_07
NRS 5857	*Oleu*	f	Lake Fetzara, Annaba; 10 Jul. 1913	Algeria	NRS	Footpad	Oleu_11
NRS 5858	*Oleu*	f	Lake Fetzara, Annaba; 10 Jul. 1913	Algeria	NRS	Footpad	Oleu_02
SMNS 39239	*Oleu*	m	Izmir; Feb 1861	Turkey	SMNS	Feathers	Oleu_01
SMNS 4785	*Oleu*	m	Gulega, 11Km west of Pahlevi; 24 Feb. 1960	Iran	SMNS	Feathers	Oleu_01
ZMA.ES1	*Oleu*	m	Jérez; 1966	Spain	ZMA	Feathers	Oleu_02
ZMA.TK1	*Oleu*	m	1969	Turkey	ZMA	Feathers	Oleu_02
ZMB 46.950	*Oleu*	f	Fetzara Lake, Annaba; 5 Jul. 1917	Algeria	ZMB	Feathers	Oleu_01
ZMB 46.951	*Oleu*	m	Fetzara Lake, Annaba; 13 Jul. 1917	Algeria	ZMB	Feathers	Oleu_02
ZMB 46.952	*Oleu*	m	Fetzara Lake, Annaba; 13 Jul.1917	Algeria	ZMB	Feathers	Oleu_01
ZMB B 892.No.1	*Oleu*	f	Fetzara Lake, Annaba; 13 Jul. 1917	Algeria	ZMB	Feathers	Oleu_02
ZMB B 892.No.2	*Oleu*	m	Fetzara Lake, Annaba; 13 Jul. 1917	Algeria	ZMB	Feathers	Oleu_02
ZMB No.3	*Oleu*	m	10 Apr. 1894	Morocco	ZMB	Feathers	Oleu_01
257M	*Oleu*	m	Vistonida Lake, Xanthi; 1999–2001	Greece	OHS	Muscle	Oleu_01
FJ3	*Oleu*	f?	Vistonida Lake, Xanthi; 1999–2002	Greece	OHS	Muscle	Oleu_01
FJ4	*Oleu*	f?	Vistonida Lake, Xanthi; 1999–2003	Greece	OHS	Muscle	Oleu_01
FJ5	*Oleu*	f?	Vistonida Lake, Xanthi; 1999–2004	Greece	OHS	Muscle	Oleu_01
FJ6	*Oleu*	f?	Vistonida Lake, Xanthi; 1999–2005	Greece	OHS	Muscle	Oleu_01
FJ7	*Oleu*	f?	Vistonida Lake, Xanthi; 1999–2006	Greece	OHS	Muscle	Oleu_04
M2	*Oleu*	m	Vistonida Lake, Xanthi; 1999–2007	Greece	OHS	Muscle	Oleu_01
53CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
54CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
59CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
60CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
61CG	*Oleu*	m	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
63CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
64CG	*Oleu*	m	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
66CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
67CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
69CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
71CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
72CG	*Oleu*	m	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
73CG	*Oleu*	?	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
75CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
76CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
77CG	*Oleu*	m	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
80CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01

Appendix A.1 (Continued)

Sample code	Organism[1]	Sex[2]	Locality and date	Country	Source[3]	Tissue type[4]	Haplotype
81CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
83CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
84CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
85CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_02
86CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
88CG	*Oleu*	f	El Hondo, Alicante; 1999	Spain	CRFES	Brain	Oleu_01
Oleu0161/01	*Oleu*	?	El Hondo, Alicante; 2000	Spain	CRFES	Muscle	Oleu_01
Oleu0343/02	*Oleu*	m	El Hondo, Alicante; 2002	Spain	CRFES	Muscle	Oleu_02
Oleu1687/01	*Oleu*	?	El Hondo, Alicante; 2001	Spain	CRFES	Muscle	Oleu_01
Oleu1688/01	*Oleu*	?	El Hondo, Alicante; 2001	Spain	CRFES	Muscle	Oleu_01
Oleu1904/02	*Oleu*	m	El Hondo, Alicante; 2002	Spain	CRFES	Muscle	Oleu_01
Oleu1906/02	*Oleu*	f	El Hondo, Alicante; 2002	Spain	CRFES	Muscle	Oleu_01
OleuAL-13	*Oleu*	f?	Rambla Morales, Almería; 2002	Spain	CMA	Muscle	Oleu_02
OleuAL-14	*Oleu*	f?	Rambla Morales, Almería; 2002	Spain	CMA	Muscle	Oleu_01
Oleu 7037121	*Oleu*	?	Lentejuela, Sevilla; 2003	Spain	EBD	Blood	Oleu_01
Oleu 7037123	*Oleu*	m	Lentejuela, Sevilla; 2003	Spain	EBD	Blood	Oleu_01
Oleu 7080040	*Oleu*	?	Lentejuela, Sevilla; 2003	Spain	EBD	Blood	Oleu_01
Oleu 7080042	*Oleu*	?	Lentejuela, Sevilla; 2003	Spain	EBD	Blood	Oleu_01
Oleu 7080043	*Oleu*	?	Lentejuela, Sevilla; 2003	Spain	EBD	Blood	Oleu_02
PND2	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1992	Spain	CRFES	M feathers	Oleu_01
PND4	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1992	Spain	CRFES	M feathers	Oleu_02
PND10	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1992	Spain	CRFES	M feathers	Oleu_01
PND11	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1992	Spain	CRFES	M feathers	Oleu_02
PND29	*Oleu*×*Ojam*	f	Albufera Adra, Almería; 1993	Spain	PND	M feathers	Oleu_01
PND33	*Oleu*×*Ojam*	f	El Hondo, Alicante; 1993	Spain	CRFES	M feathers	Oleu_01
PND34	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1993	Spain	CRFES	M feathers	Oleu_01
PND35	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1993	Spain	CRFES	M feathers	Oleu_01
PND38	*Oleu*×*Ojam*	?	Dehesa de Monreal, Toledo; 1993	Spain	PND	Brain	Oleu_02
PND39	*Oleu*×*Ojam*	m	Veta la Palma, Sevilla; 1993	Spain	PND	Brain	Oleu_02
PND42	*Oleu*×*Ojam*	m	Albufera Adra, Almería; 1993	Spain	PND	Brain	Oleu_01
PND43	*Oleu*×*Ojam*	m	Veta la Palma, Sevilla; 1993	Spain	PND	Brain	Oleu_01
PND45	*Oleu*×*Ojam*	f	Tarelo, Cádiz; 1993	Spain	PND	Brain	Oleu_01
PND51	*Oleu*×*Ojam*	m	El Hondo, Alicante; 1993	Spain	CRFES	Brain	Oleu_01
PND52	*Oleu*×*Ojam*	m	Salinas de Cerrillos, Almería; 1993	Spain	PND	Brain	Oleu_01
PND110	*Oleu*×*Ojam*	f	Laguna de Tíscar, Córdoba; 2000	Spain	CMA	Muscle	Oleu_03
PND124	*Oleu*×*Ojam*	m	El Hondo, Alicante; 2000	Spain	CRFES	Muscle	Oleu_03
PND126	*Oleu*×*Ojam*	f	El Hondo, Alicante; 2000	Spain	CRFES	Muscle	Oleu_03
PND128	*Oleu*	f	El Hondo, Alicante; 2001	Spain	CRFES	Muscle	Oleu_01
PND129	*Oleu*	f	El Hondo, Alicante; 2001	Spain	CRFES	Muscle	Oleu_02
PND130	*Oleu*	f	El Hondo, Alicante; 2001	Spain	CRFES	Muscle	Oleu_02
HybRH5018	*Oleu*×*Ojam*	?	El Hondo, Alicante; 2002	Spain	CRFES	F feathers	Oleu_03

[1] Organism: Oleu, *O. leucocephala*; *Oleu*×*Ojam*, *O.leucocephala*×*O. jamaicensis*.

[2] Sex: f, female; m, male.

[3] Individuals or institutions contributing samples (see also Acknowledgements): AIZ, Almaty Institute of Zoology, Almaty, Kazakhstan; AMNH, American Museum of Natural History, New York, USA; CMA, Consejería de Medio Ambiente de la Junta de Andalucía, Spain; CRFES, Centro de Recuperación de Fauna de El Saler, Generalitat Valenciana, Valencia, Spain (previously CPEMN); EBD, Estación Biológica de Doñana, Sevilla, Spain; ICONA, Instituto para la Conservación de la Naturaleza, Spain; INFS, Istituto Nazionale per la Fauna Selvatica, Italy; KNSM, Kostanay Natural Sciences Museum, Kostanay, Kazakhstan; MAK, Museum Alexander Koenig, Bonn, Germany; MCZ, Harvard Museum of Comparative Zoology, Cambridge, USA; MNHN, Muséum National d'Histoire Naturelle, Paris, France; NHM, Natural History Museum, Tring, UK; NRS, Naturhistoriska Riksmuseet, Stockholm, Sweden; OHS, Ornithological Hellenic Society, Greece; PND, Parque Nacional de Doñana, Spain; SMNS, Staatliches Museum für Naturkunde, Stuttgart, Germany; VCNZ, Visitor's Centre of Naurzum Zapvednik, Karamendy, southern Kustanay region, Kazakhstan; ZMA, The Zoological Museum Amsterdam, The Netherlands; ZMB, Museum für Naturkunde, Berlin, Germany.

[4] In the case of contemporary samples (1992–2003): F feathers, fresh feathers; M feathers, museum feathers.

also recommend that the population in Algeria and Tunisia be studied and considered as an additional source of birds for reintroductions in the Mediterranean region.

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