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Comparing the genetics of wild and captive populations of White-headed Ducks *Oxyura leucocephala*: consequences for recovery programmes

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The White-headed Duck is a globally threatened species historically recorded from Spain in the west to China in the east. It has suffered major population declines, local extinctions and range fragmentation. Several projects have attempted to reintroduce captive-bred birds into parts of the former range in Europe, but with little success. Two captive stocks currently exist, one originating from Pakistan in 1968 and the other originating from Spain in 1982. This study compares the suitability of these captive stocks for specific reintroduction projects by using 11 microsatellite markers and mtDNA control region sequences to assess genetic differences between captive populations and wild birds from Spain and Greece. No significant population structure was found and all microsatellite alleles recorded in captive birds originating from Pakistan were also observed in the wild Spanish population. A higher diversity of alleles was observed in wild birds from Greece than from Spain, probably due to the effects of a strong bottleneck experienced in Spain in the 1970s. Compared with wild populations, both captive stocks have suffered a significant loss of diversity in microsatellites and mitochondrial DNA owing to founder effects and/or genetic drift, and therefore may not be well suited for release programmes. We recommend the development of a more diverse captive breeding programme based on birds taken from different areas of the range, in particular by supplementing the Spanish population with birds from North Africa. Our study shows the value of molecular tools in developing conservation programmes for threatened bird species and has implications for the design of recovery programmes.

Keywords: bottleneck, drift, inbreeding, microsatellites, mitochondrial DNA, waterfowl.

Molecular genetic data are increasingly important for effective conservation and management of threatened species. In addition to allowing the identification of populations sufficiently divergent to warrant independent conservation programmes, genetic data are critical in evaluating which sources of animals are most appropriate for reintroductions in areas where a threatened species has suffered local extinctions. These are key issues for the White-headed Duck *Oxyura leucocephala*, a globally threatened species (IUCN Endangered; BirdLife International 2000) that has experienced a drastic reduction in population size and fragmentation of its Palaearctic range since 1900, as well as suffering local extinctions in several European and North African countries (Green & Anstey 1992, Green & Hughes 2001). The result has been an increasing geographical separation between remaining sedentary populations in the western Mediterranean (Spain, Algeria and Tunisia) and the mainly migratory populations spanning from the eastern Mediterranean and Black Sea coast (Greece, Bulgaria and Turkey) to as far east as Pakistan and the western tip of China (Scott & Rose 1996, Wetlands International 2006).

Various international and national action plans have been developed to promote the conservation of the White-headed Duck (e.g. Anstey 1989, Green & Hughes 1996, Li & Mundkur 2003, Hughes *et al.* 2006). Since 1985, projects have been developed to reintroduce White-headed Ducks to parts of Europe

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where the species has become extinct (Hungary, Italy and Corsica), as well as to release captive-bred ducks to reinforce the Spanish population after it declined to low numbers in the 1970s (Anstey 1989, Pereira 1991, Hughes *et al.* 2006). Two different captive breeding stocks have been available to provide birds for these release programmes. The first was established with the importation of ducks from Pakistan to the Wildfowl & Wetlands Trust (WWT) at Slimbridge, England. The WWT population has been the source of all White-headed Ducks held in captivity in Northern Europe. The second was established at the Acebuche centre at Doñana National Park in Spain with birds taken from the wild in Spain. Previous decisions as to which captive stock to use in release programmes have been hampered by the absence of information on genetic variability of different populations. Additionally, because both stocks have now been held in captivity for many generations, loss of genetic diversity is also a concern, as it can reduce the chances of establishing a wild population through reintroduction (Ballou *et al.* 1995, Frankham *et al.* 2002).

In this study, we address several questions important to conservation programmes for this threatened species. First, are there significant genetic differences between Ducks from eastern and western parts of the range that would justify the development of separate conservation programmes for each population? Secondly, which of the existing captive stocks is most appropriate for ongoing release programmes in former parts of the range such as Italy? Thirdly, is the level of genetic diversity in the existing captive stocks comparable to that of the remaining wild populations? Muñoz-Fuentes *et al.* (2005a) found the mtDNA sequence diversity in the wild population in Spain to be about half of that found prior to the population bottleneck suffered between the 1960s and 1980s. Any further loss of diversity in the captive stock established from this already depauperate wild population might reduce the prospects for successful reintroduction.

We used a panel of 11 nuclear autosomal microsatellite markers to compare birds from western (sedentary) and eastern (migratory) populations. In our analysis, birds sampled in Spain represented the western part of the range, and the WWT population, which was established with birds from Pakistan and birds sampled in Greece during the winter, represented the eastern part of the range. Birds wintering in Greece are thought to breed in Kazakhstan or Russia, the main eastern breeding area (Scott & Rose 1996, Green & Hughes 2001). Pakistan is a wintering area, and birds found there may breed in a different part of Kazakhstan than birds wintering in Greece and other parts of the eastern Mediterranean (Scott & Rose 1996, Li & Mundkur 2003). In addition, we used a combination of mitochondrial DNA (mtDNA) control region sequences and microsatellite markers to compare genetic diversity between wild and captive populations. Finally, we considered the implications of our findings for future measures to conserve White-headed Ducks.

METHODS

Samples

Samples from White-headed Ducks were collected between 1993 and 2003 from the wild in Spain ($n = 63$) and Greece ($n = 7$) and from two different avicultural collections: El Acebuche, Doñana National Park, Spain ($n = 27$), and the Wildfowl and Wetlands Trust (WWT), UK ($n = 11$; Table 1). We also included 19 White-headed x Ruddy Duck *Oxyura jamaicensis* hybrids shot in Spain that had White-headed Duck mtDNA to provide additional data on mtDNA diversity in the Spanish population (details in Muñoz-Fuentes *et al.* 2005a). The captive birds in El Acebuche are descendants of a captive stock established in 1982 from eight wild birds from Spain (Pereira 1991,

Table 1. White-headed Duck samples used in this study.

Location	N-mtDNA	N-microsatellites	Sampling date
Wild			
Spain (plus hybrids)	39 (+11)	63	1993–2003
Greece	7	6	1999–2001
Captive			
El Acebuche (Spain)	27	19	1993–2001
Wildfowl and Wetlands Trust (UK)	6	11	2003
Total	90	99	

P. Pereira pers. comm.). The captive population at WWT was sampled in 2003 and is descended from three pairs captured at Lake Khabbaki in Pakistan in 1968.

Samples for genetic analysis included blood, brain or muscle tissue, and feathers. Most samples from wild birds were taken opportunistically from individuals found dead in the field. In Spain, birds died from lead poisoning or in outbreaks of disease (Mateo *et al.* 2001, Svanberg *et al.* 2006). Blood was also taken from seven birds captured in 2003 during a ringing campaign. The above-mentioned hybrids were shot during a government control programme. In Greece, birds drowned in fishing nets while wintering in Lake Vistonida (Panayotopoulou & Green 2000). Blood samples were obtained from birds in the WWT and Acebuche captive populations.

The present analysis is based on previously reported data (Muñoz-Fuentes *et al.* 2005a, 2007) combined with additional data for the captive populations. Microsatellite data previously reported for the wild birds and one of the captive populations were used to confirm the identity of White-headed Duck alleles and to test for genetic introgression in a study of hybridization between Ruddy Ducks and White-headed Ducks in Spain (Muñoz-Fuentes *et al.* 2007). These data, however, have not previously been described in detail or used to compare genetic diversity between regions, or between wild and captive populations.

Laboratory methods

DNA was isolated following the salt-extraction procedure of Gemmell and Akiyama (1996) or using the DNeasy Tissue Kit (Qiagen). In the case of feathers, we added 30 µl of 100 mg/ml dithiothreitol (DTT) to the digestion buffer to achieve complete digestion of feather quills (Cooper 1994).

A mitochondrial DNA (mtDNA) control region fragment comprising 575 base pairs was sequenced for 90 individuals (Table 1) using primers L81 (5′-TATTTGGYTATGYAYRTCGTGCAT-3′) and H768 (5′-TATACGCMAACCGTCTCATYGAG-3′). PCR and sequencing protocols are described in Muñoz-Fuentes *et al.* (2005a). Sequence data have been deposited in EMBL/GenBank/DDBJ (accession numbers: AY836375-AY836506; EU617361-617393).

We genotyped 11 microsatellite loci developed for both Ruddy Ducks and White-headed Ducks (Muñoz-Fuentes *et al.* 2005b) in 99 individuals (Table 1). PCR conditions are described in Muñoz-Fuentes *et al.* (2005b). PCR products were electrophoresed on a MegaBACE sequencer (Amersham) and fragment sizes were determined using GENETIC PROFILER v2.0 (Amersham) by comparison with an internal size standard.

Data analysis

Haplotype diversity (*Hd*), nucleotide diversity (π) and their standard deviations were estimated using DNASP v4.0 (Rozas *et al.* 2003). TCS version 1.21 (Clement *et al.* 2000) was used to construct an unrooted parsimony network illustrating relationships among haplotypes. Individuals were divided into populations as indicated in Table 1. To test for differences in haplotype diversity, we drew from the sample of wild birds in Spain 1000 random samples equal in size to the number of birds sampled in the Spanish captive population (Acebuche). To assess population genetic differentiation, we calculated Φ_{ST} as implemented in GENALEX 6 (Peakall & Smouse 2006). Statistical significance was tested using a permutation approach, and the number of permutations was set to 9999.

We tested for evidence of genotyping errors in our microsatellite data using the programme MICRO-CHECKER version 2.2.1 (Van Oosterhout *et al.* 2004). We used the Markov chain method implemented in GENEPOP (Raymond & Rousset 1995) to test for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium. We applied the sequential Bonferroni correction (Rice 1989) to assess statistical significance when multiple simultaneous tests were performed. We used GENALEX 6 (Peakall & Smouse 2006) to calculate allele frequencies and MICROSATELLITE TOOLKIT (Park 2001) to obtain expected (H_E) and observed (H_O) heterozygosities. HP-RARE 1.0 (Kalinowski 2005) was used to correct allelic richness values for differences in sample size.

To test for differences in allelic diversity, we used allele frequency estimates for both the wild Spanish and Greek populations to generate 1000 sets of simulated genotypes, each equal in sample size to the Acebuche and WWT captive populations. To test for nuclear genetic differentiation among populations, we calculated F_{ST} using GENALEX 6 (Peakall & Smouse 2006), with significance assessed using 9999 permutations of the data. To test for evidence of a recent bottleneck, we used BOTTLENECK version 1.2.02 (Luikart & Cornuet 1998, Luikart *et al.* 1998, Piry *et al.* 1999). The analyses implemented by the software are based on the assumption that when a population goes through a bottleneck, the rarer alleles

are lost first and so we expect to find excess heterozygosity in all or most of the loci examined (Luikart & Cornuet 1998). We ran the programme only on the microsatellite data for wild White-headed Ducks from Spain, as the software requires a sample size of 20–30 diploid individuals and at least four polymorphic loci, although a minimum of 10 are recommended (Piry *et al.* 1999). We selected three different mutation models: the Infinite Alleles Model (IAM), the Stepwise Mutation Model (SMM) and the Two Phase Model (TPM). For the TPM, we chose values of 95% for the percentage of SMM in the model and variance = 12, as recommended by Piry *et al.* (1999) for microsatellite loci. To test the sensitivity of the results to these assumptions, we also used 70% SMM and variance = 30.

RESULTS

Mitochondrial DNA

Only one mtDNA haplotype was found in each of the captive populations, both of which were present in wild White-headed Ducks (Fig. 1). All the Acebuche individuals had haplotype Oleu_01, also found in 62% of wild birds from Spain and in 86% of those from Greece. The six individuals from WWT had haplotype Oleu_02, found in 30% of the wild birds analysed from Spain but in none from Greece. Haplotype Oleu_03, present in 8% of wild birds from Spain, and haplotype Oleu_04, present in one individual from Greece, were not found in the other populations. In total, three different haplotypes were recorded in wild birds from Spain and two from Greece (Fig. 1). When resampling 27 birds from the wild population in Spain, at least two haplotypes were found in all 1000 random samples, indicating that haplotype diversity in the Acebuche population was significantly lower than that in the wild population. When resampling six birds from the wild population in Greece, two haplotypes were included in 85.2% of the random samples, indicating that haplotype diversity was lower in WWT than in Greece, although not significantly so.

Overall Φ_{ST} was significant ($\Phi_{ST} = 0.365$, $P = 0.001$), indicating a difference between the four populations in haplotype frequencies. All pairwise comparisons were significant, except that wild birds from Greece were not significantly different from those from Spain or Acebuche (Table 2). Significant genetic structure is due primarily to lower haplotype diversity in the captive populations as compared to the wild populations, rather than differences in haplotype identities (Table 3).

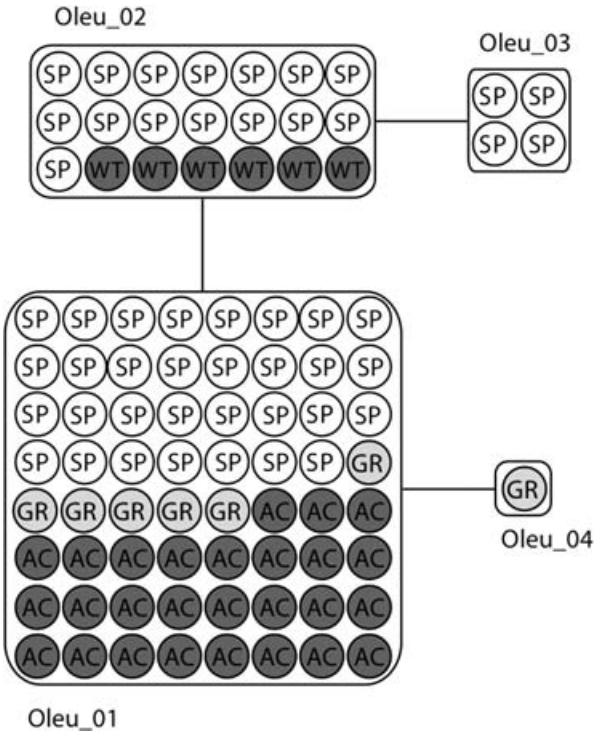

Figure 1. Haplotype network of White-headed Ducks. The shading of circles helps to identify the different populations and indicates whether the individuals belong to a wild population (white and light grey) or are captive individuals (dark grey). SP, Spain; GR, Greece; AC, Acebuche; WT, WWT.

Table 2. Pairwise population Φ_{ST} (mtDNA data, above the diagonal) and F_{ST} (microsatellites, below the diagonal) values. Significance values (P) are based on 9999 permutations. Overall $\Phi_{ST} = 0.365$, $P = 0.001$. Overall $F_{ST} = 0.105$, $P = 0.001$. $^{*}P < 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

	Spain	Greece	Acebuche	WWT
Spain	–	0.130	0.201***	0.437**
Greece	0.000	–	0.225	0.865**
Acebuche	0.102***	0.111**	–	0.865***
WWT	0.155***	0.128**	0.206***	–

Microsatellites

Among the microsatellite loci we analyzed, we found no evidence of null alleles, scoring errors due to stuttering, or large allele dropout. All loci conformed to Hardy–Weinberg expectations in all populations.

Table 3. Genetic variability in White-headed Ducks in wild birds from Spain and Greece and two captive breeding populations for mtDNA and microsatellites. *n*, sample size; No. hapl., number of haplotypes, *Hd*, haplotype diversity; π, nucleotide diversity; sd, standard deviation; AR, allelic richness (or mean number of alleles per locus); AR_C , allelic richness corrected for population size; *HO*, observed heterozygosity; *HE*, expected heterozygosity.

	mtDNA				Microsatellites				
Population	*n*	No. hapl.	*Hd* ± sd	π ± sd	*n*	AR	AR_C	H_O ± sd	H_E ± sd
Wild									
Spain	39	2	0.456 ± 0.053	0.00079 ± 0.00009	63	1.50	1.46	0.188 ± 0.016	0.183 ± 0.067
Greece	7	2	0.286 ± 0.196	0.00050 ± 0.00034	6	1.70	1.70	0.150 ± 0.046	0.199 ± 0.081
Spain, including hybrids	50	3	0.530 ± 0.054	0.00110 ± 0.00015	–				
Captive									
Acebuche	27	1	0	0	19	1.30	1.16	0.055 ± 0.017	0.052 ± 0.041
WWT	6	1	0	0	11	1.20	1.20	0.082 ± 0.026	0.078 ± 0.052

The allele frequencies for each locus and population are given in Table 4. Evidence for linkage disequilibrium between Oxy4 and Oxy10 was found in three populations (Spain, Greece and Acebuche; $P < 0.05$), whereas it was not possible to test for linkage disequilibrium for these two loci in the WWT population as both loci were monomorphic (Table 4). We therefore excluded Oxy4 from the genetic structure analysis, but included it in allele counts.

Wild ducks from Greece and Spain had higher allelic richness and observed and expected heterozygosities than the captive populations. The mean number of alleles per locus (or allelic richness) and the expected heterozygosity were highest in ducks from Greece (Table 3), despite the small sample size for this population. Microsatellite alleles in captive ducks were a subset of those present in wild birds and, in general, the frequencies found at polymorphic loci in the captive populations were similar to those found in the wild populations (Table 4).

All 11 loci included alleles that were shared among all four populations, but allelic diversity varied significantly. We found 20 different alleles among six wild birds from Greece, whereas only 17 were found among 63 wild birds from Spain, indicating that allelic diversity is higher in the eastern population (Table 4). The four rare alleles found in the Greek population were confirmed by replicate PCR reactions. The captive populations had fewer alleles: 15 were found in Acebuche ($n = 19$), whereas only 11 alleles were found in the WWT population ($n = 11$). In random resampling of genotypes from the wild Spanish population, 17 alleles were recorded in 97.2% of 1000 replicates, whereas 16 alleles were found in the remaining 2.8% of replicates, indicating that the number of alleles in the wild population is significantly greater than in the Acebuche population. Similarly,

Table 4. Allelic frequencies in two wild and two captive (Acebuche and WWT) populations of White-headed Ducks, per locus and population. Sample size, *n*, is indicated below the population name. Locus name is in bold and alleles are in italics, designated by their size in base pairs. Private alleles (present only in one population) appear in bold.

Population	Spain	Greece	Acebuche	WWT
n	63	6	19	11
Oxy3				
189	0.180	0.167	–	0.273
191	0.820	0.750	1.000	0.727
193	–	**0.083**	–	–
Oxy4				
236	0.627	0.667	0.750	1.000
238	0.373	0.250	0.250	–
240	–	0.083	–	–
Oxy6				
247	0.333	0.333	0.026	0.227
249	0.667	0.667	0.974	0.773
Oxy10				
158	0.627	0.667	0.722	1.000
160	0.373	0.250	0.278	–
162	–	**0.083**	–	–
Oxy11				
194	0.667	0.667	0.972	1.000
198	0.333	0.250	0.028	–
200	–	**0.083**	–	–
Oxy13				
208	1.000	1.000	1.000	1.000
Oxy15				
235	1.000	1.000	1.000	1.000
Oxy17				
209	1.000	1.000	1.000	1.000
Oxy19				
218	1.000	1.000	1.000	1.000
Oxy1				
150	1.000	1.000	1.000	1.000
Oxy14				
131	0.911	1.000	1.000	1.000
133	**0.089**	–	–	–
Total no. of alleles	17	20	15	13

the number of alleles in the WWT population is substantially less than expected if it was a sample of the Greek population ($P < 0.001$), as 16 or more alleles were found in all 1000 replicates.

Overall F_{ST} was significant and accounted for 11% of the genetic variation ($F_{ST} = 0.105$, $P = 0.001$). Pairwise F_{ST} comparisons of populations performed with GENALEX indicated that the captive populations were significantly differentiated from each other and from the wild populations ($P < 0.05$ in all cases). Wild populations from Spain and Greece, however, were not significantly differentiated (Table 2). This result, however, could reflect insufficient statistical power to detect a low level of genetic differentiation given the small size of the Greek samples (type II error).

Our sample size of White-headed Ducks from Spain ($n = 63$) was twice as big and the number of polymorphic loci ($n = 5$) half as large as Piry *et al.* (1999) recommended for analyses in BOTTLENECK (see Methods). Given the small number of polymorphic loci in our sample, the Wilcoxon test was the only test among those implemented in BOTTLENECK that was sufficiently powerful and robust (Piry *et al.* 1999). Four or five polymorphic loci showed significant heterozygote excess regardless of the assumed mutation model ($P = 0.031$; one-tailed test as our *a priori* expectation was excess heterozygosity in the bottlenecked population; see Methods).

DISCUSSION

Biogeographical populations

Based on microsatellite data, we found no significant differentiation between wild birds from Greece and Spain. Similarly, based on mtDNA data, we found no significant differences between wild birds from Greece and captive or wild birds from Spain. Genetic diversity at microsatellite loci, however, was higher in our sample of wild eastern birds wintering in Greece ($n = 6$) than in our sample of the sedentary Spanish population ($n = 63$). This is probably a result of the population bottleneck suffered by the Spanish population in the 1970s and 1980s, with a record low count of only 22 birds registered in 1977 (Torres & Moreno-Arroyo 2000).

In an analysis of a larger sample of birds from across the range that included historical samples from museum skins, Muñoz-Fuentes *et al.* (2005a) showed that the bottleneck suffered by the Spanish population also reduced mtDNA-haplotype diversity, and that historical eastern and western populations have a higher diversity of haplotypes than the contemporary Spanish population. DNA extracted from museum specimens is usually of low quality and obtaining reliable microsatellite data for specimens that are just 30 years old is difficult (Sefc *et al.* 2003). Most available historical specimens of White-headed Ducks are about a century old (Muñoz-Fuentes *et al.* 2005a), such that reliable microsatellite results would be difficult to obtain for these samples. Thus, we used methods that have been developed to test for bottlenecks based on genetic data from a population sampled once in time and found that there is significant evidence that the White-headed Ducks from Spain went through a bottleneck based on current patterns of allelic diversity at microsatellite loci. Although the well-documented demographic bottleneck suffered by the Spanish population in the 1970s seems to be the most likely explanation for the low level of genetic diversity currently observed (Muñoz-Fuentes *et al.* 2005a), it is not possible to rule out other factors, such as long-term low effective population size and/or recurrent bottlenecks, as contributing factors.

Scott and Rose (1996) and Wetlands International (2006) recognized four separate wintering populations of White-headed Ducks: (1) Spain, (2) Algeria and Tunisia, (3) East Mediterranean, West and Southwest Asia (including Greece) and (4) South Asia (Pakistan). This designation of four separate populations was based on census data; almost no information on the movements of marked birds is available. We included birds originating from three of these populations in the current study (Spain, Greece and Pakistan) and found no evidence of the geographical structure or genetic divergence that would be expected if there had been long-term isolation of these populations. It is particularly noteworthy that all the microsatellite alleles recorded in the WWT birds, which originated from Pakistan at the eastern end of the range, were also recorded in wild birds from Spain at the extreme western end of the range (Table 4). Similarly, we did not find genetic differentiation in mtDNA between eastern and western populations in a historical study (Muñoz-Fuentes *et al.* 2005a); haplotypes recorded in Ukraine, Russia, Iraq, Iran, Afghanistan and Pakistan were also found in Spain. These results bring into question the assumption that the breeding range of birds wintering in Pakistan was historically isolated from that of birds wintering elsewhere in Asia (Scott & Rose 1996). Indeed, growing evidence suggests that ducks in Eurasia do not show strong fidelity to flyways, making the designation of biogeographical

populations questionable (Guillemain *et al.* 2005, Veen *et al.* 2005). There is an urgent need to study the migration route of White-headed Ducks breeding in Asia.

Until the extinction of White-headed Duck populations in central Europe during the 20th century (Green & Anstey 1992), it seems likely that there was regular exchange of individuals between populations in the western Mediterranean (Spain, Tunisia and Algeria) and those further east. Although we found evidence of genetic differentiation among sampled populations, all of these differences are attributable to population bottlenecks, either in the wild population in Spain (Muñoz-Fuentes *et al.* 2005a) or in the establishment of captive populations.

Taken together, our analyses suggest little or no geographic structure across the range of this species, but perhaps somewhat higher genetic diversity at present in the eastern part of the range. The strength of this inference, however, is limited by sample size; a larger sample of birds from Greece and other areas in the east is needed to test the latter result further and to make further inferences about the population structure and genetic diversity of this species over longer time scales.

Status of captive populations

Although our sample sizes are small in some cases, it is clear that genetic diversity was reduced in the captive populations: monomorphic loci were more frequent in the captive populations, there were alleles present in the wild populations that were not found in the captive ones, and private alleles were only recorded in the wild populations (Table 4). This reduction in genetic diversity is most likely due to founder effects, genetic drift and inbreeding during the 20–35 years over which the captive stocks were maintained prior to our sampling. The effect is most striking for mtDNA, where single haplotypes became fixed in each of the two captive populations. Haplotype fixation is likely to occur quickly in captive waterfowl populations. For example, only two of three females imported into the UK from Pakistan produced offspring. Similarly, a single mtDNA haplotype is found in the feral European Ruddy Duck population, which originated from a captive population founded by three females and four males, as compared with 23 haplotypes in the North American population from which they originated (Muñoz-Fuentes *et al.* 2006). In contrast, the feral Ruddy Duck population has a lower but still intermediate diversity of microsatellite alleles as compared with the North American population (Muñoz-Fuentes *et al.* 2006).

Comparisons of genetic diversity between captive populations and their respective source populations indicate a substantial reduction in allelic richness in the captive populations. Assuming that the wild population in Pakistan in the 1960s was similar in genetic diversity to the Greek population in recent years, our data suggest that the WWT population has suffered a reduction in allelic richness of about 29%. Similarly, when Acebuche and Spain are compared, the reduction in allelic richness for Acebuche is about 21% (Table 3). Compared to the respective wild populations, expected heterozygosity has also decreased by 72% in the Acebuche population and by 61% in the WWT population.

Implications for conservation programmes

Based on our results, there is no evidence of significant genetic structure that might justify the treatment of each surviving population of White-headed Ducks as a separate management unit (*sensu* Moritz 1994). It should be noted, however, that lack of structure in presumably neutral genetic markers does not rule out the possibility that recent selection in different environments across the extensive range of this species has produced some important adaptive differences between White-headed Duck populations, e.g. in migratory behaviour (Muñoz-Fuentes *et al.* 2005a). In the absence of knowledge as to whether certain ecological differences are genetic adaptations or phenotypic responses, and given that ecological adaptations in quantitative heritable traits can arise quickly if traits are under selection, mixing populations that differ in ecological characteristics should be avoided (Crandall *et al.* 2000).

Captive birds from WWT were used in a reintroduction project in Hungary (Anstey 1989), whereas birds from Acebuche have been released in Spain and Corsica (Pereira 1991, Mayol 1994). Although birds from both stocks have been considered for release in Italy, no releases into the wild have occurred to date (Gustin *et al.* 2000, Hughes *et al.* 2006). The reintroduction project in Hungary failed to establish a population in the wild (Anstey 1989). Similarly, releases in Mallorca (Balearic Islands) have so far failed to establish a self-sustaining population (Torres & Moreno-Arroyo 2000). Only males were released in Corsica in a trial of release methods, but these birds disappeared quickly. The role of releases on the Spanish mainland in the recovery of the wild population is unclear, as these efforts began when the population

was already increasing in response to effective protection from hunting (Pereira 1991). There are many potential factors that could explain the limited success of reintroduction projects for the White-headed Duck to date, but low genetic diversity of the birds released may be one of them (O'Grady *et al.* 2006, Hale & Briskie 2007).

Captive populations should, as far as possible, represent the genetic composition of the wild population, as their purpose is to be a source for reinforcing or re-establishing wild populations (Ballou *et al.* 1995). Due to their relatively small size, it is inevitable that captive populations suffer from founder effects, genetic drift, inbreeding depression and selection for the captive environment, although management can be directed to minimize these effects (Ballou *et al.* 1995, Frankham *et al.* 2002). Our results suggest that the existing captive populations of White-headed Duck have significantly reduced genetic diversity and thus may not be optimally suited for successful release in the wild. Although efforts to obtain more wild birds from Spain to broaden the Acebuche stock are underway, our results suggest that the addition of more wild birds from other populations would do more to increase the genetic diversity of captive stocks. In general, a starting population of at least 20 individuals from a population with a minimum of 1000 individuals is recommended (Frankham *et al.* 2002).

Because the current interest in reintroduction programmes centres on the establishment or reinforcement of sedentary populations, e.g. in Italy and Corsica (Hughes *et al.* 2006) or Spain, we recommend that the genetic diversity of other sedentary populations be studied as potential sources for a more diverse captive stock. For example, haplotypes that were present in the Spanish and Italian (now extinct) historical samples and that were not found in the contemporary Spanish sample, were recorded in the historical sample from Algeria (Muñoz-Fuentes *et al.* 2005a). In addition to the population in Tunisia and Algeria, there are also resident birds in Turkey and Iran (Green & Anstey 1992, Green & Hughes 2001) that are likely to provide more genetic variation than the remaining birds in Spain. The Spanish population faces unique challenges both from the consequences of a population bottleneck (Muñoz-Fuentes *et al.* 2005a) and from hybridization with the introduced Ruddy Duck (Muñoz-Fuentes *et al.* 2007). Indeed, the wild population in Spain is so genetically depauperate that even the feral European Ruddy Duck population established from only seven individuals has significantly higher levels of nuclear variation (Muñoz-Fuentes *et al.* 2006, 2007).

In conclusion, White-headed Duck populations from across the range have a high degree of similarity based on the genetic markers studied. Given no evidence of historical isolation or differentiation of these populations, current division of White-headed Ducks into biogeographical populations and flyways should be re-examined. Management and conservation plans for the White-headed Duck should aim at re-establishing connectivity of populations. Observed differences in ecological characteristics, however, should be considered when selecting individuals for reintroduction programmes or augmentation of captive and/or wild populations, so that source and recipient populations are matched to the greatest extent possible. Existing captive stocks retain critically low levels of genetic diversity and should be improved by the provision of individuals from relatively diverse wild populations and by careful management (e.g. studbooks, large captive population size) to minimize further loss of diversity.

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REFERENCES

Anstey, S. 1989. *The Status and Conservation of the White-headed Duck* Oxyura leucocephala. Slimbridge, UK: International Waterfowl and Wetlands Research Bureau (now Wetlands International).

Ballou, J.D., Gilpin, M. & Foose, T.J. 1995. *Population Management for Survival and Recovery*. New York: Columbia University Press.

Birdlife International. 2000. *Threatened Birds of the World*. Barcelona and Cambridge: Lynx Edicions and BirdLife International.

Clement, M., Posada, D. & Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657–1660.

Cooper, A. 1994. DNA from museum specimens. In Herrmann, B. & Herrmann, S. (eds) *Ancient DNA: Recovery and Analysis of Genetic Material from Paleontological, Archaeological, Museum, Medical, and Forensic Specimens*: 149–165. New York: Springer.

Crandall, K.A., Bininda-Edmonds, O.R.P., Mace, G.M. & Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**: 290–295.

Frankham, R., Ballou, J.D. & Briscoe, D.A. 2002. *Introduction to Conservation Genetics*. Cambridge: Cambridge University Press.

Gemmell, N.J. & Akiyama, S. 1996. An efficient method for the extraction of DNA from vertebrate tissue. *Trends Genet*. **12**: 338–386.

Green, A.J. & Anstey, S. 1992. The status of the White-headed Duck *Oxyura leucocephala*. *Bird Conserv. Int*. **2**: 185–200.

Green, A.J. & Hughes, B. 1996. Action plan for the White-headed Duck *Oxyura leucocephala* in Europe. In Heredia, B., Rose, L. & Painter, M. (eds) *Globally Threatened Birds in Europe. Action Plans*: 119–145. Strasbourg: Council of Europe Publishing.

Green, A.J. & Hughes, B. 2001. *Oxyura leucocephala* White-headed Duck. *BWP Update (The Journal of the Birds of the Western Palearctic)* **3**: 79–90.

Guillemain, M., Sadoul, N. & Simon, G.R. 2005. European flyway permeability and abmigration in Teal *Anas crecca*, an analysis based on ringing recoveries. *Ibis* **147**: 688–696.

Gustin, M., Rizzi, V. & Gallo-Orsi, U. 2000. White-headed Duck reintroduction in Abulia, southern Italy: 1999 update. *Threatened Waterfowl Specialist Group News* **12**: 21–25.

Hale, K.A. & Briskie, J.V. 2007. Decreased immunocompetence in a severely bottlenecked population of an endemic New Zealand bird. *Anim. Conserv*. **10**: 2–10.

Hughes, B., Robinson, J.A., Green, A.J., Li, Z.W.D. & Mundkur, T.C. (compilers) 2006. *International Single Species Action Plan for the Conservation of the White-headed Duck* Oxyura leucocephala. Bonn, Germany: CMS Technical Series No. 13 & AEWA Technical Series No. 8.

Kalinowski, S.T. 2005. HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* **5**: 187–189.

Li, Z.W.D. & Mundkur, T. 2003. *Status Overview and Recommendation for Conservation of the White-headed Duck* Oxyura leucocephala *in Central Asia*. Kuala Lumpur: Wetlands International Global Series 15. URL http://www.wetlands.org/publication.aspx?id=672aed57-06dd-45d0-979d-4053617292d7 [accessed 11 June 2008].

Luikart, G. & Cornuet, J.-M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol*. **12**: 228–237.

Luikart, G., Allendorf, F.W., Cornuet, J.-M. & Sherwin, W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered*. **89**: 238–247.

Mateo, R., Green, A.J., Jeske, C.W., Urios, V. & Gerique, C. 2001. Lead poisoning in the globally threatened Marbled Teal and White-headed Duck in Spain. *Environ. Toxicol. Chem*. **20**: 2860–2868.

Mayol, J. 1994. La malvasía en Mallorca. Consideraciones en torno a una reintroducción. *Oxyura* **7**: 109–118.

Moritz, C. 1994. Defining 'Evolutionary Significant Units' for conservation. *Trends Ecol. Evol*. **9**: 373–375.

Muñoz-Fuentes, V., Green, A.J., Negro, J.J. & Sorenson, M.D. 2005a. Population structure and loss of genetic diversity in the endangered White-headed Duck, *Oxyura leucocephala*. *Conserv. Gen*. **6**: 999–1015.

Muñoz-Fuentes, V., Gyllenstrand, N., Negro, J.J., Green, A.J. & Vilà, C. 2005b. Microsatellite markers for two Stifftail ducks: the White-headed Duck, *Oxyura leucocephala*, and the Ruddy Duck, *O. jamaicensis*. *Mol. Ecol. Notes* **5**: 263–265.

Muñoz-Fuentes, V., Green, A.J., Sorenson, M.D., Negro, J.J. & Vilà, C. 2006. The Ruddy Duck *Oxyura jamaicensis* in Europe: natural colonisation or human introduction? *Mol. Ecol*. **15**: 1441–1453.

Muñoz-Fuentes, V., Vilà, C., Green, A.J., Negro, J.J. & Sorenson, M.D. 2007. Hybridization between White-headed Ducks and introduced Ruddy Ducks in Spain. *Mol. Ecol*. **16**: 629–638.

O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D., Tonkyn, D.W. & Frankham, R. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biol. Conserv*. **133**: 42–51.

Panayotopoulou, M. & Green, A.J. 2000. White-headed Ducks in Greece. *Threatened Waterfowl Specialist Group News* **12**: 16–17.

Park, S.D.E. 2001. *Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection*. PhD Thesis, University of Dublin.

Peakall, R.O.D. & Smouse, P.E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295.

Pereira, P. 1991. Resultados del seguimiento de malvasías procedentes del programa de cría en cautividad durante el año 1990. In Martos, M.J. & Fernández Palacios, J. (eds) *Plan Rector de Uso y Gestión de las Reservas Naturales de las Lagunas de Cádiz*: 135–143. Junta de Andalucía: Consejería de Cultura y Medio Ambiente, Agencia de Medio Ambiente.

Piry, S., Luikart, G. & Cornuet, J.-M. 1999. Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered*. **90**: 502–503.

Raymond, M. & Rousset, F. 1995. GenePop version-1.2 – population genetics software for exact tests and ecumenicism. *J. Hered*. **86**: 248–249.

Rice, W.R. 1989. Analysing tables of statistical tests. *Evolution* **43**: 223–225.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X. & Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.

Scott, D.A. & Rose, P.M. 1996. *Atlas of Anatidae Populations in Africa and western Eurasia*. Wageningen: Wetlands International. URL http://www.wetlands.org/publication.aspx?id=792563ec-1b86-4f80- b5f9-170d59f6c406 [accessed 11 June 2008].

Sefc, K.M., Payne, R.B. & Sorenson, M.D. 2003. Microsatellite amplification from museum feather samples: effects of fragment size and template concentration on genotyping errors. *Auk* **120**: 982–989.

Svanberg, F., Mateo, R., Hillström, L., Green, A.J., Taggart, M.A., Raab, A. & Meharg, A.A. 2006. Lead isotopes and lead shot ingestion in the globally threatened Marbled Teal *Marmaronetta angustirostris* and White-headed Duck *Oxyura leucocephala*. *Sci. Total Environ*. **370**: 416–424.

Torres, J.A. & Moreno-Arroyo, B. 2000. La recuperación de la malvasía cabeciblanca *Oxyura leucocephala* en España durante el último decenio del siglo XX. *Oxyura* **10**: 5–51.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**: 535–538.

Veen, J., Yurlov, A.K., Delany, S.N., Mihantiev, A.I., Selivanova, M.A. & Boere, G.C. 2005. *An Atlas of Movements of Southwest Siberian Waterbirds*. Wageningen: Wetlands International.

Wetlands International. 2006. *Waterbird Population Estimates*, 4th edn. Wageningen: Wetlands International.

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