The ruddy duck *Oxyura jamaicensis* in Europe: natural colonization or human introduction?

VIOLETA MUÑOZ-FUENTES,* ANDY J. GREEN,* MICHAEL D. SORENSON,† JUAN J. NEGRO* and CARLES VILÀ‡

**Estación Biológica de Doñana (CSIC), Avda. María Luisa s/n, 41013 Sevilla, Spain, †Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA, ‡Department of Evolutionary Biology, Uppsala University, Norbyvägen 18D 75236 Uppsala, Sweden*

Abstract

Native to North America, ruddy ducks *Oxyura jamaicensis* now occur in 21 countries in the western Palaearctic (including Iceland) and their expanding population threatens the native white-headed duck, *Oxyura leucocephala*, through hybridization and possibly competition for food and nest sites. We used mitochondrial DNA sequences and nuclear microsatellites to test whether the European ruddy duck population is descended solely from the captive population in the UK, which traces to seven individuals imported from the USA in 1948, or, alternatively, has been augmented by natural dispersal of birds from North America. Limited genetic diversity in the European population is consistent with a founder population as small as seven birds. In addition, shifts in allele frequencies at several loci, presumably due to genetic drift in the founding population, result in significant differentiation between the European and North American populations. Despite the recent separation of these populations, almost all individuals could be unambiguously assigned based on their composite genotypes, to one of two distinct populations, one comprising all of the European ruddy ducks we sampled (including those from Iceland and captive birds in the UK) and the other comprising all North American samples. Our results confirm that the European ruddy duck population is likely to derive solely from the captive population in the UK and we find no evidence of recent arrivals from North America or of admixture between ruddy ducks from Europe and North America.

Keywords: biological invasions, founder effect, genetic drift, microsatellites, mitochondrial DNA, *Oxyura*, ruddy ducks

Introduction

The introduction of species to areas outside their natural range has become commonplace and is one of the major threats to biodiversity (Allendorf *et al*. 2001; Brown & Sax 2004). In addition to the potential negative effects of invasive species on ecosystem function and biodiversity, hybridization with an exotic species can lead directly to the extinction of a native species (Caughley & Gunn 1996; Rhymer & Simberloff 1996; Parker *et al*. 1999; Allendorf *et al*. 2001). Such hybridization currently threatens several bird species with extinction (BirdLife International 2000). Introduction of exotic birds is often carried out by human intervention, but in some cases birds have colonized new areas on their own accord. For example, the pied stilt, *Himantopus himantopus*, expanded from Australia to New Zealand, and subsequently hybridized with the endangered black stilt, *Himantopus novaezelandiae* (Marchant & Higgins 1990). Naturally occurring hybridization may be viewed as part of a species' evolutionary process (Arnold 1997), whereas human-mediated introductions that result in invasive species and hybridization are generally considered undesirable.

The ruddy duck, *Oxyura j. jamaicensis*, is native to North America, and over recent decades has had stable or increasing populations throughout its breeding range (Brua 2001).

Correspondence: Violeta Muñoz-Fuentes, Present address: Department of Evolutionary Biology, Uppsala University, Norbyvägen 18D, 152 36 Uppsala, Sweden, Fax: +46-(0)18-4716310; E-mail: Violeta.Munoz@ebc.uu.se

It is migratory in the north and resident in the south (Brua 2002), with a total population of *c*. 500 000 birds (Wetlands International 2002). Two other subspecies exist that inhabit South America (McCracken & Sorenson 2005). The ruddy duck now also breeds in Europe. It was first detected breeding in the wild in the UK in 1960 (Hughes 1998) and, by the year 2000, the UK population comprised *c*. 5000 individuals (Hughes *et al*. 2004). This represents a growth rate of 18% per annum. The species has now been recorded in 21 western Palearctic countries concentrated along the northern European coast, with breeding attempts in at least 11 countries (Hughes *et al*. 1999, 2004). Regular breeding takes place in Iceland, Ireland, the UK, the Netherlands, France, Spain and Morocco. Since 1984, Iceland has had a breeding population numbering up to 36 ruddy ducks that likely migrate to the UK in winter (Nielsen 1994).

The ruddy duck population in Europe has become the main threat to the survival of the endangered white-headed duck, *Oxyura leucocephala*, through hybridization and perhaps also competition for food and nest sites (BirdLife International 2000; Green & Hughes 2001). Concern for the white-headed duck has led to measures to control or eliminate ruddy ducks in Portugal, Spain, France and the UK (Hughes *et al*. 2004). Control measures have generated considerable controversy in the UK, where the ruddy duck has been welcomed as an interesting addition to the avifauna by many birdwatchers (e.g. Zonfrillo 2000).

The presence of the ruddy duck in Europe has been attributed to the importation of seven birds from the USA to the UK in 1948 for captive breeding and the subsequent escape of birds from captivity (Hughes 1998). Breeding was first recorded in the wild in 1960, and no ruddy ducks were recorded in Europe prior to the establishment of the captive population (Hughes 1998). Nevertheless, vagrants of other duck species regularly cross the Atlantic from North America (Cramp & Simmons 1977). Hence it is not surprising that opponents to control measures against ruddy ducks have argued that the growing population in Europe has been partly established by vagrants arriving through natural dispersal. This seems most plausible for birds breeding in Iceland, because some of the waterfowl species breeding there winter in North America (notably Barrow's goldeneye *Bucephala islandica*, Kear 2005).

To test whether the natural arrival of ruddy ducks from North America helped found the European population, we compared the genetic diversity of ruddy duck populations from both continents (including both feral and captive populations in Europe). We predicted that if ruddy ducks in Europe originated from a small captive population, genetic diversity should be lower in Europe as compared to North America. Only three females and four males were imported to establish the captive population in the UK in 1948 (Hudson 1976). If these were the exclusive source of the feral population, and if all of them reproduced, not more than three mtDNA haplotypes should be observed today in the European population. In addition, microsatellite alleles and genotypes found in the European population should be consistent with an origin from a small captive population and many of the same alleles should be present in the current captive population in the UK, which is comprised of direct descendants of the seven birds imported in 1948. Finally, we tested a small sample of ruddy ducks from Iceland to determine whether they are of European or North American origin.

Materials and methods

Samples

A total of 171 ruddy duck samples were collected between 1987 and 2003. Samples comprised fresh tissues, including blood, brain, muscle, or feathers. Samples from North America ($n = 67$) included 34 birds from the eastern USA, 24 from the central USA and Manitoba in Canada, and 9 from the western USA. We concentrated our sampling effort in eastern North America because this region would be the most likely source for birds dispersing naturally from North America to Europe. Samples from Europe included 29 from Great Britain, 19 from France, 39 from Spain, 3 from Iceland and 14 from two different avicultural collections in the UK (7 from the Wildfowl and Wetlands Trust, WWT, and 7 from a private collection in Monmouth). As far as it is known, these captive birds and those in other European collections are descendants of the seven birds imported to WWT from the USA in 1948 (B. Hughes, personal communication).

All ducks from the wild in Europe were shot during government-sponsored programmes to control ruddy ducks. From captive ducks blood samples were obtained. Samples from North America were taken from birds shot by hunters (samples obtained from the US Fish and Wildlife Service) or birds caught and released in other research projects. Samples from Iceland comprise the only three individuals that have been collected there to date.

DNA was isolated following the salt-extraction procedure of Gemmell & Akiyama (1996) or 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).

Sequencing of the mtDNA control region

A subset of 107 samples representing all localities was selected for sequencing domain I and part of domain II of the mitochondrial DNA (mtDNA) control region (Table 1). We used the primers L81 (5′-TATTTGGYTATGYAYRT-CGTGCAT-3′) and H768 (5′-TATACGCMAACCGTCTC-ATYGAG-3′) to amplify and sequence a fragment of 575

Table 1 Ruddy duck samples used in this study for mitochondrial DNA and nuclear microsatellite analyses

Location	N-mtDNA	N-microsatellites
North America		
Atlantic Flyway	34	31
Vermont (VT)	2	2
New York (NY)	2	2
Rhode Island (RI)	2	2
New Jersey (NJ)	5	5
Pennsylvania (PA)	3	3
Maryland (MD)	5	5
North Carolina (NC)	6	5
Georgia (GA)	2	2
Florida (FL)	7	5
Mississippi Flyway	24	13
Manitoba (MB)	19	13
Illinois (IL)	1	0
Louisiana (LA)	2	0
Texas (TX)	2	0
Pacific Flyway	9	7
Montana (MT)	1	1
Nevada (NV)	4	4
California (CA)	4	2
Europe		
Iceland (IC)	3	3
United Kingdom (UK)	8	27
France (FR)	10	18
Spain (SP)	13	30
Captive		
WWT (Wildfowl and Wetlands Trust)	3	6
Monmouth	3	7
Total	107	142

base pairs (bp). The details of polymerase chain reaction (PCR) and sequencing protocols are described in Muñoz-Fuentes *et al*. in press). Sequences were aligned by eye using SE-AL version 1.0a1 (Rambaut 1996). We found no evidence of nuclear copies (or numts) in our sequences: DNA extracts from tissues that differ in the relative number of mtDNA and nuclear copies (for example, blood and muscle tissue) provided clean and identical sequences and no double peaks were found in the electropherograms (see Sorenson & Quinn 1998) for additional information on *numts*). Sequences have been submitted to the EMBL/GenBank/DDBJ database (Accession nos AM84909–AM85003). Additionally we used sequences from McCracken & Sorenson (2005; AY747742, 43, 45–48, 50, 51, 56, 57, 60, 61).

Typing of microsatellite loci

We genotyped 11 microsatellite loci developed for both ruddy ducks and white-headed ducks (Muñoz-Fuentes *et al*. 2005) in 141 individuals (Table 1). PCRs were performed with three primers to allow standard fluorescently labelled primers to be used with different loci: we attached either an M13Reverse or a CAG tag to the 5′-end of the forward primer, and added to the amplification reaction either a labelled M13Reverse or CAG tag, respectively (Muñoz-Fuentes *et al*. 2005). PCR conditions are described in Muñoz-Fuentes *et al*. (2005). PCR products were electrophoresed on a MEGABACE sequencer (Amersham). Fragment sizes were determined using Genetic Profiler version 2.0 (Amersham) by comparison to an internal size standard.

Analysis of mitochondrial data

Haplotype diversity (*Hd*), nucleotide diversity (π), and their standard deviations were estimated using DNASP version 4.0 (Rozas *et al*. 2003). To illustrate relationships among haplotypes, we constructed an unrooted parsimony network using TCS, version 1.13 (Clement *et al*. 2000).

To assess population genetic structure, we used an analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) as implemented in ARLEQUIN 2.001 (Schneider *et al*. 2000). We calculated Φ-statistics, analogues of *F*-statistics that incorporate information about genetic distance between haplotypes, and estimated the proportion of molecular variance components due to the effects of individuals, populations and groups. Significance of both the Φ-statistics and variance components was assessed using a permutation approach. In addition to testing for differentiation between North America and Europe, we defined subpopulations based on general knowledge of migratory waterfowl movements in North America (Baldassarre & Bolen 1994) and sampling location (see Table 1 for groupings and sample sizes). Samples from Iceland and from the captive populations, because of their small number, were excluded from the AMOVAS.

To test for evidence of recent population expansion in the North American population, we constructed a mismatch distribution (Rogers & Harpending 1992) and also calculated Fu's F_S, which has greater power than other statistics for detecting population expansion. (Fu 1997; Ramos-Onsins & Rozas 2002). 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; Peck & Congdon 2004). We used ARLEQUIN 2.001 (Schneider *et al*. 2000) and DNASP version 4.0 (Rozas *et al*. 2003) to perform these calculations. We also used the coalescent-based method implemented in FLUCTUATE version 1.4 (Kuhner *et al*. 1998) to test for evidence of population expansion. The program was run several times to ensure convergence of the estimates (Kuhner *et al*. 1998).

Analysis of microsatellite data

We used the programme MICRO-CHECKER version 2.2.1 (van Oosterhout *et al*. 2004) to test for evidence of genotyping errors in our microsatellite data. We used GENALEX version 5.1 (Peakall & Smouse 2001) to calculate allele frequencies and expected (H_E) and observed (H_O) heterozygosities. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using the Markov chain method implemented in GENEPOP (Raymond & Rousset 1995). We applied Bonferroni sequential correction (Rice 1989) to assess statistical significance when multiple simultaneous tests were performed. We calculated allelic richness (mean number of alleles per locus; Leberg 2002) corrected for sample size with FSTAT version 2.9.3.2 (Goudet 1995). This correction involves calculating the mean number of alleles per locus after subsampling from each population the same number of individuals as present in the smallest sample. To test whether seven individuals originally brought to the UK from North America could account for all of the genetic diversity present in the current European population, we randomly resampled seven ruddy ducks from North America 1000 times to obtain an expected distribution for the number of alleles that could be present in the founding population.

To test for nuclear genetic differentiation among populations, we calculated F_{ST} using GENALEX version 5.1 (Peakall & Smouse 2001). We used F_{ST} instead of R_{ST} because F_{ST} estimates perform better when samples sizes are small and the number of loci is low (Gaggiotti *et al*. 1999; Sefc *et al*. 2005) and because some of the microsatellite loci we used had imperfect repeats and/or did not conform to a simple stepwise-mutation model. The individuals were divided into populations as for mtDNA (Table 1). GENALEX calculates the distribution of the variance components and *F*-statistics, F_{RT}, F_{SR} and F_{ST}. F_{RT} measures how much of the genetic diversity is explained by the grouping of the populations into regions, relative to the total genetic diversity in individuals; F_{SR} accounts for how much of the genetic diversity is explained by the grouping of individuals into populations, relative to the genetic diversity of individuals within the same region; and F_{ST} measures how much of the genetic diversity is distributed in populations relative to the total genetic diversity in individuals. Statistical significance was tested using a permutation approach, and the number of permutations was set to 999.

To test whether ruddy ducks from Iceland, the rest of Europe, and captive populations in the UK belong to a single population and to test for recent dispersal of individual birds from North America to Europe, we used the program STRUCTURE version 2.1 (Pritchard *et al*. 2000). STRUCTURE implements a Bayesian clustering method to identify the most likely number of populations (*K*) and probabilistically assign individuals to populations without using a priori information on sampling location. Two runs were completed for each value of *K* (from 1 to 5) using 30 000 steps for burn-in length and 1 000 000 steps for run length. The likelihood values were observed to converge during the runs, and the two runs for each value of *K* gave almost identical results. All individuals for which microsatellite data were available were included in these analyses. We also used the software NEWHYBRIDS version 1.1 beta (Anderson & Thompson 2002) to test for evidence of two genetically distinct groups (i.e. North American and European) and whether any individuals within the European sample might be the result of interbreeding between a ruddy duck from North America and a ruddy duck from Europe. No a priori information about the origin of individuals was entered into the analysis and, as recommended, we ran the program with different priors to explore the sensitivity of the results.

Results

Genetic diversity

Among ruddy ducks from North America, we found 18 variable sites defining 23 unique haplotypes for the 575-bp control region fragment we sequenced (Table 2). Of the 67 North American ducks for which mtDNA was sequenced, 27 (40%) had the same haplotype, Ojam_01. The remaining haplotypes were observed in one to seven individuals (Fig. 1). In contrast, only the common North American haplotype, Ojam_01, was found among European ruddy ducks, including the three birds from Iceland.

The parsimony network illustrates the relationships between haplotypes (Fig. 1). The most common haplotype, Ojam_01, occupies the central position in this network, with all the others differing from it by one to three substitutions. There was no evidence of population structure within North America: individuals from each flyway had haplotypes distributed throughout the network and, among the nine haplotypes present in more than one individual, seven were observed in different flyways (Table 2). Within a nested AMOVA framework, overall Φ_{ST} was significant ($\Phi_{ST} = 0.080$, $P = 0.044$), whereas all pairwise comparisons within North America or within Europe were nonsignificant (Table 3). A small proportion of genetic variation was due to differentiation between North American and European populations but this was not statistically significant ($\Phi_{CT} = 0.100$, $P = 0.102$). However, given the lack of structure within each continent, we also calculated pairwise Φ_{ST} between North America and Europe without considering populations inside the continents. The value obtained was highly significant ($\Phi_{ST} = 0.093$, $P = 0.000$), indicating a lack of power in the previous analysis due to small sample sizes.

Table 2 Haplotypes of ruddy ducks from North America and Europe defined by the variable sites in the 575 bp of the mtDNA control region and number of individuals with a particular haplotype. For North American states and European country abbreviations, see Table 1

	Nucleotide Position																					Geographical origin																							
																						North America																			Europe				Total	
																						Atlantic										Mississippi				Pacific									
Haplotype	1 8 8	1 0 4	1 0 7	1 1 4	1 1 7	1 2 7	1 4 8	1 4 9	1 6 5	1 6 7	1 6 8	1 7 1	1 7 4	1 7 5	1 7 7	1 7 8	2 1 5	2 6 3	3 7 3	VT	NY	RI	NJ	PA	MD	NC	GA	FL	MB	IL	LA	TX	MT	NV	CA	SP	FR	UK	IC	NAm	Eur
Ojam_01	C	A	T	C	C	C	A	C	C	A	C	C	C	C	C	C	T	T		1			2	1	3	2	1	6	9					1	1	13	10	8	3	27	34
Ojam_02	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.							1															1	
Ojam_03	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C			1									1			1							3	
Ojam_04	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.														1								1	
Ojam_05	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C						1																1	
Ojam_06	T	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	C				1																		1	
Ojam_07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C									1	1												2	
Ojam_08	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C															1							1	
Ojam_09	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C						1				1					1							3	
Ojam_10	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.					1		1			1												3	
Ojam_11	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.				1																		1	
Ojam_12	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1			1			1			1			2			1						7	
Ojam_13	.	.	C	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.			1		1																	2	
Ojam_14	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C		1																				1	
Ojam_15	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.																	1					1	
Ojam_16	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1					1			1		1										4	
Ojam_17	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.								1														1	
Ojam_18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.											1											1	
Ojam_19	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.										2												2	
Ojam_20	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.																1					1	
Ojam_21	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.											1												1	
Ojam_22	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	c											1												1	
Ojam_23	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.										1												1	
Total																				2	2	2	5	3	5	6	2	7	19	1	2	2	1	4	4	13	10	8	3	67	34

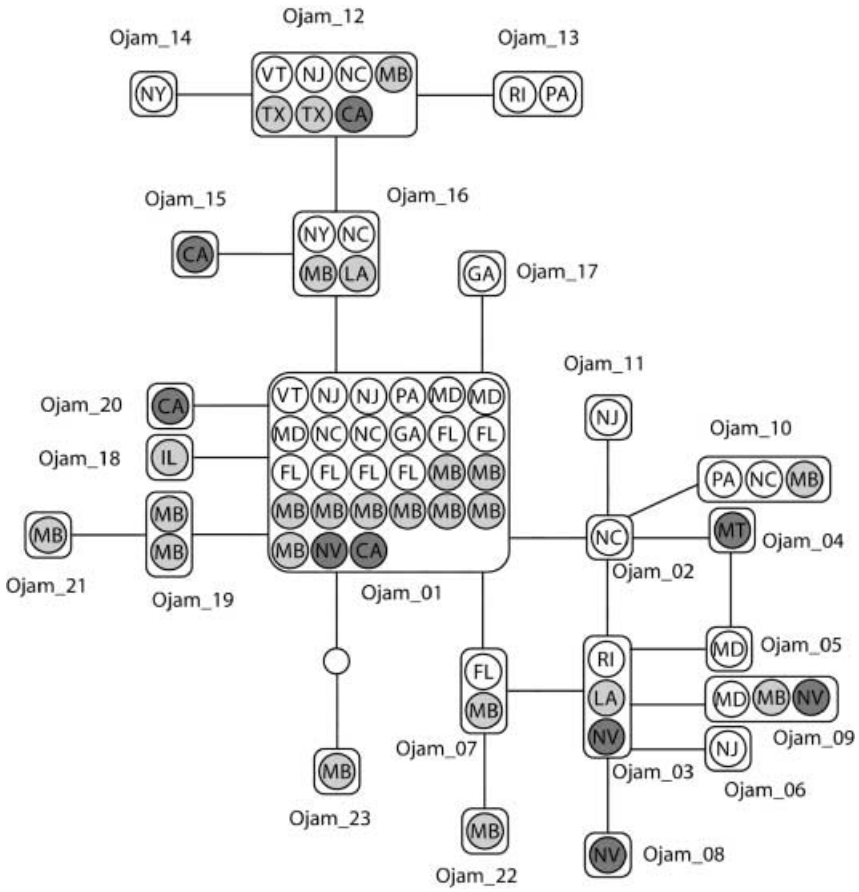

Fig. 1 Haplotype network of ruddy ducks. Only North American ruddy ducks are represented here; all the European ruddy ducks sequenced shared the same haplotype, Ojam_01. The shading of circles indicates the flyway in which each individual was collected: white, Atlantic flyway; light grey, Mississippi flyway; dark grey, Pacific flyway. The letters indicate the state of origin (see Table 1 for abbreviations).

Mississippi	Atlantic	Pacific	UK	France	Spain	
—	0.000	0.028	0.006	0.024	0.043	Mississippi
0.000	—	0.000	0.041	0.058	0.076*	Atlantic
0.000	0.000	—	0.156*	0.192*	0.237	Pacific
0.241***	0.246***	0.271***	—	0.000	0.000	UK
0.243***	0.243***	0.274***	0.002	—	0.000	France
0.294***	0.303***	0.325***	0.005	0.026	—	Spain

Table 3 Pairwise population Φ_{ST} (above the diagonal) and F_{ST} (below the diagonal) values. Significance values (P) based on 1023 permutation for Φ_{ST} and on 999 permutations for F_{ST}. Overall $\Phi_{ST} = 0.080$, $P = 0.044$. Overall $F_{ST} = 0.155$, $P = 0.001$. *$P < 0.05$; ***$P = 0.001$

Mitochondrial DNA haplotype and nucleotide diversity were relatively high in ruddy ducks from North America, with values of 0.824 and 0.00345, respectively. Similar values were observed within each flyway in North America. In contrast, no mtDNA variation was found in ruddy ducks from Europe (Table 4).

The mtDNA mismatch distribution for individuals from North America was unimodal (Fig. 2), a pattern that is compatible with a recent population expansion (Rogers & Harpending 1992). This pattern is also supported by the haplotype network (Fig. 1), in which a number of rare haplotypes radiate from a central common haplotype (Slatkin & Hudson 1991). Fu's F_S was negative and significant ($F_S = -12.30$; $P = 0.00$), a result also compatible with population expansion (Fu 1997). The expansion coefficient and exponential growth parameter also showed evidence of expansion ($S/d = 9.07$ and $g \approx 10^3$, respectively).

Among the microsatellite loci, Oxy4 did not conform to Hardy–Weinberg expectations ($P < 0.01$) in four populations (Atlantic, Mississippi, Pacific and Spain), as was the case for locus Oxy14 in two populations (Atlantic and Pacific). After sequential Bonferroni correction, however, the deviation from Hardy–Weinberg expectations was significant only for locus Oxy4 in three populations (Mississippi, Atlantic and Spain). Evidence for linkage disequilibrium between Oxy4 and Oxy10 was found in six populations (Atlantic, Mississippi, Pacific, UK, France and Spain) ($P < 0.05$). We therefore excluded Oxy4 from all analyses except allele counts. Ten additional pairs of loci (3% of 334 paired comparisons) showed some evidence of

Table 4 Genetic variability in ruddy ducks from North America, Europe and captive breeding populations for mtDNA and microsatellites

	mtDNA				Microsatellites				
Population	*n*	No. hapl.	*Hd* ± SD	π ± SD	*n*	A/locus	AR_C	H_O	H_E
North America	67	23	0.824 ± 0.044	0.00345 ± 0.00033	51	6.27	6.13	0.44	0.50
Europe (feral)	34	1	0.000	0.000	78	3.36	3.34	0.36	0.37
North America									
Atlantic	34	14	0.775 ± 0.074	0.00324 ± 0.00048	31	5.82	2.61	0.44	0.50
Mississippi	24	12	0.851 ± 0.064	0.00331 ± 0.00052	13	4.18	2.57	0.44	0.49
Pacific	9	8	0.972 ± 0.064	0.00478 ± 0.00070	7	3.36	2.51	0.44	0.48
Europe									
Spain	13	1	0.000	0.000	30	3.18	2.03	0.31	0.36
France	10	1	0.000	0.000	18	3.00	2.11	0.42	0.40
UK	8	1	0.000	0.000	27	2.91	2.01	0.38	0.36
Iceland	3	1	0.000	0.000	3	1.91	1.91	0.21	0.22
Captive									
WWT	3	1	0.000	0.000	6	2.27	2.03	0.48	0.39
Monmouth	3	1	0.000	0.000	7	2.45	2.12	0.42	0.38

n, sample size; No. hapl., number of haplotypes, Hd, haplotype diversity; π, nucleotide diversity; SD, standard deviation; A/locus, number of alleles per locus; AR_C, allelic richness corrected for sample size; H_O, observed heterozygosity; H_E, expected heterozygosity.

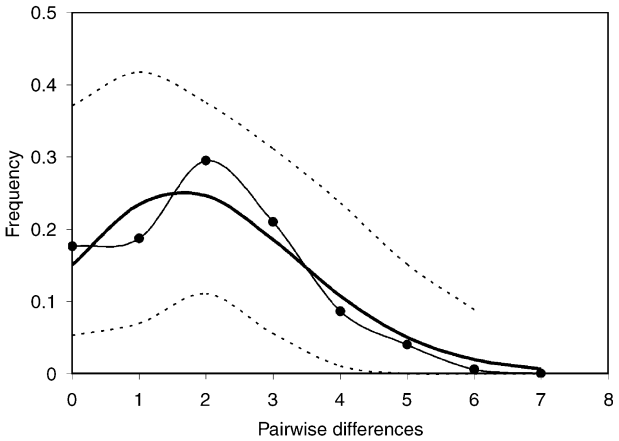

Fig. 2 Observed (thin line) mismatch distribution based on mtDNA haplotypes of North American ruddy ducks and the distribution fitted to the data (thick line) assuming population expansion. The dashed lines indicate 97.5 and 2.5 percentile values based on 1000 permutations. The observed distribution is compatible with recent population expansion.

linkage disequilibrium, but the same pairs of loci were not consistently linked in all populations (maximum of two populations). We therefore treated these as unlinked loci. The program MICRO-CHECKER detected evidence of null alleles in Oxy14 in Atlantic and in Oxy17 in Spain, but no consistent pattern was detected across populations, so we included these loci in the analyses. The allele frequencies for each locus and population are given in Appendix S1 (Supplementary material).

As with mtDNA haplotypes, a greater diversity of microsatellite alleles was present in North America than in Europe (Appendix S1, Fig. 3). Across all loci, a total of 71 alleles were found, of which 69 were present in North America and 38 were present in Europe, including both feral and captive ducks. The mean number of alleles per microsatellite locus, allelic richness and observed and expected heterozygosities were all higher in North American ruddy ducks (Table 4). The same was observed when comparing individual populations. In general, microsatellite alleles in European ruddy ducks were a subset of those found in North American ruddy ducks, although two alleles found in feral ducks from Europe were not found in our sample from North America. For many loci, the reduction in allelic diversity in Europe was accompanied by a substantial shift in the identity and/or frequencies of the most common alleles (Fig. 3).

Samples from Iceland, Europe, and captive collections in the UK generally shared similar sets of alleles. Although based on a small sample, allele frequencies in ruddy ducks from Iceland were clearly more similar to those in the feral European population than to those in North America (Fig. 3, Appendix S1). Only six alleles present in the European feral population were not present in our smaller sample of captive European populations (allele 154 in Oxy1, allele 246 in Oxy4, allele 168 in Oxy10, alleles 195 and 219 in Oxy13, and alleles 129 in Oxy14) (Appendix S1), whereas a single allele found in Monmouth was not found in feral ruddy ducks from Europe, but was present in North America.

As compared to the mitochondrial data, microsatellite data indicated greater differentiation between North American and European populations (F_{RT} = 0.276, P = 0.001), whereas no differences were found among the populations within these two groups (F_{SR} = 0.000, P = 0.475). Overall

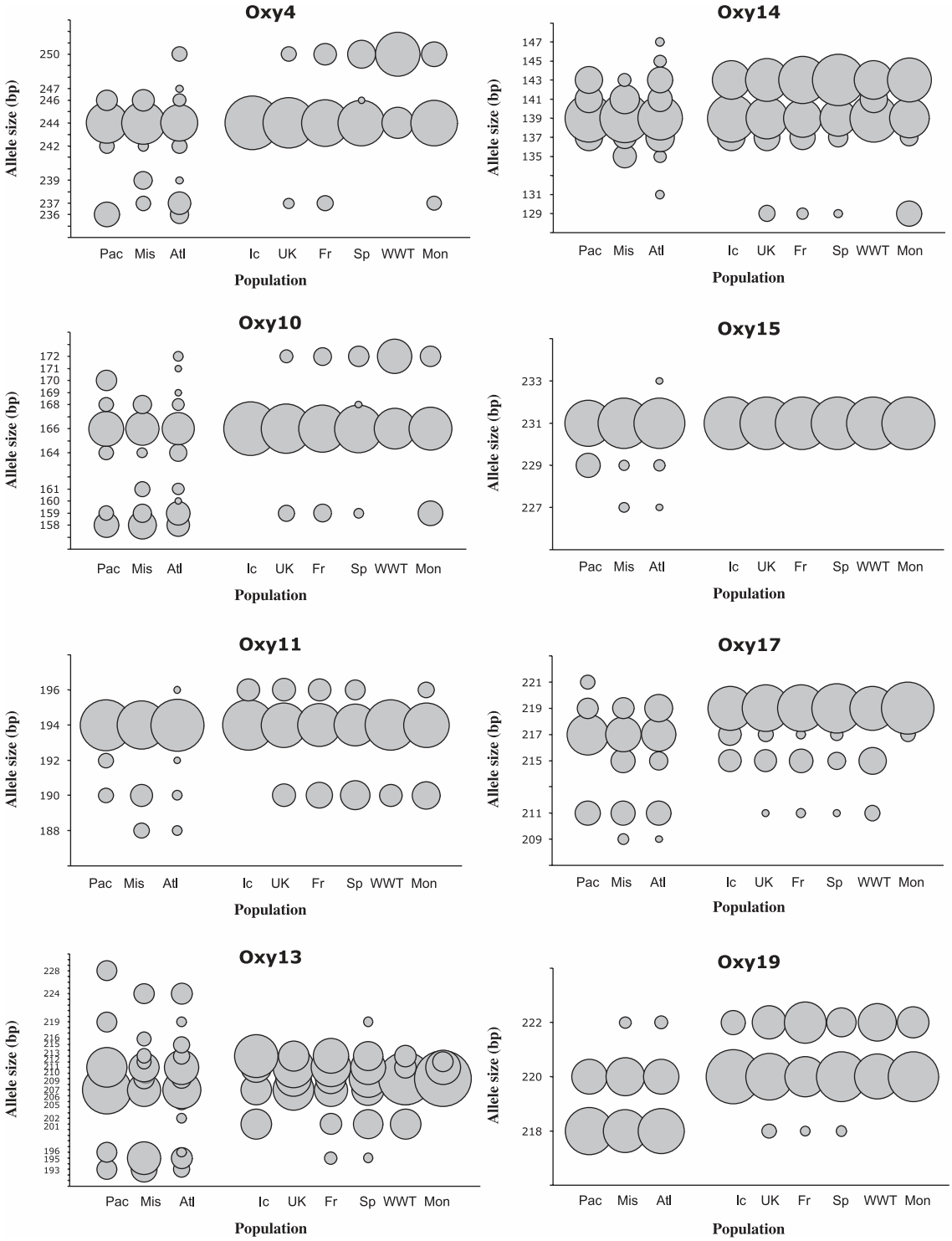

Fig. 3 Allelic frequencies for eight microsatellite loci in ruddy ducks in relation to geographical location. (Pac, Pacific; Mis, Mississippi; Atl, Atlantic; Ic, Iceland; UK, United Kingdom; Fr, France; Sp, Spain; WWT, Wildfowl and Wetlands Trust; Mon, Monmouth.) Three additional loci (Oxy1, Oxy3, Oxy6) showed lower allelic diversity in both populations. These loci had 3 and 3 alleles; 2 and 1 alleles; and 2 and 2 alleles in North America and Europe, respectively. The area of each circle is proportional to the relative frequency of each allele.

F_{ST} was also significant and accounted for 27% of the genetic variation (F_{ST} = 0.274, P = 0.001). Pairwise F_{ST} comparisons of populations were significant for all North American vs. European comparisons (P = 0.001 in all cases) (Table 3), but were not significant for pairwise comparisons among North American populations or among European populations.

Analyses with the software STRUCTURE indicated that the greatest likelihood was for K = 2 populations (estimated ln prob of data = –2394.3), with individuals sampled in North America strongly assigned to one group and both feral and captive individuals sampled in Europe strongly assigned to another group. For 93% of individuals, the inferred proportion of ancestry to their respective population was greater than 90%, and for 97% of the individuals it was greater than 80%. One bird from the UK was assigned almost equally to both groups (i.e. North America and Europe), and one bird from Spain and one from a captive breeding population were not clearly assigned (the proportion of ancestry attributed to the European population was 75% and 64%, respectively, for these two individuals). Posterior credible regions for these three individuals, however, ranged from 0 to 1, indicating that their genotypes were consistent with belonging to either of the two groups and that a lack of power rather than genetic admixture likely explains the uncertain assignment of a few birds. Some additional individuals had wide credible regions, but typically they were centred around the probability of belonging to one group or the other. Because STRUCTURE could not clearly assign these few individuals to either of the two populations, we also analysed the data with the software NEWHYBRIDS to check if these individuals could be 'hybrids' resulting from crosses between American and European birds. As with the STRUCTURE analysis, we found that ruddy ducks from North America were assigned to one group and that ruddy ducks from Europe, both feral and captive, were assigned to another group. No individuals were assigned to an intermediate group (referred to as F_1 by NEWHYBRIDS and corresponding to first-generation offspring of crosses between individuals from the two continents). Using different priors produced essentially the same results. This suggests that the genotypes of all European ducks were clearly differentiated from all the North American ducks, indicating that none of them was likely to be a recent immigrant or its offspring.

With both of the above methods, ruddy ducks from Iceland were assigned to the European population, suggesting that they originate from the European population rather than North America. Likewise, the ruddy ducks from captive populations in the UK were also assigned to the same group as feral ruddy ducks from Europe. This result is consistent with the hypothesis that the European feral population was founded by the escape of captive ducks in the UK.

The six microsatellite alleles present in the European feral population that were not found in the captive European populations (see above) might suggest that those alleles were derived from sources independent of the captive population in the UK, such as natural arrival of individuals from North America or other undocumented captive populations in Europe. However, a resampling approach indicates that seven individuals (i.e. the number of founders for the captive population) sampled from the North American population would carry a minimum of 28 and a maximum of 48 alleles, with a modal value of 38 (Fig. 4). Therefore, the number of alleles observed in captive and feral ruddy ducks from Europe (38 alleles) could be explained with just seven founders and without any additional gene flow from North America. This conclusion assumes minimal loss of alleles through drift between the founding event

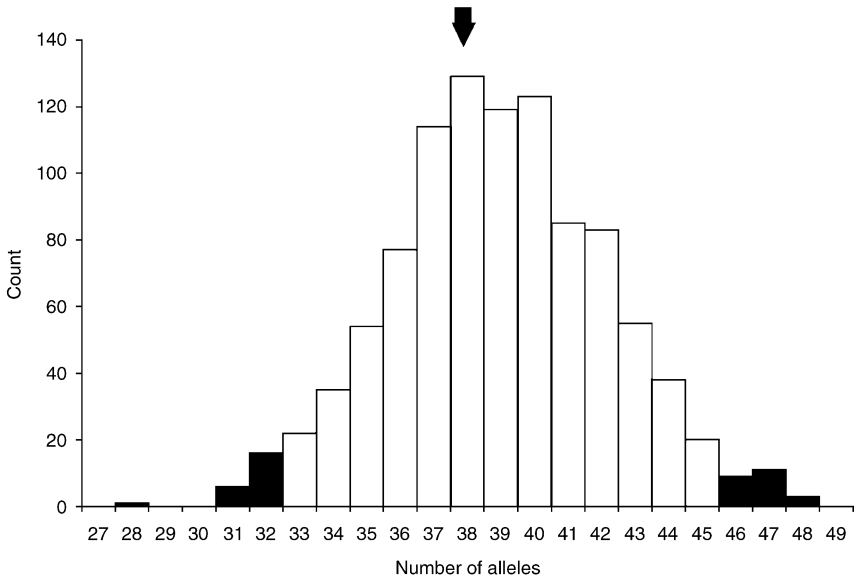

Fig. 4 Expected distribution for the number of microsatellite alleles in seven individuals randomly drawn from the North American population (results based on 1000 random samples). The arrow indicates the number of alleles found in the feral population in Europe. The 95% confidence interval (white colour) was determined by calculating the 0.025 and 0.975 percentiles (black colour).

and the present, a condition that might be satisfied if all seven founders contributed to the European population and if the founding event was followed by rapid population expansion (see Discussion). Drift within the smaller captive population subsequent to the establishment of the feral population in Europe combined with our small sample of captive individuals (n = 13) may also help to explain the somewhat larger number of alleles we documented in our sample from the feral population (n = 79 individuals).

Discussion

The ruddy duck in North America

Our analyses of ruddy ducks from North America showed no evidence of genetic structure across the geographical range of this species, either in mtDNA or microsatellite nuclear DNA. These results are consistent with band-return data for ruddy ducks (US Geological Survey unpublished data): birds ringed in one flyway are often recovered in another, suggesting a high degree of mobility and dispersal.

Our analyses also provide evidence for population expansion as suggested from the haplotype network (Fig. 1), the mismatch distribution (Fig. 2) and the negative and significant value of Fu's F_S. The expansion coefficient (S/d) and the maximum-likelihood estimate for the parameter S reached values reported in other studies as indicative of population expansions (von Haeseler *et al*. 1996; Lessa *et al*. 2003; Peck & Congdon 2004). Although tests of neutrality (*Fs*) cannot distinguish between a population expansion and a selective sweep (Fu 1997; Ramos-Onsins & Rozas 2002), our results, taken as a whole, are indicative of population expansion. Among birds, there is considerable variation in mtDNA mutation rate estimates (Lovette 2004), among which there are values as high as 20.8% divergence per million years for a small portion of the control region in geese (Quinn 1992). Given the absence of fossil evidence and the fact that our sequences comprise both highly variable and relatively conserved regions, it is difficult to establish a molecular clock. Recent expansions have also been documented for other North American birds (Milá *et al*. 2000; Hull & Girman 2005) and mammals (Brant & Ortí 2003; Lessa *et al*. 2003), possibly following the retreat of ice sheets after the last glacial maximum about 21 000 years ago (Cowling 1999) or other recent climatic changes. The lack of population genetic structure and evidence of recent expansion in North American ruddy ducks is similar to that observed in the congeneric white-headed duck *Oxyura leucocephala* in the Palaearctic (Muñoz-Fuentes *et al*. in press).

The lack of genetic structure in North American ruddy ducks is relevant to evaluating hypotheses about the origin of the European population. If, for example, ruddy ducks in the eastern USA were genetically differentiated from other populations, then natural dispersal to Europe might introduce only a limited subset of the genetic diversity present in North America. Our results, however, indicate that the European population is no more similar to ruddy ducks in the eastern USA than to those in other regions.

The ruddy duck in Europe

Considerably lower genetic diversity was found in the ruddy ducks from Europe than from North America, both in mtDNA and microsatellite markers. The only haplotype present in Europe, Ojam_01, is the most common and widespread in North America. In terms of microsatellite data, 69 alleles were found in North America whereas only 38 were found in a larger sample of ruddy ducks from Europe. Therefore, the ruddy ducks from North America exhibited higher haplotype and microsatellite diversity (number of alleles per locus, allelic richness, observed heterozygosity and expected heterozygosity). In some cases the opposite pattern is observed, such that the introduced population harbours greater genetic variability than any of the possible source populations due to repeated introductions from genetically differentiated sources (Kolbe *et al*. 2004). In the case of the ruddy duck in Europe, the low diversity suggests that the number of colonizing events/individuals is probably limited.

The level of genetic diversity in European ruddy ducks is consistent with population growth after a strong bottleneck associated with a single founding event. Our results suggest that a small number of founders from North America, bearing only a fraction of the total genetic variability of the species, gave rise to a new population in Europe. The initial founding event also explains the shifts in allele frequencies observed for several microsatellite loci and the restricted subset of North American alleles found in the European population (Fig. 3). Seven ruddy ducks, four males and three females, were brought to the WWT at Slimbridge, UK, in 1948 and approximately 90 descendants of these birds escaped between 1953 and 1973 (Hudson 1976). These appear to be the founders of the present feral population across Europe and North Africa, as well as the birds still held in captivity in Europe. It is unknown how many of these seven individuals successfully reproduced, or how many later escapes from captive populations have taken place (Rose 1993). Limited diversity in both mitochondrial haplotypes (a single haplotype in all European ruddy ducks) and microsatellite alleles is consistent with the captive population at WWT being the single source of feral European ruddy ducks.

Six alleles found in the present feral population in Europe were not found in the captive birds examined (the WWT and Monmouth groups). Despite the fact that Monmouth was founded by individuals from WWT, both of the captive collections had alleles not present in the

other, suggesting either that these alleles have been lost by drift or were not represented in the sample of birds we analysed. In the same manner, the six additional alleles found in the feral population in Europe could have been present in the seven original individuals that were imported to WWT but have since disappeared in the captive populations due to drift. Moreover, these alleles were typically at a frequency less than or equal to 6%, and therefore would have been more likely to disappear by drift than if they were highly frequent. The feral population in the UK became established between 1953 and 1973, and in 1980, it numbered approximately 1500 birds. This rapid population growth likely minimized the effects of drift (Merilä *et al*. 1996; Zeisset & Beebee 2003). In contrast, the two captive populations studied, which include a combined total of less than 100 birds, may have lost some alleles over the past 30–50 years. It is also possible that some of these rare alleles have resulted from new mutations in the European population over the last 50 years. Microsatellites have high rates of mutation, and some of the fastest evolving microsatellites have been described in birds (Primmer & Ellegren 1998; Brohede *et al*. 2002; Beck *et al*. 2003). Assuming the founders had common alleles from North America, then stepwise mutations would likely generate other alleles already present in the North American population. Therefore, drift in the captive population, minimal drift in a rapidly growing feral population, and high microsatellite mutation rates may account for the allelic diversity found in European ruddy ducks without the input of additional genetic variation from North America.

Noticeably, composite genotypes based on just 10 microsatellite loci consistently identified individual ruddy ducks as being from Europe or North America. Despite the limited amount of time these populations have had to diverge genetically (*c*. 50 years), this strong discrimination was possible as a result of the reduction in allelic diversity in ruddy ducks from Europe and substantial shifts in the identity and/or frequencies of the most common alleles at several loci (Fig. 3), consequences of the founding event. No evidence was found for the recent arrival of North American ducks to the European population or for the existence of individual birds resulting from interbreeding between a ruddy duck from Europe and a ruddy duck from North America. Therefore we find no evidence that ruddy ducks from North America contributed to the population of ruddy ducks in Europe after the initial founding event.

Contributing to this inference is the fact that ruddy ducks from Iceland and the captive-bred population are indistinguishable from the feral population in the rest of Europe, while the European population as a whole is significantly differentiated from the North American population. The assignment of ruddy ducks from Iceland to the European population indicates colonization from Europe rather than from North America and suggests that wild ruddy ducks from North America have not reached Iceland or the European mainland via Iceland in recent years. Moreover, our findings are consistent with the fact that many bird species migrate between Iceland and the UK (Wernham *et al*. 2002) and that the Icelandic biota is formed mainly by species from the Palaearctic (Tiedemann *et al*. 2004).

Summarizing, our findings indicate that the European population, including the captive and Icelandic individuals, forms a group genetically differentiated from the North American population due to shifts in allele frequencies associated with a founding event. The genetic diversity of European ruddy ducks is consistent with a founder population as small as seven birds, and we find no evidence for any recent arrival of additional ruddy ducks from North America.

Implications for conservation

In countries where the endangered white-headed duck is native, hybridization with the ruddy duck has been identified as a major threat to its survival (Hughes *et al*. 1999, 2004). Because the ruddy duck and the white-headed duck have been separate species for perhaps 1–2 million years (McCracken *et al*. 2000; McCracken & Sorenson 2005; Muñoz-Fuentes *et al*. submitted), evolving in different continents, and because we can now rule out the natural arrival of ruddy ducks from North America to Europe, ongoing efforts to eliminate introduced ruddy ducks from European countries (Torres & Moreno 2000; Hughes *et al*. 2004) should be continued in order to conserve the white-headed duck.

Acknowledgements

We greatly appreciate the assistance of everyone who provided samples for our analysis: B. Hughes, C. Gerique, C. Perennou, C. Sánchez, C. Urdiales, H. Garrido and Sylvática S.A., I. Henderson, J.L. Echevarrías, J.L. Torres-Esquivias, J. Peters, L. Barbier, N. Jarrett, O.K. Nielsen, P. Pereira, R.B. Brua and R. Cromie. This study was funded by La Consejería de Medio Ambiente de la Junta de Andalucía, Spain, a fellowship by the Spanish Ministry of Science and Education to V.M.F., a National Science Foundation grant to M.D.S., and a grant by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning to C.V. Access to the US Geological Survey banding data was provided by T.C. Michot. P. Smouse provided help with GenAlEx. B. Hughes and three anonymous reviewers provided helpful comments on the manuscript.

Supplementary material

The supplementary material is available from http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2886/MEC2886sm.htm

Appendix S1 Allelic frequencies at 11 microsatellite loci in ruddy ducks, per locus and population.

References

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution*, **16**, 613–622.

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.

Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford University Press, Oxford, UK.

Baldassarre GA, Bolen EG (1994) *Waterfowl Ecology and Management*. John Wiley & Sons, New York.

Beck NR, Double MC, Cockburn A (2003) Microsatellite evolution at two hypervariable loci revealed by extensive avian pedigrees. *Molecular Biology and Evolution*, **20**, 54–61.

BirdLife International (2000) *Threatened Birds of the world*. Lynx Edicions and BirdLife International, Barcelona, Spain, and Cambridge, UK.

Brant SV, Ortí G (2003) Phylogeography of the Northern short-tailed shrew, *Blarina brevicauda* (Insectivora: Soricidae): past fragmentation and postglacial colonization. *Molecular Ecology*, **12**, 1435–1449.

Brohede J, Primmer CR, Møller A, Ellegren H (2002) Heterogeneity in the rate and pattern of germline mutation at individual microsatellite loci. *Nucleic Acid Research*, **30**, 1997–2003.

Brown JH, Sax DF (2004) An essay on some topics concerning invasive species. *Austral Ecology*, **29**, 530–536.

Brua RB (2001) Ruddy Duck (*Oxyura jamaicensis*). In: *Birds of North America, No. 696* (eds Poole A, Gill A). The Birds of North America, Inc., Philadelphia.

Caughley G, Gunn A (1996) *Conservation Biology in Theory and Practice*. Blackwell Science, Cambridge, Massachusetts.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

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

Cowling SA (1999) Simulated effects of low atmospheric CO_2 on structure and composition of North American vegetation at the last glacial maximum. *Global Ecology and Biogeography*, **8**, 81–93.

Cramp S, Simmons KEL (1977) *Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palaearctic*. Oxford University Press, Oxford, UK.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fu Y (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513–1520.

Gemmell NJ, Akiyama S (1996) An efficient method for the extraction of DNA from vertebrate tissue. *Trends in Genetics*, **12**, 338–386.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F* statistics. *Journal of Heredity*, **86,** 485–486.

Green AJ, Hughes B (2001) *Oxyura leucocephala*. White-headed duck. *BWP Update*, **3**, 79–90.

Hudson R (1976) Ruddy ducks in Britain. *British Birds*, **69**, 132–143.

Hughes B (1998) Ruddy duck. *BWP Update*, **2**, 159–171.

Hughes B, Criado J, Delany S *et al*. (1999) *The Status of the North American Ruddy Duck Oxyura jamaicensis in the Western Palearctic: Towards an Action Plan for Eradication*. Council of Europe Publication T-PVS/Birds (99) 9. Council of Europe Publishing, Strasbourg, France.

Hughes B, Robinson JA, Green AJ, Li ZWD, Mundkur T (Compilers) (2004) *International Single Species Action Plan for the White-headed Duck Oxyura leucocephala*. The Wildfowl and Wetlands Trust, Slimbridge, UK.

Hull JM, Girman DJ (2005) Effects of Holocene climate change on the historical demography of migrating sharp-shinned hawks (*Accipiter striatus velox*) in North America. *Molecular Ecology*, **14**, 159–170.

Kear J (2005) *Bird Families of the World: Ducks, Geese, Swans and Screamers*. Oxford University Press, Oxford, UK.

Kolbe JJ, Glor RE, Rodríguez Schettino L, Chamizo Lara A, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, **149**, 429–434.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, **11**, 2445–2449.

Lessa EP, Cook JA, Patton JL (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the late Quaternary. *Proceedings of the National Academy of Sciences, USA*, **100**, 10331–10334.

Lovette IJ (2004) Mitochondrial dating and mixed support for the '2% rule' in birds. *Auk*, **121**, 1–6.

Marchant S, Higgins PJ (1990) *Handbook of Australian, New Zealand and Antarctic Birds*. Oxford University Press, Oxford, UK.

McCracken KG, Harshman J, Sorenson MD, Johnson KP (2000) Are ruddy ducks and white-headed ducks the same species? *British Birds*, **93**, 394–398.

McCracken KG, Sorenson MD (2005) Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff-tailed ducks (*Nomonyx-Oxyura*)? *Systematic Biology*, **54**, 35–55.

Merilä J, Björklund M, Baker AJ (1996) The successful founder: genetics of introduced *Carduelis chloris* (greenfinch) populations in New Zealand. *Heredity*, **77**, 410–422.

Milá B, Girman DJ, Kimura M, Smith TB (2000) Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American song bird. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 1033–1040.

Muñoz-Fuentes V, Green AJ, Negro JJ, Sorenson MD (2005) Population structure and loss of genetic diversity in the endangered white-headed duck, *Oxyura leucocephala*. *Conservation Genetics*, **6**, 999–1015.

Muñoz-Fuentes V, Gyllenstrand N, Negro JJ, Green AJ, Vilà C (2005) Microsatellite markers for two stifftail ducks: the white-headed duck, *Oxyura leucocephala*, and the ruddy duck, *O. jamaicensis*. *Molecular Ecology Notes*, **5**, 263–265.

Nielsen OK (1994) The ruddy duck (*Oxyura jamaicensis*) in Iceland. *Oxyura*, **7**, 67–73.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Parker IM, Simberloff D, Lonsdale WM *et al.* (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions*, **1**, 3–19.

Peakall R, Smouse PE (2001) *GENALEX V5: Genetic Analysis in Excel. Population genetic software for teaching and research*. Australian National University, Canberra, Australia. www.anu.edu.au/BoZo/GenAlEx.

Peck DR, Congdon BC (2004) Reconciling historical processes and population structure in the sooty tern *Sterna fuscata*. *Journal of Avian Biology*, **35**, 327–335.

Primmer CR, Ellegren H (1998) Patterns of molecular evolution in avian microsatellites. *Molecular Biology and Evolution*, **15**, 997–1008.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Quinn TW (1992) The genetic legacy of mother goose — phylogeographic patterns of lesser snow goose *Chen caerulescens* maternal lineages. *Molecular Ecology*, **1**, 105–117.

Rambaut A (1996) *SE-AL: Sequence Alignment Editor*. Department of Zoology, University of Oxford, UK.

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092–2100.

Raymond M, Rousset F (1995) GENEPOP (version 1.2.): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248–249.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rose P (1993) *Ruddy Duck European Status Report*. International Waterfowl and Wetlands Research Bureau, Slimbridge, UK.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN Version 2.000: A software for population genetics data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Sefc KM, Payne RB, Sorenson MD (2005) Genetic continuity of brood parasitic indigobird species in space and time. *Molecular Ecology*, **14**, 1407–1419.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Sorenson MD, Quinn TW (1998) Numts: a challenge for avian systematics and population biology. *Auk*, **115**, 214–221.

Tiedemann R, Paulus KB, Sheer M *et al.* (2004) Mitochondrial DNA and microsatellite variation in the eider duck (*Somateria mollissima*) indicate stepwise postglacial colonization of Europe and limited current long-distance dispersal. *Molecular Ecology*, **13**, 1481–1494.

Torres JA, Moreno-Arroyo B (2000) Presencia de la malvasía canela (*Oxyura jamaicensis*) en España. *Oxyura*, **10**, 69–78.

von Haeseler A, Sajantila A, Pääbo S (1996) The genetics archaeology of the human genome. *Nature Genetics*, **14**, 135–140.

Wernham CV, Toms MP, Marchant JH, Clark JA, Sirwardena GM, Baillie SR (2002) *Migration Atlas: Movements of the Birds of Britain and Ireland*. T. & A.D. Poyser, London, UK.

Wetlands International (2002) *Waterbird Population Estimates*, 3rd edn. Wetlands International, Wageningen, The Netherlands.

Zeisset I, Beebee TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Molecular Ecology*, **12**, 639–646.

Zonfrillo B (2000) Ruddy duck. *British Birds*, **93**, 394–396.

Violeta Muñoz is interested in conservation genetics and in applying molecular biology to understanding the biology and ecology of species. Andy Green is interested in waterfowl biology and aquatic ecology. Michael Sorenson has long-standing interests in the systematics and behavioural ecology of waterfowl and has in recent years been working on a comprehensive molecular phylogeny for the group. Juan Negro, although initially trained as a behavioural ecologist, is also interested in genetic variability issues, hybridization and genetic erosion in small populations. Carles Vilà works in conservation genetics and animal domestication.
