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Genetically distinct island populations of the Egyptian vulture (*Neophron percnopterus*)

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Abstract

The Egyptian vulture (*Neophron percnopterus*) is a species in decline throughout Europe, with the largest remaining breeding populations found in northern Spain. Iberian Peninsula populations of this species (about 1000 pairs) migrate to Africa in winter, while small populations in both the Canary and Balearic Islands (less than 40 pairs in each case) are apparently sedentary. We found that Egyptian vultures from both of these island groups were significantly differentiated from Iberian Peninsula populations (R_{ST} = 0.065–0.129, p = 0.000–0.007), using nine microsatellite loci isolated in a related species, the bearded vulture. The greatest degree of genetic differentiation was observed between the two island groups (R_{ST} = 0.279, p = 0.000). These island populations were more distinct from mainland groups than was a small sample of a well-defined separate subspecies from India (*N. p. ginginianus*; R_{ST} = 0.083–0.091, p = 0.023–0.024). This implies that these two island populations have been isolated from peninsular populations for many generations, despite the long-distance migration capabilities of the species. In constrast, populations within the Peninsula were not differentiated from one another at these microsatellite loci (R_{ST} = −0.004–0.007, p = 0.442–0.675). Introductions of Egyptian vultures from the larger northern breeding groups might therefore be appropriate in southern Spain, if necessary, but mainland birds should not be introduced to the islands if the genetic distinctiveness of these groups is to be preserved. Independent conservation plans are urgently required to protect these two island populations from extinction.

Introduction

The Egyptian vulture (*Neophron percnopterus*) is the smallest of the four vulture species inhabiting the Iberian Peninsula. The species is currently listed as "endangered" in Europe (Tucker and Heath 1994) and "vulnerable" in Spain, which harbors the largest remaining breeding populations in Europe (Blanco and González 1992). Up to 1300 breeding pairs were censused in 1989, most of them concentrated in the northern mountains, the Ebro Valley of Navarra and Aragón, Central Cordillera and Extremadura (Perea et al. 1990). By the year 2000, however, several formerly dense populations had largely vanished; up to 70% of the breeding pairs were lost in some areas, and a 30% decline was recorded overall (JL Tella, G Blanco and JA Sánchez-Zapata, unpublished data). Strong regional declines, particularly in southern Spain (Andalucía), may be largely attributable to illegal poisoning (78 deaths apparently caused by poisoning were reported between 1990-1999, Grupo de Trabajo de Ecotoxicología, unpublished report).

The Iberian Peninsula Egyptian vulture populations migrate to Spain from wintering grounds in the African Sahel region in February and March, and return to Africa in September and October, following the summer breeding season (Brown and Amadon 1968). Migratory breeding populations of Egyptian vultures also occur elsewhere in Europe, including France, Italy, Turkey, Greece, and Bulgaria. The

population in the south of France follows the same migration route as the Iberian birds (Max Gallardo, personal communication), but nothing is known about migratory movements of other European populations. Nearly all of these populations have declined drastically in recent decades (Tucker and Heath 1994). The species also exists in suitable habitat in much of eastern Africa (Brown and Amadon 1968); it is now very rare in the southern subcontinent (Mundy 1978) and the northern Magreb region (CJ Palacios, unpublished data). A separate subspecies (*Neophron percnopterus ginginianus*) has been described from south-central India, which is of smaller size than the nominate subspecies, with weaker feet and claws and an all yellow bill (Brown and Amadon 1968).

In contrast to the migratory Egyptian vulture populations of the Iberian Peninsula, small breeding groups found in the Canary and Balearic Islands are believed to be sedentary (Palacios 2000; De Pablo 2000). Sedentary populations are also found on small islands of the Arabian Sea (Socotra, Masira) and the Cape Verde archipelago, as well as on the Arabian peninsula and in India (Cramp and Simmons 1980). The species was formerly much more abundant and widespread in the Canaries; it was numerous on Grand Canary Island in the 1930s, before the port slaughterhouses were closed (Bannerman 1963). By 1987, the population had been reduced to 31–37 pairs, primarily restricted to the island of Fuerteventura; the 2001 census found 26 breeding pairs (Donázar et al. 2002a). The principal threats to this population appear to be human disturbance of nest sites, poisoned carcasses, and the proliferation of power lines, with associated electrocution mortality of Egyptian vultures (Palacios 2000). In the Balearics, the species was also formerly more abundant, breeding on both Mallorca and Menorca, but was considered exterminated on Mallorca by 1977 (Bannerman and Bannerman 1983). One breeding pair was found on Mallorca in 1993, apparently originating from Menorca (Viada and Rebassa 1993). The most recent census found 34 breeding pairs of Egyptian vultures on the island of Menorca in 1999 (De Pablo 2000).

If the island populations are truly sedentary and no significant immigration occurs, these birds are likely to be diverging genetically from mainland populations. If this is the case, separate management plans are urgently needed for the effective conservation of these small island populations. Introductions of Egyptian vultures from the mainland in order to bolster island populations may not be advisable, depending on the degree of genetic differentiation among these breeding groups. An examination of possible population genetic structure within the Peninsula is also important, particularly in light of the recent population decline in southern Spain. This population is geographically isolated from the larger breeding aggregations in the north, and could potentially be genetically distinct.

In addition to the threats posed by poison and power lines, carrion-scavenging birds are now facing possible food shortages, due to changes in Spanish law regarding disposal of livestock remains. Egyptian vultures at communal roosts in northern Spain were strongly dependent upon livestock waste dumps for feeding (Donázar et al. 1996). This species is likely to suffer more serious declines in the future, as many of these dumps are destined to be closed. As a result, conservation attention has been increasingly focused on these birds (e.g., Verón 2001). In addition to ongoing studies of habitat use and factors responsible for population declines, an understanding of Egyptian vulture population genetic structure is critical to the design of an effective conservation program for this species.

Methods

Sampling

Blood samples were obtained from Egyptian vulture nestlings during banding campaigns conducted between 1995 and 2000 in Bardenas (Navarra; n = 30), Monegros (Aragón; n = 30), Cádiz/Málaga (Andalucía; n = 9), Menorca (Balearic Islands; n = 7) and Fuerteventura (Canary Islands; n = 13; see Figure 1 for locations). Each of these samples represents one offspring of a unique reproductive pair. Because the same pair may re-use the same nest site for many years, we included only new nest sites in our population samples from successive years. Within a population, the distance between nest sites ranged from 200m to about 12 km. The Egyptian vulture is unusual among vultures in that it often lays two eggs, although brood reduction frequently occurs (Donázar and Ceballos 1989). When both siblings survived to sampling, we used only one in our analysis. Because the island populations were of particular interest, we obtained additional samples (n = 15) from juvenile Egyptian vultures (one to three years old, based on plumage) captured at a communal

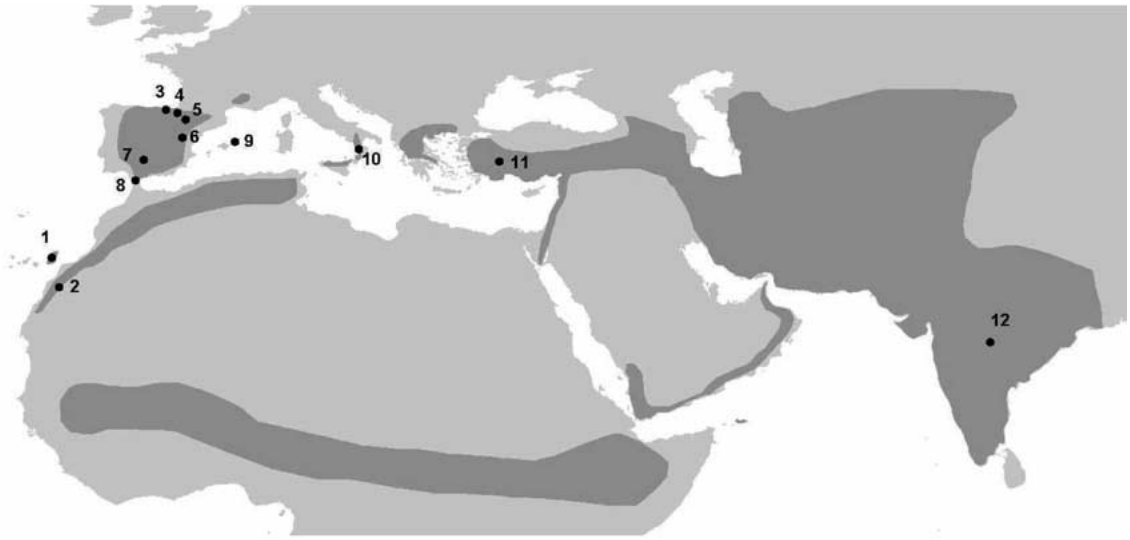

Figure 1. Distribution of the Egyptian vulture (*Neophron percnopterus*) in the Palearctic. Birds sampled for this study originated in: (1) Canary Islands, (2) Sahara (Morocco), (3) Vizcaya, (4) Navarra, (5) Monegros, (6) Teruel, (7) Cordoba, (8) Cádiz, (9) Menorca, (10) Italy, (11) Turkey, and (12) India. Population samples large enough for pairwise comparisons include the Canary Islands (1), Navarra (4), Monegros (5), Andalucia (8), Menorca (9), and India (12).

roost on Fuerteventura. No comparable samples were available from Menorca.

In order to evaluate the importance of genetic differences among Spanish populations, we used six individuals of the Indian subspecies (*N. p. ginginianus*) for comparison. These samples were obtained from a captive breeding colony (Zoo Mulhouse, France) and included two juveniles and four adults (excluding known parents of the young birds). The exact location of origin of the adult birds within the Indian subcontinent was unknown. Additional samples were obtained opportunistically in numbers too small for population comparisons: three nestlings from a small population in Vizcaya (Pais Vasco), and one each from isolated nests in Córdoba (Andalucía) and Teruél (Aragón), three adult birds from Turkey, one juvenile from Sicily, and a captive individual known to originate from the Spanish Sahara (now Morocco).

DNA extraction

DNA extractions were performed by adding 300 μl of digestion buffer (100 mM NaCl, 50 mM Tris-HCl [pH = 8], 1% SDS, 50 mM EDTA) to 100 μl of whole blood, adding 10 μl proteinase k (20 mg/ml), and incubating samples overnight (or longer) at 37 °C on a rotating rack. Following digestion, an equal volume of 5M LiCl was added to each sample and mixed gently. Then two extractions with chloroform:isoamyl alcohol (24:1) were performed and DNA was precipitated in the final supernatant by the addition of two volumes of cold 100% ethanol. After several hours in the freezer, samples were centrifuged again for 15 m at 13,000 rpm to form a DNA pellet, which was washed twice with 70% ethanol, air-dried, re-suspended in 50–100 μl TE and stored at 4 °C. DNA concentrations were measured using a Hoefer DyNA Quant 200 fluorometer, following manufacturer's instructions, and diluted to approximately 50 ng/μl.

Microsatellite loci amplification and analysis

Microsatellite loci were isolated in the bearded vulture (*Gypaetus barbatus*) and the resulting primers were shown to amplify polymorphic loci in the Egyptian vulture by Gautschi et al. (2000). Nine of these loci were used in this study, and PCR conditions were optimized for each locus in this species (Table 1). One primer in each pair was then labeled with one of three fluorescent dyes (FAM, TET and HEX; Progenetic S.L., Barcelona, Spain). Three loci with non-overlapping allele size ranges were marked with each dye, to allow all nine loci to be evaluated simultaneously. Individual locus amplifications were carried out in a PTC-100TM Thermocycler (MJ Research, Inc.). PCR reaction mixes of 20 μl volume contained

Table 1. PCR conditions for microsatellite loci amplification in the Egyptian vulture, and the number of alleles found in this study of 120 individuals

Locus[a]	Repeat motif[a]	$MgCl_2$	Annealing temp[b]	# alleles
BV6	$(CA)_{11}$	2 mM	59 °C	7
BV9	$(TA)_6(CA)_{11}$	1.5 mM	59 °C	5
BV11	$(CA)_{22}$	1.5 mM	59 °C	2
BV12	$(CA)_{15}$	1.5 mM	TD60/50 °C	3
BV13	$(CA)_{16}$	2 mM	TD60/50 °C	5
BV14	$(CA)_{16}$	2 mM	59 °C	2
BV16	$(GA)_3(CA)_3$ $A_{13}(GA)_{13}$	2 mM	59 °C	5
BV17	$(CA)_{11}$	1.5 mM	TD60/50 °C	2
BV20	$(CA)_{13}$	2 mM	TD60/50 °C	3

[a]from Gautschi et al. (2000).
[b]Programs were run as follows: 59 °C: 2 m at 94 °C, followed by 34 cycles of 30 s at 92 °C, 30 s at annealing temperature, 30 s at 72 °C, and then 5 m at 72 °; TD60/50 °C: 2 m at 94 °C, followed by 30 s at 92 °C, 30 s at initial annealing temp (subtract 1 °C per cycle until final annealing temp reached), 1 m at 72 °C, 40 cycles overall, and then 5 m at 72 °.

1 × PCR buffer (without $MgCl_2$), 1.5–2 mM $MgCl_2$ (see Table 1), 0.1 mg/ml BSA, 0.25 mM dNTPs, 0.25 μM each of forward and reverse primers, 0.5 U/μl Taq polymerase, and approximately 100 ng DNA. Presence of a PCR product of the expected size was verified using agarose gel electrophoresis and ethidium bromide staining.

For each sample, PCR products from all nine amplifications were mixed into a final volume of 125 μl, using milliQ water and 2.5–10 μl product, depending on amplification strength (as visualized in the gel). Two microliters of this diluted mixture was then added to 12 μl formamide + 0.25 μl TAMRA-500 size standard (Applied Biosystems), denatured for 2 m at 95 °C, and loaded into the capillary system of an ABI Prism 310 Genetic Analyzer automated sequencer, using POP4 polymer and 1X EDTA buffer (Applied Biosystems). Allele sizes were determined using the GeneScan software, based on comparison with the TAMRA size standard.

Statistical analysis

Analysis of population differentiation was performed with R_{ST}Calc (version 2.2, Goodman 1997), based on Slatkin's (1995) measure of genetic differentiation using microsatellite data. This program includes corrections for differences in sample size among populations, and differences in allele size variance among loci, both of which were substantial in our data set. Fstat (version 2.9.1, Goudet 2000), and Genepop (web version 3.1, Raymond and Rousset 1995) provided traditional F_{ST} estimates (Weir and Cockerham 1984), as well as genetic diversity indices, tests for deviation from Hardy-Weinberg equilibrium, heterozygote deficit and linkage disequilibrium among loci. The sequential Bonferroni correction for multiple tests was applied to estimates of statistical significance where appropriate (Rice 1989).

Results

Allele number and frequency

The number of alleles found at each of these microsatellite loci, in our sample of 120 Egyptian vultures, ranged from two to seven (Table 1). These were generally fewer than the number of alleles found at these same loci in a sample of 30 bearded vultures, from which these markers were isolated (Gautschi et al. 2000). While this finding is perhaps indicative of ascertainment bias (Ellegren et al. 1995), it does not affect conclusions about differences in variability among populations of the Egyptian vulture. Of 1080 PCRs (120 individuals × nine loci), only 16 (1.5%) failed to yield a product after repeated attempts. These amplification failures involved seven of the nine loci, and are therefore unlikely to be explained by the presence of null alleles (Pemberton et al. 1995), although BV6 failed to amplify most frequently ($n = 6$).

Of the nine loci, two (BV11 and BV17) were monomorphic in some populations, and nearly so overall. Analysis of population differentiation was therefore performed both including and excluding these two loci. A third locus (BV6) was also nearly fixed for a single allele in several populations, although six other relatively rare alleles were detected. Three loci with two or three alleles each, and three others with five alleles each, showed clear differences in allele frequency across populations (Figure 2). The sample from the Indian subspecies, although composed of only six individuals, included two unique ("private") alleles, at BV20 and BV13. Private alleles at two other loci were detected in the larger samples ($n = 30$) from Navarra (BV9) and Aragón (BV16; Figure 2). No private alleles were found in samples from the Balearic or Canary Islands, and no additional alleles were found in the few individuals originating from isolated nests in Spain, or from Italy, Turkey or the Spanish Sahara (data not shown).

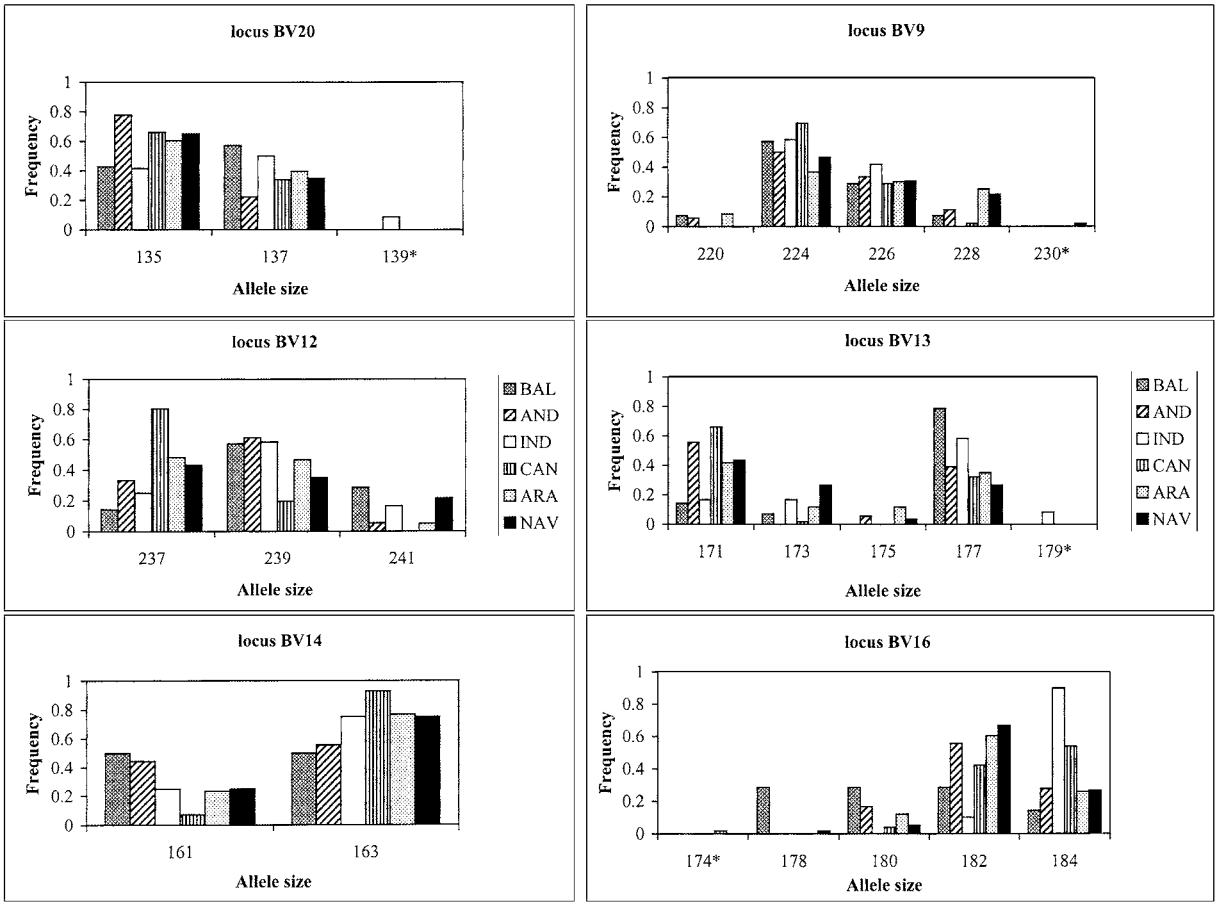

Figure 2. Allele frequencies at six of the nine microsatellite loci used in this study, in 6 populations of Egyptian vultures. Allele sizes are in base pairs; private alleles are marked with an asterisk. Populations and sample sizes as follows: BAL = Balearic Islands (n = 7), AND = Andalucía (n = 9), IND = India (n = 6), CAN = Canary Islands (n = 28), ARA = Aragón (n = 30), NAV = Navarra (n = 30).

For the population comparisons, 110 individuals were available. The 15 individuals from the communal roost did not differ in allele frequency at any locus from the 13 birds from known nest sites in the Canary Islands (log-likelihood G test, all p-values > 0.06, Goudet et al. 1996). Therefore, we included all 28 birds from this site in the population comparisons, although this sample may include some related individuals.

Genetic variability

The two small island populations of Egyptian vultures showed different levels of genetic variability at these microsatellite loci. Rare alleles were generally absent from the Canaries but present in the Balearic Island birds (Figure 2). Mean expected heterozygosity (H_e) and allelic richness (a measure of the number of alleles independent of sample size; El Mousadik and Petit 1996) were highest in the Balearic Islands population, and all nine loci were polymorphic despite the small sample available from this location (n = 7). In contrast, the Canary Islands population (including unrelated birds only; n = 13) showed significantly lower mean values of H_e than the Peninsular populations (Table 2). Mean allelic richness was also lowest in the Canaries, and two of the nine loci were monomorphic in this population (Table 2).

Linkage disequilibrium

All possible comparisons between pairs of loci in each population ($36 \times 6 = 216$ comparisons) yielded only six cases of significant ($p < 0.05$) linkage disequilibrium. Following Bonferroni correction for multiple tests, none of these values retained signifi-

Table 2. Measures of genetic diversity (number of polymorphic loci, observed and expected heterozygosity, and mean allelic richness [AR]) based on 9 microsatellite loci for six Egyptian vulture populations (abbreviations as in Figure 1). N = number of individuals; only unrelated birds (from known independent nests) were included

Population	N	# poly. loci	Mean H_o	Mean H_e[a]	Mean AR[b]
AND	9	8	0.290	0.431*	2.31*
ARA	30	9	0.410	0.432*	2.41*
NAV	30	9	0.403	0.416*	2.31*
BAL	7	9	0.468	0.523*	2.81*
CAN	13	7	0.308	**0.288**	**1.75**
IND	6	7	0.374	0.390	2.29

[a]Two-tailed *t*-tests for differences in heterozygosities were performed as described by Nei and Kumar (2000); [b]Allelic richness adjusted for sample size (El Mousadik and Petit 1996).
*Values significantly different ($p < 0.05$) from that of the Canary Island population (in **bold**). Other comparisons were not significantly different.

Table 3. Pairwise R_{ST} (above diagonal) and F_{ST} (below diagonal) estimates based on analysis of 9 microsatellite loci in 6 populations of Egyptian vultures (abbreviations and sample sizes as in Figure 1). Significance was determined by permutation analysis (n = 5000); numbers in **bold** indicate p-value < 0.003 (significant after Bonferroni correction)

	AND	ARA	NAV	BAL	CAN	IND
AND	–	0.004	0.007	0.080	0.096**	0.085
ARA	−0.001	–	−0.004	0.101**	**0.065**	0.083*
NAV	0.015	−0.001	–	**0.129**	**0.093**	0.091*
BAL	0.089**	**0.120**	**0.148**	–	**0.279**	0.166*
CAN	0.113**	**0.077**	**0.077**	**0.295**	–	**0.182**
IND	0.067	0.082**	0.100	0.096*	0.161*	–

*$p < 0.05$, **$p < 0.01$.

cance. In addition, the six cases (two each from the Canaries, Aragón and Navarra) all involved different pairs of loci. We therefore concluded that there was no evidence of significant linkage disequilibrium among these microsatellite loci, and that they could be appropriately treated as independent.

Hardy-Weinberg equilibrium

We tested for deviations from Hardy-Weinberg equilibrium at each locus, in each of the six populations. Because the Canary Island population sample may include some related individuals (see above), we used only the 13 unrelated birds from known nest sites in this analysis. Significant departures from HW equilibrium were obtained for locus BV16 ($p = 0.005$) and for the Navarra population ($p = 0.021$; Louis and Dempster 1987). In global tests of heterozygote excess and deficit (combining all loci for each population and all populations for each locus), there was no evidence of heterozygote excess (all p-values > 0.142; Guo and Thompson 1992). Heterozygote deficit was suggested in two populations (Andalucía, $p < 0.001$ and Navarra, $p = 0.007$) and for one locus (BV16, $p < 0.001$).

Following Bonferroni correction for multiple tests, only the locus BV16 showed a significant heterozygote deficit. This deficit was consistent across populations, and largely responsible for the apparent lack of heterozygous individuals in Andalucía and Navarra. The apparent heterozygote deficit at BV16 could be attributable to the presence of null alleles at this locus (Pemberton et al. 1995), or to our inability to distinguish alleles that may differ by a single base pair (the repeat unit of this microsatellite includes a poly-A segment; see Table 1). Due to the potential difficulties in assigning allele sizes, and the possible deviation from HW equilibrium, we conducted analyses of population differentiation both including and excluding this locus.

Population differentiation

The island populations of Egyptian vultures from both the Balearics and Canaries were clearly distinguished from the other populations based on allele frequencies at these microsatellite loci (Figure 2). Two measures of pair-wise population differentiation, R_{ST} (based on variance in allele size = number of repeat units), and F_{ST} (based on allele frequency differences regardless of size), generally produced similar results (Table 3). R_{ST} and F_{ST} values were high ($\geq$0.08) for all comparisons with the Balearic population, although significant differences were found only for those comparisons with populations of larger sample size (Canaries, Aragón and Navarra; Table 3). The Canary Island population was significantly differentiated from all others, including India and Andalucía (Table 3).

Some estimates of differentiation between populations increased when the nearly monomorphic loci BV11 and BV17 were omitted, and confidence in these estimates improved (data not shown). However, no additional pairwise population comparisons achieved statistical significance. Similarly, omission of the problematic locus BV16 did not change any of our conclusions, but only strengthened the support for existing pairwise differences (data not shown).

Table 4. Standard genetic distance (Nei 1972; above diagonal) and $(\delta\mu)^2$ (Goldstein et al. 1995; below diagonal) based on 9 microsatellite loci for 6 populations of Egyptian vultures (abbreviations and sample sizes as in Figure 1)

	AND	ARA	NAV	BAL	CAN	IND
AND	–	0.003	0.012	0.091	0.061	0.067
ARA	0.078	–	−0.001	0.111	0.040	0.057
NAV	0.333	0.114	–	0.129	0.041	0.065
BAL	0.305	0.472	0.947	–	0.212	0.080
CAN	0.474	0.214	0.084	1.253	–	0.071
IND	0.752	0.417	0.246	1.224	0.278	–

In every case, the largest differences observed were between the two island populations. In contrast, the Iberian Peninsula populations of Andalucía, Aragón and Navarra did not show any evidence of genetic differentiation from one another under any circumstances. The separate subspecies from India was characterized by relatively high R_{ST} and F_{ST} values (0.07–0.18; Table 3), but due to small sample size (n = 6), failed to distinguish itself significantly from most populations.

Standard genetic distances (Nei 1972) and $(\delta\mu)^2$ estimates (Goldstein et al. 1995) between populations generally confirmed results from the R_{ST} and F_{ST} analysis (Table 4). Maximal distance separated the two island populations; and the Balearic Islands population was quite distant from all others (Table 4). In contrast, the Canary Island vultures were not as distant from those of the Iberian peninsula. Standard distances suggested that the Indian subspecies was about equidistant from all other populations, while $(\delta\mu)^2$ estimates indicated a much larger distance between Indian and Balearic birds (Table 4). $(\delta\mu)^2$ estimates also suggested a large distance between some peninsular populations (0.333 for Andalucía and Navarra; Table 4), which was not supported by any of the other analyses. Clustering and phylogenetic analyses produced trees with inconsistent and weakly supported topologies (data not shown).

Discussion

The Egyptian vulture from the Canary Islands has been proposed as a unique subspecies, based on differences in morphometric measurements and mtDNA control region sequences, as compared to Iberian Peninsula populations (Donázar et al. 2002b). Canary Island birds are significantly larger than mainland vultures (tail feathers and wingchord 4–8% longer), and control region sequences from Fuerteventura birds consistently formed a monophyletic group within the non-Indian Egyptian vultures (Donázar et al. 2002b). The results of this study support the idea of limited gene flow between Canarian and other populations, as the Fuerteventura birds differed from all other populations in allele frequency at these microsatellite loci (Table 3). This implies that the sedentary population in the Canary Islands has not received significant reproductive input from mainland migrants for many generations, despite the long-distance dispersal capabilities of this species. The pairwise R_{ST} and F_{ST} estimates between Canary Island birds and those from the Peninsula were similar to comparisons with the Indian subspecies (Table 3).

An independent conservation plan is urgently required to prevent the genetically and morphologically distinct Egyptian vulture population on the island of Fuerteventura from going extinct, as only 26 breeding pairs remain (Donázar et al. 2002a). This population has experienced a low reproductive rate in recent years compared to populations in northern Spain and France (0.5 fledglings/pair/year, vs. 0.8–1.1, Palacios 2000; Donázar et al. 2002a). Given the very small and sedentary nature of the Canary Island population, inbreeding may be a possibility at this site. Our data provided no evidence of heterozygote deficit in the Fuerteventura population, when only unrelated birds (n = 13) were considered. However, significantly lower mean heterozygosity was found in the 13 Canary Island individuals (Table 2). In addition, mtDNA control region sequences from the Fuerteventura population were much less variable than were those from the larger Iberian Peninsula populations (Donázar et al. 2002b). Genetic similarity between parents (even in the absence of incestuous breeding) has been associated with hatching failure in other species (e.g., Bensch et al. 1994).

The Egyptian vulture population on Fuerteventura is probably descended from a small number of founders arriving in the Canary Islands from the Iberian Peninsula. This is supported by the fact that all microsatellite alleles found in Canary Island birds were also found in Peninsular vultures, whereas several rare alleles found in the Peninsular birds were absent from our Canarian sample of similar size. In a study of the Laysan finch *Telespiza cantans*, Tarr et al. (1998) documented a reduced number of polymorphic loci and smaller number of microsatellite alleles in

three small translocated populations, as compared to the known source population. In addition, higher estimates of F_{ST} and R_{ST} were found for island populations that had experienced severe size reductions (Tarr et al. 1998). This "founder effect" (random genetic drift accompanying the establishment of a new population from an established one; Hartl and Clark 1989) likely explains the similar pattern of genetic variation we observed for the Canary Island vultures.

Founder effect should also be reflected in genetic distance estimates. Standard genetic distance measures between the Canary Islands and the peninsular populations were only slightly lower than those separating the Indian subspecies from those populations; Table 4). The $(\delta\mu)^2$ distance estimates were uniformly higher and less consistent than standard distances (Table 4). Although $(\delta\mu)^2$ distances were specifically designed for use with microsatellite data, they are generally less useful than traditional genetic distance measures for phylogenetic inference (Takezaki and Nei 1996), perhaps because of a bias toward expansion mutations, and a mutation rate that correlates with heterozygosity (Amos et al. 1996).

The small sample sizes available from India, the Balearic Islands and Andalucía clearly limited our ability to distinguish these populations from the others. The Indian subspecies did not differ significantly in allele frequency from many of the other populations (Table 3). This was true despite the presence of two private alleles among the six Indian individuals, and relatively high R_{ST} and F_{ST} estimates for comparisons with the Indian population (Table 3). These microsatellite markers were relatively low in diversity and resolving power (perhaps because they were isolated in another vulture species), as compared to similar studies in other birds of prey (e.g., Nesje et al. 2000). Other genetic data strongly supported subspecific status for two of these same birds from India, which were clearly distinguished as a separate clade in an analysis of 11 Egyptian vulture mtDNA control region sequences (Donázar et al. 2002b).

The discrepancy between the results based on mtDNA and microsatellites is not unexpected, given the four-fold lower effective population size and enhanced effect of genetic drift for mtDNA, with respect to nuclear markers (Birky et al. 1983). Buonaccorsi et al. (2001) found that F_{ST} estimates for blue marlin populations from the Atlantic and Pacific were nearly four times greater when based on mitochondrial DNA as compared to microsatellite data. This difference could be explained by variation in mode of inheritance and mutation rate alone, without invoking selection or sex-biased dispersal. While mtDNA sequence data provide more reliable information on long-term population history, microsatellites yield more precise estimates of current gene flow (Buonaccorsi et al. 2001). Using mtDNA sequence data, two Egyptian vultures from the Canary Islands were distinguished as a separate clade, while two birds from Menorca were not clearly distinguished from Peninsular individuals (Donázar et al. 2002b). This suggests that the isolation of the vulture population in the Balearics may be a more recent event.

Although we sampled only seven individuals from the Balearics, these birds were much more distinct from the larger peninsular populations than were the six Indian individuals (Table 3). The population on Menorca is also at high risk for extinction with approximately 34 breeding pairs (De Pablo 2000), and in need of a separate management plan, as recent gene flow from the Iberian Peninsula has evidently been minimal. Some effort should now be made to investigate possible morphological and/or behavioral differences between these birds and those of the Peninsula. In addition, it would be interesting to obtain an estimate of fledging success for the Balearic vultures. The seven individuals from Menorca showed high levels of genetic variation compared to the other populations (Table 2), suggesting that this island population may have been founded by more individuals than was the Canary Island population, or by founders of diverse genetic backgrounds.

The greatest differentiation was observed between the island populations of Menorca and Fuerteventura (Table 3), as would be expected for two isolated groups diverging along independent trajectories. On the other hand, the separate Iberian Peninsula breeding groups may apparently be considered as one large, panmictic population. Allele frequencies in the Aragón and Navarra populations were quite similar, and this was reflected in very small (or negative) estimates of population differentiation between the two (Table 3). Although our sample from Andalucía was quite small, there was no evidence that significant differentiation from either Aragón or Navarra would be achieved with more extensive sampling (Table 3). From a practical standpoint, these results imply that introductions of Egyptian vultures from the populations in northern Spain to declining populations in Andalucía would be appropriate, if necessary. In contrast, if the goal of a conservation program is to

preserve genetic distinctiveness and ongoing evolutionary processes, introductions of mainland birds to the island populations should be discouraged. Given that the Canary Island birds differ from mainland vultures in microsatellite and mtDNA allele frequencies, morphology and behavior, they are likely to be both genetically and ecologically non-exchangeable (Crandall et al. 2000).

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