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Genetic diversity and differentiation between the two remaining populations of the critically endangered Mediterranean monk seal

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Abstract

The Mediterranean monk seal *Monachus monachus*, is a critically-endangered species of which only two populations, separated by *c*. 4000 km, remain: the eastern Mediterranean (150–300 individuals) and the Atlantic/western Sahara populations (100–130 individuals). We measured current levels of nuclear genetic variation at 24 microsatellite loci in 12 seals from the eastern Mediterranean and 98 seals from the western Sahara population and assessed differences between them. In both populations, genetic variation was found to be low, with mean allelic richness for the loci polymorphic in the species of 2.09 and 1.96, respectively. For most loci, the observed allele frequency distributions in both populations were discontinuous and the size ranges similar. The eastern Mediterranean population had 14 private alleles and the western Sahara had 18, but with a much larger sample size. Highly significant differences in allele frequencies between the two populations were found for 14 out of 17 loci. F_{ST} between the two populations was 0.578 and the estimated number of migrants per generation was 0.046, both clearly indicating substantial genetic differentiation. From a conservation perspective, these results suggest that each population may act as a source for introducing additional genetic variation into the other population.

Introduction

In classical times (~2500 BP), Mediterranean monk seals *Monachus monachus,* were abundant and distributed continuously from the Black and Mediterranean seas to the temperate and subtropical waters of the eastern North Atlantic (Johnson & Lavigne, 1999). Today, the Mediterranean monk seal is one of the world's most critically endangered mammals (IUCN, 2004). Only two populations of considerable size remain: (1) one inhabiting the eastern Mediterranean (Greece and Turkey), composed of 150–300 individuals (Reijnders, Verriopoulos & Brasseur, 1997), with seals in small fragmented groups scattered among islands in the Ionian (Panou, Jacobs & Panos, 1993) and Aegean seas (Adamantopoulou, Androukaki & Kotomatas, 1999), as well as on islands and in remote locations of the Turkish coast (Guçu, Guçu & Orek, 2003; Güçlüsoy *et al*., 2004), (2) that located at the peninsula of Cap Blanc, in the western Sahara, composed of 100–130 individuals (Forcada, Hammond & Aguilar, 1999). The latter is the only cohesive, large aggregation of the species and also the only colony that retains the social structure thought natural to the species. These two populations are located more than 4000 km apart, and only a few, extremely small groups of seals occur in the intervening region: in the Madeira archipelago ($n \approx 30$; Pires, Neves & Karamanlidis, 2007) and on the Mediterranean coast of Morocco and western Algeria ($n \approx 11$–15; Johnson *et al*., 2006).

In the eastern Mediterranean, the most severe decline occurred during the Roman era and was apparently due to intensive exploitation for oil, fur and medical products. Killing by fishermen also appears to have been common and, interestingly, for the same reasons that it occurs today–perceived damage to fishing gear and loss of catch. Deliberate aggression associated with fishing continues to be the main cause of death of adult seals in the eastern Mediterranean region (Androukaki *et al*., 1999; Guçu *et al*., 2003). In the western Mediterranean and Atlantic, the decline occurred later (Marchessaux, 1989) and was primarily due to commercial hunting by Spanish and Portuguese sealers. During the 15th and 16th centuries, the number of seals became so low that sealing was no longer sustainable and was therefore discontinued (Monod, 1923). Since then, the few remaining breeding colonies of monk seal have been further reduced by other human-related pressures, such as destruction or disturbance of shoreline

habitat, fishery related reduction of prey availability, pollution and deliberate aggression by fishermen (Aguilar, 1999). As a consequence, the Mediterranean monk seal is currently on the brink of extinction.

Small, isolated populations are generally considered to be susceptible to both stochastic demographic risks (Franklin, 1980; Lande & Shannon, 1996) and genetic risks that can reduce fitness and compromise long-term persistence (Hedrick, 2000) by, for example, limiting a population's ability to mount a variable response to parasites or pathogens (O'Brien & Evermann, 1988). These effects can be particularly severe when small population size is the consequence of a recent reduction in effective population size, or bottleneck (Weber *et al.*, 2000; Larson *et al.*, 2002), as inbreeding depression is most severe then and is associated with low fitness (Jimenez *et al.*, 1994; Keller *et al.*, 1994; Madsen, Stille & Shine, 1996). Nevertheless, some species of pinnipeds have been able to recover spectacularly from very low population sizes (Stewart *et al.*, 1994; Atkinson, 1997; Gerber & Hilborn, 2001).

Two previous studies of the western Sahara population of monk seal found low levels of genetic variation in both the nuclear and mitochondrial DNA (mtDNA) genomes (Stanley & Harwood, 1997; Pastor *et al.*, 2004). Little mtDNA variation was also found in four animals from Greece (Harwood *et al.*, 1996; Stanley & Harwood, 1997). In addition, Harwood *et al.* (1996) sequenced a 444 bp portion of the mtDNA control region (D-loop) and identified two variable sites that defined two haplotypes: the first was found in all samples ($n = 3$) from the Atlantic and the other in those from Greece ($n = 4$).

This latter finding is difficult to assess in terms of a population demographic trajectory because variation at the mtDNA level may be lost relatively rapidly while significant nuclear diversity is retained (Avise, 1994) and mtDNA does not detect male-mediated gene flow, because of its maternal inheritance (Moritz, 1994). Phocids are known to range over large areas; the closely related elephant seals *Mirounga angustirostris* and *Mirounga leonina*, migrate distances of up to 5200 km twice annually (Stewart & Delong, 1995; Hindell & McMahon, 2000). In addition, most phocids are polygynous (Riedman, 1990), and so male-mediated gene flow may be important, because few dispersing males that then successfully reproduce can prevent the genetic divergence of two geographically distant colonies. Although no long-distance migration movements have ever been described for the species, there is little information available on the dispersal capabilities of the Mediterranean monk seal. Its reproductive system is also unknown, but behavioural and genetic analysis point to mild polygyny (Pastor *et al.*, 1998; Guçu *et al.*, 2003), and the potential for active, male-mediated gene flow cannot therefore be excluded.

The objectives of the present study are to evaluate the level of nuclear genetic variability in the eastern Mediterranean monk seal population assess whether there is current gene flow between the two monk seal populations, and estimate the extent of genetic differentiation between the two populations. To address these questions, we examined variation of 24 microsatellite loci in 12 seals from the eastern Mediterranean and in 98 seals from the western Sahara population.

Material and methods

Sample collection

Eastern Mediterranean region

Samples of skin were collected from 12 individuals (five pups, two juveniles and five adults) during 1995–1999 in four geographic areas: the Cyclades, Dodecanese, north and eastern Aegean Island groups (Fig. 1). Tissues were obtained during necropsy of seals that had died from various reasons and gender was known for all sampled individuals (nine females and three males). The samples were preserved frozen until analysis. Samples of fur from nine seals were also collected but good-quality DNA for microsatellite analysis was not obtained.

Western Sahara region

Skin samples were collected during 1994–1999 from the rear flippers of 98 pups during tagging activities. Samples were initially preserved in 20% DMSO saturated with salt and were then kept frozen until analysis. The gender was known for 91 (40 females and 51 males) of the 98 pups. Some of these samples ($n = 52$) were analysed previously (Pastor *et al.*, 2004).

Genetic analysis

DNA was extracted using a standard protocol involving proteinase K digestion, followed by phenol/chloroform separation and a final precipitation with ethanol (Sambrook, Fritsch & Maniatis, 1989). We evaluated 24 microsatellite loci originally isolated in other pinnipeds, including grey seal *Halichoerus grypus*, harbour seal *Phoca vitulina,* northern elephant seal *M. angustirostris* and the walrus, *Odobenus rosmarus*. Original references and accession numbers for these loci are in (Allen *et al.*, 1995; Coltman, Bowen & Wright, 1996; Gemmell *et al.*, 1997; Buchanan *et al.*, 1998; Goodman, 1998; Pastor *et al.*, 2004). These loci included eight that were monomorphic and 15 that were polymorphic in the previous study of the Western Sahara colony (Pastor *et al.*, 2004), as well as three loci that had not been evaluated previously in Mediterranean monk seals. PCR was performed with fluorescent end-labelled primers in a 10 μL volume and 1 μL of the product was then mixed with 2 μL formamide and 0.5 μL of TAMRA-labelled size standard. This mixture was then loaded on a 6% acrylamide gel and electrophoresed on an ABI 377 automated sequencer (Applied Biosystems). Allele sizes were estimated with Genotyper Software (Applied Biosystems, Foster City, CA, USA).

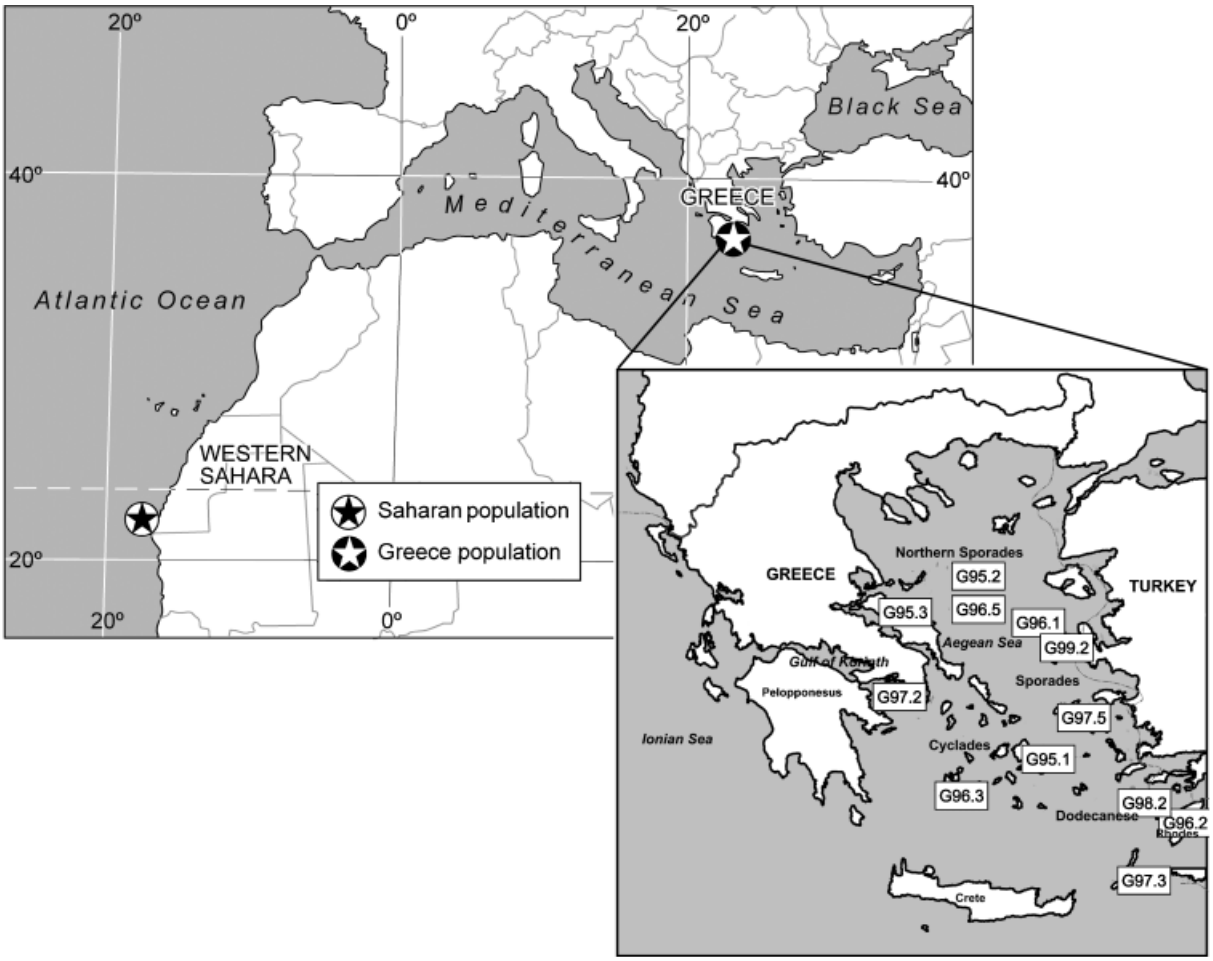

Figure 1 Sampling sites of the Saharan and Greek populations of the Mediterranean monk seal.

Statistical analysis

We assessed genetic variability in each population as (1) the proportion of polymorphic loci; (2) the mean number of alleles for polymorphic loci (allelic diversity); (3) allelic richness, which is the number of observed alleles corrected for sample size; (4) the observed heterozygosity (H_o), which then was compared with the unbiased estimate of heterozygosity (H_e) expected under Hardy–Weinberg (H–W) assumptions (Nei, 1978). For loci known to be X-linked (Coltman *et al*., 1996; Gemmell *et al*., 1997; Pastor *et al*., 2004), we excluded males from statistical analysis.

We examined possible departures from H–W equilibrium by calculating exact significance probabilities following the procedure described by Louis & Dempster (1987). We also evaluated heterozygote deficit and excess using an exact test procedure. When fewer than five alleles were present, we calculated an exact *P*-value. Otherwise, we used the method of Guo & Thompson (1992) to estimate the *P*-value. We also evaluated linkage disequilibria using an exact test, with sequential Bonferroni's correction for multiple comparisons (Rice, 1989). These analyses were performed using the algorithms used in the FSTAT (Goudet, 1995) and GENEPOP (Raymond & Rousset, 1995*a*) software packages.

The limited number of samples available for the eastern Mediterranean population precluded a proper statistical analysis of population bottlenecks. We therefore used the qualitative, graphical method of Luikart *et al*. (1998), which looks for the deficit of rare alleles characteristic of recently bottlenecked populations. Evaluation of population bottlenecks in the western Sahara population was described previously (Pastor *et al*., 2004).

We assessed population genetic differentiation between the two monk seal populations using several methods. Because of the small sample size of the Mediterranean population (Gaggiotti *et al*., 1999) and the large difference in sample size between the two (see Goudet *et al*., 1996), we used Weir & Cockerham's (1984) estimator of F_{ST}, with the confidence interval obtained by bootstrapping over loci, and the genic differentiation method of Raymond & Rousset (1995*b*) to detect significant differences in allele frequencies. We also estimated the number of migrants per generation,

Table 1 Genetic diversity measured by observed number of alleles (A_o), allelic richness (A_r), expected (H_e) and observed (H_o) heterozygosities, and results of tests for deviations of Hardy–Weinberg (H–W) equilibrium and for heterozygote deficit and excess

			Diversity				P (SE)		
Loci	*n*	Range	A_o	A_r	H_e	H_o	H–W E	H_e deficit	H_e excess
Eastern Mediterranean									
HG1.3	8	216–222	3	2.88	0.54	0.63	0.40	0.78	0.41
HG3.6	7	83–103	5	5.00	0.76	0.71	0.16 (0.01)	0.10 (0.01)	0.93 (0.01)
HG6.1	11	144–158	2	1.96	0.25	0.09	0.14	0.14	1.00
HG6.3	7	210	1	1.00	–	–	–	–	–
SGPV9	9	160	1	1.00	–	–	–	–	–
SGPV10	10	122–124	2	2.00	0.34	0.2	0.31	0.31	0.99
SGPV11	9	154–156	2	2.00	0.37	0	0.01*	0.01*	1
SGPV16	7	117–119	2	2.00	0.14	0.14	–	–	–
SGPV17[a]	10	138–144	3	2.00	0.40	0	0.01*	0.01*	1
PVC63[a]	8	118–126	4	4.00	0.58	0.63	1	0.66	0.52
PVC78	9	146–148	2	1.70	0.11	0.11	–	–	–
TBPV2	9	b–d	3	2.56	0.22	0.22	1	1	0.94
MA11C	10	197–212	4	3.38	0.43	0.5	1	1	0.52
Mean polymorphic loci			2.62	2.68	0.32	0.23			
Western Sahara									
HG1.3	79	220–232	3	2.14	0.25	0.27	0.32	0.82	0.30
HG3.6	95	87–99	4	3.07	0.62	0.62	0.05	0.01*	0.30
HG6.1	81	144–164	5	2.38	0.27	0.24	0.01	0.01* (0.01)	1.00 (0.01)
HG6.3	88	227–231	2	1.85	0.21	0.19	0.60	0.36	0.90
SGPV9	86	160–166	2	2.00	0.45	0.44	1.00	0.55	0.64
SGPV10	81	122–124	2	2.00	0.39	0.44	0.22	0.13	0.95
SGPV11	88	154–156	2	1.88	0.24	0.21	0.19	0.19	0.81
SGPV16	66	117–119	2	2.00	0.48	0.55	0.31	0.91	0.20
SGPV17[a]	43	140–148	2	1.67	0.13	0.14	1.00	1.00	0.83
PVC63[a]	39	118–124	3	2.71	0.57	0.54	0.19	0.47	0.54
PVC78	83	144–150	3	2.79	0.46	0.45	0.76	0.47	0.55
TBPV2	61	a–d	4	3.20	0.67	0.48	0.01	0.01*	0.99
MA11C	80	197–212	3	1.61	0.10	0.08	0.08	0.08	0.98
Mean polymorphic loci			2.85	1.96	0.38	0.35			

[a]X-linked loci for which males have been excluded from calculations.
*Statistical significance.

Nm, using the private allele method (Slatkin, 1985). Finally, we estimated Nei's D genetic distance between the two populations for comparative purposes. All analyses were performed using the GENEPOP (Raymond & Rousset, 1995*a*) and Genetix (Belkhir *et al*., 2004) software packages.

Results

Eastern Mediterranean region

Allele sizes in the eastern Mediterranean population were similar to those described for the same loci in other pinniped species (Table 1 and Fig. 2). However, of the 24 loci examined, 13 (55%) were monomorphic and 11 (45%) were polymorphic. The mean allelic diversity for the polymorphic loci was 2.62 and was 1.87 when all loci were considered. The mean allelic richness, when all loci polymorphic in the species were considered, was 2.09. Of the polymorphic loci, five had two alleles, three had three alleles, two had four alleles and one locus had five alleles (Fig. 2). For most of these loci, the size range was large and discontinuous. No significant linkage disequilibrium was detected and all loci conformed to H–W expectations, except for loci SGPV11 and SGPv17. SGPv17 is X-linked, indicating that some animals may have had gender incorrectly identified, and no heterozygotes were observed for the di-allelic locus SGPv11 (154 and 156). Interestingly, the two homozygous individuals for the rare allele (156) were both from the Cyclades. The graphical representation of allele frequencies for all 11 polymorphic loci showed a deficit of rare alleles (i.e. frequency <0.1; data not shown), as is expected for recently bottlenecked populations.

Western Sahara region

Of the 24 loci genotyped, 11 (45%) were monomorphic, and 13 (55%) were polymorphic, of which six loci had two alleles, four had three alleles, two had four alleles and one locus had five alleles (Table 1). Few new alleles were detected that were not present in the 52 seals genotyped by Pastor *et al*. (2004): two new alleles at Hg1.3, one at Hg3.6, 3 at

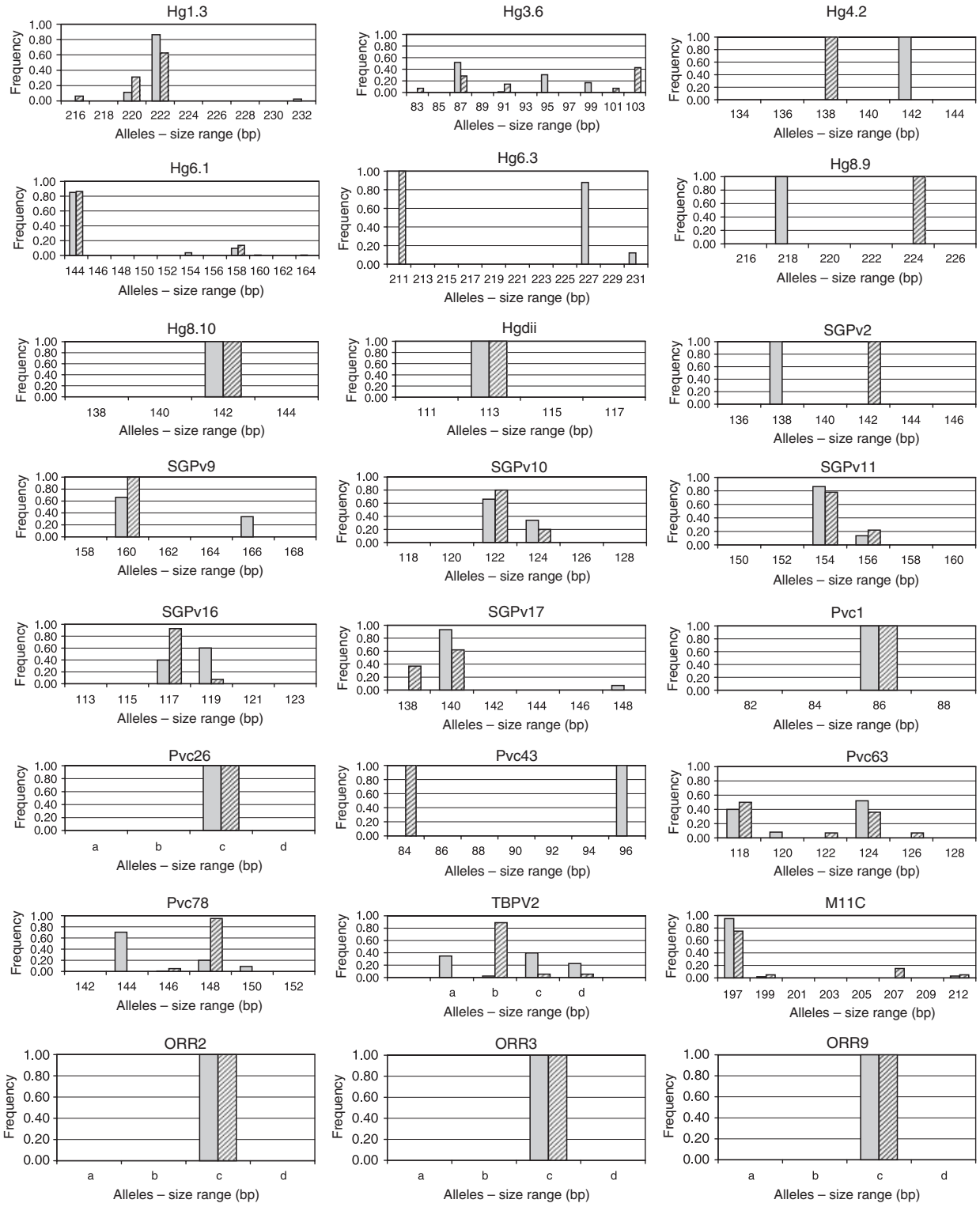

Figure 2 Allele frequencies for 24 microsatellite loci analysed in the western Sahara population (in grey) and in the eastern Mediterranean population (hatched).

Hg6.1, one at TBPv2 and one at MA11C2. The mean allelic diversity for the polymorphic loci was 2.85 and 2.00 when all loci were considered. The mean allelic richness was 1.96. The allele frequency distribution was also large and discontinuous for most of the loci. Three loci (Hg3.6, Hg6.1 and TBPv2) had a heterozygote deficit. No significant linkage disequilibrium was detected after Bonferroni's correction for multiple tests.

Variation in the species

Allelic diversity for the species as a whole was low (3.75), as was allelic diversity for all loci variable in the species (2.32). Seven loci (Hg8.10, Hgdii, Pvc1, Pvc26, Orr2, Orr3, Orr9) were monomorphic, one locus (Hg 3.6) had 7 alleles and the rest had from five to two alleles. The allele size range for the entire species was larger for all loci than in each of the populations separately. Of note is that the allele frequency distribution for the polymorphic loci (Hg3.6, Pvc63, Pvc78, TBPv2) was almost continuous in the combined data. Only loci with little or no variation in at least one of the two populations (e.g. Hg6.1, Hg6.3, Pvc43) still had large gaps in the distribution when combined.

Comparison between populations

For all 24 loci, the two populations shared 52% of the alleles. For the shared polymorphic loci, the most common allele was the same in both populations for 58% of these loci. Four loci (Hg4.2, Hg8.9, SGPv2, Pvc43) were monomorphic in both populations, but fixed for a different allele (Fig. 2). The western Sahara population had 18 private alleles and the eastern Mediterranean population had 14. There were significant allele frequency differences between the two populations for 14 out of 17 shared polymorphic loci. In addition, the estimated number of migrants per generation, after correction for size, was 0.047, F_{ST} between the two populations was 0.578 (95%, CI: 0.365–0.749) and Nei's D was 0.740, all clearly indicating substantial genetic differentiation.

Discussion

Nuclear genetic variation in the two remaining populations of the Mediterranean monk seal was found to be extremely low. In the western Sahara colony, the increased sample size and number of loci analysed relative to a previous study (Pastor *et al.*, 2004) resulted in only a negligible increase in allelic variation detected. Low levels of variation in this population are attributed to its demographic history, with commercial hunting drastically reducing population size during the 17th century (Marchessaux, 1989; Pastor *et al.*, 2004) and more recent habitat destruction further reducing its size. In the eastern Mediterranean region, monk seals were subject to widespread killing in the Greco-Roman and Byzantine ages, with the surviving population subsequently facing severe habitat fragmentation, pollution, reduction in prey availability by overfishing and direct mortality by fishermen (Johnson & Lavigne, 1999; Johnson, 2004). The deficit of rare alleles, found with the qualitative analysis of the distribution of allele frequencies and the discontinuous allele frequency distributions, both suggest a recent reduction in effective population size and consequent loss of genetic diversity (Luikart *et al.*, 1998; Garza & Williamson, 2001). It is possible that some of the lack of diversity is due to ascertainment bias, as the microsatellite loci used in this study were originally discovered in other species. However, the effect of ascertainment bias should be negligible relative to that from the recent demographic history, because we surveyed all available pinniped microsatellite loci, including those that were monomorphic in the source species, which, in some cases, proved to be polymorphic in the Mediterranean monk seal (Gemmell *et al.* 1997; Pastor *et al.*, 2004).

The allele size range for most loci is similar in the two populations, and some of the intermediate-sized alleles missing in one population are present in the other. In the species as a whole, some loci had an almost continuous allele frequency distribution, similar to that observed in closely related species not known to have undergone dramatic reductions in population size, such as grey seals or harbour seals (Allen *et al.*, 1995; Goodman, 1998). This suggests that, in the recent past, Mediterranean monk seals constituted a single, large population with some amount of gene flow extending from the Atlantic to the Black sea. Until the 1950s, small groups of monk seals persisted along much of the northern Mediterranean coastline of North Africa, as well as in France and Spain (Sergeant *et al.*, 1978; Duguy, 1979; Marchessaux, 1989; Israëls, 1992), and so the recent historical record is consistent with this hypothesis.

However, highly significant differences in allele frequencies were found between the two sample sets. This is consistent with the general principle that remnant patch populations often preserve more genetic variance than would a single population of the same total size (Gilpin, 1999). In addition, the two monk seal populations had a large number of private alleles and the F_{ST} (0.578) and Nei's D (0.740) estimates between them were large. This implies that current gene flow is highly restricted, a result consistent with a previous study on the mtDNA control region, which found a different haplotype fixed in each population (Harwood *et al.*, 1996). Stanley & Harwood (1997) sequenced 350 bp of the more conserved portion of the mtDNA (the *cytb* gene) in samples from Greece and the Atlantic and found no sequence variation. This, along with the observation that the control region haplotypes differed at only two nucleotides, argues against the existence of two distinct subspecies or any deep phylogenetic divide between the two remaining populations. However, the fact that the rare allele (156) at locus SGPv17 was found only in two homozygous individuals from the same site (Cyclades) may be indicative of the existence of some past genetic structure.

Conservation implications

Low genetic variability is associated with low population fitness and lack of adaptability to changing environments, at least in model systems (Lynch, 1991; Frankham, 1995).

Although external causes, such as lack of a suitable habitat (González *et al*., 2002) or storms (Gazo *et al*., 2000), are implicated in the low reproductive (Gazo *et al*., 1999) and high pup mortality rates (Gazo *et al*., 2000) in the western Sahara monk seal population, the low observed heterozygosity may be contributing to further reduce the reproductive rate (Gazo *et al*., 1999) and/or increase the pup mortality rate (Gazo *et al*., 2000). These traits are unusual in pinnipeds but are often found in terrestrial mammal populations subject to severe genetic erosion (Bulger & Hamilton, 1988; Dietz & Baker, 1993; Packer & Pusey, 1993; Pusey & Wolf, 1996; Mansfield & Land, 2002) and may explain the lack of demographic recovery of the western Sahara population, despite the absence of significance human pressure in recent decades (Aguilar, 1999).

The observation of highly significant differentiation between the western Sahara and the eastern Mediterranean populations of Mediterranean monk seal indicates that they have preserved different allelic variants at many loci in the face of intense genetic drift due to population size reduction. Because these microsatellite alleles are presumably linked to chromosomal segments with functional genes, it is likely that they also preserve different sets of functionally significant genetic variation. Given the extremely small sizes and reduced genetic diversity in both the remaining populations, extensive inbreeding is inevitable, thereby increasing the threat of extinction. Translocation of individual monk seals between the two populations may potentially reduce inbreeding, through a 'genetic rescue' effect (Tallmon, Luikart & Waples, 2004). In a number of taxonomically diverse vertebrate taxa, such as the adder *Vipera berus*, grey wolf *Canis lupus* and Florida panther *Puma concolor coryi*, the translocation of a relatively small number of individuals into isolated and severely inbred wild populations has been followed by rapid demographic growth and presumably by reduced threat of extinction, at least due to demographic processes (Madsen *et al*., 1999; Mansfield & Land, 2002; Vilà *et al*., 2002). This is consistent with the hypothesis that few individuals are needed to prevent inbreeding depression and serve as the basis for 'genetic rescue' (Tallmon *et al*., 2004). However, such short-term demographic benefits can come at the expense of longer-term population health, particularly if adaptive differences between populations exist. Given the tenuous nature of survival for this species, a more comprehensive evaluation of the consequences of such translocations, so as to ensure a broader 'genetic restoration' (Hedrick, 2005), is prudent and should be performed before any such management action is undertaken.

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References

Adamantopoulou, S., Androukaki, E. & Kotomatas, S. (1999). The distribution of the Mediterranean monk seal in Greece based on an information network. *Contr. Zoogeogr. Ecol. East Med. Reg*. **I**, 399–404.

Aguilar, A. (1999). Status of Mediterranean monk seal (*Monachus monachus*) populations. In *RAC-SPA, United Nations Environment Program (UNEP)*: 1–60. Tunis: Aloès Editions.

Allen, P.J., Amos, W., Pomeroy, P.P. & Twiss, D. (1995). Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Mol. Ecol*. **4**, 653–662.

Androukaki, E., Adamantopoulou, S., Dendrinos, P., Tounta, E. & Kotomatas, S. (1999). Causes of mortality in the Mediterranean Monk seal (*Monachus monachus*) in Greece. *Contr. Zoogeogr. Ecol. East Med. Reg*. **1**, 405–411.

Atkinson, S. (1997). Reproductive biology of seals. *J. Repr. Fertil*. **2**, 175–194.

Avise, J.C. (1994). *Molecular markers, natural history and evolution*. New York: Chapman & Hall.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (2004). *GENETIX 4.05: logiciel sous Windows^{TM} pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000*. Montpellier, France: Université de Montpellier II.

Buchanan, F.C., Maies, L.D., Thue, T.D., De March, B.G.E. & Stewart, R.E.A. (1998). Microsatellites from the Atlantic walrus *Odobenus rosmarus rosmarus*. *Mol. Ecol*. **7**, 1083–1090.

Bulger, J. & Hamilton, W.J.I. (1988). Inbreeding and reproductive success in a natural chacma baboon *Papio cynocephalus ursinus* population. *Anim. Behav*. **36**, 574–578.

Coltman, D.W., Bowen, W.D. & Wright, J.M. (1996). PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. *Mol. Ecol*. **5**, 161–163.

Dietz, J.M. & Baker, A.J. (1993). Polygyny and female reproductive success in golden lion tamarins, *Leontopithecus rosalia*. *Anim. Behav*. **46**, 1067–1078.

Duguy, R. (1979). Situation report on the monk seal. *Aquat. Mamm*. **7**, 21–22.

Forcada, J., Hammond, P.S. & Aguilar, A. (1999). Status of the Mediterranean monk seal *Monachus monachus* in the western Sahara and the implications of a mass mortality event. *Marine Ecol. Prog. Ser*. **188**, 249–261.

Frankham, R. (1995). Conservation genetics. *Ann. Rev. Genetics* **29**, 305–327.

Franklin, I.R. (1980). Evolutionary change in small populations. In *Conservation biology: an evolutionary – ecological perspective*: 135–149. Soulé, M.E. & Wilcox, B.A. (Eds). Massachussets: Sinauer Associates.

Gaggiotti, O.E., Lange, O., Rassmann, K. & Gliddon, C. (1999). A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Mol. Ecol.* **8**, 1513–1521.

Garza, J.C. & Williamson, E. (2001). Detection of reduction in population size using data from microsatellite loci. *Mol. Ecol.* **10**, 305–318.

Gazo, M., Aparicio, F., Cedenilla, M.A., Layna, J.F. & González, L.M. (2000). Pup survival in the Mediterranean monk seal (*Monachus monachus*) colony at Cabo Blanco Peninsula (Western Sahara – Mauritania). *Mar. Mammal. Sci.* **16**, 158–168.

Gazo, M., Aparicio, F., Cedenilla, M.A., Layna, J.F., González, L.M. & Aguilar, A. (1999). Pupping season, perinatal sex ratio and natality rates of the Mediterranean monk seal (*Monachus monachus*) from the Cabo Blanco colony. *J. Zool. (Lond.)* **249**, 393–401.

Gemmell, N.J., Allen, P.J., Goodman, S.J. & Reed, J.Z. (1997). Interspecific microsatellite markers for the study of pinniped populations. *Mol. Ecol.* **6**, 661–666.

Gerber, L.R. & Hilborn, R. (2001). Catastrophic events and recovery from low densities in populations of otariids: implications for risk of extinction. *Mammal Rev.* **31**, 131–150.

Gilpin, M.E. (1999). Spatial structure and population vulnerability. In *Viable populations for conservation*: 125–139. Soulé, M.E. (Ed.). Cambridge: Cambridge University Press.

González, L.M., Cedenilla, M.A., de Larrinoa, P.F., Layna, J.F. & Aparicio, F. (2002). Changes in the breeding variables of the Mediterranean monk seal (*Monachus monachus*) colony of Cabo Blanco peninsula after a mass mortality episode. *Mammalia* **66**, 173–182.

Goodman, S.J. (1998). Patterns of extensive genetic differentiation and variation among European harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorhisms. *Mol. Biol. Evol.* **15**, 104–118.

Goudet, J. (1995). FSTAT (Version 12): A computer program to calculate F-statistics. *J. Hered.* **86**, 485–486.

Goudet, J., Raymond, M., de Meeüs, T. & Rousset, F. (1996). Testing differentiation in diploid populations. *Genetics* **144**, 1933–1940.

Güçlüsoy, H., Kiraç, C.O., Veryeri, N.O. & Savas, Y. (2004). Status of the Mediterranean Monk Seal, *Monachus monachus* (Hermann, 1779) in the coastal waters of Turkey. *Eur J. Fish. Aquat. Sci.* **21**, 201–210.

Guçu, A.C., Guçu, G. & Orek, H. (2003). Habitat use and preliminary demographic evaluation of the critically endangered Mediterranean monk seal (*Monachus monachus*) in the Cilician Basin (Eastern Mediterranean). *Biol. Conserv.* **116**, 417–431.

Guo, S.W. & Thompson, E.A. (1992). Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372.

Harwood, J., Stanley, H.F., Beudels, M.O. & Vanderlinden, C. (1996). Metapopulation dynamics of the Mediterranean monk seal. In *Metapopulations and wildlife conservation*: 241–256. Cullough, D.Mc. (Ed.). California: Island Press.

Hedrick, P.W. (2000). *Genetics of populations*. 2nd edn. Sudbury: Jones and Bartlett Publishers.

Hedrick, P.W. (2005). 'Genetic restoration': a more comprehensive perspective than 'genetic rescue'. *TREE* **20**, 109.

Hindell, M.A. & McMahon, C.R. (2000). Elephant seals circle the globe: a record movement for a southern elephant seal (*Mirounga leonina*), from Macquarie Island to Peter 1 ØY. *Marine Mammal. Sci.* **16**, 504–507.

Israëls, L.D.E. (1992). Thirty years of Mediterranean monk seal protection, a review. *Nederlandsche Commissie voor Internationale Natuurbescherming, Mededelingen* **28**, 1–65.

IUCN (2004). *Red list of threatened species*. Gland, Switzerland: IUCN.

Jimenez, J.A., Hughes, K.A., Alaks, G., Graham, L. & Lacy, R.C. (1994). An experimental study of inbreeding depression in a natural habitat. *Science* **266**, 271–273.

Johnson, W.M. (2004). *Monk seals in post-classical history*. Leiden: Netherlands Commission for International Nature Protection.

Johnson, W.M., Karamanlidis, A.A., Dendrinos, P., Fernández, P., Gazo, M., González, L.M., Güçlüsoy, H., Pires, R. & Schnellmann, M. (2006) Monk seal fact files. Biology, behaviour, status and conservation of the Mediterranean monk seal, *Monachus monachus*. *The Monachus Guardian*

Johnson, W.M. & Lavigne, D.M. (1999). *The Mediterranean monk seal (Monachus monachus) in ancient history and literature*. Leiden: Netherlands Commission for International Nature Protection.

Keller, L.F., Arcese, P., Smith, J.N.M., Hochachka, W.M. & Stearns, S.C. (1994). Selection against inbred song sparrows during a natural population bottleneck. *Nature* **372**, 356–357.

Lande, R. & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**, 434–437.

Larson, S., Jameson, R.J., Etnier, M., Fleming, M. & Bentzen, P. (2002). Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. *Mol. Ecol.* **11**, 1899–1903.

Louis, E.J. & Dempster, E.R. (1987). An exact test for Hardy-Weinberg and multiple alleles. *Biometrics* **43**, 805–811.

Luikart, G.L., 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.

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* **45**, 622–629.

Madsen, T., Stille, B. & Shine, R. (1996). Inbreeding depression in an isolated population of adders. *Biol. Conserv.* **75**, 113–118.

Madsen, T., Shine, R., Olsson, M. & Wittzell, H. (1999). Restoration of an inbred adder population. *Nature* **402**, 34–35.

Mansfield, K.G. & Land, E.D. (2002). Cryptorchidism in Florida panthers: prevalence, features, and influence of genetic restoration. *J. Wildl. Dis.* **38**, 693–698.

Marchessaux, D. (1989). Distribution and status of the monk seal population *Monachus monachus* (Hermann, 1779). *Mammalia* **53**, 621–642.

Monod, T. (1923). Note sur la présence du *Monachus albiventer* Bodd. sur la côte saharienne. *Bull. Mus. Nat. d'Histoire Naturelle* **29**, 555–557.

Moritz, C. (1994). Applications of mitochondrial DNA analysis in conservation: a critical review. *Mol. Ecol.* **3**, 401–411.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

O'Brien, S.J. & Evermann, J.F. (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *TREE* **3**, 254–259.

Packer, C. & Pusey, A.E. (1993). Dispersal, kinship, and inbreeding in African lions. In *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*: 375–391. Thornhill, N.E. (Ed.). Chicago: University of Chicago Press.

Panou, A., Jacobs, J. & Panos, D. (1993). The endangered Mediterranean monk seal *Monachus monachus* in the Ionian sea, Greece. *Biol. Conserv.* **64**, 129–140.

Pastor, T., Garza, J.C., Allen, P.J., Amos, W. & Aguilar, A. (2004). Low genetic variability in the highly endangered Mediterranean monk seal. *J. Hered.* **95**, 291–300.

Pastor, T., Gazo, M., Aramburu, M.J., Cedenilla, M.A., Aparicio, F., Layna, J.F., Grau, E., González, L.M. & Aguilar, A. (1998). Reproductive parameters of the Mediterranean monk seal: new data for an old species. European Research on Cetaceans **12,** 306.

Pires, R., Neves, H.C. & Karamanlidis, A.A. (2007). Conservation status and priorities of the critically endangered Mediterranean monk seal *Monachus monachus* in the archipelago of Madeira. *Oryx*. (in press).

Pusey, A. & Wolf, M. (1996). Inbreeding avoidance in animals. *TREE* **11**, 201–206.

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

Raymond, M. & Rousset, F. (1995***b***). An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Reijnders, P.J.H., Verriopoulos, G. & Brasseur, S. (1997). *Status of pinnipeds relevant to the European Union*. Wageningen, The Netherlands: DLO Institute for Forestry and Nature Research (IBN-DLO).

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Riedman, M.L. (1990). *The pinnipeds. Seals, sea lions, and walruses*. Berkeley: University of California Press.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular cloning, a laboratory manual*. 2nd edn. New York: Cold Spring Harbor Laboratory Press.

Sergeant, D.E., Ronald, K., Boulva, J. & Berkes, F. (1978). The recent status of *Monachus monachus*, the Mediterranean monk seal. *Biol. Conserv.* **14**, 259–287.

Slatkin, M. (1985). Rare alleles as indicators of gene flow. *Evolution* **39**, 53–65.

Stanley, H.F. & Harwood, J. (1997). Genetic differentiation among subpopulations of the highly endangered Mediterranean monk seal. In *The role of genetics in conserving small populations*: 97–101. Tew, T.E., *et al.* (Eds). Peterborough, UK: JNCC (Joint Nature Conservation Committee).

Stewart, B.S. & Delong, R.L. (1995). Double migrations of the northern elephant seal, *Mirounga angustirostris*. *J. Mammal.* **76**, 196–205.

Stewart, B.S., Yochem, P.K., Huber, H.R., Delong, R.L., Jameson, R.J., Sydeman, W.J., Alle, S.G. & Le Boeuf, B.J. (1994). History and present status of the Northern elephant seal population. In *Elephant seals: population ecology, behavior, and physiology*: 1–27. Le Boeuf, B.J. & Laws, R.M. (Eds). Los Angeles: University of California Press.

Tallmon, D.A., Luikart, G. & Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *TREE* **19**, 489–496.

Vilà, C., Sundqvist, A.-K., Flagstad, Ø., Seddon, J., Björnerfeldt, S., Kojola, I., Casulli, A., Sand, H., Wabakken, P. & Ellegren, H. (2002). Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proc. Roy. Soc. Ser. B* **270**, 91–97.

Weber, D.S., Stewart, B.S., Garza, J.C. & Lehman, N. (2000). An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. *Curr. Biol.* **10**, 1287–1290.

Weir, B.S. & Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.