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SHORT COMMUNICATION

Major histocompatibility complex variation at three class II loci in the northern elephant seal

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Abstract

Northern elephant seals were hunted to near extinction in the 19th century, yet have recovered remarkably and now number around 175 000. We surveyed 110 seals for single-strand conformation polymorphism (SSCP) and sequence variation at three major histocompatibility (MHC) class II loci (*DQA*, *DQB* and *DRB*) to evaluate the genetic consequences of the population bottleneck at these loci vs. other well-studied genes. We found very few alleles at each MHC locus, significant variation among breeding sites for the *DQA* locus, and linkage disequilibrium between the *DQB* and *DRB* loci. Northern elephant seals are evidently inbred, although there is as yet no evidence of correlative reductions in fitness.

Keywords: *DQA*, *DQB*, *DRB*, inbreeding depression, linkage disequilibrium, major histocompatibility complex, Northern elephant seal, pinniped, population bottleneck

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Introduction

Northern elephant seals (*Mirounga angustirostris*) were hunted to near extinction in the 19th century, yet have recovered remarkably and now number around 175 000 (Cooper & Stewart 1983; Stewart *et al*. 1994). This textbook case of a severe population bottleneck was accompanied presumably by a genetic bottleneck. Indeed, no allozyme variability and only minimal variability at mitochondrial DNA (mtDNA), minisatellite and microsatellite loci has been detected (Bonnell & Selander 1974; Hoelzel *et al*. 1993; Lehman *et al*. 1993; Garza 1998; Weber *et al*. 2000; Lehman & Stewart 2002). Sequence variation at functional nuclear loci has been less investigated, although a prior survey of one class II major histocompatibility complex (MHC) locus, *DQB*, demonstrated low variation. Only two *DQB* alleles were found in 69 northern elephant seals (NES) from one population, compared with eight alleles in 109 seals from the sister taxa, the southern elephant seal (SES; *Mirounga leonina*) (Hoelzel *et al*. 1999).

Because the genetic homogeneity discovered so far in this species may belie its dramatic demographic recovery, we sought to investigate more completely the MHC variability in contemporary populations. Heterozygous advantage allows higher than expected levels of polymorphism to be maintained at loci such as the MHC. Heterosis is achieved by increased antigen recognition and presentation ability, theoretically providing heightened disease resistance (Potts & Slev 1995; Parham & Ohta 1996). Therefore, balancing selection should operate in favour of increased allelic variation, and it could be possible for this species to possess significant numbers of MHC alleles despite a lack of variation at other loci. In this report we surveyed 110 NES individuals from two populations at three MHC class II loci, *DQA*, *DQB* and *DRB*. Our aims were to determine the within-locus variation and between–loci allelic associations using single-stranded conformational polymorphism (SSCP) and direct nucleotide-sequence analysis, and to compare the results with similar data obtained in other species.

Materials and methods

Samples and loci

We processed samples from 110 seals born at the two principal breeding colonies for the NES at San Nicolas

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Island (n = 50) and San Miguel Island (n = 50) and from 10 beach-cast seals from unknown birth sites (see Weber *et al*. 2000 for sampling and DNA purification protocols). Both these islands are 50–100 km off the coast of southern California, USA. We targeted exon 2 in three different loci in MHC class II region (*DQA*, *DQB* and *DRB*) using a combined SSCP/direct-sequence analysis. Exon 2 encodes the peptide-binding region (PBR) of the MHC, the region thought to be subject the most to balancing selection. We also targeted exon 2 for the *DRB* locus from two SES from Marion Island in the southern Indian Ocean. The SES has been surveyed previously for intraspecific variation at the *DQA* (Slade *et al*. 1998) and *DQB* (Hoelzel *et al*. 1999) loci.

SSCP analysis

We processed all samples using a nonradioactive (cold) and then a radioisotope-containing (hot) polymerase chain reaction (PCR), eliminating the need to purify the template. The cold PCR was optimized to obtain a single sharp band using 1 μL of genomic DNA (*c*. 10 ng) in a 25 μL reaction volume. We used the following parameters: 0.1 U Ampli*Taq* DNA polymerase (Perkin-Elmer), the manufacturer's supplied standard buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pmol of each primer and a PCR profile of 92 °C 10′, [92 °C 1′, 55 °C 1′, 72 °C 1′] × 40, 72 °C 10′. Two microlitres of the cold PCR served as the template for the hot PCR in a 15 μL reaction volume. In the hot PCR, we used the same protocol but with 20 μM dCTP, 0.9 μCi [α^{32}P]·dCTP, and only 30 cycles. To amplify the *DQA* locus, we used primers MDQA1 (5′-CCGGATCCCAGTACACCCATGAATTTGATGG-3′) and MDQA2 (5′-CCGGATCCCCAGTGCTCCACCTTGCAGTC-3′; Slade *et al*. 1993) for the cold PCR followed by a seminested procedure with MDQA1 and GH27 (5′-CACGGATCCGGTAGCAGCGGTAGAGTTG-3′; Sharf *et al*. 1986) for the hot PCR. We repeated this two-step process with the *DQB* locus using MDQB1 (5′-GAGCTGCAGGTAGTTGTGTCTGCACAC-3′) and MDQB2 (5′-ACGGATCCATGTGCTACTTCACCAACGG-3′; Horn *et al*. 1988) and with the *DRB* locus using DRB2A (5′-AACGGGACGGAGCGGGTGCG-3′) and DRB2B (5′-TCGCCGCTGCACCAGGAAGC-3′; Garza 1998), except that the same primers were used for both the cold and hot PCRs. To confirm terminal *DRB* sequences, we also used the primers KZ77 (5′-GGATCCACTAGTAACGGCCGCC-3′) and KZ64 (5′-CGCCAGTGTGATGGATATCTGC-3′) for some of the samples.

To perform nondenaturing SSCP electrophoresis, we used a mixture of 1 μL of hot PCR template and 9 μL of stop solution (95% formamide, 10 mM NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol), which was heated at 94 °C for 3 min and cooled in ice water prior to 2.5 μL being loaded onto a prerun 100 mL SSCP gel: 25 mL MDE™ gel (BMA BioProducts), 6 mL 10× TBE and 69 mL distilled water, to which was added 400 μL fresh 10% ammonium persulphate and 40 μL TEMED. The gels were run in 0.6× TBE running buffer at room temperature 8 W for 14–15 h (depending on the locus) and visualized by phosphorimaging (Storm 860, Molecular Dynamics). Many individuals were processed twice using the SSCP procedure to avoid categorizing PCR artefacts (such as amplified *Taq* errors or recombinant sequences generated by template jumping by *Taq*) as new alleles, a problem in prior cat and dog MHC research (cf. Kennedy *et al*. 2002).

Cloning, sequencing, and data analysis

We ligated the PCR products from each locus into a cloning vector (TA or TOPO TA, Invitrogen) and transformed the vector into competent DH5α *Escherichia coli* cells. Plasmid DNA was isolated from each colony by the boiling-lysis method (Sambrook *et al*. 1989). For *DQA*, we cloned PCR products from one or two randomly chosen seals for each SSCP genotype that we discovered, and we determined the nucleotide sequence of a ~400 base pairs (bp) region encompassing the PBR for three to five clones per seal. For *DQB*, we cloned products from four randomly chosen seals for each genotype, and determined ~170 bp sequences from three to six clones per seal. For *DRB*, we cloned products from two randomly chosen seals for each genotype, and determined ~190 bp sequences from between two and seven clones per seal. For the *DRB* locus of the SES, we cloned products from each of the two seals and determined sequences from four to five clones per seal.

Successful plasmid preparations were diluted (1:500) and 1 μL served as the template for a subsequent standard 50 μL PCR with the primers listed above. We determined bidirectional nucleotide sequences of the purified PCR products (QIAquick PCR purification kit, Qiagen). Sequence determination was made by using [α-^{35}S]·dATP, Sequenase 2.0 (US Biochemical), 6% polyacrylamide/8 M urea gels and autoradiography, or on an Applied Biosystems 373 A DNA sequencer (modified for the BigDye Terminator technology). We inspected the subsequent sequences with the program SEQUENCHER version 5.2 (Gene Codes Corporation), and tested Hardy–Weinberg equilibrium and linkage dissociation with GENEPOP (http://www.cefe.cnrs-mop.fr) using allele and genotype frequencies from the SSCP data.

Results and discussion

We surveyed 110 northern elephant seals at three MHC class II loci in the variable peptide-binding regions. The comparison of the different MHC loci produced three interesting results. First, the NES has a very small number of alleles at the MHC loci studied. In the NES, we found no more than two alleles at any MHC locus in our sample of

Table 1 The genotypic compositions for each of the MHC class II loci (exon 2) surveyed in the northern elephant seal (n = 110)

Locus	Genotype	Allele(s)	Number of seals
DQA1	1 (homozygous)	*MianDQA1*01*	93
	2 (heterozygous)	*MianDQA1*01* *MianDQA1*02*	17
DQB1	1 (homozygous)	*MianDQB1*01*	66
	2 (homozygous)	*MianDQB1*02*	6
	3 (heterozygous)	*MianDQB1*01* *MianDQB1*02*	38
DRB1†	— (homozygous)	*MianDRB1*01*	110
DRB2	1 (homozygous)	*MianDRB2*01*	66
	2 (homozygous)	*MianDRB2*02*	6
	3 (heterozygous)	*MianDRB2*01* *MianDRB2*02*	38

†One allele (*DRB1*01*) in the *DRB1* locus was monomorphic in all seals.

110 seals. At the *DQA* locus, the SSCP gels provided band patterns consistent with only two alleles and two genotypes (Table 1; Fig. 1a) and we detected the rarer *MianDQA1*02* allele only in the heterozygote condition. At the *DQB* locus, in agreement with Hoelzel *et al*. (1999), the band patterns were consistent with two alleles and three genotypes (Table 1; Fig. 1b). However, at the DRB gene, the SSCP and sequence analyses revealed that the NES appears to have at least two loci, one of which, *DRB1*, is monomorphic for the *MianDRB1*01* allele (Fig. 1c), while a second, *DRB*2, is polymorphic with two alleles (Table 1). Similarly, *DRB* in the beluga (*Delphinapterus leucas*) has been reported to have two loci, one monomorphic for the allele *DeleDRB2*0601*, and the other a polymorphic locus, in which *DeleDRB1*0401* (Fig. 1c) is one of eight alleles (Murray & White 1998). The *DRB* allele from the monomorphic locus (*MianDRB1*01*) in the NES was also found in the SES (*MileDRB1*01*). In 110 NES individuals we found only three *DRB* alleles, but we found only five alleles in two SES individuals (Fig. 1c).

The three loci differed in variability at both the nucleotide and amino-acid levels. The *DQ* loci showed low overall variability, with *DQA* showing 0.5% variability at the nucleotide level (two polymorphic sites of 399 base pairs sequenced) and 1.2% at the amino acid level (only one nucleotide change leads to a detectable nonsynonymous substitution). Variability increased slightly for *DQB*, being 2.3% at the nucleotide level and 7% at the amino acid level. *DRB* was the most variable of the three loci as indicated by 8.0% variability at the nucleotide level (15 polymorphic sites in the 188 bases sequenced), 14.5% at the amino acid level and 53.8% at the 13 peptide-binding sites for the three alleles. Notably, in the *DQA* intron separating exons 2 and 3, we detected no variation between the two NES alleles or between the SES and NES (Fig. 1a). Also, a close examination of the amino acid sequences indicates that substitutions tend to be at or near the peptide binding sites, at least for the two beta-chain loci, and often involve conversions between two chemically distinct residues (Fig. 1d).

This overall lack of variability in the MHC is uncommon, even in mammalian species that have experienced bottlenecks and extreme founder events. For example, a sample of 14 Przewalski's horses had four alleles at one *DRB* locus and two at another, even though the species descended from 13 individuals (Hedrick *et al*. 1999), and the Tiburon Island bighorn sheep had five alleles in 14 individuals despite the population founder size of 20 animals (Hedrick *et al*. 2001). The drastic bottleneck of the American bison in the late 1800s left fewer than 1000 individuals (from an estimated 60 000 000), but still the population had nine *DRB* alleles in a survey of 20 animals (Mikko *et al*. 1997). Thus we think that a single population bottleneck event followed by inbreeding may not account adequately for the low genetic variability consistently observed in the NES.

Secondly, alleles at one locus, *DQA*, have a significant association with seal birth site (i.e. San Nicolas Island vs. San Miguel Island). To determine if relationships exist among genotypes at the three MHC loci, and between MHC genotypes and other factors (e.g. mtDNA genotype and location), we conducted tests of association (2×2 contingency table or $R \times C$-test of independence with the Williams correction). We performed G-tests of independence that indicated that the two possible genotypes in the mtDNA control region (Hoelzel *et al*. 1993; Weber *et al*. 2000) were not significantly associated with genotypes at all three MHC loci: *DQA* (G_{adj} = 2.36, P = 0.13) and *DQB* or *DRB* (G_{adj} = 3.69, P = 0.084). Further, we found no relationship between the rarer mtDNA genotype no. 2 seen in 28% of the seals sampled and the rarer MHC *DQB* or *DRB* allele (G_{adj} = 1.784, P = 0.16). However, with seal birth site we did find a significant association with *DQA* alleles as indicated by the G-test (G_{adj} = 6.032, $P < 0.025$), although this comparison has a relatively high probability of giving a Type I error. The rare *DQA* allele, *DQA1*02*, was found in seals from both colonies but only in the heterozygous condition. Eleven seals at San Nicolas Island had this allele compared with only two at San Miguel Island. This could be simply a result of island-specific founder events. In fact, MHC genotypes frequencies were very close to expected Hardy–Weinberg proportions (Table 2), and an exact test failed to detect a deviation from HWEQ (P = 1 because there were no *DQA1*02*/*DQA1*02* genotypes detected). In contrast, alleles at both *DQB* and *DRB* loci were independent of location (G_{adj} = 1.667, P = 0.17). For *DQB*, the observed genotype

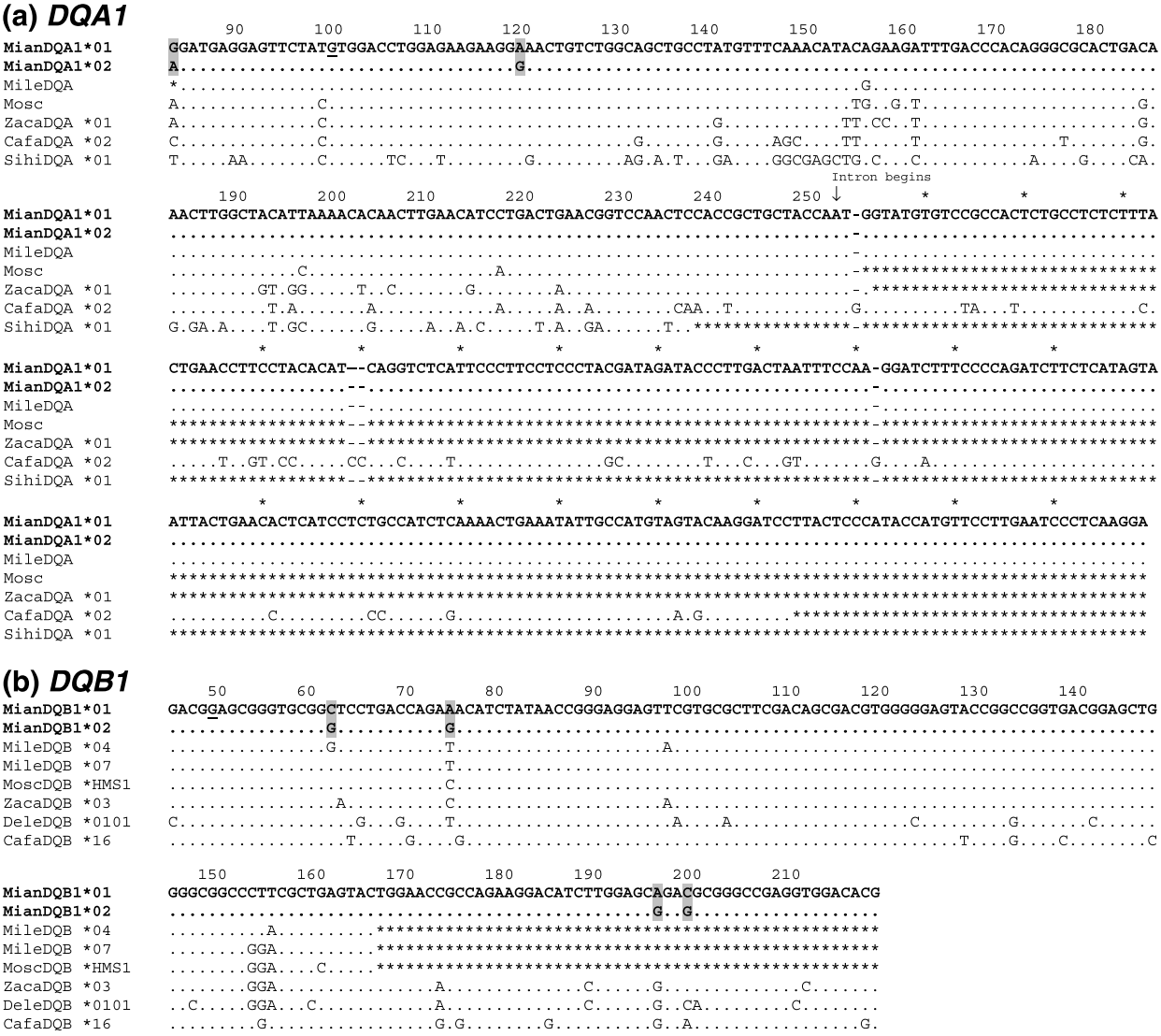

Fig. 1 MHC Class II variability in the northern elephant seal. (a) DNA sequence alignment for the *DQA1* exon 2 found in 110 modern NES (*Mian*). The SSCP analysis showed two genotypes: 1, a homozygote of *MianDQA1*01*; and 2, a heterozygote of *MianDQA1*01* and *MianDQA1*02*. NES nucleotide sequences were compared with *Mile* = southern elephant seal (*Mirounga leonina*; GenBank Accession no. U91907, this study); *Mosc* = Hawaiian monk seal (*Monachus schauinslandi*; AF093799); *Zaca* = California sea lion (*Zalophus californianus*; AF502560, Bowen *et al*. 2002); *Cafa* = domestic dog (*Canis familiaris*; U47857); and *Sihi* = cotton rat (*Sigmodon hispidus*; AF155924, Pfau *et al*. 1999). Asterisks in the sequence line indicate missing data; dots signify bases that are identical at that position to top sequence. Shading denotes nucleotide changes between NES alleles. Arrow signifies the start of the intron. Nucleotide numbering scheme based on the complete exon 2 sequence by homology to the human sequence (human position no. 100 underlined). (b) DNA sequence alignment for the *DQB1* exon 2 (human position no. 50 underlined). The SSCP analysis showed two genotypes: 1, a homozygote of *MianDQB1*01*; 2, a homozygote of *MianDQB1*02*; and 3, a heterozygote of *MianDQB1*01* and *MianDQB1*02*. NES sequences were compared with the southern elephant seal (AF111034 for *MileDQB1*04* and AF111037 for *MileDQB1*07*, Hoelzel *et al*. 1999), Hawaiian monk seal (AY007203), California sea lion (AF503399, Bowen *et al*. 2002), beluga (U16986, Murray *et al*. 1995) and domestic dog (AF043161). (c) DNA sequence alignment for the *DRB* exon 2 (human position no. 80 underlined). SSCP results determined that *DRB* for the northern elephant seal has two genes comprising three genotypes: 1, both loci homozygous, for the alleles *MianDRB1*01* and *MianDRB2*01*; 2, both loci homozygous, for the alleles *MianDRB1*01* and *MianDRB2*02*; and 3, homozygous for *MianDRB1*01* and heterozygous for *MianDRB2*01* and *MianDRB2*02*. NES sequences were compared with the southern elephant seal (*Mile* alleles; this study); beluga (AF012935 for *DeleDRB1*0401* (polymorphic locus) and AF012928 for *DeleDRB2*0601* (monomorphic locus), Murray & White 1998); and domestic dog (AJ311091). (d) Amino-acid translations of the NES MHC alleles above, starting with the second nucleotides shown in (a, b, c). Inferred peptide binding sites are indicated by the symbol + below the last sequence for each *DQA*, *DQB* and *DRB* (homology with mouse, Brown *et al*. 1988, 1993); underlined indicated the approximate area of the MHC alpha helix in contact with the peptides. Shading denotes amino acid polymorphisms between the different alleles for the NES. Arrow above the *DQA* sequence signifies the start of the intron. Numbers above *DQA* residues are based on the cotton rat (*SihiDQA1*, Pfau *et al*. 1999); numbers above *DQB* and *DRB* residues are based on the beluga (*DeleDQB* and *DeleDRB*, Murray *et al*. 1995).

(c) *DRB*

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                    80        90       100       110       120       130       140       150       160       170
MianDRB1*01         GCTCCTGGTCAGAAACATCTATAACGGGGAGGAGGTCTCGCGCTTCGACAGCGACGTGGGGGAGTTCCGGCCGGTGACGGAGCTGGGGCGGCCGGACGCT
MianDRB2*01         ........AT..GT.TT..................T..GT.....................................................
MianDRB2*02         ........AT..GT.TT..................T..GT.....................................................CTT....
MileDRB*01          ..........................................................................................
MileDRB*02          .T...........T...........C........T..GT.....................................................
MileDRB*03          .......AC....T...........C........TA.GT.....................A.....................CTC....
MileDRB*04          ...............T..................TA.GT.....................................................
MileDRB*05          .TA..........G.....................A.....................................................
DeleDRB1*0401       ....G...AT...T.....................TA.GT.....A..............C....A....G...A...C........C....G.AC...C
DeleDRB2*0601       .TA.A..AC....C.....................TA.GT.....................C....A....A.......C........C....G.AC...C
CafaDRB1*01504      ...T.....G...G...........C........CA.GT.....................G....C........C........C......

                    180       190       200       210       220       200       210       220       230
MianDRB1*01         GAGTACTGGAACCGCCAGAAGGACATCTTGGAGCGGAGGCGGGCCGAGGTGGACACGGTGTGCAGACACAACTACCCGGTGGTTGAGA
MianDRB2*01         ........C.............................C.................G.............................
MianDRB2*02         ........C.............................C.................G.............................
MileDRB*01          ..........................................................................................
MileDRB*02          ........................T..A......A..C.................G.............................
MileDRB*03          ..........................................................................................
MileDRB*04          ........C.....................................................................G.....G.....
MileDRB*05          ........................T..A......A..C........C.............................A...........
DeleDRB1*0401       ............A...........C..C......A..AC.......C.C........TAC..............GG...T..G....
DeleDRB2*0601       ............A...........C..C......A..AA.A....A...C.......TAC.....G.........GG..CT..G...C
CafaDRB1*01504      ............G.G.........GC.........A.....................................GG....A......
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(d) amino-acid alignments

DQA1

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                                                                                                  Intron begins
                    30        40        50        60        70        80                          ↓
MianDQA1*01         DEEFYVDLEKKETVWQLPMFQTYRRFDPQGALTNLATLKHNLNILTERSNSTAATN
MianDQA1*02         ...........G.........................................
MileDQA             ....................G.....................................
Mosc                ......................GG.......R....S......M...........
ZacaDQA *01         ..................V...FTS.......R...VG.LT..V..K.........
CafaDQA *02         ................R..V.S.F.S.......R...II.Q....M.K...Q.....
SihiDQA1*01         .......SD......RI.E.GELTS.....G.QDI..A.Y..E.M.K...******
                                    + + +  +   +   +  +  +   +
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DQB1

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                    21        30        40        50        60        70
MianDQB1*01         TERVRLLTRNIYNREEFVRFDSDVGEYRPVTELGRPFAEYWNRQKDILEQTRAEVDT
MianDQB1*02         .....V...D.....................................RR......
MileDQB1*04         .....V...Y......Y...................Y...*****************
MileDQB1*07         .........Y.........................D...*****************
MoscDQB*HMS1        .........H.........................D.Q.*****************
ZacaDQB *03         .....H...H......Y...................D.....S......R....A..
DeleDQB *0101       ......VS.Y......L.H.........A.......D.....S......R....L..
CafaDQB             .........S................F.A.......V.....G..E...RK.....R
                         + + +      ++                  ++           +  +
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DRB

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                        30        40        50        60        70        80
MianDRB1*01         LLVRNIYNGEEVSRFDSDVGEFRPVTELGRPDAEYWNRQKDILERRRAEVDTVCRHNYPVVE
MianDRB2*01         ..D.YF.....FV.....................C..........T.....R..........
MianDRB2*02         ..D.YF.....VS.....................F.....................................
MileDBR*01          ..........................................................................
MileDBR*02          F...YF..R..FV.............................FM.QT.....R..........
MileDBR*03          ..T.Y...R..YV.........Y.........S..............................
MileDBR*04          ....YF.....YV.......................C.......................R.G.
MileDBR05(039-12 Y...D......VT.............................FM.QT..A.........Q...
DeleDBRB1*0401      .VD.Y......YV.Y......Y.AE.....RT.....S...L..QN..AL........G...
DeleDRB2*0601       YMT.H......YV........Y.T......RT.....S...L..QKQ.KA........GA..
CafaDRB1*01504      ....D...R..HV..........A.............G..EL..Q.............G.I.
                      + +      ++                    +   +      +  ++  +   +   +   +
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Fig. 1 *Continued*

frequencies were also not significantly different from Hardy–Weinberg expectations (Table 2; $\chi^2 = 0.0336$, $P = 0.79$). We found the *DRB* locus to have the same frequencies as *DQB* (Table 2) and thus is also in HWEQ (see below). An excess of heterozygotes was not indicated at any of the three loci (Table 2).

We reported earlier that there was no association between seal birth site and mtDNA control-region genotype (Weber *et al*. 2000). In addition, a survey of 14 variable microsatellite loci from many of the same seals failed to detect any significant deviation from HWEQ nor any significant differences in allele frequencies between San Nicolas and San Miguel Island populations when all loci were considered jointly (Garza 1998). Yet the intriguing possibility remains that colony differences in fitness may exist for MHC genotypes, perhaps conferred merely by the single detectable amino-acid difference in *DQA* alleles. Differential historic exposure to infectious disease might explain

Table 2 Allele and genotype frequencies and heterozygosities at the three MHC class II loci and a mtDNA locus for the northern elephant seal (n = 110)

Locus	No. of alleles	Allele freq. (observed)	H_{EXP}	H_{OBS}	Genotype frequency	
					observed	expected
DQA1	2	0.077/0.923	0.142	0.154		
Genotype 1					0.845	0.853
Genotype 2					0.155	0.142
DQB1	2	0.227/0.773	0.350	0.345		
Genotype 1					0.600	0.600
Genotype 2					0.055	0.051
Genotype 3					0.345	0.351
DRB1	1	1.0	—	—		
DRB2	2	0.227/0.773	0.350	0.345		
Genotype 1					0.600	0.600
Genotype 2					0.055	0.051
Genotype 3					0.345	0.351
Post-mtDNA†	2	0.72/0.28	—	0.41		
Pre-mtDNA†	5	—	—	0.90		

†Mitochondrial DNA variation was analysed from the control region in 149 postbottleneck (modern) seals and from seven prebottleneck seals living at or before the last known bottleneck of 1892 (Hoelzel *et al*. 1993; Weber *et al*. 2000); observed heterozygosity values for mtDNA are given as gene diversity (H_E).

such patterns, although we are not aware of any substantive evidence that would yet support this hypothesis.

Our third and final result is that *DQB* and *DRB* showed complete linkage disequilibrium. Genotypes at the *DQA* locus were independent of genotypes at either *DQB* or *DRB* loci (G_{adj} = 2.40, P = 0.12), but alleles at *DQB* and *DRB* had a highly significant association (G_{adj} = 154.9, $P < 0.001$). Sixty-six NES that were homozygous for *DRB2*01* were also homozygous for *DQB1*01*, and six seals were also homozygous for both *DRB2*02* and *DQB1*02*. The remaining 38 seals that were heterozygous at *DRB*2 were also heterozygous at *DQB*1, indicating linkage between these two loci. All cloned *DRB* PCR products had the *MianDRB1*01* allele. As there was a direct relationship between *DQB* and *DRB* (Table 2), *DQB* appears to be linked to the polymorphic locus of *DRB*2. Gene duplication and subsequent divergence can be inferred from the identities of six nucleotides clustered together near the beginning of the *DRB* sequence (Fig. 1c) that are identical in DRB2 alleles but distinct in the DRB1 allele. This same area of six nucleotides may also have been the result of a gene conversion event.

A nonrandom association of alleles may caused by population bottlenecks that sustain the effects of prior population events for long periods if linkage is tight (Hedrick 2000). This could explain the association we found between the *DQB* and *DRB* genotypes in the NES, a species that has been reduced to small numbers at least once and perhaps several times (Lehman & Stewart 2002). Similarly, a prior significant bottleneck for the St Lawrence beluga population (Reeves & Mitchell 1984; O'Corry-Crowe & Lowry 1997) may explain the linkage disequilibrium for the same two MHC loci and subsequent reduction in haplotypes in this species (Murray *et al*. 1999). Alternatively, of course, the linkage of the *DQB* and *DRB* loci may be attributed to a small chromosomal distance such that recombination has not had time to disrupt the observed MHC haplotypes. These loci are separated by only ~85 kb in humans with no apparent recombination hot spots inbetween (Cullen *et al*. 2002). Small selection intensities at this distance could result in significant linkage disequilibrium, but the pinniped MHC has not yet been subjected to a fine-scale mapping analysis.

Although the NES has clearly suffered a drastic demographic constriction, from these data and prior studies it is still unclear what ecological consequences, if any, will come about from the loss of genetic variation that resulted. We speculate that the NES founder population may have started out with little genetic variation after speciation from the SES lineage. The NES then experienced a series of bottlenecks which facilitated a periodic purging of deleterious alleles, enhanced by particular aspects of its natural history (e.g. male–male competition, extreme polygyny, small effective population size, site fidelity, etc. all of which can hinder the production and maintenance of genetic variation). We advance the hypothesis that the NES may be an example of a species 'adapted to low genetic variability'. It is clearly inbred, but has overcome the apparent effects of inbreeding depression. As postulated for certain other species (e.g. Potts *et al*. 1994; Seddon &

Baverstock 1999), high MHC polymorphism may not be necessary for the long-term population vitality in the NES. On the other hand, Lento *et al*. (2003) suggested a correlation between an epizootic and low MHC variation at the *DQB* locus (two alleles in 61 sea lions) in Hooker's sea lions. As yet, no epizootics have occurred in northern elephant seals, in contrast to substantial epidemics in other pinniped populations (e.g. Laws & Taylor 1957; Geraci *et al*. 1982; Osterhaus *et al*. 1998, etc.). Monitoring of the responses of NES populations to new or re-emergent pathogens will be critical to evaluating this hypothesis.

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This research is part of a University at Albany PhD dissertation for Diana Weber on the investigations of bottlenecks in pinnipeds with Niles Lehman. D. S. W. is interested in studying the effects of bottlenecks, inbreeding and other ecological pressures on species and in particular marine organisms. B. S. S. is a Senior Research Biologist at Hubbs-SeaWorld Research Institute in San Diego, California specializing in studies of marine mammal demography, ecology, physiology and evolution. J. S. is currently an Assistant Professor in Residence in the Forensic Research Laboratory at the University of Connecticut. N. L. is currently an Associate Professor in the Department of Chemistry at Portland State University focusing on molecular–evolutionary questions at many levels.
