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MHC gene configuration variation in geographically disparate populations of California sea lions (*Zalophus californianus*)

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Abstract

Major histocompatibility complex (MHC) class II DRB genotypes were examined in two geographically isolated populations of California sea lions (*Zalophus californianus*) (Gulf of California and California coastal Pacific Ocean). Genomic DNA from 227 California sea lions was examined using eight sequence-specific primer (SSP) pairs flanking the putative peptide-binding site. A total of 40 different *Zaca-DRB* genotype configurations were identified among the 227 individuals. Using SSP-PCR, significant differences were found between coastal California and Gulf of California *Zalophus* populations in numbers of DRB sequences per individual and configuration of sequences within individuals. Additionally, unique local patterns of MHC diversity were identified among the Midriff Island animals. These population differences are consistent with either ecologically distinct patterns of selection pressures and/or geographical isolation. The consequences of these partitioned MHC configurations at the population level are as yet unknown; however, the worldwide increase in emerging marine diseases lends urgency to their examination.

Keywords: California sea lion, class II MHC, DRB, Gulf of California, Pacific Ocean, pathogen

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Introduction

The concept of associations between functionally relevant genetic diversity and ecosystem processes is increasingly accepted (Meyer & Thomson 2001; Cohen 2002). Functional diversity is particularly important in the genes of the immune system, such as those in the major histocompatibility complex (MHC), which encode a set of transmembrane proteins critical to the generation of immune responses (Paul 1999; Klein & Sato 2000a, b). The variety of MHC-encoded proteins in an individual ultimately determines the repertoire of foreign peptides to which that animal is capable of responding and, at the population level, reflects the historic influence of pathogen pressures and breeding biology (Zinkernagel 1979; Reizis *et al*. 1998). For these reasons, analysis of the MHC provides a mechanism to relate functionally important genetic diversity at the population level with environmental stresses (Cohen 2002).

Recent studies in California sea lions (*Zalophus californianus*) (CSL) have described a multilocus class II MHC (*Zaca-DRB*) gene family with a pattern of variability and multiplicity that would favour a broad scope of peptide presentation (Bowen *et al*. 2002, 2004). *Zaca-DRB* constitutes a gene family, comprised of eight unique sequence patterns, each of which exhibits limited variability, and are

Study performed jointly in the laboratories of Stott and Johnson
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present in variable configurations between individuals; assignment of these sequences to loci or alleles was not possible. This unusual mechanism for generating MHC *DRB* diversity is similar to that recently described in the macaque (Doxiadis *et al*. 2001) and differs from human and murine MHC systems which have a limited number of loci each with highly variable alleles. In order to establish an immunogenetic framework upon which to study potential ecological influences on CSL MHC diversity, a comparative study of *Zaca-DRB* genes was performed between animals in the Gulf of California, Baja, Mexico, and coastal California populations.

In this study, we examined MHC gene configurations and multiplicity between and within coastal California and Gulf of California *Zalophus* populations. This geographical isolation of the Midriff Island sea lions can provide a model system with which to examine the effects of potentially differential ecological pressures on MHC diversity in this species.

Materials and methods

Study sites

All free-ranging CSL were randomly selected at rookeries.

Midriff Islands, Gulf of California, Mexico: This region harbours approximately 30 000 CSL with most rookeries occurring in the northern half of the Gulf. Sea lions were sampled from six rookeries located in the Midriff Islands area: El Rasita (n = 17), El Partida (n = 16), Los Cantiles (n = 18), El Granito (n = 19), Los Machos (n = 18), and El Coloradito (n = 10) (Fig. 1).

San Miguel Island, Pacific coastal southern California: Sea lions were sampled from San Miguel Island (pups, n = 47; adults, n = 32) in the Channel Islands, California, USA (Fig. 1). San Miguel Island is one of the two largest breeding grounds for CSL and is occupied by over 100 000 individuals during peak breeding season.

Northern California stranded sea lions: Sea lions stranded on the northern California coast were sampled at The Marine Mammal Center (TMMC) at the time of necropsy. Fifty animals were sampled with the apparent cause of death including cancer, gunshot, domoic acid poisoning and shark bite. CSL from TMMC could not be distinguished based on *Zaca-DRB* genotype from San Miguel Island sea lions and were therefore considered representative of the broader Pacific population (Bowen *et al*. in press).

Animals and sample preparation

Caudal gluteal venous blood samples or skin clips from flippers were collected from 98 CSL pups from the Midriff Islands in 2000 and 2001; 47 pups or juveniles in San Miguel Island in 2001 and 2002; 32 adults in San Miguel Island in 2001, 2002 and 2003; and 50 stranded adults at TMMC in 2001, 2002 and 2003 (58 of the 79 SMI individuals were analysed previously and reported in Bowen *et al*. 2004). Blood samples were collected into EDTA tubes (Vacutainer CPT, Becton Dickinson) and used for rapid isolation of peripheral blood leucocytes (PBLs). Whole blood was subjected to NH_4KCl lysis and PBLs were frozen at –80 °C pending DNA isolation. DNA from PBLs and skin clips was extracted by silica-based gel membranes combined with microspin technology (DNeasy, QIAGEN).

Fig. 1 Geographic locations of the Midriff Islands (El Rasito, El Partida, Los Cantiles, El Granito, Los Machos, and El Coloradito) in the Gulf of California, San Miguel Island off the California coast and The Marine Mammal Center on the central California coast. Approximate location indicated by +.

Examination of Zaca-DRB *sequence configurations using sequence-specific primers*

Genomic DNA was examined with eight sequence-specific primer pairs (SSP) flanking the putative peptide-binding site using an intercalating fluorescent dye PCR (Bowen *et al*. 2004). Each reaction contained 500 ng DNA in 25 µL volumes with 20 pmol SSP, Tris-Cl, KCl $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$ (pH 8.7), dNTPs, HotStar *Taq* DNA polymerase (Quantitect SYBR Green PCR Master Mix, QIAGEN), and 0.5 unit uracil-N-glycosylase (Roche). Amplifications were performed in an iCycler (Bio-Rad) under the following conditions: 2 min at 50 °C, followed by 15 min at 95 °C,

and 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, with a final extension step of 72 °C for 10 min. Reaction specificity was monitored by melting curve analysis using a final data acquisition phase of 60 cycles of 65 °C for 30 s and verified by direct sequencing of randomly selected amplicons (Bowen *et al*. 2004).

The predictive value of individual *Zaca-DRB* genes was determined by logistic regression, polychotomous logistic regression with a generalized logit model, and correspondence analysis (SAS). Standard *t*-tests were used to determine differences in number of unique sequences present between populations (Hintze 2001: Number Cruncher Statistical System; NCSS Statistical, Utah 84037).

Results

Zaca-DRB *sequence configurations*

The prevalence of each sequence was not uniform between the Midriff Islands and the broader Pacific (San Miguel Island and TMMC), nor even among individual rookeries in the Midriff Islands (Table 1). Forty different *Zaca-DRB* configurations were identified in the 227 sea lions (Table 2); 29 of the 40 identified were in Midriff Islands pups, 21 in San Miguel Island pups, 15 in San Miguel Island adults and 19 in TMMC adult sea lions. Eleven configurations were unique to the Midriff Islands population, eight were unique to the San Miguel Island pups and one was unique to the San Miguel Island adults. Only nine configurations were common to all groups.

Numbers of Zaca-DRB *sequences*

Total numbers of *Zaca-DRB* sequences per individual were less in San Miguel Island pups ($x = 5.7$) compared with San Miguel Island adults ($x = 6.4$) ($t = 2.355$, $P = 0.01$), and TMMC adults ($x = 6.2$) ($t = 2.644$, $P = 0.004$). The total number of *Zaca-DRB* sequences per individual in the Gulf of California pups ($x = 5.5$) was not different from San Miguel Island pups ($t = 0.807$, $P = 0.21$).

Logistic regression

Multinomial logistic regression was used to identify unique configurations of *Zaca-DRB* sequences which could be used to discriminate between groups of CSL in the Midriff Islands and in the Pacific (San Miguel Island and The Marine Mammal Center). The frequencies of *Zaca-DRB.A* and *Zaca-DRB.H* were significantly lower in the Gulf of California than in the Pacific (*Zaca-DRB.A*: $\chi^2 = 13.06$, $P = 0.0003$; *Zaca-DRB.H*: $\chi^2 = 10.61$, $P = 0.0011$). Polychotomous logistic regression with a generalized logit model was used to identify *Zaca-DRB* sequences which could be used to discriminate among rookeries in the Midriff Islands (*Zaca-DRB.A*: $\chi^2 = 9.85$, $P = 0.07$; *Zaca-DRB.D*: $\chi^2 = 10.52$, $P = 0.06$; *Zaca-DRB.G*: $\chi^2 = 9.94$, $P = 0.07$); results approach significance.

Discussion

The present study focused on establishing an immunogenetic foundation upon which to study effectively isolated populations of CSL. This was accomplished by characterizing class II MHC diversity within populations of CSL located in the Gulf of California and off the central California coast.

Marked MHC genotype differences were identified between sea lions in the Gulf of California and those in coastal California. The distinction between these two broad CSL populations is supported by historic geographical boundaries and preliminary MHC investigations that show a low number of shared *Zaca-DRB* sequences between animals from the Midriff Islands and those from the coastal Pacific (Bowen *et al*. 2004). While the MHC differences between the Gulf and California populations were anticipated, the striking pattern of class II MHC variation between sea lions from rookeries in close proximity within the Midriff Islands was unexpected.

Theoretical predictions and numerous empirical studies suggest that, in general, small, insular populations experience more dramatic effects of neutral processes than

Table 1 Frequency (%) of *Zaca-DRB* sequences as a function of geographical location

Zaca-DRB sequence	TMMC ($n = 50$)	SMI pups ($n = 47$)	SMI adults ($n = 32$)	El Granito ($n = 19$)	Los Cantiles ($n = 18$)	Los Machos ($n = 18$)	El Coloradito ($n = 10$)	El Partida ($n = 16$)	El Rasito ($n = 17$)
A	48	68	66	21	56	17	20	38	47
B	68	43	66	68	72	61	50	38	41
C	68	64	75	37	56	44	70	69	71
D	72	57	69	68	61	78	80	31	53
E	100	100	97	95	100	100	100	100	100
F	100	94	100	100	94	100	100	94	100
G	66	55	72	79	56	44	100	63	71
H	100	96	100	79	89	72	90	94	76

Table 2 Genotype presence as a function of geographical location

Genotype	El Granito $n = 19$	El Coloradito $n = 10$	El Partida $n = 16$	El Rasito $n = 17$	Los Cantiles $n = 18$	Los Machos $n = 18$	SMI pups $n = 47$	SMI adults $n = 32$	TMMC $n = 50$
ABCDEF	1				1				
ABCDEFGH		1					4	10	6
ABCDEFH					1		1	1	2
ABCEFG				1					
ABCEFGH					2			2	2
ABCEFH							1		1
ABEF							1		
ABEFGH					1				1
ABEFH	1		2		1		1	2	2
ACDEF					1				
ACDEFGH				2	2	2	6	2	2
ACDEFH							2		3
ACDEGH							1		
ACEF				1					
ACEFGH	2	1	2	3	1		8	2	3
ACEFH			1			1		1	2
ADEFH			1	1			4	1	
AE							1		
AEFH							2		
BCDEFGH	2	2		1				2	6
BCDEFH					1				1
BCDEGH							1		
BCEFGH	1		2	2	1	2	4		1
BDEF	3					4	1		
BDEFGH	2	2		1	2		1	1	6
BDEFH	2		1	1	3	4	4	3	5
BEF			1	1		1			
BEFH							1		
BEFGH	1								1
CDEFG		1							
CDEFGH		1	3	2		3	1	1	4
CEFGH	1	1	2					2	1
CEFH							1		
CEGH			1						
CFGH								1	
DEF	1			1					
DEFGH		1				1			
DEFH	1						1	1	1
DFH	1								
EGH					1				

their mainland counterparts at neutral or nearly neutral genetic loci (Seddon & Baverstock 1999; Klein & Sato 2000a, b). Genetic drift acting upon these small, Midriff Island populations could ultimately force the partitioning of MHC genotypes observed between rookeries. An alternate, although not necessarily mutually exclusive explanation of the observed patterns of MHC variation among rookeries, is that of structuring in the source population (Seddon & Baverstock 1999). Local variations in the distribution and frequencies of sequences could be the result of unique MHC genotypes present in the source populations at the time of colonization or due to hunting-induced bottleneck events (Zavala-Gonzalez & Mellink 2000). Partitioning of genotypes and sequence frequencies could be further exacerbated by the high site fidelity and resource partitioning evidenced in the Midriff Island populations (Garcia-Rodriguez & Aurioles-Gamboa 2004).

It is well established that the polymorphism at MHC loci is selectively maintained, and there is evidence that infectious disease plays an important role in this selection (Hughes & Nei 1988; Hedrick & Kim 2000). Pathogen-driven selection operates when specific alleles confer enhanced protection or susceptibility to a pathogen (Hughes & Nei 1988; Paterson *et al*. 1998; Hedrick & Kim 2000; Jeffery &

Bangham 2000; Meyer & Thomson 2001). Many pathogens exhibit an epidemic pattern of infection (i.e. fluctuating intensity of selection), leading to temporal and geographical changes in selection pressure on the host (Jeffery & Bangham 2000). While it cannot be determined if selection has acted on specific MHC sequences in these subpopulations, evidence supporting differential pathogen pressures as an explanation for these MHC patterns would be supported by Acevedo-Whitehouse *et al*. (2003). Prevalence of leptospirosis serovars (a debilitating and often fatal bacterial infection in sea lions and other mammals) was significantly different among selected Midriff Island rookeries and suggestive of the presence of enzootic host-adapted serovars (Acevedo-Whitehouse *et al*. 2003). Crowding in rookeries as well as the presence of different assemblages of terrestrial inhabitants could also influence such differences in seroprevalence. In addition to these interrookery differences, leptospirosis serovars differ between Gulf of California and Pacific populations (Godinez *et al*. 1999).

The identification of geographical differences in the CSL MHC described here should stimulate additional studies into the potential ecological pressures at play in this system. The consequences of partitioned MHC configurations at the population level are as yet unknown; however, the worldwide increase in emerging marine diseases lends urgency to their examination.

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We are interested in the effects of ecological pressures on disease dynamics in marine organisms, specifically, the influence of anthropogenic and natural stressors on immune function and disease state.
