THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE HISPID COTTON RAT (*SIGMODON HISPIDUS*): MOLECULAR EVOLUTION, EVOLUTIONARY TOXICOLOGY, AND PATTERNS OF GEOGRAPHIC VARIATION

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CHAPTER I

INTRODUCTION: THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE HISPID COTTON RAT (*SIGMODON HISPIDUS*)

The major histocompatibility complex (MHC) is a multigene family that plays a key role in the immune response of vertebrates. The MHC is one of the most extensively studied genetic complexes, and much is known about the structure and function of these genes. Most of these studies, however, have focused on a few species of laboratory animals. Only recently have wild populations of other species been the subject of investigation. The unique nature of MHC genes makes them useful in a variety of disciplines including evolutionary biology, immunology, population and conservation genetics, and systematics.

MHC molecules act as receptors that bind protein fragments (peptides) within cells and transport those peptides to the cell surface where they are displayed for recognition by T-lymphocytes. Both self and non-self peptides are bound indiscriminately, but an immune response is initiated by the T-lymphocytes only if the peptides are recognized as non-self. There are two subfamilies of MHC molecules, class I and II, which have different patterns of expression and peptide-binding abilities and thus play different roles in immune response. Class I MHC molecules are expressed on the surfaces of most cells and present peptides to cytotoxic T-cells (Bjorkman and Parham 1990). These molecules bind peptides of 8 - 10 amino acids in length. Class II MHC molecules have a more limited expression, mostly on monocytes, macrophages, dendritic cells, and B-lymphocytes (Huston 1997, Neefjes and Momburg 1993, Trowsdale 1993), and can bind larger peptides ranging from 12 - 24 amino acids in

length. Class I molecules predominantly bind peptides derived from intracellular infectious agents such as viruses, whereas class II molecules mostly bind extracellularly derived peptides from bacteria and other parasites (Cresswell 1994, Germain and Margulies 1993). Amino acid residues found in the region of the MHC molecule that contacts bound peptides, the peptide-binding region (PBR), typically are highly variable (Hughes and Yeager 1998). The corresponding nucleotide positions of MHC genes encoding these molecules are, of course, highly polymorphic.

Most mammals are highly polymorphic at functional MHC loci: including American bison (*Bison bison*; Mikko et al. 1997), rhesus macaques (*Macaca mulatta*; Slierendregt et al. 1995), red deer (*Cervus elaphus*; Swarbrick et al. 1995), and white-tailed deer (*Odocoileus virginianus*; Van Den Bussche et al. 1999). However, several species and populations have very low levels of variability or even complete monomorphism, including European beaver (*Castor fiber*; Ellegren et al. 1993), beluga, fin, and sei whales (*Delphinapterus leucas, Balaenoptera physalus, B. borealis*; Murray et al. 1999, Trowsdale et al. 1989), moose (*Alces alces*; Mikko and Andersson 1995, Ellegren et al. 1996), southern elephant seal (*Mirounga leonina*; Slade 1992), island populations of *Mus* (Figueroa et al. 1986), and Asiatic lion and cheetah (*Acinonyx jubatus* and *Panthera leo*; Yuhki and O'Brien 1990).

The normally high level of MHC polymorphism is thought to be maintained by balancing selection acting at specific amino acid residues (Hughes and Nei 1988, Takahata et al. 1992). The neutral theory (Kimura 1983) predicts that the rate of nucleotide substitutions will be the same for each nucleotide position. In protein coding regions, the mutation rate should differ between synonymous and non-synonymous sites because most non-synonymous mutations are deleterious and are eliminated by purifying

selection (Nei 1987). However, if balancing selection is acting to maintain genetic diversity at a locus, the number of non-synonymous substitutions will be greater than the number of synonymous substitutions in the affected codons (Hughes and Nei 1988, Klein et al. 1993). This is the pattern of substitution typically found at functional MHC loci (Hughes and Yeager 1998).

Because most MHC loci apparently are acted on by balancing selection, alleles can be retained for longer periods of time than they could under neutral selection. The result is that alleles can be shared among recently diverged species, and over longer periods of time some allelic lineages show closer relationships among species (or even among genera) than within species (Klein et al. 1998). This phenomena, known as trans-species persistence, is common at MHC loci (Graser et al. 1996, Trtkova et al. 1995).

Sigmodon hispidus is a common rodent occurring throughout the southern United States. Because it is often locally abundant it has been the subject of diverse ecological studies (Cameron and Spencer 1981) and has been explored for potential use in biomonitoring of hazardous waste sites (Lochmiller et al. 1999, McBee et al.1987, McMurry et al. 1999). *S. hispidus* also is used in medical research as the primary small animal model of human respiratory syncytial virus and parainfluenza virus type 3 infections, and is the only known small animal model of human adenovirus type 5 infection (Prince et al. 1978, Ginsberg et al. 1989, Porter et al. 1991, Prince et al. 1993, Prince and Porter 1996, Coe and Prince 1996). Because of the frequent use of *S. hispidus* in research and the likely relationship between MHC, pathogens, and population regulation (Apanius et al. 1997, Lochmiller 1996, Lochmiller and Dabbert 1993) an understanding of MHC evolution, diversity, and geographic variation in this species

should provide important insight into the patterns and processes revealed by these studies.

The following chapters address molecular evolution of an MHC locus (*Mhc-DQA* exon-2) in *S. hispidus*, the relationship between environmental contamination and *Mhc-DQA* genetic diversity, and the genetic diversity of this locus across a large portion of the geographic distribution of *S. hispidus*. These chapters are formatted for submission to *Immunogenetics* (Chapter II), *Environmental Toxicology and Chemistry* (Chapter III), and *Molecular Ecology* (Chapter IV).

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CHAPTER II

ALLELIC DIVERSITY AT THE *MHC-DQA* LOCUS IN COTTON RATS (*SIGMODON HISPIDUS*) AND A COMPARISON OF *DQA* SEQUENCES WITHIN THE FAMILY MURIDAE (MAMMALIA: RODENTIA)

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Abstract–The cotton rat (*Sigmodon hispidus*) is a common murid rodent of the southern United States, Mexico, and Central America. Using single-strand conformation polymorphism analysis and DNA sequencing techniques, 26 *Mhc-DQA* exon-2 alleles were detected among 406 *S. hispidus* from 13 localities in the United States and Costa Rica. These alleles represent a single locus exhibiting a high level of polymorphism. Nucleotide and amino acid distance values among *DQA* alleles of *S. hispidus* were higher than those within *Mus musculus* and species of *Rattus*. Although the distribution of polymorphic amino acid positions among alleles of *S. hispidus* was similar to that of *Mus* and *Rattus*, some positions were more variable in *S. hispidus*. Comparisons of nonsynonymous and synonymous substitutions indicated a trend toward higher numbers of nonsynonymous substitutions; however, this difference was not statistically significant among *S. hispidus* alleles. To examine evolution of *DQA* alleles within Muridae, we performed a phylogenetic analysis that included *DQA* alleles from *S. hispidus*, *Peromyscus leucopus*, *M. musculus*, *R. norvegicus*, and six Australian species of *Rattus*. Results depicted monophyly for each genus. This concordance between species and gene trees represents a lack of evidence for trans-species persistence of alleles among these

genera.

Key words–Major histocompatibility complex, *DQA*, *Sigmodon hispidus*, Muridae, allelic diversity

Introduction

The major histocompatibility complex (MHC) is a large, multigene complex that plays a pivotal role in the immune response of vertebrates (Klein 1987). The MHC was first described and studied in detail in laboratory mice (Gorer 1936). When numerous associations between MHC polymorphism and disease susceptibility were detected in humans, mice, and chickens (Klein 1986; Longenecker and Mosmann 1981; Ryder et al. 1981), a major research emphasis was initiated to characterize MHC polymorphism in domesticated and laboratory animals. This work indicated that the MHC is the most polymorphic genetic system thus far detected in vertebrates, with high allelic diversity and high levels of sequence divergence among alleles. It also has been noted that MHC loci often exhibit their highest levels of polymorphism in the functionally important peptide binding region (PBR; Benoist et al. 1983; Jonsson et al. 1989). This high level of variability is thought to be maintained by some form of balancing selection acting to maintain diversity of the functional properties of MHC molecules, thus providing protection against a diversity of pathogens (Wakeland et al. 1990; Hughes et al. 1994). It is thought that balancing selection results in persistence of allelic lineages over long periods of time, and certain taxa exhibit trans-species persistence of allelic lineages where some alleles are more similar between species than within (Klein 1987).

MHC variability in natural populations is of great interest to evolutionary

biologists because of the high levels of polymorphism typically observed. Consequently, representative species of several mammalian orders, including Artiodactyla, Carnivora, Cetacea, Primates, and Rodentia, have been characterized for MHC allelic diversity. These studies have documented aspects of MHC variability that had gone undetected in domesticated or laboratory animals. Some species exhibit extremely high levels of MHC polymorphism (Mikko et al. 1997; Slierendregt et al. 1995; Swarbrick et al. 1995; Van Den Bussche et al. 1999; Yuhki and O'Brien 1997), whereas others are characterized by very low levels (Ellegren et al. 1993; Mikko and Andersson 1995; Murray and White 1998). Even in species characterized by high levels of MHC polymorphism, some populations are monomorphic or oligomorphic (Klein 1987). Several hypotheses have been proposed to explain the varying levels of MHC diversity among populations (Hedrick 1994; Klein 1987). Only the 'trans-species persistence' hypothesis seems to provide a universal explanation for differences in levels of MHC variability and other characters of the MHC in natural populations (Klein 1987). According to this hypothesis, variability within and among populations or species is directly related to the interplay between effective population size, time of divergence, and intensity of selection acting at a particular locus (Klein et al. 1993). Studies of MHC evolution across taxonomically diverse organisms are necessary to understand the relative importance of these hypotheses for explaining MHC variability in natural populations.

Muridae is the largest mammalian family, with 281 extant genera and 1326 species (Muser and Carleton 1993). The natural distribution of the family is world-wide except for some Arctic mountains, portions of the West Indies, New Zealand, and many oceanic islands. Because of human activity, many murids have become established in areas where they did not occur naturally (Nowak 1991). Because of the diversity and

broad geographic distribution of murid taxa, studies of MHC within this family should provide insight into levels of variability, effects of varied environments on MHC allelic diversity, and trans-species persistence of allelic lineages.

The cotton rat (*Sigmodon hispidus*) is a common murid found throughout the southern United States and southward through Central America to northern South America (Cameron and Spencer 1981). We describe here 26 class II *DQA* exon-2 alleles from *S. hispidus*. To examine allelic diversity of *S. hispidus* in relation to other murid rodents and assess trans-species persistence of allelic lineages, we include orthologous alleles from three additional taxa: *Peromyscus*, which occurs with *Sigmodon* in the subfamily Sigmodontinae, and *Rattus* and *Mus* from the subfamily Murinae.

Materials and Methods

Allelic diversity was characterized by examining 406 cotton rats from 13 localities in the United States and Costa Rica. Genomic DNA was extracted from muscle or liver tissue following Longmire et al. (1997). PCR amplification of *DQA* exon-2 was performed using primers PeleDQAex2-F (5'-ACAGCTGACCATGTTGGCGCCTA-3') and PeleDQAex2-R (5'-CACGTACCATTGGTAGCTGGGGTA-3'), which were designed from a published *DQA* sequence of *Peromyscus leucopus* (Crew and Bates 1996). All individuals were screened using single-strand conformation polymorphism analysis (SSCP; Orita et al. 1989) to identify allelic diversity at the *DQA* exon-2 locus. PCR amplification, SSCP analysis, and DNA sequencing were conducted as described in Pfau et al. (1999). Nomenclature of *S. hispidus DQA* (*Sihi -DQA*) exon-2 alleles follows Klein et al. (1990).

The following sequences were used for comparative and phylogenetic analyses

(GenBank accession numbers in parentheses): *Mus musculus* (K01922 - K01924, K01926, M11356 - M11358), *Rattus norvegicus* (L11338 - L11342, X14879), six species of Australian *Rattus*, including *Rattus tunneyi* (AF041070), *R. colletti* (AF041071), *R. leucopus* (AF041072, AF041073), *R. villosissi* (AF041074), *R. fuscipes* (AF041075, AF041076), and *Rattus* sp. nov. (AF041077), and *Peromyscus leucopus* (U34805). *Homo sapiens* (M17236, M17237), *Canis familiaris* (U61400), *Alopex lagopus* (Z26591), *Ovis aries* (M33304), and *Sus scrofa* (M29938) sequences were used as the outgroup in phylogenetic analysis.

The program MEGA 1.01 (Kumar et al. 1993) was used to calculate nucleotide distance values and relative frequencies of synonymous (d_S) and nonsynonymous (d_N) substitutions by the method of Nei and Gojobori (1986) with the Jukes and Cantor (1969) correction for multiple substitutions. A neighbor-joining phylogenetic tree (Saitou and Nei 1987) was constructed from genetic distance values corrected for multiple substitutions (Kimura 1980) under the minimum evolution criteria (Nei 1991; Saitou 1991). Following the recommendation of Swofford et al. (1996), the single neighbor-joining tree was used as the starting tree for a heuristic search using tree bisection-reconstruction and minimum evolution options in PAUP*4.0 (Swofford 1998).

Results

SSCP and DNA sequence analysis revealed 26 unique *Sihi-DQA* exon-2 sequences among the 406 cotton rats examined. The sequences correspond to positions 20 - 232 of the complete exon (Fig. 1 and 2). All sequences were confirmed by comparing SSCP banding patterns of cloned PCR products with those of the individual from which the allele was isolated. Additional confirmation was provided by the

occurrence of each variant sequence in more than one individual. These *Sihi-DQA* sequences have been deposited in GenBank under accession numbers AF155914–AF155924 and AF279850–AF279864. Of the 406 individuals examined by SSCP, none demonstrated more than four bands, which would be expected from a heterozygous individual, indicating that only one *DQA* locus was amplified by this set of primers.

Nucleotide distance values among all pairwise comparisons of *Sihi-DQA* alleles, corrected for multiple substitutions, ranged from 0.47% to 18.55%, and amino acid distance values ranged from 0.00% to 28.57%. Nonsynonymous substitutions at peptide binding sites (PBS) among the 26 *Sihi-DQA* alleles occurred at higher frequencies than synonymous substitutions but the difference was not significant ($d_N = 0.1713 \pm 0.0343$; $d_S = 0.0870 \pm 0.0569$; $z = 1.27$; $P > 0.2$). Observed values of d_N were higher than d_S for PBS in *Mus* and *Rattus* as well (Table 1), but the difference was significant only in *R. norvegicus* ($z = 2.96$, $P < 0.01$). Comparisons of nucleotide, amino acid, synonymous, and nonsynonymous substitutions in murid rodents are presented in Table 1. Although each of the four rodent groups had many unique amino acids, there was extensive sharing of amino acids (Table 2). Number and distribution of polymorphic amino acid positions among *Sihi-DQA* alleles were similar to those detected among *DQA* alleles from other murid rodents (Table 2, Fig. 3). One notable difference was the high level of amino acid polymorphism at positions 11, 62, and 68 of *Sigmodon* (Fig. 2 and 3) relative to *Mus* and *Rattus*. Also, comparisons among Australian *Rattus* species show position 52 to be highly polymorphic in this group, whereas position 32 was monomorphic in all taxa except Australian *Rattus*.

Phylogenetic analysis of *DQA* exon-2 alleles among *Sigmodon*, *Peromyscus*, *Mus*,

and *Rattus* resulted in a single shortest tree (Fig. 4). The 26 alleles of *S. hispidus* formed a monophyletic clade composed of five distinct sub-clades. Alleles from *Peromyscus*, *Mus*, and *Rattus* also formed monophyletic clades.

Discussion

This study demonstrated high levels of polymorphism among *DQA* exon-2 alleles of *S. hispidus*. Nucleotide and amino acid distance values among *DQA* alleles were higher in *Sigmodon* than in the other rodent taxa examined, and they also were higher than reported for the MHC locus *Eb* in several species of *Mus* (She et al. 1990). However, they were lower than observed at *DRB* loci in cervids (Van Den Bussche et al. 1999). In contrast to previous studies of *DQA* (Hughes and Hughes 1995), comparisons of nonsynonymous (d_N) versus synonymous (d_S) substitutions among *Sihi-DQA* alleles revealed no significant difference (Table 1). When peptide binding sites were examined separately, d_N was greater than d_S as expected if balancing selection has been acting at these positions. However, possibly because of the small number of peptide binding sites compared this difference was not statistically significant. For all other taxa examined, d_N was greater than d_S but was statistically significant only in *R. norvegicus*. The low ratios of d_N to d_S seen here may indicate that balancing selection has only a minor effect at this locus in some rodent species.

Phylogenetic analysis (Fig. 4) showed no evidence of shared allelic lineages (trans-species persistence) among genera. Evolutionary relationships within Muridae are unclear (Musser and Carleton 1993), but some data on divergence times are available. Catzeflis et al. (1993) estimated from DNA-DNA hybridization that the subfamilies Murinae (including *Rattus* and *Mus*) and Sigmodontinae (including *Sigmodon* and

Peromyscus) diverged approximately 30 MYA. Estimates for divergence time between *Rattus* and *Mus* vary from 10-15 MYA to 22-38 MYA based on the fossil record and molecular data, respectively (Frye and Hedges 1995). Theoretically, the upper limit for two taxa to share alleles is 30 - 40 MY (Takahata and Nei 1990), near the divergence times of *Rattus/Mus* and *Sigmodon*, and possibly all three taxa. In addition to large divergence times among these genera, our lack of evidence for trans-species persistence of allelic lineages could be attributed to small sample size of murid species other than *S. hispidus*. Low effective population sizes relative to divergence times and associated stochastic losses of allelic lineages (Hughes et al. 1994) may also contribute to the absence of trans-species persistence. Finally, low selection pressure on this locus may have reduced the period of time over which allelic lineages were maintained. Trans-species sharing of allelic lineages among genera has been documented in most studies of MHC evolution in non-rodent taxa (Edwards et al. 1997; Klein et al. 1993; Trtková et al. 1993; Swarbrick et al. 1995; Van Den Bussche et al. 1999), but to our knowledge no evidence of this phenomena exists for rodents. Further studies of closely related genera of rodents are needed to determine if this is due simply to the small number of rodent taxa examined or, more interestingly, differences in population history and demography of rodents relative to non-rodent taxa.

As noted by Seddon and Baverstock (1998), nucleotide and amino acid distance values were slightly lower for the eight alleles of Australian *Rattus* than for alleles of *R. norvegicus*. This observation seems striking considering that the sequences of Australian *Rattus* represent six species, whereas the *R. norvegicus* sequences represent only two strains of laboratory rats. Additionally, *M. musculus* and the outbred, natural populations of *S. hispidus* exhibit slightly higher levels of diversity (Table 1). The low values of

nucleotide and amino acid diversity among Australian *Rattus* species, together with the small number of unique amino acid residues, are intriguing.

There are several examples of taxa with limited MHC variability (Ellegren et al. 1993; Mikko and Andersson 1995; Murray and White 1998), but to our knowledge relatively low levels of diversity like that of Australian *Rattus* never have been reported for any other group of species. Explanations for low diversity in some species include population bottlenecks (or small size of founding populations) and reduced pathogen-driven selection (Ellegren et al. 1996). Founder effect is a likely explanation for relatively low *DQA* diversity of Australian *Rattus* considering their route of dispersal from Asia. It appears that at least two species of *Rattus* entered Australia and New Guinea about one MYA and later diverged into the species found today (Watts and Aslin 1981). Our results suggest that a more thorough examination of MHC diversity in naturally occurring populations of *Rattus* from Australia and mainland Asia could provide insight into the evolution of this genus as it colonized Australia and add to our knowledge of the natural history of MHC in general.

Because *S. hispidus* has such a broad geographic distribution in the New World and, as a taxon, is exposed to considerable environmental variation, a more thorough analysis of geographic variation of polymorphism, allelic frequencies, and evolution of MHC in this species should prove insightful. Such information should provide additional data for understanding the evolutionary forces maintaining MHC polymorphism in natural populations.

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Table 1. Mean nucleotide distances and estimates of synonymous (d_S) and nonsynonymous (d_N) substitutions for peptide binding (PBS) and non-peptide binding sites (non-PBS) for pairwise comparisons among rodent *DQA* exon-2 alleles. Amino acid distances are also given. All distance values expressed as percentage. Means of standard errors in parentheses.

			PBS			Non-PBS		
Pairwise comparison (*n*)	Nucleotide	Amino acid	d_N	d_S	d_N/d_S	d_N	d_S	d_N/d_S
Sigmodon hispidus (325)	9.05 (2.11)	16.50 (4.31)	17.13 (3.43)	8.70 (5.69)	1.97	6.51 (1.20)	9.23 (3.22)	0.71
Rattus norvegicus (21)	6.80 (1.80)	13.31 (3.83)	16.18 (4.26)	0.90 (2.91)	17.98	4.70 (1.36)	4.58 (2.54)	1.03
Australian *Rattus* (28)	6.33 (1.75)	10.60 (3.50)	15.64 (4.06)	8.94 (7.06)	1.75	2.04 (0.88)	10.13 (4.03)	0.20
Mus musculus (28)	6.63 (1.82)	12.96 (3.98)	17.00 (3.58)	7.16 (5.42)	2.37	3.66 (0.99)	4.06 (2.12)	0.90

Table 2. Polymorphic amino acid positions for *DQA* exon-2 sequences of murid rodents. Asterisks indicate peptide binding sites (Brown, 1993); dash indicates amino acid deletion. Amino acid position is based on a complete exon-2 sequence.

Position	*Mus musculus*	Australian *Rattus*	*Rattus norvegicus*	*Sigmodon hispidus*
10	IT	IL	I	ILM
11*	STV	NS	ENT	ANSTV
12	V	M	MV	FIM
13	Y	Y	Y	NY
14	Q	Q	Q	HQ
15	S	Y	Y	S
16	P	Y	HY	H
17	G	E	EK	KNQ
18	D	SP	S	P
19	I	R	RK	N
22*	FY	FY	FY	Y
23	T	T	T	IT
24*	FH	FL	FH	F
28	G	G	G	DGN
29	D	D	D	DN
31*	LW	EKL	EIKR	EKL
32*	F	FL	F	F
33	Y	Y	SY	Y

Table 2. Continued.

Position	*Mus musculus*	Australian *Rattus*	*Rattus norvegicus*	*Sigmodon hispidus*
36	L	L	L	SL
39	K	K	K	KR
40	EK	E	E	E
41	T	T	NT	T
42	IV	I	I	IV
43*	W	W	W	W
44	MR	R	R	KR
45	L	I	I	I
49	AG	G	G	AG
50	Q	Q	Q	EK
51*	L	LQ	L	L
52	AIRT	LT	IT	ART
53*	SLR	INS	S	NS
55*	DE	D	D	D
58*	G	G	G	GKR
59	G	G	AG	G
61	Q	Q	QR	Q
62*	NE	NE	NE	DEHNQST
63	I	I	IM	IL
64	A	A	A	AS

Table 2. Continued.

Position	*Mus musculus*	Australian *Rattus*	*Rattus norvegicus*	*Sigmodon hispidus*
65*	ATV	IT	IT	AIT
66	EGV	AV	AI	AV
68*	HY	HY	HY	HQRSY
69*	NT	N	N	NS
70	L	L	L	LW
71	GE	E	E	EK
72*	IGV	IS	IL	AIT
73	LW	LM	L	LM
74	T	M	IM	IT
75	K	K	K	EK
76*	R	R-	R-	ER
77	S	S	S	ST

Figure legends

Figure 1. Nucleotide sequences of *DQA* exon-2 alleles from *Sigmodon hispidus*. Numbering of nucleotide positions follows that of a complete exon.

Figure 2. Amino acid sequences of *DQA* exon-2 alleles from *Sigmodon hispidus*. Numbering of amino acids and position of β-sheet and α-helix strands follows Brown et al. (1988). Amino acids forming the pepide binding sites (+) follow Brown et al. (1993).

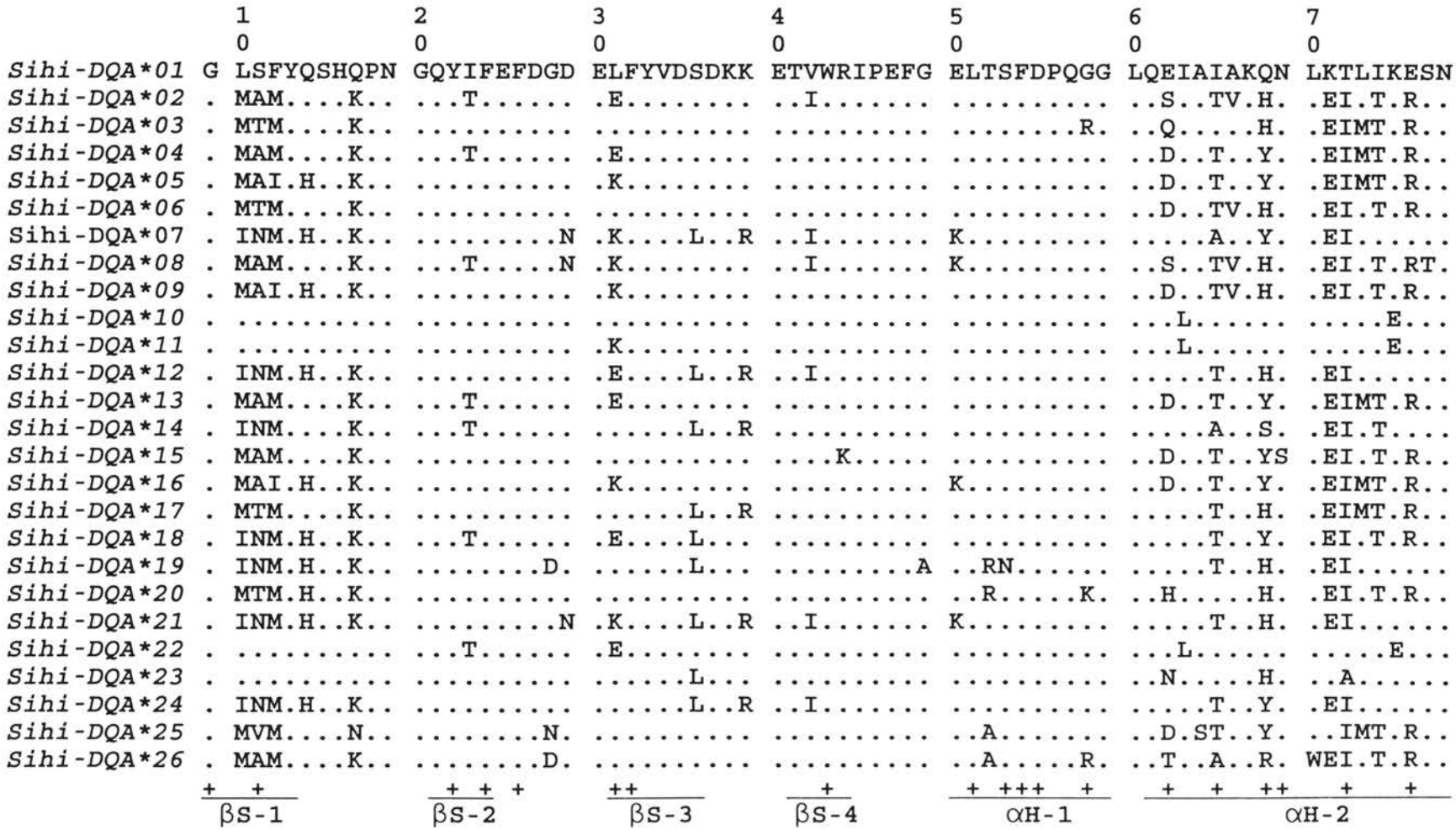
Figure 3. Amino acid variability plot (Wu and Kabat 1970) for derived amino acid sequences of *DQA* exon-2 from *Sigmodon hispidus*, *Mus musculus*, *Rattus norvegicus*, and Australian *Rattus* species. Black bars indicate peptide binding sites (Brown et al. 1993); numbers along the X-axis denote amino acid position based on a complete exon-2 sequence.

Figure 4. Neighbor-joining tree for DQA exon-2 sequences of muroid rodents. DQA exon-2 sequences of *Canis familiaris* (dog), *Alopex lagopus* (Alla), *Sus scrofa* (pig), *Ovis aries* (Ovar), and *Homo sapiens* (human) were used to root the tree. Sihi = *Sigmodon hispidus*, Mumu = *Mus musculus*, Pele = *Peromyscus leucopus*, Rano = *Rattus norvegicus*. The remaining symbols represent six species of Australian *Rattus*; Raco = *R. colletti*, Rafu = *R. fuscipes*, Rale = *R. leucopus*, Rasp = *Rattus* sp. nov., Ratu = *R. tunneyi*, Ravi = *R. villosissi*. GenBank accession numbers from all previously described *DQA* alleles follow the species designation.

```
            2             3            4            5             6            7            8
            0             0            0            0             0            0            0
Sihi-DQA*01 T GGT TTA AGC TTC TAT CAG TCT CAT CAA CCC AAC GGC CAG TAC ATA TTT GAA TTT GAT GGT GAT GAG TTG
Sihi-DQA*02 . ... A.G GCT A.G ... ... ... ... A.. ... ... ... ... ... .C. ... ... ... ... ... ... ... GA.
Sihi-DQA*03 . ... A.G .CT A.G ... ... ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*04 . ... A.G GCT A.G ... ... ... ... A.. ... ..T ... ... ... .C. ... ... ... ... ... ... ... GA.
Sihi-DQA*05 . ... A.G GCT A.A ... ..C ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... AA.
Sihi-DQA*06 . ... A.G .CT A.G ... ... ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*07 . ... A.. .AT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... A.. ... AA.
Sihi-DQA*08 . ... A.G GCT A.G ... ... ... ... A.. ... ... ... ... ... .C. ... ... ... ... ... A.. ... AA.
Sihi-DQA*09 . ... A.G GCT A.A ... ..C ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... AA.
Sihi-DQA*10 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*11 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A AA.
Sihi-DQA*12 . ... A.. .AT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... GA.
Sihi-DQA*13 . ... A.G GCT A.G ... ... ... ... A.. ... ..T ... ... ... .C. ... ... ... ... ... ... ... GA.
Sihi-DQA*14 . ... A.. .AT A.G ... ... ... ... A.. ... ..T ... ... ... .C. ... ... ... ... ... ... ... ...
Sihi-DQA*15 . ... A.G GCT A.G ... ... ... ... A.. ... ... ..T ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*16 . ... A.G GCT A.A ... ..C ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... AA.
Sihi-DQA*17 . ... A.G .CT A.G ... ... ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*18 . ... A.. .AT A.G ... ..C ... ... A.. ... ... ... ... ... .C. ... ... ... ... ... ... ... GA.
Sihi-DQA*19 . ... A.. .AT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... .A. ... ... ...
Sihi-DQA*20 . ... A.G .CT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*21 . ... A.. .AT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... A.. ... AA.
Sihi-DQA*22 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ... ... ... ... ... ... GA.
Sihi-DQA*23 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*24 . ... A.. .AT A.G ... ..C ... ... A.. ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*25 . ... A.G GTT A.G ... ... ... ... A.C ... ... ... ... ... ... ... ... ... ... AA. ... ... ...
Sihi-DQA*26 . ... A.G GCT A.G ... ... ... ... A.. ... ..T ... ... ... ... ... ... ... ... .A. ... ... ...

                        1            1            1             1            1            1             1
            9           0            1            2             3            4            5             6
            0           0            0            0             0            0            0             0
Sihi-DQA*01 TTC TAC GTG GAC TCG GAT AAG AAG GAG ACT GTC TGG AGG ATT CCT GAG TTT GGC GAG CTG ACA AGC TTT GAC
Sihi-DQA*02 ... ... ... ... ..A ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*03 ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*04 ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*05 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*06 ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*07 ... ..T ... ... .T. ... ... .G. ... ... A.. ... ... ... ... ... ... ... A.. ... ... ... ... ...
Sihi-DQA*08 ... ..T ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... A.. ... ... ... ... ...
Sihi-DQA*09 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*10 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*11 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*12 ... ..T ... ... .T. ... ... .G. ... ... A.. ... ... ... ..C ... ... ... ... ... ... ... ... ...
Sihi-DQA*13 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*14 ... ... ... ... .T. ... ... .G. ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ...
Sihi-DQA*15 ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*16 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ...
Sihi-DQA*17 ... ... ... ... .T. ... ... .G. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*18 ... ..T ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*19 ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ... .G. .A. ... ...
Sihi-DQA*20 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ... ...
Sihi-DQA*21 ... ..T ... ... .T. ... ... .G. ... ... A.. ... ... ... ... ... ... ... A.. ... ... ... ... ...
Sihi-DQA*22 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*23 ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Sihi-DQA*24 ... ... ... ... .T. ... ... .G. ... ... A.. ... ... ... ..C ... ... ... ... ... ... ... ... ...
Sihi-DQA*25 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ...
Sihi-DQA*26 ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ...

                     1            1             1            2            2             2            2
                     7            8             9            0            1             2            3
                     0            0             0            0            0             0            0
Sihi-DQA*01 CCA CAA GGC GGA CTG CAA GAG ATA GCT ATA GCA AAA CAG AAC TTA AAA ACC CTG ATT AAA GAG TCC AAT TC
Sihi-DQA*02 ... ... ... ... ... ... AGC ... ... .C. .T. ... ..C ... ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*03 ... ... A.A ... ... ... C.. ... ... ... ... ... ..C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*04 ... ... ..A ... ... ... ..C ... ... .C. ... ... T.C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*05 ... ... ..A ... ... ... ..C ... ... .C. ... ... T.C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*06 ... ... ... ... ... ... ..C ... ... .C. .T. ... ..C ... ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*07 ... ... ... ... ... ... ... ... ... GC. ... ... T.C ... ..G G.. .T. ... ... ... ... ... ... ..
Sihi-DQA*08 ... ... ... ... ... ... AGC ... ... .C. .T. ... ..C ... ..G G.. .T. ... .C. ..G AG. A.. ... ..
Sihi-DQA*09 ... ... ... ... ... ... ..C ... ... .C. .T. ... ..C ... ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*10 ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ..
Sihi-DQA*11 ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ..
Sihi-DQA*12 ... ... ... ... ... ... ... ... ... .C. ... ... ..C ... ..G G.. .T. ... ... ... ... ... ... ..
Sihi-DQA*13 ... ... ..A ... ... ... ..C ... ... .C. ... ... T.C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*14 ... ... ..A ... ... ... ... ... ... GC. ... ... TCC ... ..G G.. .T. ... .C. ... ... ... ... ..
Sihi-DQA*15 ... ... ..A ... ... ... ..C ... ... .C. ... ... T.C .G. ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*16 ... ... ..A ... ... ... ..C ... ... .C. ... ... T.C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*17 ... ... ... ... ... ... ... ... ... .C. ... ... ..C ... ..G G.. .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*18 ... ... ... ... ... ... ... ... ... .C. ... ... T.T ... ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*19 ..T ... ... ... ... ... ... ... ... .C. ... ... ..C ... ..G G.. .T. ... ... ... ... ... ... ..
Sihi-DQA*20 ... ... AAA ... ... ... C.C ... ... ... ... ... ..C ... ..G G.. .T. ... .C. ..G AG. ... ... ..
Sihi-DQA*21 ... ... ... ... ... ... ... ... ... .C. ... ... ..C ... ..G G.. .T. ... ... ... ... ... ... ..
Sihi-DQA*22 ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ..
Sihi-DQA*23 ... ... ... ... ... ... A.C ... ... ... ... ... ..C ... ... ... G.. ... ... ... ... ... ... ..
Sihi-DQA*24 ... ... ... ... ... ... ... ... ... .C. ... ... T.C ... ..G G.. .T. ... ... ... ... ... ... ..
Sihi-DQA*25 ... ... ..T ... ... ... ..C ... T.. .C. ... ... T.C ... ..G ... .T. A.. .C. ..G AG. ... ... ..
Sihi-DQA*26 ... ... A.A ... ... ... ACC ... ..G GC. ... ... .GC ... .GG G.. .T. ... .C. ..G AG. ... ... ..
```


1 2 3 4 5 6 7
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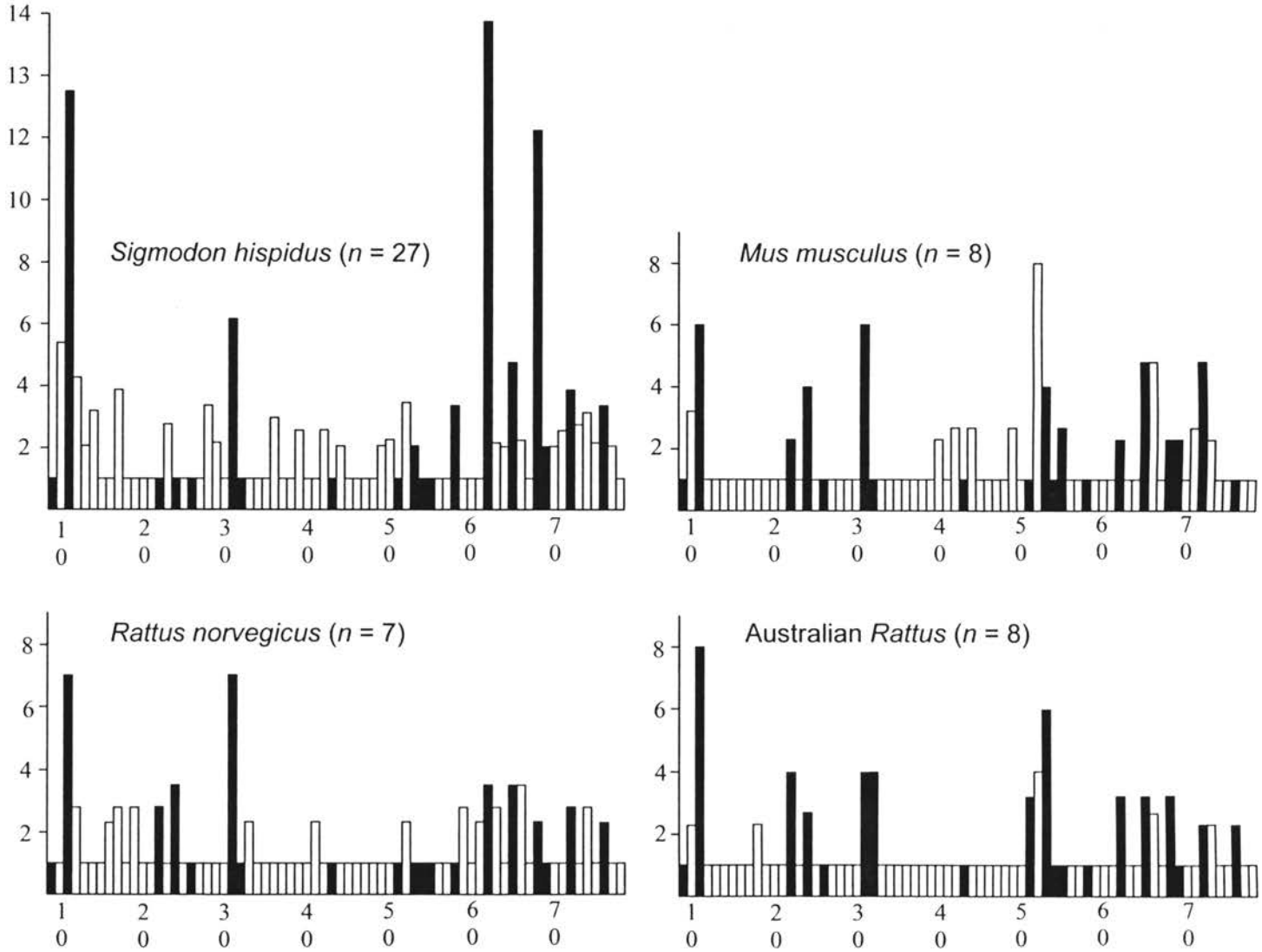
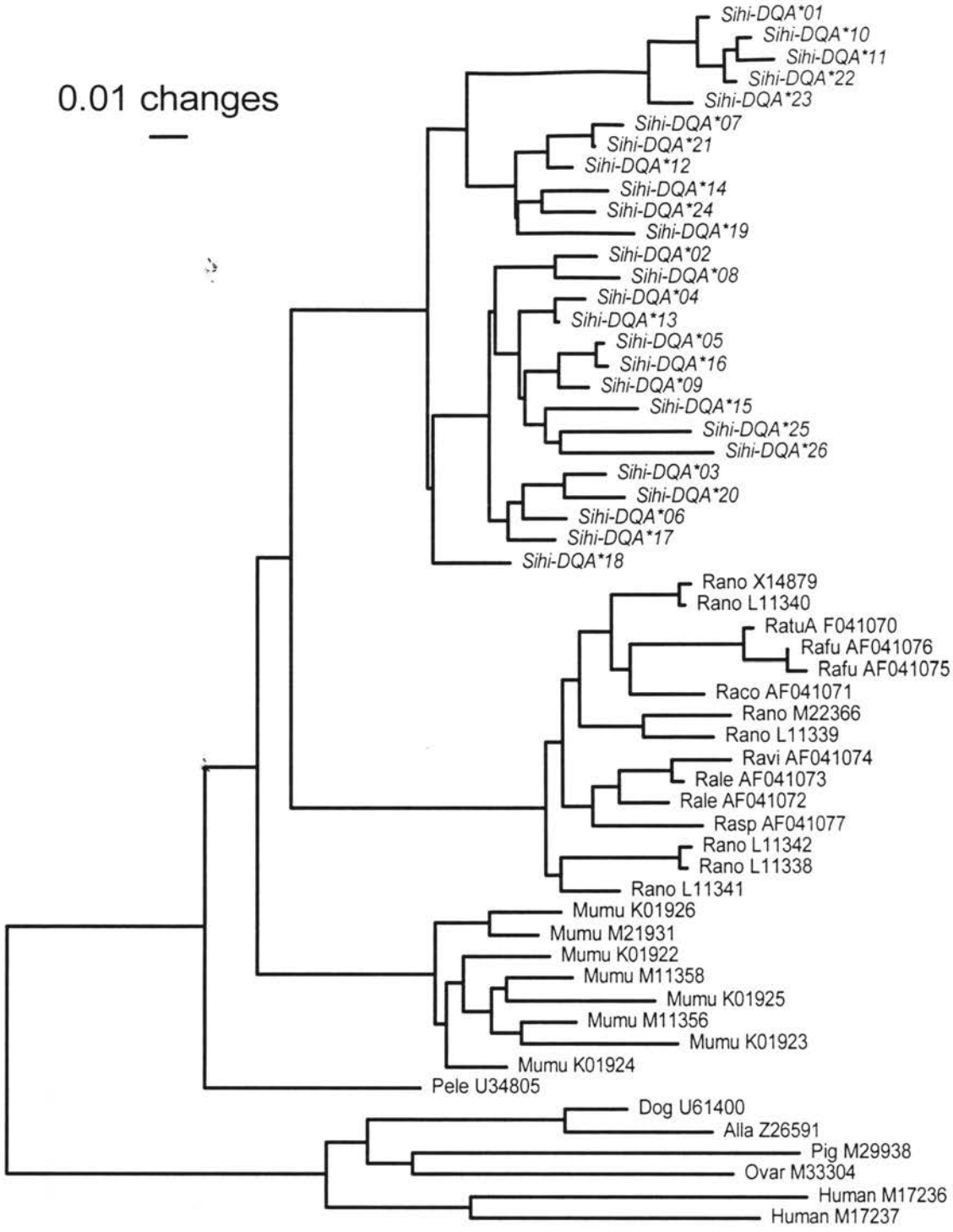
Sihi-DQA*02 . MAM....K.. ...T..... .E..... ..I..... ....S..TV.H. .EI.T.R..
Sihi-DQA*03 . MTM....K.. ....R. ..Q.....H. .EIMT.R..
Sihi-DQA*04 . MAM....K.. ...T..... .E..... ....D..T..Y. .EIMT.R..
Sihi-DQA*05 . MAI.H..K.. ..... .K..... ....D..T..Y. .EIMT.R..
Sihi-DQA*06 . MTM....K.. ....D..TV.H. .EI.T.R..
Sihi-DQA*07 . INM.H..K.. .....N .K....L..R ..I..... K..... .....A..Y. .EI.....
Sihi-DQA*08 . MAM....K.. ...T.....N .K..... ..I..... K..... ..S..TV.H. .EI.T.RT.
Sihi-DQA*09 . MAI.H..K.. ..... .K..... ....D..TV.H. .EI.T.R..
Sihi-DQA*10 . ...L..... .....E...
Sihi-DQA*11 . ... .K..... ....L..... .....E...
Sihi-DQA*12 . INM.H..K.. ..... .E....L..R ..I..... ....T..H. .EI.....
Sihi-DQA*13 . MAM....K.. ...T..... .E..... ....D..T..Y. .EIMT.R..
Sihi-DQA*14 . INM....K.. ...T..... .....L..R ....A..S. .EI.T....
Sihi-DQA*15 . MAM....K.. ....K..... ....D..T..YS .EI.T.R..
Sihi-DQA*16 . MAI.H..K.. ..... .K..... ..... K..... ..D..T..Y. .EIMT.R..
Sihi-DQA*17 . MTM....K.. ....L..R ....T..H. .EIMT.R..
Sihi-DQA*18 . INM.H..K.. ...T..... .E....L... ....T..Y. .EI.T.R..
Sihi-DQA*19 . INM.H..K.. .....D. .....L... .....A ..RN..... .....T..H. .EI.....
Sihi-DQA*20 . MTM.H..K.. ....R.....K. ..H.....H. .EI.T.R..
Sihi-DQA*21 . INM.H..K.. .....N .K....L..R ..I..... K..... .....T..H. .EI.....
Sihi-DQA*22 . ...T..... .E..... ....L..... .....E...
Sihi-DQA*23 . ...L... ....N.....H. ..A.....
Sihi-DQA*24 . INM.H..K.. ....L..R ..I..... ....T..Y. .EI.....
Sihi-DQA*25 . MVM....N.. .....N. ....A..... ..D.ST..Y. ..IMT.R..
Sihi-DQA*26 . MAM....K.. .....D. ....A.....R. ..T..A..R. WEI.T.R..
+ + + + + ++ + + +++ + + + ++ + +
βS-1 βS-2 βS-3 βS-4 αH-1 αH-2


Sigmodon hispidus (n = 27)
Mus musculus (n = 8)
Rattus norvegicus (n = 7)
Australian Rattus (n = 8)


0.01 changes
Sihi-DQA*01
Sihi-DQA*10
Sihi-DQA*11
Sihi-DQA*22
Sihi-DQA*23
Sihi-DQA*07
Sihi-DQA*21
Sihi-DQA*12
Sihi-DQA*14
Sihi-DQA*24
Sihi-DQA*19
Sihi-DQA*02
Sihi-DQA*08
Sihi-DQA*04
Sihi-DQA*13
Sihi-DQA*05
Sihi-DQA*16
Sihi-DQA*09
Sihi-DQA*15
Sihi-DQA*25
Sihi-DQA*26
Sihi-DQA*03
Sihi-DQA*20
Sihi-DQA*06
Sihi-DQA*17
Sihi-DQA*18
Rano X14879
Rano L11340
RatuA F041070
Rafu AF041076
Rafu AF041075
Raco AF041071
Rano M22366
Rano L11339
Ravi AF041074
Rale AF041073
Rale AF041072
Rasp AF041077
Rano L11342
Rano L11338
Rano L11341
Mumu K01926
Mumu M21931
Mumu K01922
Mumu M11358
Mumu K01925
Mumu M11356
Mumu K01923
Mumu K01924
Pele U34805
Dog U61400
Alla Z26591
Pig M29938
Ovar M33304
Human M17236
Human M17237

CHAPTER III

GENETIC DIVERSITY OF THE MAJOR HISTOCOMPATIBILITY COMPLEX OF COTTON RATS (*SIGMODON HISPIDUS*) INHABITING AN OIL REFINERY COMPLEX

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Abstract–Analysis of genetic diversity is being used increasingly to assess the impact of environmental contamination on natural populations. We examined genetic diversity of an immune-response gene within the major histocompatibility complex in cotton rats (*Sigmodon hispidus*) inhabiting an oil-refinery complex. Genetic diversity of *MHC-DQA* exon-2 was examined within and among contaminated and noncontaminated reference grids. The level of gene diversity within contaminated grids (0.748) was lower than within reference grids (0.818) but the difference was not statistically significant ($P > 0.5$). Analysis of molecular variance (AMOVA), pairwise F_{ST}-values, and hierarchical clustering failed to reveal population genetic structure related to contamination. Results of this study indicate that either the level of contaminant-induced selection is insignificant at this MHC locus or that gene flow from surrounding areas has obliterated the effects of selection.

Keywords–population genetics, major histocompatibility complex, genetic diversity, selection, evolutionary toxicology

INTRODUCTION

Environmental contamination by anthropogenic chemicals has long been known to affect both individual organisms and overall health of local ecosystems. However, only recently has the importance of long-term evolutionary effects of such contamination been recognized [1]. Exposure to genotoxic agents in the environment can reduce fitness of individuals with sensitive genotypes, thereby causing changes in genotypic frequencies and genetic diversity within populations [2]. These genetic changes in populations represent effects of environmental contamination that result from adaptation of populations to polluted environments [1]. Such changes have the potential to affect both the short-term well-being and long-term evolutionary fate of local populations. Results of studies examining protein loci [3-11] and randomly amplified polymorphic DNA (RAPDs) [11-15] suggest that selection is operating on populations stressed by natural events and chemical contaminants. However, the relationship between genetic biomarkers and physiological response to environmental contamination typically is not known.

Relatively few studies have examined environmental-genetic correlations in which the function and role of loci under investigation are well understood. Genes involved in immune response have great potential for such studies. Environmental and chemical stressors can suppress immune function [16-22], which in turn may lead to increased susceptibility to disease. This alteration in immunocompetence likely will lead to enhanced selection against individuals genetically less resistant to naturally occurring pathogens. The result can be a population adapted to a locally and temporally restricted suite of pathogens but at risk for future outbreaks of 'new' diseases and parasites. For example, Langefors et al. [23] examined diversity of an immune response gene in

managed populations of Atlantic salmon and noted that two hatchery populations with lowest heterozygosity had the highest rates of a fatal syndrome affecting the yolk-sac stage.

Many genes critical to immune response lie within the major histocompatibility complex (MHC). Molecules encoded by MHC genes bind peptides (including antigens derived from pathogens) and present them on cell surfaces to T-cells. If the peptide is recognized as foreign, an immune response is initiated. Amino acid residues in the peptide-binding region of MHC molecules show the highest levels of polymorphism [24]. Variation in this region can influence the type of peptides that can be successfully bound. The large amount of variability usually seen in stress response among individuals may be due in part to the large degree of genetic variability in immunocompetent genes such as the MHC [25]. Selection acting on genotypes more susceptible to certain stressors likely would lead to changes in the immunogenetic structure of a population [20,26,27].

Our objective was to study the relationship between potential anthropogenic, environmental stressors and population genetic structure of MHC genes in the hispid cotton rat (*Sigmodon hispidus*). To compare contaminated and noncontaminated reference sites, we examined *S. hispidus* collected on, and adjacent to, an oil refinery complex that has been declared a Superfund Site (Oklahoma Oil Refinery, Caddo County, Oklahoma). EPA reports indicate that the site is contaminated with organic compounds, caustic wastes, and heavy metals [28]. Effects of contaminants at this site have been investigated to determine immunotoxicity, genotoxicity, and metabolic toxicity of contaminants and the demographic structure and composition of small-mammal populations [29]. Several measures of immune response in cotton rats showed significant differences between contaminated and noncontaminated reference sites [30].

Additionally, field monitoring of resident populations showed lower densities and decreased survival on contaminated sites [31]. On the basis of studies correlating effects of immunotoxicants with MHC variability [32,33], we hypothesized that genotypic and allelic frequencies in populations of cotton rats from contaminated areas would differ from those in uncontaminated areas, and that the former would show reduced genetic variation.

MATERIALS AND METHODS

Animals used in this study were collected at the Oklahoma Refining Company, Caddo County, Oklahoma; a hazardous waste site on the Environmental Protection Agency's National Priorities List (Superfund Site). Contaminated sites were located adjacent to pits used for storing oil-processing wastes, oil-sludge sedimentation ponds, and on land treatment areas used in processing oil/sludge from sedimentation ponds. These sites were selected because of their high levels of contamination with complex mixtures of heavy metals and organic hydrocarbons [28,34]. Reference sites were located within 7 km of the refinery, had no history of prior contamination, and were ecologically similar to contaminated sites. Approximately thirty adult hispid cotton rats *(S. hispidus)* were examined from each of the three contaminated sites (grids 2, 3, and 4) and three noncontaminated reference sites (grids 1, 5, and 6) for a total of 177 individuals. Several health parameters (including immune function) were examined in a previous study [30] using these same individuals.

Genomic DNA was extracted from muscle or liver tissue following Longmire et al. [35]. PCR amplification of the functionally important peptide binding region (exon-2) of a class II MHC gene known as *DQA* was performed using primers PeleDQAex2-F (5'-

ACAGCTGACCATGTTGGCGCCTA-3') and PeleDQAex2-R (5'-CACGTACCATTGGTAGCTGGGGTA-3'), which were designed from a published *DQA* sequence of *Peromyscus leucopus* [36]. We used single-strand conformation polymorphism (SSCP) analysis [37] to examine genetic diversity of cotton rats from contaminated and reference sites. PCR amplification, SSCP analysis, and DNA sequencing were conducted as described in [38]. Nomenclature of *S. hispidus DQA* (*Sihi -DQA*) exon-2 alleles follows Klein et al. [39].

Analyses of genetic diversity were conducted using Arlequin 1.1 [40]. Allelic frequencies, observed heterozygosity, and gene diversity (along with its sampling variance [41]) were calculated for each grid. Genotypic frequencies were tested for conformation to Hardy-Weinberg equilibrium using 100,000 steps in the Markov chain and 1,000 dememorization steps. Partitioning of genetic diversity within and among grids and contaminated and reference groups was examined by an analysis of molecular variance (AMOVA) [42]. Divergence of allelic frequencies among all pairwise comparisons of grids was estimated using pairwise F_{ST}-values. F_{ST}-values range from zero to one, the higher the value the greater the degree of genetic divergence. Slightly negative values are sometimes obtained due to the statistical methodology used, and such values usually reflect absence of genetic structure [43]. Significance of F_{ST}-values was tested using 10,000 permutations. Hierarchical analysis of population divergence was conducted using pairwise F_{ST}-values in the clustering algorithm of Holsinger and Mason-Gamer [44]. Each node of the resulting tree provides the F_{ST}-value (along with its significance) indicating the degree of population divergence. A sequential Bonferonni correction [45] was applied whenever multiple statistical tests were conducted.

RESULTS

The *Mhc-DQA* exon-2 locus examined in this study exhibited substantial levels of polymorphism, with 13 alleles identified from the 177 individuals examined. Based on a larger-scale study examining MHC allelic diversity in cotton rats from a larger geographic area [46], no unique alleles were detected at any of the six grids although one allele occurred on only one of the six grids in this study. DNA sequences of these alleles were reported elsewhere [38,46] and were deposited in GeneBank under accession numbers AF155914–AF155942 and AF279850–AF279864. After Bonferroni correction, significant deviation from Hardy-Weinberg equilibrium ($\alpha = 0.05$) was observed for one noncontaminated reference grid which showed a marked deficiency of heterozygotes (Table 1). Observed heterozygosity ranged from 0.586 in grid 1 to 0.833 in grid 6 and gene diversity ranged from 0.645 in grid 3 to 0.851 in grid 1 (Table 1). With the grids combined into contaminated and reference groups, gene diversity was 0.748 ± 0.029 and 0.818 ± 0.022, respectively. Although lower on contaminated areas, the difference in gene diversity was not statistically significant ($t = 0.30$, $P > 0.5$)

Analysis of molecular variance (AMOVA) showed that 98.17% of observed variation was accounted for by differences among individuals within grids and 1.54% was accounted for by differences among grids within contaminated and reference groups. Only 0.29% of the variation was attributable to differences between contaminated and reference groups. Following sequential Bonferroni correction, there was no significant genetic divergence among grids (Table 2) based on pairwise F_{ST}-values and a hierarchical clustering dendrogram (Fig. 1).

DISCUSSION

Several laboratory studies have examined the effects of immunotoxicants on MHC documenting reducing gene expression or increased susceptibility of certain genotypes to immunochallenge. For example, when exposed to mercuric chloride and tetrachlorodibenzo-p-dioxin, strains of mice having certain MHC genotypes were found to be more sensitive to immunochallenge [47]. Mice exposed to 3-methylcholanthrene were more susceptible to *Trichinella* infection, and this susceptibility was correlated with MHC genotype [33]. Prevalence of berylliosis, an inflammatory lung disease in humans caused by exposure to the metal beryllium, is more prevalent in individuals having a certain mutation in an MHC gene [48]. Studies such as these indicate that selection may affect MHC genetic diversity of cotton rat populations inhabiting contaminated areas. The present study however, revealed no significant differences in genetic diversity or allelic frequencies among contaminated and reference grids.

The failure to detect significant differences among our contaminated and reference grids could be due to gene flow from surrounding noncontaminated areas or the absence of selection at a level that could be detected with our sample sizes. The challenge of using this approach in assessing effects of environmental contamination on natural populations is that evolutionary processes are ongoing, quite complex, and often antagonistic. In particular, gene flow can greatly reduce or completely obliterate effects of selection.

This is the first assessment of the impact of environmental contamination on genetic diversity of an immune-response gene. Although we found no significant effects, the potential for such effects should be explored further, especially considering the link between contaminants, immunocompetence, and disease resistance. As noted by

Bickham and Smolen [1], it is important to understand all levels at which environmental contaminants affect populations, from the molecular level to the population level. Immunocompetence has been used successfully as a biomarker at the individual level, and effects of environmental contamination on immunologic health of wildlife species have been relatively well documented [19,49,21,50]. If alterations in immunocompetence documented by studies such as these result in loss of genetic diversity or selection for certain genotypes, this would represent population level effects of evolutionary change, adding another important component to our understanding of the impact of environmental contaminants.

Acknowledgment–Support for R. Pfau was through a Presidential Research Fellowship from Oklahoma State University, Environmental Institute. Funding for this project was provided in part by American Society of Mammalogists and Sigma-Xi Grants-in-Aid of Research and the Theodore Roosevelt Memorial Fund to R. Pfau, College of Arts and Sciences and Department of Zoology, Oklahoma State University (R. Van Den Bussche) and United States Air Force Office of Scientific Research #F49620-95-1-0249 to R. Lochmiller and K. McBee. We thank T. Echelle and U. Melcher for comments on this manuscript, S. Hoofer for his assistance in the laboratory, and S. McMurry for his critical role early in the project.

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Table 1. Number of individuals, number of alleles, allelic frequencies, observed heterozygosity (H_O), gene diversity (H_E), and level of significance for deviations from Hardy-Weinberg equilibrium (P_{HW}) in each grid. An asterisk indicates table-wide significance ($\propto = 0.05$) following adjustment for multiple tests using the sequential Bonferroni method.

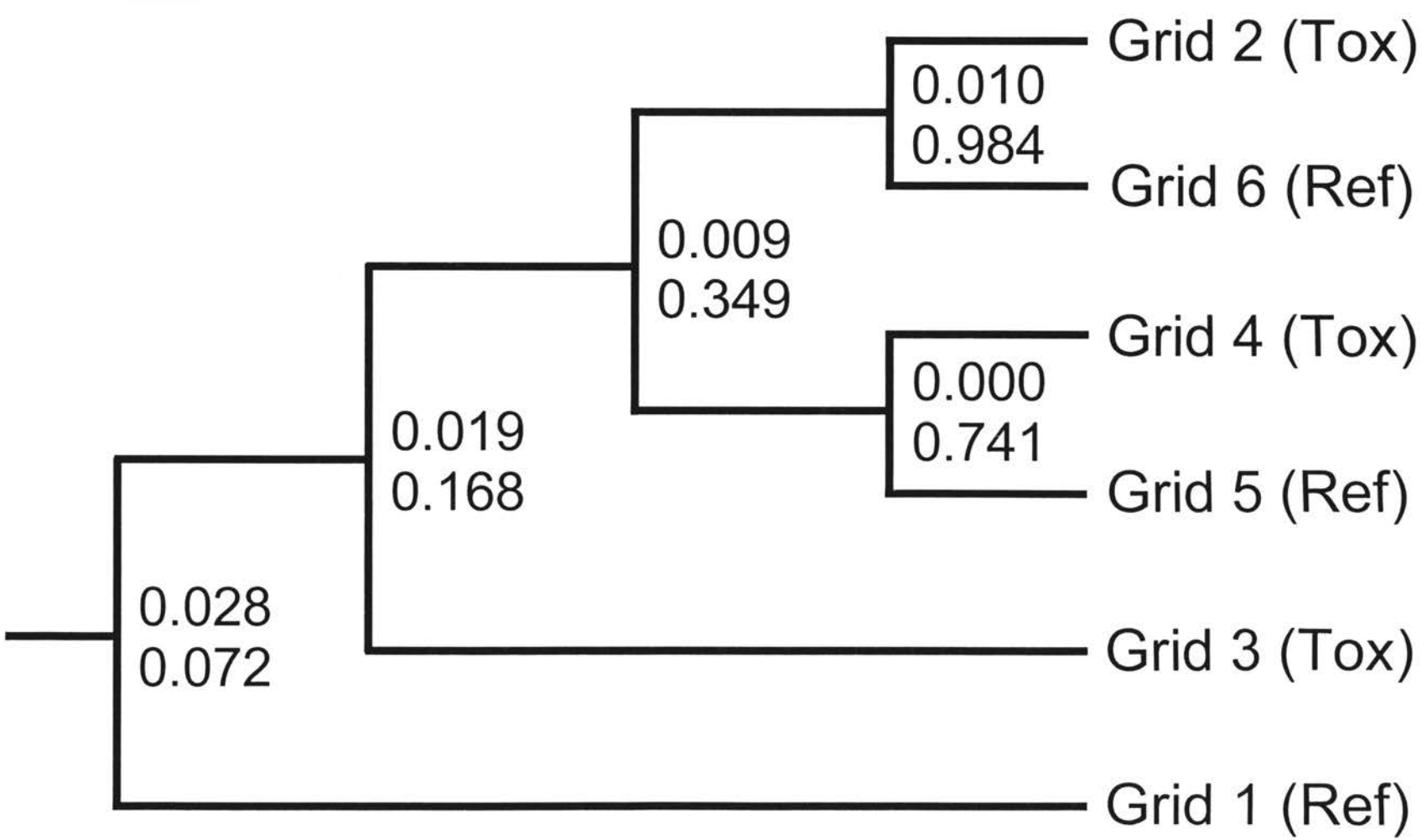
	Contaminated			Reference		
	Grid 2	Grid 3	Grid 4	Grid 1	Grid 5	Grid 6
No. of Individuals	28	30	30	29	30	30
No. of Alleles	11	10	11	10	10	11
Allelic Frequencies						
*Sihi-DQA*01*	0.393	0.583	0.400	0.293	0.467	0.350
*Sihi-DQA*02*	0.071	0.017	0.050	0.103	0.100	0.033
*Sihi-DQA*03*	0.054	0.067	0.133	---	0.067	0.050
*Sihi-DQA*04*	0.036	0.050	---	0.086	---	0.050
*Sihi-DQA*05*	0.054	0.067	0.067	0.190	0.050	0.067
*Sihi-DQA*06*	0.018	---	0.017	0.103	0.050	0.050
*Sihi-DQA*07*	0.018	0.017	0.050	0.052	0.117	0.050
*Sihi-DQA*08*	0.250	0.100	0.133	0.086	0.100	0.200
*Sihi-DQA*09*	---	---	0.017	---	---	---
*Sihi-DQA*10*	0.036	0.033	---	0.052	0.017	0.067
*Sihi-DQA*11*	0.036	0.017	0.100	0.017	0.107	---
*Sihi-DQA*14*	---	---	0.017	---	0.107	0.033
*Sihi-DQA*26*	0.036	0.050	0.017	0.017	---	0.050
H_O	0.750	0.633	0.667	0.586	0.700	0.833
H_E	0.781	0.645	0.797	0.851	0.751	0.828
P_{HW}	0.301	0.484	0.049	0.002*	0.040	0.432

Table 2. F_{ST}-values (above the diagonal) and level of significance (P; below the diagonal) for each pairwise comparison of grids.

	Grid 1	Grid 2	Grid 3	Grid 4	Grid 5	Grid 6
Grid 1		0.025	0.063	0.027	0.026	0.010
Grid 2	0.204		0.027	0.001	0.011	-0.010
Grid 3	0.017	0.162		0.020	0.011	0.031
Grid 4	0.162	0.671	0.251		0.000	0.004
Grid 5	0.194	0.419	0.437	0.759		0.009
Grid 6	0.491	0.998	0.148	0.651	0.535	

Figure legends

Fig. 1. Hierarchical clustering analysis of genetic divergence in *Sigmodon hispidus* inhabiting contaminated and reference areas. The number above the branch at each node is the distance (F_{ST}) between its two daughter nodes. The associated *P*-value (below the branch) is the significance of divergence based on 10,000 permutations.


Grid 2 (Tox)
0.010
0.984
Grid 6 (Ref)
0.009
0.349
Grid 4 (Tox)
0.000
0.741
Grid 5 (Ref)
0.019
0.168
0.028
0.072
Grid 3 (Tox)
Grid 1 (Ref)

CHAPTER IV

POPULATION GENETICS OF THE HISPID COTTON RAT (*SIGMODON HISPIDUS*): PATTERNS OF GENETIC DIVERSITY AT THE MAJOR HISTOCOMPATIBILITY COMPLEX

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Abstract

We examined MHC variability across a portion of the geographic distribution of *S. hispidus* to characterize MHC diversity in this species. Specifically, we examined MHC diversity within a disjunct population of *S. hispidus* and a population that may represent an undescribed species. We also tested the hypothesis that populations within the United States are at equilibrium with regard to gene flow and genetic drift. Using SSCP analysis we identified 25 alleles from 246 individuals. Analysis of molecular variance (AMOVA) revealed that 83.7% of observed variation was accounted for by within population diversity and 16.3% was accounted for by among population divergence. A disjunct population in Arizona was fixed for a single allele, and a Costa Rica population, which may represent an undescribed species, was quite divergent based on allelic composition. There was considerable divergence among some populations within the main portion of the geographic distribution of *S. hispidus* in the U.S. However, there was no significant pattern of isolation-by-distance.

Keywords: genetic diversity, major histocompatibility complex, *Sigmodon hispidis*,

population genetics

Introduction

The hispid cotton rat, *Sigmodon hispidus*, has one of the largest geographic distributions of small mammals in the Americas, ranging from southern Nebraska to northern South America and from the east coast of the United States to southeastern California. Despite its broad geographic distribution and common use in ecological, medical, and biomonitoring research, relatively little is known regarding genetic variability of *S. hispidus*. Previous allozyme surveys (Johnson et al. 1972, McClenaghan 1977, McClenaghan and Gaines 1981, Reed 1988) detected low levels of variation and little or no geographic partitioning. Examination of blood serum protein variation across the geographic distribution of *S. hispidus* (including some populations in Mexico now known to be *S. mascotensis* and *S. arizonae*; Johnson et al. 1972, Zimmerman 1970) revealed unique electromorph patterns suggestive of geographic variation (Dalby and Lillevik 1969). However, these patterns did not correspond to those for karyotypic variation described by Zimmerman (1970) and, as noted by Johnson et al. (1972), may have been a methodological artifact. A recent phylogenetic analysis of DNA sequence variation in the mitochondrial cytochrome B gene did, however, detect significant geographic partitioning, which was interpreted as indicating that *S. hispidus* is paraphyletic and that a minimum of three species may exist (Peppers and Bradley 2000). Overall, these findings indicate that additional studies using highly variable genetic markers are needed to fully characterize patterns of genetic diversity and taxonomy of *S. hispidus*.

Genes of the major histocompatibility complex (MHC) are often highly

polymorphic and increasingly are being used to investigate population genetic structure and to clarify relationships and evolutionary history of closely related species (Hedrick and Parker 1998, Langefors et al. 1998, Klein et al. 1997). The objective of our study was to examine MHC variability across a portion of the geographic distribution of *S. hispidus* to characterize MHC diversity in this species and to assess the usefulness of this genetic marker in identifying genetic partitioning among populations. Specifically, we examined MHC diversity in a disjunct population of *S. hispidus* in Arizona, a population in Costa Rica that may represent an undescribed species, and tested the hypothesis that populations in the United States are at equilibrium with regard to gene flow and genetic drift.

Materials and Methods

Specimens or tissue samples were obtained from 13 localities (Table 1) representing much of the western distribution of *S. hispidus* in the United States, including Missouri, Kansas, Louisiana, Oklahoma, Texas, New Mexico, and Arizona. Samples were also acquired from the Puntarenas Province, Costa Rica.

Genomic DNA was extracted from muscle or liver tissue following Longmire et al. (1997). PCR amplification of *DQA* exon-2 (which codes for the peptide binding region) was performed using primers PeleDQAex2-F (5'-ACAGCTGACCATGTTGGCGCCTA-3') and PeleDQAex2-R (5'-CACGTACCATTGGTAGCTGGGGTA-3'), which were designed from a published *DQA* sequence of *Peromyscus leucopus* (Crew and Bates 1996). To examine genetic diversity we used single-strand conformation polymorphism analysis (SSCP; Orita et al. 1989). PCR amplification, SSCP analysis, and DNA sequencing were conducted as described in

Pfau et al. (1999). Nomenclature of *S. hispidus DQA* (*Sihi -DQA*) exon-2 alleles follows Klein et al. (1990).

Analyses of genetic diversity were conducted using Arlequin 1.1 (Schneider et al. 1997). Allelic frequencies and gene diversity (expected heterozygosity; H_E) were calculated for each population. Genotypic distributions were tested for conformation to Hardy-Weinberg equilibrium using 100,000 steps in the Markov chain and 1,000 dememorization steps. Genetic structure was examined by an analysis of molecular variance (AMOVA; Excoffier et al 1992) and pairwise F_{ST}. Significance of F_{ST}-values was tested using 10,000 permutations. Hierarchical analysis of population divergence was conducted using pairwise F_{ST}-values in the clustering algorithm of Holsinger and Mason-Gamer (1996; p. 633). Each node of the resulting tree provides the F_{ST}-value (along with its significance) indicating the degree of population divergence. A sequential Bonferonni correction (Rice 1989) was applied whenever multiple statistical tests were conducted. Isolation-by-distance was tested using the Mantel test as implemented in GENEPOP 3.1 (Raymond and Rousset 1995) with 10,000 permutations. Because the Arizona population is isolated, and substantial barriers to gene flow exist between the United States and Costa Rica populations, these two populations were excluded from the isolation-by-distance analysis.

Results

The *Mhc-DQA* exon-2 locus in *S. hispidus* exhibited substantial levels of polymorphism, with 25 alleles identified from 246 individuals examined. Number of alleles per population ranged from one in Arizona to 15 in Costa Rica and frequencies of these alleles varied substantially among populations (Table 2). The predominant allele

was *DQA*01* which occurred at the highest frequency in six of the 13 populations. DNA sequences were reported elsewhere, including Pfau et al. 1999 and Pfau 2000, and were deposited in GeneBank under accession numbers AF155914–AF155942 and AF279850–AF279864. After sequential Bonferonni correction, significant deviation from Hardy-Weinberg equilibrium ($\propto = 0.05$) was observed only in Ellis Co., Kansas which had a marked deficiency of heterozygotes. Gene diversity was high in most populations and ranged from 0.000 in Arizona to 0.911 in Costa Rica (Table 1).

Analysis of molecular variance (AMOVA) revealed that 83.7% of observed variation was accounted for by within-population diversity and 16.3% was accounted for by among-population diversity. Pairwise F_{ST}-values documented significant genetic divergence among many populations (Table 3), but there was no significant correlation of genetic divergence with geographic distance ($P = 0.090$). Correspondingly, although cluster analysis separated populations into five significantly distinct groups, these groupings were not completely concordant with geographic distance (Fig. 1). For example, Las Palomas, Missouri, and Fort Bliss group together despite their substantial geographic separation.

Discussion

The high level of MHC polymorphism and significant genetic divergence among populations revealed by this study contrasts with previous allozyme surveys and indicates that this MHC locus provides a sensitive marker for revealing population structure in *S. hispidus*. Our study confirms the reduced levels of genetic diversity previously reported for the isolated population in the lower Colorado River valley (McClenaghan 1980) and suggests that some demes of that population are even more genetically depauperate than

suggested by the allozyme survey. Reduced variability of this disjunct population is likely the result of founder effect or genetic drift subsequent to geographic isolation. Additionally, the significant divergence of the Costa Rica population, due to its very different allelic composition, lends support to the idea that this population may represent a different species (Peppers and Bradley 2000).

In the absence of barriers to dispersal, an equilibrium will develop between gene flow and genetic drift, resulting in a pattern of isolation-by-distance (Slatkin 1994a, Slatkin 1994b, Hutchison and Templeton 1999). Although *S. hispidus* occurs more or less continuously throughout the main portion of its range in the United States, results of this study indicate an absence of isolation-by-distance, suggesting a lack of genetic equilibrium in at least some areas included in our study. Based on pairwise F_{ST}-values (Table 3, Fig. 1), it appears that the Mississippi River is a substantial barrier to gene flow as indicated by the Baton Rouge population which is significantly divergent from its nearest neighboring sampled populations west of the Mississippi River (Bossier Parish, Richland Creek, and Peach Point). The Mississippi River in Louisiana also has been shown to be a barrier to gene flow in *Sciurus niger* and *Sciurus carolinensis* (Moncrief 1993).

Reanalysis with the Baton Rouge population removed results in a more highly significant pattern of isolation-by-distance ($P = 0.046$). Given the almost continuous distribution of suitable habitat in this region and the lack of obvious barriers to dispersal, this pattern is not surprising. Although not statistically significant following Bonferroni correction, there is also substantial divergence between Peach Point and Las Palomas and between Bossier Parish and Richland Creek, all of which are west of the Mississippi River. Rivers along the gulf coast of Texas may contribute to reduced gene flow among

the former two populations; however, there are no obvious barriers to dispersal between Bossier Parish and Richland Creek unless habitat heterogeneity is playing a role in reducing dispersal.

It should be noted that balancing selection, which is thought to act at MHC loci (Hughes and Yeager 1998), tends to counteract the effects of genetic drift by maintaining polymorphism within populations (Takahata and Nei 1990). The interplay of balancing selection and genetic drift on MHC genetic diversity was examined in island populations of the Australian bush rat, *Rattus fuscipes* (Seddon and Baverstock 1999). Although populations on two islands showed greater than expected heterozygosity under neutrality, genetic drift remained as a dominant force in shaping the genetic structure of the majority of island populations. The extent to which balancing selection is influencing regional population structure of *S. hispidus* is not known.

Populations of *S. hispidus* are known to undergo extreme fluctuations in density (Cleveland 1964, Haines 1963, Odum 1955), at least in some portions of their geographic range, with population sizes often dropping to low numbers. Density patterns seem to be related to temperature extremes, rainfall cycles, associated changes in habitat quality (Joule and Cameron 1975, Langley and Shure 1988), and possibly outbreaks of disease and parasitism (Cleveland 1971, Fleharty et al. 1972, Goertz 1964, Haines 1971). Because of the potential association of parasites and pathogens with population fluctuations, along with the occurrence of *S. hispidus* in diverse ecological regions, it is tempting to speculate on effects of regional and temporal adaptation to variable parasite and pathogen loads on partitioning of MHC genetic diversity. The MHC is intimately tied to immune response, and it is frequently assumed that MHC genotype can influence resistance to specific pathogens. This has been substantiated by a variety of studies

(Wassom and Kelly 1990). However, the long persistence times of MHC allelic lineages is strong evidence against selection acting on specific alleles in local populations which would normally result in divergence of allelic frequencies among those populations (Klein et al. 1997). The potential effect of selection on observed patterns of MHC genetic diversity in *S. hispidus* will have to await future studies examining neutral loci, including microsatellites, and additional populations.

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Table 1. Locality, sample size, number of alleles, gene diversity (H_E), and level of significance for deviations from Hardy-Weinberg equilibrium (P_{HW}) for 13 populations of *Sigmodon hispidus*. An asterisk indicates table-wide statistical significance ($\propto = 0.05$) following adjustment for multiple tests using the sequential Bonferroni method.

Locality (acronym)	Sample size	Alleles	H_E	P_{HW}
Yuma Co., Arizona (AZ)	13	1	0.000	---
Fort Bliss, Otarro Co., New Mexico (FB)	15	5	0.729	0.022
Hays, Ellis Co., Kansas (HK)	24	5	0.726	0.000*
Caddo Co., Oklahoma (CO)	30	11	0.828	0.422
Las Palomas WMA, Willacy Co., Texas (LP)	10	7	0.868	0.535
Tallgrass Prairie Nature Preserve, Osage Co., Oklahoma (TP)	30	10	0.855	0.050
Peach Point WMA, Brazoria Co., Texas (PP)	16	4	0.528	0.865
Richland Creek WMA, Freestone Co., Texas (RC)	20	8	0.697	0.702
Eufala WMA, Okmulgee Co., Oklahoma (EO)	20	8	0.763	0.679
Johnson Co., Missouri (MO)	8	7	0.892	0.153
Shreveport, Bossier Parish, Louisiana (BP)	9	6	0.745	0.433
Baton Rouge, East Baton Rouge Parish, Louisiana (BR)	27	10	0.811	0.129
Puntarenas Province, Costa Rica (CR)	24	15	0.908	0.162

Table 2. Distribution and frequencies of *DQA* exon-2 alleles of *Sigmodon hispidus* from 13 localities. Locality acronyms are defined in Table 1).

Allele	AZ	FB	HK	CO	LP	TP	PP	RC	EO	MO	BP	BR	CR
*Sihi-DQA*01*	---	0.267	0.438	0.350	0.100	0.267	0.656	0.525	0.450	0.125	0.278	0.037	0.042
*Sihi-DQA*02*	---	0.433	---	0.033	0.200	0.067	---	0.125	0.150	0.188	---	0.037	0.104
*Sihi-DQA*03*	---	0.133	---	0.050	---	---	0.094	---	---	---	---	0.056	---
*Sihi-DQA*04*	---	---	0.229	0.050	0.100	0.150	---	0.025	0.100	---	---	0.074	---
*Sihi-DQA*05*	---	---	---	0.067	0.250	0.067	0.031	0.025	---	---	0.444	0.019	0.063
*Sihi-DQA*06*	---	---	0.042	0.050	---	0.033	---	---	0.075	0.063	---	---	---
*Sihi-DQA*07*	---	---	---	0.050	---	---	0.219	0.125	0.025	0.188	0.111	0.019	---
*Sihi-DQA*08*	---	0.133	0.167	0.200	0.200	0.200	---	0.025	0.050	0.188	0.056	0.093	---
*Sihi-DQA*09*	---	---	---	---	---	0.033	---	0.075	---	---	---	---	---
*Sihi-DQA*10*	---	0.033	---	0.067	0.050	0.067	---	0.075	---	0.063	---	0.370	---
*Sihi-DQA*11*	1.000	---	---	---	---	0.100	---	---	0.100	---	0.056	0.185	0.042
*Sihi-DQA*12*	---	---	---	---	---	---	---	---	---	---	---	---	0.063
*Sihi-DQA*13*	---	---	---	---	0.100	---	---	---	---	---	0.056	0.111	---
*Sihi-DQA*14*	---	---	0.125	0.033	---	0.017	---	---	---	0.188	---	---	---

Table 2. Continued.

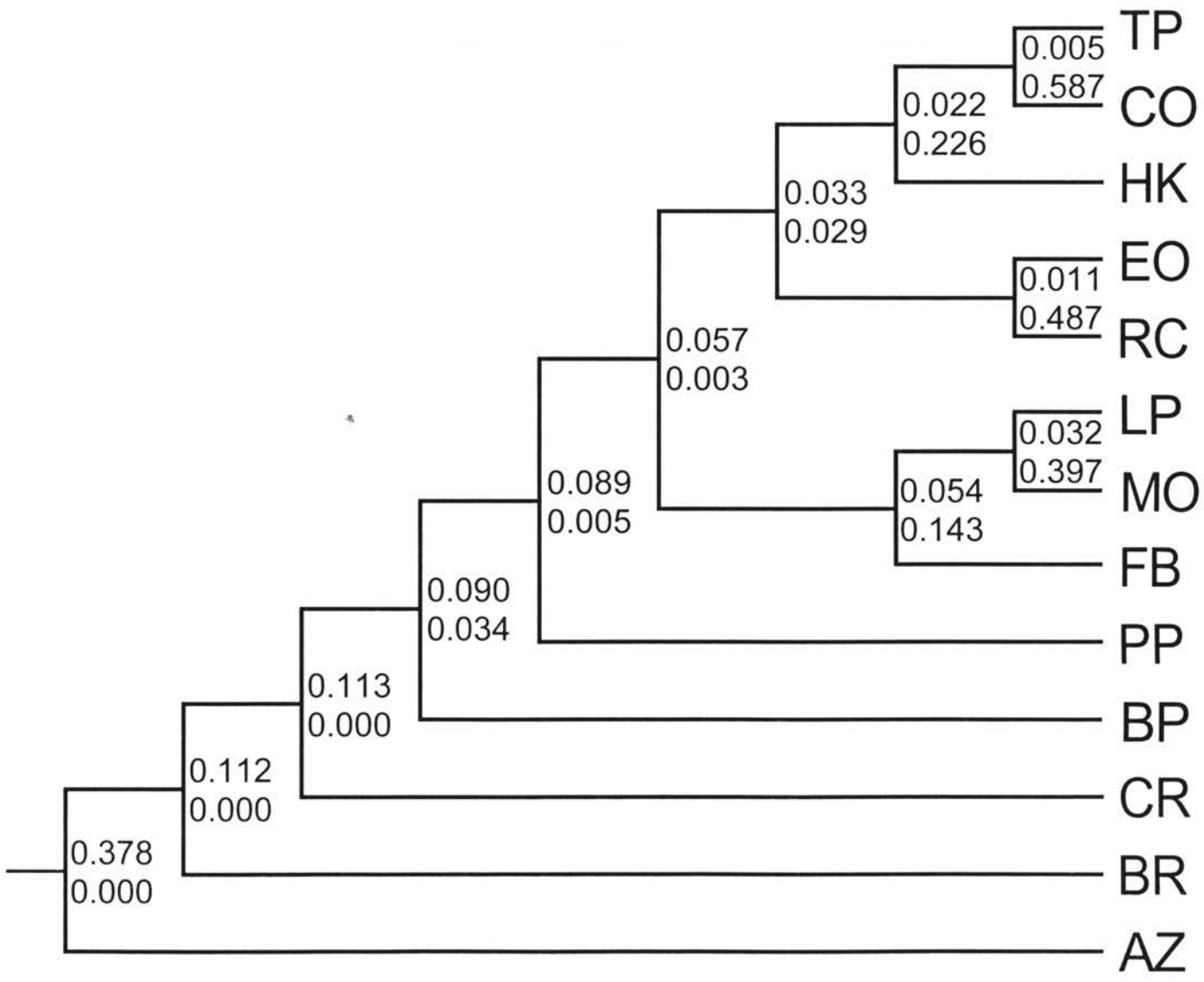
Allele	AZ	FB	HK	CO	LP	TP	PP	RC	EO	MO	BP	BR	CR
*Sihi-DQA*15*	---	---	---	---	---	---	---	---	0.050	---	---	---	0.167
*Sihi-DQA*16*	---	---	---	---	---	---	---	---	---	---	---	---	0.083
*Sihi-DQA*17*	---	---	---	---	---	---	---	---	---	---	---	---	0.042
*Sihi-DQA*19*	---	---	---	---	---	---	---	---	---	---	---	---	0.083
*Sihi-DQA*20*	---	---	---	---	---	---	---	---	---	---	---	---	0.021
*Sihi-DQA*21*	---	---	---	---	---	---	---	---	---	---	---	---	0.021
*Sihi-DQA*22*	---	---	---	---	---	---	---	---	---	---	---	---	0.021
*Sihi-DQA*23*	---	---	---	---	---	---	---	---	---	---	---	---	0.021
*Sihi-DQA*24*	---	---	---	---	---	---	---	---	---	---	---	---	0.208
*Sihi-DQA*25*	---	---	---	---	---	---	---	---	---	---	---	---	0.021
*Sihi-DQA*26*	---	---	---	0.050	---	---	---	---	---	---	---	---	---

Table 3. F_{ST}-values (above the diagonal) and level of significance (P; below the diagonal) for each pairwise comparison of localities for *Sigmodon hispidus*. Locality acronyms are defined in Table 1. An asterisk indicates table-wide statistical significance ($\propto = 0.05$) following adjustment for multiple tests with the sequential Bonferroni method.

	AZ	FB	HK	CO	LP	TP	PP	RC	EO	MO	BP	BR	CR
AZ		0.618	0.571	0.498	0.604	0.435	0.715	0.603	0.525	0.628	0.657	0.422	0.456
FB	0.000*		0.155	0.089	0.072	0.088	0.228	0.109	0.077	0.061	0.198	0.180	0.129
HK	0.000*	0.004		0.023	0.120	0.026	0.116	0.064	0.032	0.097	0.155	0.192	0.168
CO	0.000*	0.021	0.263		0.052	0.005	0.091	0.037	0.027	0.036	0.078	0.129	0.113
LP	0.000*	0.130	0.019	0.141		0.027	0.260	0.142	0.102	0.032	0.044	0.098	0.073
TP	0.000*	0.017	0.234	0.589	0.364		0.148	0.067	0.028	0.039	0.085	0.088	0.095
PP	0.000*	0.002	0.021	0.023	0.001	0.001		0.022	0.074	0.211	0.194	0.294	0.249
RC	0.000*	0.034	0.066	0.128	0.012	0.026	0.367		0.011	0.104	0.131	0.197	0.165
EO	0.000*	0.070	0.223	0.206	0.023	0.202	0.062	0.492		0.083	0.126	0.168	0.122
MO	0.000*	0.246	0.091	0.332	0.400	0.302	0.016	0.079	0.083		0.125	0.103	0.077
BP	0.000*	0.006	0.016	0.083	0.263	0.051	0.023	0.034	0.026	0.073		0.184	0.130
BR	0.000*	0.000*	0.000*	0.000*	0.026	0.001	0.000*	0.000*	0.000*	0.043	0.000*		0.129
CR	0.000*	0.000*	0.000*	0.000*	0.021	0.000*	0.000*	0.000*	0.000*	0.024	0.002	0.000*	

Figure legend

Figure 1. Hierarchical analysis of genetic divergence in *Sigmodon hispidus*. The number above the branch at each node is the distance (F_{ST}) between its two daughter nodes. The associated *P*-value (below the branch) is the significance of divergence based on 10,000 permutations. Acronyms are defined in Table 1.


0.005
0.587
TP
0.022
0.226
CO
HK
0.033
0.029
0.011
0.487
EO
RC
0.057
0.003
0.032
0.397
LP
MO
0.089
0.005
0.054
0.143
FB
0.090
0.034
PP
0.113
0.000
BP
0.112
0.000
CR
0.378
0.000
BR
AZ

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Thesis: THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE HISPID COTTON RAT (*SIGMODON HISPIDUS*): MOLECULAR EVOLUTION, EVOLUTIONARY TOXICOLOGY, AND PATTERNS OF GEOGRAPHIC VARIATION

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