Hereditas 127: 75–82 (1997)

A microsatellite-based MHC genotyping system for house mice (*Mus domesticus*)

SHAWN MEAGHER and WAYNE K. POTTS

Department of Biology, University of Utah, Salt Lake City, UT 84112, U.S.A.

Meagher, S. and Potts, W. K. 1997. A microsatellite-based MHC genotyping system for house mice (*Mus domesticus*). — *Hereditas 127*: 75–82. Lund, Sweden. ISSN 0018-0661. Accepted July 20, 1997.

Major histocompatibility complex (MHC) genes are the most polymorphic loci known for vertebrates. Although this has been known for over two decades, the selective forces maintaining this genetic diversity are unclear. Efforts to study selection on these loci in nature have been hampered because no simple MHC typing systems are available. Here, we describe and evaluate a microsatellite-based MHC genotyping system for house mice (*Mus domesticus*). Thirty-five MHC-linked microsatellite loci were tested for amplification and scoring reliability, and 21 were deemed useful. These 21 loci were efficient at discriminating among nine serologically distinct MHC haplotypes, with 52 % of microsatellite pairs providing 100 % resolution. Since these microsatellite loci are scattered across the entire MHC region, they will be effective at detecting recombinant haplotypes. The number of alleles is higher for microsatellites inside the MHC than outside it, which presumably reflects genetic hitchhiking with MHC alleles under balancing selection. This microsatellite typing system now allows testing hypotheses about the nature of selection operating on MHC genes in natural populations of *M. domesticus* and other murid rodent species.

Shawn Meagher, Department of Biology, University of Utah, Salt Lake City, UT 84112, USA. E-mail: MEAGHER@BIOLOGY.UTAH.EDU

Genes in the vertebrate major histocompatibility complex (MHC) play a critical role in immune recognition and the initiation of immune responses (KLEIN 1986). When T-lymphocytes recognize foreign antigens presented by MHC proteins on the surfaces of cells, this event triggers both the cellular and humoral immune responses. Cellular immunity involves the destruction of cells infected with intracellular parasites by cytotoxic T-cells, and humoral immunity involves the B-cell production of antibodies aimed at neutralizing extracellular invaders by B-cells.

MHC genes are highly polymorphic due to the action of diversifying and balancing natural selection, but the exact nature of this selection is unclear (APANIUS et al. 1997). Due to their critical role in immunity, it is generally assumed that the selective pressures affecting MHC diversity come directly from infectious diseases, but the form of that selection is debated (HUGHES and NEI 1992; SLADE and MCCALLUM 1992; POTTS and SLEV 1995). In addition to parasite-mediated selection, it has been shown that female house mice (*Mus domesticus*) prefer to mate with MHC-dissimilar males (POTTS et al. 1991), and these behaviors could also maintain MHC diversity (HEDRICK 1992). MHC-based mating patterns may have evolved so that individuals can produce disease resistant MHC-heterozygous offspring or to allow them to avoid mating with relatives that could result in inbreeding depression in their offspring (POTTS and WAKELAND 1993). There is also some evidence that non-random spontaneous abortion might favor MHC diversity in some species (ALBERTS and OBER 1993).

Since natural selection operates in natural populations, an important, underutilized approach to understanding the selective forces affecting MHC genes is to test for associations between MHC genotypes and variables such as disease resistance or mate choice in wild populations. The barrier to doing this has been the lack of a simple and inexpensive MHC genotyping system for any animal species. Characterizing MHC loci in new species is a massive undertaking. In the few species for whom the MHC antigen-presenting loci are fully characterized, it would be an extremely labor intensive task to obtain a complete genotype for all of an individual's MHC loci (its haplotype) with any of the currently available methods (for example, SSCP, DGGE (POTTS 1996), and allele specific probes (ALLEN et al. 1994)). Thus, population studies in which all MHC genes are typed are generally not feasible. Consequently, all non-serological MHC studies in natural animal populations have been restricted to the use of one or a few loci (references in EDWARDS and POTTS 1996). However, because recombination generally creates linkage equilibrium across the MHC region (KLITZ and THOMPSON 1987), a single locus will be a poor marker for the rest of the antigen-presenting loci, and this will often provide inaccurate haplotype information.

The discovery of hypervariable microsatellite loci densely distributed across all vertebrate chromosomes, including the MHC region, now makes it possible to obtain MHC haplotypes indirectly by genotyping a series of these loci spread across the MHC. Microsatellites are useful for discriminating

alleles at single loci in the MHC region of mice (SAHA et al. 1993), humans (ABRAHAM et al. 1993), cattle (ELLEGREN et al. 1993; DAVIES et al. 1994) and sheep (OUTTERIDGE et al. 1996). Furthermore, CROUAU-ROY et al. (1996) found that genotypes at three linked microsatellite loci were identical in a sample of humans sharing the same MHC haplotype, suggesting that associations between MHC genes and linked microsatellites may persist for thousands of years in unrecombined chromosomes, and so may be useful for distinguishing distinct MHC haplotypes.

The bulk of our data about how MHC genes affect immunological mechanisms, parasite resistance, and mate choice comes from *M. domesticus*. Microsatellite-based MHC typing is now feasible for this species because of the recent description of over 7000 microsatellites for *Mus* (DIETRICH et al. 1996). Here, we test the utility of MHC-linked microsatellite loci for MHC genotyping by evaluating their ability to discriminate among nine known haplotypes from inbred strains of house mice, and a haplotype from each of three other *Mus* species.

MATERIALS AND METHODS

MHC haplotypes and microsatellite loci

Nine serologically defined MHC haplotypes were chosen to test the discriminating power of microsatellite loci (Table 1). These nine haplotypes are distinct from one another at their *K*, A_β, A_α, E_β, *D*, and *L* loci (KLEIN et al. 1983). These antigen-presenting MHC genes are clustered together with other immunological and non-immunological genes in a small region of Chromosome (Chr) 17 in *Mus domesticus* (Fig. 1). Mouse strains carrying these nine haplotypes were chosen because they are commonly used in research (WASSOM and KELLY 1990; ATCHLEY and FITCH 1994) and they differ in both background and MHC genes. Three additional inbred strains from other *Mus* species were chosen as outgroups to evaluate the degree of conservation of the microsatellite loci (Table 1). In order of increasing phylogenetic distance from *M. domesticus*, they were *M. castaneus*, *M. spretus*, and *M. caroli* (LUNDRIGAN and TUCKER 1994; SILVER 1995). DNA from all 12 inbred strains was purchased from the Jackson Laboratory.

Thirty-five microsatellite loci closely linked to the MHC were surveyed in this study (Fig. 1). The microsatellites are found between 18.00 to 22.50 cM from the centromere, while the highly polymorphic antigen-presenting MHC loci are found 18.43 to 19.13 cM from the centromere (HAMVAS et al. 1996). One microsatellite within E_β ("*Saha*", Fig. 1) is described by SANT'ANGELO et al. (1991) and SAHA et al. (1993), and the rest are described by DIETRICH et al. (1996) and were purchased from Research Genetics, Inc.

Genotyping

Microsatellite loci were amplified via the polymerase chain reaction (PCR) using *Taq* polymerase, 0.13 μM primers, and 1.5 mM Mg^{++} buffer (Boehringer-Mannheim), consisting of 35 cycles of 30 s each at 94°C, 55°C, and 72°C. The first cycle was preceded by a denaturing step of 2 min at 94°C, and the last cycle was followed by an extended annealing period of 10 min at 72°C. PCR amplification products were separated on 7 % polyacrylamide sequencing gels and disclosed by ethidium bromide staining and UV illumination (POTTS 1996). PCR amplification of all 35 microsatellite loci was examined initially using annealing temperatures of 50°C and 55°C (twice at each temperature) on a subset of MHC haplotypes (*b*, *d*, *k*, and *q*). Loci that either amplified poorly or were difficult to score were not pursued further. Loci that amplified well were then used to genotype all 12 inbred strains. PCR products were electrophoresed two times: First, the strains' relative migration distances were observed, then similar-sized products were run in adjacent lanes, in order of decreasing speed (lineup gels) to corroborate their relative sizes. We were able to distinguish between PCR products that differed in size by two base pairs, based on product sizes reported for *M. castaneus*, *M. spretus*, C57BL/6J, and DBA/2J (Research Genetics, Inc.).

RESULTS

Of the 35 microsatellite loci, 21 were scorable among all nine *M. domesticus* MHC haplotypes (Table 1). Twenty-three *Mit* markers amplified; however, *D17Mit31*, *32*, *33*, and *34* amplify the same $(CA)_n$ repeat locus (they share the same left primer but have different right primers), which left 20 interpretable *Mit* loci and *Saha*. Ten loci (*D17Mit147*, *230*, *214*, *231*, *64*, *104*, *11*, *Nds2*, and *Nds3*) amplified too weakly to interpret, and one locus (*D17Mit16*) amplified well, but unusual band patterns for two haplotypes (*r* and *v*) made it difficult to score. Genotypes (relative PCR product sizes) for the 21 loci among all 12 mouse strains are presented in Table 1. Summary statistics for the number of alleles among *M. domesticus* strains are in Table 2.

Phylogeny and amplification

All 21 loci that produced unambiguous genotypes in *M. domesticus* were also scorable in *M. castaneus* and *M. spretus*, but 5/21 loci (24 %) did not amplify, or were uninterpretable in *M. caroli* (at least four PCR

Table 1. *Locus names, repeat units, and relative PCR product sizes (higher numbers reflect larger fragment sizes) for 21 MHC-linked microsatellite loci among 12 inbred strains of mice. ? = sequence data unavailable, NA = no amplification*

Locus	Repeat(s)	Species, MHC haplotype and strain												# of alleles
		Mus caroli	*Mus spretus*	*Mus castaneus*	*Mus domesticus*									
		caroli	spretus	CAST/Ei	*b* C57BL/6J	*d* DBA/2J	*f* A.CA/Sn	*k* CBA/J	*p* P/J	*q* SWR/J	*r* RIIIS/J	*s* SJL/J	*v* SM/J	
63	GT	1	5	2	3	3	4	4	2	4	3	4	3	5
82	CA	3	10	1	2	6	2	2	8	5	9	4	7	10
28	CA	3	8	1	10	4	5	2	5	9	7	6	8	10
62	GT	9	3	10	7	1	4	4	2	8	5	6	6	10
102	CA	3	6	3	5	1	2	2	1	1	1	4	1	6
103	CA	9	1	2	4	8	6	5	3	7	7	5	2	9
21	CA	1	8	2	8	9	2	5	4	3	4	6	7	9
22	GT, CAGA	8	4	7	3	9	4	3	5	1	6	2	5	9
Saha	TGGA, GGCA	5	5	2	2	6	3	2	1	4	2	3	2	6
34	CA	NA	4	6	4	7	2	1	1	4	8	3	5	8
83	AT	NA	1	3	4	3	2	3	8	5	6	6	7	8
13	?	1	6	5	7	5	4	4	1	3	2	8	4	8
125	CA	NA	5	3	2	2	1	4	7	3	6	3	2	7
24	CA, CCT	9	1	3	6	2	4	5	1	6	7	8	6	9
148	CA	3	6	8	4	6	1	2	4	4	7	5	4	8
233	CA	7	1	5	4	2	5	3	3	4	6	2	3	7
234	CA	NA	1	4	3	5	2	3	2	3	2	3	3	5
126	CA	NA	1	2	4	4	3	2	4	4	5	4	4	5
105	CA	6	4	1	2	2	5	3	3	2	1	2	2	6
124	CA	1	2	4	3	5	6	7	3	5	8	5	3	8
176	GT, GA	5	6	2	5	4	1	6	7	4	3	4	5	7

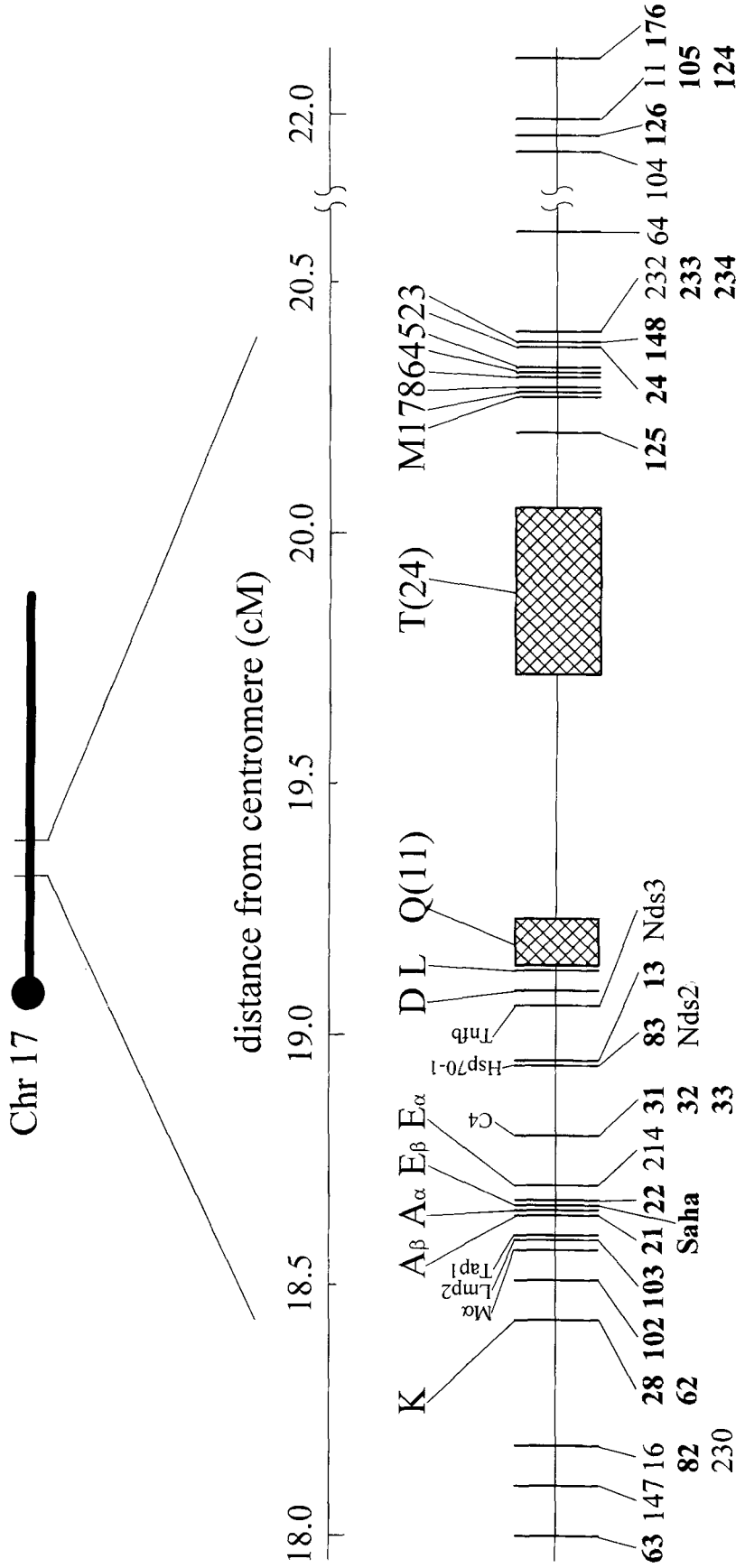

Fig. 1. Relative positions of microsatellite loci and selected major histocompatibility complex (MHC) genes on *Mus domesticus* Chr 17. Microsatellite loci below line (D17Mit designation omitted) and MHC genes above. Microsatellite loci mapped to the same position are listed in columns; those that produce interpretable genotypes are in bold. *K*, A_β, A_α, E_β, E_α, *D*, and *L* = polymorphic except E_x, antigen-presenting molecules; *Q*, *T*, and *M* = low diversity, potential antigen-presenting molecules, with 11 and 24 genes in the *Q* and *T* regions; *Mα*, *Lmp2*, *Tap1* = genes involved in antigen processing; *C4* = complement protein; *Hsp70-1* = heat shock protein; *Tnfb* = tumor necrosis factor β (cytokine). Scale is distance from centromere (cM) from HAMVAS et al. (1996).

attempts). *Mus caroli* had either no amplification or unique alleles (not shared with any *M. domesticus* strain) at 19/21 microsatellite loci. *Mus spretus* and *M. castaneus* were unique at 14 and 11 loci, respectively, which are similar to the proportion of loci at which *M. domesticus* strains were unique relative to each other (median = 10, range = 6–14). These patterns are consistent with the increasing phylogenetic distance of *M. castaneus*, *M. spretus*, and *M. caroli* from *M. domesticus*.

Allele number for microsatellites in relation to distance from polymorphic MHC loci

There were more alleles at loci inside the MHC than outside the region. The average number of alleles (*M. domesticus* strains only) is higher for loci between the *K* and *D* loci, than those loci either proximal or distal to this region (Table 2; Mann-Whitney $U = 145.0$, $p = 0.015$).

Table 2. *Summary statistics of number of alleles for 21 microsatellite loci among nine inbred strains of Mus domesticus. "Within MHC" is defined as those loci found within the region bounded by two flanking polymorphic antigen-presenting loci (K and L). "Flanking MHC" is defined as the region surrounding the "Within MHC" region*

Locus	Number of alleles		
	Total	Within MHC	Flanking MHC
63	3		3
82	7		7
28	8	8	
62	7	7	
102	4	4	
103	7	7	
21	8	8	
22	7	7	
Saha	5	5	
34	7	7	
83	7	7	
13	7	7	
125	6		6
24	7		7
148	6		6
233	5		5
234	3		3
126	4		4
105	4		4
124	5		5
176	6		6
Median	6	7	5
Mean	5.86	6.70	5.09
Minimum	3	4	3
Maximum	8	8	7

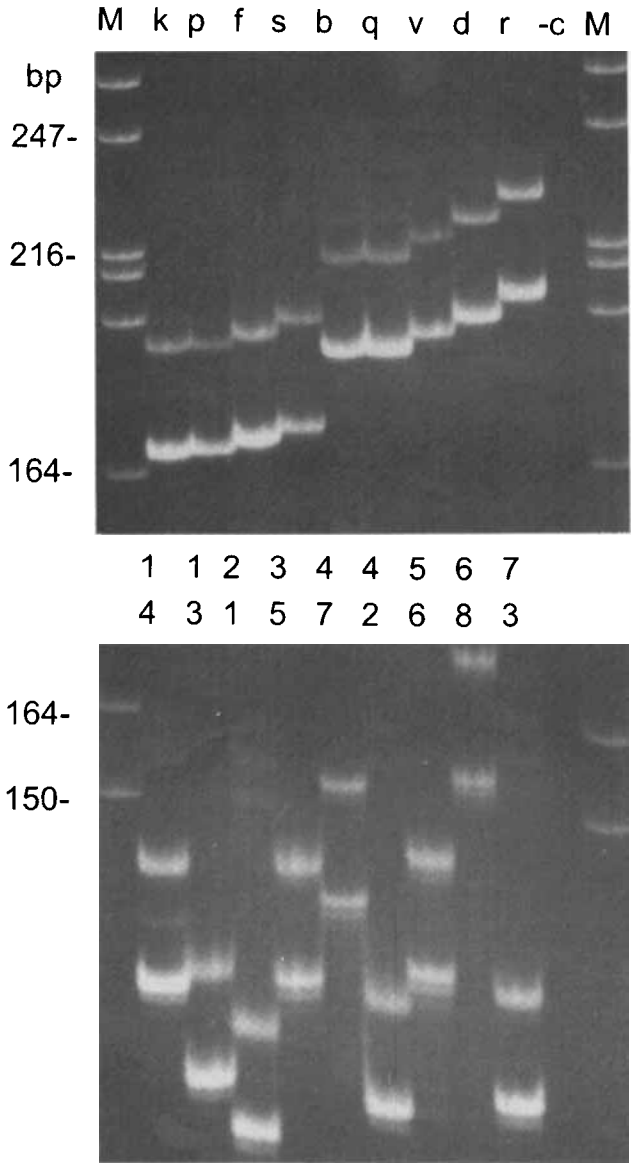

Fig. 2. Example of two microsatellite loci that together discriminate nine major histocompatibility complex (MHC) haplotypes. Ethidium bromide-stained native polyacrylamide (7 %) gel of PCR products for *D17Mit33* (above) and *D17Mit21* (below). Labels: M = marker (size standard), lower-case letters (k–r) = MHC haplotypes, -c = negative control (PCR reaction mixture without DNA); numbers between gels = relative migration speeds of PCR products, bp = base pairs. Each lane shows two bands; the faster running band is the actual allele while the slower is an artifact of dinucleotide microsatellites run on native gels.

Haplotype discrimination

No single microsatellite locus discriminated all nine MHC haplotypes, but 52 % (109/210) of all two-locus combinations did (Fig. 2, 3). Since there are 1330 combinations of three loci, to evaluate their discrimination efficiency, we randomly chose three non-overlapping pairs of loci that did not discriminate all nine

haplotypes and combined them with the remaining 19 loci. Of these 57 triplets, 84% (48/57) distinguished all of the haplotypes. This is, of course, an underestimate for the resolving power of three-locus combinations, since it excludes triplets that include two-locus pairs that already provide 100% resolution.

Because of their higher polymorphism, combinations of loci from within the MHC were better at discriminating MHC haplotypes. For 45 pairs in which both microsatellites were within the MHC, 80% (36/45) discriminated all nine haplotypes. When one locus was within the MHC, 57% (63/110) of two-locus combinations discriminated all nine, and when both loci were outside the MHC, only 18% (10/55) of the pairs distinguished all of the haplotypes.

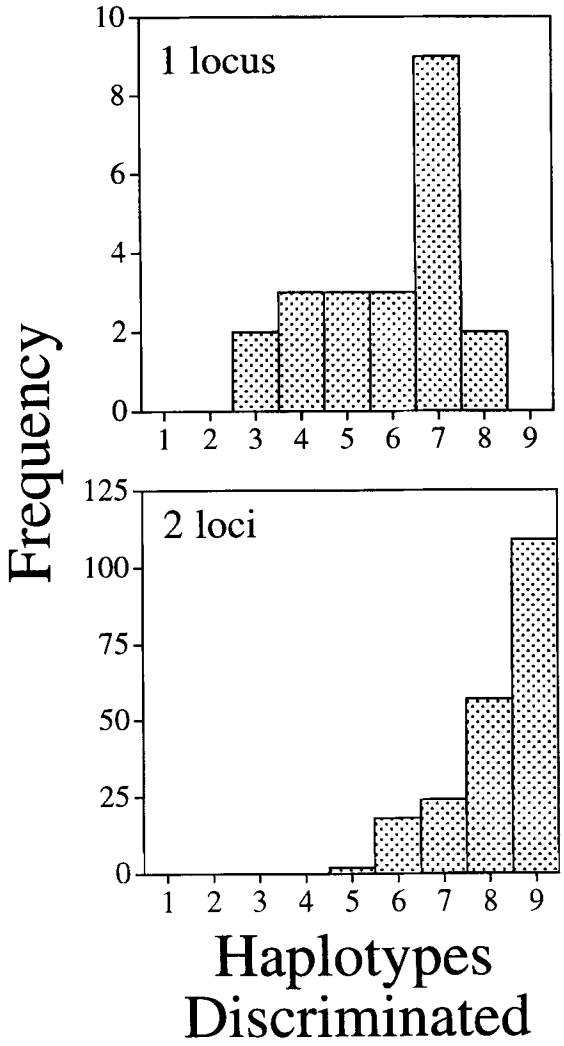

Fig. 3. Histograms of the number of nine major histocompatibility complex haplotypes discriminated by one and two MHC-linked microsatellite loci. Upper panel = discrimination by 21 single microsatellite loci. Lower panel = discrimination by 210 two-locus combinations.

DISCUSSION

This study is significant because it provides a simple and inexpensive method for inferring complete MHC haplotypes (genotypes for all MHC loci) for *Mus* individuals. We confirmed that a series of microsatellites in and near the *M. domesticus* MHC are powerful at discriminating haplotypes and that microsatellite allele numbers are higher for loci nearer to polymorphic antigen-presenting loci. This method for identifying distinct MHC haplotypes now makes it possible to investigate the evolutionary forces acting on MHC genes in wild populations of *M. domesticus* and other murid rodents.

Discriminating MHC haplotypes

Combinations of MHC-linked microsatellites could easily discriminate among nine MHC haplotypes. No single microsatellite distinguished all nine haplotypes, but 52% of all microsatellite pairs could (Fig. 3). This is consistent with findings that microsatellites are useful for distinguishing MHC genotypes at individual loci (ABRAHAM et al. 1993; CROUAU-ROY et al. 1996), and suggests that a combination of microsatellites provides a practical method for determining MHC haplotypes. One advantage to using a series of microsatellites is that recombinant MHC haplotypes can be identified, which is not the case for single locus MHC typing systems. This attribute is especially important if the MHC gene(s) relevant to a particular component of fitness are not known prior to beginning a study. Ideally, a field study using this system should utilize a combination of at least three or four microsatellites that are both flanking and internal to the MHC to identify haplotypes and recombinant haplotypes. While recombinant genotypes can be identified, this method cannot identify intragenic recombination nor gene conversion events, both of which are known to affect the MHC (EHRLICH and GYLLENSTEN 1991; SHE et al. 1991). The magnitude of this practical problem is probably small, but quantification will require a comparison of MHC gene sequences among microsatellite-defined MHC haplotypes from natural populations.

Microsatellite polymorphism near MHC

We found that microsatellites nearest the polymorphic antigen-presenting loci had more alleles than microsatellites outside this region (Table 2). Since inbred mouse strains were originally derived from wild mouse populations, the number of alleles among these strains is probably indicative of levels of polymorphism at the same loci in natural populations of *M. domesticus*. The higher diversity at microsatellite loci nearest MHC genes is consistent with increased

levels of polymorphism in MHC-linked allozyme genes (NADEAU et al. 1982), and may be due to "hitchhiking" of neutral microsatellite alleles on selectively maintained MHC alleles (Ellegren et al. 1993). While most recent attention to genetic hitchhiking has focused on how directional selection at one locus will reduce neutral genetic diversity at linked genes (BRAVERMAN et al. 1995), neutral diversity is expected to be higher near loci where natural selection maintains multiple alleles (SVED 1983; NADEAU and COLLINS 1983; SLATKIN 1995).

Future research

MHC gene polymorphism is one of few well-documented cases of adaptive molecular variation. Several lines of evidence indicate that the diversity of MHC genes is maintained by natural selection (APANIUS et al. 1997). In order to determine what selective forces maintain this diversity, we must now measure the effects of MHC genotypes on fitness in wild populations. Two potential types of studies include testing whether parasite loads, mating patterns, or both are associated with multi-locus MHC genotypes. To date, such studies have been performed only on humans (for example HILL et al. 1991; OBER et al. in press), and it is difficult to know how well modern human circumstances reflect the conditions under which MHC diversity evolved. The multi-locus microsatellite MHC typing system we present here provides the opportunity to perform similar field studies, as well as experimental studies, with *M. domesticus*. Since most data on the effects of MHC genes on both disease resistance (WASSOM and KELLY 1990) and mate choice (POTTS et al. 1991) have been collected from this species, we are now in the powerful position of being able to tell whether MHC genes have similar effects on these attributes in the real world.

Finally, microsatellite loci may be conserved between mammal species for up to 20 million years (SCHLOTTERER et al. 1991). Since 76 % of the loci we examined worked well for *M. caroli*, a species believed to have diverged from *M. domesticus* nearly four million years ago (SILVER 1995), this specific MHC genotyping system may prove useful for the study of MHC evolution in other rodents, particularly those in the family Muridae, which accounts for nearly 25 % of all mammal species.

ACKNOWLEDGMENT

We thank Anthony Baker for teaching SM lab techniques, and Linda Morrison, Michele Franz, Caroline Krater, and Ed King for last-minute help with lab work and analysis. This study was conducted while SM was an NSF fellow and WKP was supported by grants from NSF and NIH.

REFERENCES

Abraham LJ, Marley JV, Nedospasov SA, Cambon-Thomsen A, Crouau-Roy B, Dawkins RL and Giphart MJ, (1993). Microsatellite, restriction fragment-length polymorphism, and sequence-specific oligonucleotide typing of the tumor necrosis factor region. Hum. Immunol. 38: 17–23.

Alberts SC and Ober C, (1993). Genetic variability in the major histocompatibility complex: A review of non-pathogen-mediated selective mechanisms. Yearb. Phys. Anthropol. 36: 71–89.

Allen M, Liu L and Gyllensten U, (1994). A comprehensive polymerase chain reaction-oligonucleotide typing system for the HLA class I A locus. Hum. Immunol. 40: 25–32.

Apanius VA, Penn D, Slev PR, Ruff LR and Potts WK, (1997). The nature of selection on the major histocompatibility complex. Crit. Rev. Immunol. 17: 179–224.

Atchley WR and Fitch WM, (1994). Gene trees and the origins of inbred strains of mice. Science 254: 554–558.

Braverman JM, Hudson RR, Kaplan NL, Langley CH and Stephan W, (1995). The hitchhiking effect on the site frequency spectrum of DNA polymorphisms. Genetics 140: 783–796.

Crouau-Roy B, Bouzekri N, Carcassi C, Clayton J, Contu L and Cambon-Thomsen A, (1996). Strong association between microsatellites and an HLA-B, DR haplotype (B18-DR3): implication for microsatellite evolution. Immunogenetics 43: 255–260.

Davies CJ, Joosten I, Andersson L, Arreins MA, Bernoco D, Bissumbhar B, Byrns G, van Eijk MJT, Kristensen B, Lewin HA, Mikko S, Morgan ALG, Muggli-Cockett NE, Nilsson PR, Oliver RA, Park CA, van der Poel JJ, Polli M, Spooner RL and Stewart JA, (1994). Polymorphism of bovine MHC class II genes. Joint report of the fifth international bovine lymphocyte antigen (BoLA) workshop, Interlaken, Switzerland, 1 August 1992. Eur. J. Immunogen. 21: 259–289.

Dietrich WF, Miller J, Steen R, Merchant MA, Damron-Boles D, Husain Z, Dredge R, Daly MJ, Ingalls KA, O'Connor TJ, Evans CA, DeAngelis MM, Levinson DM, Kruglyak L, Goodman N, Copeland NG, Jenkins NA, Hawkins TL, Stein L, Page DC and Lander ES, (1996). A comprehensive genetic map of the mouse genome. Nature 380: 149–152.

Edwards SV and Potts WK, (1996). Polymorphism of genes in the major histocompatibility complex (MHC): Implications for conservation genetics of vertebrates. In: Molecular Genetic Approaches in Conservation (eds TB Smith and RK Wayne) Oxford University Press, New York p. 214–237.

Ehrlich HA and Gyllensten UB, (1991). Shared epitopes among HLA class II alleles: Gene conversion, common ancestry and balancing selection. Immunol. Today 12: 411–414.

Ellegren H, Davies C and Andersson L, (1993). Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene: implications for microsatellite stability and PCR-based DRB3 typing. Anim. Genet. 24: 269–275

Hamvas R, Trachtulec Z, Forejt J, Williams RW, Arzt K, Fischer-Lindahl K and Silver L, (1996). Mouse chromosome 17. Mamm. Genome 6: S281–S299.

Hedrick PW, (1992). Female choice and variation in the major histocompatibility complex. Genetics 132: 575–581.

Hill AVS, Allsopp CEM, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, Bennett S, Brewster D, McMichael AJ and Greenwood BM, (1991). Common West African HLA antigens are associated with protection from severe malaria. Nature 352: 595–600.

Hughes AL and Nei M, (1992). Models of host-parasite interaction and MHC polymorphism. Genetics 132: 863–864.

Klein J, (1986). Natural History of the Major Histocompatibility Complex. John Wiley & Sons, New York.

Klein J, Figueroa F, and David CS, (1983). H-2 Haplotypes, genes and antigens: Second listing II. The H-2 complex. Immunogenetics 17: 553–596.

Klitz W and Thomson G, (1987). Disequilibrium pattern analysis. II. Application to Danish HLA-A and B locus data. Genetics 116: 633–643.

Lundrigan BL and Tucker PK, (1994) Tracing paternal ancestry in mice, using the Y-linked, sex-determining locus, Sry. Mol. Biol. Evol. 11: 483–492.

Nadeau JH and Collins RL, (1983). Does associative overdominance account for the extensive polymorphism of H-2-linked loci? Genetics 105: 241–244.

Nadeau JH, Collins RL and Klein J, (1982). Organization and evolution of the mammalian genome: I. Polymorphism of H-2 linked loci. Genetics 102: 583–598.

Ober C, Weitkamp LR, Cox N, Dytch H, Kostyu D and Elias S, (1997). HLA and mate choice in humans. Am. J. Hum. Genet. (in press).

Outteridge PM, Andersson L, Douch PGC, Green RS, Gwakisa PS, Hohenhaus PS and Mikko S, (1996). The PCR-typing of MHC-DRB genes in the sheep using primers for an intronic microsatellite: Application to nematode parasite resistance. Immunol. Cell Biol. 74: 330–336.

Potts WK, (1996). PCR-based cloning across large taxonomic distances and polymorphism detection: MHC as a case study. In: Molecular Zoology (eds JD Ferraris and S Palumbi) John Wiley, New York, p. 181–194.

Potts WK and Slev PR, (1995). Pathogen-based models favoring MHC genetic diversity. Immunol. Rev. 143: 181–197.

Potts WK and Wakeland EK, (1993). The evolution of MHC diversity: A tale of incest, pestilence and sexual preference. Trends Genet. 9: 408–412.

Potts WK, Manning CJ and Wakeland EK, (1991). Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature 352: 619–621.

Saha BK, Shields JJ, Miller RD, Hansen TH and Shreffler DC, (1993). A highly polymorphic microsatellite in the class II Eb gene allows tracing of major histocompatibility complex evolution in mouse. Proc. Natl. Acad. Sci. USA 90: 5312–5316.

Sant'Angelo D, Heine D, and Passmore H, (1991). Diversity and evolution at the Eb recombinational hotspot in the mouse. In: Molecular Evolution of the Major Histocompatibility Complex (eds J Klein and D Klein) Springer-Verlag, Berlin, p. 473–482.

Schlotterer C, Amos B, and Tautz D, (1991). Conservation of polymorphic simple sequence loci in cetacean species. Nature 354: 63–65.

She JX, Boehme S, Wang TW, Bonhomme F and Wakeland EK, (1991). Amplification of major histocompatibility complex class II gene diversity by intra-exonic recombination. Proc. Natl. Acad. Sci. USA 88: 453–457.

Silver LM, (1995). Mouse Genetics. Oxford University Press, New York.

Slade RW and McCallum HI, (1992). Overdominant vs. frequency-dependent selection at MHC loci. Genetics 132: 861–862.

Slatkin M, (1995). Hitchhiking and associative overdominance at a microsatellite locus. Mol. Biol. Evol. 12: 473–480.

Sved JA, (1983). Does natural selection increase or decrease variability at linked loci? Genetics 105: 239–240.

Wassom DL and Kelly EAB, (1990). The role of the major histocompatibility complex in resistance to parasite infections. Crit. Rev. Immunol. 10: 31–52.