Major Histocompatibility Complex Haplotypes

in the Nokota Horse

Cornell Veterinary Leadership Program

The Antczak Lab

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Cornell University
College of Veterinary Medicine
Baker Institute for Animal Health

Overview

I. **Nokota Horse**: an introduction

II. **The Antczak Lab** & the Nokota Horse Project

III. **My Summer Project:** characterize Nokota horse MHC diversity using microsatellites

AIM:

to determine the **diversity** of **MHC haplotypes** in the Nokota horse using **microsatellites**.

Nokota Horses

- Descendants from feral horses at Theodore Roosevelt National Park, in North Dakota, USA.
- Mostly unknown genetic ancestry
  - *National Park:* ranch horses x domestic breeds
  - *Nokota Horse Conservancy:* link to horses from Chief Sitting Bull (Lakota)?
- Name: **No**rth Da**kota** → **Nokota**
- Population size: < 1,000 in 2006

Antczak lab & the Nokota horse project

AIM
to study the genetic makeup of the Nokota horse

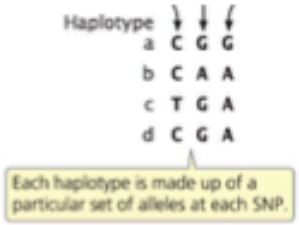
METHOD
SNP assay

Single Nucleotide Polymorphism

- Loci with alleles that differ at a single basepair.
- Occur once every 1,000 basepairs on average.
- Occur in both exons and introns.

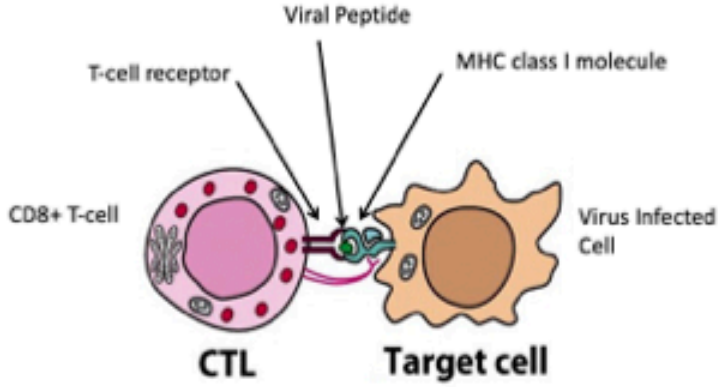
Haplotype

= constellation of alleles at a gene in a single region that is inherited.



Major Histocompatibility Complex (MHC)

- Encodes many immune system genes, like **antigen presenting MHC Class I and II genes**, that shape the epitope-specificity of T-cell responses.
- MHC is both:
  - **Polygenic** (different genes involved)
  - **Polymorphic** (different alleles)
- **Heterozygosity** at MHC **increases an individual's adaptive immunity** to pathogens.
- MHC haplotypes database: *what is t*

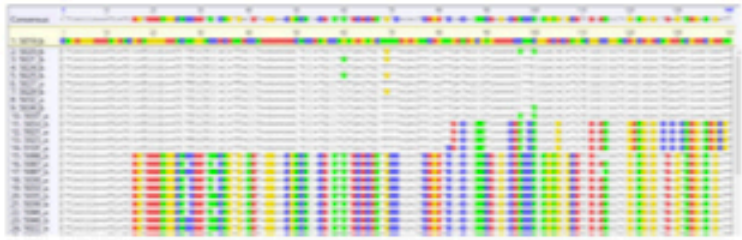


SNP analysis of MHC class I, II & III

AIM:
to determine the **diversity** of **MHC haplotypes** in the Nokota horse

94 unrelated Nokota horses
(blood samples)

DNA extraction
with Qiagen DNEasy kit



SNP analysis of MHC class I, II & III

1. Neogen GGP70 SNP array
2. Phasing with SHAPEIT

My project: confirming the SNP haplotypes with microsatellites

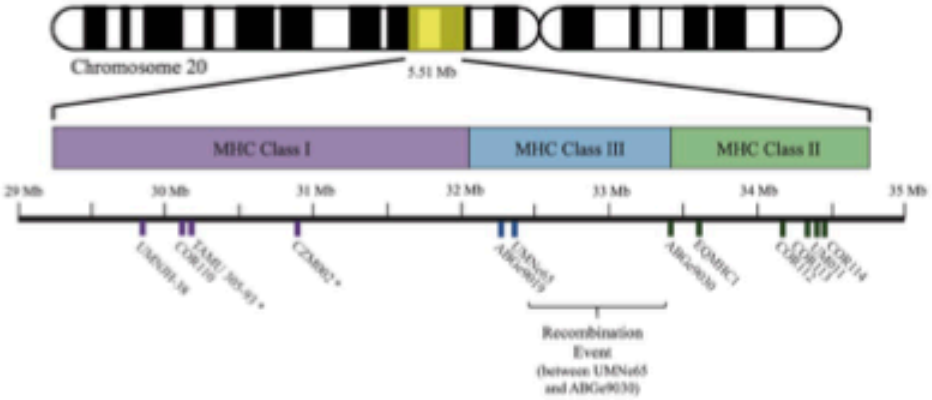
Microsatellites

- Simple, repeating sequences of 2 – 6 basepairs.
- Can be repeated 3 – 100 times.
- Amount of repeats can differ between individuals.

SNP analysis was confirmed with microsatellites

Selection of 45 Nokota horses

- MHC homozygotes
- MHC heterozygotes for the two most common haplotypes (A & B)

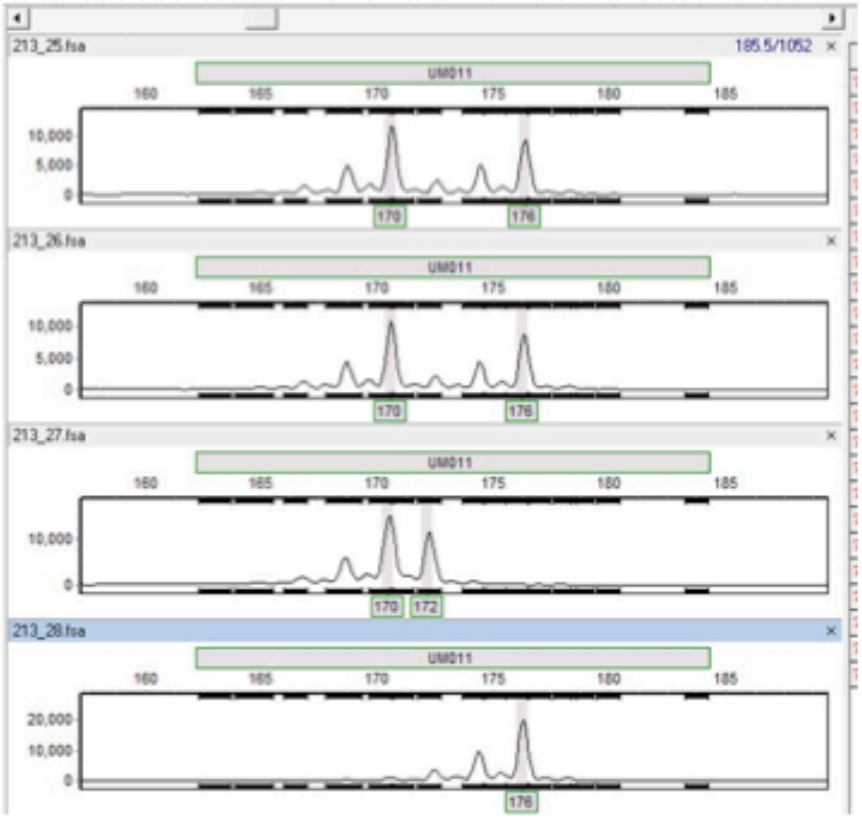


Intra-MHC microsatellite testing

- Fluorescently labeled PCR primers for 12 loci
- PCR fragments were electrophoresed on a ABI3700 at the Cornell BioResource Center

(Holmes et al., 2019; Sadeghi et al., 2018).

Calling microsatellites alleles using Genemarker Software



1. Heterozygote (170 x 176)

2. Heterozygote (170 x 176)

3. Heterozygote (170 x 172)

4. Homozygote (176 x 176)

MHC haplotype calling with microsatellites

		I	I	I	I	III	III	II	II	II	II	II	II	
		UMNJH-38	COR110	305-93	CZM002	ABGe9019	UMNe65	ABGe9030	EQMHC1	COR112	COR113	UM011	COR114	
	Acc. #	29.814.528	30.140.839	30.197.833	30.925.265	32.249.535	32.339.323	33.454.044	33.599.372	34.192.010	34.390.587	34.419.882	34.426.065	
1.	5021_A/C	163	207	343	251	312	261	211	192	262	268	176	247	ELA-A3b
		163	207	343	251	312	261	**	196	264	266	170	245	C
2.	5025_A/C	163	207	343	251	312	261	211	192	262	268	176	247	ELA-A3b
		163	207	343	251	312	261	**	196	264	266	170	245	C
3.	5038_D/E	156	207	345	247	307	255	**	196	264	266	170	245	D
		156	209	343	261	316	249	211	184	252	280	172	253	E
4.	5045_A/A	163	207	343	251	312	261	211	192	262	268	176	247	ELA-A3b
		163	207	343	251	312	261	211	192	262	268	176	247	ELA-A3b
5.	5046_B/B	156	221	342	259	305	259	205	194	260	274	172	247	B
		156	221	342	259	305	259	205	194	260	274	172	247	B

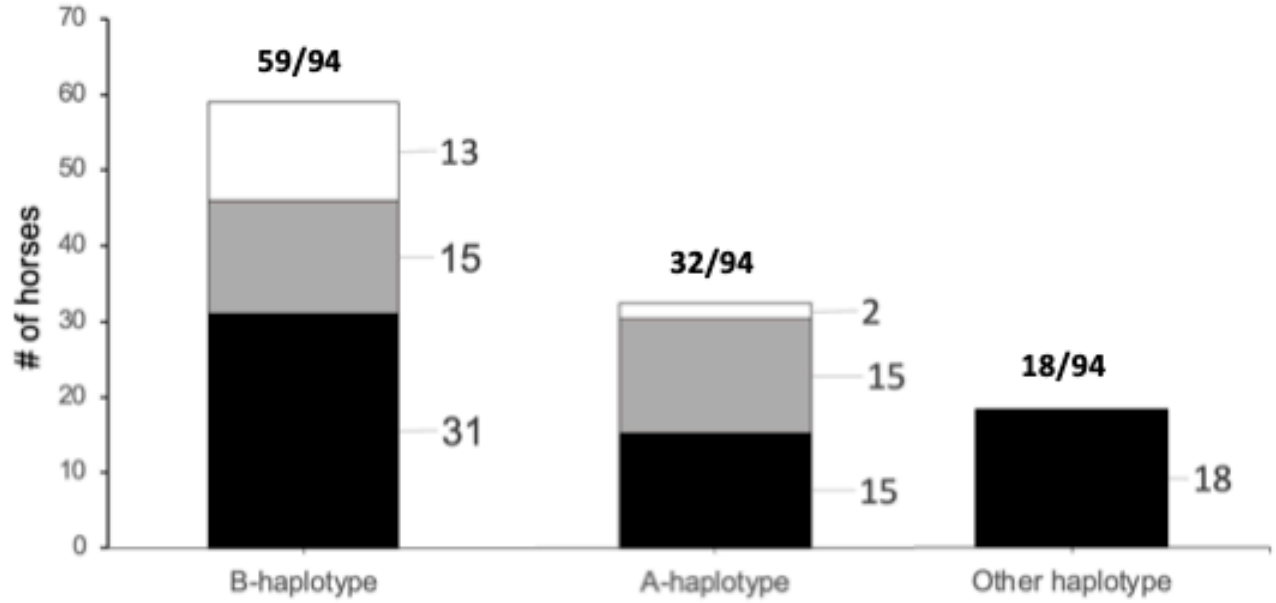
1. Microsatellites confirm SNP analysis is correct:

12 different haplotypes, 9 of which are new

#	Haplotype	Notes
1	A	Previously seen as: **ELA-A3, associated with Thoroughbreds** Second most common haplotype in Nokota Horses (17.5%)
2	B	Most common haplotype in Nokota Horses (38.3%)
3	C	Recombination of A-haplotype (shared class I region) Shared class III-II region with D- and K-haplotype
4	D	Shared class III-II region with C- and K-haplotype
5	E	Unique, never observed before
6	F	Previously seen as: **COR212, associated with Arabian Horses**

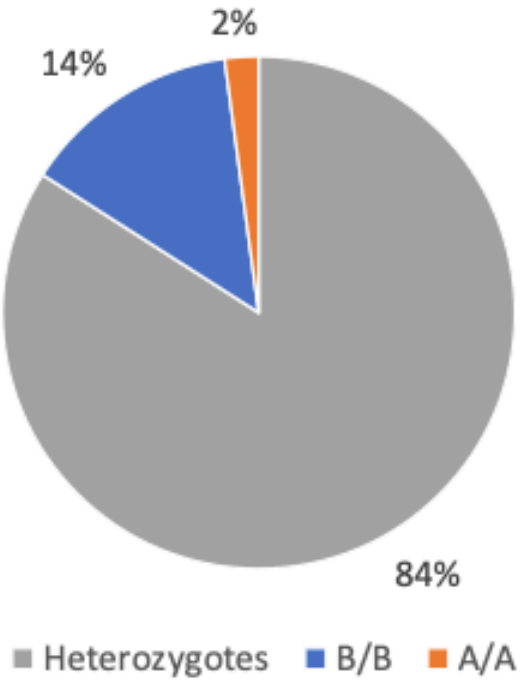
#	Haplotype	Notes
7	G	Previously seen as: **QHnov04, associated with Quarter Horses**
8	H	Unique, never observed before
9	I	Unique, never observed before
10	J	Unique, never observed before
11	K	Shared class III-II region with C- and D-haplotype
12	L	Unique, never observed before

2. SNP analysis detects 2 haplotypes at high frequency



***White** = MHC homozygotes, **grey** = A/B MHC heterozygotes, **black** = B/x, A/x, and x/y heterozygotes.*

3. We found a high level of homozygosity: 16%



Persian Arabians: 3 out of 124 horses → **2.4%**
(Sadeghi et al., 2018)

Icelandic Horses: 1 out of 156 horses → **< 1%**
(Holmes et al., 2019)

Take-aways

- SNP assay is a reliable method to call MHC haplotypes, as confirmed by microsatellite testing.

- Nokota horses have low MHC diversity and a high level of homozygosity compared to other horse breeds.

What's next?

- Y-chromosome paternal ancestry tracing for genetic reconstruction of the Nokota horse breed.

- Compare the Nokota MHC with different horse breeds to get a broader understanding of MHC diversity in the horse.

Contributions

Cornell University
College of Veterinary Medicine
Baker Institute for Animal Health

The Antczak Lab

Dr. Doug Antczak

Don Miller

Maya Kulikowski

Scott Hoffay

Brandon Garcia

..... and all the horses, donkey's and hinny's at the McConville and Kleburg barn

Leadership Program

Dr. John Parker

Dr. Gerlinde van de Walle

Dr. David Fraser

Elaine Lu

Fellow Leadership Scholars

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Thank you for your attention!