

# A Study of Genetic Diversity in a Rare U. S. Pig Breed - The Mulefoot Pig

Paul Kapke, postdoctoral associate,  
Hans Peter Jorgensen, executive director, Institute  
for Agricultural Biodiversity,  
Max F. Rothschild, professor,  
Department of Animal Science

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### Summary and Implications

The genetic relatedness of a foundation herd of Mulefoot pigs was determined using highly informative anonymous genetic markers. Using this information, breeding strategies can be developed to preserve the gene pool of this endangered pig breed.

### Introduction

Mulefoot pigs (Figure 1) are a breed of pigs developed in the United States. Their exact origin is uncertain although they were a popular breed in the Mississippi Valley during the late 1800s. One theory concerning their origin is that they arose from a cross between a Berkshire boar and a native razorback sow. They are characterized by having a syndactyl (non-cloven) foot and are usually all black but can have some white points. They are sometimes referred to as Ozark hogs. Although syndactylism is occasionally seen in pigs, this trait is the one feature that distinguishes this breed. At one time there were three breed societies associated with Mulefoot pigs and more than 235 registered herds. Over the years, the popularity of the breed has declined; it is on the verge of extinction. The last Mulefoot pig registry closed in 1975.

The Institute for Agricultural Biodiversity, a non-profit organization located in Decorah, Iowa, has started a genetic diversity park in association with Luther College. The mission of the park is to preserve endangered farm animal breeds of economic and/or cultural importance. According to Hans Peter Jorgensen, the park director, as many as 27 breeds of mammalian livestock have become extinct since 1900. The purpose of the park and its conservation program is to prevent further loss of agricultural animal breeds. The Mulefoot pig represents a portion of the pig population gene pool and it is facing extinction. The park is interested in preserving the Mulefoot pig breed for future generations. The genetic diversity park obtained a portion of what was believed to be the last pure bred Mulefoot pig herd in the world from a farmer in Missouri.

To determine how closely individuals within a breed are related, genetic relatedness must be determined. Microsatellites are anonymous polymorphic pieces of DNA. They are the genetic markers of choice to use in determining genetic relatedness within an animal pool because they are usually very different among animals and are highly informative. Microsatellites also are passed from generation to generation in a stable Mendelian fashion and are easy to use in the laboratory. The microsatellite markers are usually

dinucleotide repeats having a repeat motif present in from 5-150 copies. Each microsatellite locus is present in >5 allelic forms within an animal population. One can determine the genetic relatedness between two animals by comparing the microsatellite alleles for each locus in the two animals. As the number of microsatellite markers having the same allelic form increases between the two animals, so too does their genetic relatedness.

### Material and Methods

Genotyping was carried out using either radiolabelled primers and the alleles visualized by autoradiography or fluorescently labeled primers and analysis carried out on an ABI sequencing apparatus (Applied Biosystems, Foster City, CA). Standard methods for genotyping were used to amplify and score allelic patterns within the Mulefoot pig herd. Fluorescently labeled microsatellites were analyzed on a Power Mac 7100 using Genescan and GeneTyper software (ABI, Foster City, CA). All alleles were checked using family material. All alleles were scored twice to verify accuracy.

### Results and Discussion

There were 15 foundation animals and 3 offspring in this herd. The animals were genotyped by ISU for The Institute for Agricultural Biodiversity in order to determine which animals were most closely related. The rationale was to identify which animals were the least similar so they could be bred to each other. The animals were genotyped for 11 different genetic markers on 10 different chromosomes. Listed in Table 1 are the pigs with their respective genotypes for each genetic marker. Most genetic markers used for typing the pigs showed only 3 alleles (five alleles for microsatellite Sw210 and only two alleles for Swr435 or ESR). The large number of homozygous animals may suggest that there are null alleles (allelic forms that don't amplify) but this cannot be confirmed without a pedigree tree to follow segregation of the alleles. Surprisingly, one animal had the B allele for ESR. Previous work from our laboratory has found this allele to be relatively rare (Rothschild et al., 1996).

### Acknowledgments

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### References

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**Table 1. Mulefoot pig genotypes<sup>a</sup>.**

chromosome	1	2	3	4	5	6	7	13	14	X
markers	Swr48 ESR	Sw240	Sw72	S0097	S0005	S0003	S0025	Pit1	Sw210	S0511
sex/animal										
F Z-7	2,2	1,2	1,1	1,3	1,2	1,3	0,0	2,2	3,4	1,2
F 6R	1,2	1,2	1,3	2,3	2,2	2,2	0,0	1,1	3,3	3,3
F 2	2,2	1,2	1,3	1,1	1,2	1,1	1,1	2,2	3,3	2,3
F 3	2,2	1,2	1,1	1,3	1,2	1,1	1,1	2,2	3,3	2,3
F 16	2,2	1,2	3,3	1,2	1,2	1,2	1,1	1,2	1,3	3,3
F 14	2,2	2,3	1,3	1,2	0,0	1,3	1,1	1,2	2,3	3,3
F 12	2,2	2,2	1,3	1,3	1,1	1,3	1,1	1,2	2,3	1,3
M 18-R	2,2	1,3	1,1	1,3	1,2	1,1	1,2	1,2	2,3	2,2
F 4	2,2	1,2	1,2	1,3	2,2	1,1	1,2	1,2	2,3	2,3
M 2	2,2	1,2	1,2	2,3	0,0	1,1	1,2	2,2	1,3	2,2
M 1	2,2	1,2	2,3	1,3	1,1	1,2	1,2	1,2	2,3	2,2
F 8	2,2	2,2	3,3	1,2	2,2	1,2	1,2	2,2	2,3	2,3
F 10	2,2	2,2	1,3	2,3	1,2	1,1	2,2	1,2	1,3	2,3
F Z-3	2,2	2,2	2,3	3,3	2,2	1,3	2,2	1,2	1,2	2,3
M Z-1	2,2	2,2	1,3	2,3	1,2	1,1	2,2	1,2	0,0	3,3
<u>Offspring:</u>										
16-3	2,2	2,2	3,3	1,1	1,1	1,2	1,1	2,2	1,3	2,3
4-5	2,2	2,2	2,3	1,2	1,2	1,1	1,2	1,2	2,3	2,3
10-9	2,2	1,2	1,3	1,3	1,2	1,1	2,2	1,2	2,3	2,2
No. alleles	2	3	3	3	3	3	3	2	5	3

<sup>a</sup> alleles with a 0 description are null alleles