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# Identification of Putative Origins of Introduced Pigs in Indiana Using Nuclear Microsatellite Markers and Oral History

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**ABSTRACT:** Feral swine (*Sus scrofa*) have been introduced throughout North America from various global locations (Mayer and Brisbin 1991). In some cases, sources for feral swine are provided through historical records, but for many newly established populations, the origins of feral swine are not known. Understanding the origins and dispersal patterns of feral swine is an important management consideration, because of the introduction of diseases to new locations, for prosecution of individuals who have transported feral swine across state lines, and for allocating swine removal efforts appropriately to address swine translocations within a state or a management area.

**Key Words:** Indiana, microsatellite markers, swine origin

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## INTRODUCTION

Oral history can be useful for understanding feral swine origins, but individual accounts and even official records may be incomplete or inaccurate. Molecular techniques are also a useful tool for identifying the origins of feral swine, but interpreting the results of DNA analyses without field-level context can lead to incomplete explanations. However, the combination of oral histories and molecular analyses can provide a more complete picture of natural and anthropogenic dispersal of swine at local, state, and national scales (McCann 2012).

We collected tissue samples from 25 feral swine occurring in seven counties in Indiana and processed them for a panel of fourteen microsatellite loci. We compared these samples to microsatellite genotypes from 158 tissue samples taken from free-living feral swine originating in 28 other U.S. states as part of the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services' National Wildlife Diseases Surveillance Program (McCann 2012). All wild pig tissue samples were processed for molecular data at an ex-

ternal laboratory (GeneSeek, Lincoln, Nebraska, USA). Genomic DNA was extracted with proprietary protocols, and samples were genotyped for fourteen microsatellite loci identified by the International Society for Animal Genetics for diversity studies of pigs and from USDA program MARC with multiplex polymerase chain reaction (PCR) followed by fragment length scoring on a LICOR 4200 (LI-COR Biosciences, Lincoln, Nebraska, USA). Nuclear microsatellite data were output in spreadsheet format and sent to University of North Dakota.

We used the Bayesian clustering program STRUCTURE (v2.3.3; Pritchard et al. 2000) to delineate population membership based on multi-locus genotypes. In program STRUCTURE, we estimated  $K$ , the number of populations, with ten independent runs of  $K = 1 - 12$  with 100,000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in period of 10,000 MCMC iterations. We used the default settings with the admixture ancestry model and correlated allele frequencies among populations. We averaged the ten log-likelihood scores for each value of  $K$  and calculated the standard deviation. Next, we inferred the most likely number of clusters by calculating  $\Delta K$  after Evanno et al. (2005). We then performed a “nested” analysis of our dataset, where identified groupings were subjected to additional analyses to elucidate population substructure as suggested by Gao et al. (2011).

To determine the putative origins of feral swine in Indiana and their relatedness to populations in the US, we used a combination of publications (Gispon et al. 1998, Mayer and Brisbin 1991), public records, and first- and second-hand information from individuals involved in the illegal introduction of feral swine to Indiana. This information was then compared to results of DNA analyses to give a more complete picture of introductions in Indiana and provide an explanation of dispersal pathways.

Genetic analyses revealed three distinct molecular clusters of feral swine in Indiana, which roughly match the oral history of introduction to the state provided by private individuals and wildlife professionals. The first group consisted almost entirely of feral swine from Jackson and Lawrence Counties in Indiana and animals from locations in North America where Eurasian wild boar have been introduced and

have hybridized with feral pigs; in Great Smoky Mountains National Park, Central California, and near Corbin’s Park New Hampshire (Mayer and Brisbin 1991). The one exception was a single wild hog shot in Harrison County, Indiana. Oral reports suggest that pigs were introduced to Jackson and Lawrence Counties from Louisiana, where Eurasian wild boar and feral pigs have also hybridized (Mayer and Brisbin 1991, 2009). Though no direct link between feral pigs in Indiana and those from Louisiana can be supported with our findings, a common ancestry through Eurasian wild boar introduced to North America is likely for these animals.

The second molecular group consisted of feral pigs from throughout the US and animals from Wayne County Indiana that were legally imported during the late 1990s and early 2000s for a fenced-hunting operation. This hunting club subsequently closed due to financial problems, and the pigs were illegally released. During the same period, a commercial livestock facility was destroyed by fire and domestic swine were released with only a few being recaptured. These domestic pigs reportedly mated with swine from the hunting club to form the basis of the population in east central Indiana. In this case, the poorly resolved association of pigs sampled from Wayne County, Indiana with numerous feral swine from other states is explained, at least in part, by oral introduction histories that suggest considerable genetic admixture.

The third molecular grouping consisted of feral Vietnamese pot-bellied pigs (based on morphological appearance) collected from Pike, Putnam, and Lake Counties, Indiana and feral swine sampled from nine other states throughout the US. The exact origin of these pot-bellied pigs is unknown, but it is thought that they were intentionally released, possibly because of the expense of keeping pigs as pets. Ten to twenty reports per year of feral swine sighted or shot in Indiana are pot-bellied pigs, and pot-bellied pigs have been found running feral in numerous other states (McCann, unpublished data). Currently, it is unclear to what extent pot-bellied pigs are contributing to feral pig numbers, but the association of these animals with feral swine from other states supports some Asian ancestry for intro-

duced pigs in Indiana and other parts of the United States.

Our molecular findings generally supported suspected modes of introduction for wild pigs in Indiana, suggesting that molecular techniques are a viable tool for elucidating introduction histories. However, oral histories were critical for interpreting molecular findings and inferring potential management solutions. Ongoing research will include collecting additional samples from more geographic areas in Indiana and throughout the US to generate a larger frame of reference for genotype comparison useful for further resolving relationships among feral swine populations.

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