

# Genetic relatedness of feral pigs in the United States: national and regional perspectives with implications for management

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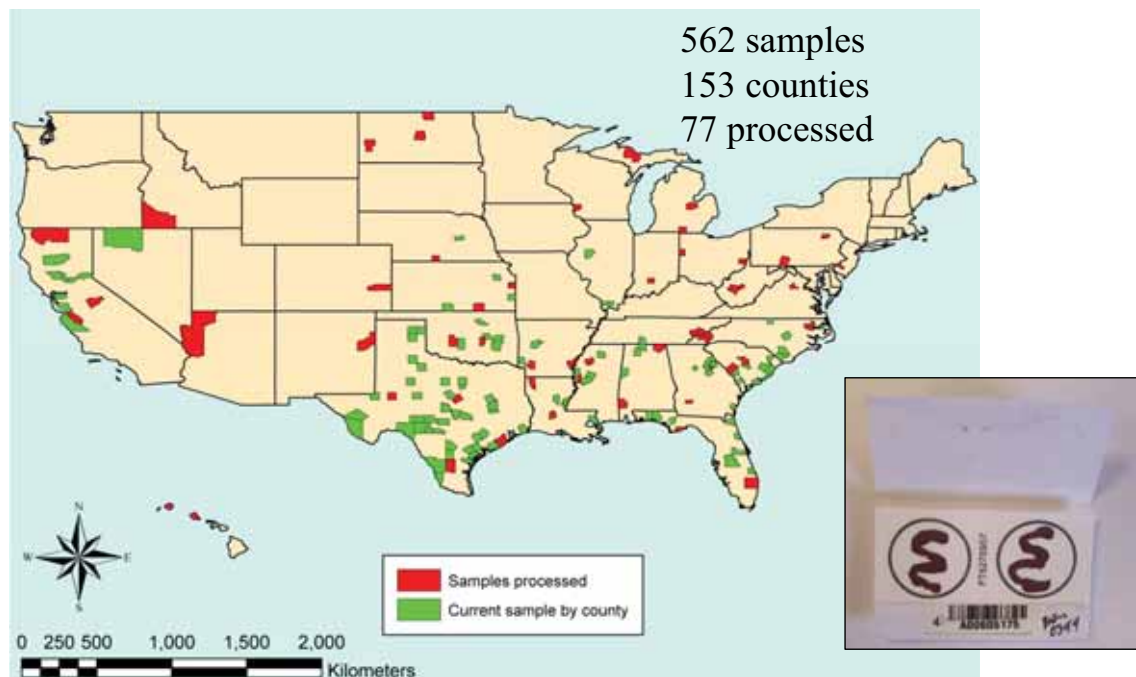
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## Objectives

1. Determine relatedness and origins of pig populations in U.S.
2. Identify source populations for new introductions



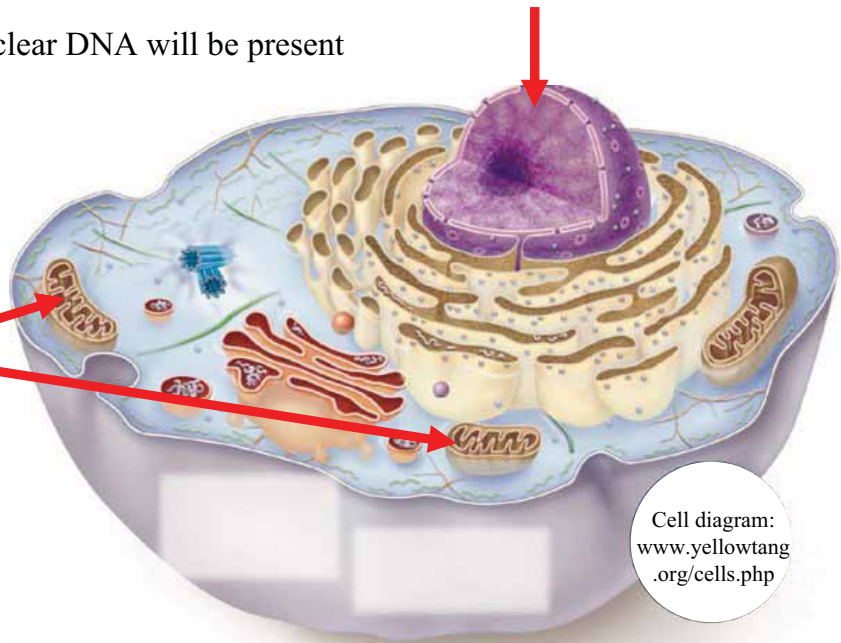
# Background on DNA

*Given a typical animal cell:*

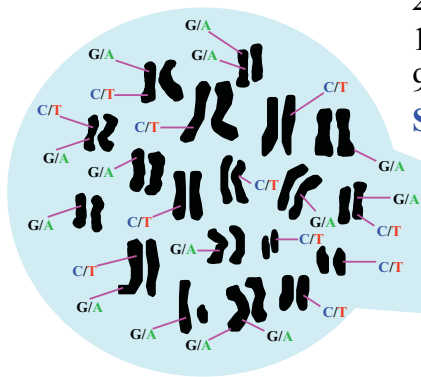
Both mitochondrial and nuclear DNA will be present

**Mitochondria**

**Nucleus**



Cell diagram:  
[www.yellowtang.org/cells.php](http://www.yellowtang.org/cells.php)



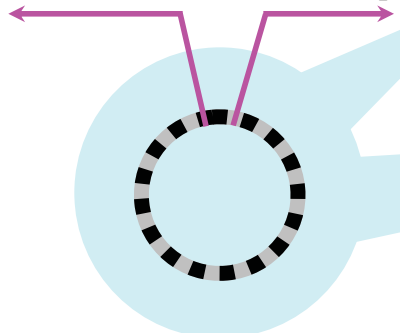
2.7 billion base pair nuclear genome

19 paired chromosomes

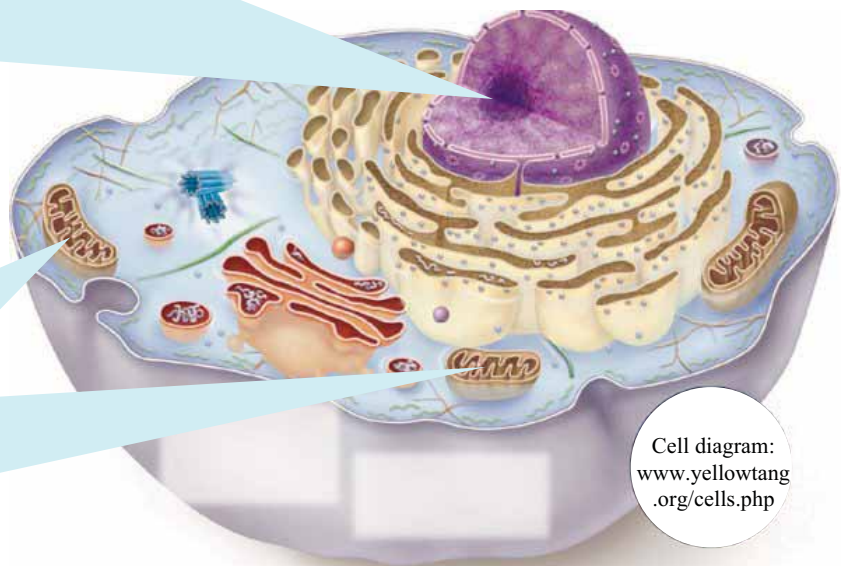
96 locations (loci) where bases vary

**SNP** – single nucleotide polymorphism

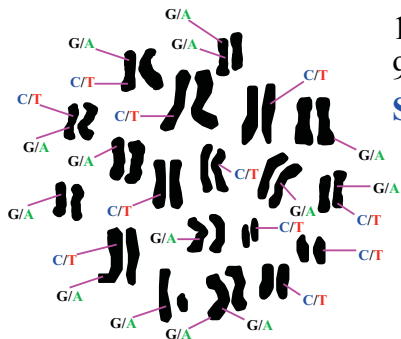
**DNA sequence**  
CTCAAGCCAAT...~400bp



16,000 base pair genome  
Single circular Mitochondrial DNA



Cell diagram:  
[www.yellowtang.org/cells.php](http://www.yellowtang.org/cells.php)



2.7 billion base pair nuclear genome  
 19 paired chromosomes  
 96 locations (loci) where bases vary  
**SNP** – single nucleotide polymorphism

### Key points of nuclear SNPs

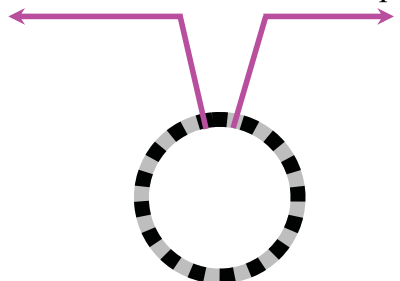
**Inherited from both sexes**

- track introductions of male & female pigs

**Multiple markers**

- high resolution of relationships
- informative at large-scale & small-scale

**DNA sequence**  
 CTCAAGCCAAT...~400bp



### Key points of MtDNA

**Maternally inherited**

- cannot track male pig introductions

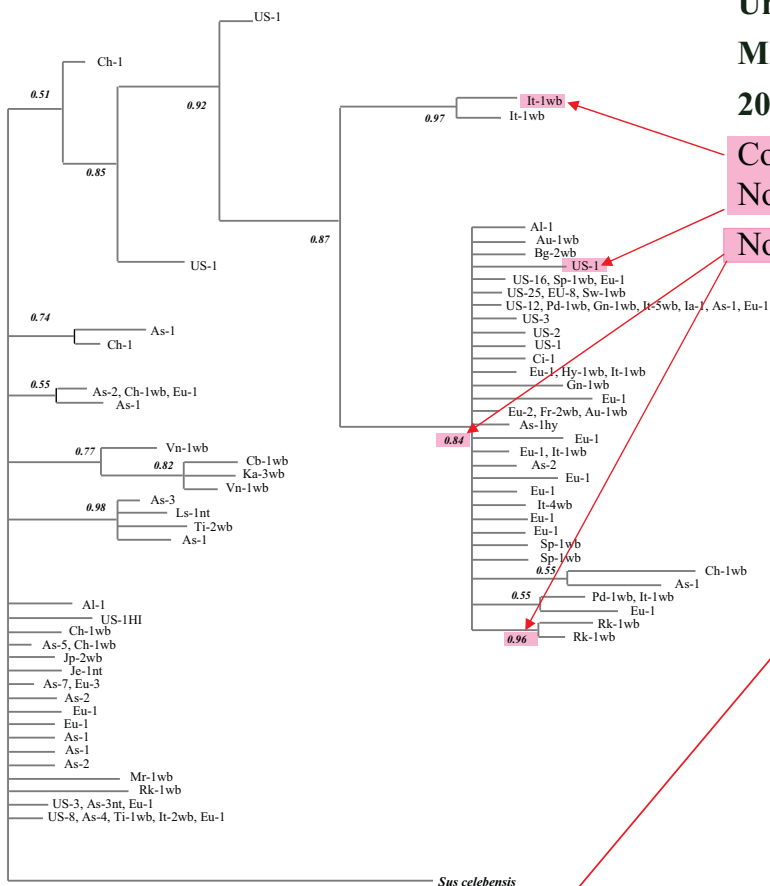
**Single marker**

- low resolution of relationships

**Quickly evolving**

- useful for large-scale population genetics

16,000 base pair genome  
 Single circular Mitochondrial DNA



### Unrooted Phylogram

MrBayes 3.1 GTR model

200 sequences MtDNA ~400bp

Country or other origin abbreviated  
 No. samples from each location

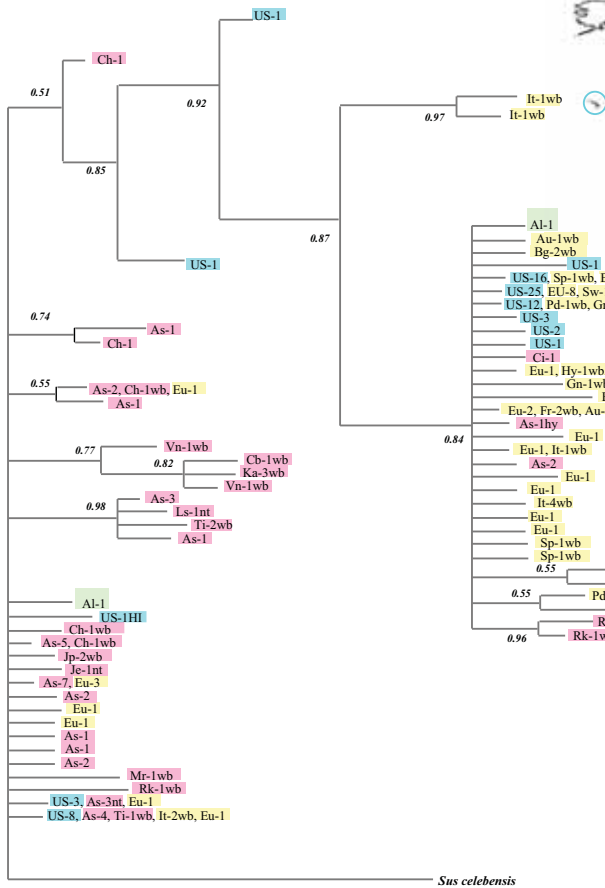
No. indicate confidence in split

This tree is based on variation along a 400 base pair region of the MtDNA D-loop obtained from 77 feral pig sequences processed from the United States and 133 published sequences from around the world accessed online at Genbank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

Country of origin is abbreviated and number of samples from each locale is indicated. Published sequence reportedly from "wild" populations are further designated with a "wb".

Information on the Bayesian analysis and Markov Chain Monte Carlo simulation is indicated below the tree along with a measure of scale for genetic distance.

-ln likelihood = 1377.743  
 10,000,000 generations 0.1



Asia & South Pacific

Europe

USA

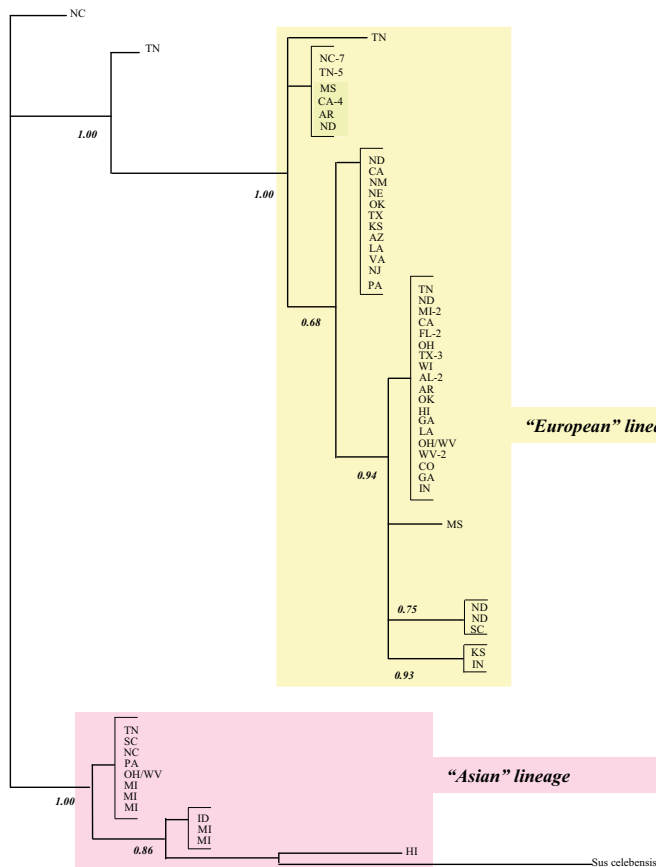
Australia

Indications are that we have a strong “Asian” lineage (left side of tree) and a “European” lineage (right side of tree). Presence of animals from European and Asian origin in both locations suggests interchange of animals between geographic locations. United States feral pig populations appear to have both Asian and European origins.

The lack of association of the two US samples at the top of the tree with any known population, and their position in the tree, points to a possible geographic origin between the European and Asian areas for which published sequences were available. These animals were collected from Great Smoky Mountains National Park, where a hybrid Eurasian wild boar population is known to exist. The origin of the founding wild stock for this population is uncertain.

-ln likelihood = 1377.743  
10,000,000 generations 0.1

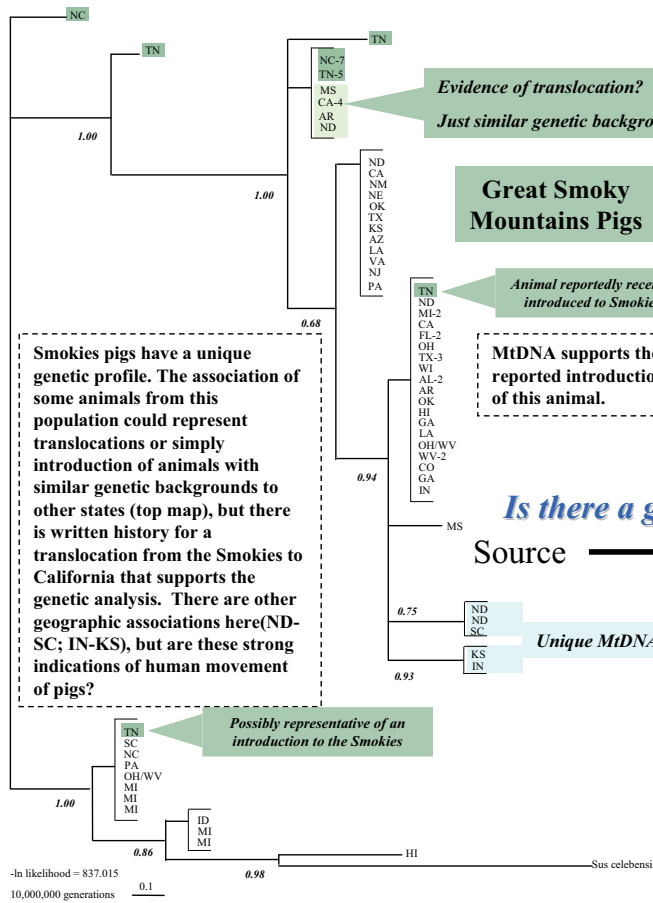
## Mitochondrial Sequence – U.S. populations 77 individuals



This tree represents U.S. feral pig samples and was produced with MrBayes 3.1 under the same evolutionary model as described previously. Note the similar structure to the tree representing the world sample and the resolution of what we consider “European” and “Asian” lineages.

-ln likelihood = 837.015  
10,000,000 generations 0.1

# Mitochondrial Sequence – U.S. populations 77 individuals



Smokies pigs have a unique genetic profile. The association of some animals from this population could represent translocations or simply introduction of animals with similar genetic backgrounds to other states (top map), but there is written history for a translocation from the Smokies to California that supports the genetic analysis. There are other geographic associations here (ND-SC; IN-KS), but are these strong indications of human movement of pigs?

Evidence of translocation?  
Just similar genetic background?

Great Smoky Mountains Pigs

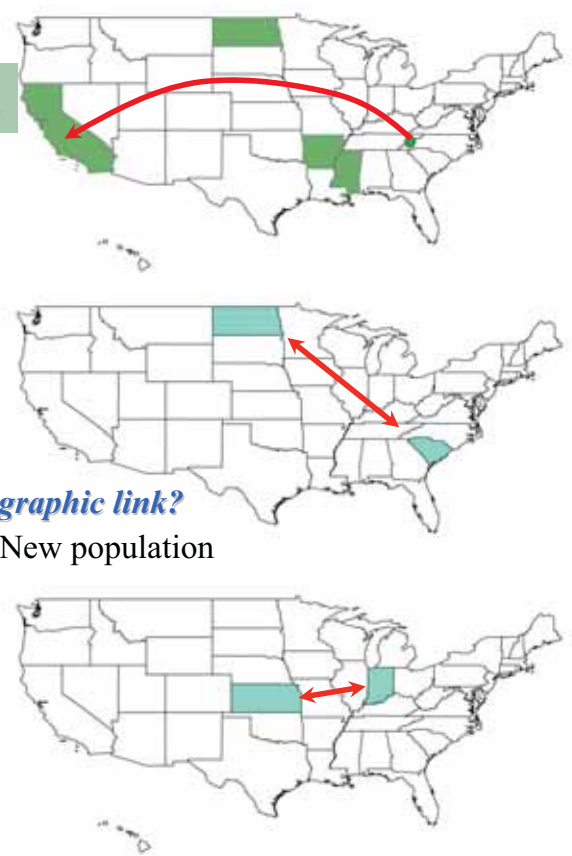
Animal reportedly recently introduced to Smokies

MtDNA supports the reported introduction of this animal.

Unique MtDNA

Possibly representative of an introduction to the Smokies

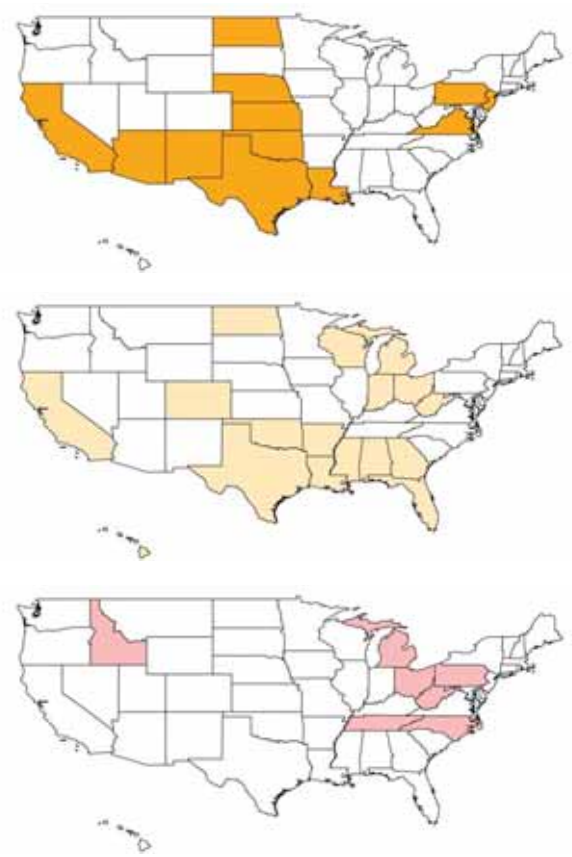
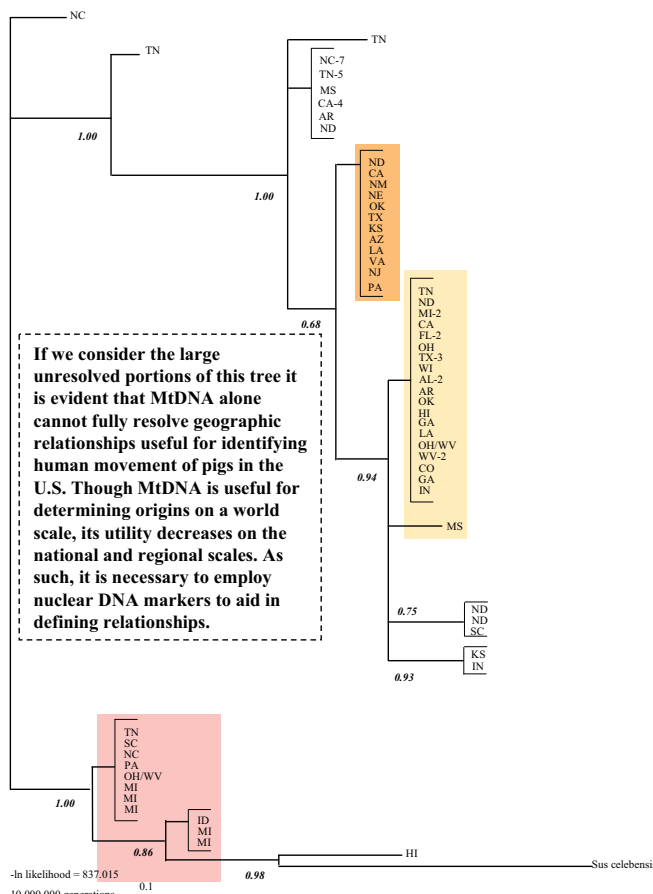
Is there a geographic link?  
Source → New population



If we consider the large unresolved portions of this tree it is evident that MtDNA alone cannot fully resolve geographic relationships useful for identifying human movement of pigs in the U.S. Though MtDNA is useful for determining origins on a world scale, its utility decreases on the national and regional scales. As such, it is necessary to employ nuclear DNA markers to aid in defining relationships.

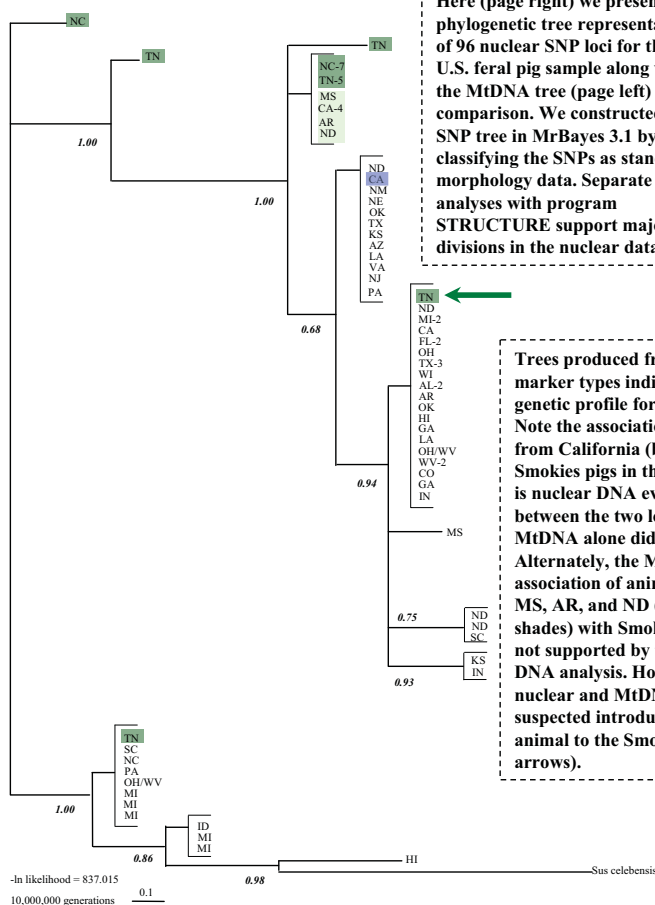
$-\ln \text{likelihood} = 837,015$   
10,000,000 generations

# Mitochondrial Sequence – U.S. populations 77 individuals



$-\ln \text{likelihood} = 837,015$   
10,000,000 generations

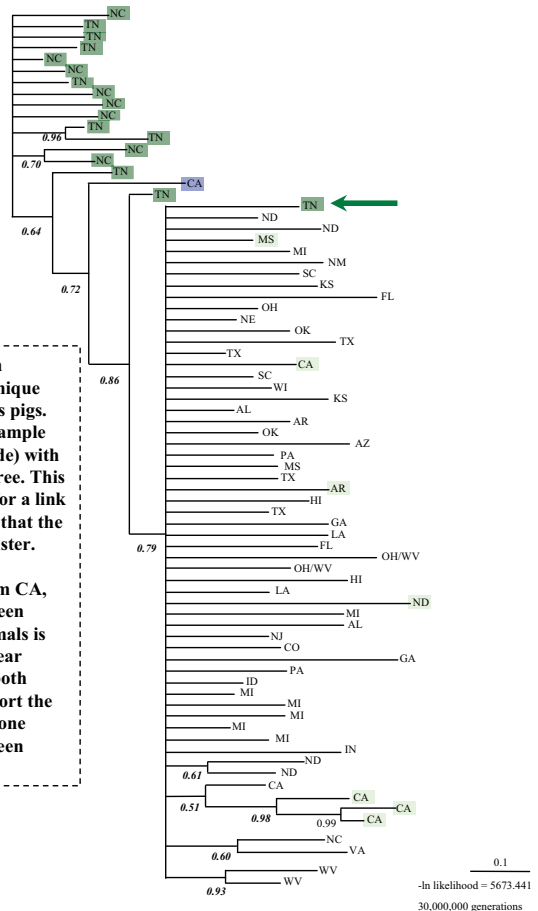
## Mitochondrial Sequence



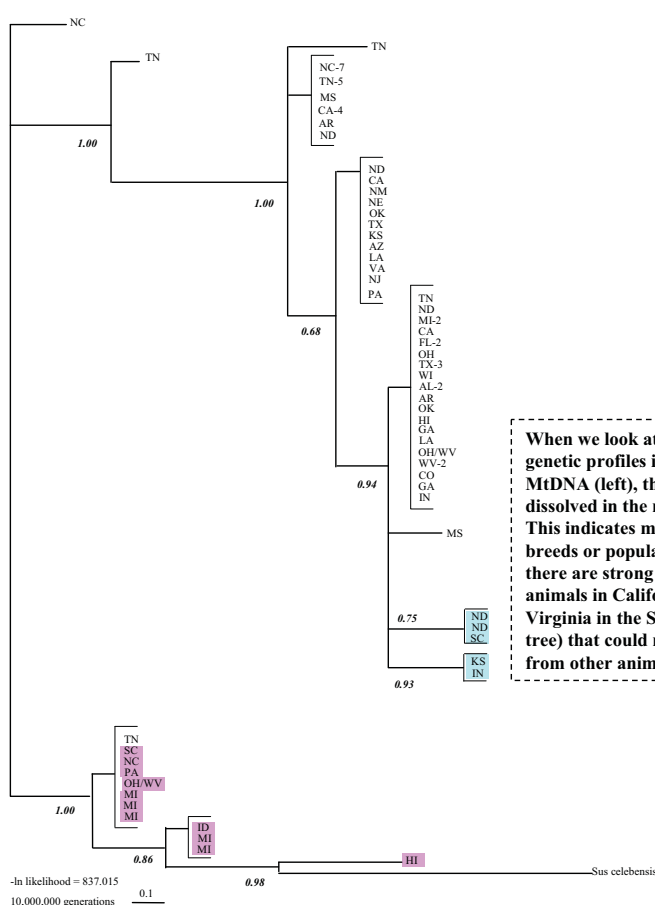
Here (page right) we present a phylogenetic tree representative of 96 nuclear SNP loci for the U.S. feral pig sample along with the MtDNA tree (page left) for comparison. We constructed the SNP tree in MrBayes 3.1 by classifying the SNPs as standard morphology data. Separate analyses with program STRUCTURE support major divisions in the nuclear dataset.

Trees produced from both marker types indicate a unique genetic profile for Smokies pigs. Note the association of a sample from California (blue shade) with Smokies pigs in the SNP tree. This is nuclear DNA evidence for a link between the two locations that the MtDNA alone did not register. Alternately, the MtDNA association of animals from CA, MS, AR, and ND (light green shades) with Smokies animals is not supported by the nuclear DNA analysis. However, both nuclear and MtDNA support the suspected introduction of one animal to the Smokies (green arrows).

## SNP 96 Loci

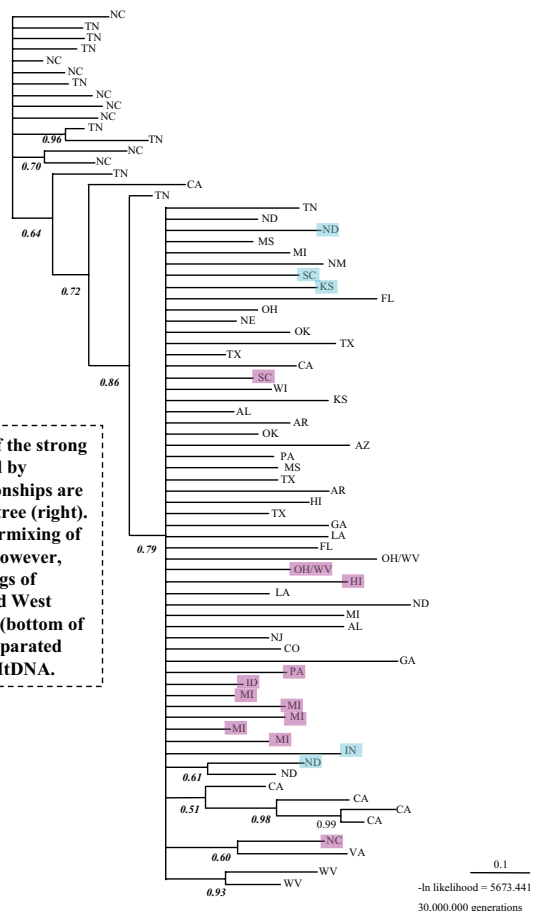


## Mitochondrial Sequence

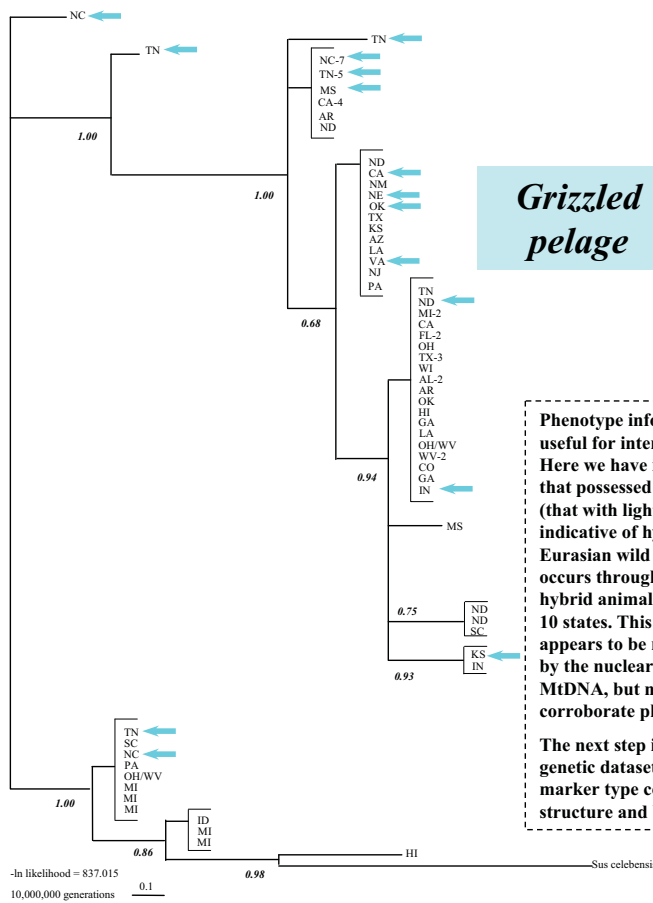


When we look at some of the strong genetic profiles indicated by MtDNA (left), the relationships are dissolved in the nuclear tree (right). This indicates much intermixing of breeds or populations. However, there are strong groupings of animals in California and West Virginia in the SNP tree (bottom of tree) that could not be separated from other animals by MtDNA.

## SNP 96 Loci



## Mitochondrial Sequence

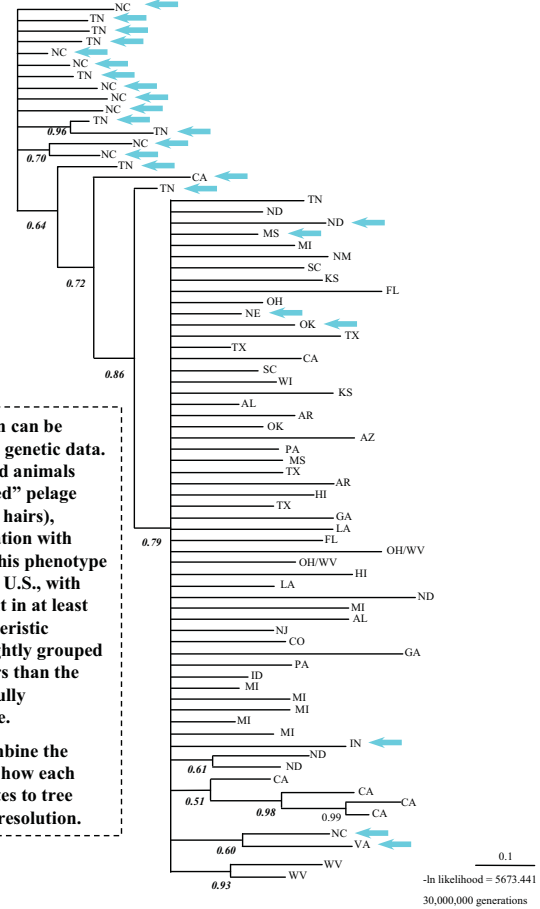


*Grizzled pelage*

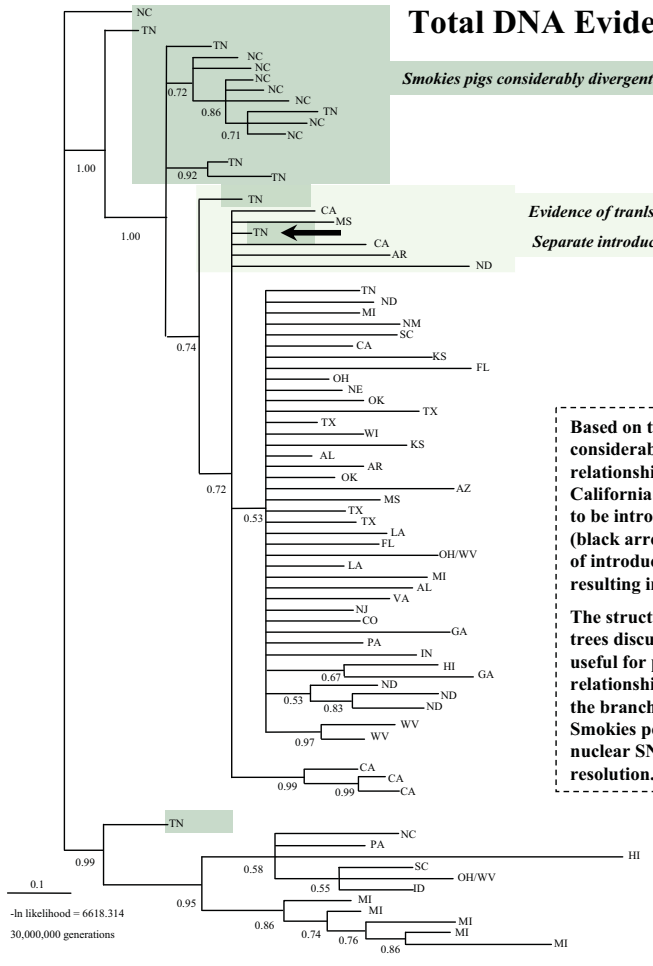
Phenotype information can be useful for interpreting genetic data. Here we have indicated animals that possessed "grizzled" pelage (that with light-tipped hairs), indicative of hybridization with Eurasian wild boar. This phenotype occurs throughout the U.S., with hybrid animals present in at least 10 states. This characteristic appears to be more tightly grouped by the nuclear markers than the MtDNA, but neither fully corroborate phenotype.

The next step is to combine the genetic datasets to see how each marker type contributes to tree structure and branch resolution.

## SNP 96 Loci



## Total DNA Evidence – MtDNA + SNP



*Smokies pigs considerably divergent from other US populations*

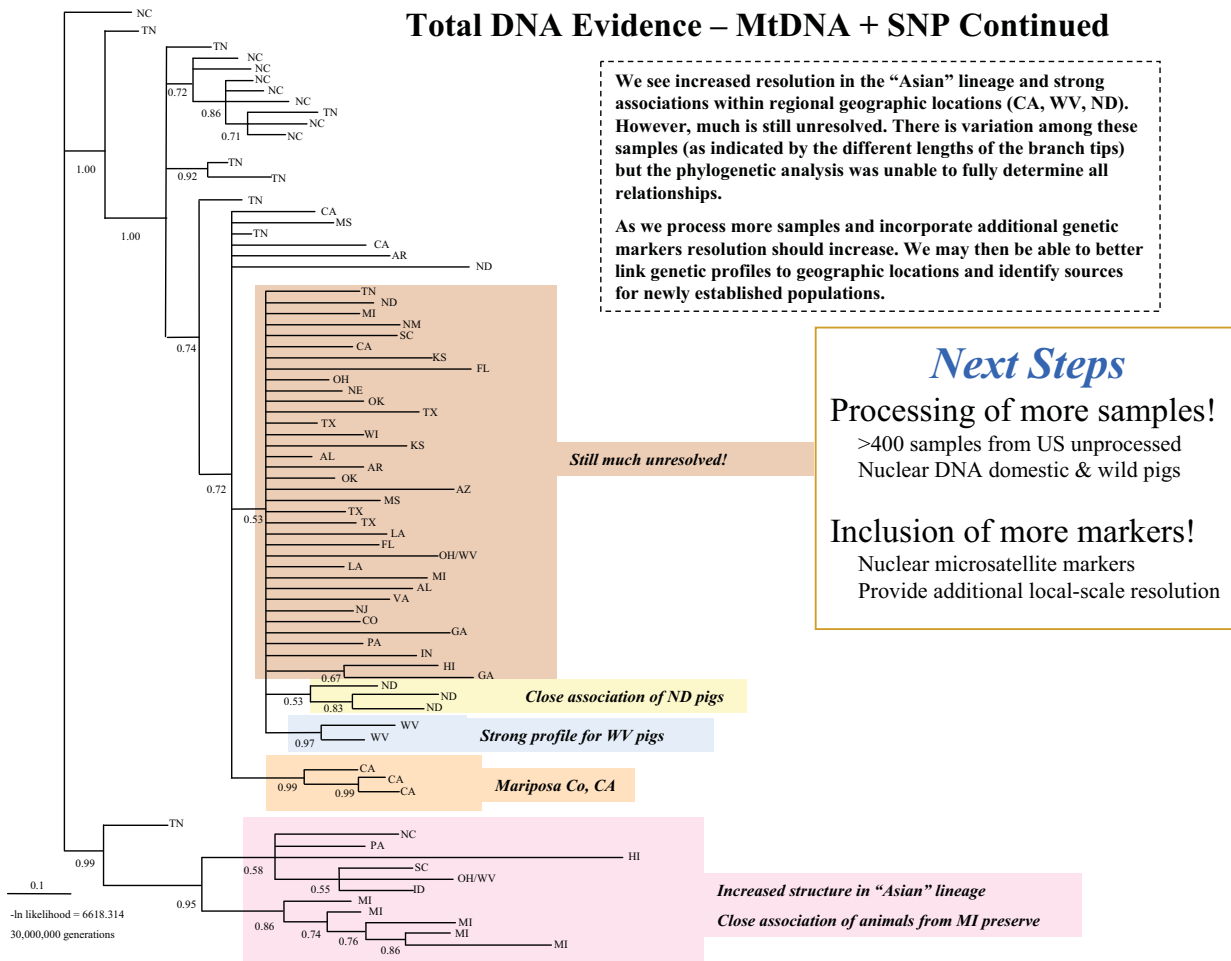
*Evidence of translocation events (e.g. Smokies to CA)  
Separate introduction of "wild" stock*

Based on total DNA evidence, Smokies pigs are considerably divergent. However, some geographic relationships once again indicate translocation events to California and other states. The animal that was thought to be introduced to the Smokies also falls out in this group (black arrow), which may indicate that it is the offspring of introduced pigs that have bred with Smokies pig, resulting in some level of nuclear DNA introgression.

The structure of this tree is similar to the other MtDNA trees discussed. As such, it seems that MtDNA is most useful for providing a "backbone" for resolving relationships. Alternately, we see increased resolution at the branch ends in the "Asian" lineage and within the Smokies population. This is evidence of the ability of nuclear SNP data to provide a high level of relationship resolution.

Here we present a phylogenetic tree representative of both MtDNA sequence and 96 nuclear SNP loci for U.S. feral pigs. We constructed this tree in MrBayes 3.1 by partitioning the data set so that sequence data were assessed under an evolutionary model and SNP data were processed as standard morphology characters. Rates were allowed to vary across datasets. By pulling both marker types into the same program we were able to assess how each contributed to our understanding of feral pig relatedness.

## Total DNA Evidence – MtDNA + SNP Continued



## Key Findings

U.S. feral populations are of European and Asian origins

Written histories of translocation (e.g. Smokies – California) corroborated

Unique genetic profiles (ND, WV, CA, Asian lineage, Smokies) present

*- useful for identifying sources of newly established populations*

A combination of Mitochondrial and Nuclear markers is necessary

**Tremendous opportunity for improved management of feral swine!**

*- identification of domestic and wild origins*

*- tracking and stopping translocations*

*- tracking potential for spread of disease*

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