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Worldwide Phylogeography of Wild Boar Reveals Multiple Centers of Pig Domestication

Greger Larson,[1]* Keith Dobney,[2] Umberto Albarella,[3] Meiying Fang,[4] Elizabeth Matisoo-Smith,[5] Judith Robins,[5] Stewart Lowden,[6] Heather Finlayson,[7] Tina Brand,[8] Eske Willerslev,[1] Peter Rowley-Conwy,[2] Leif Andersson,[4] Alan Cooper[1]*†

Mitochondrial DNA (mtDNA) sequences from 686 wild and domestic pig specimens place the origin of wild boar in island Southeast Asia (ISEA), where they dispersed across Eurasia. Previous morphological and genetic evidence suggested pig domestication took place in a limited number of locations (principally the Near East and Far East). In contrast, new genetic data reveal multiple centers of domestication across Eurasia and that European, rather than Near Eastern, wild boar are the principal source of modern European domestic pigs.

The domestication of plants and animals led to one of the most important socioeconomic transitions in human history, yet little is known about whether the process took place in a limited number of geographic regions or was a more widespread innovation involving multiple, independent "events." Wild boar were important prey animals for early hunter-gatherers across wide areas of Eurasia (*1*) until the early Holocene, when this predator-prey relation radically shifted as they, and several other large mammals, were domesticated. An extensive zooarcheological record suggests that pigs were first domesticated ~9000 years ago in the Near East (*2*), whereas more recent molecular and archaeological evidence suggests a second, independent domestication in the Far East (*3, 4*). In eastern Anatolia, several sites record gradual changes in pig morphology and demographic profile (principally a reduction in certain tooth dimensions and the increased predominance of younger animals in archaeological assemblages) (*5, 6*) over several millennia, and these have been taken to represent the domestication process in situ. Although the independent domestication of wild boar in Europe has been suggested (*7*), others have concluded that, like cattle (*8*) and sheep, pigs derived from Near Eastern genetic stock were imported by Neolithic farmers into Europe (*9*).

The wild progenitors of many Eurasian domesticates are either extinct [e.g., the aurochs (*8*) and the wild horse (*10*)] or have little or no phylogeographic structure [e.g., the wolf (*11*)]. Consequently, the broad distribution of surviving wild boar populations across the Old World provides a unique opportunity to analyze the origins of modern domestic lineages. Previous studies (*3, 12*) have identified three divergent clusters of *Sus scrofa* mitochondrial sequences, one Asian clade and two European groups, of which one consists solely of Italian wild boar. Both the Asian and European groups contain domestic breeds, yet molecular clock estimates indicate the split between the two gr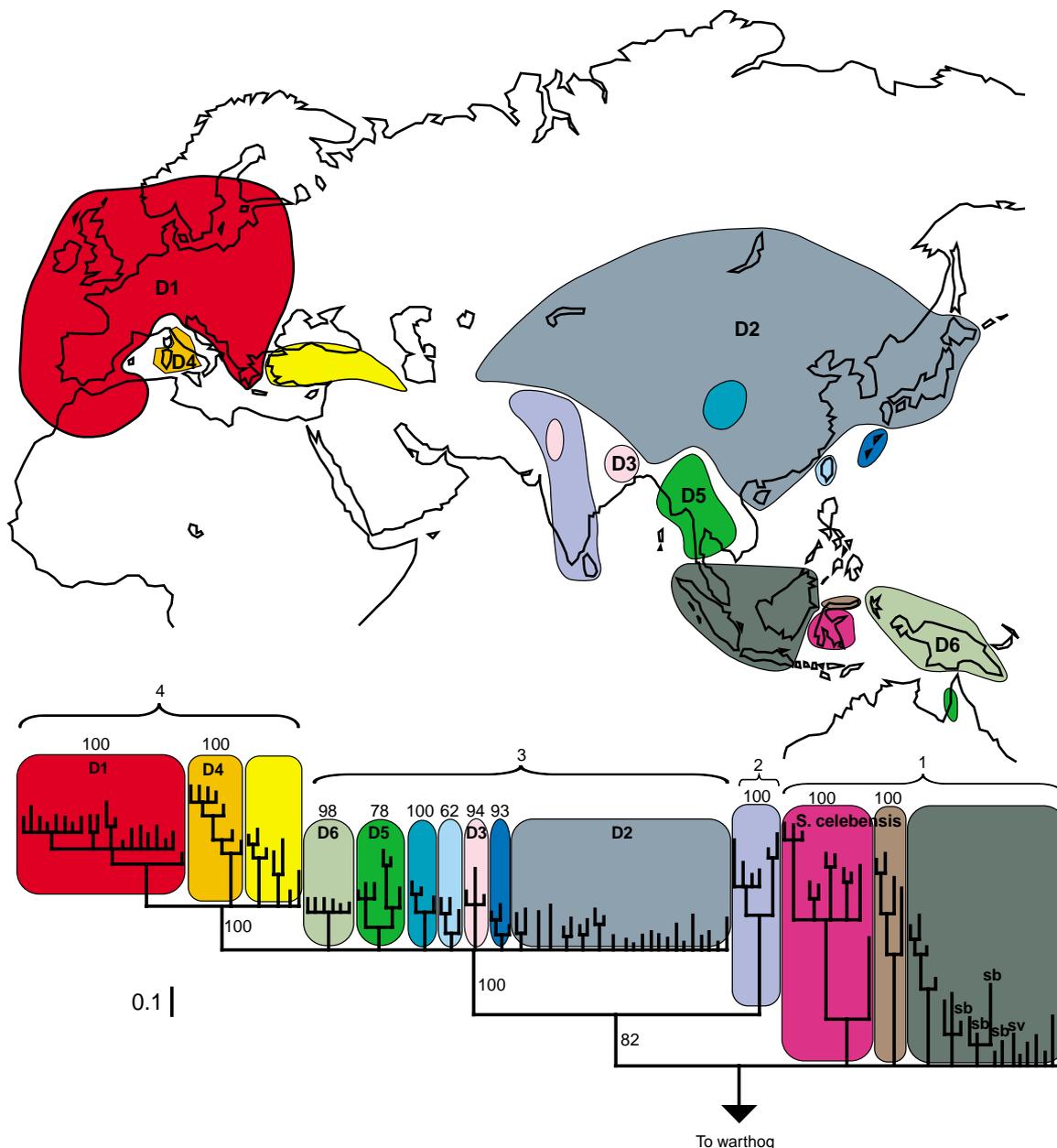oups significantly predates evidence for pig domestication, which suggests independent domestication events in each area from divergent wild boar lineages (*3, 12*).

To investigate the relationships between domestic pigs and indigenous wild boar across their range, we sequenced 663 base pairs (bp) of the mitochondrial control region from 165 wild and feral pigs primarily from museum specimens, using appropriate ancient-DNA methods (*13*), and from 58 domestic pigs. An additional 463 individual pig sequences were obtained from GenBank, and phylogenetic analyses were performed using Bayesian Monte Carlo–Markov chain (MCMC) (*14*) and median-joining networks (*15*). The consensus tree (Fig. 1) shows that the basal lineages of *S. scrofa* occur in western island Southeast Asia (ISEA). An initial dispersal from this area into the Indian subcontinent was followed by subsequent radiations into East Asia and a final, progressive spread across Eurasia into Western Europe. The marked East-West split among wild boar is consistent with morpho-

[1]Henry Wellcome Ancient Biomolecules Centre, University of Oxford, Department of Zoology, South Parks Road OX1 3PS, UK. [2]Department of Archaeology, University of Durham, South Road, Durham DH1 3L, UK. [3]Department of Archaeology, University of Sheffield, West Street, Sheffield S1 4ET, UK. [4]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences and Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala Biomedical Center, Box 597, SE-75124 Uppsala, Sweden. [5]Department of Anthropology and Allan Wilson Centre for Molecular Ecology and Evolution, University of Auckland, P.B. 92019, Auckland, New Zealand. [6]Jurox Pty Limited, 85 Gardiners Road, Rutherford, NSW, 2320, Australia. [7]Department of Genomics and Bioinformatics, Roslin Institute, Roslin, Midlothian, EH25 9PS, UK. [8]Department of Evolutionary Biology, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen O, Denmark.

*To whom correspondence should be addressed. E-mail: greger.larson@zoo.ox.ac.uk (G.L.) and alan.cooper@adelaide.edu.au (A.C.)
†Present address: School of Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

logically based studies, which have highlighted the distinctiveness of the Southeast Asian forms (*Sus scrofa vittatus*) in particular (*16*). Interestingly, fewer than 2% of wild boar specimens (*13*) violate the otherwise consistent correlation between phylogeny and geography, and these are likely the result of past, human-mediated introgression and/or dispersal. This strong phylogeographic structure provides an opportunity to trace the geographic origins of domestic pig lineages.

Modern domestic European pig breeds fall within the general European cluster (excluding recently "improved" breeds known to have been crossbred with Asian varieties over the past 200 years) and lack any affinity with wild boar lineages present in the Middle East

(Fig. 1). The complete lack of Turkish, Armenian, or Iranian signatures within modern European breeds implies that, even if domesticated Near Eastern pigs entered central Europe with early Near Eastern farmers, those lineages have left no descendants among modern European domestic pigs. Network analysis (Fig. 2) provides further resolution of the closely related European haplotypes and reveals two core lineages (A+C), separated by a transversion, each of which is surrounded by a starlike pattern consistent with a recent population expansion, analogous to that seen in cattle (*8*). Statistical analyses (Fu and Li's *F* and Tajima's *D*) were consistent with a population expansion for both wild and domestic pigs in Europe and Asia, although statistical significance was

only reached for Fu and Li's *F* in the domestic populations (*13*). Unlike cattle, however, the two core pig haplotypes are only present in Europe (or European-derived populations), which indicates the independent domestication of at least two European wild boar lineages, although the intermingling of wild boar and domestic pig sequences throughout the network suggests that numerous additional wild boar lineages may also have been domesticated. The only region in which both core haplotypes are indigenous is Germany, which suggests that central Europe may have been a center for early European pig domestication. It is noteworthy that only two of the haplotypes on the network are found within both wild boar and domestic pigs; this indicates that the wild boar sampled

in this study are unlikely to be derived from feral animals.

Network analysis of Asian domestic and wild haplotypes (Fig. 3) shows starlike rapid expansion patterns similar to those in Europe but less marked. Two haplotypes (GX and AE) found in wild boar (morphologically wild individuals from the Chinese provinces of Gansu and Hunan) are shared by several East Asian domestic pigs, although the diffuse nature of the network is also compatible with domestication of more than just these two wild boar lineages. Unlike the rather ambiguous zooarcheological record for China, where claims for early pig domestication have recently been questioned (4), these data clearly support recent genetic studies that have identified at least one pig domestication event in East Asia (12). Haplotypes I, FH, and L (Fig. 3) include feral and domestic pigs found across Asia, Australia, New Zealand, Hawaii, the United States, and Europe and appear to have been the primary haplotypes that were distributed worldwide. It is interesting that a genetically distinctive subclade of central Chinese wild boar, indigenous to Sichuan, Shanxi, and Shensi provinces, has not contributed to any of the modern Asian domestic breeds sampled (Fig. 1).

The presence of an Indian domestic pig sequence within the cluster of phylogeographically differentiated wild boar lineages from India (D3 in Fig. 1) indicates the existence of yet another independent domestication event and supports claims for pig domestication in India (16). Numerous introduced Australian pigs from the west coast of Cape York have a haplotype that falls within a cluster of differentiated wild boar from Burma and Thailand (D5 in Fig. 1). The presence of an exotic nematode and a Melanesian tick on the pigs also suggest that some pigs in Cape York are of Southeast Asian stock (17). The absence of any historical evidence for the introduction of Southeast Asian wild boar to Northern Australia, combined with the coat color variation in these feral pig populations, strongly supports their domestic origin and, therefore, identifies this part of Southeast Asia as yet another region where independent pig domestication has occurred.

The strong phylogeographic structure of pig mitochondrial sequences also provides clear evidence for human-mediated dispersal of this species. For example, S. scrofa was not present on the Mediterranean islands of Corsica and Sardinia before the seventh millennium B.C. (18). Thus, the extant populations of so-called miniature "wild boar" found on these islands has long been interpreted as a human-aided, prehistoric introduction of wild or domesticated (now feral) pigs. Indeed, all but 2 of the 15 wild boar sampled on both islands appear near the center of the European haplotype network (fig. S2). Two anomalous sequences on Sardinia, however, share a close affinity to the phylogenetically distinctive Italian wild boar clade found in Maremma (D4 in Fig. 1), the only region where native Italian wild boar populations have not been replaced by more recent introductions (19). Although the European lineages on Sardinia may represent recent introductions and/or introgressions, the presence of the distinctive Italian signature in at least two Sardinian pigs supports the idea of an early Italian origin. If the ancestors of these two pigs were originally introduced as domestic animals (that have since gone feral), these individuals must represent a lineage of pigs independently domesticated on the Italian mainland.

Our data also have implications for conflicting hypotheses regarding Pacific prehistory. Although the dates for the first human settlements in Near Oceania (the region from New Guinea to the Solomon Islands) range between 30,000 and 40,000 years ago, undisputed evidence of domesticated animals (pigs, dogs, and chickens) does not appear in the region until about 3500 to 3300 years before the present (B.P.), in association with the appearance of the Lapita cultural complex (20). Claims of pre-Lapita pigs as old as 10,000 B.P. have been made (21), and although pigs were probably present by 6000 B.P. (22), all of these dates remain contentious and have yet to be validated through carbon dating (20). The New Guinea pig haplotypes in this study cluster with pigs from Hawaii, Vanuatu, and Halmahera in a monophyletic group (here termed the "Pacific

Fig. 2. A parsimonious median-joining network depicting the relationships between European domestic breeds and European wild boar located solely in the red portion of Fig. 1 labeled D1. Node sizes are proportional to haplotype frequencies in the dataset. Colors within the nodes: yellow, domestic; green, wild; orange, feral; blue, unknown status; and red, inferred intermediate haplotypes not represented by any sampled pigs. Lines between nodes represent a single nucleotide change, except where perpendicular hashes represent single changes.
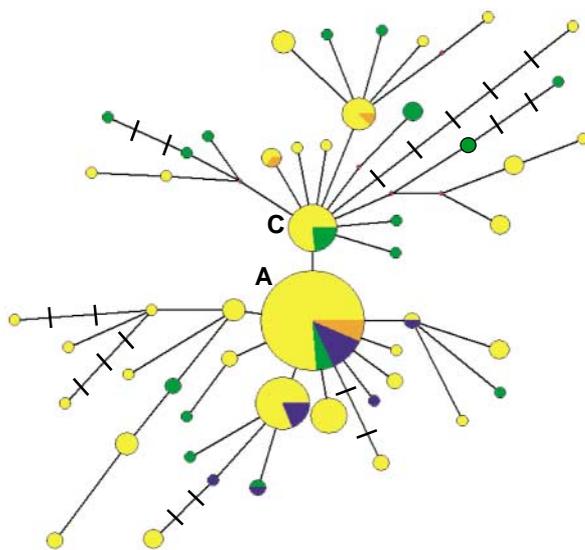
Fig. 3. A median-joining network depicting the relationships between Asian domestic breeds and closely related Asian wild boar located solely in D3 (Fig. 1). Colors are identical to Fig. 2, except gray pie segments denote samples identified as wild boar but more likely feral pigs (13). Only two haplotypes (AE and GX) contain both Asian domestic breeds and definitive Chinese wild boar (from Gansu and Hunan provinces, China). Japanese wild boar clusters are shown within the three shaded regions. Haplotypes I, FH, and L collectively contain feral and domestic pigs found in Asia, Australia, New Zealand, Hawaii, the United States, and Europe.
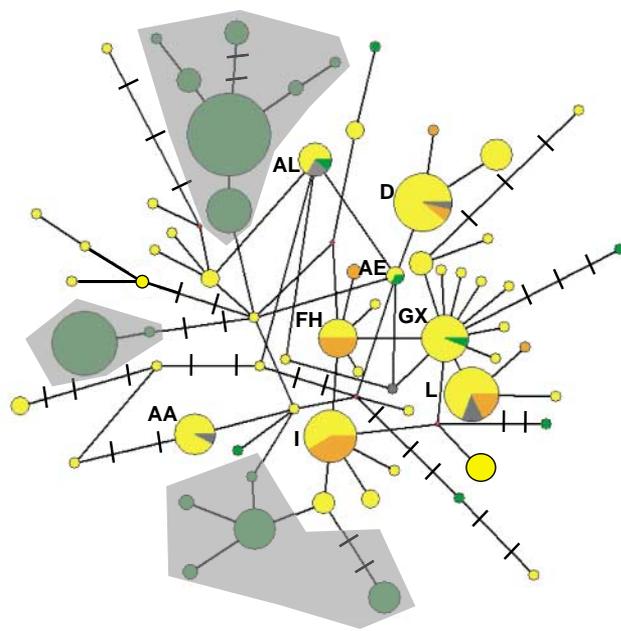
clade") within the large Eastern Eurasian cluster and are well separated from any other individuals, domestic or wild (D6 in Fig. 1). This evidence is consistent with a Lapita dispersal from Near to Remote Oceania, but the lack of any genetic affinity between this group and Taiwanese wild boar (Fig. 1) offers no support for the "Out-of-Taiwan" model (*23*) of human and pig dispersal into Near Oceania. This evidence also supports the importance of Halmahera, which has been shown to be the origin of Remote Oceanic populations of the Pacific rat (*Rattus exulans*) transported by Lapita peoples (*24*) and the origin of the human mitochondrial DNA (mtDNA) marker known as the "Polynesian motif" (*25*). The additional lack of affinity of the New Guinea pigs with *Sus celebensis* (the indigenous Sulawesi wild boar, also found on offshore islands including Halmahera) rules out a significant *S. celebensis* maternal genetic input as suggested by Groves (*16*).

The lack of an obvious genetic source population on either mainland or island Southeast Asia from which pigs in the Pacific clade were drawn is intriguing and may suggest either the existence of indigenous *S. scrofa* in Wallacea or an early human-mediated introduction from elsewhere in ISEA currently not sampled by our study. In either case, then ISEA must be considered another independent center of pig domestication. Interestingly, the two clades of *S. celebensis* (Fig. 1 and fig. S1) demonstrate that this group is not monophyletic. In fact, the North-South geographic partitioning of the two strongly implies two independent invasions of wild boar onto Sulawesi, a pattern identical to that reported for other Sulawesi fauna including macaques (*26*), shrews (*27*), and bovids (*28*). Lastly, an ISEA origin of the other sister taxa of *S. scrofa* (Javan warty pig, *S. verrucosus*, and the bearded pig, *S. barbatus*) is supported by the phylogenetic tree, although the paraphyletic arrangement provides no support for current species designations. Thus, further studies using both mtDNA and nuclear DNA are required to resolve the phylogenetic status of these taxa.

The genetic evidence presented in this study provides clear proof for multiple centers of domestication across Eurasia. To further examine the domestication of pigs, the zooarcheological records of Europe, India, Southeast Asia, and ISEA should be explored in more detail.

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High-Throughput Mapping of a Dynamic Signaling Network in Mammalian Cells

Miriam Barrios-Rodiles,[1] Kevin R. Brown,[2] Barish Ozdamar,[1,3] Rohit Bose,[1,3] Zhong Liu,[1] Robert S. Donovan,[1] Fukiko Shinjo,[1] Yongmei Liu,[1] Joanna Dembowy,[1,3] Ian W. Taylor,[1,3] Valbona Luga,[1,3] Natasa Przulj,[4] Mark Robinson,[5] Harukazu Suzuki,[6] Yoshihide Hayashizaki,[6] Igor Jurisica,[2,4,7] Jeffrey L. Wrana[1,3]*

Signaling pathways transmit information through protein interaction networks that are dynamically regulated by complex extracellular cues. We developed LUMIER (for luminescence-based mammalian interactome mapping), an automated high-throughput technology, to map protein-protein interaction networks systematically in mammalian cells and applied it to the transforming growth factor–β (TGFβ) pathway. Analysis using self-organizing maps and *k*-means clustering identified links of the TGFβ pathway to the p21-activated kinase (PAK) network, to the polarity complex, and to Occludin, a structural component of tight junctions. We show that Occludin regulates TGFβ type I receptor localization for efficient TGFβ-dependent dissolution of tight junctions during epithelial-to-mesenchymal transitions.

Dynamic protein-protein interactions (PPIs) are key for cell signaling and dictate the timing and intensity of network outputs. Systematic mapping of PPI networks has thus far focused on static analyses in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans* (*1–5*). To begin building an understanding of how signaling networks convey information in vertebrates, we developed high-throughput

LUMIER to systematically map PPIs in mammalian cells. This strategy uses *Renilla* luciferase enzyme (RL) fused to proteins of interest, which are then coexpressed with individual Flag-tagged partners in mammalian cells. Their interactions were determined by performing an RL enzymatic assay on immunoprecipitates using an antibody against Flag (Fig. 1A). As a model for a systematic study of cell signaling, we focused on the