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Archaeogenomic insights into commensalism and regional variation in pig management in Neolithic northwest Europe

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The relationship between humans and pigs has changed dramatically since their domestication in southwest Asia and subsequent human-induced introduction into Europe. Introgression between incoming southwest Asian pigs and European boar resulted in the gradual replacement of southwest Asian ancestry in European pigs. However, we currently lack genomic data required to explore the regional trajectories, nature, and extent of contact between European boar and pigs that led to this turnover, and how this process was facilitated by human activity. We addressed this deficit by sequencing four Mesolithic boar and seven Neolithic pig samples from six archaeological sites in the Netherlands and Britain ranging from the Mesolithic (5500 BCE) to Neolithic (2500 BCE). Our data show that despite continuous gene flow with European boar, Neolithic European pigs show varying levels of southwest Asian ancestry. The low and varying southwest Asian ancestry in pigs from the Early Neolithic Dutch settlement Swifterbant indicates a high contribution of wild ancestry. The genetic profile, enriched $\delta^{15}N$ values, on-site presence, and wide size distribution of Swifterbant *Sus scrofa* suggest a commensal relationship. Runs of homozygosity (ROH) imply that both closed-breeding and free-ranging management occurred in Neolithic communities, where the former showed an extreme burden of long segments of ROH. We further show selection signatures, associated with coat color and behavior, in Neolithic herds despite recurrent wild gene flow. Altogether, our results show distinct husbandry practices through space and time in Neolithic Europe, with heavy reliance on boar recruitment via the commensal pathway in northwest Europe.

Neolithic | pigs | animal husbandry | wild boar | human-animal relationships

Significance

The genomic makeup of pigs changed rapidly after their introduction from southwest Asia to Europe, with domestic southwest Asian ancestry largely replaced by European wild boar ancestry. The role of humans in this process remains elusive. To address this, we sequenced Mesolithic boar and Neolithic pigs from northwest Europe. Neolithic pig management was not uniform but ranged from closed husbandry to free-ranging commensal relationships. Despite this, southwest Asian ancestry preserved suggesting genetic continuity. Genomic changes between Neolithic European pigs and modern boar indicate strong selective pressures counteracting wild gene flow. Altogether, our data from the earliest dated northwest European farming settlement of Swifterbant indicate the recruitment of local wild boar into the anthropogenic niche through the commensal pathway.

Human–pig relationships have changed dramatically over the last 10,000 y (1–3). For millennia, hunter-gatherers across Eurasia, notably in northwest Europe, relied on wild boar for subsistence and ritual practices (4–7). Predomestication wild boar exploitation strategies varied through time and space, e.g., from targeting large individuals to targeting family groups of females and young adults (3, 7–9). Recurrent commensal interactions between humans and wild boar led to the independent domestication of pigs in southwest Asia (~8500 BCE) (10) and China (~4000 BCE) (11). Southwest Asian domesticated pigs were introduced into Europe *circa* 6500 BCE accompanying farming communities and had reached the Paris Basin by 5000 BCE (12, 13). This spread involved contact between incoming domestic pigs and local European wild boar, resulting in wild gene flow which gradually replaced the southwest Asian ancestry in managed herds (12, 14).

Little is known about the frequency and extent of contact between European wild boar and incoming domestic pigs and the socioeconomic factors underlying these processes. For example, in northern Europe (the Netherlands, northern Germany, Denmark, and Belgium; ~5500 to 4500 BCE), predominantly inhabited by hunter-gatherers, the practice of hunting wild boar persisted for roughly a millennium (5–7) despite continuous contact with neighboring farming communities (15–19). However, domestication signatures (e.g., southwest Asian mitochondrial haplotypes and mutations causing black coat color) have been detected in a Mesolithic context in northern Germany, indicating domestic-to-wild introgression (5). This process seems to have continued for millennia (12, 14), altering the genetic makeup of both domestic and wild populations. The consensus is that some form of loose management provided the opportunity for recurrent gene flow between domestic pigs and wild boar (5, 14, 20). The persistence of a mutation in the *MC1R* gene [associated with the change from camouflage to black coat color in pigs (21)], originating in southwest Asia (14), implies that human-mediated selection and controlled breeding occurred alongside continual admixture. Selective pressures,

The authors declare no competing interest.

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whether adaptive or human-mediated, have been important processes shaping both prehistoric and modern European domestic pigs.

Here, we explore the genetic variation in Mesolithic European wild boar and Neolithic European pigs to assess how human–mediated control and continual admixture affected their genomic landscape and selective signatures. We obtained genome-wide data from 11 archaeological samples of *Sus scrofa* from five archaeological sites in the Netherlands (5540 to 3380 BCE), and one in Britain (2525 to 2470 BCE) (Fig. 1*A* and *SI Appendix*, Fig. S1 and Table S1). These sites constitute ideal case studies because of their heavy economic reliance on *Sus scrofa* (7, 22–24), proximity and contact with neighboring farming communities (15, 17, 18) and earliest evidence for crop cultivation (25) and animal husbandry (24) beyond the expansion of Neolithic cultures in Central Europe. The chosen sites include a Mesolithic context (Hardinxveld-Polderweg and Hardinxveld-De

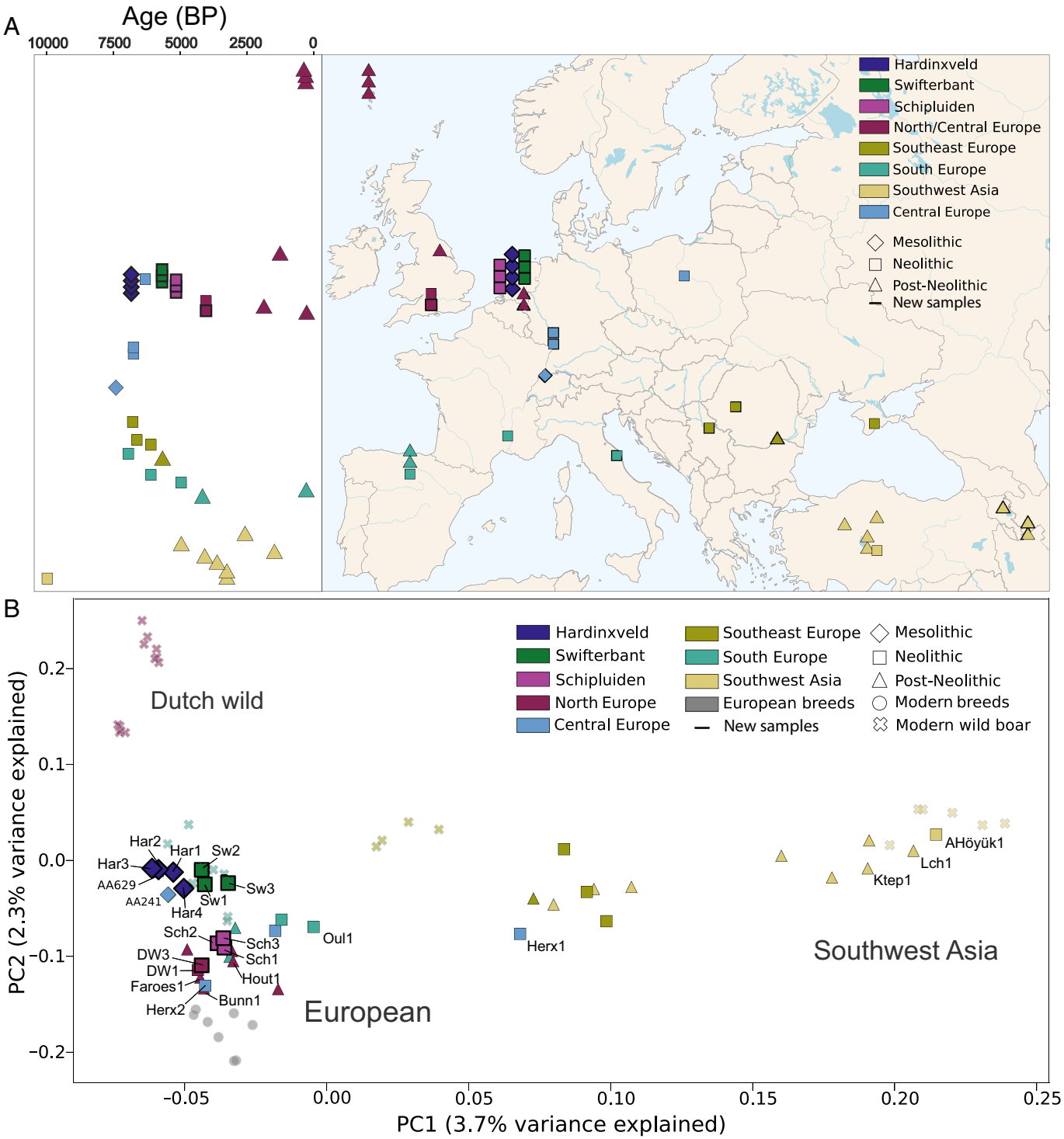

Fig. 1. Temporal and geographical distribution of ancient samples and a projected Principal Component Analysis (PCA). (*A*) Temporal and geographical distribution of ancient samples used in this study; Symbols denote chronological periods and colors denote geographical origins. New samples are shown with a bold black outline. (*B*) A PCA projecting low-coverage ancient samples on the variation of modern and high-coverage ancient individuals, showing principal components 1 and 2. The PCA shows structured variation that mirrors the geographical distribution, where the newly introduced samples (bold outline) cluster within the European variation. The PCA excludes Asian wild boar and domestic breeds with Asian influence (*SI Appendix*, Fig. S7). New samples are shown with a bold black outline.

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bruin), Early Neolithic context with a combination of hunter-gatherer and farming subsistence strategies (Swifterbant S2 and S3) and two younger Neolithic contexts (Schipluiden and Durrington Walls).

Results and Discussion

Genome Sequencing of Archaeological European *Sus scrofa*. DNA was extracted from 11 *Sus scrofa* petrous bones (Fig. 1*A* and *SI Appendix*, Fig. S1 and Table S1). Namely, from the Netherlands, four samples were obtained from the earliest occupation phases at the Mesolithic Hardinxveld-Polderweg [5450 to 5300 BCE (26)] and Hardinxveld-De Bruin [5400 to 5120 BCE (26)], hereafter labeled collectively as "Hardinxveld" (*SI Appendix*, Table S1). Additionally, three samples were obtained from the Early Neolithic Swifterbant sites S2 (4300 to 4000 BCE) and S3 [4220 to 4050 BCE (27)], hereafter labeled "Swifterbant" (*SI Appendix*, Table S1) and three were obtained from the Late Neolithic site of Schipluiden [3630 to 3380 BCE (18)] (*SI Appendix*, Table S1). Finally, one sample was obtained from the Late Neolithic henge of Durrington Walls [2525 to 2470 BCE (28)], in Britain (*SI Appendix*, Table S1). Of these samples, molecular sex was determined, nine were identified as female and two as male (*SI Appendix*, *Molecular Sex Identification* and Table S1). Genomic coverage ranged between 0.03× and 6.16×, with a median of 0.8× (*SI Appendix*, Table S1). Molecular damage and read length distributions are consistent with the ancient origin of the recovered genomes (*SI Appendix*, Figs. S2-S3). This dataset doubles the number of medium-coverage (1 to 6.2×) Neolithic European genomes and introduces Mesolithic European wild boar genomes above 0.1×. These new data (n = 11) were analyzed alongside previously published ancient (n = 72) and modern (n = 78) sequences (14, 29–31) (Dataset S1 and *SI Appendix*, Table S2).

Mesolithic European Wild Boar Show Genetic Discontinuity with Modern Dutch Wild Boar. Modern European wild boar variation is often used to infer the demographic population history of boar and pig populations. However, genetic variation in Mesolithic European wild boar before and during the early stages of farming is poorly understood. Our four Mesolithic Hardinxveld wild boar genomes from the Netherlands (Har1–4: 5450 to 5120 BCE) offer the opportunity to explore genetic variation in a Mesolithic context contemporary with the first European farming culture in the northern plains, the LinearBandKeramik culture (LBK).

The mitochondrial phylogeny illustrates the admixture between European wild and domestic individuals, as the European mitochondrial clade includes both wild and domestic individuals from ancient and modern contexts (*SI Appendix*, Fig. S4). The Mesolithic Hardinxveld wild boar fall within the European mitochondrial clade, consistent with matrilineal recruitment of wild boar into managed herds. An autosomal neighbor-joining phylogenetic reconstruction distinguishes between European wild and domestic genomes, where the European wild boar clade separated from the European domestic clade, the Mesolithic Hardinxveld wild boar form a cluster within the European wild boar clade (*SI Appendix*, Figs. S5 and S6).

A PCA shows that the Hardinxveld wild boar (Fig. 1*B* and *SI Appendix*, Fig. S7*C*) cluster with a Mesolithic Swiss wild boar (AA241: ~6000 BCE), a Neolithic Italian wild boar (AA629: Late Neolithic) and modern Spanish, French, and northern Italian/Swiss wild boar, indicating genetic continuity from the Mesolithic (6000 to 5100 BCE) to present day. Interestingly, modern Dutch wild boar form their own cluster on PC2, away from the Mesolithic Hardinxveld and modern central European wild boar (Fig. 1*B*), suggesting a shift in the ancestry of modern Dutch wild boar since the Mesolithic. One plausible event that could account for this shift is the (near) extinction of wild boar in the Netherlands during the 19th century, driven by overhunting and habitat fragmentation caused by deforestation that has been going on since the High Medieval period (32–35). Followed by their reintroduction from Germany in the early 20th century, since their reintroduction the wild boar population in the Netherlands has experienced repeated cycles of decline and growth, primarily due to direct human intervention (32). These population turnovers and strong bottlenecks most likely explain the genomic variation we observe in modern Dutch wild boar and why it moved away from the Mesolithic variation. This highlights why modern wild boar populations are imperfect analogies for their prehistoric counterparts.

Ancestry modeling (ADMIXTURE) shows that the Hardinxveld genomes have a completely European boar ancestral profile, indicating that gene flow from domestic to wild was entirely absent or not strong enough to detect in the wild boar population surrounding the Hardinxveld region at the end of the 6th millennium BCE (Fig. 2 and *SI Appendix*, Fig. S8). In addition, one Hardinxveld wild boar (Har3) was homozygous wild type for the D124N mutation in the *MC1R* gene. This specific mutation is associated with a change in pigmentation that shifts the coat color phenotype from the wild-type brown, which provides natural camouflage, to a derived black phenotype (21). The black phenotype, linked to a specific derived allele, is often associated with a domestic status. The homozygous wild status of the Hardinxveld boar (Har3) indicates that it exhibited the descriptive brown camouflage coat color associated with wild boar (Dataset S1), further indicating the lack of gene flow. This is intriguing, because archaeological evidence shows contact between Hardinxveld hunter-gatherers and neighboring Neolithic communities (15, 18).

Neolithic Northwest European *Sus scrofa* Show Signs of Commensalism. The Early Neolithic Dutch Swifterbant settlement (S2 and S3; 4300 to 4000 BCE), the earliest known Neolithic settlement in the Dutch wetlands, is often described with its mixed subsistence strategies, consisting of both wild and domestic resources (25, 36). While osteometrics and stable isotope analysis indicate that people at Swifterbant practiced cattle husbandry (24), tillage marks show conclusive evidence for crop cultivation (25), but ample fish, waterfowl, and beaver remains show that wild resources of the surrounded wetlands were also utilized (22). However, human–pig relationships in Swifterbant remain ambiguous, despite the fact that ~60% (of the number of identified specimens) of the mammalian remains were from *Sus scrofa* (24). Moreover, *Sus* in Swifterbant have a wide size distribution, with outliers on either end (22, 24). The size distribution of *Sus scrofa* in Swifterbant do not indicate distinct wild and managed domestic populations, which one would expect from a domestic population like Schipluiden and Durrington Walls (23, 37). Archeological evidence from the later site of Schipluiden (3630 to 3380 BCE), the first year-round settlement in the Dutch wetlands, does show this divide between domestic pig and wild boar populations (37). The subsistence strategies of Schipluiden are predominantly focused on domestic resources, where cattle is the dominant species in the mammalian remains, with pigs being second (37, 38). The subsistence strategy and culture in Schipluiden is similar to contemporaneous Neolithic settlements (18).

Our three individuals from Swifterbant (Sw1-3: 4300 to 4000 BCE), three individuals from Schipluiden (Sch1-3:3630 to 3380 BCE) and one individual from Durrington Walls (DW3: 2525 to 2470 BCE) all possess European wild boar mitochondrial haplotypes (*SI Appendix*, Fig. S4), in agreement with previously shown matrilineal recruitment of wild boar into managed herds (14). An autosomal phylogenetic reconstruction illustrates that the Swifterbant

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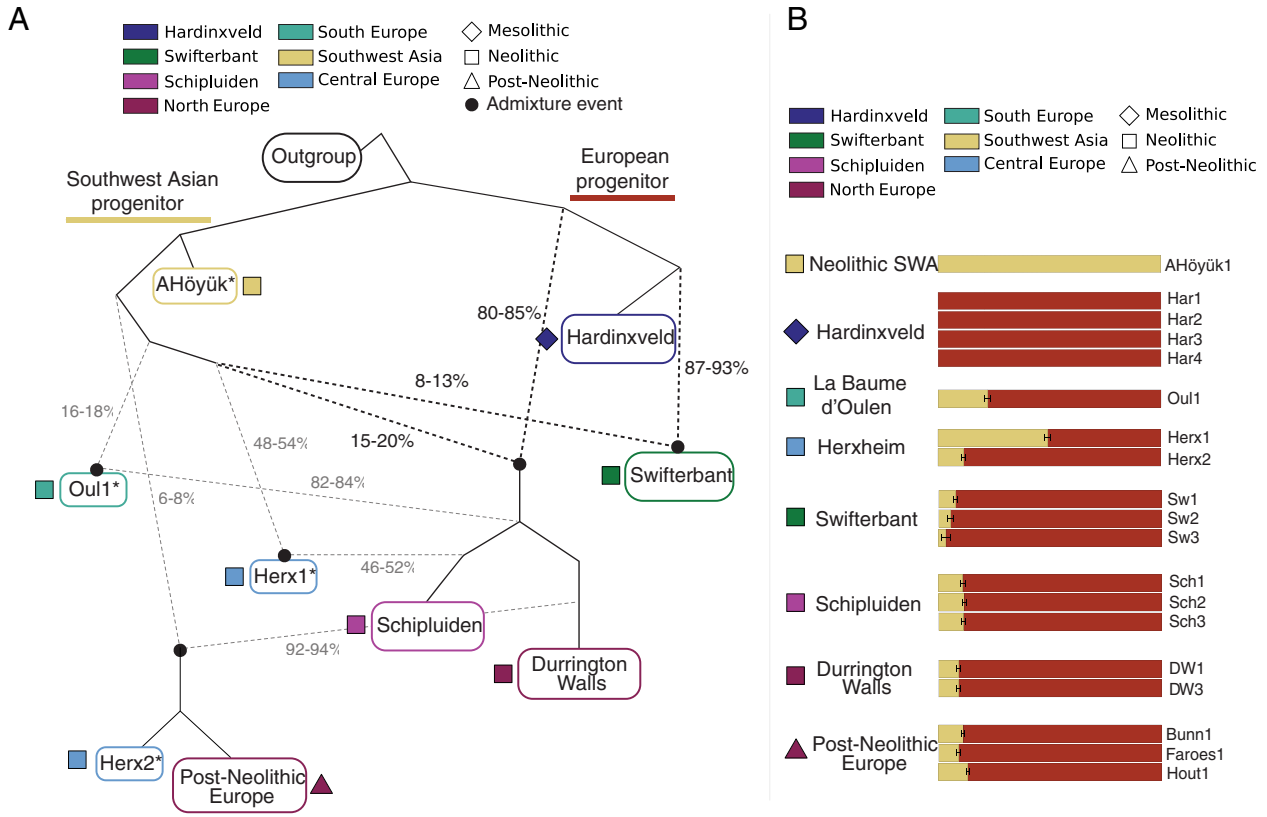

Fig. 2. Admixture graph of ancient Sus scrofa and an ADMIXTURE analysis. (*A*) An admixture graph reconstructing the population history and gene flow events (m = 5) of Neolithic and post-Neolithic Sus scrofa. Dashed lines indicate (bidirectional) admixture events, with five migration events as optimal (*SI Appendix*); symbols denote chronological periods and color indicates geographical regions. * Indicates single individuals. (*B*) An ADMIXTURE analysis modeling the analyzed genomes as mosaics of hypothetical ancestral genomes. Symbols denote temporal periods and color indicates geographical regions. The colors on the bar charts are associated with ancestry, where southwest Asian (SWA) ancestry are in yellow and European ancestry are in red. The ADMIXTURE analysis excluded Asian wild boar and domestic breeds (*SI Appendix*, Fig. S8).

individuals and the Mesolithic wild boar form a subclade within the larger Dutch, Spanish, and French wild boar clade (*SI Appendix*, Figs. S5 and S6). The phylogeny places the Schipluiden individuals as a basal sister group to this clade. It is notable that the two Swifterbant individuals do not form a monophyletic grouping. However, gene flow can complicate the interpretation of basal relationships in phylogenies (39, 40) and gene flow between European wild boar and southwest Asian domestic pigs in the Neolithic has previously been demonstrated (14). This could explain the trend observed between the Schipluiden individuals and Hardinxveld wild boar. The newly sequenced Durrington Walls individual (DW3) and the previously sequenced individual (DW1) (14) cluster together outside of the "wild boar" clade.

A PCA shows a similar trend, where the Swifterbant individuals fall on the outside of the Mesolithic Hardinxveld group (Fig. 1*B*). However, the Late Neolithic Schipluiden individuals fall more toward the Durrington Walls, the Early Neolithic LBK Herxheim (Herx2: 5340 to 4900 BCE) and post–Neolithic European individuals. They form a group positioned away from the European wild boar toward local modern European breeds (Fig. 1*B* and *SI Appendix*, Fig. S7 *B* and *C*). The autosomal phylogenetic analysis and PCA positioning indicate distinct genetic variation and possible gene flow events, though further research is necessary to confirm and clarify these patterns.

To formally test for southwest Asian ancestry in Swifterbant and Schipluiden we performed a *D*-statistic of the form: *D*(*Outgroup, Neolithic Anatolian wild; X, Hardinxveld wild*), where X stands for Mesolithic and Neolithic individuals. This *D*-statistic (significance threshold Z >= 3) indicated that all Neolithic European individuals tested possessed southwest Asian ancestry. This affinity is least pronounced in the Swifterbant individuals (Sw1-3) and most pronounced in an Early Neolithic LBK Herxheim individual (Herx1: 5340 to 4900 BCE; *SI Appendix*, Fig. S9). Mesolithic D-statistics involving a Swiss wild boar (AA241: ~6000 BCE), were not significant as expected, indicating a lack of southwest Asian ancestry in this individual; however, the Mesolithic Swiss wild boar has low coverage. In the future other high-coverage Mesolithic wild boar should be sequenced to test this hypothesis.

To further investigate gene flow and potential genetic affinity to southwest Asian pigs we inferred the amount of local boar and southwest Asian ancestry in an ADMIXTURE analysis, where the Swifterbant individuals all possess high levels of European ancestry and low, variable amounts of southwest Asian ancestry (Sw1 7.5%, Sw2 5.4%, Sw3 3.3%). Southwest Asian ancestry in Schipluiden was higher and less variable than in the Swifterbant individuals (10.9 to 11.5%; Fig. 2*B* and *SI Appendix*, Fig. S8 *B* and *C*). A *qpGraph* analysis corroborates southwest Asian ancestry in Swifterbant and Schipluiden, where an optimum of five admixture events was found (Fig. 2*A* and *SI Appendix*, Figs. S10 and S11 and Table S3.1). While these admixture graphs do not have enough power to capture continuous or repeated pulses, due to low sample size, they do imply an intricate network of gene flow in European *Sus* populations in the Neolithic and presence of southwest Asian ancestry in Swifterbant and Schipluiden (*SI Appendix*, Table S3.2).

High-resolution data from key sites and regions are crucial to understand how human–pig relationships shaped this introgression. Swifterbant, the earliest well-dated Neolithic settlement in northern Europe, provides the ideal case study due to the high frequency of

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Sus scrofa remains recovered from the site. While the Swifterbant *Sus scrofa* carry predominantly local boar ancestry, the persistence of ~3.3 to 7.5% southwest Asian ancestry and the movement away from the Mesolithic wild boar in all three individuals suggest that they did not derive from a purely wild boar ancestral stock. Biometric (22, 24), stable isotope, (24) and contextual coprolite data (41) corroborate this interpretation. Sw2 with a southwest Asian ancestry of ~5.4% exhibits enriched δ^{15}N values of +9.22‰ (*SI Appendix*, Fig. S12 and Table S1), clustering with small-bodied *Sus scrofa* and morphologically domestic cattle with similarly enriched δ^{15}N values (24). Enriched δ^{15}N values are commonly interpreted as signatures of a human–mediated diet (42). *Sus scrofa* coprolites from Swifterbant deposits contain fish and cereal microremains as well as sheep hair (41), strongly suggesting that *Sus scrofa* benefited from this sedentary anthropogenic niche.

The combination of evidence for varying southwest Asian ancestry, movement away from the Mesolithic wild boar toward the domestic spectrum in a PCA, the high frequency (60% of the Number of Identified Specimens in the mammalian assemblage), on or near-site presence, human–mediated diet and intermediate size distribution of *Sus scrofa* all point at complex human–pig relationships in Swifterbant. This relationship between *Sus scrofa* and humans in Swifterbant was most likely of a commensal nature, where wild boar were attracted to anthropogenic environments and potentially their domestic counterparts, adapting to a new anthropogenic niche. This behavior is not uncommon for wild boar, it has been shown to occur in prehistoric times, and even today (1, 43, 44).

In this sense, human–boar–pig interactions in and around Swifterbant were not dissimilar to the process of initial pig domestication in southwest Asia (2, 10), where pigs gradually adapted to novel anthropological niches and were allowed to roam freely in and outside of the settlement, interbreeding with wild boar. In Swifterbant, the community practiced agriculture, managed cattle, and kept domestic pigs, likely free-roaming. The precise dynamics of pig–human relations in this setting is difficult to surmise. A possible hypothesis is that the free-roaming of *Sus scrofa* around the settlement and the incorporation of wild boar into the domesticated cohort were deliberate management strategies, akin to practices observed among indigenous populations in New Guinea (45). However, unlike the sex-biased interactions seen in New Guinea, where domestic sows primarily mate with male wild boar, mitochondrial and rapid genomic replacement in Europe (12, 14) suggests that interactions at Swifterbant involved both male and female wild boar. Closer to home, ethnographic research in Europe shows that control over the mobility and reproduction of *Sus* sp. varies depending on management strategies. Free-ranging pig husbandry in Iberia, Italy, Serbia, and Greece focuses on preventing wild boar from interbreeding with their domestic stock, due to the undesirable effects (e.g., lower growth rate and leaner meat) (46–50). However, in Greece free-ranging pig husbandry is becoming more lenient, allowing domestic pigs to roam freely and interbreed with wild boar (48). This shift is likely due to the reduced reliance on local agriculture and a focus on subsistence farming rather than commercial production (48). An ethnographic study on urban wild boar in Spain highlights the plasticity of human–boar relationships, illustrating how wild boar continue to exploit anthropogenic niches even in modern societies (51). Notably, reliance on pigs increased toward the end of the fifth and start of the fourth millennium throughout Central and North Europe (52–54) and was paired with an increase in the mean size of domestic pigs (52, 55). This indicates that the commensal relationship observed in Swifterbant is not an isolated event but it shaped human–pig relationships throughout Central and northern Europe.

In contrast, the tight placement of the Schipluiden individuals (3630 to 3380 BCE) on a PCA, their similar ADMIXTURE ancestral components and distinct size distributions suggest a more controlled managed environment. This is in line with previous zooarchaeological research (37), mirroring Neolithic settlements like Durrington Walls. However, the Schipluiden pigs show diversity in their *MC1R* mutations, Sch1 is heterozygous, Sch2 is homozygous for the domestic type, and Sch3 is homozygous for the wild type. The latter shows the wild brown camouflage color, while the former two show the domestic black coat color (Dataset S1). This together with zooarchaeological evidence suggests that wild boar were still exploited and most likely incorporated in the domestic stock in Schipluiden (37), but to a much more limited extent than in the previous millennium.

Runs of Homozygosity (ROH) Indicate Community-Specific Management Strategies in Neolithic Europe. ROH provide a good proxy to assess population dynamics and the possibility of inbreeding. Small to medium ROH (1.0 to 4.0 Mb) are indicative of a bottleneck or a prolonged small population size, whereas large ROH (>4.0 Mb) are often indicative of recent inbreeding (56). A ROH analysis was performed on diploid genotypes to allow for heterozygote calls, something that is not feasible with the single read sampling approach typically taken in ancient genome studies. ROH analysis was performed on a dataset of 62 moderns and 11 ancient samples (*SI Appendix*, *Diploid Variant Calling* and *Runs of Homozygosity*). To mitigate allelic dropout caused by differences in sequencing coverage (*SI Appendix*, Fig. S13 and Table S4), all individuals were downsampled to 4.4×. ROH detection was performed using multiple complementary methods: plink, with settings allowing either no heterozygotes or a single heterozygote per sliding window; a window-based heterozygosity rate analysis; and ROHan (57). The latter two had a heterozygosity rate threshold (*SI Appendix*, Table S5). A detailed discussion is provided in *SI Appendix*, *Run of Homozygosity* analysis section.

The low number and cumulative sum of ROH in a Neolithic Anatolian wild boar (AHöyük1: 8350 to 8050 BCE) suggest that the wild population which was domesticated in southwest Asia was likely from a relatively outbred population (Fig. 3, *SI Appendix*, Figs. S14–S16, and Dataset S2). In contrast, Early Neolithic Central and South European pigs show both higher numbers and cumulative sums of ROH than the Neolithic Anatolian wild boar. 21.5% (Oul1: Cardial Culture, 5450 to 4950 BCE; *SI Appendix*, *Archaeological Sites*) to 26.1% (Herx1: LBK Culture, 5340 to 4900 BCE; *SI Appendix*, *Archaeological Sites*) of their autosomes consist of ROH larger than 1 Mb, with 4.7% (Oul1) and 3.7% (Herx1) consisting of ROH >4 Mb (Fig. 3*A* and Dataset S2). The relatively high number of small- to medium-sized ROH observed in the Neolithic Cardial Culture pig from la Baume d'Oulen in France (Oul1) and a Neolithic LBK pig from Herxheim Germany (Herx1) suggests a bottleneck (Fig. 3*B*), and is indicative of a history of inbreeding; The plausibility of inbreeding is even more evident under the more lenient approach (Fig. 3*C* and *SI Appendix*, Figs. S14 and S17–S20; as detailed in *SI Appendix*, *Run of Homozygosity* analysis). This bottleneck effect has been earlier observed in human populations that suffered from small population size, isolation, and inbred marriages (58, 59). Multiple studies have shown that a bottleneck is mostly observed in small and medium-sized ROH, whereas inbreeding is mostly observed in large ROH (58–60). Moreover, studies on nonhumans also found an effect of population history on ROH, where higher sums and numbers of small to medium-sized ROH (1 to 4 Mb) were found in bottlenecked or island populations compared to populations with large population sizes (60–67). This potential bottleneck could have been the result of relatively small numbers of pigs being introduced from southwest Asia into Europe, a possibility supported by

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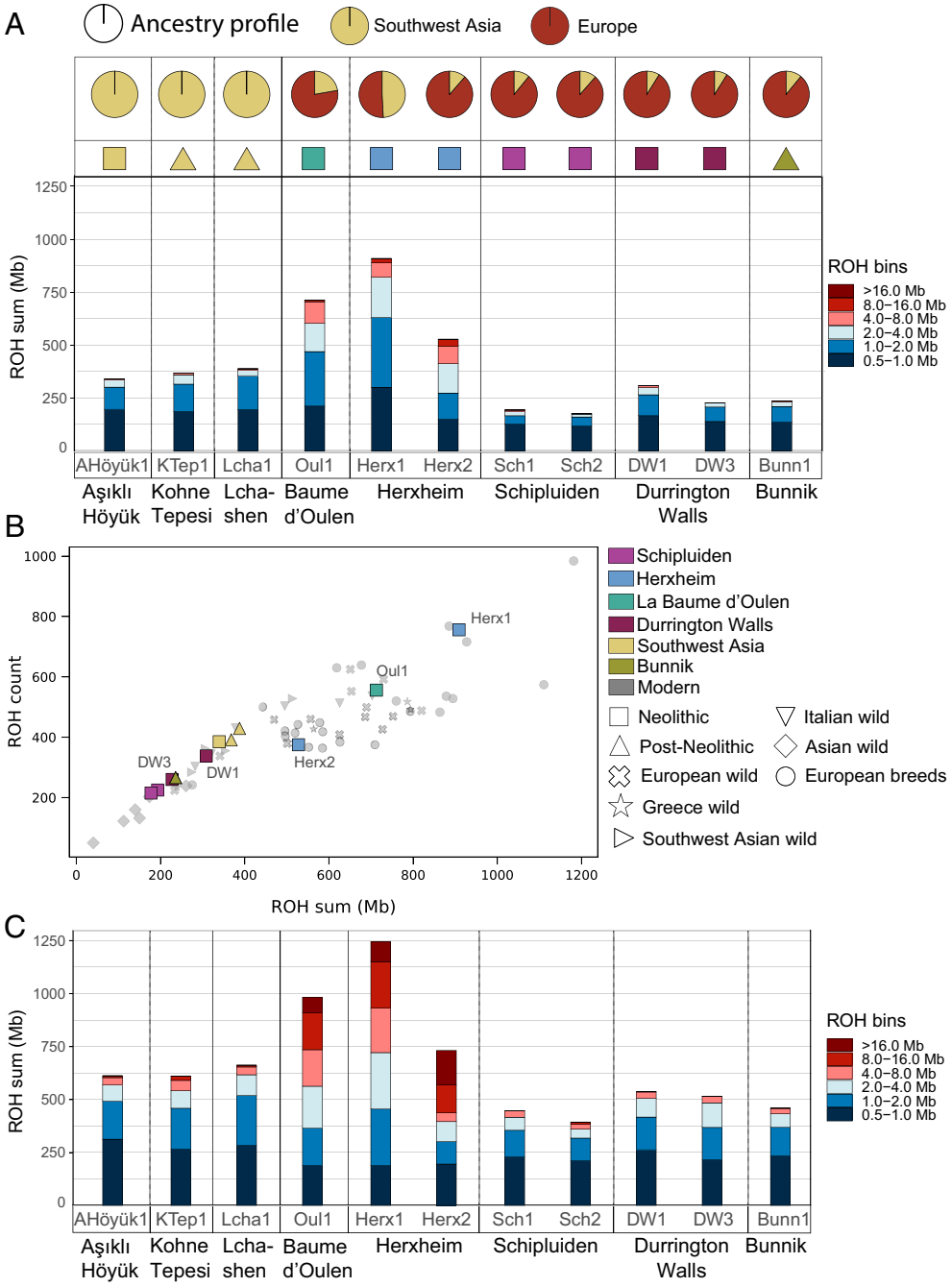

Fig. 3. ROH demographics of ancient Sus scrofa. (*A*) ROH profiles of 11 ancient Sus scrofa, from stringent ROH (0 het). Pie charts denote ancestry percentages from ADMIXTURE. Symbols denote temporal periods and color indicates geographical regions. The colors in the bar charts indicate different ROH bin sizes. The ROH analysis shows that early Neolithic settlements (Oul1, Herx1-2) show an increased level of long ROH, whereas later Neolithic settlements such as Schipluiden and Durrington Walls show low numbers of long ROH. (*B*) Distribution of ROH counts against the sum of ROH >1 Mb. Samples on the left of the dashed line on the *x* axis indicate an excess of large ROH while samples in the upper range of the *y* axis indicate an excess of ROH. (*C*) ROH profiles of 11 ancient Sus scrofa, from lenient ROH (1 het). The colors in the bar charts. indicate different ROH bin sizes. The ROH analysis shows that early Neolithic settlements (Oul1, Herx1-2) show an increased level of long ROH, whereas later Neolithic settlements such as Schipluiden and Durrington Walls show low numbers of long ROH.

zooarchaeological research which shows that settlements close to the original expansion zone (Anatolia and southeast Europe) have few or even no pigs (52, 53, 68).

Another explanation is that the local European wild boar population was small at the beginning of the Holocene. Indeed, demographic history shows that European wild boar populations underwent population declines during the Late Pleistocene, which lasted until the last glacial maximum (69). Furthermore, wild boar occurrences in the archaeological record of Europe show that from 47,000 y ago until 11,000 y ago the archaeological record of wild boar was relatively low (70). However, an increase in European wild boar was observed starting from ~11,000 y ago (70), indicating that ROH > 4 Mb would have been broken down due to recombination and would most likely not have prevailed in wild populations ~3,000 y later (60, 71). Thus, the most likely explanation for the ROH patterns we observe is a bottleneck associated with the initial migration from southwest Asia.

Modern European wild boar populations tested in this study show elevated levels of number and cumulative sum of ROH (*SI Appendix*, Fig. S21), being the most extreme in the Dutch wild boar population, which has previously been shown to have suffered from recurrent bottlenecks (32, 34, 35). Furthermore, a study on European wild boar from different geographical regions (Netherlands,

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Switzerland/northern Italy, and Greece) showed high amounts of inbreeding and recent pig hybridization, which were suggested relating to recent anthropogenic impact instead of natural processes (72). Consequently, the ROH patterns observed in our modern wild boar populations are most probably related to more recent anthropogenic factors. This again, highlights that modern European wild boar populations are imperfect analogies for their prehistoric counterparts.

Herx2, another Neolithic LBK pig from Herxheim shows the highest cumulative sum for ROH > 8 Mb (Fig. 3 *A* and *C*, *SI Appendix*, Figs. S14, S22, and S23, and Dataset S2). The low number of ROH and more specifically the low number of small- to medium-sized ROH compared to Herx1 and Oul1 suggest no past bottleneck event. Instead, Herx2 has 3 long ROH >8 Mb (Fig. 3*A* and Dataset S2), with the largest being 14 Mb, which indicates that Herx2 has related ancestors in its recent genealogy (73). Moreover, Herx2 falls on the right side of Fig. 3*B*, indicating a larger cumulative length of ROH compared to the number of ROH, which is indicative of inbreeding, and is also observed in human populations with severe inbreeding (58, 59, 74), livestock species (75, 76) and wild populations (77, 78). Additionally, when a more lenient approach to ROH detection is employed—permitting one heterozygous site per window (as detailed in *SI Appendix*, *Run of Homozygosity* analysis)—Herx2 exhibits five ROH segments exceeding 16 Mb (ranging from 24.71 to 43.55 Mb; *SI Appendix*, Figs. S23 and S24). This observation strongly suggests inbreeding events or mating between closely related individuals (73, 74).

In contrast to the Early Neolithic pigs (la Baume d'Oulen and Herxheim) the Late Neolithic Schipluiden pigs have a low number of ROH and the lowest cumulative sum of ROH of the ancient individuals tested, with little to no ROH > 4 Mb (Fig. 3, *SI Appendix*, Figs. S14 and S25–S28, and Dataset S2; Swifterbant was excluded due to lack of coverage). This might be attributed to the continual admixture with European wild boar or potentially the incorporation of wild boar into the domestic stock, resulting in a continual large source population. Admixture between populations with varying ancestry has been observed to lower large and medium-sized ROH in cattle (79, 80), goats (61, 76), killer whales, (77) and humans (56, 58). Even with the more lenient approach, the Schipluiden individuals exhibit the lowest cumulative sum of ROH, with the addition of ROH > 4 Mb (Fig. 3*C*). There is a slight difference in ROH profiles of the Durrington Walls pigs, DW1 shows a slightly larger number and cumulative sum of ROH compared to DW3 (Fig. 3 *A* and *B*). Due to the small sample size it is difficult to determine whether this is due to stochasticity. Moreover, this difference between the Durrington Walls pigs is less with the more lenient approach (Fig. 3*C* and *SI Appendix*, Fig. S14). Overall their ROH profile is similar to the Schipluiden individuals (Fig. 3, *SI Appendix*, Figs. S25–S32, and Dataset S2). A low number and cumulative sum of ROH was inferred in an Iron Age Dutch pig (Bunn1), showing a similar profile as the Schipluiden individuals in both the stringent and more lenient approach (Fig. 3 and *SI Appendix*, Figs. S14, S33, and S34).

These inbreeding patterns demonstrate a genomic impact of human-controlled pig management in Early Neolithic South/Central Europe and subsequent shift to more loose management in the Late Neolithic in northern Europe. Long ROH (>4 Mb) in Early Neolithic Cardial and LBK pigs suggest strong signatures of inbreeding, indicating that these Early Neolithic communities had a degree of control on the breeding of pigs. Less is known about the specifics of this control over breeding, i.e. whether this control necessitated direct intentional breeding of pigs with specific phenotypes or it was unintentional and a consequence of a continuous small population size. Moreover, wild boar were hunted at both la Baume d'Oulen and Herxheim (14, 81), showing that there was contact between humans and wild boar. The most plausible scenario is that these individuals were part of a more closed form of management, with more control over breeding compared to the free-range loose management seen later in the Neolithic (3600 to 2500 BCE).

Selective Pressures Throughout Neolithic Europe Result in Genomic Changes. Genomic changes that occurred in prehistoric domestic populations undergoing substantial gene flow with their wild counterparts are poorly understood. We identified outlier regions (0.1% Fst values) between eight Neolithic European genomes and 27 modern wild boar genomes from Europe (n = 24) and southwest Asia (n = 3) (*SI Appendix*, Table S6). 46 outlier regions indicated 57 genes located in or near these peaks (Fig. 4 and *SI Appendix*, Table S6.2).

Two outlier genes are associated with coat color variation (SI-Fst; Dataset S3 for GO terms). One of these genes is *KITLG*, linked to coat pattern variations in modern pigs (21), goats, and cattle (82, 83), and was also identified in a similar analysis in Neolithic goat populations (84). Another gene, *RAPGEF2*, linked to coat color variation in pigs, is in close genomic proximity and in the same molecular pathway as *KIT* (85). Coat color variation is a phenotypic trait that is often associated with domestication and has been found in several studies of ancient domesticates (14, 84, 86–88). In pigs, a mutation in the *MC1R* gene, resulting in black coat color, compared to the brown camouflage coat color, was present from the onset of the Neolithic (14). The *MC1R* mutation is heterogeneous within our dataset (Dataset S1), an effect of the continuous admixture with wild boar in the Neolithic, which may explain the absence of a strong signal in the Fst analysis. Altogether, this indicates that coloration traits in pigs were selected for and maintained by early farmers, potentially for either practical (e.g., to distinguish pigs/herds), or for aesthetic reasons.

Six outlier genes were associated with adaptive responses to occupying anthropogenic niches. Of these outlier genes, *GRIK2*, *NRG1*, and *PRKN* are associated with behavioral responses in animals (e.g., anxiety, response to external stimuli, social interaction; Dataset S3 for GO terms) to human-mediated stressors (89–91). In particular, *GRIK2* has been suggested to play a major role in the domestication of rabbits (92), ducks (93), and dogs (94). Two outlier genes are associated with microbiota profiles in pigs (*MUC2* and *THSD7B*; Dataset S3 for GO terms); both genes are involved in host control in gut microbial communities (95, 96). Additionally, the gene *N4BP1* is associated with antiviral response (97). These genes' differential genetic variation between Neolithic European pigs and modern wild boar might be related to the degree of proximity to

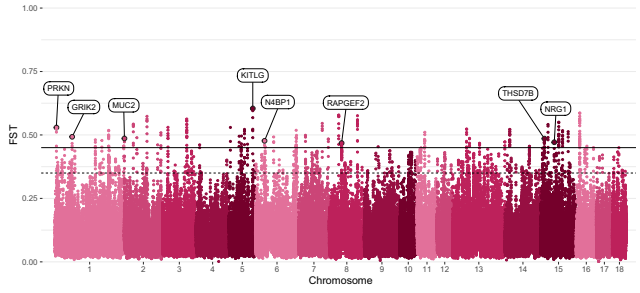

Fig. 4. Genome-wide distributions of Fst values between Neolithic European genomes and modern European (24) and southwest Asian (3) wild boar. The 18 autosomes are plotted along the *x* axis and the genetic differentiation coefficient (Fst) values are plotted along the *y* axis. Chromosomes are indicated by different colors. The dashed line represents a suggestive threshold of 0.5 percentile and the solid line represents the threshold of 0.1 percentile. The outlier genes mentioned are marked.

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humans and their microbial and viral communities. The evolution of pathogens such as *Salmonella enterica* has likely been shaped by the long-term interaction between humans and pigs (98), and we see possible reciprocal effects in Neolithic pigs.

Conclusion

The Neolithic transition in Europe, in particular the relationship between humans and pigs, cannot be generalized and is rather a mosaic of intricate processes. Our findings indicate distinct practices in space and time with varying human–pig–boar relationships. In Early Neolithic Central/South European communities (5450 to 4900 BCE) we observed closed breeding practices of most likely small pig herds, resulting in inbreeding. Whereas in the Early Neolithic Swifterbant community (4300 to 4000 BCE) a commensal relationship between humans and wild boar is observed. The farming settlement of Swifterbant provided a niche with elements crucial for this commensal relationship. The genomic, stable isotope, zooarchaeological, and coprolite data corroborate the inference that refuse from anthropogenic activities, associated with sedentary agriculture in combination with human behavior created the socioecological context that allowed this commensal relationship. The consistent genetic profiles of Late Neolithic northwest European pigs (3630 to 2500 BCE) suggest control over breeding in these communities, indicating that the human–pig relationships became more human-focused; despite this shift, interbreeding with wild boar is still observed. We also observe distinct selection pressures related to coat color, behavior, and pathogens, despite continuous gene flow with wild boar. We argue that the reliance on wild boar, as commensal relationships or as a more controlled husbandry practice, is most likely the largest factor in the near complete genomic replacement of southwest Asian ancestry in European domestic pigs.

Our findings offer invaluable insights into early human–pig relationships in key regions and reveal distinct trajectories across Europe during the Neolithic. However, our dataset contains geographical and chronological gaps. Broader sampling in Neolithic domestic and wild populations for paleogenomic research and (bio)archaeological analyses will be imperative to understand the chronological and geographical extent of pig domestication reoccurring through the commensal pathway in Europe.

Materials and Methods

A detailed description of the materials and methods is provided in *SI Appendix*. A brief summary is presented here. DNA was extracted from eleven petrous bones obtained from five archaeological sites in the Netherlands and England (*SI Appendix*, Table S1). Stable isotope analysis followed a modified protocol (99). DNA extraction employed a step-by-step protocol (100), involving a dilute bleach wash and proteinase digestion, followed by DNA treated with USER enzyme, and construction of double-stranded DNA libraries (101). Sequencing was performed on an Illumina NovaSeq 6000 (2 × 100 bp). Reads were trimmed and aligned to the Sscrofa 11.1 pig reference genome (102) using bwa aln (103) with relaxed parameters for ancient DNA, while modern samples were aligned using bwa mem.

Diploid and pseudohaploid transversion biallelic SNP exploration using both modern and ancient wild and domestic genomes, using dedicated pipelines (*SI Appendix*). Pseudohaploid genotypes were used for autosomal phylogenetic reconstruction [using plink 1.9 (104) and PHYLIP (105)], a projection PCA using smartPCA (106), an admixture analysis using ADMIXTURE (107), and an admixture graph analysis using ADMIXTOOLS2 qpGraph (108). *D*-statistics, *Fst* outlier analysis, and mitochondrial phylogeny reconstruction were performed using the ANGSD toolkit (109), restricted to transversions. The mitochondrial phylogeny was constructed with RAxML (110).

ROH exploration was performed using diploid genotypes and BAM files from samples >4.4× coverage. ROH exploration was performed using three previously established methods 1) plink 1.9 homozyg, 2) a sliding window approach (111), and 3) ROHan (57), applied to both "high-coverage" and downsampled (4.4×) genomes. Seven test configurations were evaluated, including plink homozyg approaches (high-coverage with 1 heterozygote allowed, downsampled with 0 heterozygotes allowed, and downsampled with 1 heterozygote allowed), sliding window datasets (reduced and all-sites, both downsampled), and ROHan datasets (high-coverage and downsampled). Further details and exploration are provided in *SI Appendix*, *Run of Homozygosity*.

For *Fst* outlier detection, Neolithic, modern wild, and modern domestic genomes were grouped together (*SI Appendix*, Table S6.1), all genomes were >2× coverage. *Fst* outlier detection was performed using the ANGSD toolkit with 50 kb sliding windows and 10 kb steps. Windows exhibiting high divergence (*Fst* ≥ 0.99 quantile) were further investigated.

Data, Materials, and Software Availability. Sequence reads of data are available through the ENA under accession number PRJEB83109. Previously published ancient data used in this study are available under accession number PRJEB30282 (14, 29–31) and are detailed in Dataset S1. Sources for previously published modern genomic data analyzed here are listed in *SI Appendix*, Table S2.

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