

## ORIGINAL RESEARCH

# Walking together: artificial and natural selection in traditional husbandry of feral pigs

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evolutionary trajectory; feral swine; free-range pig; genomics; traditional pig husbandry; *Sus scrofa*; wild boar.

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**Abstract**

The history of *Homo sapiens* is studded with many events promoting relationships with wild animals changing their evolutionary path or impacting their adaptation. Artificial selection is recognized as the product of planned actions aimed to annex useful species into the anthropic niche. However, the effect of humans on animal evolutionary trajectories is diversified and cannot be assigned to a single driver. We characterized the genomes of feral pigs managed by different traditional husbandry practices to infer about the combining effect of artificial and natural selection. Whole genome characterization showed a clear distinctiveness of Sardinian wild boars (*Sus scrofa*) from free-range pig and domestic pig (*Sus domesticus*) populations, while Eurasian wild boars and hybrids are closely related, also in agreement with allelic frequency. In the Southern Italy system, we found 7 SNPs putatively under selection, associated with genomic regions including genes mainly involved in body weight control and feeding behavior, muscle growth and development, and adipocyte proliferation. Considering Sardinian wild boar and free-range pigs, over 3000 SNPs were found putatively under selection, and the genomic regions in which these SNPs fall include genes linked mainly to litter size and number of teats. The screening of genomic variability was useful to characterize feral pigs and wild boars from Southern Italy and Sardinia and the relationships between them, highlighting the effect of a peculiar artificial selection that modulates its weightiness due to the concomitant natural selection. In particular, the traditional Sardinian pig husbandry seems to act pushing down gene flow towards wild boar while favoring adaptations to life in the wild, creating a unique genetic pattern in free-range pigs, different both from the domestic and the wild genetic makeup. Our contribution opens up a discussion on the current European policy for the management of free-range pigs, the effective conservation actions for diversity in Suidae forms and their consequent impacts on biodiversity.

**Introduction**

Since its appearance, *Sus scrofa* (Linnaeus, 1758) has undergone a tangled evolution (Price, 2020).

Domestication, hybridization between domestic and wild animals (Frantz et al., 2019) and escapes from captivity of domesticated animals becoming feral (Petrelli et al., 2021) lead to a variety of interbreeding natural populations, differently named in the scientific literature. For example, the process of feralization in pigs (*Sus domesticus*) takes place through intentional mechanisms, including animal abandonment, free-ranging or farming practices, or unintentional events such as animal escape from the captivity, leading to the so-called endoferality *sensu* (Gering et al., 2019). In other cases, hybridization arises among pig

populations due to spontaneous admixture of their members with wild boar, the so-called exoferality (Gering et al., 2019).

Although the term 'endoferal' (domestic animal returned to wild) and 'exoferal' (domestic animal hybridized with the wild form) are recently widely used by specialists (Gering et al., 2019), in this study our populations are not fully compliant with this nomenclature. Thus, we used 'free-range pig' (probably endoferal) and 'hybrid' (probably exoferals) according to definition proposed by (Price, 2020).

The continuous reproductive interactions between free-range pig or hybrid populations and wild boar also have unwanted effects throughout the disappearance of the diversity pattern of wild boars in the Palearctic (De Jong et al., 2023). Indeed, some phenotypic and behavioral characters are likely to be

transmitted from pig to wild boar creating maladaptive responses (Mary *et al.*, 2022). Described examples exist for the sense of smell (Buglione *et al.*, 2024; Maselli, Ripa, *et al.*, 2014) and fertility (Fulgione *et al.*, 2016), one of the main factors that probably is at the basis of the demographic emergencies involving wild boars all over the world (Barmenlo *et al.*, 2024; Fulgione & Buglione, 2022).

Similarly, feral genomes may be introgressed by wild characters which would alter their evolutionary trajectory planned by humans.

Although it is intuitive to believe that where humans interact with natural populations the greatest admixture between forms is created, here we demonstrate that, in some circumstances, human may broaden the existing gap between wild and domestic forms that came back to the wild.

We focus on two emblematic case study areas: Southern Italy and Sardinia. In both these regions, the practice of free-ranging domestic pigs in the non-managed environment (hereafter traditional pig husbandry) was crucial for the economy of local rural communities; nowadays, however, it has been completely abandoned in Southern Italy since 1950s, whereas it is still widely practiced in Sardinia.

In both study sites, populations of wild boars and wild pigs can be found (Fig. 1): in Southern Italy, populations of wild pigs are represented by the hybrids, descendants from cross-breeding between free-range pigs and Eurasian wild boars. In Sardinia, free-range pig populations under extensive husbandry are completely free to wander into the bush, having a total control on food, recovery sites, and mate ([Price, 2020] and our personal observations), even with Sardinian wild form. In this contribution, we collected empirical evidence to demonstrate the hypothesis that hybridization phenomena are avoided by some traditional pig husbandry practices in Sardinia. This depends on free-range pigs need to be well adapted to the natural environment but in a different way from the wild boar. They must show the characteristics that make them 'attractive'

to humans (i.e., uncryptic coat, high fat content, high fertility), while retaining the ability to live in the wild. This creates a sort of artificial selection that freezes gene flow with the wild boar and pushes towards adaptations to wildlife (where natural selection reigns). By doing so, this type of traditional pig husbandry practice in Sardinia determines a peculiar evolutionary trajectory, favoring a unique genome makeup in free-range pig that is much different from the domestic and wild forms.

### The history of traditional pig husbandry in southern Italian (generating the current hybrid population)

There are lots of evidence of traditional pig husbandry (free-range practices) in Southern Italy. The most ancient one can be attributed to the Greek historian Polybius, who states: 'Even in Italy, pig farmers do not take their animals to pasture, as is customary in Greece, but they drive them in groups to the sound of trumpets' (Polybius, XII, 4, 5–6). The Latins called these musical instruments *buccine* or *tube*, and among the Campanian peoples they were known as *tofe* since the Oscan times (Soppelsa, 2016; Polybius, 1976).

The Roman agricultural writer Varro provides information on the optimal sex ratio for pig breeding, which must be 1:10 (males:females), as well as on the number of individuals that make up a herd (between 100 and 150). In confirmation of this, agronomist Nicola Onorati states that in the Kingdom of Naples, the pig herd taken to pasture consisted of 100 to 150 individuals and was known in vernacular terms as *morra* (Onorati, 1806). With such large groups left to range freely, hybridization events were very likely. In fact, 'The Ancients [i.e., Romans] used to mate pigs with wild boars, and the offspring were called hybridi' (Onorati, 1806).

Several authors describe the most appreciated traits for breeding. Varro praises the sow with an elongated body, wide abdomen, small head, and short legs, preferably uniformly colored



**Figure 1** Wild boars family in which the effect of hybridization is visible (photo by Photo by Domenico Fulgione).

rather than mottled. As for males, in addition to the small head and short legs, he notes a preference for erect hair on the cervix, which should be large. Another author, Columella, additionally advises for males a low abdomen, short nails, broad and glandular neck, short and upturned snout. He also suggests paying attention to the 'lineage' and geographic origin. Pliny reports for sows that individuals with 12 breasts/udders should be preferred (Pliny XI, 95, 233); moreover, Juvenal states that the Latins considered white sows more fertile (Juvenalis *et al.*, 2004, VI, 177). Onorati distinguishes between hairless pigs and bristly ones: 'In Campania and the surrounding regions, we have hairless pigs, known as the Teano breed; in other provinces, one can see bristly pigs, both black and white, with curly bristles', referring to the famous *pelatiello* variety, also known as *puorco cu' 'e sciuacqua* due to the prominent wattles (*bargiglioni*) (Soppelsa, 2016).

In the Roman society, it was possible to let pigs freely in non-managed woodland areas owned by the *dominus*, upon payment of taxes like the *glandaticus*, which consisted of a portion of the gathered acorns, or the *decima porcorum*, a tenth of the free-range pigs (Baruzzi & Montanari, 1981). These areas were adjacent to vast non-managed territories where wild boars were found. During the 4th century CE, *communia* were established, allowing grazing rights (*ius pascendi*). During the Middle Ages, with the invasion of the Lombards, there was a significant increase in the number of pig individuals raised in the wild. In that period, the semi-nomadic customs of the invaders led to a preference for pastoralism over agriculture, as it is evident in the Edict of Rothari in 643 CE (Bluhme, 1920).

During the Middle Ages, the woodlands was of great importance as a feeding ground for pigs, indeed it were not quantified in terms of surface area rather in terms of the number of individuals the area could feed. Every year an employee of the feudal lord estimated the acorn yield necessary to fatten the livestock, set as '*grassa*' (fat), became synonymous of wealth and prosperity (Beccu, 2000). In this period, pig herds ranged in the wild were gathered in groups from several dozens to hundreds of animals, led by a swineherd and a leader boar, called *sonorpair* by the Lombards, or a sow (*ducaria*) with a bell around its neck (Baruzzi & Montanari, 1981). Free-ranging pig husbandry allowed only for a brief winter pause, during which the animals were housed in pigpens.

A remnant of free-ranging pig husbandry is also attached to the history of the famous *purciello 'e sant'Antuono*, a pig the community raised for the Antonine monks, that was allowed to roam freely in the cities. This tradition persisted in the Kingdom of Naples until the 17th century (Soppelsa, 2016).

Lastly, oral testimonies report that, until the mid-20th century, farmers in Campania would let sows free at night to mate with wild boars to strengthen the breed however subsequently these practices were over.

### The history of Sardinian traditional pig husbandry (generating the current free-range pig population)

Archaeological evidence suggests that swine feralization occurred in Sardinia in ancient times, possibly dating back to the

early Neolithic period (Frantz *et al.*, 2019; Lega *et al.*, 2017; Maselli *et al.*, 2016). According to palaeontological data, after the extinction of a *Sus sondaari*, endemic to Sardinia, no swine species populated the island for a considerable period of time (Palombo *et al.*, 2012; van der Made, 1999; Vigne, 1989; Wilkens, 2012). Additionally, from the middle Pleistocene, the island's large mammal fauna 'was endemic and impoverished and only included a few large mammals' (Albarella *et al.*, 2006), none of them belonging to the *Sus* genus (Vigne, 1989). Wild boars and/or domesticated pigs reappeared on the island in the early Neolithic, not before the 7th millennium BCE (Larson *et al.*, 2005; Vigne, 1989). While it is possible that boars, being good swimmers, may have covered the distance between the Italian coastline and Sardinia (Lega *et al.*, 2017), the most consistent hypothesis is that humans introduced the species from the mainland during the first colonization of the territory, as the findings of *Sus* archaeological remains mainly in association with clearly allochthonous domestic livestock suggest (Albarella *et al.*, 2006).

The 'wild' morphological characteristics of Sardinian and Corsican boars, which have been recognized as an autonomous subspecies (*Sus scrofa meridionalis*), might be reconciled with their domestic origin by hypothesizing that 'the ancestors of these pigs escaped from captivity very early in prehistory when domestication had not yet had any major morphological effect on their morphology' (Albarella *et al.*, 2006). This would assign a central role to human activities in shaping the evolutionary history of this (then incipient) subspecies from very ancient times, a trend that is still ongoing, as the current interactions between Sardinian wild pigs and local human populations demonstrate.

Therefore, the first episode of feralization may have occurred soon after the arrival of the first human settlers on the island, as early as 6000 years BCE. Since then, the relationship between humans and pigs has remained central to the island's economy, continuing until the present day.

Traditional pig husbandry has been continuously practiced by locals in both Sardinia and Corsica with a wide range of strategies recorded throughout the two islands. According to (Albarella *et al.*, 2007), these practices range from the controlled hunting of wild animals to the intensive stock-breeding of improved domestic breeds. Such diversity likely depends on both local environmental constraints and cultural peculiarity, as well as the small size of Sardinian and Corsican specimens, which is probably a case of insular dwarfism.

One of the most common forms of traditional pig husbandry practiced in Sardinia today consists in pigs are allowed to live in a semi-wild condition for most of the year, roaming around vast territories of up to 50 hectares and mostly self-sustaining with the products of woodlands and pastures. The level of control exerted by the human owners varies slightly in different areas, ranging from daily contact to provide food to the livestock, to a system of very little control, limited to two to three human interventions per year. Although enclosed breeding is practiced, it is difficult to differentiate clearly the two forms of herding.

Moreover, it is important to note that, due to this type of management, 'crossbreeding between wild (or feral) and domestic animals occurs regularly today, and must have occurred even more in the past, when free-range systems of

pig-keeping were more or less the rule' (cf. Ferrero della Marmora, 1839; Manca Dell'Arca & Marci, 2005).

However, swine herders claim that the management of free-range pigs become more difficult if they acquire wild boar characteristics. To maintain the individuals more docile and with coat color detectable in the wild, owners usually resort to a form of artificial selection by slaughtering newborns that show clear morphological signs of hybridization. Furthermore, they sometimes use castration (practiced both on male and female specimens). Finally, it should also be considered that in Sardinia there are no natural predators that could threaten ferals, and this can lead to the emergence of maladaptive phenotypes.

## Materials and methods

### Study area and sample collection

The sampling areas were established to depict two study systems: the Sardinian system is represented by the upland of Golgo, in the Western part of the Ogliastra region (40°5.21' N/9°40.2' E, Sardinia, Italy) where Sardinian wild boars and free-range pigs live free in sympatry (Fig. 2).

The Southern Italy system, on the other hand, was represented by the Campania region (40°30' N/15°16' E, Southern Italy), where Eurasian wild boars and hybrids co-occurred in wild (Fig. 2). Both free-range pigs and hybrids show a phenotype with clear traits derived from the domestic form, such as non-cryptic (i.e. white, black or spotted) coat coloration (Petrelli *et al.*, 2021, 2023).

From 2021 to 2022, we conducted an extensive field survey collecting 8 Sardinian wild boars, 5 free-range pigs, 16 Eurasian wild boars and 14 hybrids. Free-range pigs correspond

exactly to the domestic pig breed autochthonous from Sardinia. The hybrids from Southern Italy derived from breeding of a variety of autochthonous pig breeds, usually range free on pasture until 1950s, among which Palatella and Nero Casertano were the most widespread and used.

We sampled animals aged  $\geq 1$  year, according to phenotype and dentation for wild boars and hybrids, and also herders' information for Sardinian free-range pigs.

The wild individuals were collected during legal hunts in accordance with Italian National laws (157/92 and 394/91 Laws), and all field protocols were approved by the Ministry of Environment (ISPRA, protocol number 24581 20/07/2014). The good health of animals was evaluated by a veterinary attending our sampling procedures.

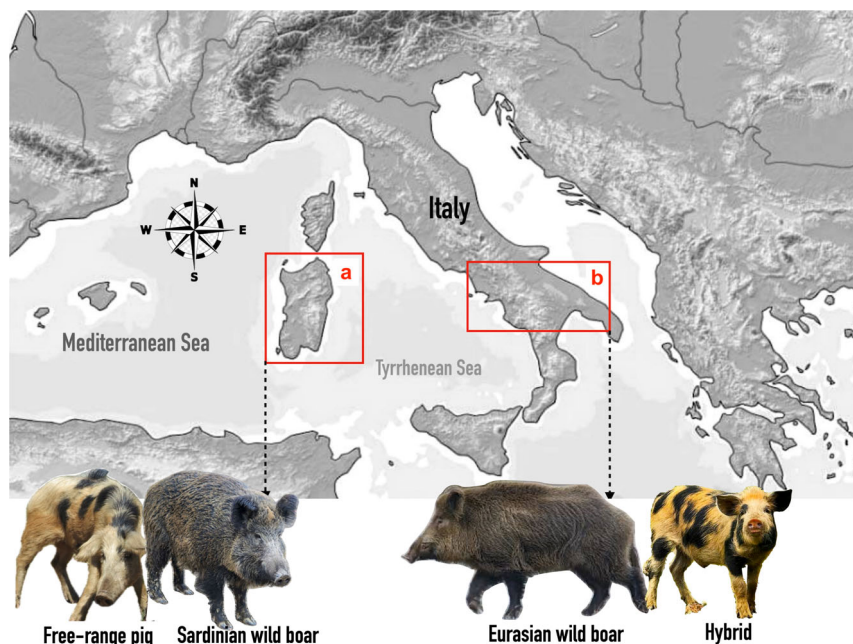
In addition, we sampled 5 adult domestic pigs of large white breed (Indigenous Genetic Type Registry by the Italian National Association of Swine Breeders), which is the most widespread, commercialized and affected by artificial selection pig breed in Southern Italy and Sardinia.

We collected ~5 cm sections of muscle tissue with sterilized equipment immediately after death of the animals. Samples were placed in sterile tubes with 99.6% ethanol and then immediately stored at  $-20^{\circ}\text{C}$ , until laboratory processing.

### Genomic characterization of populations

DNA isolation from tissues was performed using the Qiagen DNeasy 96 Blood & Tissue Kit (QIAGEN GmbH Valencia, CA, USA) according to manufacturer's instructions.

We genotyped the samples using the Illumina Porcine SNP (Single Nucleotide Polymorphism) 60 BeadChip (Ramos *et al.*, 2009) at the Genomix4Life Srl (<http://www.genomix4life.com/it/>).



**Figure 2** Study area. (a) Sardinia and (b) Southern Italy (Campania region). The corresponding phenotypes sampled in the two areas were reported in the inserts.

The quality control of raw data was assessed using PLINK 1.9 (Purcell *et al.*, 2007), removing sites with at least 10% missing genotype rate, genotypes with a minor allele frequency (MAF) lower than 0.05, in linkage disequilibrium (LD,  $r^2 > 0.2$ ) and with Hardy–Weinberg threshold of 0.0000001. We retained 38 K SNPs to use in the structure analyses, resulting in 25 365 SNPs for principal component analysis (PCA).

PCA was carried out in PLINK 1.9 and visualized by R programming language (R Core Team, 2013) using the plot3d package (Soetaert, 2024).

To detect signatures of selection on genomes, we calculated the fixation index ( $F_{ST}$ ) (Pritchard *et al.*, 2000; Weir & Cockerham, 1984) as measure of group differentiation per locus in PLINK 1.9. Then, we identified the candidate genes under selection using biomaRt and ‘ssscrofa\_gene\_ensembl’ as reference database, considering a region of 500 kb around the SNP of interest.

### Admixture analysis

We estimated the maximum likelihood of individual ancestries from multilocus SNP genotype dataset using a Bayesian clustering method in STRUCTURE v. 2.3.4 (Pritchard *et al.*, 2000), run with a burn-in period of 500 steps and 10 000 repetitions of Markov Chain Monte Carlo (MCMC). We tested  $K$  ranging from 1 to 7, in five independent runs, using the recessive alleles model of ancestry, and the selection of the most appropriate number of clusters that best fits the dataset was performed in STRUCTURE Harvester (Earl & vonHoldt, 2012), according to (Buglione *et al.*, 2020) using to posterior probability of data  $\ln P(D)$  (Garnier *et al.*, 2004), the admixture parameter  $\alpha$  (Pritchard *et al.*, 2000), and according to (Evanno *et al.*, 2005) method.

### Run of homozygosity

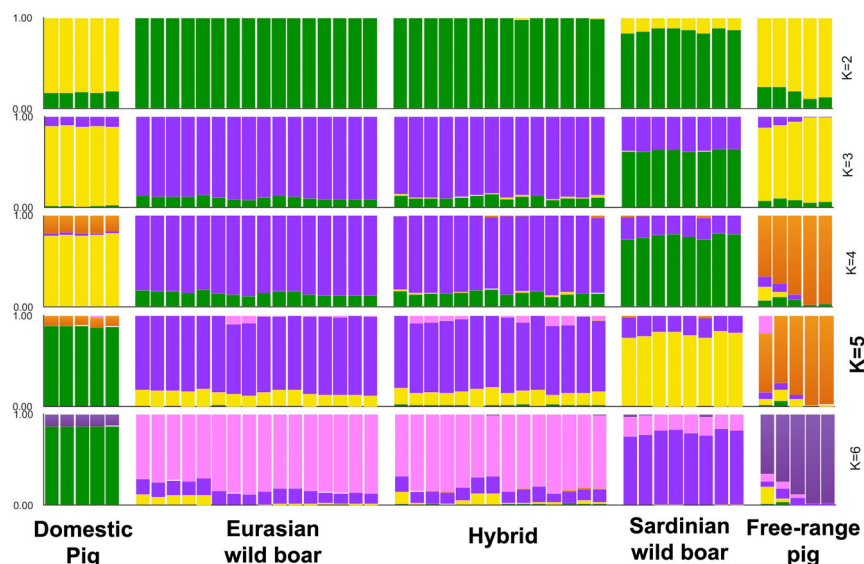
Runs of homozygosity (ROH) were calculated using detectRUNS package v. 4.2.3 (Biscarini *et al.*, 2018) in R according to sliding-window method Purcell *et al.* (2007), setting minimum length of the run = 500 kb with minimum of SNPs = 50. We estimated the number and length of ROHs per each group and calculated the individual inbreeding coefficients on a per-chromosome basis from runs of homozygosity (Frho) (Schiavo *et al.*, 2020).

### Results

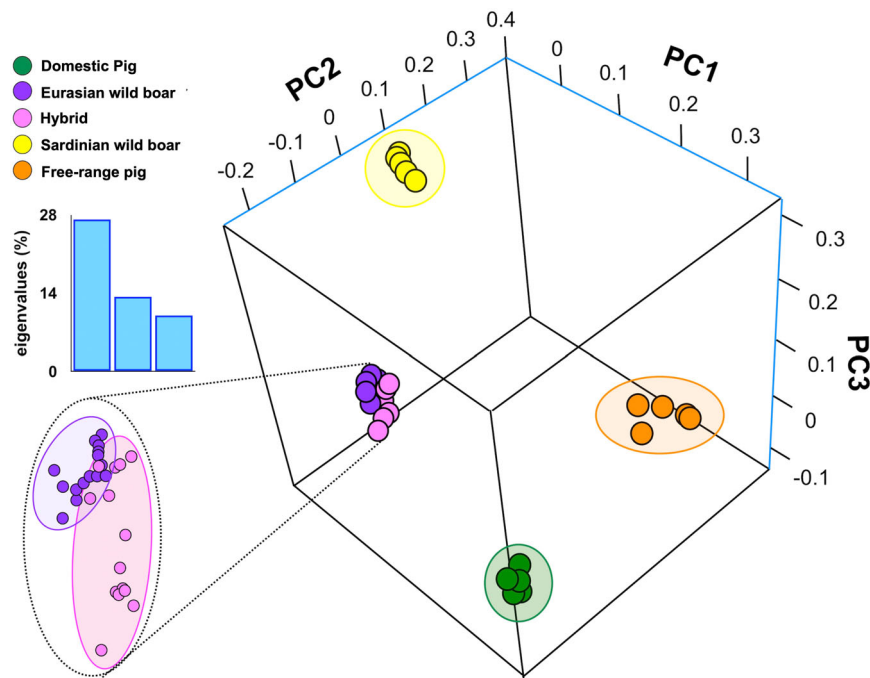
To characterize our five swine populations, we developed a structure analysis which confirmed the existence of five distinct genetic groups. Indeed, admixture analysis showed that  $K = 5$  was the most likely value to describe population clusterization, according to posterior probability of data, and Evanno method (Figure S1, Tables S1 and S2).

A total of 48 genotypes were unambiguously allocated in the five putative clusters  $K$  (Fig. 3). In particular, there is a clear assignment of Sardinian (free-range pig and wild boar) and domestic populations, while the populations of wild boars and hybrids from Southern Italy seem to be strongly interpenetrated with each other.

To better unravel the reciprocal relationships between these five populations, we ordered the genotypes on a three-dimensional space defined by the first three main components. The variation represented by the first three principal components (50.57%) evidenced that hybrids and Eurasian wild boars are closely related, showing a markedly mixed sub-population (Fig. 4). This was also in agreement with difference in allelic frequency quantified using  $F_{ST}$  in the SNPs



**Figure 3** Estimated population structure based on the analysis of SNPs according to STRUCTURE (from  $K = 2$  to  $K = 6$ ). Each bar represents a sample analyzed. The number of populations is  $K = 5$ .



**Figure 4** Principal Component Analysis (PCA) performed with 25 365 SNPs. Each genotype was represented by a dot, colored according to category. Circles show the well-defined spatial groups.

(Table 1). Indeed, signatures of selection showed that the lowest  $F_{ST}$  value was between Eurasian wild boar and hybrid (Table 1). Instead, the values of  $F_{ST}$  were higher for domestic pig when compared with all the other forms, and for free-range pig with respect to the other forms. Interestingly, the comparison between free-range pig and Sardinian wild boar also returns the highest  $F_{ST}$  value ( $F_{ST} = 0.315$ ), (Table 1) suggesting they are affected by strong selection signature, as also emerged from Manhattan plot of  $F_{ST}$  (Fig. 5). The number of SNPs over the threshold line of significance is higher in Sardinian system (Fig. 5a, Table S3) compared to Southern Italy population (Fig. 5b, Table S4).

The  $F_{ST}$  analysis to test selection between the two wild boar forms (Sardinian and Eurasian wild boar) (Fig. 5c, Table S6) and the two feral forms (free-range pig and hybrid) (Fig. 5d, Table S6) highlights a higher number of SNPs above the threshold line of significance for  $F_{ST}$  for the second comparison (Fig. 5d), probably as a consequence of combination of natural and artificial selection.

### Inferring genes under putative selection and related GO terms

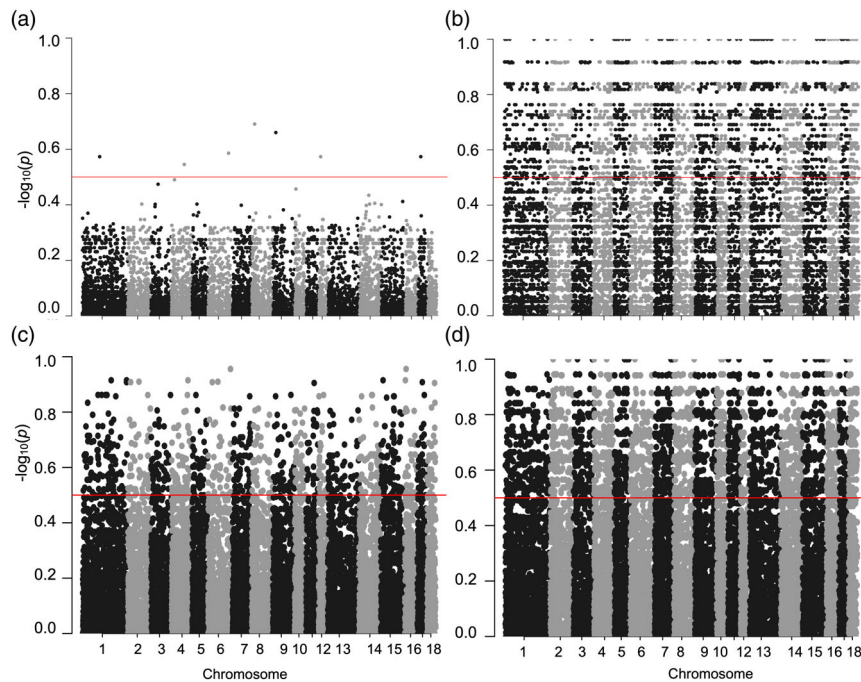
Comparative SNPs analysis considering hybrid versus Eurasian wild boar revealed seven SNPs, of total 25 366, above the threshold of  $F_{ST} = 0.5$  (Fig. 5a). These SNPs fall in genomic regions associated with 26 genes putatively being under selection (Tables S3 and S7). These genes are linked mainly to body weight control and feeding behavior, such as the NEGR1 (neurotrophin growth regulator 1 gene) gene (Kim et al., 2017;

**Table 1** Matrix of pairwise  $F_{ST}$  (Weir & Cockerham, 1984) values between *Sus scrofa* categories based on SNP data

	Hybrid	Sardinian wild boar	Free-range pig	Domestic pig
Eurasian wild boar	0.0155067	0.164389	0.303314	0.291278
Hybrid	—	0.155705	0.275867	0.262421
Sardinian wild boar		—	0.315016	0.304908
Free-range pig			—	0.252609

Lee et al., 2011; Singh et al., 2019), with mRNAs translation, for example ZCCHC4 (the zinc finger CCHC-type 4) gene (Ma et al., 2019) as well as with muscle growth and development, and adipocyte proliferation and differentiation, for example DLG2 (the discs large MAGUK scaffold protein 2) gene (Ardestani et al., 2018; Lee et al., 2023).

Considering the Sardinian system, the comparison between wild boar and free-range pig showed 25 352 SNPs in total, of which 3787 are above the threshold line of  $F_{ST}$  significance (Fig. 5b). In this case, the genomic regions in which these SNPs fall include genes ( $n = 5014$ ) mainly linked to litter size and number of teats, such as ELF5 (E74-like factor 5), SOX9 (SRV-Box Transcription Factor 9), STAT3 (Signal transducer and activator of transcription 3) genes (Martins et al., 2022) (Table S4). The same analysis performed comparing Sardinian system and Southern Italian systems showed that they shared three putatively genes under selection (Fig. S2), named PAK5 (p21 (RAC1) activated kinase 5),



**Figure 5** Manhattan plots of  $F_{ST}$  above the threshold line (red horizontal line: 0.5) showing SNPs under potential selection. (a) Hybrid versus Eurasian wild boar. (b) Free-range pig versus Sardinian wild boar. (c) Eurasian wild boar versus Sardinian wild boar. (d) hybrid versus free-range pig.

SNAP25 (synaptosome-associated protein 25) and ANKEF1 (Ankyrin Repeat And EF-Hand Domain Containing 1) genes (Table S7), which are mainly associated with neuronal morphology, modulation of synapse plasticity, proliferation and functionality (Dan *et al.*, 2002; Kawasaki & Kretsinger, 2017; Kumar *et al.*, 2017; Najera *et al.*, 2019).

### Runs of homozygosity indicate level of variability

We identified 1274 ROHs across five different groups. Eurasian wild boar, Sardinian wild boar and hybrid showed the higher number of shorter ROHs (Table 2), while free-range pig populations had fewer number of shorter ROHs ( $n = 30$ ; 0–6 Mbs), suggesting a more recent bottleneck probably associated with the contingents of animals that swineherds released in historical times.

The analysis of the SNPs along all the chromosomes useful for the estimation of the inbreeding coefficient highlights a

rather uniform distribution of the homozygous regions except for the domestic pigs which highlight the lowest Frho levels (Fig. 6).

The wild forms, Eurasian and Sardinian wild boars, and hybrids, showed a similar pattern, while free-range pigs differ in higher median values and a wider variation gap (Fig. 6).

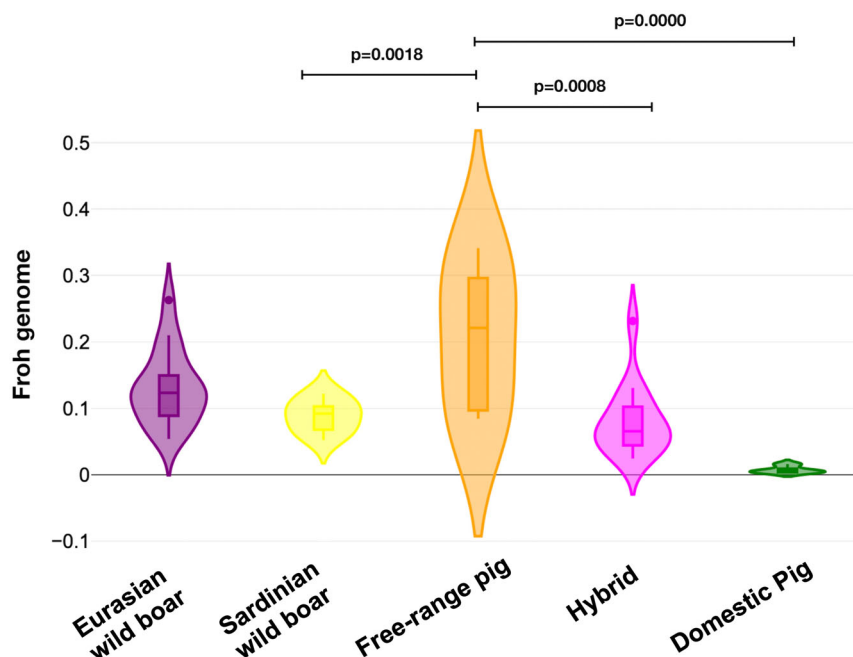
One-way ANOVA test and  $F$  statistic on Froh genome data suggest statistically significant differences between the groups ( $P$ -value < 0.05;  $f$ -ratio value: 9.0643) (Table S8). In particular, the Tukey's HSD (honestly significant difference) showed significant values considering the comparison between Sardinian wild boar versus free-range pig, free-range pig versus hybrid and free-range pig versus domestic pig (Table S9).

### Discussion

Human impact on natural systems has been studied through various points of view and with different levels of detail (Goudie, 2018). In particular, domestication represents a key turning

**Table 2** Number and length (Mbp) of Runs of homozygosity for each group

Mbps	Eurasian wild boar	Sardinian wild boar	Free-range pig	Hybrid	Domestic pig
0–6	234	126	30	153	2
6–12	225	75	52	110	6
12–24	76	26	35	50	1
24–48	30	4	11	12	NA
>48	1	1	13	1	NA



**Figure 6** Individual inbreeding/consanguinity coefficients estimated on a per-chromosome basis from runs of homozygosity plotted by group. Statistically significant value  $P$ -value  $<0.05$  (One-Way ANOVA and Tukey's HSD (honestly significant difference)).

point in human history. The fact that evolutionary trajectory of both animals and plants were changed, opens interesting insights on pathways that have led to modification in terms of morphology, reproduction, and behavior (Larson & Fuller, 2014) in the domesticated phenotype compared to their respective wild ancestors.

The effect humans exert on domesticated populations can occur in different ways and, at times, it can involve the interaction with the wild form, where it still exists.

In this contribution we compared, for the first time, two traditional pig husbandry practices and analyzed their evolutionary effects on the animal genome, which results in an analysis of wild management. Our study system highlighted an extraordinary complexity interplay of natural and artificial selection, affecting gene flow among pig forms as well as their evolutionary path.

In Sardinia, where free-range pigs could give rise to admixed populations with wild boars, there is no evidence of hybrids. Instead, they are detected in Southern Italy where traditional pig husbandry practices were abandoned around the 1950s and to date the domestic breeds are well isolated from the wild compartment.

Pig husbandry in Sardinia is based on solid traditional practices that apply a very strong selection on newborns showing wild characteristics (such as striped coat color); this human-induced barrier, probably, with some behavioral differences between piglets, limits gene flow and the onset of hybrid forms. Indeed, in Sardinia, traditional pig husbandry is based on practices in which artificial selection pushes phenotypes that are attractive to human populations (docile, fat, not very cryptic), but also capable of living in a wild environment,

obtaining food and water and finding shelter. In our hypothesis, swineherd management determines a mixing of selections leading to animals with human desirable traits while able to live wild. This is simplified by no large natural predators on the island, such as the wolf (*Canis lupus*).

This mixed selection (natural and artificial selection) affects the genome variability. Indeed, low inbreeding and low rate of homozygous regions were detected on free-range pigs, well distinguishable from wild boars. Differently, hybrids from Southern Italy showed lower level of variability and a reduced genetic distance from wild populations.

Analysis of the signature of selection revealed that the greater number of SNPs above the threshold line of  $F_{ST}$  significance was detectable in the Sardinian system (wild boar vs. free-range pig). This suggests a consistent effect of mixing selection compared to Southern Italy system where only natural selection acts. The Sardinian population probably carries on the genome both the effects of traditional pig husbandry and of the environment in which they spend most of their lives.

Comparing the signature of selection between Sardinian system and Southern Italian systems, it results they shared three putatively under selection genes, which are mainly associated with neuronal morphology, proliferation and functionality. This evidence suggests that, to face natural selection, both the free-range pig and hybrid need to remodulate their ability (derived to a domestic ancestor) to perceive and respond to the environmental stimuli, as we observed already for the sense of smell (Buglione *et al.*, 2024; Fulgione *et al.*, 2017; Maselli, Polese, *et al.*, 2014; Petrelli *et al.*, 2021).

However, the effect of traditional pig husbandry in Sardinian directorates the evolution of this free-range pig population, as



also suggested by the significant different levels of inbreeding emerging comparing free-range pig and other populations, both domestic and wild.

Moreover, the presence of selective signals on genes encoding for crucial enzymes in the melanogenesis pathway is associated with coat pigmentation. These observations probably find an explanation in the practices of killing piglets which do not have a coat color agreeable to human needs (Sardinian shepherds).

In conclusion, our empirical evidence suggests a tangle relationship between different forms of swine (wild and domesticated) and human animal management, rising a new and unexplored evolutionary trajectory. There are several and various ways to manage pigs in the wild across the world (Risch *et al.*, 2021) and our research launches a broader investigation, necessary to understand if a model of 'return to wild' shared among different feral population exists.

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## Author contributions

DF and MB conceived the ideas, took care of data curation, write the original draft and led the writing of the manuscript. DF, MB and SA designed methodology and analyzed the data; DF, MB and ER collected the samples; OS and SB collected historical information; DF gave the resources. All authors contributed critically to the drafts and gave final approval for publication. This research was partially supported by Program of Funding of University Research (FRA) 2021 of University of Naples Federico II: CUP E25F21000320001 : PG/2021/0034364 del 06/04/2021.

## Conflicts of interest

The authors declare no conflicts of interest.

## Data availability statement

Subset of SNP data is available from FigShare at <https://doi.org/10.6084/m9.figshare.28105262>.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Graphical methods to the detection of the best number of  $K$  groups elaborated in STRUCTURE Harvester (Earl & vonHoldt, 2012). (A) Mean  $L(K)$  ( $\pm$  SD) over 5 runs for each  $K$  value. (B) Rate of change of the likelihood distribution (mean  $\pm$  SD) calculated as  $L'(K) = L(K) - L(K-1)$ . (C) Absolute values of the second order rate of change of the likelihood distribution (mean  $\pm$  SD) calculated according to the formula:  $|L''(K)| = |L'(K+1) - L'(K)|$ . (D)  $\Delta K$  calculated as  $\Delta K = m |L''(K)| / s[L(K)]$ .

**Figure S2.** Symmetric Venn diagram of shared and exclusive genes putatively under selection (means genes included in the genomic regions in which SNPs above the threshold line of significance of  $F_{ST}$  fall) elaborated by Venn Diagram Tool freely available on the web (<https://bioinformatics.psb.ugent.be/webtools/Venn/>); see Table S7 for details. EW, European wild boar; FRP, free-range pig; HY, hybrid; SW, Sardinian wild boar.

**Table S1.** Raw STRUCTURE output.

**Table S2.** The Evanno table output.

**Table S3.** Genes associated to genomic regions in which fall SNPs with  $F_{ST}$  above the threshold line: 0.5 considering Southern Italy system (Eurasian wild boar vs. hybrids).

**Table S4.** Genes associated to genomic regions in which fall SNPs with  $F_{ST}$  above the threshold line: 0.5 considering Sardinian system (Sardinian wild boar vs. free-range pig).

**Table S5.** Genes associated to SNP with  $F_{ST}$  above the threshold line: 0.5 considering Eurasian wild boar and Sardinian wild boar.

**Table S6.** Genes associated to SNP with  $F_{ST}$  above the threshold line: 0.5 considering hybrids and free-range pigs.

**Table S7.** Output of Symmetric Venn diagram of shared and exclusive genes putatively under selection (means genes included in the genomic regions in which SNPs above the threshold line of significance of  $F_{ST}$  fall) elaborated by Venn Diagram Tool freely available on the web (<https://bioinformatics.psb.ugent.be/webtools/Venn/>); see also Figure S2. EW, European wild boar; FRP, free-range pig; HY, hybrid; SW, Sardinian wild boar. \*For the genes missing of external\_gene\_name is reported the ensembl\_gene\_id.

**Table S8.** One-Way ANOVA between groups. The result is significant at  $P < 0.05$ .

**Table S9.** Post Hoc Tukey HSD (honestly significant difference): T1, Eurasian wild boar; T2, Sardinian wild boar; T3, free-range pig; T4, hybrid; T5, domestic pig.