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Morphometric and aDNA study of charred plant remains found in the Monteverdi medieval castle, Civitella Paganico, Grosseto, Italy

Claudio Milanesi [a,*], Alessandro Sebastiani [b], Alessandro Carabia [c], Rita Vignani [d], Francesca Antonucci [e], Simona Violino [e], Monica Scali [d], Giampiero Cai [a], Mauro Cresti [a], Claudia Moricca [f]

[a] Department of Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy
[b] Department of Classics, University at Buffalo (SUNY), USA
[c] University of Birmingham, Birmingham, UK
[d] Department of Life Sciences, University of Siena, San Miniato, Via Aldo Moro 2, 53100 Siena, Italy
[e] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA) - Centro di ricerca Ingegneria e Trasformazioni agroalimentari, Via della Pascolare 16, 00015 Monterotondo (Rome), Italy
[f] Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

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ABSTRACT

The castle of Monteverdi is a medieval settlement in central Italy, located on a hill that once controlled the communication routes between the Tyrrhenian coast and Mount Amiata. The remains of the castle's walls cover the flat summit of a large ovoid ridge, enclosing a series of structures. The excavation focused on one of these areas, which was characterized by the presence of a small oven, revealing numerous charred carpological and xylological remains dating from the mid to late 12th century. These were subject of a multidisciplinary study, which included morphological, morphometric and genetic investigations, with the aim of obtaining information about human-environment interactions, particularly in relation to the exploited cereal grains. Traditional archaeobotanical analyses allowed us to identify a range of plants available at the site, such as cereals (mostly barley and naked wheats), pulses and weeds (represented by *Lolium temulentum*). Woody taxa correspond to the typical vegetation of Maremma (Tuscany, Grosseto, Italy). Subsequently, through geometric morphometry, the profiles of a selection of ancient and modern cereal of *Triticum* caryopses were extracted, and their average shapes were analyzed by comparing Euclidean and genetic distances. This allowed for the assessment of homologies and differences with some current cultivars of the same species. Finally, molecular analyses successfully applied to charred material made it possible to identify grains of *Triticum aestivum/durum* with genetic characteristics comparable to some accessions still cultivated in Italy today.

1. Introduction

By the 12th century, the rural landscape of central Italy, and especially Tuscany, had undergone profound transformations shaped by demographic growth, the assertion of aristocratic power, and shifting patterns of settlement and land use. This period marked a decisive phase in the long trajectory from dispersed early medieval hamlets to fortified hilltop communities, known as *castelli*, which often replaced or incorporated earlier wooden villages established between the 7th and 10th centuries (Francovich, 2007). The process of *incastellamento* – far from homogeneous – led to the nucleation of populations into strategically located sites, often accompanied by the construction of stone fortifications, seigneurial residences, and increasingly organized layouts, as seen at sites like Rocca San Silvestro, Scarlino, and Montarrenti. This territorial consolidation paralleled the transition from estate-based seigneuries to more structured forms of territorial lordship, in which aristocratic families sought to control agricultural resources and surplus production (Francovich, 2007; Bianchi, 2024). Agriculture during this time was shaped by efforts to intensify production and optimize land use. Estate structures such as *casali* and *poderi* in both urban hinterlands and rural zones were increasingly used for the cultivation of cereals, often in combination with animal husbandry, particularly the use of

* Corresponding author.
E-mail addresses: claudio.milanesi@unisi.it (C. Milanesi), claudia.moricca@uniroma1.it (C. Moricca).

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draught animals for ploughing and land clearance (Tabarrini, 2024). Granarie – often built in perishable materials – played a central role in storing surplus grain for both consumption and market exchange, though by the 12th century, their symbolic placement in dominant parts of settlements seems to have given way to more practical architectural priorities (Bianchi, 2024). Simultaneously, livestock practices, particularly sheep and goat herding, expanded significantly, especially along transhumance routes connecting upland and lowland pastures. In areas like Grosseto and the Maremma, the rising presence of ovicaprids in faunal assemblages reflects broader economic strategies aimed at consolidating elite control over land and pastoral resources (Vanni, 2015).

The work of historians in reconstructing the past can be aided by several technical and scientific disciplines. In this field, the analysis of ancient caryopses can, in certain cases, allow the study of cultivated species as well as human plant diet and provide insights into aspects of the past natural environment. Seeds, fruits, and charred wood found *in situ* are important for reconstructing diet and resource exploitation (Pearsall, 2015). Specialist morphological and molecular analyses can provide more specific information about plant domestication and their spread across different geographical areas (Peleg et al., 2011; Geleta & Ortiz 2016; Milanesi et al., 2016; Sabato et al., 2019; Bouby et al., 2021).

The preservation of ancient DNA (aDNA) in carpological charred remains is very challenging, and archaeogenetic studies have highlighted significant limitations in this regard (Palmer et al., 2012), requiring appropriate control experiments before proceeding with aDNA isolation. The application of geometric morphometrics, captures the overall geometry of shapes and sizes of the remains. As noted by Bonhomme et al. (2017), charred archaeological wheat kernels suitable for morphometric analysis should be well preserved (i.e., progressively charred and at relatively low temperatures. Geometric differences could therefore determine the characteristics of the parent species from the derived one (Milanesi et al., 2011), and the application of morphometric analysis protocols to cereal kernels can both confirm identification obtained with traditional methods (Ros et al., 2014) and shed light on their geographic origin (López-Merino et al., 2015; Milanesi et al., 2021; Rivera et al., 2024, Jeanty et al. (2023). Once the best-preserved carpological remains have been identified, archaeobotanical research on aDNA can be initiated using specific molecular protocols (Kistler, 2018). Such investigations are also useful for confirming correlations between the genotypes of traditional cultivars (Jlassi et al., 2021). The availability of molecular markers (Adhikari et al., 2017, Guan et al., 2020) represents an effective tool not only for studying aDNA (Bunning et al., 2012; Fernández et al., 2013; Fornaciari et al., 2018; Sykes et al., 2019) but also for analyzing plant domestication. Interestingly, SSR markers target the genome of a single species, making them quite effective for identifying individual species in aDNA mixtures (Kowalczyk et al., 2018). This feature is very useful for ruling out nonspecific amplifications even in the case of aDNA from multiple sources or in the case of paleontological or archaeobotanical samples that may have been contaminated during field or laboratory procedures. SSR genotyping aims to amplify the *Triticum* A genome, producing DNA fragments corresponding to Triticum alleles only if the correct specific genomic targets are identified (Vieira et al., 2016). Recent studies confirm the possibility of obtaining information despite sample degradation (Del Gaudio et al., 2013) demonstrating the effectiveness of using SSR markers to characterize wheat resources distributed at the local or regional level (Galal et al., 2023; Vasile et al., 2023).

In this study, we propose multidisciplinary (morphological, morphometric, and genetic) investigations conducted on carpological and anthracological remains recovered from the medieval castle of Monteverdi (Province of Grosseto, Tuscany, Italy), with the aim of obtaining information about human-environment interactions, both in terms of food plants and availability of timber. A focus was then placed on the exploited cereal grains. Geometric morphometry was used to compare the shape profiles of modern *Triticum aestivum* caryopses, modern *Triticum monococcum* caryopses, and archaeological *Triticum aestivum/durum* caryopses. Molecular analyses of *Triticum aestivum/durum* caryopses showed homologies with traditional wheat varieties still cultivated and protected by the regional Tuscan agency

1.1. The site

The fortified medieval settlement of the Castle of Monteverdi (42°55′55″N 11°18′28″E) is located on a hill (ca. 130 m a.s.l.) that controlled the communication routes between the Tyrrhenian coast and Mount Amiata (Tuscany, Italy). It lies within the inland territory of Grosseto, an area characterized by the presence of major watercourses, including the Ombrone River (Fig. 1). This river features continuous riparian vegetation zones and a semi-natural course. The Paganico Valley, in the Grosseto hinterland, is marked by a predominantly agricultural landscape, with fields of varying sizes, yet consistently structured by a network of Mediterranean scrub hedgerows punctuated by individual trees, usually oaks. Wooded areas are clearly identifiable in the higher elevations of the landscape. The hilly terrain is characterized by occasionally extensive woodland areas of Mediterranean scrub, with a predominance of holm oak (*Quercus ilex* L.) and Turkey oak (*Quercus cerris* L.), pinewoods, chestnut groves, and isolated beech stands (Regione Toscana, 2015). At an altitude between 300 and 800 m, a belt of Turkey oak forests can be found, situated between the sclerophyllous vegetation and the montane zone of chestnut and beech woods. These form transitional woodlands with beech forests at higher elevations. Isolated stands of beech trees at lower altitudes are common, typically found in cool valleys and along riverbanks (Arrigoni et al., 1990). Specifically, the site discussed in this article is located on the Hill of Monteverdi, an area of extensively developed agriculture, predominantly devoted to farming activities (Comune di Civitella Paganico, 2023). In fact, in the Municipality of Civitella Paganico, mature coppices are subject to cattle grazing, with significant effects on their structure and floristic composition (Arrigoni et al., 1990).

The Castle of Monteverdi was strategically important due to the presence of a medieval bridge that crossed the Ombrone River, allowing the Maremma region of Tuscany to connect with other major roads like the Via Francigena. It was part of the larger estates of the Ardengheschi family and remained under their control until 1280, when the properties of the Abbey of Ardenghesca were sold to Siena. Within a few years, the castle was abandoned, and its walls were dismantled to support the construction of the town walls of Paganico (Farinelli, 2015). The medieval castle consists of a ruined oval-shaped curtain wall approximately

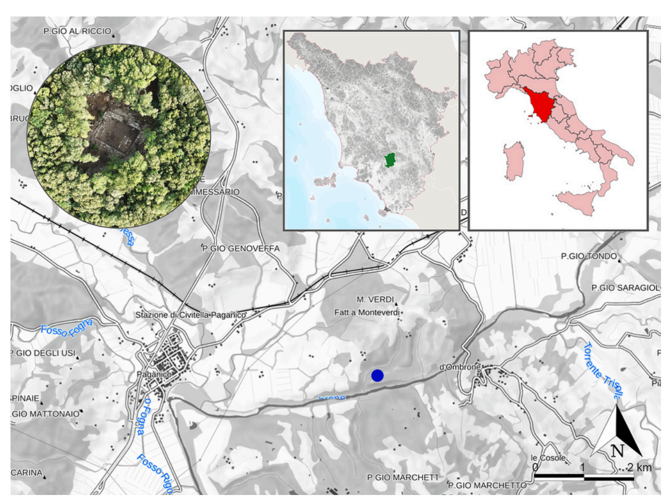

Fig. 1. The geographic location of the castle (blue dot) on the Monteverdi hill and within Tuscany (the green area indicates the Municipality of Civitella Paganico). Aerial view of the site in the top right corner (Base map: Regione Toscana – https://www502.regione.toscana.it/geoscopio/cartoteca.html).

110 m by 30 m and is situated at the top of the ridge overlooking the Ombrone River (Hobart & Carabia, 2020; 2022). In their current state, the ruins of the old settlement are partially visible, being covered by a dense forest of holm oaks (*Q. ilex*) and vegetation typical of the Mediterranean maquis.

The site reveals at least a dozen structures, two of which, located at the eastern end of the ridge, constitute the keep with a tower (Area 1000), while at least six other structures are part of a development that leans against the inner side of the northern curtain wall (Fig. 2 a). Among these, Area 2000 (Fig. 2 b) features remains of dry-stone walls about 1.5 m high, built with roughly hewn stones and held together by interlocking and gravity (Fig. 2 c). The room subject of this study was adjacent to the inner wall of the northern enclosure. The environment had a small oven, likely used for baking or drying, as foodstuffs were stored in large, ventilated spaces, and heating them in very small spaces was not recommended for their preservation and storage. The *in-situ* recovery of fragments of a vessel used for baking bread and flatbreads seems to support this hypothesis.

The excavation initially proved to be straightforward, and after uncovering a collapse layer, a floor surface (stratigraphic unit – SU 37) was discovered. This represents the last phase of use of the space. After removing SU 37, poor in carpological remains and related to the final phase of the environment, a very dark stratigraphy, rich in charred remains, emerged. This new stratigraphic sequence documents a series of life phases of Area 2000, predating the formation of the SU 37 floor and WSU 27, but succeeding the construction of wall stratigraphic units (WSU) 18, 43, and 46 (the surrounding wall, Fig. 2). The phase of the environment used for storing foodstuffs has been preliminarily dated to between the mid and late 12th century based on a preliminary analysis of the pottery found in the stratigraphy, an analysis that was confirmed by radiocarbon dating of a fragment of carbonized beam in SU 49 (NW corner), very close to SU 55 and SU 67, which yielded a date between 1150 and 1271 CE (Fig. 3). For clarity, the stratigraphic diagram (Supplementary Material 1) illustrates the spatial relationships between SUs and wall stratigraphic units (WSUs) within Area 2000.

2. Materials and methods

2.1. Sampling and preliminary analyses

The methodological approach for charred grain isolation was undertaken to select archaeobotanical samples that could be most promising for molecular analysis. After clearing the surface consisting of small trees and shrubs and removing an initial layer of collapsed stones, rubble, and disturbed deposits, the excavated site revealed an original black soil layer rich in wood fragments and carbonized seeds and fruits. The samples were wrapped by a thin layer of sediment that could be easily removed in the laboratory after washing with sterile water and UV sterilization. We proceeded by hand-picking, selecting one by one the charred caryopses that appeared well-preserved, chosen as most suitable

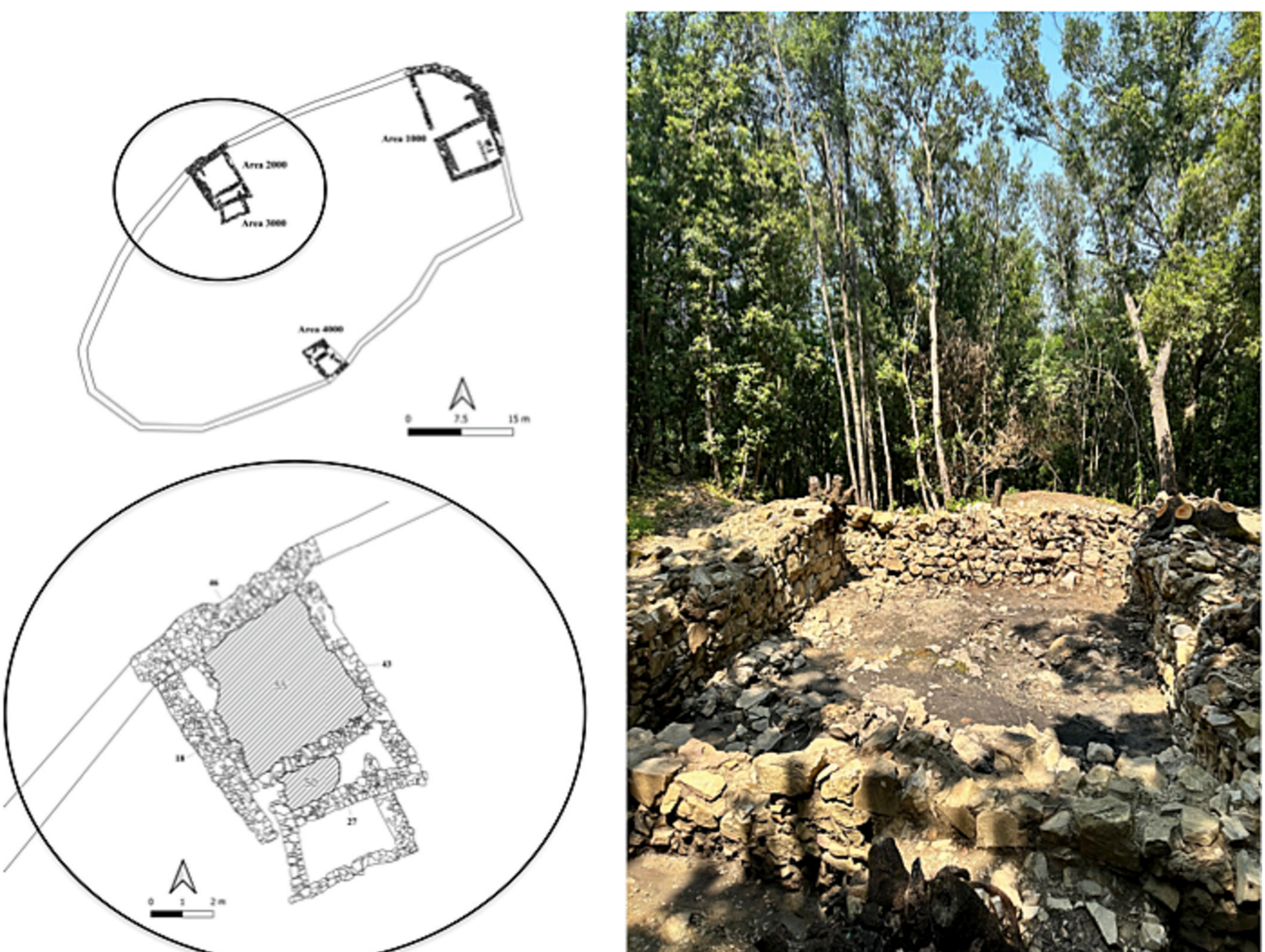

Fig. 2. A) area 2000 within the excavation of the monteverdi castle; b) the mentioned stratigraphic units within area 2000; c) a photograph of area 2000.

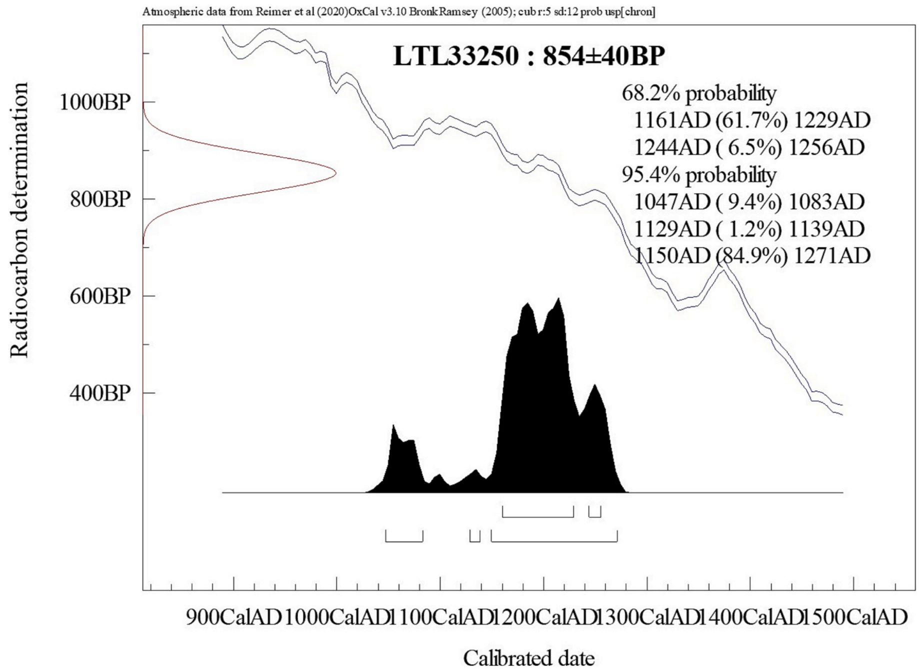

Fig. 3. Radiocarbon dating of a charred fragment of *Quercus robur* beam fragment from SU 49, very close to (SU) 55 and 67 which returned a date between 1150 and 1271 CE.

for the subsequent DNA analysis. Our investigations focus on stratigraphic units 55 and 67, which showed abundant, intact, and well-preserved archaeobotanical materials, in contrast to stratigraphy with low concentrations. Regarding carpological and anthracological analysis remains, dry sieving was performed using stacked sieves with progressively smaller mesh sizes (10, 5, and 2 mm). This allowed the separation of fragments by size class and the study of all fragments larger than 2 mm. Separated carpological remains were observed using a Zeiss Stemi 305 microscope (8x to 40x magnification) and identified with the aid of atlases such as Sabato and Peña-Chocarro (2021), Jacomet (2006), and online resources. Botanical nomenclature follows Galasso et al. (2024). The wooden fragments were identified by examining the diagnostic sections (transverse, tangential, and radial) using a differential interference contrast microscope (Nomarski) and reference atlases (Schweingruber, 1990; Cambini, 1967). The nomenclature for the oaks follows Cambini (1967).

2.2. Morphometric analysis

A total of 97 selected (i.e., grains with most conform to morphological coherence shapes) wheats caryopses were analysed. Digital images of the caryopses in dorsal position were acquired against a white, illuminated background to maximize the outlines, with a high-resolution scanner. The overall caryopses shape was studied by elliptic Fourier analysis on the extracted outline coordinates (for the procedure see Costa et al., 2008). As reported by Loy et al. (2000), this method consists of decomposing a curve into a sum of harmonically related ellipses. In addition, the mean-harmonics outlines for all the cultivar (both modern and ancient) were extracted. Euclidean distances among the mean caryopses shape configurations of each cultivar (both modern and ancient) belonging to the same taxon, were calculated with the complete linkage algorithm (Costa et al., 2010) considering morphological and molecular data. Results were plotted as dendrograms, and the Mantel test (10 000 permutations; r coefficient) was used to measure the significance of differences between pairs of distance matrices [i.e. morphological and molecular distance matrices for each cultivar (both modern and ancient)]. The results of analysis of morphological differences were compared with previously computed molecular distances to verify the presence of homologies between ancient and modern caryopses.

2.3. Genetic analysis

Each sample was divided into two parts to perform at least two independent extractions, amplifications, and sequences to verify the consistency of the results. These precautions are essential to be reasonably certain that the DNA being analyzed is indeed extracted from the ancient remains. For the purification of DNA from ancient caryopses, we followed a simplified extraction protocol using the DNeasy Plant Extraxction Kit (Qiagen), reducing all volumes by a factor of 10 and performing an overnight extraction at 50 °C. Carpological samples were processed in amounts of 20 mg each, comparable to lyophilized material. The extracted material was resuspended in ultrapure sterile water. DNA from traditional wheat varieties (*Triticum aestivum* L.) was extracted from leaves following the kit instructions without volume reduction. This procedure allowed us to avoid routine analytical methods to check DNA quality before amplification, thus saving DNA template from genotyping. The varieties Autonomia B, Sieve, and the species *T. monococcum* L. used for genetic comparisons are ancient grains from the germplasm of the Tuscan region (https://germoplasma.arsia.toscana.it/Germo_Img/14_1_1533031619.pdf), which is dedicated to enhancing traditional genomic heritage. The quality of extracted DNA was checked using routine techniques for leaf material, while for DNA from ancient carpological remains, due to the limited quantity available, we decided to verify the quality of the purified material using capillary electrophoresis with specific markers for the *Triticum* genome. Ten SSR (Simple Sequence Repeats) markers were selected through capillary electrophoresis (CE): gwm63, gwm186, gwm126, gwm312, gwm205, WMC20, WMC50, WMC283, WMC713, WMC24. The markers and critical PCR conditions are indicated by available databases for *Triticum aestivum* (https://wheat.pw.usda.gov; https://webtom.cabgrid.res.in/wheatssr/). General PCR amplification conditions are described in Vignani et al. (1996). PCRs were set up under sterile conditions using a biohazard hood, making sure to treat ancient samples separately from contemporary wheat varieties, according to guidelines for avoiding contamination, manipulating them with extra care under a laminar hood. SSR site polymorphism was examined using capillary electrophoresis, based on laser scanning of fluorescently labeled DNA fragments. Genotyping was performed on a DNA sequencer and a fragment analyzer ABI3130 (Life Technologies). Fluorescently labeled amplified fragments were assessed using the free GeneMarker software. The discriminative power of the test in resolving varieties was estimated by calculating the probability of identity (PI) with the IDENTITY v.4.0 program (Wagner & Sefc, 1999) for each set of SSR markers used, as described by Sefc et al., (2000).

Specifically, to improve the experimental strategy, we chose for genetic comparisons cultivars with traditional genomic configurations that the Tuscany region has committed to preserving and protecting to avoid losing an ancient genomic heritage (http://terreregionali.toscana.it). These are grains selected from traditional cultivars to be protected and distinguished from selective grain cultivations aimed at the food industry. Thus, it seems plausible that the archaeological material, potentially subjected to fewer anthropogenic selective pressures than varieties cultivated over the years, shows an elective affinity with markers mapping in the oldest version of the *Triticum* genome.

3. Results

Through morphological observations using optical microscopy, it was possible to identify a total of 1134 carpological remains preserved through carbonization, attributable to 13 different taxa (Table 1). The sample from stratigraphic unit 67 shows a qualitatively greater variability (12 taxa) compared to the sample from SU 55 (9 taxa). In both samples, the carpological remains found are mainly attributable to cereals, except for darnel (*Lolium temulentum* L.), a common weed in cultivated fields whose grains are similar in size to those of cereals. Additionally, in SU 67, there are seeds of peas (*Lathyrus oleraceus* Lam.), lentils (*Vicia lens* (L.) Coss. & Germ.), and other unidentified legumes. An endocarp of Mediterranean medlar (*Craetagus azalorus* L.) was also found. It is interesting to note that despite a similar quantity of grains, SU 55 is characterized by an abundance of *Hordeum vulgare* L., while in SU 67, grains of *Triticum aestivum/durum* prevail.

Regarding the anthracological remains (Table 2), 52 fragments of charred wood were studied and identified, 44 from SU 55 and 8 from SU 67. In SU 55, there is an abundance of deciduous oaks (*Quercus* sect. *robur* – 35 fragments), but five fragments of *Ostrya/Carpinus* and two of evergreen oaks (*Quercus* sect. *suber*) were also identified. In contrast, SU 67 presents greater taxonomic diversity. Here, ash (*Fraxinus* sp.) is the most abundant taxon (2 fragments), followed by *Acer* sp. (maple), Fabaceae Faboideae, *Quercus* sect. *cerris* (semi-deciduous oaks), *Quercus* sect. *suber*, and *Quercus* sp.

Table 1
Number of carpological remains found in the studied samples. "fr." indicates fragments, while "indet." means unidentified, indicating that a more precise identification could not be reached.

Taxon	Type of remain	Area 2000 SU 55	SU 67
Avena sativa	caryopsis	2	2
Craetagus azalorus	fruit	0	1
Hordeum vulgare	caryopsis	295	37
	caryopsis fr.	47	6
Lathyrus oleraceus	seed	1	3
Lolium temulentum	caryopsis	6	17
Triticum aestivum/durum	caryopsis	123	423
Triticum turgidum subsp. *dicoccon*	caryopsis	8	39
Triticum monococcum	caryopsis	45	6
Triticum sp.	caryopsis fr.	28	0
Cereals indet	caryopsis fr.	26	6
Vicia lens	seed	0	2
Vicia/Lathyrus sp.	seed	0	6
	seed fr.	0	2
Pulses indet.	seed fr.	0	3
	TOTAL	581	553

Table 2
Anthracological assemblage of the studied samples, number of studied fragments. "Indet." indicates fragments that were not identifiable. The fragments are divided by size class, based on the mesh size of the sieve on which they were collected.

	Area 2000 SU 55 >10 mm	5–10 mm	2–5 mm	SU 67 2–5 mm	TOTAL
Acer sp.				1	1
Fabaceae Faboideae				1	1
Fraxinus sp.				2	2
Ostrya/Carpinus sp.			5		5
Quercus sect. *robur*	19	13	3		35
Quercus sect. *cerris*				1	1
Quercus sect. *suber*			2	1	3
Quercus sp.				1	1
Indet.			2	1	3
TOTAL	19	13	12	8	52

Table 3 shows the allele sizes obtained through PCR amplification of DNA extracted from archaeological seeds and contemporary traditional grains, after separation of the amplicon by capillary electrophoresis (CE). The observed sizes for SSR markers in the archaeological seeds and contemporary grains matched at the WMC20 marker, suggesting a correct amplification of the ancient DNA. Notably, in SU 55, there is a perfect match with the einkorn wheat (*T. monococcum* L. ssp. *monococcum*) and Sieve used as reference (112 bp; see Fig. 4), while in SU 67, there is a match with traditional modern grains (Autonomia B) for the 114 bp allele. Even if the DNA from each sample was tested at 10 SSR markers as listed in Table 3, only the WMC20 produced a readable allelic size within the expected range in the archaeological charred samples. The binning of the allele observed in the electropherogram graphs both in fresh and archaeological samples seem quite convincing and informative (Fig. 5). The polymorphism between the archaeological samples and living wheat species is well described in Table 3.

Although both morphometric and molecular analyses yielded very similar results for both distances (Fig. 5), the modern (i.e., *Triticum aestivum* cv. Sieve, Autonomia B, and *Triticum monococcum*) and archaeological (i.e., *Triticum aestivum/durum* from SU 55 and SY 67) wheat grains exhibited completely different patterns. Specifically, the samples were divided into two distinct groups. The first group consisted of all the modern caryopses, and the second group comprised the ancient ones.

Fig. 6 shows the average profiles for all the samples (i.e., the modern *Triticum aestivum cv.* Sieve, Autonomia B, and *Triticum monococcum*, and the archaeological *Triticum aestivum/durum* from SU 55 and SU 67). These shape differences are not easily perceivable visually, but such discrimination can be accurately achieved through quantitative image analysis techniques.

4. Discussion

The demographic decline recorded in central Italy during the 11th century experienced a reversal, possibly linked to changes in agricultural techniques that promoted the expansion of cultivated areas and subsequent economic recovery (Cortonesi & Passigli, 2016). With deforestation, also indicated by pollen analyses from sediment cores, there was a significant development in agriculture (Mensing et al., 2015). Cereal crops gained much more importance, and the consumption of meat, which became a social privilege increasingly reserved for the nobility, declined. The diet of the middle-class population, predominantly vegetarian, was largely based on the consumption of bread, which became a widely consumed product (Cortonesi & Passigli, 2016). The establishment of fortified villages and hamlets was widespread, with toponymy studies highlighting that geographical names attributed during the Middle Ages often encapsulated images of the surrounding environment (Tichelaar, 2017). Thus, the name "Monteverdi", meaning

Table 3
Allelic sizes obtained through PCR amplification of DNA extracted from ancient grains and contemporary wheat leaves.

SSR marker	Gwm 63	Gwm 186	Gwm 126	Gwm 312	Gwm 205	WMC 20	WMC 50	WMC 283	WMC 713	WMC 24
SU 55						112				
SU 67						114				
Autonomia B	255–271	127	193	209–214	134–151	110–114	134–212	146–152	118–128	112
					(range)			(range)	(range)	
Sieve	261–270	121–137	193	214–220	134–155	110–112	141–145	93–150	105–124	
					(range)			(range)	(range)	
Triticum	236–270		193	183	137–145	112		83–108	97–122	248–255
monococcum					(range)			(range)	(range)	

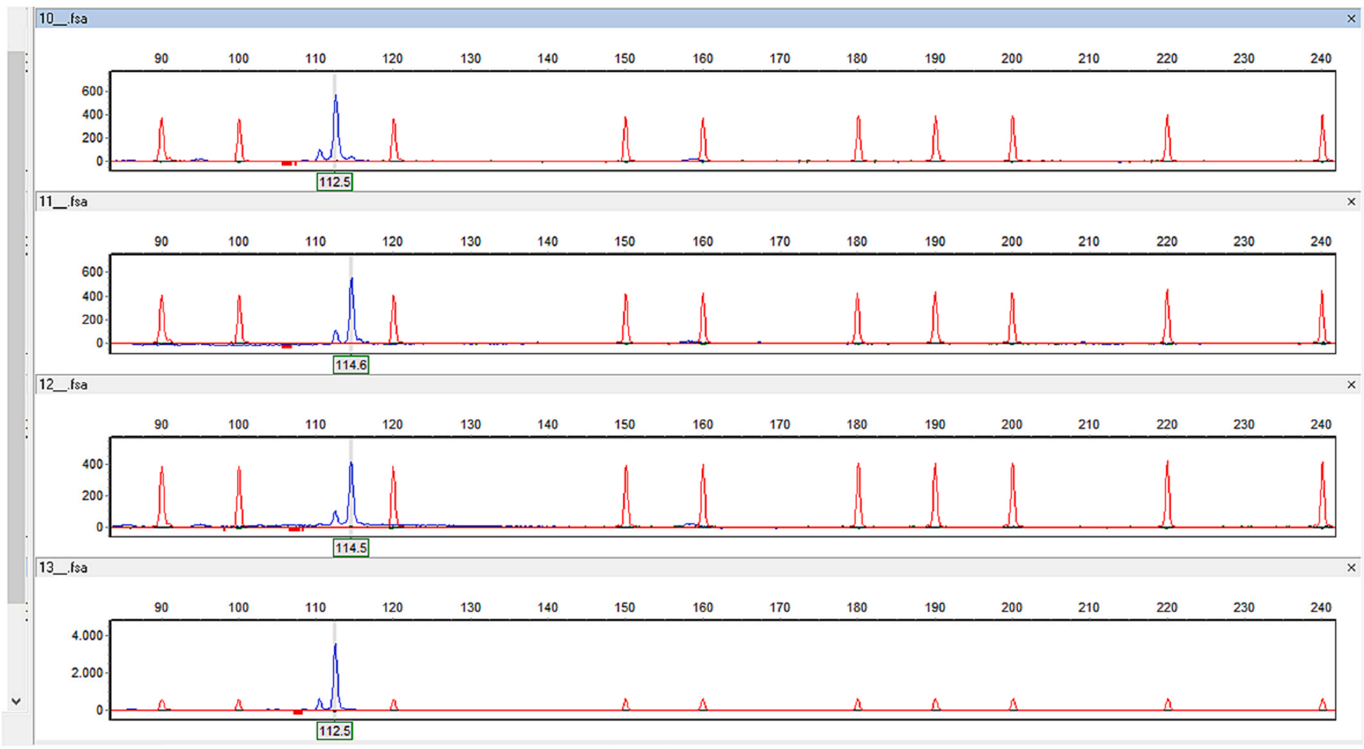

Fig. 4. Amplification at the WMC20 locus of ancient grains. From the top: SU 55, SU 67, SU 67 (technical replicate), SU 55 (technical replicate). The allelic size is shared for this genetic locus with some traditional wheat varieties (SU 67) and with einkorn wheat (SU 55).

"green mountain" likely reflected the presence of evergreen trees. The increase in evergreen oaks in the 10th-11th century is in fact also visible in pollen spectra from Grosseto province (Clò et al., 2024). The Black Death pandemic, which hit Italy in 1348, rapidly reverted the scenario, with palynological data confirming field abandonment and discernible reforestation (Izdebski et al., 2022).

The use of different plant proxies, such as carpology, anthracology, palynology, as well as morphometry and the study of ancient DNA, allows for a broader perspective and the reconstruction of various aspects of the human-environment relationship in past societies (e.g., Fornaciari et al., 2018; Moricca et al., 2021; Bosi et al., 2022; Sarigu et al., 2022; Masi et al., 2024). The geographical area of Monteverdi is particularly interesting as it is set on the margins of a predominantly agricultural fertile area. The traditional identification of seeds and fruits, based on the morphological characteristics of specific taxa, is a fundamental step in archaeobotanical studies, allowing for the identification of plants present in a particular archaeological context. These are economic and quick analyses that do not require pre-treatment. However, it is not always possible to identify remains at the species level. This is usually due to two factors: poor preservation (Pearsall, 2015), or few interspecific morphological differences and significant intraspecific variability (Tarongi et al., 2024).

The recovered plant remains provide insights into the resources available at the fortified medieval settlement of *Castellaraccio di Monteverdi* (Castle of Monteverdi) during the mid- to late-12th century. Although we are aware that visual recovery is heavily biased towards remains that are either larger or accumulated in caches (Pearsall, 2015), our study represents a contribution to the state of art of medieval archaeobotany in Italy. In fact, according to the BRAIN database (brainplants.successoterra.net; Mercuri et al., 2024), plant macroremains dating to the Middle Ages in Tuscany have only been studied in twelve sites (carpological remains were found in only five of these), only one of which is in the province of Grosseto. Due to the limitations posed by the chosen sampling method, we will discuss our assemblages solely from a qualitative perspective.

The numerous charred carpological remains give information about the food plants available at the site. These are represented by cereals (mainly *Hordeum vulgare* – barley, and *Triticum aestivum/durum* – naked wheat), but also include pulses, such as *Lathyrus oleraceus* (pea) and *Vicia lens* (lentil). An endocarp of medlar (*Crataegus azalorus*) was also

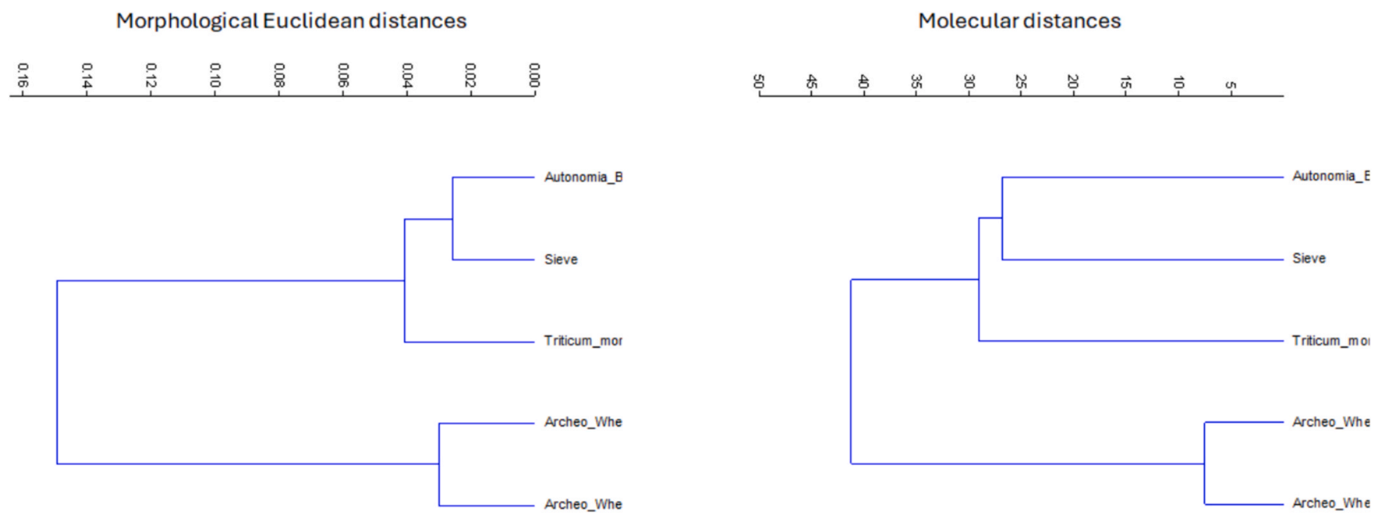

Fig. 5. Dendrograms for morphological Euclidean (left) and molecular analyses (right) obtained by matrices for morphological Euclidean and molecular distances, treated with the complete linkage algorithm. The samples analyzed were: for modern seeds, *Triticum aestivum* cv. Sieve, Autonomia B, and *Triticum monococcum*; for ancient ones (Arche Whe), *Triticum aestivum/durum* SU 55 and SU 67.

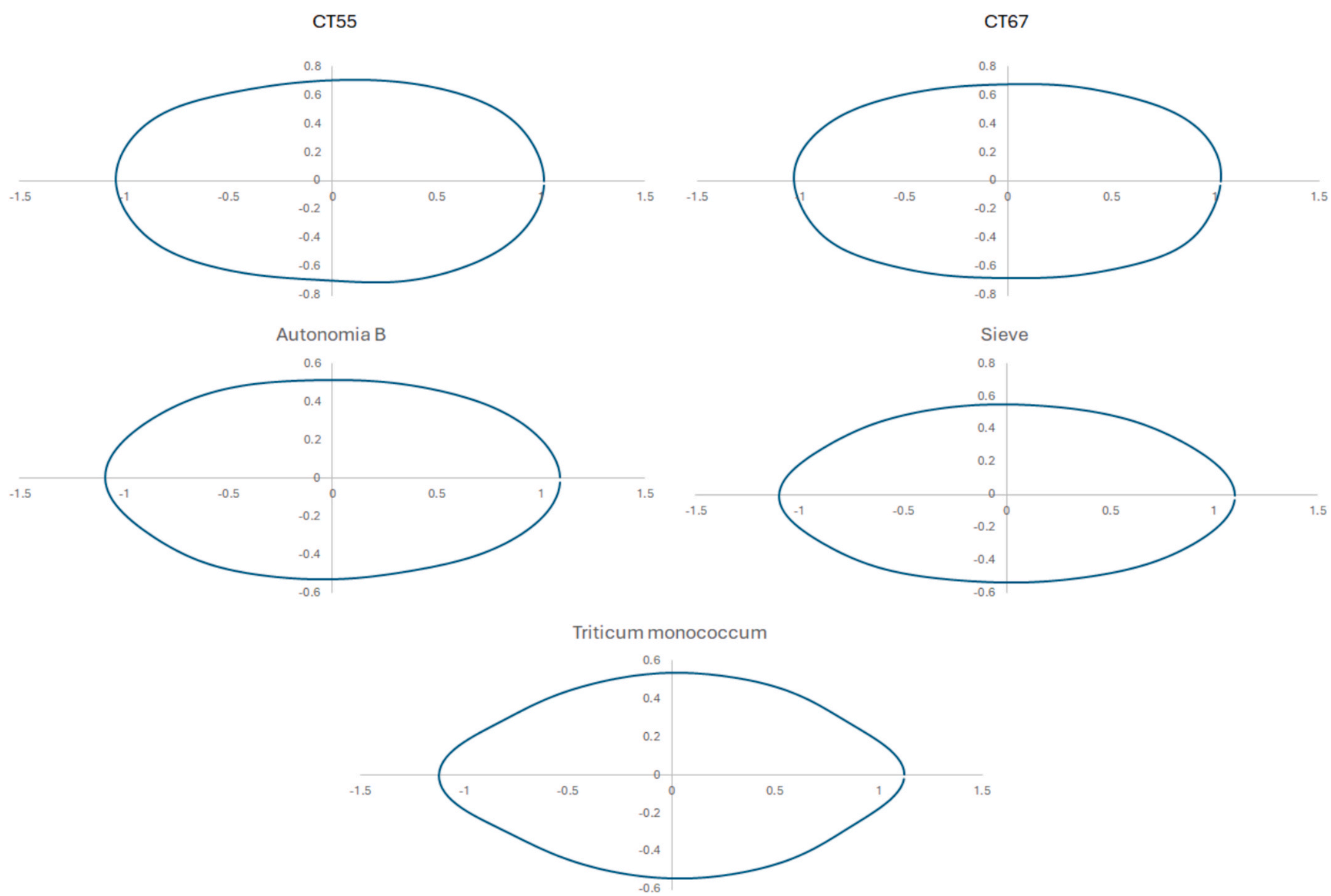

Fig. 6. Mean harmonic outlines for all the samples (i.e., modern caryopses *Triticum aestivum* cv. Sieve, Autonomia B, and *Triticum monococcum*, and for ancient ones, *Triticum aestivum/durum* SU 55 and SU 67).

found. Finally, weeds of cultivated fields are represented by darnel (*Lolium temulentum*), whose grains are of similar size to those of cereals.

The best-preserved grains, which had undergone minimal fire damage, belong to the *Triticum* genus. They were accumulated in small pits or scattered on the layers, possibly as residual activity from the drying process (Bianchi et al., 2011; Bianchi & Grassi, 2013). The structure found in SU 79, interpreted as a small oven due to the presence of fragments of a vessel probably used for bread-making near SU 55 and SU 67, might explain the richness in archaeobotanical materials of these stratigraphic units. These findings are coherent with those collected from the 10th century samples of the nearby Medieval castle of Rocchette Pannocchieschi, in the province of Grosseto (Buonincontri et al., 2013a). Here, cereals represent most of the assemblage, with naked wheats prevailing. Similarly to the case of Monteverdi, pulses are represented by peas and lentils. However, *Vicia sativa* L. and *Ervilia sativa* Link, not identified in the castle of Monteverdi, were found at Rocchette Pannocchieschi.

The anthracological assemblage reveals the presence of deciduous (*Quercus* sect. *robur*), semi-evergreen (*Quercus* sect. *cerris*) and evergreen (*Quercus* sect. *suber*) oaks, as well as hop-hornbeam (*Ostrya/Carpinus*), ash (*Fraxinus* sp.), maple (*Acer* sp.), and Fabaceae Faboideae. These are taxa that reflect the current vegetation of the woodlands of the Grosseto Maremma (Arrigoni et al., 1990; Selvi, 2010). They are all recorded in the northern hill-planitial phytogeographical sector of Maremma, delimited to the north by the Pecora river and to the south by the Ombrone river (Selvi, 2010). It is therefore possible to hypothesize that the inhabitants of the Monteverdi castle exploited local resources. Anthracological data from the castle of Monteverdi are generally coherent with data collected in other Medieval castles in Tuscany, with deciduous oak fragments being found in Rocchette Pannocchieschi (GR) (Buonincontri et al., 2013a), Gorfigliano (LU) (Montanari and Scipioni, 2004), Donoratico (LI) (Buonincontri et al., 2013b) and Campiglia Marittima (LI) (Di Pasquale, 2003). Evergreen oaks and maple were identified in three out of the four castles, *Ostrya/Carpinus* in two, while Fabaceae only at Gorfigliano. These differences in the wood assemblages might be influenced by the sampling procedure, which might have limited the number of taxa retrieved in Monteverdi. Nonetheless, a similar number of taxa was also recorded at Campiglia Marittima and Rocchette Pannocchieschi. Another factor to consider is given by the vegetation surrounding each site. For example, Donoratico is found much closer to the sea, which is reflected by the presence of numerous Mediterranean maquis taxa in the anthracological assemblage.

Despite of the DNA from each sample having been examined at the ten SSR markers indicated in Table 3, only the WMC20 marker generated a readable allelic size within the expected range in the archaeological charred grain. The local bread wheat strain “Sieve” and *T. monococcum* ssp. *monococcum* share the observed allele size of 112 bp, while the local bread wheat strain “Autonomia B” shares the allele size of 114 bp. The electropherogram graphs of both fresh and archeological samples show reliable binning of the allele, which appears to be very informative and compelling (Fig. 5). It is not surprising that only one SSR marker produced a correct amplification product, since the performance of the SSR testing, especially on degraded DNA templates, largely depends on unknown factors, which might include the specific integrity of the tagged site and the degree of purity of the total DNA extracted out of “difficult” samples and possible contaminants interfering with the PCR efficiency.

Finally, the morphometric analysis allowed us to appreciate the harmonic carpological profiles of the caryopses, which differ from modern ones. The data obtained through quantitative analysis techniques show the profile of multiple samples combined into a single image, highlighting how their carbonization, preservation, time, and biodeterioration can transform the artifact. Charred archaeological plant materials are resistant to biodeterioration but may be subjected to: (a) reducing conditions, with morphometric measurements calibrated considering this factor; and (b) swelling or deformities due to high heat that can alter material identification, as reported in the work by Bonhomme et al. (2017). While experimental charring of reference material would be indicated, the study by Bonhomme et al. (2017) shows that there is little overlap between species shape at a charring temperature of 230 °C, which gradually increases with the increase of temperature. In our case, the carbonization temperature does not appear to have exceeded 230–260 °C, and for morphometric observations, materials were selected that, under optical microscopy, did not show swelling or morphological alterations. The similarities between the dendrograms of the morphometric and molecular analyses are particularly interesting. Moreover, considering the high cost of molecular analysis materials, morphometry, which is much cheaper, confirms its potential as a preliminary investigative tool for the classification and selection of both ancient and modern grain cultivars, before proceeding to more demanding and expensive analyses.

5. Conclusions

The archaeobotanical evidence at the fortified settlement of *Castellaraccio di Monteverdi* provide insights into human-environment interactions in southern Tuscany during the 12th century. The charred carpological remains are mostly refereable to diet, constituted primarily of cereals (mostly barley and naked wheats), supplemented by pulses and occasional fruits like medlar. Although these data derive from hand-picked samples and need to be interepreted with caution, they are consistent with evidence for a bread-based diet during the medieval period, particularly among the non-elite population, as described in historical sources. The richness of plant remains in specific stratigraphic units, in association with baking-related features such as a small oven, suggests localized domestic food preparation activities, possibly related to bread-making. The carpological dataset aligns with evidence from other contemporary sites in the region, such as Roccette Pannocchieschi, although some differences in pulse taxa were observed.

Anthracological analysis indicates the exploitation of surrounding woodland resources, with a predominance of deciduous oaks, along with hop-hornbeam, ash, maple, evergreen and semi-deciduous oaks, and Fabaceae Faboideae. These taxa correspond to the vegetation typical of the Grosseto Maremma and are consistent with findings from other medieval castles in Tuscany. Minor differences may be due to sampling procedures or localized ecological contexts.

The integration of morphometric and molecular analyses has proven particularly valuable. While DNA preservation is limited, the single successful SSR marker (WMC20) produced reliable data, revealing allele sizes compatible with known local wheat strains. The morphometric profiles of the well-preserved charred caryopses reinforce these results and confirm themselves to be a low-cost, complementary method for varietal identification.

This study contributes new data to the still limited archaeobotanical record for medieval Tuscany, particularly in the province of Grosseto. It demonstratesthe high value of combining carpological, anthracological, molecular and morphometric approaches to reconstruct human-enviroment interactions in rural medieval contexts.

CRediT authorship contribution statement

Claudio Milanesi: Supervision, Formal analysis. **Alessandro Sebastiani:** Investigation. **Alessandro Carabia:** Data curation. **Rita Vignani:** Investigation. **Francesca Antonucci:** Data curation. **Simona Violino:** Data curation. **Monica Scali:** Formal analysis. **Giampiero Cai:** Methodology. **Mauro Cresti:** Methodology. **Claudia Moricca:** Methodology, Investigation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2025.105397.

Data availability

Data will be made available on request.

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