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Unexpected morphological diversity in ancient dogs compared to modern relatives

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Dogs are among the most variable species today, but little is known about the morphological variability in the early phases of their history. The Neolithic transition to farming may have resulted in an early morphological diversification as a result of changes in the anthropic environment or intentional selection on specific morphologies. Here, we describe the variability and modularity in mandible form by comparing 525 dog mandibles from European archaeological sites ranging from 8100 to 3000 cal. BC to a reference sample of modern dogs, wolves, and dingoes. We use three-dimensional geometric morphometrics to quantify the form of complete and fragmented mandibles. We demonstrate that an important morphological variability already existed before the Bronze Age in Europe, yet the largest, smallest, most brachycephalic or dolichocephalic extant dogs have no equivalent in the archaeological sample, resulting in a lower variation compared to modern relatives. The covariation between the anterior and posterior parts of the mandible is lower in archaeological dogs, suggesting a low degree of intentional human selection in early periods. The mandible of modern and ancient dogs differs in functionally important areas, possibly reflecting differences in diet, competition, or the implication of ancient dogs in hunting or defence.

1. Background

Although they descend from the same ancestor (the grey wolf; [1]), extant dogs are among the most variable species on the planet, both in terms of size and proportions [2]. This tremendous variability is the result of rapid phenotypic changes in response to strong intentional selection by humans over the last 200 years (defined herein as 'modern' times). This selection on specific physical or behavioural traits to satisfy functional needs (e.g. for work, hunting or running) or for strictly aesthetic reasons led to the creation of breeds (defined by standards established since the nineteenth century) and to the diversity of forms observed today [3].

While numerous studies have explored the genetic basis of the phenotypic variability in extant dogs (e.g. [4–10]), very little is known about their morphological variability in the past in Europe. Considering that the notion of breed refers to a very recent event, the terms ‘type’ or ‘morphotype’ (characterized by different cranial proportions) are more relevant to describe head shape in ancient dogs. A division into dolichocephalic (elongated and narrow skull, akin to greyhounds), brachycephalic (broad and short skull, akin to mastiffs or bulldogs) and intermediate mesocephalic types is thus often used. Different morphotypes appear identifiable in Western Europe as early as the Metal Ages (i.e. from the second millennium cal. BC), and this phenomenon intensified during the Middle Ages and modern times [11,12]. Few studies have explored morphological variability in earlier periods, yet different sizes of dogs are described as early as the late Upper Palaeolithic in Western Europe [13,14]. However, the early phases of domestication are particularly complex to consider given the uncertainty surrounding the place and timing of domestication [10,15–17] and the difficulty of distinguishing wolves from early dogs owing to the scarcity and the fragmented nature of these early canid remains [18]. The strong morphological similarity between early dogs and wolves [19–21], and possible hybridization between them [1,3] render this even more complex.

The period from the Mesolithic onwards (i.e. from the tenth millennium cal. BC) is of great interest to explore the morphological diversity of dogs in Europe. First, because from this period onwards dogs show a marked phenotypic divergence from their ancestors and size seems to clearly distinguish them [13,22–24]. Second, dog remains become recurring in European sites ([9,24–28]), their frequency increasing during the fifth millennium cal. BC throughout Europe (e.g. [22,24,29–33]). Third, genetic data show that modern dogs originate from at least five ancestral dog lineages which diversified by *ca* 9000 years BC [10], several of them being responsible for early and successive gene flow which made up the genetic history of ancient dogs in Europe [6,10]. In parallel, the increasing sedentarization and subsequent transition to farming during the Neolithic in Western Eurasia (i.e. from the middle tenth millennium cal. BC) may have involved intentional selection of certain phenotypes (in terms of size or proportions) associated with specific tasks (hunting, guarding). In Europe, from the Mesolithic onwards, dogs were widely eaten, and their bones were occasionally used for the manufacturing of ornaments. Dogs were also the subject of other social or symbolic considerations as attested by the burial of complete bodies, sometimes in close association with human deposits. It is difficult to demonstrate their role in hunting or for the protection of camps or villages, but their scarce presence in prehistoric settlements, where they were neither eaten nor buried, could be an indication. In that respect, the vast diversity in the roles played by dogs at that time [11,22,25,29,30,34] may have been accompanied by an early morphological diversification, long before the Metal Ages.

The morphological and genetic diversity of dogs during this time period in Western Eurasia has been explored to some degree. Genetic data providing information on coat colour [5], mitochondrial haplogroup [6], or their ability to digest starch [10,35] have been published. Osteometric data are abundant, however, these consist of linear measurements which only provide information on the stature and gracility of the animals (e.g. [22–24,28,36]) and do not allow us to explore the morphological variability of canids beyond size. Thus, to date, there is no large-scale study describing the morphological variability of European prehistoric dogs. Given the considerable number of mandibles in archaeological assemblages, and the fact that mandibular shape has been proven to be a good indicator of cranial shape and thus of the overall head morphotype in modern dogs [37], this bone is a promising object to explore morphological variability in the head of ancient dogs (yet not systematically correlated to the morphology of the complete body in modern dogs). Additionally, as significant and strong relationships between the mandible and the masticatory muscles have been demonstrated in modern dogs [37,38], mandible form may be used to make functional inferences on archaeological specimens.

With the present study, we aimed at describing the overall morphological variability in the mandible of a very large sample of European prehistoric dogs (defined herein as ‘ancient’ dogs) and compare them to a sample of modern dogs. We included dogs from the period ranging from the Mesolithic to the end of the Romanian Chalcolithic (*circa* 8100–3000 cal. BC) to avoid the possible misidentification of wolves, dogs, or their hybrids in the early phases of the domestication process. Three-dimensional geometric morphometric methods were used to describe mandible form in a sample of 525 archaeological mandibles of dogs and eight wolves from 27 sites in Western Europe and Romania (figure 1*a*). Given that mandibles are rarely complete in the archaeological record, making it more challenging to have a complete representation of an extensive sample from ancient remains, we used 10 different protocols corresponding to the most common fragmentation patterns in our sample (figure 1*c*, electronic supplementary material, text and figure S1). For each fragment, the variability of ancient dogs was compared to variability evident in a sample of 66 modern dogs of various breeds, dingoes and captive wolves. We used multivariate statistical tools to quantify the variability in both size and shape. Given the diversity in status of these prehistoric dogs we expected some variability in ancient dogs, yet less so than in modern dogs submitted to intensive intentional human selection. As dingoes have evolved separately over the last 3600–5000 years (the estimated time of first introduction to Australia, mostly likely as a result of transport by Asian seafarers [39]), we predicted that their form would be intermediate between that of the ancient and modern dogs. Assuming that prehistoric dogs were subjected primarily to natural constraints while the anatomy of modern dogs is limited by strong developmental constraints [2,40,41], we expect the mandible of ancient dogs to be more plastic (i.e. to show morphological traits in response to their environment and allowing them to survive in new conditions [42]). We thus tested the relationship (i.e. covariation, integration) between the anterior (supporting the teeth and thus more conservative) and posterior part (supporting the masticatory muscles and thus more akin to represent functional variability showing adaptation to environmental constraints) of the mandible (figure 1*b*) and expect it to be lower (i.e. more modular, less integrated) in ancient dogs compared to modern relatives.

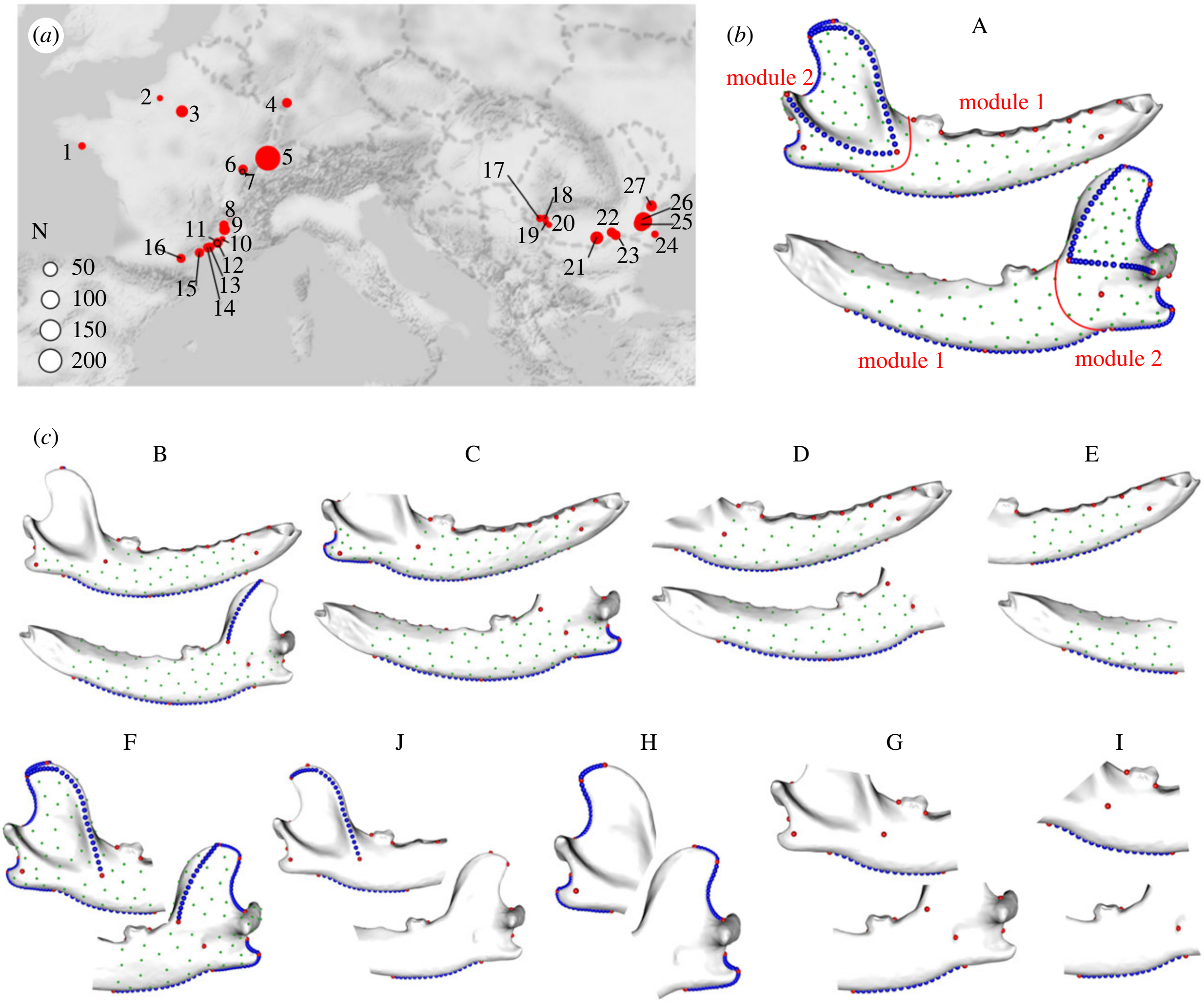

Figure 1. Origin and fragmentation patterns of the archaeological mandibles. (*a*) Map showing selected sites dating from the Mesolithic to the end of the Romanian Chalcolithic in Western Europe and Romania. Site key, chrono-cultural attribution and dating are presented in the electronic supplementary material, dataset S1. (*b*,*c*) Three-dimensional landmarks considered in the geometric morphometric analyses for the most complete (*b*) or fragmented (*c*) mandibles, matching the 10 subsets of fragments (letters A to J), represented on lateral (top image) or medial (bottom image) views of the mandible. Anatomical landmarks are in red, sliding semi-landmarks of curves are in blue, sliding semi-landmarks of surfaces are in green. 'Module 1' and 'module 2' represent the two modules used in the modularity tests performed on the complete mandibles. Definitions of the anatomical landmarks are provided in the electronic supplementary material, table S2. (Online version in colour.)

2. Methods

A full description of the materials and methods used is provided in the electronic supplementary material, supplementary text.

We quantified the shape of 525 dog and eight wolf mandibles from 27 archaeological sites dated to between the Mesolithic and the end of the Romanian Chalcolithic across Western Europe and Romania (figure 1*a*; electronic supplementary material, table S1). The distinction between dogs and wolves was made following a size criterion [13,14,22,35] (see the electronic supplementary material, text and figure S2). The modern sample constituted 66 dogs of various breeds (see the electronic supplementary material, dataset S1), most being represented by only one or two specimens with the exception of beagles ($n = 20$). Modern dogs were classified into brachycephalic (short-headed), mesocephalic, or dolichocephalic (long-headed) based on the cephalic index, i.e. the relative proportion of skull breadth to skull length [43]. We also quantified the shape of the mandibles of eight captive wolves and eight wild Australian dingoes. Only specimens with the first molar completely erupted (i.e. non-juvenile specimens) were considered for analyses.

We used photogrammetry to obtain three-dimensional models of the mandibles. Next, we quantified morphological variation using three-dimensional geometric morphometrics. Ten different landmarking protocols (figure 1*b*,*c*; electronic supplementary material, tables S2 and S3) were considered to take into account a maximum number of mandibles and mandible fragments, based on the fragmentation patterns most represented in the archaeological sample [44] (see the electronic supplementary material, figure S1).

A suite of statistical analyses (landmark sliding, Procrustes superimposition, Student *t*-tests, ANOVAs, Procrustes ANOVAs, principal component analyses (PCA), discriminant analyses through canonical variate analyses (CVA), etc.) were carried out in R v. 4.0.0 (2020-04-24) using the packages Morpho and geomorph (see the electronic supplementary material, supplementary text). We tested for differences in the means or the variability in centroid size, differences in shape, or allometry-free shape and explored the covariation between the anterior and posterior parts of the mandible using modularity tests (figure 1*b*).

3. Results

In the following, we focus primarily on the results based on the complete mandibles. The results of descriptive statistics for the 10 fragments are shown in table 1 and illustrated in figures 2 and 3, as well as in the electronic supplementary material, figures S3–S11 and S13. The results of the parallel analyses performed on a reduced number of landmarks are reported in the electronic supplementary material, table S3.

(a) Variability in mandible size

Ancient dogs are significantly less variable in terms of mandible centroid size and tend to have smaller mandibles, on average, than modern dogs, having a mean mandible size comparable to modern beagles (figure 2*c*, table 1). The difference in size is significant for all mandible fragments analysed (table 1). However, the range of sizes in ancient dogs was smaller than in the modern sample, with the largest brachycephalic modern dogs and the smallest toy dogs having no equivalent in the archaeological sample, which was expected. All modern wolves have mandibles larger than the largest one in ancient dogs, according to all fragments except the smallest (fragments G and I), for which there is some overlap between dogs and wolves (electronic supplementary material, figures S8C and S10C). Dingoes have mandibles of intermediate sizes, between those of dogs and wolves (figure 2*c*).

(b) Variability in mandible shape

Ancient dogs have significantly less variability in mandible shape or allometry-free shape than modern dogs (table 1), demonstrating that the differences in morphological variability depend on more than just differences in size. The results are significant for all fragments except the smallest (fragments G and I). In ancient dogs, a reduced part of the variability in shape is related to size compared to modern dogs (table 1).

This greater variability in shape in modern dogs is observable on the first two axes of the PCA for most fragments and on the trees depicting morphological similarity (figure 2*a*,*b*; electronic supplementary material, figures S3 to S11). For the most complete mandibles (fragment A), low values of the first principal component (PC1; 21.9% of total variability) describe a relatively smaller jaw angle and smaller coronoid process, while high values describe a larger area for the masseteric fossa. Low values of second principal component (PC2; 13.5% of total variability) describe a shorter and more curved mandible with a greater ramus depth, while high values for PC2 describe a flatter, shallower mandible. The first PC correlates with centroid size (PC1: $R^2 = 0.25$, $p < 0.001$; PC2: $p = 0.16$; figure 2*a*): the dogs with the smallest mandibles (therefore the smallest dogs in terms of body size) are generally positioned to the left of the scatterplot and the biggest dogs tend to be positioned towards the right. Modern dogs contribute strongly to the variability described by the first two axes, and some modern breeds extend beyond the variability observed in ancient dogs, in particular large brachycephalic breeds (such as rottweilers, pitbulls, bulldogs, leonberger, boxer, mastiff), extremely dolichocephalic dogs (such as borzoi), and small toy dogs (papillon, chihuahua, dachshund). The most dolichocephalic modern dogs have higher PC2 values (appear towards the top of the scatterplot), while the most brachycephalic dogs have higher PC1 values and lower PC2 values and separate out towards the bottom right of the scatterplot. Most ancient dogs overlap at the centre of the scatterplot with modern normocephalic breeds, modern dingoes and modern or ancient wolves. Interestingly, some ancient dogs occupy a unique part of the morphospace not occupied by modern canids on the negative part of PC1 (figure 2*a*).

The trees based on morphological distances (figure 2*b*) support the distinction between ancient and modern dogs and provide a more refined view on the positioning of ancient dogs compared to their modern counterparts. Modern and ancient dogs tend to be on separate branches and some branches (e.g. figure 2*b*, branches b and c) contain only ancient dogs. One branch clearly isolates the most brachycephalic modern dogs (figure 2*b*, branch a), distinct from the ancient dogs. Most dolichocephalic modern dogs (figure 2*b*, branch f) are similarly distinguished from most ancient dogs. By contrast, the mandible shape of some modern breeds (figure 2*b*, branch b: mastiff, figure 2*b*, branch d: husky, pomeranian, bichon teneriffe, sloughi) appears more similar to that of ancient dogs. The beagles ($n = 20$) are nearly all grouped on the same branch (figure 2*b*, branch e) in the middle of the ancient dogs. Modern dingoes group into three separate branches on this tree, mostly with modern and ancient wolves, but some are close to modern dogs and one is in the midst of ancient dogs. Most modern and ancient wolves isolate on a separate branch (figure 2*b*, branch g), distinct from all ancient dogs except one (over 127). These observations are confirmed when the sample size is increased by conducting the same analyses with fragment B ($n = 226$; electronic supplementary material, figure S3) and even other, smaller, fragments (electronic supplementary material, figures S4–S11). Not surprisingly, the analyses conducted on the smallest fragments G and I (electronic supplementary material, figures S8 and S10) lead to a tree without clear structure.

Ancient dogs significantly differ from modern dogs in mean mandible shape ($p < 0.001$ for all fragments, table 1). The discriminant analyses (CVA) performed on all fragments result in an excellent classification rate (over 87%, table 1), reinforcing the observation that ancient and modern dogs differ in shape, and that the differences involve different anatomical features located on all regions of the mandible. Ancient dogs had more robust (body of the mandible being taller under the carnassial) and curved mandibles, with a straighter coronoid process, a more developed angular process, and a shallower masseteric fossa (figure 3; electronic supplementary material, figure S13).

(c) Modularity of the mandible

The results of the modularity tests indicate significant modular structure in shape and allometry-free shape for all groups ($p < 0.001$, table 2). The coefficient ratio (CR) coefficient is much lower in ancient dogs compared to modern dogs both when considering the shape (Z-score = 11.3, $p < 0.001$) or the allometry-free shape (Z-score = 10.5, $p < 0.001$), suggesting that mandible is more modular (i.e. less integrated) in ancient dogs (the anterior and posterior part of the mandibles covary less). The preliminary results obtained for the ancient and modern wolves and modern dingoes further suggest that modularity in modern dogs is not different from that of wolves or dingoes.

4. Discussion

Our analyses clearly demonstrated that the morphological diversity in the head (the mandible being a proxy of overall head shape [37]) of European dogs was considerable well

Table 1. Results of the statistical analyses performed on all fragments to compare the variance or mean in mandible size, shape and allometry-free shape between modern ($n = 66$) and ancient dogs (sample size and the proportion of the total archaeological sample for each fragment is reported in the table). (The sample sizes of ancient wolves used for visualization in the principal component analyses and trees depicting morphological similarity for each fragment are also reported. CVA: canonical variate analysis; *p*: *p*-value; R^2 coefficient of correlation; PVa: Procrustes variance of ancient dogs; PVm: Procrustes variance of modern dogs.)

	sample size		mandible size		mandible shape			allometries		mandible allometry-free shape		
fragment	ancient dogs	ancient wolves	variance	mean	disparity	mean	CVA	ancient	modern	disparity	mean	CVA
A	127–24%	4	$p < 0.001$	$p < 0.001$	PVa 0.0039 PVm 0.0069 $p < 0.001$	R^2 0.12 $p < 0.001$	99.5%	R^2 0.027 $p < 0.001$	R^2 0.082 $p < 0.001$	PVa 0.0038 PVm 0.0065 $p < 0.001$	R^2 0.093 $p < 0.001$	83%
B	227–43%	4	$p < 0.001$	$p < 0.001$	PVa 0.0038 PVm 0.0065 $p < 0.001$	R^2 0.091 $p < 0.001$	99%	R^2 0.028 $p < 0.001$	R^2 0.079 $p < 0.001$	PVa 0.0037 PVm 0.0062 $p < 0.001$	R^2 0.077 $p < 0.001$	94%
C	216–41%	4	$p < 0.001$	$p = 0.006$	PVa 0.0034 PVm 0.0054 $p < 0.001$	R^2 0.080 $p < 0.001$	98%	R^2 0.029 $p < 0.001$	R^2 0.097 $p < 0.001$	PVa 0.0033 PVm 0.0049 $p < 0.001$	R^2 0.074 $p < 0.001$	95%
D	392–75%	6	$p < 0.001$	$p = 0.004$	PVa 0.0051 PVm 0.0068 $p = 0.004$	R^2 0.047 $p < 0.001$	99%	R^2 0.033 $p < 0.001$	R^2 0.051 $p < 0.001$	PVa 0.0049 PVm 0.0066 $p = 0.003$	R^2 0.043 $p < 0.001$	98%
E	437–83%	7	$p < 0.001$	$p < 0.001$	PVa 0.013 PVm 0.018 $p = 0.009$	R^2 0.050 $p < 0.001$	99%	R^2 0.14 $p < 0.001$	R^2 0.12 $p < 0.001$	PVa 0.011 PVm 0.017 $p = 0.003$	R^2 0.023 $p < 0.001$	96%
F	155–29%	5	$p < 0.001$	$p < 0.001$	PVa 0.0062 PVm 0.0076 $p = 0.013$	R^2 0.088 $p < 0.001$	97%	R^2 0.021 $p < 0.001$	R^2 0.082 $p < 0.001$	PVa 0.0061 PVm 0.0075 $p = 0.037$	R^2 0.068 $p < 0.001$	83%
G	386–74%	6	$p < 0.001$	$p = 0.017$	$p = 0.11$	R^2 0.053 $p < 0.001$	97%	R^2 0.053 $p < 0.001$	R^2 0.062 $p < 0.001$	PVa 0.0096 PVm 0.017 $p = 0.029$	R^2 0.053 $p < 0.001$	96%
H	160–30%	5	$p < 0.001$	$p < 0.001$	PVa 0.0035 PVm 0.0053 $p < 0.001$	R^2 0.056 $p < 0.001$	92%	R^2 0.0099 $p < 0.13$	R^2 0.093 $p < 0.001$	PVa 0.0035 PVm 0.0048 $p = 0.002$	R^2 0.041 $p < 0.001$	83%
I	488–93%	7	$p < 0.001$	$p = 0.21$	$p = 0.9$	R^2 0.021 $p < 0.001$	93% 98% ancient 62% modern	R^2 0.023 $p < 0.001$	R^2 0.051 $p < 0.001$	PVa 0.010 PVm 0.013 = 0.032	R^2 0.025 $p < 0.001$	93% 98% ancient 62% modern
J	215–41%	5	$p < 0.001$	$p < 0.001$	PVa 0.0044 PVm 0.0058 $p < 0.001$	R^2 0.11 $p < 0.001$	97%	R^2 0.027 $p < 0.001$	R^2 0.097 $p < 0.001$	PVa 0.0043 PVm 0.0056 $p = 0.009$	R^2 0.075 $p < 0.001$	91%
total	525	8										

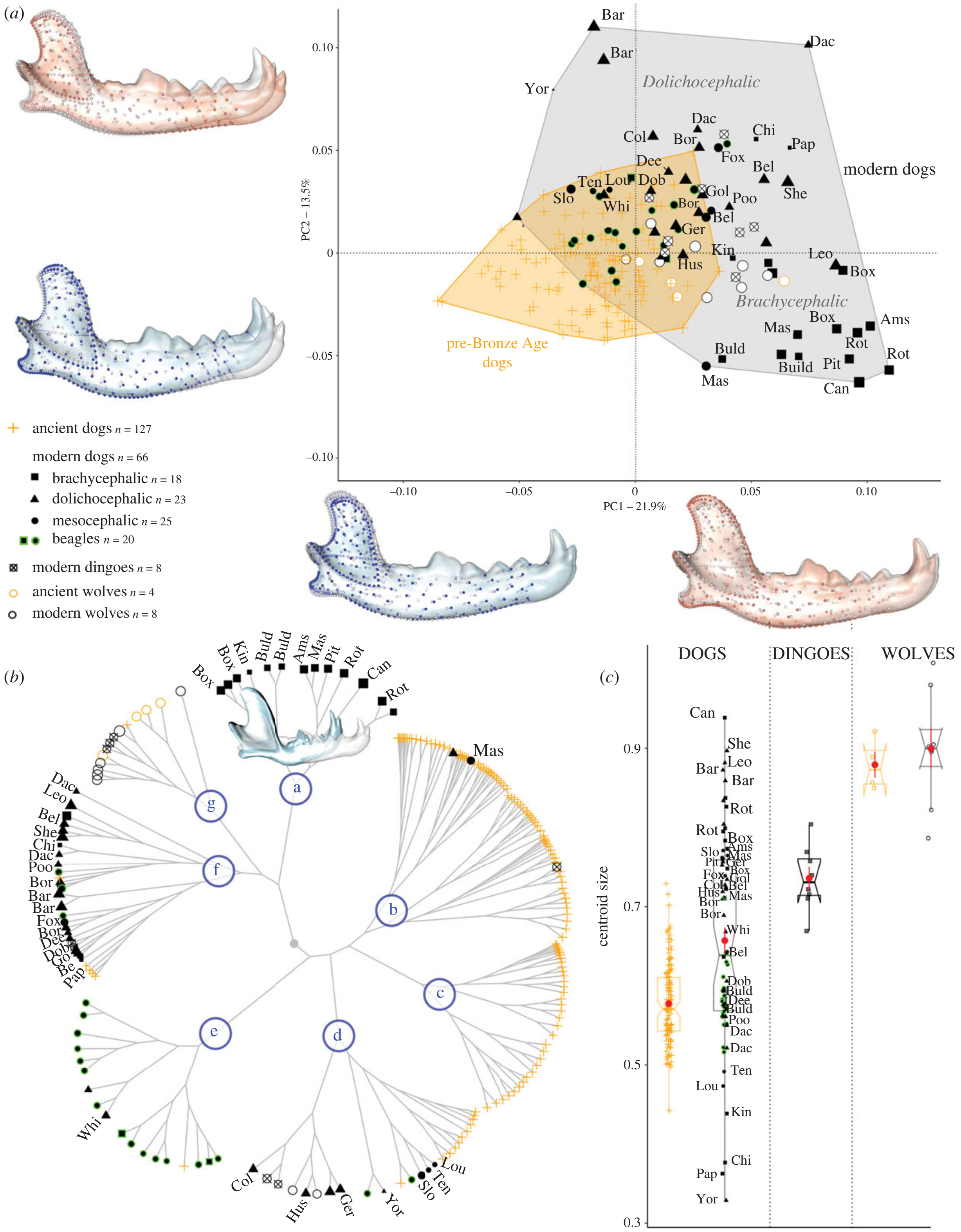

Figure 2. Visualization of the variability in mandible shape and size in ancient dogs with complete mandibles (fragment A), with comparison with ancient wolves and modern *Canis*. (*a*) Principal component (PC) analyses on modern and ancient specimens with fragment A (66 modern dogs, eight modern dingoes, eight modern wolves, 127 ancient dogs and four ancient wolves). Icon size is proportional to the $\log_{10}$ of the centroid size. Deformations at the minimum and maximum of the PC axes are represented in blue and pink, respectively. (*b*) Tree depicting the morphological similarity between mandibles based on pairwise Euclidean distances. Icon size is proportional to the $\log_{10}$ of the centroid size. (*c*) Boxplot of the centroid sizes of modern and ancient canids. Ancient canids are orange, modern canids are in black. The labels of modern dogs indicate the breed (key is presented in the electronic supplementary material, dataset S1). Groups (a), (b), (c), (d), (e), (f) and (g) are mentioned in the text. (Online version in colour.)

before the Bronze Age, yet lower than in modern dogs. In ancient dogs, mandible size varied from very small, similar to some modern small dogs such as the pomeranian or dachshund, to larger sizes compatible with modern large dogs such as the husky, golden retriever or German shepherd. However, the mandible size of ancient dogs was always much smaller than that of wolves and on average corresponds to that of modern beagles. Mandible shape varied

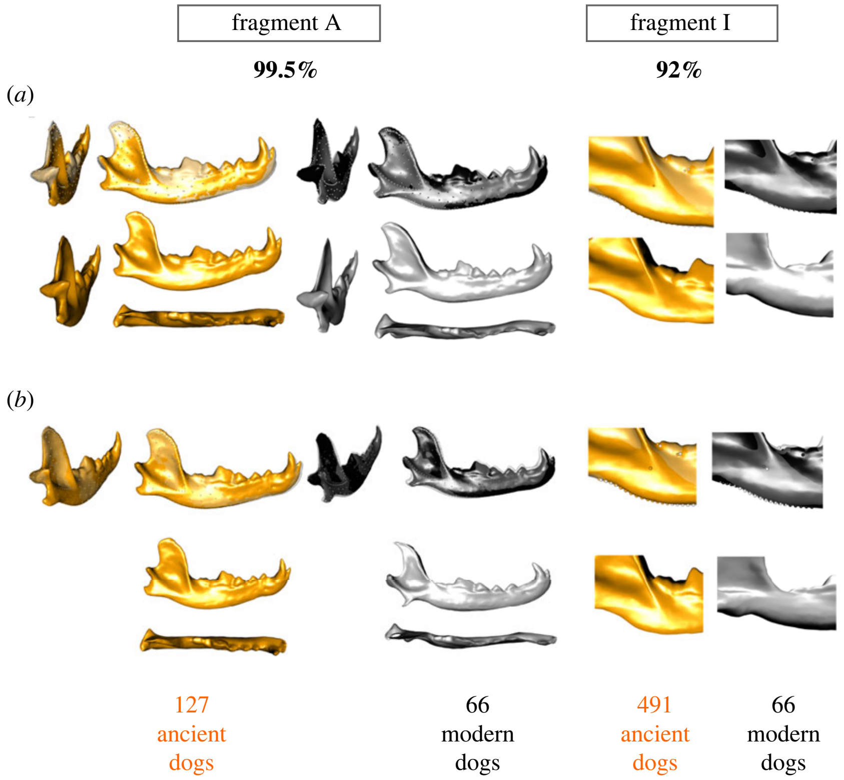

Figure 3. Visualization of the differences between modern (black/grey) and ancient (orange) dogs, based on the results of the CVA performed on mandible shapes (*a*) or allometry-free shapes (*b*) for fragments A and I. The sample size and percentage of cross-validation are indicated. On the upper line, shapes at the minimum and maximum of CV scores are superimposed to the mean shape of the CVA and vectors of deformations between the two shapes are represented. On the lower line, deformations from the mean shape to the minimum or maximum of the CV scores are magnified by 3. See the electronic supplementary material, figure S13 for visualizations with other fragments. (Online version in colour.)

Table 2. Results of the modularity tests performed on the complete mandibles. (*n*: sample size; CR: CR coefficient or covariation ratio, quantifying the degree of modularity; *p*-value: empirically calculated *p*-value from the resampling procedure; effect size: the multivariate effect size associated with the covariance ratio.)

		shape			allometry-free shape		
	n	CR	*p*-value	effect size	CR	*p*-value	effect size
ancient dog	127	0.60	0.002	−15.2	0.60	0.002	−15.0
modern dog	66	0.85	0.002	−12.8	0.85	0.002	−12.7
ancient wolf	4	0.94	0.002	−11.7	0.93	0.002	−12.6
modern wolf	8	0.88	0.002	−13.0	0.86	0.002	−14.6
modern dingo	8	0.82	0.002	−14.2	0.83	0.002	−14.1

from shapes similar to those of ancient or modern wolves or dingoes, to those of modern meso-dolichocephalic dogs (e.g. beagles, sloughi, bichon Tenerife, pomeranian, mastiff, shepherd dogs). As expected, no extreme forms were observed among the ancient dogs: the very brachycephalic (pitbull, amstaff, boxer, bulldog etc.) and very dolichocephalic (borzoi) modern dogs have no equivalent in ancient times. There was nevertheless substantial morphological variability in ancient dogs, indicating that dogs had diverse head shapes as early as the Neolithic in Europe. Yet, this diversification probably did not result from a drastic selection similar to the intentional breed selection over the past 150 years. The lower variability in ancient dogs, as well as the relative short morphological distance between some dingoes and ancient dogs or between some ancient dogs and wolves or modern dogs of average morphologies suggests minimal intentional human selection for specific morphotypes. In other words, if the first farmers selected for distinct morphotypes, they did not favour extreme morphologies.

Interestingly, clear differences in the mean shapes between the ancient and modern samples were discovered. This separation is not influenced by taphonomic or pathological processes (e.g. exostosis associated with dental disease). Indeed, only the most complete mandibles were included in analyses with fragment A. Additionally, the parallel analyses performed with only anatomical landmarks (surface landmarks being more likely to be impacted by bone deformation) led to similar results (see the electronic supplementary material, figure S12 and table S3). Neither it is influenced by ontogeny as the youngest specimens were removed from the analyses (see methods). Our modern sample covers a wide range of shapes (all morphological types including hypertypes are represented), however, is not exhaustive and lacks some landraces of dogs, such as those from Asia, or village dogs. Further analyses are thus needed before concluding that the unique shapes observed in ancient dogs definitively have no equivalent in modern dogs, and represent shapes that have disappeared.

Our results are based on a large number of ancient mandibles (227), supported by fragments A and B (which are the most representative of the complete form of the mandible), and are generally confirmed when including data for smaller fragments (table 1). The two smallest fragments (G and I) lead to less conclusive results, probably because these fragments represent only a very small part of the mandible and are thus less representative of the complete mandible shape. This is supported by the distribution of the ancient dogs with complete mandibles in the PCA based on fragments I and G (electronic supplementary material, figures S8 and S10). Considering the composition of the archaeological sample (electronic supplementary material figure S1, and table S1), the conclusions drawn are especially valid for the Middle Neolithic in Western Europe and the Late Chalcolithic in Romania. The earliest (Mesolithic and Early Neolithic) phases are poorly represented owing to the scarcity of dog bones and their fragmentation (electronic supplementary material, figure S1, and table S1). It thus cannot be excluded that during these early phases some other forms existed, possibly adding to the group of ancient dogs extending outside the variability of our modern sample. Further samples from these early periods would enable us to further explore this variability.

The anatomical differences between modern and ancient dogs may partly be explained by a change in the genetic composition of dog populations through time. Mitochondrial haplogroups C and D were dominant in Europe before the Bronze Age (63% and 20%, respectively), whereas they represent only around 11% and 3% of extant European dogs, respectively [15]. Haplogroups A and B currently overwhelmingly dominate (64% and 22%, respectively) in Europe. This change results from complex migrations, replacements and admixture through time and space. Considering the distinct geographical distribution of the different maternal lineages before the Neolithic period [6,15] each may be characterized by specific phenotypic traits, yet this remains unstudied to date. They may also reflect differences in the anthropic environment or the way of life of dogs. Given that the most discriminant traits are located in areas of functional importance (robustness and curvature of the mandibular body, size of the angular and coronoid processes, shallowness of the masseteric fossa), this may have resulted in differences in jaw function and bite force [45]. The differences in the curvature of the body of the mandible under the carnassial suggests a greater importance of the role of the temporal muscle in ancient dogs (figure 3*a*,*b*). This may be related to diet of these dogs which were probably feeding on more tough and hard foods than most modern dogs, thus placing very different demands on mandibular function and form (e.g. [46,47]). Additionally, a greater bite force and/or implication of the temporalis muscle would have been useful in dogs used for defence or hunting.

Our analyses further show that the relationship between the anterior and posterior parts of the mandible was less strong in ancient dogs compared to modern dogs (or captive wolves and commensal dingoes, but the low sample size did not allow us to draw conclusions for these animals). The strong integration in the mandible of modern dogs, despite intense artificial selection, is concordant with previous comparisons between modern dogs and wolves [40] or other wild canids such as the red fox [33], and may be explained by developmental factors constraining mandible shape in canids [2]. This is consistent with previous studies evidencing similar patterns of developmental integration in the skull of domestic/wild pairs of mammals [2,40,41]. The lower integration in ancient dogs may be owing to the fact that they were more submitted to natural (environmental) constraints allowing some parts of the mandible to be more variable compared to modern dogs. In particular, the mandibular ramus (providing attachment for masticatory muscles) is more likely to adapt quickly to natural constraints and functional demands, related to, for example, diet, than the mandibular corpus (bearing the teeth, which are much more conservative). The overall variability in diet in ancient dogs probably being higher, this may have selected for more variability in the shape of the mandibular ramus. Such a difference in integration related to artificial selection was previously demonstrated in the long bones of modern horses and donkeys [48], and by comparing the mandible of dogs and commensal red foxes [33], but never, to our knowledge, in the mandible of other domestic/wild mammal pairs.

The observed differences in mean shape and integration of the mandible between modern and ancient dogs and the fact that we did not find any extreme shapes in prehistoric dogs support the hypothesis that the first farmers did not exert strong selection on dogs for aesthetic or utilitarian reasons that might have constrained the anatomy (and modularity) of the mandible. Dogs were most likely free-ranging and closer to a commensal lifestyle, as observed in some extant tribes of hunter–gatherers where humans share their diet with dogs [49]. This is further suggested by isotopic data for some Neolithic dogs [29]. Additional feral dogs probably subjected to constraints closer to ancient dogs (i.e. less intentional human selection and more natural constraints) are needed to investigate this possibility further.

Now that the global variability in dogs prior to the Bronze Age has been described overall, it would be of interest to confront morphological data with genetic, isotopic or contextual data (i.e. whether dogs were eaten or buried complete, or in accordance with the mode of subsistence relying on hunting or herding) to explore what drove diversity within the archaeological sample. This would allow more profound insights into how the changes in human societies were accompanied by changes in the morphology of dogs through time and space. Palaeogenetic data might, for example, allow us to test whether the acquisition of the ability to digest starch was accompanied by changes in mandible form. Additional contextual data would further enable a better understanding of when the morphological diversification started: as soon as hunter–gatherers settled, with the farming transition, or later with the complexification of Neolithic societies?

5. Conclusion

In summary, our comparison of mandible form between European dogs from the Mesolithic to the end of the Romanian Chalcolithic and modern breeds highlighted an unexpected diversity in dogs before the Bronze Age, suggesting that dogs showed significant morphological diversity in the head as early as the Neolithic. Additionally, ancient dogs have mandible shapes that differ, on average, from modern dogs, which may be partly related to the changes in the genetic composition of dog populations through time, but also suggests changes in lifestyle and access to food, considering that the anatomical differences are located in areas of functional importance. This, added to the fact that no extreme shapes were observed in ancient dogs and that the integration between the anterior and posterior parts of the mandibles is lower in ancient dogs, suggests that if the first farmers selected for distinct morphotypes, they did not favour extreme morphologies unlike the results of recent breed selection.

Data accessibility. All data used for the analyses (three-dimensional models of the mandibles, R scripts and datasets) are available in Dryad Digital Repository: https://doi.org/10.5061/dryad.gqnk98sqd [50]. Detailed information about the sample and methods are provided in the electronic supplementary material [51].

Authors' contributions. C.B.: data curation, formal analysis, investigation, methodology, visualization, writing—original draft; A.B.: data curation, resources, writing—review and editing; R-M..A.: resources; V.F.: resources, writing—review and editing; C.B.: resources; A.B.: resources, writing—review and editing; F.C.: resources, writing—review and editing; M.G.: resources; V.R.: resources, writing—review and editing; P.A.F.: data curation, resources, writing—review and editing; C.G.: data curation, resources, writing—review and editing; T.L.K.: resources; C.C.: conceptualization; A.F.: conceptualization, writing—review and editing; A.T.: conceptualization, project administration, resources; R.C.: conceptualization, investigation, methodology, software, supervision, validation, writing—review and editing; A.H.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—original draft; S.B.: conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—original draft.

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