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The life history of European Middle Pleistocene equids: first insights from bone histology

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Abstract

Evolutionary trends in body size are a central issue of study in Paleontology. However, and despite body size being one of the most important life history traits of an animal, iconic size-decrease trends such as the one experienced by *Equus* during the European Pleistocene has never been analysed under a life history framework. Here, we studied the metapodial bone histology of two large Middle Pleistocene species (*Equus mosbachensis* and *Equus steinheimensis*) to reconstruct key life history traits that correlate with body size (e.g. size at birth, growth rate), and compare them with that of smaller extant *Equus* (*Equus grevyi*, *Equus quagga*, *Equus zebra* and *Equus hemionus*). Our results show that neonatal size of these Middle Pleistocene equids fits predictions from body mass scaling. We estimate a similar age of epiphyseal fusion for the metapodia of *E. mosbachensis* and *E. steinheimensis* and that of extant equids. Our findings also reveal that extinct equids grew at higher rates than extant *Equus*. This result conforms to the predictions of LH theory on environments with different levels of resource availability and provides a new framework of study for body size shifts on European Pleistocene equids.

Keywords: Bone histology, body size, life history, growth rate, Pleistocene, *Equus*

Introduction

The family Equidae is of special relevance for Palaeontology and evolution (MacFadden 1992; Janis 2007). The abundance of equid remains in the fossil record has facilitated the tracking of evolutionary changes in the clade (Orlando 2015), positioning Equidae as a classic example of macroevolution (MacFadden 2005; Janis 2007). Along with modifications in dentition and number of digits (Simpson 1953; Azzaroli 1992; MacFadden 1992; Strömberg 2006; Janis 2007; Cantalapiedra et al. 2017; McHorse et al. 2017), this mammalian group experienced significant variations in size during its evolutionary history (Simpson 1953; MacFadden 1986; Eisenmann 1991; Forsten 1991a; MacFadden 1992; Alberdi et al. 1995; Guthrie 2003; Ortiz-Jaureguizar and Alberdi 2003; Shoemaker and Clauset 2014; Cantalapiedra et al. 2017). Generally, a phylogenetical large-scale trend towards ever increasing body size is observed (Cope's law, Stanley (1973)), although several clades of the family experienced size reduction (MacFadden 1986; Forsten 1991a; Forsten 1991b; MacFadden 1992; Alberdi et al. 1995; Alberdi et al. 1998). One of these dwarfing tendencies occurred during the evolution of the genus *Equus* in the Old World (Forsten 1991b; Alberdi et al. 1995). A size shift towards a smaller body size is described for the first monodactyl horses (stenonoid horses) (Alberdi et al. 1995; Alberdi et al. 1998) that arrived in Eurasia between 3.0 – 2.5 Ma ago (Lindsay et al. 1980; Azzaroli 1983) (Fig. 1). A second migratory event (1 – 0.8 Ma ago) introduced the equid caballoid forms in Europe (Alberdi and Bonadonna 1988), which also experienced size reduction during their Pleistocene evolution in this continent (Forsten 1991b; Alberdi et al. 1995) (Fig. 1). The size decrease trend continued throughout the Holocene (Forsten 1988; Forsten 1991b), resulting in a smaller body size of extant *Equus* (Ernest 2003) in comparison to their Pleistocene relatives (Alberdi et al. 1995) (Fig. 1).

Body size is one of the most important characteristics of an animal, as it tightly correlates with its physiology (Kleiber 1932; Peters 1983; McNab 1990), life history (Blueweiss et al. 1978; Western 1979; Calder 1984) and ecology (Damuth 1981; Peters 1983; Eisenberg 1990). Due to the close relationship between body size and the ecological conditions of the ecosystem, the size shift observed in Pleistocene equids has usually been related to the climatic and resource variations occurring during that epoch (Forsten 1991b; Alberdi et al. 1995; Cantalapiedra et al. 2017). Demographic (population density) and behavioural (social structure) characteristics of the species have also been proposed to explain the body size differences observed between European Pleistocene horses (Saarinen et al. 2016). Body size is also a key life history trait that correlates with other biological traits (Peters 1983; Calder 1984; Damuth and MacFadden 1990), but no previous research has investigated the body size variations observed in *Equus* within a life history framework. In fossil mammals, the histological analysis of bones is known to provide valuable insights into their life history strategy (Köhler and Moyà-Solà 2009; Köhler 2010; Marín-Moratalla et al. 2011; Martínez-Maza et al. 2014; Amson et al. 2015; Kolb et al. 2015; Moncunill-Solé et al. 2016; Orlandi-Oliveras et al. 2016; Orlandi-Oliveras et al. 2018). The kind of bone tissue type, its vascularization, and the bone growth marks (BGMs) present within a bone cortex, record the pace of growth and development of the species (Chinsamy-Turan 2005; Huttenlocker et al. 2013; Lee et al. 2013; Woodward et al. 2013). The detailed study of these histological features, hence, allows the inference of the key life history longevity (Köhler 2010; Marín-Moratalla et al. 2011; Martínez-Maza et al. 2014; Moncunill-Solé et al. 2016; Orlandi-Oliveras et al. 2016), growth rate (Padian et al. 2001; Köhler and Moyà-Solà 2009; Cubo et al. 2012; Amson et al. 2015; Orlandi-Oliveras et al. 2018) and age at maturity (Köhler and Moyà-Solà 2009; Marín-Moratalla et al. 2011; Woodward et al. 2015; Jordana et al. 2016; Nacarino-Meneses et al. 2016; Orlandi-Oliveras et al. 2018; Calderón et al. 2019) in extant and extinct vertebrates.

In the present research, we aim to investigate whether the body size shift observed in the *Equus* lineage is related to changes in the life history of the species. We focus our study in the European Middle Pleistocene species *Equus steinheimensis* and *Equus mosbachensis*. Both extinct caballoid horses were described for the first time at Steinheim an der Murr and Mosbach Sands respectively, two classical Middle Pleistocene localities of Germany (Adam 1954; Forsten 1999; Koenigswald et al. 2007; Van Asperen 2013). Previous studies based on measurements of their dental and postcranial remains have reported differences in body size for these extinct equids (Eisenmann 1991; Forsten 1999). While *E. steinheimensis* is considered a medium-sized caballoid horse (Eisenmann 1991; Forsten 1999), *E. mosbachensis* is one of the largest equids that can be found in the Pleistocene fossil record of Europe (Eisenmann 1991; Alberdi et al. 1995). From bone histology, we reconstruct their pace of life and compare it with that of extant *Equus*, as both Middle Pleistocene equids greatly exceeded in size extant representatives of the group (Alberdi et al. 1995; Forsten 1999; Ernest 2003).

Thus, we also examine here the bone histology of the extant equid species of Asiatic wild ass (*Equus hemionus*), Grevyi's zebra (*Equus grevyi*), Plains zebra (*Equus quagga*) and mountain zebra (*Equus zebra*). These four extant taxa are the most appropriate ones for comparison with our fossil sample, as they cover almost all habitats, body size and life histories within extant wild *Equus* (Grubb 1981; Penzhorn 1988; Churcher 1993; Nowak 1999; Ernest 2003; Novellie 2008; Moehlman et al. 2013; Kaczensky et al. 2015; Orlando 2015; King and Moehlman 2016).

Material and methods

Equid sample

We analysed postcranial fossil remains of *E. steinheimensis* and *E. mosbachensis* belonging to the collections of the Staatliches Museum für Naturkunde (Stuttgart, Germany) (Table 1). Specifically, isolated metapodial bones (metacarpi and metatarsi) were used for palaeohistological analyses, while isolated phalanges and metacarpi were analysed for body mass estimations. The material of *E. steinheimensis* comes from Steinheim an der Murr site (Steinheim, henceforward), a late Middle Pleistocene locality (Van Asperen 2013) situated in south-west Germany (Fig. 2). Steinheim fossil site presents a mix of glacial and interglacial fauna (Adam 1954) due to a sampling bias during the recovery of the remains and/or to the coexistence of species with flexible adaptations (Pushkina et al. 2014). Although the dating of Steinheim site is, hence, controversial, it is thought to generally correlate with the Holsteinian Interglacial (MIS 11) (Van Asperen 2013). The sample of *E. mosbachensis* belongs to Mosbach Sands, an early Middle Pleistocene site (Koenigswald et al. 2007) located in Wiesbaden (Germany) (Fig. 2). This German classical site is divided into two different stratigraphic ages based on lithological and paleontological criteria (Maul et al. 2000). The *E. mosbachensis* sample analysed here belongs to the Mosbach 2 fauna, which is known to present interglacial conditions (Koenigswald et al. 2007) and to correlate with the Cromerian Interglacial (MIS 13, 15) (Maul et al. 2000; Kahlke et al. 2011).

We also studied an adult metapodial sample of extant *E. hemionus*, *E. grevyi*, *E. quagga* and *E. zebra* (Table 1) for comparison. Specimens of *E. hemionus* are stored at the Museum of Domesticated Animals (Halle, Germany) and lived wild in the Gobi Desert (Table 1). They were found killed by poachers and collected during the

Mongolian-German Biological Expeditions (2001 – 2006) (Schöpke et al. 2012). The *E. grevyi* individual belongs to the collections of the Zoological Institute of Hamburg University (Germany) (Table 1), after living captive in the Hagenbeck Zoo (Hamburg, Germany) (Table 1). Finally, *E. quagga* and *E. zebra* specimens of the study lived semi-captive in the Réserve Africaine de Sigean (Sigean, France) and are part of the collections of the Institut Català de Paleontologia (Barcelona, Spain) (Table 1).

Preparation and analysis of histological thin-sections

We sectioned 18 metapodia of extant and extinct equids: 4 metapodia of *E. steinheimensis*, 3 metapodia of *E. mosbachensis*, 4 metapodia of *E. hemionus*, 1 metatarsus of *E. grevyi*, 2 metapodia of *E. quagga* and 4 metapodia of *E. zebra* (Table 1). To avoid damaging of the most valuable fossil specimens, only fossil metapodia fragmented at the mid-shaft level were used in the present study. The preparation of the histological thin-sections followed standard procedures in our laboratory (Köhler et al. 2012; Nacarino-Meneses et al. 2016). In all extant and extinct exemplars, a chunk of 2 – 3 cm was extracted from the mid-diaphysis of each bone and embedded in an epoxy resin (Araldite 2020). Afterwards, this block was cut into two halves with a low speed diamond saw (IsoMet, Buehler). The mid-shaft surface of each block was later polished using a Metaserv®250 (Buehler) or carborundum powder and fixed to a frosted glass with ultraviolet-curing glue (Loctite 358). Each sample was then cut and grounded with a diamond saw (PetroThin, Buehler) and polished again using the Metaserv®250 (Buehler) or carborundum powder to obtain histological thin-sections of 100 – 120 μm thick. Finally, histological slices obtained from fossil samples were dehydrated in alcohol, bathed in a histological clearing agent (Histo-Clear II) and mounted using DPX mounting medium (Scharlau). Thin-sections prepared from extant specimens were covered with a mix of oils (Lamm 2013) to improve their view under the microscope.

Samples were analysed under polarized light in a Zeiss Scope.A1 microscope and photographed with the camera incorporated on it (AxioCam ICc5). We also observed the histological thin-sections under polarized light with a ¼λ filter, which helps on the identification and visualization of the bone tissue and the bone growth marks (BGMs) (Turner-Walker and Mays 2008). Histological description of bone tissue types and vascular organization follows classical bibliography (Francillon-Vieillot et al. 1990; de Margerie et al. 2002; Huttenlocker et al. 2013). Regarding BGMs, both cyclical and non-cyclical features were identified in our metapodial equid sample. Cyclical BGMs (CGMs) record annual cycles of growth in response to hormonal and environmental cycles (Castanet et al. 1993; Köhler et al. 2012). From their study, we inferred the minimum longevity of extinct equid species by counting the total number of CGMs within a bone cross-section (Nacarino-Meneses et al. 2016). A non-cyclical BGM related to birth (Nacarino-Meneses and Köhler 2018) was also recognized in our metapodial sample. We considered this neonatal line (NL) as time zero in growth reconstructions. The perimeter of each BGM, regardless of its cyclicality, was calculated using Image J software and the results were plotted to reconstruct the pattern of metapodial growth (Bybee et al. 2006; Woodward et al. 2013). From the obtained metapodial growth curves, we estimated the timing of epiphyseal fusion in these bones for each fossil species following Nacarino-Meneses et al. (2016a).

Adult and neonatal body size estimation

We analysed metacarpi and phalanges of *E. steinheimensis* and *E. mosbachensis* to estimate their adult body mass, a proxy of its body size (Damuth and MacFadden 1990). For each species, we measured the proximal depth on the first phalanx and the distal minimal depth of the lateral condyle on the third metacarpus (Eisenmann et al. 1988), as these linear measurements present the best correlation with body mass in extant equids (Alberdi et al. 1995). Measurements were taken using a digital electronic precision calliper (0.05 mm error). We used the equations provided by Alberdi et al. (1995) to perform body mass estimations. These data were based on allometric models expressed as the power function $y = ax^b$ (Damuth and MacFadden 1990) and logarithmically transformed to obtain a linear relationship ($\ln y = \ln a + b \ln x$) (Peters 1983); where y is body mass, x is the measurement taken on the bone, b is the allometric coefficient and a is a constant (Alberdi et al. 1995).

We also estimated the size at birth of extinct and extant equids from the analysis of the NL found in their metapodia (Nacarino-Meneses and Köhler 2018). Alberdi et al. (1995) did not found a significant correlation between the measurements taken at the mid-diaphysis on the metapodia of extant *Equus* (measures 3 and 4 according to Eisenmann et al. (1988)) and their adult body mass. Because histological slices of the present study were prepared at this level of the diaphysis, we cannot use measurements of the NL to infer body weight at birth. Moreover, equations of Alberdi et al. (1995) are calculated for adult individuals. Thus, they are little useful for estimating body mass at earlier ontogenetic stages (Köhler 2010). We decided, then, to represent size at birth as a linear measurement instead of on terms of body mass, by using the perimeter of the NL as a proxy of the neonatal size.

Results

Bone histology is generally better preserved in Steinheim (Fig. 3A, C; Fig. 4A, B) than in Mosbach samples (Fig. 3B, D; Fig. 4C, D). Although several histological slides from both fossil sites present several microtaphonomical alterations (Fig. 3; Fig. 4), it is still possible to recognize the primary bone tissue and the BGMs in all fossil specimens studied (Fig. 3; Fig. 4). The description and identification of the diagenetic alterations of the bone microstructure is, however, beyond the scope of the present research.

Primary bone histology

Metapodial bones of *E. steinheimensis* and *E. mosbachensis* are mainly composed of fibrolamellar bone (Fig. 3A–D), the same as in extant equids (Fig. 3E–H). Most of the vascular canals identified in extinct species are oriented longitudinally (Fig. 3A, B). However, both fossil equids also present multiple circular and radial canals that interconnect primary osteons (Fig. 3A, B). Therefore, we found a qualitatively higher number of vascular canals in fossil (Fig. 3A, B) than in extant metapodia (Fig. 3E–H).

Metacarpi of *E. steinheimensis* do not show an external fundamental system (EFS) in their outermost cortex (Fig. 3C). This avascular and lamellar bone tissue (Huttenlocker et al. 2013) is recognized, however, in the metatarsi of this species and in metacarpi and metatarsi of *E. mosbachensis* (Fig. 3D).

Skeletochronology

Up to five cyclical BGMs are identified in the metapodia of *E. steinheimensis* (Fig. 4A, B). In *E. mosbachensis*, the highest number of cyclical BGMs is found in the metacarpus IPS96017 (Fig. 4C). This specimen presents six cyclical BGMs within the primary bone tissue (Fig. 4C). Besides, all fossil metapodia show a non-cyclical BGM in the most internal cortex (Fig. 4) that corresponds to the NL observed in extant *Equus* (Nacarino-Meneses and Köhler 2018).

From the study of cyclical and non-cyclical BGMs, we reconstruct the metapodial growth pattern of *E. steinheimensis* and *E. mosbachensis* (Fig. 5). As Figure 5 shows, metacarpus (Fig. 5A) and metatarsus (Fig. 5B) of extinct equids follow the same growth pattern as the metapodia of extant *E. hemionus*, *E. grevyi*, *E. quagga and E. zebra* (Fig. 5). In both fossil and extant species, a decrease in growth rate at the second year of life is observed (Fig. 5). Growth reconstruction also shows, however, a steeper slope in fossil than in extant samples (Fig. 5), both during the phase of highest growth rate and during residual growth (Fig. 5). These differences in growth rate are unlikely related to sex differences, as equid bones from males and females are known to grow similarly (Nacarino-Meneses et al. 2016).

Adult and neonatal body size

We estimated the adult weight of fossil equid species from measurements on metacarpi and phalanges (Table 2). A mean adult body mass of 469.3 kg is obtained for *E. steinheimensis*, while adult *E. mosbachensis* is predicted to weight around 607.4 kg (Table 2; Fig. 1).

The perimeter of the NL (Fig. 4) is used here to provide a rough estimation of the size at birth for the Pleistocene species studied (Table 3). As Table 3 shows, extinct equids present a larger size at birth than extant *Equus*. The mid-shaft perimeter of the metacarpi and metatarsi of a neonate *E. mosbachensis* measures between 83.65 and 90.86 mm (Table 3, Fig. 4), while that of *E. steinheimensis* does around 80 mm (Table 3, Fig. 4). Perimeters of neonate metapodia in extant species are considerably smaller, as they vary between 50 – 60 mm in *E. hemionus*, around 75 mm in *E. grevyi*, and *ca.* 70 mm in *E. quagga* and *E. zebra* (Table 3).

Discussion

Evolutionary trends in body size of European extinct *Equus* are widely studied (Eisenmann 1991; Forsten 1991b; Alberdi et al. 1995; Cantalapiedra et al. 2017), but no previous research has investigated its relationship with the life history of the species. In the present research, we reconstruct several life history traits of the extinct caballoid horses *E. steinheimensis* and *E. mosbachensis* for the first time, providing a unique life history framework for the analysis of the size shifts observed in European Pleistocene equids.

Body size of European Middle Pleistocene **Equus**

Body size is usually represented by body mass (i.e. the weight of an individual) because this measure does not consider the shape of the organisms (Saarinen et al. 2016). The exhaustive study of Alberdi et al. (1995) reported adult body mass estimations for the most important Plio-Pleistocene *Equus* species of Europe, Africa and America. Here, we expand their database by calculating the body weight of *E. steinheimensis*. Our results show that its mean body mass is around 470 kg (Table 2), which is similar to that reported for the Late Pleistocene species *Equus germanicus* (Fig. 1) (Alberdi et al. 1995). This finding agrees with a previous study by Forsten (1999), where she indicated similarities in metapodial size between *E. steinheimensis* and latter caballoid equids such as *E. germanicus*. Our adult body mass estimations for this equid also matches previously published data about the weight of Steinheim horses (Saarinen et al. 2016). We further estimated body mass of *E. mosbachensis* from measurements on its metacarpi and phalanges (Table 2). We obtained a mean body weight of 607 kg for this early Middle Pleistocene species (Table 2). As expected, this result matches previous body mass estimations of 671.15 kg for *E. mosbachensis* (Fig. 1) (Alberdi et al. 1995) that were obtained from measurements on fossil material recovered from the same locality (Mosbach Sands fossil site) but housed at other European collections (Eisenmann 1979).

Size at birth is one of the most important life history traits of mammals (Stearns 1992), although this information is still lacking for most extinct equid taxa. In the present study, we obtained an estimation of this attribute for the Middle Pleistocene equids of the analysis (Table 3) based on the perimeter of the NL (Nacarino-Meneses and Köhler 2018) identified in their metapodia (Fig. 4). Our findings reveal that *E. mosbachensis* presents the largest size at birth within the equids studied, followed by *E. steinheimensis*, *E. grevyi*, *E. zebra*, *E. quagga* and *E. hemionus* (Table 3). *E. mosbachensis* is also the largest *Equus* of the study (Table 2, Fig. 1), while the Asiatic wild ass is the smallest (230 kg, Ernest 2003). *E. steinheimensis* (Table 2, Fig. 1), *E. grevyi* (384 kg, Ernest 2003), *E. zebra* (296 kg, Ernest 2003) and *E. quagga* (257 kg, Ernest 2003) present intermediate adult weights between *E. mosbachensis* and *E. hemionus*. Therefore, our results on neonatal body size of extinct equids (Table 3) are the ones expected by scaling, as they agree with the known correlation between size at birth and adult body size (Blueweiss et al. 1978; Calder 1984).

Growth rate of European Middle Pleistocene Equus

Growth rate inferences made from bone histology are usually based on the combined analysis of the bone matrix and its vascularization (Amprino 1947; de Margerie et al. 2002; Chinsamy-Turan 2005; Huttenlocker et al. 2013; Lee et al. 2013). Our histological study of *E. steinheimensis* and *E. mosbachensis* has not revealed differences in bone tissue type and/or vascularization between both extinct species (Fig. 3A, B), which indicates similar rates of bone formation in these Pleistocene equids (de Margerie et al. 2002; Lee et al. 2013). We have observed, however, a higher number of vascular canals in their metapodia (Fig. 3A, B) than in extant *Equus's* (Fig. 3E – H). According to de Margerie et al. (2002), this suggests a higher rate of bone deposition in the Pleistocene equids analysed, which could be generally interpreted as a higher growth rate. Growth curves have also been commonly used to report differences in growth rate between sexes (Marín-Moratalla et al. 2013), taxa (Erickson et al. 2001; Erickson 2005; Orlandi-Oliveras et al. 2018) and even between animals under different ecological regimes (Marín-Moratalla et al. 2013; Nacarino-Meneses et al. 2016; Calderón et al. 2019). The slope of the metapodial growth curves obtained here for extinct equids is steeper than those of *E. hemionus*, *E. grevyi*, *E. quagga* and *E. zebra* (Fig. 5), suggesting higher rates of growth for *E. steinheimensis* and *E. mosbachensis* in comparison with these extant species. Therefore, our metapodial growth reconstruction (Fig. 5) supports the histological inference (Fig. 3A, B) of higher rates of growth in *E. steinheimensis* and *E. mosbachensis* than in extant *Equus*.

Age at maturity and longevity of European Middle Pleistocene Equus

Estimates about the age at maturity in fossil taxa use to rely on the identification of the lamellar and avascular bone tissue that is deposited in the outer cortex of the bones: the external fundamental system (EFS) (Chinsamy-Turan 2005; Marín-Moratalla et al. 2013; Calderón et al. 2019). The timing of deposition of this tissue in equid and ruminant femora is known to correlate with the age at first reproduction of the species (Jordana et al. 2016; Nacarino-Meneses et al. 2016), as it is supposed to record the life history trade-off between growth and reproduction (Stearns 1992). The significance of deposition of the EFS in other bones, however, is poorly known. Although several authors have suggested a relationship between the presence of EFS in metapodia and the skeletal maturity of the species (Martínez-Maza et al. 2014), the appearance of this tissue type in these bones is just likely related with the cessation of periosteal/radial bone growth (Nacarino-Meneses et al. 2016). Therefore, the lack of EFS in metacarpi of *E. steinheimensis* (Fig. 3C) might just indicates a prolonged periosteal bone growth in this Pleistocene species. Alternatively, inferences on skeletal maturity can be made from growth reconstructions, as growth curves provide reliable information about the cessation of longitudinal bone growth (Nacarino-Meneses et al. 2016). The decrease in growth rate observed at the second year of life in metapodia of Pleistocene and extant species (Fig. 5) suggests a similar age of epiphyseal fusion in these bones for both groups of equids (Nacarino-Meneses et al. 2016). However, we cannot use this finding to infer an absolute age at skeletal maturity for *E. mosbachensis* and *E. steinheimensis* because metapodia fuse their epiphyses very early in ontogeny (Silver 1963). Future histological studies on other long bones that finish later their growth, such as the femur or the tibia (Silver 1963), will be necessary to obtain information about this biological trait in the extinct taxa studied. These forthcoming studies would also shed light on the onset of sexual maturity in these Middle Pleistocene species, by analysing the timing of deposition of the EFS in their femora (Nacarino-Meneses et al. 2016).

The total number of CGMs within a bone cortex is known to broadly match the age at death of an individual (Castanet et al. 1993). Hence, several studies have analysed these histological features on ancient bones to estimate longevity on extinct mammals (Köhler and Moyà-Solà 2009; Marín-Moratalla et al. 2011; Moncunill-Solé et al. 2016; Orlandi-Oliveras et al. 2016). Because mammals present asymptotic growth (Lee et al. 2013) and the identification of CGMs within the EFS is sometimes a challenging issue (Woodward et al. 2013), this methodology always yields a minimum longevity in this group of vertebrates (Castanet et al. 2004; Castanet 2006; Calderón et al. 2019). Moreover, femur is the most valuable bone for skeletochronological studies (Horner et al. 1999; García-Martínez et al. 2011; Nacarino-Meneses et al. 2016), although metapodia is known to yield accurate individual age estimations in equids (Martínez-Maza et al. 2014; Nacarino-Meneses et al. 2016). In our Pleistocene equid sample, we have identified a maximum of 5 and 6 CGMs in the metapodial cortex of *E. steinheimensis* and *E. mosbachensis* respectively (Fig. 4). These results suggest that the minimum longevity of *E. steinheimensis* is 5 years, while it is of 6 years in *E. mosbachensis*. Although the number of CGMs identified in the metapodial cortex of these extinct equids is similar to that found in the limb bones of *E. hemionus* (Nacarino-

Meneses et al. 2016), the small sample size of the present research limits us to perform a comparison with enough confidence between the longevity of extinct species (*E. steinheimensis* and *E. mosbachensis*) and that of extant equids. Further studies with a larger sample size are necessary, hence, to estimate the longevity of these Middle Pleistocene species.

Size variation in Old World* Equus*: environment and life history

Our life history reconstruction of *E. mosbachensis* and *E. steinheimensis* indicates that these Middle Pleistocene species grew at higher rates than extant horses (Fig. 3 and 5). As resource availability is the main selection pressure acting on individual growth rate (Palkovacs 2003), the difference in adult body size between extant and Middle Pleistocene equids would likely be influenced by the resource availability of each habitat. On the one hand, paleoenvironmental reconstructions on Mosbach (Cromerian interglacial (MIS 13, 15), Kahlke et al. (2011)) suggest open steppe landscapes interrupted by warm and humid episodes with extended forests to be the main habitats at this fossil site (Maul et al. 2000; Kahlke et al. 2011). Steinheim horse sample, on the other hand, is thought to have been originated in the Holsteinian interglacial (MIS11) (Van Asperen 2013), which is characterized by temperate, humid and forested environments (Nitychoruk et al. 2005). Specifically, Steinheim site during this interglacial is thought to present a temperate humid habitat of woodland and shrubland (Pushkina et al. 2014). Extant Grevy’s zebra also occurs in grasslands and shrublands, but its habitat is considered as arid or semiarid (Rubenstein et al. 2016) with a negative mean annual climatic water balance (Schulz and Kaiser 2013). The habitat of *E. zebra,* who dwells in mountainous and escarpment areas (Novellie 2008), also presents a negative mean annual climatic water balance (Schulz and Kaiser 2013). Thus, the habitat of both *E. grevyi* and *E. zebra* can be considered as poor-resourced in comparison with that of *E. steinheimensis* or *E. mosbachensis*. The drier habitat of the Grevy’s and the mountain zebra probably forces them to grow at lower rates than extinct *Equus* (Fig. 3 and 5) which, following the model of Palkovacs (2003), finally results in a smaller adult body size for *E. grevyi* and *E. zebra* than for Middle Pleistocene taxa. A similar reasoning applies to *E. hemionus* and *E. quagga*, which also grow at lower rates than *E. steinheimensis* and *E. mosbachensis* (Fig. 3 and 5). The Asiatic wild ass, on the one hand, is endemic of the Gobi desert, one of the most arid habitats of the world (Kaczensky et al. 2015). *Equus quagga*, on the other hand, lives in the open savannah and open woodland of several African countries (King and Moehlman 2016) that are likely poor-resourced in comparison with Middle Pleistocene environments. Growth rate of both *E. hemionus* and *E. quagga*, hence, is probably restricted by the environmental conditions of their habitat, as it is their final body size.

Along with modifications in growth rate, the life history model proposed by Palkovacs (2003) postulates that age at sexual maturity also influences adult body size. However, and as previously mentioned, we could not obtain information about this LH trait from our histological analysis due to the characteristic of the sample studied (e.g. lack of femora). Following this model (Palkovacs 2003), we would expect that the fast-growing Middle Pleistocene equids present an advance in their age at sexual maturity in comparison with extant taxa, although future histological studies on femora (Nacarino-Meneses et al. 2016) must be done to confirm this preliminary hypothesis. Interestingly, the hypothesis of an earlier sexual maturation in the Middle Pleistocene species *E. mosbachensis* and *E. steinheimensis* conforms to the predictions of life history theory in environments where there is a high extrinsic mortality pressure (i.e. predation) (Roff 1992; Stearns 1992; Roff 2002). In these circumstances, theory predicts that organisms achieve sexual maturity early to reduce the time of exposure to juvenile mortality (Stearns 2000). Paleontological and ecological data indeed suggest differences in levels of extrinsic mortality between Middle Pleistocene equids and extant species. Thus, while important potential predators for equids such as felids (*Lynx issiodorensis*, *Panthera gombaszoegensis*, *Panthera leo fossilis*, *Panthera pardus*, *Acinonyx pardinensis*, *Homotherium*), canids (*Canis lupus mosbachensis*, *Cuon alpinus priscus*, *Cuon dubius stehlini*) and hyaenids (*Pliocrocuta perrieri*, *Crocuta spelaea*) have been found in Mosbach or Steinheim sites (Adam 1954; Kahlke 1961; Kahlke 1975), the extant *E. grevyi*, *E. hemionus*, *E. quagga* and *E. zebra* are threatened by a few number of carnivore species (Grubb 1981; Penzhorn 1988; Churcher 1993; Feh et al. 2001).

Conclusions

Our study infers, for the first time, several life history traits of European Middle Pleistocene *Equus* from the histological analysis of their metapodia. Bone histology and growth reconstruction performed in these bones indicate higher growth rates for the extinct *E. mosbachensis* and *E. steinheimensis* in comparison with extant representatives of the group (*E. hemionus*, *E. grevyi*, *E. quagga* and *E. zebra*). Their occurrence in temperate and humid habitats during European interglacials explains the higher growth rates found in these extinct equids, as they dwelt in resource-richer environments than those inhabited today by the extant Asiatic wild ass and the Grevyi's, mountain and plains zebra. The resource levels of each habitat seem to be, thus, the main selection pressure acting upon individual growth rate and finally determining body size in *Equus*. The influence of other key life history traits such as age at maturity in determining the adult body size of these mammals could not be assessed in the present research, although some hypotheses are proposed. Our first approach to the skeletochronology of these Middle Pleistocene equids suggests a similar age at epiphyseal fusion for the metapodia in *E. mosbachensis* and *E. steinheimensis* and extant *Equus* and yield a minimum age at death for these extinct species. In the present study, we also provide estimates of the adult (in terms of body mass) and the neonatal body size (considering the perimeter of the NL as a proxy of this size) of *E. mosbachensis* and *E. steinheimensis*. Our results match previous adult weight estimations for these extinct equids and show that their size at birth is the expected from allometric scaling.

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Declaration of interest statement

The authors declare that there is no conflict of interest.

References

Adam KD. 1954. Die mittelpleistozänen Faunen von Steinheim an der Murr (Württemberg). Quaternaria. 1:131–144.

Alberdi MT, Bonadonna FP. 1988. Equidae (Perissodactyla, Mammalia): extinctions subsequent to the climatic changes. Rev Española Paleontol. 3:39–43.

Alberdi MT, Ortiz-Jaureguizar E, Prado JL. 1998. A quantitative review of European stenonoid horses. J Paleontol. 72(2):371–387. doi:10.1017/S0022336000036350.

Alberdi MT, Prado JL, Ortiz-Jaureguizar E. 1995. Patterns of body size changes in fossil and living Equini (Perissodactyla). Biol J Linn Soc. 54:349–370.

Amprino R. 1947. La structure du tissu osseux envisagée comme expression de différences dans la vitesse de l'accroissement. Arch Biol. 58:315–330.

Amson E, Kolb C, Scheyer TM, Sánchez-Villagra MR. 2015. Growth and life history of Middle Miocene deer (Mammalia, Cervidae) based on bone histology. Comptes Rendus - Palevol. 14(8):637–645. doi:10.1016/j.crpv.2015.07.001.

Van Asperen EN. 2013. Position of the Steinheim interglacial sequence within the marine oxygen isotope record based on mammal biostratigraphy. Quat Int. 292:33–42. doi:10.1016/j.quaint.2012.10.045.

Azzaroli A. 1983. Quaternary mammals and the "end-Villafranchian" dispersal event -

A turning point in the history of Eurasia. Palaeogeogr Palaeoclimatol Palaeoecol. 44(1–2):117–139. doi:10.1016/0031-0182(83)90008-1.

Azzaroli A. 1992. Ascent and decline of monodactyl equids: a case for prehistoric overkill. Ann Zool Fennici. 28:151–163.

Blueweiss L, Fox H, Kudzma V, Nakashima D, Peters R, Sams S. 1978. Relationships between body size and some life history parameters. Oecologia. 37:257–272.

Bybee PJ, Lee AH, Lamm E-T. 2006. Sizing the Jurassic theropod dinosaur *Allosaurus*: assessing growth strategy and evolution of ontogenetic scaling of limbs. J Morphol. 267:347–359. doi:10.1002/jmor.

Calder WAI. 1984. Size, Function and Life History. New York: Dover Publications.

Calderón T, DeMiguel D, Arnold W, Stalder G, Köhler M. 2019. Calibration of life history traits with epiphyseal closure, dental eruption and bone histology in captive and wild red deer. J Anat. Online ver. doi:10.1111/joa.13016.

Cantalapiedra JL, Prado JL, Hernández Fernández M, Alberdi MT. 2017. Decoupled ecomorphological evolution and diversification in Neogene-Quaternary horses. Science (80-). 355(6325):627–630. doi:10.1126/science.aag1772.

Castanet J. 2006. Time recording in bone microstructures of endothermic animals; functional relationships. Comptes Rendus - Palevol. 5(3–4):629–636. doi:10.1016/j.crpv.2005.10.006.

Castanet J, Croci S, Aujard F, Perret M, Cubo J, de Margerie E. 2004. Lines of arrested growth in bone and age estimation in a small primate: *Microcebus murinus*. J Zool. 263(1):31–39. doi:10.1017/S0952836904004844.

Castanet J, Francillon-Vieillot H, Meunier F, de Ricqlès A. 1993. Bone and individual aging. In: Hall BK, editor. Bone: A Treatise, Vol. 7. Boca Raton: CRC Press. p. 245–283.

Chinsamy-Turan A. 2005. The microstructure of dinosaur bone. Deciphering biology with fine-scale techniques. Baltimore and London: The Johns Hopkins University Press.

Churcher C. 1993. *Equus grevyi*. Mamm Species. 453:1–9.

Cubo J, le Roy N, Martinez-Maza C, Montes L. 2012. Paleohistological estimation of bone growth rate in extinct archosaurs. Paleobiology. 38(2):335–349. doi:10.5061/dryad.j2m25n82.

Damuth J. 1981. Population density and body size in mammals. Nature. 290:699–700.

Damuth J, MacFadden BJ. 1990. Body size in mammalian Paleobiology. Estimations and biological implications. Cambridge: Cambridge University Press.

Eisenberg JF. 1990. The behavioral/ecological significance of body size in the Mammalia. In: Damuth J, MacFadden BJ, editors. Body size in mammalian Paleobiology. Estimations and biological implications. Cambridge: Cambridge University Press. p. 25–37.

Eisenmann V. 1979. Les métapodes d'*Equus* sensu lato (Mammalia, Périssodactyla). Geobios. 12(6):863–886. doi:10.1016/S0016-6995(79)80004-2.

Eisenmann V. 1991. Les chevaux quaternaires européens (Mammalia, Perissodactyla). Taille, typologie, biostratigraphie et taxonomie. Geobios. 24(6):747–759.

Eisenmann V, Alberdi MT, de Giuli C, Staesche U. 1988. Studying fossil horses. Collected papers after the "New York International Hipparion Conference, 1981". Volume I: Methodology. Woodburne M, Sondaar P, editors. Leiden: E.J. Brill.

Erickson GM. 2005. Assessing dinosaur growth patterns: A microscopic revolution. Trends Ecol Evol. 20(12):677–684. doi:10.1016/j.tree.2005.08.012.

Erickson GM, Rogers KC, Yerby SA. 2001. Dinosaurian growth patterns and rapid avian growth rates. Nature. 412(6845):429–433. doi:10.1038/35086558.

Ernest SKM. 2003. Life history characteristics of placental nonvolant mammals. Ecology. 84(12):3402. doi:10.1890/11-1341.1.

Feh C, Munkhtuya B, Enkhbold S, Sukhbaatar T. 2001. Ecology and social structure of the Gobi khulan *Equus hemionus* subsp. in the Gobi B National Park, Mongolia. Biol Conserv. 101:51–61.

Forsten A. 1988. The small caballoid horse of the upper Pleistocene and Holocene. J Anim Breed Genet. 105(1–6):161–176. doi:10.1111/j.1439-0388.1988.tb00288.x.

Forsten A. 1991a. Size trends in Holarctic anchitherines (Mammalia, Equidae). J Paleontol. 65(1):147–159.

Forsten A. 1991b. Size decrease in Pleistocene-Holocene true or caballoid horses of Europe. Mammalia. 55(3):407–420. doi:10.1515/mamm.1991.55.3.407.

Forsten A. 1999. The horses (genus *Equus*) from the Middle Pleistocene of Steinheim, Germany. In: Haynes G, Klimowicz J, Reumer JWF, editors. Mammoths and the Mammoth Fauna: Studies of an Extinct Ecosystem. Deinsea 6. p. 147–154.

Francillon-Vieillot H, de Buffrénil V, Castanet J, Géraudie J, Meunier FJ, Sire JY, Zylberberg L, de Ricqlès A. 1990. Microstructure and mineralization of vertebrate skeletal tissues. In: Carter JG, editor. Skeletal biomineralization: patterns, processes and evolutionary trends. New York: Van Nostrand Reinhold. p. 471–530.

García-Martínez R, Marín-Moratalla N, Jordana X, Köhler M. 2011. The ontogeny of bone growth in two species of dormice: reconstructing life history traits. Comptes Rendus - Palevol. 10(5–6):489–498. doi:10.1016/j.crpv.2011.03.011.

Grubb P. 1981. *Equus burchelli*. Mamm Species. 157:1–9.

Guthrie RD. 2003. Rapid body size decline in Alaskan Pleistocene horses before extinction. Nature. 426(6963):169–171. doi:10.1038/nature02098.

Horner JR, de Ricqlès A, Padian K. 1999. Variation in dinosaur skeletochronology indicators: implications for age assessment and physiology. Paleobiology. 25(3):295–304. doi:10.1666/0094-8373(1999)025<0295:VIDSII>2.3.CO;2.

Huttenlocker AK, Woodward HN, Hall BK. 2013. The biology of bone. In: Padian K, Lamm E-T, editors. Bone histology of fossil tetrapods: advancing methods, analysis, and interpretation. Berkeley: University of California Press. p. 13–34.

Janis C. 2007. The Horse Series. In: Regal B, editor. Icons of Evolution. Westport: Greenwood Press. p. 251–280.

Jordana X, Marín-Moratalla N, Moncunill-Solé B, Nacarino-Meneses C, Köhler M. 2016. Ontogenetic changes in the histological features of zonal bone tissue of ruminants: a quantitative approach. Comptes Rendus - Palevol. 15(1–2):255–266. doi:10.1016/j.crpv.2015.03.008.

Kaczensky P, Lkhagvasuren B, Pereladova O, Hemami M, Bouskila A. 2015. *Equus hemionus*. IUCN Red List Threat Species. e.T7951A45.

Kahlke HD. 1975. The Macro-faunas of Continental Europe during the Middle Pleistocene: Stratigraphic sequence and problems of intercorrelation. In: Butzer KW, Isaac GL, editors. After the Australopithecines. Stratigraphy, Ecology, and Culture Change in the Middle Pleistocene. The Hague: Mouton Publishers. p. 309–374.

Kahlke RD, García N, Kostopoulos DS, Lacombat F, Lister AM, Mazza PPA, Spassov N, Titov V V. 2011. Western Palaearctic palaeoenvironmental conditions during the Early and early Middle Pleistocene inferred from large mammal communities, and implications for hominin dispersal in Europe. Quat Sci Rev. 30(11–12):1368–1395. doi:10.1016/j.quascirev.2010.07.020.

Kahlke VH-D. 1961. Revision der Säugetierfaunen der klassischen deutschen Pleistozän-Fundstellen von Süßenborn, Mosbach und Taubach. Geologie. 10:493–532.

King SRB, Moehlman PD. 2016. *Equus quagga*. IUCN Red List Threat Species.:e.T41013A45172424.

Kleiber M. 1932. Body size and metabolism. Hilgardia. 6(11):315–353. doi:10.1017/CBO9781107415324.004.

Koenigswald W V, Smith BH, Keller T. 2007. Supernumerary teeth in a subadult rhino mandible (*Stephanorhinus hundsheirnensis*) from the middle Pleistocene of Mosbach in Wiesbaden (Germany). Paläontologische Zeitschrift. 81:416–428.

Köhler M. 2010. Fast or slow? The evolution of life history traits associated with insular dwarfing. In: Pérez-Mellado V, Ramon C, editors. Islands and Evolution. Vol. 19. Maó: Institut Menorquí d'Estudis. Recerca. p. 261–280.

Köhler M, Marín-Moratalla N, Jordana X, Aanes R. 2012. Seasonal bone growth and physiology in endotherms shed light on dinosaur physiology. Nature. 487:358–361. doi:10.1038/nature11264.

Köhler M, Moyà-Solà S. 2009. Physiological and life history strategies of a fossil large mammal in a resource-limited environment. Proc Natl Acad Sci U S A. 106(48):20354–20358. doi:10.1073/pnas.0813385106.

Kolb C, Scheyer TM, Veischegger K, Van den Hoek Ostende LW, Hayashi S, Sánchez-Villagra MR. 2015. Mammalian bone palaeohistology: a survey and new data with emphasis on island forms. PeerJ. 3:e1358. doi:10.7717/peerj.1358.

Lamm E-T. 2013. Preparation and sectioning of specimens. In: Padian K, Lamm E-T, editors. Bone histology of fossil tetrapods: advancing methods, analysis, and interpretation. Berkeley: University of California Press. p. 55–160.

Lee AH, Huttenlocker AK, Padian K, Woodward HN. 2013. Analysis of growth rates. In: Padian K, Lamm E-T, editors. Bone histology of fossil tetrapods: advancing methods, analysis, and interpretation. Berkeley: University of California Press. p. 217–251.

Lindsay EH, Opdyke ND, Johnson NM. 1980. Pliocene dispersal of the horse *Equus* and late Cenozoic mammalian dispersal events. Nature. 287(5778):135–138. doi:10.1038/287135a0.

MacFadden BJ. 1986. Fossil horses from “Eohippus” (*Hyracotherium*) to *Equus*: scaling, Cope’s Law, and the evolution of body size. Paleobiology. 12(4):355–369. doi:10.1017/S0094837300003109.

MacFadden BJ. 1992. Fossil horses. Systematics, Paleobiology, and Evolution of the Family Equidae. Cambridge: Cambridge University Press.

MacFadden BJ. 2005. Fossil Horses - Evidence for Evolution. Science (80-). 307:1728–1730. doi:307/5716/1728 [pii] 10.1126/science.1105458.

de Margerie E, Cubo J, Castanet J. 2002. Bone typology and growth rate: Testing and quantifying “Amprino’s rule” in the mallard (*Anas platyrhynchos*). Comptes Rendus - Biol. 325(3):221–230. doi:10.1016/S1631-0691(02)01429-4.

Marín-Moratalla N, Jordana X, García-Martínez R, Köhler M. 2011. Tracing the evolution of fitness components in fossil bovids under different selective regimes. Comptes Rendus - Palevol. 10(5–6):469–478. doi:10.1016/j.crpv.2011.03.007.

Marín-Moratalla N, Jordana X, Köhler M. 2013. Bone histology as an approach to providing data on certain key life history traits in mammals: implications for conservation biology. Mamm Biol. 78(6):422–429. doi:10.1016/j.mambio.2013.07.079.

Martínez-Maza C, Alberdi MT, Nieto-Diaz M, Prado JL. 2014. Life-history traits of the miocene *Hipparion concudense* (Spain) inferred from bone histological structure. PLoS One. 9(8):e103708. doi:10.1371/journal.pone.0103708.

Maul LC, Rekovets L, Heinrich W-D, Keller T, Storch G. 2000. *Arvicola mosbachensis* (Schmidtgen 1991) of Mosbach 2: a basic sample for the early evolution of the genus and a reference for further biostratigraphical studies. Senckenbergiana lethaea. 80:129–147.

McHorse BK, Biewener AA, Pierce SE. 2017. Mechanics of evolutionary digit reduction in fossil horses (Equidae). Proc R Soc B Biol Sci. 284(1861):20171174. doi:10.1098/rspb.2017.1174.

McNab BK. 1990. The physiological significance of body size. In: Damuth J, MacFadden BJ, editors. Body size in mammalian Paleobiology. Estimations and biological implications. Cambridge: Cambridge University Press. p. 11–23.

Moehlman PD, Rubenstein DI, Kebede F. 2013. *Equus grevyi*. IUCN Red List Threat Species.:e.T7950A21070406. doi:10.2305/IUCN.UK.2013-1.RLTS.T7950A21070406.en.

Moncunill-Solé B, Orlandi-Oliveras G, Jordana X, Rook L, Köhler M. 2016. First approach of the life history of *Prolagus apricenicus* (Ochotonidae, Lagomorpha) from Terre Rosse sites (Gargano, Italy) using body mass estimation and paleohistological analysis. Comptes Rendus - Palevol. 15(1–2):227–237. doi:10.1016/j.crpv.2015.04.004.

Nacarino-Meneses C, Jordana X, Köhler M. 2016. Histological variability in the limb bones of the Asiatic wild ass and its significance for life history inferences. PeerJ. 4(10):e2580. doi:10.7717/peerj.2580.

Nacarino-Meneses C, Köhler M. 2018. Limb bone histology records birth in mammals. PLoS One. 13(6):e0198511.

Nitychoruk J, Bińka K, Hoefs J, Ruppert H, Schneider J. 2005. Climate reconstruction for the Holsteinian Interglacial in eastern Poland and its comparison with isotopic data from Marine Isotope Stage 11. Quat Sci Rev. 24(5–6):631–644. doi:10.1016/j.quascirev.2004.07.023.

Novellie P. 2008. *Equus zebra*. IUCN Red List Threat Species.:e.T7960A12876787.

Nowak RM. 1999. Walker's mammals of the world. Baltimore and London: The Johns Hopkins University Press.

Orlandi-Oliveras G, Jordana X, Moncunill-Solé B, Köhler M. 2016. Bone histology of the giant fossil dormouse *Hypnomys onicensis* (Gliridae, Rodentia) from Balearic Islands. Comptes Rendus - Palevol. 15(1–2):238–244. doi:10.1016/j.crpv.2015.05.001.

Orlandi-Oliveras G, Nacarino-Meneses C, Koufos GD, Köhler M. 2018. Bone histology provides insights into the life history mechanisms underlying dwarfing in hipparionins. Sci Rep. 8(1):17203. doi:10.1038/s41598-018-35347-x.

Orlando L. 2015. Equids. Curr Biol.:R973–R978. doi:10.1016/j.cub.2015.09.005.

Ortiz-Jaureguizar E, Alberdi MT. 2003. The pattern of body mass changes in the Hipparionini (Perissodactyla, Equidae) of the Iberian Peninsula during the Upper Miocene-Upper Pliocene. Coloquios Paleontol. 1:499–509.

Padian K, de Ricqlès A, Horner JR. 2001. Dinosaurian growth rates and bird origins. Nature. 412(6845):405–408. doi:10.1038/35086500.

Palkovacs EP. 2003. Explaining adaptive shifts in body size on islands: a life history approach. Oikos. 103(1):37–44. doi:10.1034/j.1600-0706.2003.12502.x.

Penzhorn BL. 1988. *Equus zebra*. Mamm Species. 314:1–7.

Peters RH. 1983. The ecological implications of body size. Cambridge: Cambridge University Press.

Pushkina D, Bocherens H, Ziegler R. 2014. Unexpected palaeoecological features of the

Middle and Late Pleistocene large herbivores in southwestern Germany revealed by stable isotopic abundances in tooth enamel. Quat Int. 339–340:164–178. doi:10.1016/j.quaint.2013.12.033.

Roff DA. 1992. The evolution of life histories. Theory and analysis. New York: Chapman and Hall.

Roff DA. 2002. Life history evolution. Sunderland: Sinauer Associates.

Rubenstein D, Low Mackey B, Davidson Z, Kebede F, King SR. 2016. *Equus grevyi*. IUCN Red List Threat Species.:e.T7950A89624491.

Saarinen J, Eronen J, Fortelius M, Seppä H, Lister AM. 2016. Patterns of diet and body mass of large ungulates from the Pleistocene of Western Europe, and their relation to vegetation. Palaeontol Electron. 19(3):1–58.

Schöpke K, Stubbe A, Stubbe M, Batsaikhan N, Schafberg R. 2012. Morphology and variation of the Asiatic wild ass (*Equus hemionus hemionus*). Erforsch Biol Ressourcen der Mongolei. 12:77–84.

Schulz E, Kaiser TM. 2013. Historical distribution, habitat requirements and feeding ecology of the genus *Equus* (Perissodactyla). Mamm Rev. 43(2):111–123. doi:10.1111/j.1365-2907.2012.00210.x.

Shoemaker L, Clauset A. 2014. Body mass evolution and diversification within horses (family Equidae). Ecol Lett. 17(2):211–220. doi:10.1111/ele.12221.

Silver IA. 1963. The aging of domestic animals. In: Brothwell D, Higgs E, editors. Science in Archaeology: a comprehensive survey of progress and research. New York: Basic Books. p. 250–268.

Simpson GG. 1953. The major features of evolution. New York: Columbia University Press.

Stanley SM. 1973. An explanation for Cope’s rule. Evolution (N Y). 27:1–26.

Stearns SC. 1992. The evolution of life histories. New York: Oxford University Press.

Stearns SC. 2000. Life history evolution: successes, limitations, and prospects. 87:476–

486.

Strömberg CAE. 2006. Evolution of hypsodonty in equids: testing a hypothesis of adaptation. Paleobiology. 32(2):236–258.

Turner-Walker G, Mays S. 2008. Histological studies on ancient bone. In: Pinhasi R, Mays S, editors. Advances in human paleopathology. Chichester: John Wiley & Sons, Ltd. p. 121–146.

Western D. 1979. Size, life history and ecology in mammals. Afr J Ecol. 17(4):185–204. doi:10.1111/j.1365-2028.1979.tb00256.x.

Woodward HN, Freedman Fowler EA, Farlow JO, Horner JR. 2015. *Maiasaura*, a model organism for extinct vertebrate population biology: a large sample statistical assessment of growth dynamics and survivorship. Paleobiology. 41(04):503–527. doi:10.1017/pab.2015.19.

Woodward HN, Padian K, Lee AH. 2013. Skeletochronology. In: Padian K, Lamm E-T, editors. Bone histology of fossil tetrapods: advancing methods, analysis, and interpretation. Berkeley: University of California Press. p. 195–215.

Figure 1. Size-decrease trend experienced by caballoid (circles) and stenonoid (triangles) horses during the Pleistocene (data from Alberdi et al. 1995; Ernest 2003; Cantalapiedra et al. 2017). Body mass estimates of *Equus mosbachensis* (black star) and *Equus steinheimensis* (white star) is also shown. Black circles and triangles = extinct *Equus* species; 75% grey triangles = zebras; 25% grey triangles = asses.

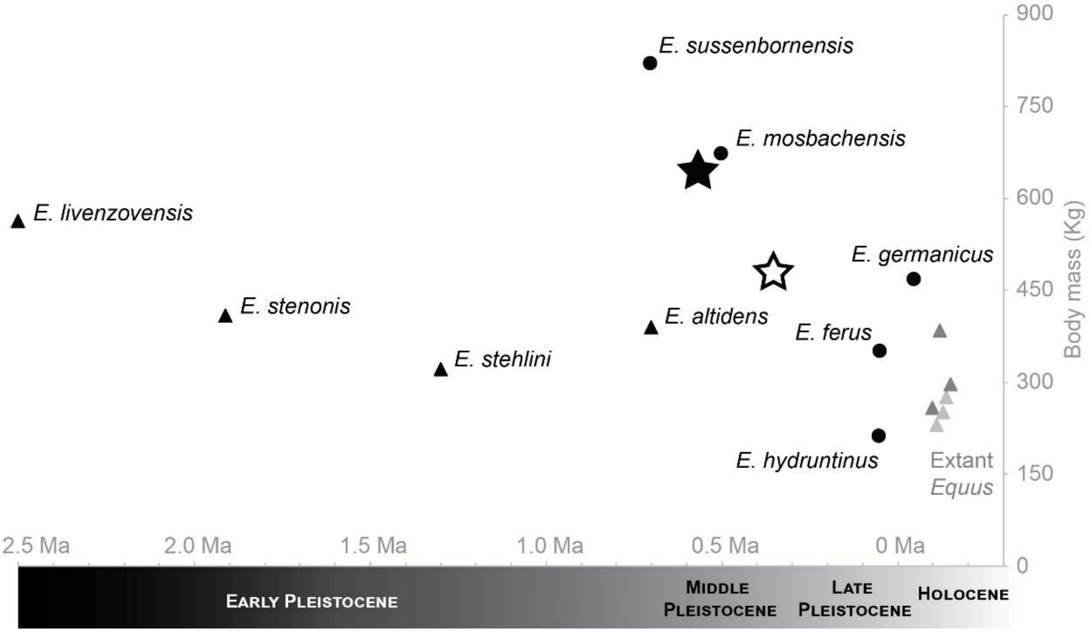

Figure 2. Map of Germany showing the provenance of the fossil sample. M = Mosbach Sands; S = Steinheim an der Murr.

Figure 3. Metapodial bone histology of European Middle Pleistocene (a–d) and extant *Equus* (e–h). (a) Metacarpal bone cortex of *Equus steinheimensis* (IPS96013). (b) Metatarsal bone cortex of *Equus mosbachensis* (IPS96014). (c) Metacarpal bone cortex of *Equus steinheimensis* (IPS96011). (d) Metacarpal bone cortex of *Equus mosbachensis* (IPS96017) (e) Metacarpal bone cortex of *Equus hemionus* (IPS83877). (f) Metatarsal bone cortex of *Equus grevyi* (IPS84963). (g) Metacarpal bone cortex of *Equus quagga* (IPS116319). (h) Metatarsal bone cortex of *Equus zebra* (IPS116321). CVC = circular vascular canal; EFS = external fundamental system; FLC = fibrolamellar complex; LCV = longitudinal vascular canal; RVC = radial vascular canal. Scale bars = 200 μm.

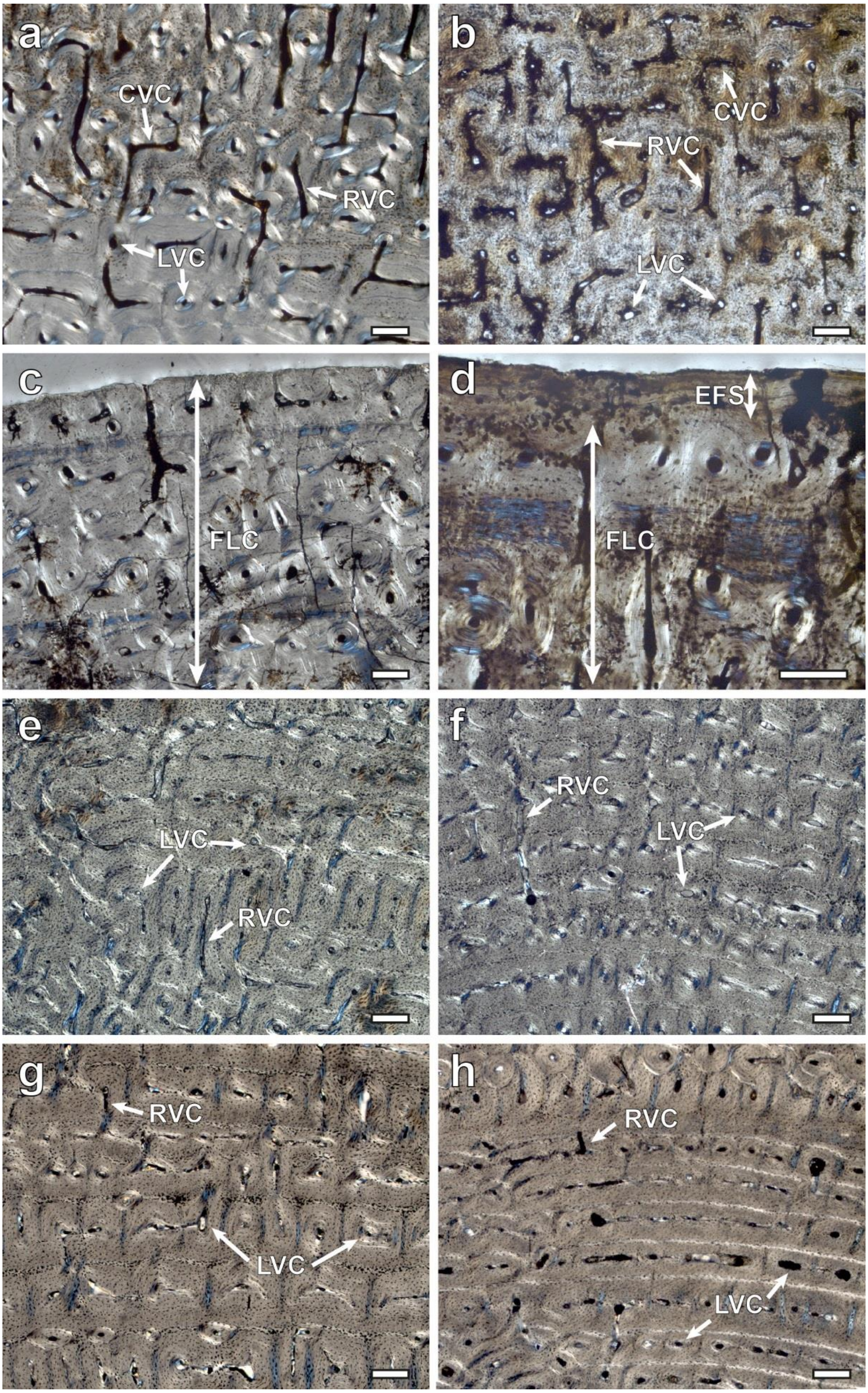
a
CVC
RVC
LVC
b
CVC
RVC
LVC
c
FLC
d
EFS
FLC
e
LVC
RVC
f
RVC
LVC
g
RVC
LVC
h
RVC
LVC

Figure 4. Bone growth marks identified in the metapodia of *Equus steinheimensis* (a,b) and *Equus mosbachensis* (c,d). (a) Metacarpal bone cortex of *Equus steinheimensis* IPS96013. (b) Metatarsal bone cortex of *Equus steinheimensis* IPS96010. (c) Metacarpal bone cortex of *Equus mosbachensis* IPS96017. (d) Metatarsal bone cortex of *Equus mosbachensis* IPS96014. White arrows = cyclical bone growth marks; black arrows = non-cyclical bone growth marks (neonatal line – NL). Scale bars = 1 mm.

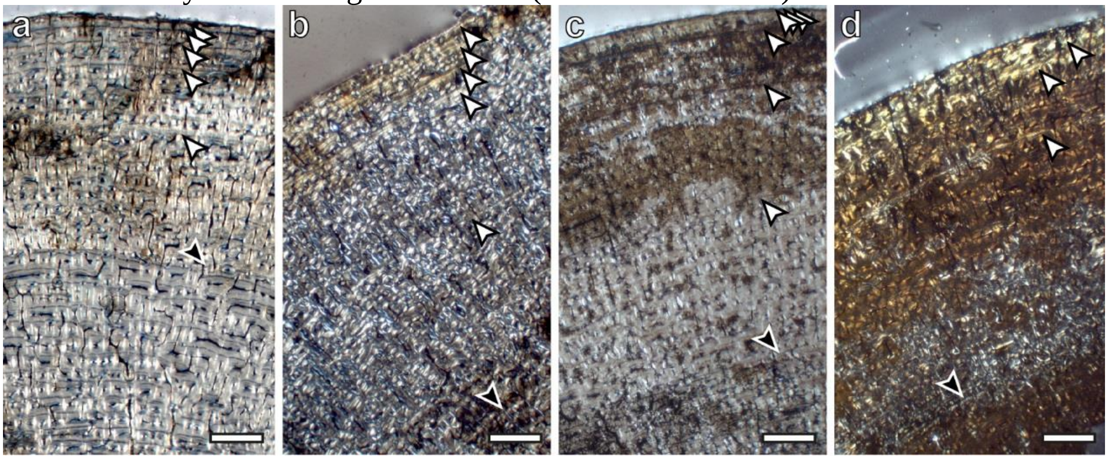

Figure 5. Metapodial growth of European Middle Pleistocene (continuous lines) and extant (dashed lines) Equus. Bone perimeter (mm, ordinate axis) is plotted against estimated age (years, abscissa axis) to obtain growth curves. (a) Growth curves obtained from the metacarpus. (b) Growth curves obtained from the metatarsus. Legend is shown at the bottom of the figure.

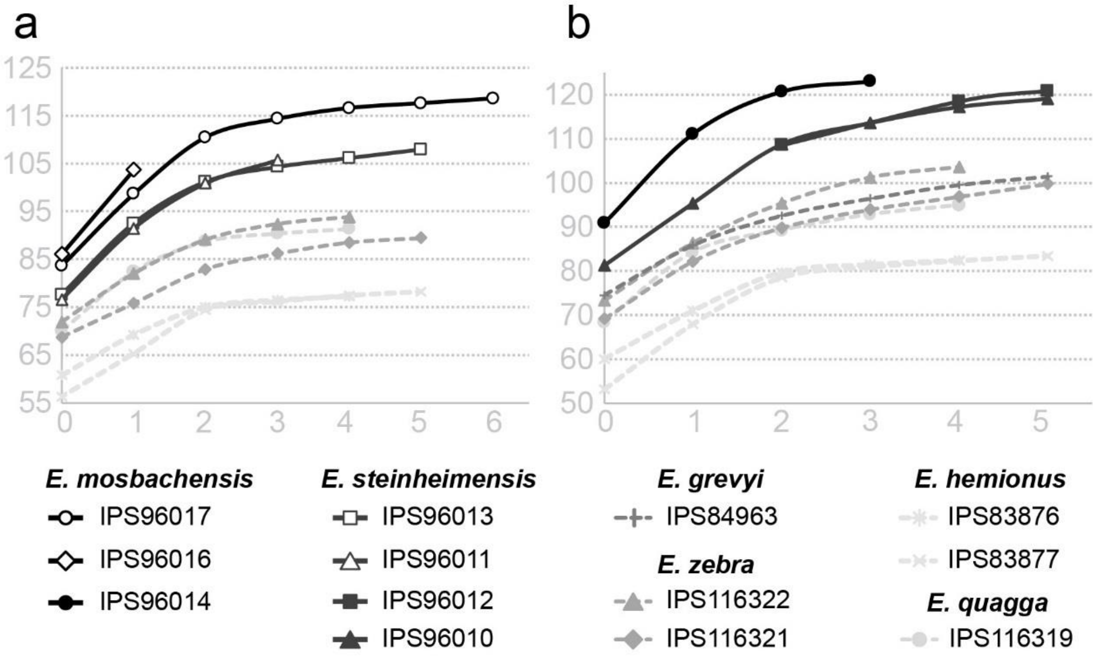

Table 1. Sample studied for bone histology. Mc = metacarpus; Mt = metatarsus; SMNS = Staatliches Museum für Naturkunde Stuttgart (Stuttgart, Germany); MDA = Museum of Domesticated Animals (Halle, Germany); ZIHU = Zoological Institute of Hamburg University (Hamburg, Germany); ICP = Institut Català de Paleontologia (Barcelona, Spain). The star (*) indicates fossil species.

Species	**Code**	**Collection code**	**Bone**	**Site/Habitat**	**Institution**
*E. steinheimensis**	IPS96010	32917/23	Mt	Steinheim an der Murr	SMNS
	IPS96011	32803/61	Mc	Steinheim an der Murr	SMNS
	IPS96012	32803/235	Mt	Steinheim an der Murr	SMNS
	IPS96013	32803/321	Mc	Steinheim an der Murr	SMNS
*E. mosbachensis**	IPS96014	32850/87	Mt	Mosbach Sands	SMNS
	IPS96016	32850/6	Mc	Mosbach Sands	SMNS
	IPS96017	32850/112	Mc	Mosbach Sands	SMNS
E. hemionus	IPS83876	225	Mc, Mt	Gobi Desert	MDA
	IPS83877	381	Mc, Mt	Gobi Desert	MDA
E. grevyi	IPS84963	7111	Mt	Hagenbeck Zoo	ZIHU
E. quagga	IPS116319	A16/033	Mc, Mt	Réserve Africaine de Sigean	ICP
E. zebra	IPS116321	A15/213	Mc, Mt	Réserve Africaine de Sigean	ICP
	IPS116322	A16/003	Mc, Mt	Réserve Africaine de Sigean	ICP

Table 2. Adult body weight estimation (kg) in extinct *Equus*. n = number of samples, SD = standard deviation.

Species	**Locality**	**Metacarpus**		**Phalanx**		**Mean ± SD (n)**
		n	**Mean ± SD**	**n**	**Mean ± SD**	
E. steinheimensis	Steinheim an der Murr	18	478.7 ± 63.1	36	464.7 ± 131.2	469.3 ± 112.6 (54)
E. mosbachensis	Mosbach Sands	7	595.6 ± 82	9	616.54 ± 275.2	607.4 ± 207.8 (16)

Table 3. Size at birth in extant and extinct Equus. The perimeter of the neonatal line (NL), considered as a proxy of the size at birth, is shown. The star (*) indicates fossil species.

Species	Code	Perimeter of the NL (mm)	
		Metacarpus	Metatarsus
*E. steinheimensis**	IPS96010	-	81.26
	IPS96011	76.55	-
	IPS96013	77.59	-
*E. mosbachensis**	IPS96014	-	90.86
	IPS96016	86.02	-
	IPS96017	83.65	-
E. hemionus	IPS83876	60.77	60.04
	IPS83877	56.29	53.09
E. grevyi	IPS84963	-	74.15
E. quagga	IPS116319	69.98	68.35
E. zebra	IPS116321	68.70	69.13
	IPS116322	71.92	73.29