
This version is available at https://ddd.uab.cat/record/239869

under the terms of the [BY-NC-ND] license
The life history of European Middle Pleistocene equids: first insights from bone histology

Carmen Nacarino-Meneses\textsuperscript{1,2,*} and Guillem Orlandi-Oliveras\textsuperscript{1}

\textsuperscript{1}Evolutionary paleobiology department, Institut Català de Paleontologia Miquel Crusafont (ICP), Campus de la Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain.

\textsuperscript{2}Department of Biological Sciences, University of Cape Town, Private Bag X3, Rhodes Gift, 7700 South Africa

*Corresponding author: Dr. Carmen Nacarino-Meneses

Address: University of Cape Town, Department of Biological Sciences, Private Bag X3, Rhodes Gift, 7700 South Africa

Email: carmen.nacarino@gmail.com

ORCiD: orcid.org/0000-0003-2123-8758
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 caballine 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 caballloid 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
caballloid 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
Thus, we also examine here the bone histology of the extant equid species of Asiatic wild ass \textit{(Equus hemionus)}, Grevyi’s zebra \textit{(Equus grevyi)}, Plains zebra \textit{(Equus quagga)} and mountain zebra \textit{(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 \textit{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 \textit{E. steinheimensis} and \textit{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 \textit{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 \textit{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 \textit{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 \textit{E. hemionus}, \textit{E. grevyi}, \textit{E. quagga} and \textit{E. zebra} (Table 1) for comparison. Specimens of \textit{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 cyclicity, 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 find 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-Menéndez 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 caballid 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 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
skeletocronology 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.

**Acknowledgments**

We thank Reinhard Ziegler for access to the collections of the Staatliches
Museum für Naturkunde (Stuttgart, Germany) and for permission to cut the metapodia
of *E. steinheimensis* and *E. mosbachensis*. We are also grateful to Renate Schafberg,
Thomas Kaiser and for loans and permission to cut bones from the collections of the
Museum of Domesticated Animals of the Martin-Luther-University Halle-Wittenberg
(Halle, Saale, Germany) and the Zoological Institute of Hamburg University (Hamburg,
Germany) respectively. We thank B. Lamglait (currently at Université de Montréal,
Canada) and the Réserve Africaine de Sigean (Sigean, France) for donation of aged
equid specimens. Manuel Fernández and Gemma Prats-Muñoz are acknowledged for
the preparation of the histological thin-sections of the study. We are also in debt to
Meike Köhler for discussion on this paper and the revision of earlier versions of the
manuscript. Thanks are extended to Xavier Jordana, Alexandra Houssaye and one
anonymous reviewer for their feedback, which resulted in significant improvements of
this manuscript.

**Funding details**
This work was supported by the Spanish Ministry of Economy and Competitiveness under Grant CGL-2015-63777, Grant BES-2013-066335 (to C.N.-M.) and Grant EEBB-I-16-10546 (to C.N.-M.); the Government of Catalonia under Grant 2014-SGR-1207, Grant CERCA Programme and Grant 2016FI_B00202 AGAUR (to G.O.-O); and the DST-NRF Centre of Excellence in Palaeosciences under Grant COE2019-PD03 (to C.N.-M.). Opinions expressed and conclusions arrived at, are those of the author and are not necessarily attributed to the CoE.

**Declaration of interest statement**

The authors declare that there is no conflict of interest.

**References**


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


Köhler M, Marín-Moratalla N, Jordana X, Aanes R. 2012. Seasonal bone growth and
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

editors. Bone histology of fossil tetrapods: advancing methods, analysis, and

In: Padian K, Lamm E-T, editors. Bone histology of fossil tetrapods: advancing
251.

Lindsay EH, Opdyke ND, Johnson NM. 1980. Pliocene dispersal of the horse Equus
doi:10.1038/287135a0.

MacFadden BJ. 1986. Fossil horses from “Eohippus” (Hyracotherium) to Equus:
doi:10.1017/S0094837300003109.

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


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 -


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


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.
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Code</th>
<th>Collection code</th>
<th>Bone</th>
<th>Site/Habitat</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. steinheimensis</em></td>
<td>IPS96010</td>
<td>32917/23</td>
<td>Mt</td>
<td>Steinheim an der Murr</td>
<td>SMNS</td>
</tr>
<tr>
<td></td>
<td>IPS96011</td>
<td>32803/61</td>
<td>Mc</td>
<td>Steinheim an der Murr</td>
<td>SMNS</td>
</tr>
<tr>
<td></td>
<td>IPS96012</td>
<td>32803/235</td>
<td>Mt</td>
<td>Steinheim an der Murr</td>
<td>SMNS</td>
</tr>
<tr>
<td></td>
<td>IPS96013</td>
<td>32803/321</td>
<td>Mc</td>
<td>Steinheim an der Murr</td>
<td>SMNS</td>
</tr>
<tr>
<td><em>E. mosbachensis</em></td>
<td>IPS96014</td>
<td>32850/87</td>
<td>Mt</td>
<td>Mosbach Sands</td>
<td>SMNS</td>
</tr>
<tr>
<td></td>
<td>IPS96016</td>
<td>32850/6</td>
<td>Mc</td>
<td>Mosbach Sands</td>
<td>SMNS</td>
</tr>
<tr>
<td></td>
<td>IPS96017</td>
<td>32850/112</td>
<td>Mc</td>
<td>Mosbach Sands</td>
<td>SMNS</td>
</tr>
<tr>
<td><em>E. hemionus</em></td>
<td>IPS83876</td>
<td>225</td>
<td>Mc, Mt</td>
<td>Gobi Desert</td>
<td>MDA</td>
</tr>
<tr>
<td></td>
<td>IPS83877</td>
<td>381</td>
<td>Mc, Mt</td>
<td>Gobi Desert</td>
<td>MDA</td>
</tr>
<tr>
<td><em>E. grevy</em></td>
<td>IPS84963</td>
<td>7111</td>
<td>Mt</td>
<td>Hagenbeck Zoo</td>
<td>ZIHU</td>
</tr>
<tr>
<td><em>E. quagga</em></td>
<td>IPS116319</td>
<td>A16/033</td>
<td>Mc, Mt</td>
<td>Réserve Africaine de Sigean</td>
<td>ICP</td>
</tr>
<tr>
<td><em>E. zebra</em></td>
<td>IPS116321</td>
<td>A15/213</td>
<td>Mc, Mt</td>
<td>Réserve Africaine de Sigean</td>
<td>ICP</td>
</tr>
<tr>
<td></td>
<td>IPS116322</td>
<td>A16/003</td>
<td>Mc, Mt</td>
<td>Réserve Africaine de Sigean</td>
<td>ICP</td>
</tr>
</tbody>
</table>
Table 2. Adult body weight estimation (kg) in extinct *Equus*. \( n \) = number of samples, SD = standard deviation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Metacarpus</th>
<th>Phalanx</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( n )</td>
<td>( n )</td>
<td>( n )</td>
</tr>
<tr>
<td><em>E. steinheimensis</em></td>
<td>Steinheim an der Murr</td>
<td>18</td>
<td>36</td>
<td>469.3 ± 112.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>478.7 ± 63.1</td>
<td>464.7 ± 131.2</td>
<td>469.3 ± 112.6</td>
</tr>
<tr>
<td><em>E. mosbachensis</em></td>
<td>Mosbach Sands</td>
<td>7</td>
<td>9</td>
<td>607.4 ± 207.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>595.6 ± 82</td>
<td>616.54 ± 275.2</td>
<td>607.4 ± 207.8</td>
</tr>
</tbody>
</table>


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.

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