General Palaeontology, Systematics and Evolution (Palaeopathology)

Surviving in a predator-free environment: Hints from a bone remodelling process in a dwarf Pleistocene deer from Crete

Survivre dans un environnement sans prédateurs : considérations sur un processus de remodelage osseux dans un cerf nain du Pléistocène de Crète

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ABSTRACT

Islands have been regarded by scientists as a living laboratory of evolution, and a prime target for the study of forces influencing evolution. This research aims to investigate whether a dwarf deer (Candiacervus ropalophorus), that suffered a traumatic fracture of its metatarsal, might have survived, despite the broken limb, in the Cretan free-predator environment for a time longer than commonly expected on mainland. The metatarsal shows a healed complete and oblique fracture at level of the distal part of the diaphysis. Both macroscopic and X-ray examinations show the impressive new bone formation that occurred in the post-trauma period. The size of osseous callus, covering the fracture line and the surface next to the lesion, suggests that the deer has survived several months after the traumatic injury. In a mainland context, an injured deer would hardly have survived for such a long period, because any movement constantly irritated the fracture, dramatically reducing performance or willingness to move and the gait speed, and hampering any escape.

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RÉSUMÉ

Les îles ont été considérées comme des laboratoires vivants de la biodiversité et une cible pour l’étude des forces qui influent sur l’évolution. Cette recherche vise à déterminer si un cerf nain du Pléistocène de Crète (Candiacervus ropalophorus) qui a subi une fracture de son métatarsien aurait pu survivre, en dépit de sa jambe cassée, dans un environnement sans prédateurs pour un temps plus long que prévu sur le continent. L’os métatarsien montre une fracture complète et oblique au niveau de la diaphyse. Les examens macroscopiques et aux rayons X montrent l’impressionnante formation d’os nouveau qui s’est produite dans la période post-traumatique. La taille de ce cal osseux, couvrant la ligne de fracture, suggère que le cerf a survécu durant plusieurs mois au traumatisme, même si tout mouvement...
1. Introduction

Studies on palaeopathology of fossil bones, dating back to the beginning of the last century, mainly deal with investigations on human and dinosaur bones or on domestic animals from archaeological sites, while only a few concern Pleistocene mammals (see e.g. Acosta Hospitaleche et al., 2012; Aufderheide and Conrado, 1998; Bartosiewicz, 2008; Bartosiewicz and Gal, 2013; Bell and Coria, 2013; Bishop, 2011; Bricknell, 1987; Brothwell, 2008; Buffetaut et al., 2007; De Boer et al., 2013; Duckler and Van Valkenburgh, 1998; Everhart, 2008; Fisk and Macho, 1992; Franklin, 2011; Johnson and Milburn, 1982; Jordana et al., 2011; Kallal et al., 2012; Kierdorf et al., 2012; Lingham-Sojiar, 2004; Martin, 2013; Martin Sander, 1992; Moodie, 1917, 1923; Ortner, 2002; Rega et al., 2012; Rothschild and Martin, 1993; Salesa et al., 2014; Tanke and Rothschild, 2010; TASNáDI-KUBACSKA, 1962; Telldahl, 2012a,b; Vann and Thomas, 2006; Wu and Schepartz, 2009).

During the last two decades, studies on palaeohistology and palaeopathology have been receiving increasing attention, at least partially reflecting the recent development and changing emphasis in the field of palaeoecology. Studying factors causing pathological diseases in past wild animals could, indeed, provide valuable hints for a better understanding of their life-style, behaviour and niche occupancy, in turn providing additional data to reconstruct predator-prey relationships and some aspects of population/ecosystem dynamics (see among others Gardner and Smith, 2006; Katsura, 2004; Palmqvist et al., 1999; Rothschild and Tanke, 1992; Rothschild et al., 2001; Salesa et al., 2014; Tanke and Farke, 2006). In particular, “survival of animals with injuries or diseases limiting their mobility and function suggests the possibility of “a support system” which allowed their survival” (Rothschild and Tanke, 1992, p. 73).

Therefore, studies of palaeopathology in insular endemic mammals may help determining whether resources availability, and ecological interaction and displacement or the release from predator pressure are among the major factors driving evolution and survival in isolated environments (see e.g. Lomolino et al., 2013 and references therein).

Here, we present a case study of a healed fracture of a still undescribed metatarsal of Candiacervus ropalophorus (the smallest among the endemic Pleistocene deer species from Crete, eastern Mediterranean), found in the latest Middle (?) – Late Pleistocene (see below) deposits of Bate cave (Rethymnon, northern Crete) (Capasso Barbato, 1990, 1992; Kotsakis, 1977; Kotsakis et al., 1976).

2. Material and methods

2.1. Bate cave and its faunal record

The metatarsal (MPUR/V coll. Bate S50) is part of a rich fossil sample collected in the seventies by a team of Sapienza University of Rome (Italy) in an unexplored cavity (named “Bate cave” by the Italian discoverers), located in the north Cretan coast, not far from the Zourida gorge, in the cliffs below the Rethymnon-Chania National Road (Fig. 1). The material is currently stored in the Museum of Palaeontology, Department of Earth Sciences Sapienza University (Rome, Italy).

The vertebrate fauna retrieved from the chaotic fossiliferous layers filling Bate cave includes a few remains of amphibians, reptiles (Bufo cf. B. viridis, Testudo marginata cretensis, Lacerta cf. L. erhardi, Coluber cf. C. gemonensis in Kotsakis, 1977), small mammals (Mus minutus) and four deer species: Candiacervus ex gr. C. ropalophorus (sensu Palombo et al., 2008), the smallest and the most abundant, and three larger deer species, Candiacervus cretensis, Candiacervus dorothensis (= Candiacervus sp. V. De Vos, 1979),...
and Candiacervus major (= Candiacervus sp. VI, De Vos, 1979) (Capasso Barbato, 1989). The latest two species have been reported only from Bate cave, while in this cave a fifth endemic Cretan species, Candiacervus rethymnensis, seems to be absent. The fossil assemblage of Bate cave belongs to the youngest Pleistocene Cretan fauna complex, the so-called Mus minotaurus “sub-zone” (Mayhew, 1977). The age of the fossiliferous deposit is not firmly established because of the inconsistency of dates obtained by different authors/methodologies (see Lax, 1996). Belluomini and Delitala (1983, 1988) indicated an age from 63,500 ± 20% to 69,000 ± 20% years (Amino Acid Racemization, AAR, datings on deer bones), while Reese et al. (1966, p. 47) argue that AAR dates done on bones are “very much less reliable than tooth enamel.” Two AAR dates on deer teeth are indeed significantly older: 152,000 ± 20% to 105,000 ± 20%, suggesting a late Middle to early Late Pleistocene age for the deposits of Bate cave (Reese et al., 1966).

The bone studied here (Fig. 2) is a left metatarsal belonging to the group of the smallest deer (cf. Capasso Barbato, 1989), whose dimensions partially overlap the dimensional range of the richest samples of the smallest deer found in Crete [i.e. samples from Simonelli (Malatesta, 1980), Gerani 4 and Liko caves (De Vos, 1979, 1984)]. This might make the identification of the sample from Bate cave questionable. The smallest Cretan deer, indeed, shows a large variation in size, proportions and in skull, teeth and especially antler morphology. This prompted some research to tentatively split them into four different species/morphotypes (C. ropalophorus, Candiacervus sp. Ila, Candiacervus sp. Ilb, Candiacervus sp. IIC) (Dermitzakis et al., 1987 and De Vos, 1987; De Vos, 1979, 1984, 2000; Van der Geer et al., 2010). Such a high level of specific diversity seems, however, improbable, and the hypothesis that differences in skull and antler morphology could depend on increased intra-specific variation, as sometime observed in insular species, cannot be discarded (cf. e.g. Caloi and Palombo, 1995; Capasso Barbato, 1990; Van der Geer et al., 2014; Palombo et al., 2008 for a discussion).

Dimensions and proportions of the metatarsal studied here (see below) (Figs. 2, 3; Table 1) suggest it could be identified as C. ropalophorus, as supported by a comparison with the rich Late Pleistocene metatarsal samples from Gerani 4 (C. ropalophorus) (AAR date 70,000 ± 20% years, ESR date 100,600 ± 20% years, Reese et al., 1966) and Liko sites (with Candiacervus spp. II) (AAR dates from 85,000 ± 20% to 105,000 ± 20% years, Reese et al., 1966) (De Vos, 1979), as well as with the Late Pleistocene sample from Simonelli cave (= “Megaceros cretensis”, Malatesta, 1980) (AAR and ESR dates ranging from 21,500 ± 20% to 81,000 ± 20%, Reese et al., 1966).

2.2. Methods

Dimensions and proportion of the metatarsal (MPUR/V coll. Bate S50) (Figs. 2, 3; Table 1) were compared with those of the smallest deer from Bate cave (Capasso Barbato, 1989) and Simonelli cave (Malatesta, 1980) to confirm its specific identification (see above) and evaluate the extent of modifications caused by the fracture.

The morphological exam of the lesion was performed by direct observation (Figs. 2, 4) and by means of X-ray examination (Fig. 5). This approach permitted to study the inner part of the bone, visualizing the medullary cavity in the diaphysis and the possible fracture line in the bone structure and inferring factor causing the pathological bone alteration. Digital X-ray analysis was conducted in the Radiological Laboratory of the Veterinary Hospital, in the Department of Veterinary Medicine of Sassari University (Italy). Digital radiographic images were obtained through anterior-posterior and lateral-medial projections of X-rays using a Radiological Unit CR 30-X (Agfa, Mortsel, Belgium) with the following parameters: 120 kVp and 120 mA. In addition, a micromorphometric analysis was made to study the inner part of the lesion area with particular attention to the shape of the medullary cavity and its three-dimensional reconstruction. The area of the osseous callus was scanned with a micro-CT scanner (Skyscan 1172, Aartselaar, Belgium) in the Biophysical Laboratory of the University of Sassari (Italy). The scanner was equipped with the X-ray detector: 11 Megapixel, 12 bit digital X-ray camera with 24 × 36 mm field of view.

3. Results

The left metatarsal of Candiacervus ropalophorus from Bate cave belongs to an adult individual, as it shows complete epiphyseal fusion (Fig. 2). Its dimensions indicate that the specimen is among the smallest and most robust specimens found at Bate cave, while at Simonelli cave a few specimens show smaller dimension and more robust proportions, confirming the large variation in size and proportion of bones of small-sized Cretan deer (Fig. 3, Table 1). Compared with the specimens from Bate cave, the metatarsal shows a more robust proximal epiphysis, sensibly expanded antero-posteriorly. This feature possibly relates to the modified general shape caused by the fracture that displaced the distal part of the bone.
The bone presents a healed, complete oblique fracture of the diaphysis, slightly distal to the mid-shaft. The fractured area shows a considerable overriding of the two main fragments, the distal part of the diaphysis being positioned laterally and displaced posterior to the proximal one. As a result, the distal shaft and the epiphysis is misaligned by at least 22 degrees from the original axis in the cranial view and at least 19 degrees in the lateral view (Fig. 4). The slipping reduces the metatarsal length of about 2 cm. The misaligned axis of the bone ant the entity of the displacement of the two broken segments, on one hand confirms that a complete fracture occurred, on the other that the fracture was likely open and a tearing of soft tissues and skin occurred.

What was the accident that caused the fracture is difficult to say. However, it could be hypothesized that the fracture has been provoked by either a sudden twisting movement or an accidental fall after a jump, as may be supported by the hypothesis that *C. ropalophorus* inhabited rocky environments, similarly to the extant Cretan goat *Capra aegagrus cretica* (Van der Geer et al., 2006, 2010).

Considering the fact that at the level of the fracture, no musculature surrounds metapodial bones in deer (only tendons are present) and that the distal part of the metatarsal, where even tendons are present, significantly slipped laterally, the hypothesis that the fracture was open may be considered realistic. In the shank of long bones of most artiodactyls, indeed, the tendons are localized in the cranial side (terminal tendons of extensor muscles) and in the plantar side (terminal tendons of flexor muscles), and only a thin layer of subcutaneous connective tissue separates the skin from the metapodial bone in the lateral and
medial sides of the diaphysis. Therefore, the medial and lateral sides of metapodial bones may be more exposed to traumatic events.

The fracture of the *C. ropalophorus* metatarsal was entirely healed. Both a direct observation and X-ray examination show the impressive new bone formation that occurred in the post-trauma period (Figs. 2 and 5).

The localization, shape, size and symmetry of the osseous callus confirm it was a consequence of a traumatic fracture and that the bone remodelling process depends neither on a bone proliferative process as a consequence of a periostium reaction due to chronic inflammatory events (e.g. periostitis or osteoperiostitis), nor on tumoral osteogenic process (e.g. osteoma and osteosarcoma, respectively benign and malignant tumours of bones). In the case of any tumoral process, the surface of the osseous callus would be porous, whereas in the case of periostium reaction the lesion would be quite localized.

X-ray images indicate that the remodelling process developed while biomechanical stresses continued to solicit movements at the fracture line level, suggesting that the animal was actively moving in spite of the important injury affecting its hind limb, because without movements the callus osseous would have been much less voluminous (Claes et al., 1998).

Assuming that the fracture was open with skin laceration, the hypothesis that the remodelling process was so important because of the simultaneous presence of an infection of the soft tissue surrounding the bone caused by pathogens seems reasonable (Urist et al., 1954). How long the stress of walking with a broken leg delayed the healing process it is difficult to hypothesize, though the size of the osseous callus suggests that the healing process lasted at least 4–5 months.

The extensive remodelling process modified the bone structure and the shape, position and direction of the diaphysis canals. The micro–CT images show a light passage from the canal typical of ruminants (doubled because the metapodial bone originated ontogenetically and phylogenetically from two distinct bones) in a labyrinth of canals becoming smaller (Fig. 5).

The rough surface of the callus and the extremely limited presence of smoothing signs indicate that the process of fracture remodelling had not appreciably progressed at the time when the deer died.

4. Discussion

Traumatic lesions constitute an important research field in the study of palaeopathology because they could provide useful insights in reconstructing animal behaviour and palaeoecology. Studies on this field go back to the 17 centuries, when the German naturalist Johann Friedrich Esper (1774) correctly described a fracture in a femur of a cave bear affected by osteosarcoma. Fractures in extinct animals have been reported ever since the Late Palaeozoic, as documented, for instance, by the healed fracture in a radius of the Permian synapsid *Dimetrodon* (Moodie, 1923).

In wild animals, fractures sometime occur as a result of accidental falls, though most long bone fractures are the result of bending and/or torsional loading, whose strengths scale similarly across a broad range of animals, in spite of differences in scaling of length and external diameter of bones (Selker and Carter, 1989).
months. The time necessary for the complete replacement mainly depends on the age (faster in juvenile than adult individuals), the type of bone tissue (more rapid in cancellous bone, slower in compact cortical bone), the kind of bone (slower in tibia than other bones) and the fracture type (slower in horizontal fracture, more rapid in helicoidal fracture) (Newton and Nunamaker, 1985). As a result, the healing process in bone fractures is a complex process that requires good general health and nutrition to be achieved rapidly. The immobilization of the fractured bone accelerates healing, while any factor disturbing bone immobility, as well as infections and other pathological conditions, significantly delays the healing process.

Contrary to poikilothermic animals (amphibians and many reptiles) that can stay for weeks or months without eating (Janssens, 1970), in endothermic animals, such as mammals, the immobilization of fractured limb bones is virtually impossible in the wild due to the need of moving at least for the minimum resource exploitation necessary to survive. Under the best conditions (immobilization by a circular external fixator, juvenile age and good nutrition), a fracture of metapodial bones in a young deer in its first year heals in five weeks (Aitken-Palmer et al., 2012). In the wild, this period could be longer. Indeed, movements constantly irritate the fracture line, causing production of more callus. Moreover, fractures of limb bones, particularly autopodium bones more exposed to traumatic injuries, are the most common in terrestrial mammals. The fracture of the metatarsal of the Cretan deer is in line with the high frequency of this kind of fracture in cervids. Nisbet et al. (2010), for instance, reported in a clinical study on twenty roe deer (Capreolus capreolus) affected by traumatic injuries, that among the 11 identified fractures, 3 affected metapodials. All fractures of the metapodials were open, with a tearing of the surrounding soft tissue and skin. A case of a spontaneous complete healing of an open metatarsal fracture has been also described in an extant white-tailed deer (Odocoileus virginianus) (Withrow, 1982).

The extent of the complete fracture in the Cretan metatarsal, the displacement suffered by the two broken parts, and the enormous development of the callus suggest that also in this case the fracture was open. This condition certainly caused the entry of pathogens. As a result, a severe inflammatory process likely developed, further increasing the disease of the injured deer.

The complete healing of the extensive trauma in the Cretan specimen, long-lasting despite the functional impact of the pathological consolidation, is an indirect evidence of the peculiar life condition of the animal in its insular ecosystem where predators were absent and the pressure for resources was expected to be low because Cretan herbivores of Mus “biozone” (elephant, and large, middle-sized and small deer) likely differed in their niche specialization (see below).

The fracture did not cause the immobility of the dwarf deer that moved at least to find food, being facilitated in this by the high pain threshold the ruminants generally have (Waterman-Pearson, 2006). Any movement constantly irritated the broken metatarsal, delaying the healing of the fracture. This joined with the difficulty firstly to place the hind leg and then the lameness due to the shortening of the
limb, dramatically reduced performance or willingness to move and the gait speed, hampering any escape and possibly reducing the animal’s home range. A survival in such conditions would be highly problematic for a mainland deer having to compete for food and to face large carnivorous predators. The low biodiversity of insular faunas and the disharmonic ecological structure of mammalian communities, changing the common rules of competition-coevolution dynamics, may increase the chance of survival of individuals suffering from some diseases. In an insular species-poor biota lacking predators, where ecological release should be prevalent, a lower level of inter-specific competition might attenuate the effect on population density and intra-specific competition caused by the absence of predators (e.g. Abrams, 2000). This was likely true of the Pleistocene fauna of Crete, lacking large mammalian predators and with a low number of herbivore species competing each other for resources.

Crete has been isolated from surrounding mainland since the end of the Pliocene by large barriers (van Hinsbergen and Meulenkamp, 2006), and most, if not all, non-volant vertebrate settlers reached the island by over-sea, sweepstake dispersal (Sondaar, 1977). As a result, the island was inhabited throughout the Pleistocene by unbalanced, disharmonic and impoverished vertebrate faunas (i.e. lacking large terrestrial mammalian carnivores, the only documented carnivore on Crete being a Late Pleistocene otter Lutrogale cretensis), counting mammals that managed to survive active or passive overwater dispersal (rodents, insectivores, elephants, deer, hippopotamuses), which enabled them to establish viable populations on the island. In this peculiar island ecosystem, the presence of a number of available ecological niches, proper on mainland to species absent on the island, triggered the radiative evolution of deer settlers. A new competition-coevolution equilibrium developed. Life-history traits of mammals evolved differently than in continental ecosystems. Deer partitioned resources and reduced competition by specializing for having different body size, habitat and trophic requirements as suggested by both the peculiar morphological traits of skulls, teeth, and limb bones and by the different Cretan deer species/morphotypes that coexisted on the same territory (Caloi and Palombo, 1995, 1996; De Vos, 2000; De Vos and Van der Geer, 2002; Van der Geer et al., 2010).

Key factors in interpreting the reason why C. ropalophorus from Bate cave survived for a fairly long time in spite of the severe injury of its hind limb depend on the interplay among resource availability and intra- and inter-specific competition that, in turn, affected the population density of the ecological group the small Cretan deer belongs to.

At the time when C. ropalophorus broke its left hind limb, the mammalian fauna consisted of deer species with a different diet and inhabiting different environments, a dwarf elephant (Palaeoloxodon creutzburgi), a giant mouse (Mus minotaurus), and a shrew (Crocidura zimmermanni), the only Cretan Pleistocene mammal surviving today. Therefore, adult deer of C. ex gr. C. ropalophorus [having nearly the same size, similar dietary behaviour (e.g. grassy food or prickly bushes) and inhabiting similar environments (e.g. rocky hill), as suggested by De Vos and Van der Geer (2002)] had neither potential competitors nor predators, the impact of the endemic near-flightless (Atheno cretensis) being negligible, and the presence of a second endemic raptor (Aquila according to Weesie, 1988, but Aves incertae sedis in Louchart et al., 2005) not substantiated because it is based on a single fragment, lacking...
diagnostic features. Accordingly, it is rational to suppose that inter-specific competition was low and individuals within each herbivorous species could efficiently exploit resources. More important (though difficult to ascertain) was the role of intra-specific competition, which regulates population growth in a density-dependent manner and tends to favor the individuals with high resource use efficiency and competition ability when resources are insufficient and competition occurs. Taking into account that available data on vegetation cover during the Late Pleistocene in the Aegean area indicate that forests were present even during the Last Glacial Maximum (i.e. on Lesbo island) (Bottema and Sarpaki, 2003; Digerfeldt et al., 2000; Fletcher et al., 2010; Margari et al., 2009), the hypothesis of a presence on Crete of diversified environments, including Zelkova forests (Kozlowski et al., 2014), an environment fairly rich in nutrients, seems reasonable. If so, it might be speculated that the competition among individuals of Candiacervus ex gr. C. ropalophorus was low, even though the absence of predators likely increased the population density. However, studies of mortality profile of small Cretan deer from both Gerani 4 (C. ropalophorus) and Liko (Candiacervus sp. II) caves carried out by Van der Geer et al. (2014), would suggest “a nutrient-poor environment supporting relatively few individuals” (Van der Geer et al., 2014, p. 21). Assuming as true this hypothesis, the competition would have increased, reducing the chance of survival of a severely crippled individual. The healed fracture of the metatarsal from Bate cave, however, indicates that the animal was reasonably healthy, as confirmed by the absence of any abnormal morphology of pathological origin, as conversely found in deer from Mavro Mouri cave (Rhythmon), most of which suffered of an osteopathy caused by a long-term malnutrition (Dermitzakis et al., 2006).

All things considered, the hypothesis that the crippled deer of Bate cave survived because throughout the time the fracture healed the competition for resources was low seems plausible. Moreover, taking into account that the reduced motility of C. ropalophorus from Bate cave limited its home range, the hypothesis that it died sometime after the fracture healed because a long dry season (or other unfavourable climatic conditions) negatively affected the vulnerable rocky environment it inhabited, thus triggering a starvation period, may be plausible. Other factors, however, could have caused the death of the draft deer before any conspicuous remodelling of the bony callus had occurred.

5. Conclusion

The complete and oblique fracture affecting a metatarsal of C. ropalophorus from Bate cave displaced laterally and posteriorly the distal epiphysis, reducing the metatarsal length of at least 2 cm (10.52% of the inferred total length). Both macroscopic observation and X-ray examination show the impressive new bone formation that occurred in the post-trauma period with the development of a massive callus with a coarse surface. The size of osseous callus suggests that the deer has survived several months after the traumatic injury, though any movement constantly irritated the open fracture. This, coupled with impaired locomotory ability due to the reduced length of the left hind limb, dramatically reduced performance or willingness to move and the gait speed, and hampering any escape. Survival in such a condition would be highly problematic for a deer having to compete for food and to face large carnivorous predators, as happens for species living on mainland. The loss of biodiversity of Pleistocene Cretan fauna and the unbalanced functional diversity of mammalian communities, in particular the absence of predators, the inferred low intra-guild competition, changing the common role of competition-coevolution dynamics, likely increased the chance of survival of this small deer.

The size and extension of the bony callus indicate that the animal had survived the metatarsal fracture for some months in environmental conditions providing enough resources for permitting the bone tissue to regenerate, though the poor remodelling suggests that no long time elapsed between the fracture healing and the death of the deer. Which among various factors caused its death is difficult to say, though taking into account the reduced motility of C. ropalophorus from Bate cave limited its home range, the hypothesis that a contrasted seasonal climate induced a starvation that in turn caused its death, cannot be discarded.

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