TAXONOMIC AND ECOLOGIC IMPLICATIONS OF MAMMOTH MOLAR MORPHOLOGY AS MEASURED VIA COMPUTED TOMOGRAPHY

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ABSTRACT

Two Late Pleistocene species of *Mammuthus*, *M. columbi* and *M. primigenius*, prove difficult to identify on the basis of their third molar (M3) morphology alone due to the effects of dental wear. A newly-erupted, relatively unworn M3 exhibits drastically different characters than that tooth would after a lifetime of wear. On a highly-worn molar, the lophs that comprise the occlusal surface are more broadly spaced and the enamel ridges thicken in comparison to these respective characters on an unworn molar. Since *Mammuthus* taxonomy depends on the lamellar frequency (# of lophs/decimeter of occlusal surface) and enamel thickness of the third molar, given the effects of wear it becomes apparent that these taxonomic characters are variable throughout the tooth’s life. Therefore, employing static taxonomic identifications that are based on dynamic attributes is a fundamentally flawed practice.

To help resolve the relationship between *M. columbi* and *M. primigenius*, I quantified the proportions of the characters that comprise the occlusal surface of *Mammuthus* third molars. Using computed tomography (CT), I digitized a sample of teeth from both species, creating models of continual wear via the removal of slices from the occlusal surface to the base of the crown. At each time slice, I calculated the occlusal enamel percentage, enamel thickness, and lamellar frequency of the exposed surface of the tooth. I then examined the relationship between relative wear percentage and dental characters to determine if there was a separation between the two species of mammoth with wear. My results demonstrate a prevalence of intraspecific variation, making a consistent separation of species difficult. In the absence of accompanying cranial morphologies or molecular data, delineation of the North American mammoth species based solely on molar morphology remains challenging, if not impossible. Additionally, the scatter in enamel values in *M. columbi* molars is indicative of a less phenotypically stable organism, suggesting that *M. primigenius* was the mammoth with a more highly conserved niche.
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Introduction

The Late Pleistocene of North America was a highly diverse environment characterized by a variety of ecosystems and an abundance of mammalian megafauna (Guthrie, 1982). The climate was characterized by repeated glacial cycles, reflected by the periodic swelling and shrinking of the Cordilleran (Booth et al., 2004) and Laurentide (Mickelson and Colgan, 2004) Ice Sheets. Among the most iconic Pleistocene fauna, the genus *Mammuthus* was a successful and a geographically widespread taxon (Agenbroad, 1984, 2005; Madden, 1983, 1985; Mead et al., 1986; Pérez-Crespo et al., 2012). Mammoths are members of the Elephantidae family, which includes numerous extinct taxa and two extant genera – *Loxodonta* (African elephants) and *Elephas* (Asian elephants) (Thomas and Lister, 2001). The species of *Mammuthus* in Pleistocene North America represent a two-pronged, chronologically separate radiation from Siberia (Agenbroad, 2005). This thesis follows the logic of Agenbroad (2003) in recognizing only four species of North American mammoths: *M. meridionalis*; *M. columbi*; *M. exilis*; and *M. primigenius*. Only *M. columbi* and *M. primigenius* are considered to have inhabited mainland North America during the Wisconsinan Glaciation (85,000 – 11,000 years BP), the final stage of the Late Pleistocene (Figure 1).

The earliest known *Mammuthus* migrants to North America entered by way of the Beringian Land Bridge around 2 million years ago (Agenbroad, 2005). Along with other distinctive mammal fauna, the appearance of *Mammuthus* in North American deposits is used to delineate units of the Irvingtonian Land Mammal Age (1,800,000 – 240,000 years BP) (Bell et al., 2004). Fragmentary remains of these ancestral mammoths makes taxonomic identification difficult, with some researchers (Webb and Dudley, 1995; Madden, 1983; Madden, 1995) referring to the species as *M. hayi* and others (Maglio, 1973; Todd and Roth, 1996; Agenbroad, 2005) designating the migrant as *M. meridionalis*. I follow most North American researchers in using the latter name. A stout, warm-adapted species, *M. meridionalis* is the first representative of the American mammoth lineage, giving rise to the Columbian mammoth (*Mammuthus*
columbi). Some time following their emergence, certain populations of *M. columbi* became trapped on islands off the coast of California, yielding the pygmy island species *M. exilis*. These three species, then, constitute the first major radiation of *Mammuthus* to the New World.

Woolly mammoths (*Mammuthus primigenius*) evolved first in Siberia (from a population of either *M. meridionalis* or the more recent *M. trogontherii*) and migrated to North America via the Bering Land Bridge at a more recent exposure of the transit ca. 200,000 years BP (Agenbroad, 2005) (Figure 2). Beringia remained viable throughout the Pleistocene, such that there was intermittent interaction between the Eurasian and American populations of the species. Debruyne and others (2008) demonstrated that Asian and western Beringian populations were replaced by New World populations during the Terminal Pleistocene. This study, which used ancient-DNA evidence, suggests a robust and well-established North American woolly mammoth lineage.

The two Late Pleistocene mammoths were adapted for distinctive and disparate ecological roles. Due to discoveries of frozen *M. primigenius* carcasses found in the tundra of Siberia and Alaska (Guthrie, 1990), we know a great deal more about the soft tissue anatomy of the woolly mammoth than that of the Columbian mammoth. *Mammuthus primigenius* was a stout organism, shorter than the modern African elephant and reaching shoulder heights of up to 3.2 meters (Anders and von Koenigswald, 2013). An undercoat of thick, insulating fur underlay a pelage of coarse guard hairs, and with a subcutaneous layer of fat up to 9 cm thick, the woolly mammoth was well-adapted to the colder steppe environment just south of the ice sheets (Kubiak, 1982). Stomach and intestinal contents recovered from preserved *M. primigenius* specimens indicate a diet predominantly comprised of graminoids (van Geel et al., 2008), but supplemented with browse (Bocherens et al., 1996; Rivals et al., 2010) or at times dominated by forbs (Willerslev et al., 2014). *Mammuthus columbi* appears to have been adapted to warmer climates, with remains of this taxon found well south of the terminal Wisconsinan moraine in areas surrounding the Great Lakes, the American Southwest, and throughout Florida. The Columbian mammoth stood up to 4.0 meters tall, making it significantly larger than the Woolly mammoth. Coprolite remains recovered from
caves in the Colorado Plateau and assigned to *M. columbi* suggest a diet comprised of approximately 95% grasses, with lesser amounts of sedge, birch, spruce, rose, sagebrush, and cactus (Mead et al., 1986; Mead and Agenbroad, 1992). These and other deposits from similar caves are signs of a mixed environment, perhaps from a large, dry area interspersed with rivers where wetland plants could grow. In general, *M. primigenius* preferred the arctic steppe, tundra, and forest/woodland ecotone, while *M. columbi* likely preferred a “steppe/savanna/parkland” habitat ((Graham, 2001): 707).

The primary method of identifying species of mammoth is based upon two different characters of the morphology of the third molar (M3). The first character, lamellar frequency, is the number of plates within a ten centimeter interval on the occlusal surface of the tooth. The second character is the thickness of the enamel ridges of a given plate. *Mammuthus columbi* typically exhibit a lamellar frequency (LF) of 5 to 9 plates/dm and an enamel thickness (ET) of 1.5 to 3.0 mm, while *Mammuthus primigenius* are characterized by an LF of 7 to 12 plates/dm and an ET of 1.0 to 2.0 mm (Maglio, 1973) (Figure 2). The range in overlap of these characters prompted Maglio (1973) to state that "the two taxa are often impossible to recognize on dental characters alone." Complicating the matter of assigning a species to mammoth molar material are the effects of dental wear. The enamel plates that comprise each molar tend to space out towards the base of the crown, and the enamel shell surrounding the dentine fill tends to thicken (Graham, 1986). This phenomenon increases the measured value of the ET and decreases the LF as the tooth matures. Thus, although *M. columbi* molars tend to have thicker enamel and more widespread enamel lophs than their woolly relatives, an older *M. primigenius* specimen can exhibit worn molars that appear more similar to young Columbian mammoth teeth (Smith and Graham, 2012).

Therein lies the problem: employing static species parameters to a dynamic system renders the method of differentiating species unreliable. One might forego the sole use of molars to define species altogether; however, the fact remains that molars are the most commonly recovered skeletal fragments of these organisms, and without a method of defining species using solely these remains, many collections would be left with only a reliable genus designation placed upon the specimens. It is therefore in our best
interest to define a new method of phylogenetic relations that can account for the dynamic nature of the teeth. In a previous study (Smith and Graham, 2012), we incorporated wear stages of *M. columbi* and *M. primigenius* molars in a taxonomic reanalysis of a mammoth from the Newton Site of Bradford County, Pennsylvania. The wear stages used in that study came from a review of African elephant (*Loxodonta africana*) lower jaws and are based on the relative arrangement of cheek teeth and the amount of wear experienced by the teeth (Laws, 1966). However, the relative age stages of Laws (1966) are not linear and several years of age can be incorporated with each relative wear stage. To this end, it would be preferable to use a method of wear that would allow for a more precise incremental method of analysis. As put so well by Todd and Roth (1996, p. 201), “A re-evaluation of elephant taxonomy that takes account of the heightened phenotypic plasticity in this group will yield a sounder basis for evolutionary inferences.”

With the ever-expanding field of CT paleontology yielding promising results (see review by Cunningham et al. (2014)), I decided to employ this method towards resolving some of the confusion surrounding *Mammuthus* phylogenetics. By rendering a 3-dimensional virtual model of molars of the two Late Pleistocene mammoths, a dynamic model of wear similar to the real-world wear pattern the tooth would have been subject to could be created. Measuring the percentage of enamel comprising the occlusal surface of the tooth at various time intervals should determine whether a difference in dental characters exists between *M. columbi* and *M. primigenius*, especially in light of dental wear. If a disparity in characters exists, then a separation of taxa will be verified, and a standard reference can be defined to differentiate species.

Although CT scanning is a relatively quick and simple process, it can be financially demanding and the process of data quantification requires a certain level of technical expertise and time, not to mention a license for 3D visualization software, which can also be quite expensive. The average researcher may prefer a process of 2D data quantification, or a reference with real fossil material. For these reasons, this project quantifies enamel using FIJI, a free image software available online. FIJI can be downloaded by any researcher with an internet connection, requires little technical expertise or
computing power, and can be used for data quantification of individual 2D tomographic slices or photographs of fossil material. Famoso and Davis (2014), for example, utilized ImageJ (a bare-bones version of FIJI) and digital photographs to quantify enamel complexity of North American Equid molars. The method utilized in this study should also be easily extendable for use with photographs of mammoth molars, without need for CT imaging. Thus, although the primary aim of this project is to measure dental characters in three dimensions, it should also prove useful for phylogenetic researchers without access to such amenities.

This study was completed in part to fulfill the requirements of a Master’s thesis at Penn State University. Thus, the scope of this project is largely exploratory. Should this work prove useful to subsequent researchers, a larger and more complete sample size may be desirable to obtain a more robust and statistically significant result.

### Background

To help frame the aims of this study, some accompanying information on the morphology, dentition, origins, and ecology of the Elephantidae is warranted. Note that I will refer to members of the Elephantidae family collectively as “elephants” in this paper, a convention set by Maglio (1973). This background will begin with an overview of the elephant skeleton. I will then cover elephantid dentition in depth, as understanding how elephants process their food is of paramount importance in a discussion of their ecology and taxonomy. This will segue into a discussion of elephantid evolutionary biology, covering herbivorous ecology and how the expansion of grasslands in the late Miocene set the stage for the emergence of the elephants. The background will culminate with a detailed discussion of mammoth phylogenetics and the problems inherent in our current system of defining the North American species, highlighting our need of a method that can better account for the dynamic system of mammoth tooth morphology.
Elephantidae Morphology

Elephants are large-bodied vertebrates characterized by tusks and large muscular trunks. As subungulates, a group of organisms morphologically similar to ungulates but with apparently more primitive origins, they walk on the tips of their toes, which are supported by a fatty pad and protected by hooves. The skeleton is relatively inflexible and is characterized by vertically oriented legs and a rigid, nearly horizontal spine offering support for a heavy body (Haynes, 1991). The body mass is carried on legs that are like columns or pillars; when the limb is extended, the upper and lower bones of the leg align vertically with each other. The length of the humerus and ulna combined (foreleg) is less than the length of the femur and tibia (rear-leg). However, the addition of the scapula causes the forelimbs to be functionally longer than the rear limbs. The height of the forelimb varies among the Elephantidae, growing longer in some taxa. This phenomenon may have been an adaptation to feeding, locomotor efficiency, or it may have coevolved with tusk size, in order to keep the tusks from dragging on the ground (Haynes, 1991). This latter claim is backed by the observation that species such as *M. columbi*, *M. primigenius*, and *M. trogontherii* all had both larger-than-normal forequarters and enormous tusks.

The body profiles of mammoths and modern elephants differ markedly in limb proportions and the lengths of vertebral spines (Haynes, 1991). In *Mammuthus* the longest spinal processes are located at about the position of the front shoulders and are slanted backwards. This creates a sloping back shape, which differs significantly from the other Elephantidae genera. For example, the posterior end in *Loxodonta* is raised higher than the central axis, giving it a “saddle-shaped” back. The body profile of *Elephas* is nearly opposite this, with a “humped” back shape due to raised lumbar spinal processes. The neck is short in all taxa, but the added length of the trunk extends the animal’s reach by 1-2 meters and makes up for the difficulty of getting the mouth down to the ground for feeding or drinking. Modern elephants cannot turn their head sideways very far, as was very likely the case with *Mammuthus* (Haynes, 1991).
The long axis of the mammoth skull is oriented more vertically than the other proboscidean taxa, a result of the evolutionary changes associated with the jaws and teeth (Lister et al., 2005). Compared to other mammalian taxa, the elephant’s orbits are placed anteriorly, the nasals and frontals are placed posteriorly, the tusks and maxillaries are enlarged, and the parietals are greatly elevated. Additional specializations are detailed by Maglio (1973). The head of *Mammuthus* has a high single-domed crown, which in profile is much higher above the neck than is the top of the skull of *Loxodonta*, but similar to the shape of the head and neck in *Elephas* (Haynes, 1991). The trunk, tusks, and skull evolved together. As the front incisors enlarged into two pairs of tusks or a massive single pair, the skull became shorter and higher in counterbalance. The animal’s increasing size and short neck took the mouth high above the ground, so a trunk was needed for feeding (Lister et al., 2005).

**Elephantidae Dentition**

Mammalian dental development, or odontogenesis, is a process that begins in the alveolus, or tooth socket, of the mandible (lower jaw), premaxilla or maxilla (upper jaw). Teeth are composite structures, and are in most mammals comprised of an inner layer of dentine surrounded by enamel on the crown and cementum in the root. The enamel is formed from the maturation and division of ameloblasts, cells which derive from oral epithelium tissue from the embryonic ectoderm. Dentine, meanwhile, is deposited from odontoblasts, cells of neural crest origin laying below the ameloblasts. Cementum comes from the follicular cells surrounding the base of the root. Odontogenesis occurs in two stages and results in two sets of teeth: an initial, deciduous (or primary) set and a second, permanent set. Deciduous teeth may be present in a neonate or may develop shortly after birth, and dislodge from the jaw upon displacement from the permanent teeth, which commonly erupt during adolescence and remain in the jaw until death. In humans, odontogenesis is a fairly rapid process, with permanent tooth crown formation completing between 12-16 years and root formation following between 18-25 years (Ash and Wheeler,
In most eutherian mammals, teeth form below their eventual positioning in the jaw and erupt vertically through the gums following mineralization.

Eutherian mammals grow four types of teeth, each adapted for its own role in chewing. The incisors, commonly found at the anterior end of the mouth rooted in the premaxilla, are narrow-edged teeth used for nipping or cutting. Incisors are commonly larger in herbivorous mammals and reduced in carnivorous ones. Posterior to the incisors, rooted in the anterior-most portion of the maxilla, are the canines - a set of relatively long, conical teeth that developed for the primary use of holding and tearing apart food. Some canines may appear more flattened and resemble incisors (“incisiform”), while some may be elongated and widened to be used as weapons (as in the Quaternary saber-toothed Felidae genera *Homotherium* and *Smilodon*). The remaining two types of teeth may be collectively referred to as ‘cheek teeth,’ and are the premolars and the molars. The most posterior and complicated teeth are the molars, which are the primary means of processing food in the mouth. Molars have attained a wide range of morphologies in mammals, each tied directly to the manner in which the organism processes its food. Between the canines and the molars are the premolars, which display properties of both teeth and so may be considered transitional teeth. Premolars allow food to be further processed before being transferred to the molars, instead of directly from the canines to the molars. Although any or all of these teeth may be found in a mammal’s jaw, most species have modified, added, or lost elements in order to more efficiently process their food.

Elephantidae dentition is highly unique and is one of the defining characteristics of the clade. All derived members of the family, including the genera *Mammuthus, Loxodonta,* and *Elephas,* exhibit a pair of upper incisors, which are modified into elongate tusks, and six functional cheek teeth per jaw quadrant. The cheek teeth are homologous to the last three deciduous premolars (dp2, dp3, dp4) and the three permanent molars (M1, M2, M3), and are morphologically similar to one another (Todd and Roth, 1996). Some authors do not accept the homology of the elephant teeth with deciduous premolars so they merely number the teeth sequentially (M1-M6) (see Haynes, 1991) for discussion; however, the premolar
terminology will be used here. Each molar is comprised of a series of lamellae, or plates, stacked in series, one plate after another like the pages of a book. Each lamellae is a shell of tough enamel surrounding a core of softer dentine. Adjacent plates are not in contact with one another; rather, the space between is filled with a matrix of cementum (Figure 3). A worn molar exposes the enamel as ridges rising higher than the surrounding dentine and cementum. Plates appear on the rather flat occlusal (chewing) surface of each molar as a series of enamel lophs, with the long axis of each loph oriented perpendicular to the long axis of the molar (Figure 4). The number of plates making up any given tooth depends on (1) the position in the eruption sequence, with later molars being longer, broader, and composed of more lamellae, and (2) the species, with more geologically recent species generally exhibiting more lophs per molar (Maglio, 1972; Todd and Roth, 1996). This unique arrangement of dental characters has earned elephants their own category of tooth morphology, aptly named loxodonty.

The cheek teeth of elephants are the largest of any vertebrate known; as such, members of the family routinely have only one or two cheek teeth per quadrant functioning at any given time (Ungar, 2010). A heterochronic delay in posterior tooth eruption has resulted from this enlargement, as has an unusual tooth replacement pattern that is characteristic of elephants. The first two premolars (dp2 and dp3) erupt vertically and are present in the jaws of a neonate; following this, the remaining molars mineralize sequentially in the posterior portion of the jaws, moving forward and erupting simultaneously (Todd and Roth, 1996). Tooth formation is a prolonged process, and the anterior portion of the molar is generally in occlusion before mineralization of the posterior part of the tooth is complete (Metcalfe et al., 2010). The tooth eruption sequence for teeth dp4, M1, M2 and M3 is thus horizontal, and has been likened to “slow movement on a conveyor belt” ((Shoshani et al., 2006): 17299). The erupting molars move towards the anterior region of the jaw, where their functional use ends and they are expectorated by dislodging from the jaw when the gum recedes from the tooth. Most elephants die before the M3, the final tooth, is worn down completely. Some captive Asian elephants have been known to survive to about 80 years of age, dying from starvation related to the M3 dislodging (Sikes, 1971).
Because of their unique eruption sequence, the relative arrangement of check teeth in an elephantid, in addition to the amount of wear the tooth has been subject to, can be used to ascertain the age of the animal at death. Richard M. Laws, in a review of 385 lower jaws of the African elephant (*Loxodonta africana*), defined 36 age groupings of elephants utilizing these criteria (Laws, 1966). This method has been extended to prescribe relative ages to other, closely related taxa of the Elephantidae family (e.g., Harington, 1980; Kirillova et al., 2012) and American mastodons (*Mammut americanum*) (Saunders, 1977; Haynes, 1991), although it has been argued that this can lead to under- or overestimates of the age of the specimen in question because of inconsistencies between tusk growth increments and cheek teeth wear stages (Rountrey et al., 2012) as well as differences in the rate of dental plate formation in these sister taxa (Dirks et al., 2012; Metcalfe and Longstaffe, 2012).

The elephant chewing method is unique among the vertebrates and must therefore must be considered independently from other food processing mechanisms (Maglio, 1972). The arrangement of lophs on the occlusal surfaces of the upper and lower molars cause the enamel ridges of each tooth to meet obliquely during occlusion. This effectively forms a shear angle between the ridges to clip food in a manner similar to the blades of a pair of scissors. The maintenance of enamel ridges and the spaces separating adjacent lophs is critical in sustaining this apparatus and optimizing masticatory efficiency (Maglio, 1972). In the earliest elephants and gomphotheres, the molar plates were V-shaped in sagittal section, compressing and pinching out towards the base of the crown. Such a configuration would serve to reduce the effective shearing function as the tooth wore because the spacing between adjacent lophs would gradually decrease. Therefore, in order to maintain shearing efficiency, the valley bases separating enamel lophs broadens in the most derived elephants while the enamel plates thickens towards the base of the crown.

There are both upper and lower limits to the thickness of an enamel plate. If the thickness surpasses the upper limit, the pressure (force per unit area) applied during mastication will not be sufficient to rupture the elastic fibers of the food, and the energy expended by the elephant will be wasted.
On the other hand, the lower limit is defined by the mechanical limits of the strength of enamel. Enamel in mammoth molars is comprised of three layers of varying thickness in cross-section, with each layer characterized by a unique stacking pattern of enamel prisms (Ferretti, 2003). Later *Mammuthus* taxa were able to maintain the structural integrity of their enamel ridges despite an overall thinning of the enamel because of the relative packing of these three layers. For example, Ferretti (2003) was able to show that the relative thickness of the middle and most wear-resistant layer increased in *M. primigenius* relative to the earlier Eurasian *M. rumanus* even as the overall enamel thickness decreased between these two taxa. The mammoth lineage thus acquired a means to fit more lamella in a single molar without compromising the shearing effectiveness of their masticatory apparatus – packing plates more tightly together while maintaining the distance between adjacent plates via thinning the less shear-resistant enamel layers.

Upper and lower elephantid molars do not erupt unidirectionally. As the molar begins to erupt, it rotates anteriorly, exposing a greater number of enamel lophs per occlusal distance (Maglio, 1973). The angle between the occlusal plane and the plane normal to the long axis of the enamel plates therefore decreases over the life of a molar. More derived species of elephantid have, in general, a larger initial eruption angle than basal species; however, intraspecific variation in molar eruption ensures that this angle is not easily predictable nor constant. In this study, for example, angle of eruption for samples varied between 43.89° and 70.62° (Table 2) and did not directly correlate with degree of wear.

**Elephantidae Evolutionary Biology**

Although some basal relatives have been found dating as far back as the Late Paleocene (Gheerbrant, 2009), the Elephantidae family is first formally recognized in the late Miocene (6.2-5.6 Ma) of Africa, its emergence thought to be tied to a shift in the masticatory apparatus from the more primitive gomphotheres (Todd and Roth, 1996; Kalb et al., 1996; Maglio, 1973). Maglio (1972) completely and exhaustively covers this evolutionary transition, which will be briefly summarized below, especially in
the context of niche divergence of the Elephantidae clade in the face of interspecific competition. An understanding of the two strategies of herbivorous food processing, grinding and shearing, is necessary here. Grinding refers to the process of crushing material between two broad, occluding surfaces; shearing refers to the cutting or clipping of material across a sharp edge, usually between an upper and lower tooth. Browsers, those organisms whose diet consists predominantly of foliage, young shoots and twigs, employ grinding to obtain a bolus. Grazers, on the other hand, rely on shearing to process a diet of almost exclusively grasses (Ungar, 2010).

The dynamic masticatory process of the gomphotheres may have preadapted their evolutionary descendants for the emergence of grasses. Newly-erupted gomphothere molars consisted of three to four pairs of rounded cusps in series, with each molar displaying a lingual and a labial row of cones. The rounded cones allowed browse to be ground within the enamel valleys and dentine basins of the molar. When partially worn, the sharp edges of enamel surrounding these dentine basins served as excellent shear surfaces; continued wear led to adjoined dentine basins and worn enamel ridges, suggesting that horizontal shearing became more predominant later in life. Chewing within an individual gomphothere thus shifted from grinding early in life, to a combination of grinding and shearing in adults, to predominately shearing in mature adults. Therefore, although these organisms appear to have been opportunistic browsers, the necessary mechanics for grazing were already in place before the emergence of the Elephantidae (Maglio, 1972).

The appearance, adaptive radiation and geographic expansion of the Elephantidae closely mirrors that of the rapidly-expanding C₄ grasses of the late Miocene between 7 and 5 Ma (Ségalen et al., 2007; Cerling et al., 1993). C₃ grasses, those in which the first product of carbon fixation involves a 3-carbon molecule (phosphoglyceric acid), are adapted to cool season establishment and growth in a wide range of moistures. C₄ grasses, on the other hand, initially produce a 4-carbon molecule (either malate or aspartic acid) before entering the Calvin Cycle, allowing them to more efficiently fix CO₂ in warm to hot climates under drier conditions (Cerling et al., 1993). Grasses may be either C₃ or C₄; however, C₄ grasses
appeared in the Oligocene and began to rapidly expand in the Late Miocene, suggesting a time of lower atmospheric CO$_2$ levels favoring their method of carbon fixation (Cerling et al., 1993).

Since they are unable to move away from potential herbivores, grasses (family Poaceae) have evolved a variety of mechanisms to reduce the impact of herbivory on reproduction and survival. A quick generation time allows grasses to develop and complete reproduction before being consumed by potential herbivores. When reproduction is compete, most grasses are able to disperse their seeds via wind currents or passing animals. This ensures that they are able to disperse quickly and in a wide geographic radius. The intercalary meristem is a tissue situated at the base of grass leaves where cells capable of division and elongation are located. By retaining the intercalary meristem close to the ground, grasses are able to continually grow and rebound quickly even after being consumed by an herbivore. Narrow leaves allow grasses to devote more surface area per geographic area to capturing sunlight for photosynthesis; as a result, a loss of a portion of the leaves to herbivory does not halt the plant’s ability to acquire energy. These adaptations allowed grasses to survive and eventually dominate plant biomass even in the face of extreme herbivory by the ungulates.

Because of their role as specialized grazers, it seems likely that the elephant’s expansion is due to the rapid expansion of open grazing habitat and the relative ease with which a population of gomphotheres could have shifted to a diet consisting almost entirely of grass. Supporting this claim, studies of the paleodiet of 19-17.5 Ma gomphotheres from Namibia yielded a $\delta^{13}$C below -8‰, indicating a diet based on C$_3$ plants (Ségalen et al., 2007); by contrast, later populations of both gomphotheres and elephants from Kenya yield a $\delta^{13}$C averaging -2‰, suggesting a C$_4$-dominated diet in both groups by 5 Ma (Cerling et al., 2005). Accompanying this shift in diet would have necessarily been a reliance on the mostly horizontal shearing aspect of mastication, a far more efficient manner of clipping and processing grasses (Maglio, 1973). Although both gomphotheres and elephants appear to have switched to a predominately C$_4$ plant diet, only the teeth of elephants underwent adaptations to more efficiently process
that abrasive food source. Gomphothere teeth were largely unchanged until their extinction in Africa about 4 Ma (Cerling et al., 2005).

Proliferating throughout Africa in the late Miocene through early Pliocene, the Elephantidae diversified into at least six genera: the basal Primelephas and Stegotetrabelodon, the end-Pleistocene extinction victims Mammuthus and Palaeoloxodon, and the extant Loxodonta and Elephas (Todd, 2010) (Figure 5). Following their emergence, some of these genera sequentially emigrated from Africa – Elephas (3.7 Ma), Mammuthus (3.5 Ma), and Palaeoloxodon (2.5 Ma) (Todd, 2010). Primelephas and Stegotetrabelodon never emigrated and went extinct by 4 Ma, while Loxodonta remains an exclusively African fauna today.

The Mammuthus lineage underwent more derivation of their dentition than either Loxodonta or Elephas, with most of this evolutionary change beginning about 1 Ma (Maglio, 1972; Todd and Roth, 1996). The clade originated in Africa and subsequently dispersed throughout Eurasia (in a single radiation) and North America (in two, chronologically disparate radiations) (Figure 6). The African M. subplanifrons gave rise to M. africanavus, an apparent evolutionary dead-end, and the Eurasian M. rumanus in the Pliocene. Throughout Eurasia, M. meridionalis and M. trogontherii remains are found in Pliocene to early Pleistocene rocks. By 1.8 Ma, M. columbi remains are found in northwestern North America, indicating the first occurrence of the Elephantidae in North America. Other Proboscidea, including the Mammutidae and Gomphotheriidae, were already established in North America prior to the arrival of the Elephantidae.

Mammoths quickly outcompeted the gomphotheres in North America. Recent isotopic work on the diet of Pleistocene gomphotheres from South America shows that some populations were feeding generalists, capable of consuming and processing wood elements, leaves and grasses, while others may have relied on a diet of both C\textsubscript{3} and C\textsubscript{4} grasses, solely C\textsubscript{3} grasses, or solely C\textsubscript{4} grasses (Asevedo et al., 2012; Sanchez et al., 2004). Supporting this conclusion is the success of colonist gomphotheres when closed forests dominated North America (~18 Ma), the high diversity of endemic gomphotheres when the
forests were replaced by woodland savannas (~13 Ma), and the subsequent decline in gomphothere taxa beginning with the rise of open grasslands in the early Hemphillian (~10 Ma) (Lambert, 1996). Because they offer a wide diversity of vegetation and resources, woodland savannas can support a greater diversity of herbivores than either closed forests or open savannas. As a result, this ecological pattern of succession had the effect of enlarging and subsequently shrinking the available niche space. The appearance of grasslands in North America and the opening of the Beringian land bridge during an interglacial phase with a low in eustatic sea level set the stage for the success of *Mammuthus*.

The Irvingtonian pioneer *M. meridionalis*, possessing a dentition already highly adapted for grazing, easily outcompeted the remaining gomphothere species (Lambert, 1996). A temperate climatic regime, high forage availability, and abundant water supply favored the extension and diversification of the American mammoth lineage, whose distribution in the peak Wisconsinan reflected an adaptation to the grasslands of the central and western portions of North America (Agenbroad, 2005; Dudley, 1996). *M. primigenius*, the proboscidean that achieved the most highly efficient grazing dentition, expanded into North America ca. 200,000 years BP. Both the earlier and later mammoth lineages co-existed on the North American mainland although they occupied different geographic areas and temperature ranges.

The mastodons were largely unaffected by the arrival of the mammoths. *Mammut americanum* intestinal remains from Ohio imply a mixed browsing and grazing diet (Lepper et al., 1991), and δ¹³C values from tooth enamel have corroborated these results (Baumann and Crowley, 2015). Mastodons appear to have had a seasonal preference for browse as indicated by δ¹³C values on long bone remains (Fisher, 1996). Additionally, the rounded cusps of mastodon molars serve as excellent grinding surfaces for processing the physically less-defended structure of shoots and twigs (as compared to grasses). Thus, mastodons appear to have been predominately browsers and did not compete for the same food source as mammoths. It is likely that the gomphotheres were outcompeted on two sides, being capable of eating both browse and grass but being less efficient than the mastodons or mammoths at processing either. This
has not been conclusively tested, but fossil evidence of the extirpation of North American gomphotheres during or just after the arrival of mastodons and mammoths makes for a compelling hypothesis.

Beginning in the late Pleistocene and continuing into the Holocene, North America saw the loss of a wide diversity of mammals, including mammoths and mastodons as well as saber-toothed cats, glyptodons, ground sloths, Irish elk, cave bears, short-faced bears, horses, and camels (Guthrie, 1982). Some estimates have placed the total loss of mammalian diversity at 15% - 42% of all species before industrialized society began (Carrasco et al., 2009). The underlying cause behind these extinctions is still very much an active area of scientific debate. Because much of this loss began around the time humans arrived in North America, some researchers have suggested that anthropogenic impact is the primary driver of these extinctions (Alroy, 2001; Barnosky et al., 2004). Exploitation by prehistoric humans is in particular noted to be coincident with the loss of the pygmy *M. exilis* on California’s Channel Islands (Agenbroad, 2005). Additionally, the end of the Wisconsinan Glaciation led to rapid climate change and shifts in species composition of plant communities (Guthrie, 1982; Willerslev et al., 2014). Whether directly or indirectly, these continental-scale climatic shifts have also been implicated as a catalyst for extinction (Guthrie, 2003; Shapiro et al., 2004). Some populations of *M. primigenius* are known to have survived on Bering Sea island refugia into the Holocene (Vartanyan et al., 1993; Guthrie, 2004); however, there are no extant members of *Mammuthus*. No proboscideans inhabit North America today, and *Loxodonta* and *Elephas* are the sole surviving genera of the Elephantidae family.

**Challenges in Mammoth Phylogenetics**

Earlier *Mammuthus* species are characterized by less derived teeth that are morphologically more similar to the teeth of *Elephas* at that time; thus, for these early species the dorsally elongate skull and strongly curved and twisted tusks are the more useful taxonomic indicators (Maglio, 1973). As the genus progressed and molars became more derived, *Mammuthus* teeth became much more distinctive, making it
possible to use the dentition to distinguish the two genera. *Elephas* molars tend to have much thicker enamel and the lophs are tightly folded, and the degree of plate packing is not nearly as great as in *Mammuthus*. Generally, in the Pliocene only one species of *Mammuthus* is recognized per continent (Figure 6); thus, distinguishing between species is not a difficult task if one has appropriate time constraints on fossil material. However, chronologically disparate radiations in the Pleistocene lead to multiple species co-existing on Eurasia and North America. Separating between these species, then, becomes the more prudent objective for the paleobiologist.

There are no clear differences in postcranial morphology to help distinguish the late Pleistocene species of mammoths (Haynes, 1991); thus, the two most diagnostic characters for separating *Mammuthus* species as outlined by Maglio (1973) are the lamellar frequency (LF) and enamel thickness (ET) of the occlusal surface of the molars. The LF is, by definition, the number of lophs appearing over a ten centimeter interval on the occlusal plane, and is expected to range between 5 and 9 plates/dm for *M. columbi* and between 7 and 12 plates/dm for *M. primigenius* (Maglio, 1973). However, as is clear to any researcher observing a mammoth molar, the spacing of enamel plates is not constant anywhere in the tooth. On the occlusal surface, the lophs squeeze together more tightly where the tooth is concave (buccal side) and span a greater distance apart where the tooth is convex (lingual side). Newly erupted lophs are smaller and more tightly packed posteriorly, while they are larger and more spaced out anteriorly. Additionally, the LF value at the base of the crown is often lower than the LF at the occlusal surface. An established method of obtaining an accurate average measure of LF is to measure the LF at four locations: the base and apex on both the buccal and lingual sides of the molar (Maglio, 1973). This has long been considered the best means of estimating the central value of the LF, although a highly worn tooth will return distorted LF values. Because of the many problems associated with LF and difficulty in maintaining consistency among systematists, lamellar frequency alone is probably not the most robust method for separating fossil *Mammuthus* specimens.
The enamel thickness (ET) is the width of the enamel sheaths surrounding a plate, and ranges from 1.5-3.0 mm for *M. columbi* and from 1.0-2.0 mm for *M. primigenius* (Maglio, 1973). ET is more quantitatively robust than LF; however, there are a number of caveats when defining ET on a molar. Because the occlusal plane is inclined at an angle with respect to the vertical axis of the plates, the surface on which ET values are measured represents an oblique cut through the enamel casing. The enamel thus appears thicker than it is, so ET must be measured perpendicular to the vertical axis of the ridge, not perpendicular to the occlusal plane itself. The thickness of a singular enamel loph is not constant on the occlusal surface, as it locally pinches out in spots and is generally thicker at the peripheries than in the center. Additionally, newly-erupted posterior lophs generally expose either amalgamated sheaths (increasing the measured ET) or the thinner vertical apices of the sheaths (decreasing ET), while highly-worn anterior lophs may be conjoined or too eroded to return accurate ET values. A more accurate reading of enamel thickness should come from lophs in the middle of the occlusal surface. To ensure accurate results, Aguirre (1969) suggested averaging a series of measurements along the crown as well as reporting the maximum range of variation for each molar. This study follows suit in providing an average enamel thickness of the occlusal plane using a plug-in for FIJI (See: Materials and Methods).

To incorporate more quantitative methods into a historically fairly subjective field, I introduce here the character of occlusal enamel percentage (OEP). OEP accounts for varying sizes of occlusal surface area (OA) amongst molars. It is acquired by determining the occlusal enamel area (OEA) of a molar and dividing that value by the total area of the occlusal surface. The resultant value can be multiplied by 100 to put it in terms of percent per unit area. Using OEP in conjunction with average ET standardizes the heterogeneity of enamel lophs distributed across the occlusal surface, which exist at disparate stages of mineralization and therefore disparate local enamel thicknesses. Together, OEP, LF and ET provide a more quantitative method of measuring interspecific and intraspecific variation in *Mammuthus* individuals.
Project Goals

The primary goal of this study is to determine whether one is able to use internal molar morphology to separate the Late Pleistocene species of mammoth. The null hypothesis that this study seeks to accept or reject is that intraspecific variation obstructs one’s ability to delineate species. If internal inspection of OEP, ET, and LF reveals a different trend with wear for *M. columbi* as opposed to *M. primigenius*, it will be evidence that the amount of variation between species is greater than the amount of variation within species. On the other hand, if a clear disparity in trends with wear is not observed, then it is evidence that *M. columbi* and *M. primigenius* individuals vary in their tooth morphology too much for molars to be considered reliable taxonomic tools on their own.

Determining whether rate of wear varies with degree of wear (i.e., do molars wear faster or slower over time?) is a secondary goal of this project. The rate of wear of a plate is expected to be inversely proportional to the thickness of its enamel sheaths because enamel is the hardest component of dental tissue. The occlusal enamel area (OEA) of a molar is the masticatory functional surface area of that molar in contact with the masticatory functional surface area of an opposing molar. Since the areas of contact in elephants are the enamel ridges, and since occlusal pressure is indirectly proportional to the area of contact between teeth (Rensberger, 1973), it follows that higher pressures result from a lower OEA. Thus, a lower OEA might also indicate a lesser rate of wear, while a higher OEA should indicate a tooth that is more rapidly wearing. If the rate of wear increases over time, one might expect that *Mammuthus* individuals would preferentially select less abrasive food sources later in life.

Materials and Methods

X-ray computed tomography (CT) is a non-destructive method of obtaining images of objects, wherein the reconstructed image stack can be virtually sectioned to view the internal architecture of the
object. CT scanners transmit high energy photon beams, or x-rays, from a source, through the object of interest, to a detector. The x-rays can pass through materials, but their intensity is attenuated along the way. Some materials are more attenuating than others (higher density, higher atomic number, thicker, etc.), so the transmitted x-rays make a pattern that mimics the internal makeup of the object. As a result, digital x-ray images are maps of x-ray attenuation; essentially an array of pixels, each with its own unique number, which is related to the absorption of x-rays as they pass through the material.

CT scanning has been used in paleontology for a variety of purposes, but the reasoning has generally been the same: by constructing a digital model of a real-world fossil, researchers can view the internal construction of fossils without destroying the fossils themselves. The fossils can thus be saved for posterity, yet their internal makeup can be understood and insight gleaned from the models themselves. Although the method is used to inspect a variety of fossil material, the technique is especially vital for use in vertebrate paleontology because specimens are often described on the basis of a single fossil, which is usually delicate and irreplaceable. One of the first uses of CT scans for 3 dimensional internal inspection of fossil material was over 30 years ago, in a Science article showcasing the cranial cavity of the Miocene ungulate *Stenopsochoerus* in 2-mm serial CT data (Conroy and Vannier, 1984). Employing CT in vertebrate paleontology since then has yielded remarkable information, from the coevolution of avian brain size and flight (Balanoff et al., 2013) to the structure and dynamics of dinosaur nasal cavities and airflow (Bourke et al., 2014) to an inspection of micromammal enamel proportions and hypsodonty (van Dam et al., 2011). Angelone et al. (2014) used micro-CT scans to understand the ontogenetic development of the premolars and molars of lagomorphs, demonstrating that this method could be used to address taxonomic and phylogenetic relationships among taxa via 3D models of cheek teeth.

A total of six specimens were scanned: three *Mammuthus primigenius* molars and three *Mammuthus columbi* molars (Table 1, 2). All of these were upper molars in order to remain consistent. Three specimens per species was chosen because it represents a number large enough to allow for variation within taxa, yet small enough to remain financially and functionally feasible for this study. The
collections sampled for this study were selected because each of the molars for the Woolly mammoth and the Columbian mammoth were discovered during the same field season in the same geographic locale (Alaska, 1933 and Colorado, 1961-62, respectively), and both collections represent Late Pleistocene specimens deposited at or just before the final retreat of the Wisconsinan ice sheets. Prior to scanning the molars, standard surficial information was collected for each tooth, including length, width, plate number, LF, and ET (Table 2).

The *M. primigenius* molars are housed at the American Museum of Natural History (AMNH) in New York City (Table 1). They were collected in the summer of 1933 by Charles Frick and the Frick Expedition during their tour around the deposits outside of Fairbanks, Alaska. The fossils were among thousands of specimens recovered from the outwash of perpetually frozen Pleistocene silt cliffs, which were being removed by giant hydraulic rams to expose the gold-rich gravels they overlaid (Galusha, 1975). Although it is difficult to ascribe individual specimens to an exact geographic locale due to the relative paucity of field reports from the Frick Expedition, Péwé (1975) has noted that “…all taxa reported from the perennially frozen silt in the Fairbanks area are represented in silt of Wisconsinan age.” This is due to the observation that most fossil material recovered around Fairbanks comes from retransported valley-bottom silt and organic material of Wisconsinan age, which was deposited after the vertebrate-bearing loess facies of Illinoian age was predominantly removed (Péwé and Reger, 1989; Péwé, 1975). Without official radiocarbon dates, an exact date cannot be reported for the molars used in this study; however, Guthrie (2006) reported dates on *Mammuthus* material from the same area ranging from 14,023 ± 98 years BP to 46,348 ± 2712 years BP, further suggesting a Wisconsinan or post-Wisconsinan date for these specimens.

The *M. columbi* molars are housed at the National Museum of Natural History (NMNH) in Washington, D.C. (Table 1), and were collected between 1961 and 1962 during initial excavations of the Lamb Spring Site near Littleton, Colorado. The Lamb Spring Site is located 3 km east of the Front Range of the Colorado Rockies, in and adjacent to an inactive spring vent between the South Platte River and
Plum Creek. The teeth were found in a layer of “very dark gray brown silty clay with locally abundant swirls and pockets of white sand” containing a host of disarticulated *M. columbi* bone material (Mandryk, 1998). Significant stratigraphic mixing abounds at the site, but the bone bed layer of the Lamb Spring site has been inferred to be reworked channel-fill sediment, with two main depositional events between 26-25 ka and 14-11 ka. Radiometric dates of mammoth bone collagen and plant material from the layer containing the teeth have been reported of 11,735 ± 95, 12,750 ± 150, and 13,140 ± 1000 years BP (Mandryk, 1998; Rancier et al., 1982).

Scanning of the *M. columbi* molars was conducted on location at the NMNH CT Laboratory. The specimens were scanned on a Siemens SOMATOM 6 CT Scanner. In order to remain as consistent as possible during the data acquisition process, the *M. primigenius* molars were also scanned on a medical CT scanner. Because the AMNH has an industrial scanner, but not a medical scanner, the specimens were borrowed and scanned at the Mount Nittany Medical Center in State College, PA on a GE LightSpeed VCT CT Scanner. The scan specifications used for these images are summarized in Table 1.

The scanning process was conducted on medical CT scanners, rather than industrial or microCT scanners, for a number of reasons. First, medical scanners allow for rapid and easy scanning, and are capable of covering an entire sample quite quickly – a matter of seconds in a typical patient. Second, in medical scanners the distance from the photon source to the photon detector is always twice the distance from the photon source to the sample; i.e. medical scanners have 2:1 magnification, a ratio that is fixed by the geometry of the scanner itself. This ratio is quite important for larger and thicker samples, to prevent beam hardening and allow for complete internal detection. Third, whereas industrial scanners have generally higher energy (voltage) ranges, medical scanners are capable of enormous power (current) output. Higher power allows more wavelengths of light to penetrate a sample, allowing for greater contrast at the expense of resolution. Since the goal of this project is to distinguish between characters of different densities (enamel, dentine, and cementum), an image displaying greater contrast, rather than resolution, is more appropriate.
A two-dimensional survey of the images collected from scans was carried out using the free image software FIJI (available at http://fiji.sc/Fiji). FIJI is an enhanced version of ImageJ, an open source Java image processing program superseding NIH Image, and includes many powerful image rendering and data quantification plugins. Raw image files from each scan were imported as an image stack that reflected a top-down view of the molar, oriented normal to the occlusal surface. In these images, enamel displays higher gray values than the rest of the molar because it is a denser, and therefore more attenuating, material than dentine or cementum. The slice thickness for each scan was 0.5 mm; thus, moving through subsequent scans in an image stack is akin to moving at 0.5 mm intervals below the occlusal surface.

The occlusal enamel area (OEA) and the occlusal enamel percentage (OEP) were quantified for each slice to calculate the proportions of enamel per unit area throughout the molar. This is possible in FIJI by converting the raw image files to a median filtered binary image stack, with every pixel above a specified threshold gray value being recomputed to a value of 1, and every pixel below the threshold gray value assigned a value of 0. The “Analyze Particles” tool then adds up the total area of pixels with a value of 1 for each image to acquire the thresholded area of the tooth. If, for a given image stack, this analysis is performed twice, with the first defined threshold being a gray value just below the first gray value of molar material, and the second defined threshold being the gray value between enamel and the rest of the molar, it is possible to calculate the total OA on the first analysis and the OEA on the second analysis. Dividing the latter value by the former value and multiplying by 100 will then return the occlusal enamel percentage (OEP) of the occlusal surface. If this process is repeated for all tomographic slices of the molar, the result is an estimate of how the proportion of enamel varies at 0.5 mm intervals through the crown.

The quantification tools available in FIJI through the BoneJ plug-in were used to determine how the average enamel thickness varies throughout the tooth (Doube et al., 2010). The thickness tool in BoneJ is run on a binary image. A sphere is fit inside the material of interest, the diameter of which is the
true thickness (Hildebrand and Rüegsegger, 1997). The analysis was run on one image at a time, and was computed every ten slices, or at 5 millimeter intervals through the crown. Lamellar frequency was acquired in FIJI by measuring the average number of lophs along a 10 centimeter anteroposterior axis of the tooth beginning at the penultimate loph (after Maglio, 1973). As with enamel thickness, these values were collected at 5 millimeter intervals.

Every subsequent tomographic slice through a molar can be considered a slightly more progressive state of wear experienced by the molar in life. Each image in a stack (along with its respective OEA, OEP, ET and LF) was considered to represent a particular “relative wear percentage” of between 0% (at the first slice) and 100% (at the final slice). This relative wear percentage was then plotted against each quantitative character in a series of graphs that shows how these characters change through the crown. These graphs are described in the Data and Results and appear in the Appendix. To help display any apparent trends in the data, a locally weighted polynomial regression, or Loess curve, was fit to the plots. Each Loess curve was then bounded by 95% confidence intervals.

The image stack for each specimen was imported into the 3D analysis software AVIZO (http://www.fei.com/software/avizo3d/) to create the visualizations used in this study. AVIZO is capable of constructing volume renderings, orthoslices and isosurfaces of 3D data acquired via CT, micro-CT, and Magnetic Resonance Imaging (MRI), and can also be used for more powerful data quantification methods than those available in open-source programs such as FIJI. Some of the values included in Table 2 (e.g., tooth volume and enamel volume) were acquired using AVIZO’s data quantification tools. Visualizations are included in the Appendix.

**Data and Results**

Quantitative data was obtained for OEA, OEP, ET, and LF throughout each of the 6 molar image stacks. OEP showed various wear patterns for each molar, which are explained more in depth in
subsequent paragraphs and are shown on the primary y-axes in Figures 7 and 8. Enamel thickness was split roughly down the middle, with 3 teeth showing little to no change in value and 3 teeth showing an either positive or negative correlation of ET with wear percentage. These patterns are also explained in the succeeding paragraphs and are plotted on the upper secondary Y-axes in Figures 7 and 8. Lamellar frequency showed disparate patterns with wear and is further explained below. The robustness of LF measurements must be viewed critically, as these values by their very nature tend to be more subjective than either OEP or ET. They are included in Figures 7 and 8 on the lower secondary y-axis and are considered in detail below. OEA was considered independently of the other variables to address the question of whether wear rate accelerates or decelerates over time. OEA is plotted on the y-axes of Figures 9 and 10 against relative wear; Figure 9 is the OEA vs wear in Columbian molars, and Figure 10 is the OEA vs wear in Woolly molars. Figure 11 shows occlusal surface photographs of the scanned molars and Figure 12 is a comparison of the relative wear stages in the samples at 20%, 40%, 60% and 80% wear.

The Columbian mammoth molar wear pattern (Figure 7) is more erratic than the Woolly mammoth molar wear pattern (Figure 8), but some trends can be observed amongst the various specimens. One molar (Field ID: S-61-NN340) trends towards a decreasing enamel percentage on the occlusal surface as the tooth wears, beginning at around 45% enamel, and ending around 15% enamel. The other two specimens (Field IDs: S-62-59 and S-109-61) follow similar wear patterns, beginning at a high OEP, wearing to a low in enamel percentage at approximately ¼ wear, peaking at the mid-coronal plane, and dropping again briefly before a final peak in percent enamel when the tooth is close to fully worn. Enamel thicknesses increase drastically (from a low of 3.0 mm to a high of over 4.5 mm) in specimen S-61-NN340 beginning around 70% wear. Outside of this phenomenon, enamel thickness only moderately varies, with little positive or negative trend, for the Columbian mammoth molars. Lamellar frequency was between 8 and 6 lophs/dm for all slices in the Columbian molars, and the maximum variation within a molar was limited to 1 loph/dm. One molar (Field ID: S-61-NN340) had a consistent
LF of 6 throughout the image stack, while the other two specimens began at a higher LF, decreased in value by 1 when approximately 50% worn, and subsequently increased back to the originally measured LF value near the base of the crown.

In general, the pattern in enamel percentage comprising the occlusal surface of Woolly mammoth molars seems to be more directed than the pattern in Columbian mammoth molars. For two *M. primigenius* samples (Catalog #s: F:AM 144552 and F:AM 144545), as tomographic slices are removed and wear approximated, the surface of the molar becomes increasingly dominated by enamel. The third *M. primigenius* molar (Catalog #: F:AM 144529) increases OEP rapidly (to a peak of almost 50% enamel) until it is approximately ¼ worn, then dips to a low of 25% enamel through the mid-coronal plane, subsequently peaks at 50% enamel when approximately ¾ worn, and then rapidly drops to a low in enamel percentage. The two *M. primigenius* molars with increasing OEP also show a positive correlation in ET across the same wear percentages. F:AM 144529 is characterized by a slight decrease in ET with wear, although the values never decrease below 2.0 mm ET. Lamellar frequency for the Woolly molars varied between 9 and 6 lophs/dm with a maximum variation within a single sample of 2 lophs/dm. Two specimens began at higher LF values and subsequently dropped to lower values when approximately 50% worn. Sample F:AM 144529 followed a sinusoidal curve, beginning at 7 lophs/dm, dropping in value, subsequently rising, and returning to a value of 7 lophs/dm near a higher wear stage.

The wear pattern in OEA varies between the Columbian and Woolly mammoth molars. For all samples of *M. columbi* molars, OEA begins at a higher value near the top of the crown and generally decreases to a low with wear, with a lower average value closer to 100% wear. Sample S-109-61 follows a slight sinusoidal curve, but generally adheres to the trend described above. For all samples of *M. primigenius* molars, OEA begins at a relatively low value, increases (at times drastically, i.e. Catalog # F:AM 144529) to a high around the mid-way point of the crown, and decreases again following this spike to a low in OEA when nearly completely worn. These trends are apparent in each sample, and therefore
indicate a prevalent difference between the two species. Because of the similarities in molar sizes of the two collections (Table 2), the OEA should not be due to a smaller or larger molar in either species.

Figure 12 is a collection of screen shots in AVIZO of the occlusal surfaces of the molars at four relative wear stages – 20%, 40%, 60%, and 80%. It was created by orienting each tooth in coronal view and removing tomographic slices proportional to 1/5 of the total image stack for each image. For example, if the image stack for the *M columbi* molar S-62-59 was comprised of 560 images, the removal of 112 of these images would show the tooth at a 20% wear stage. If the image stack for *M. columbi* molar S-61-NN340 was comprised of 500 images, the removal of 200 of these images would be a representation of the tooth at 40% wear. By standardizing this method, I am able to show each molar at the same relative wear stage despite differences in molar size and absolute (Laws, 1966) wear stage. Because of the differences in scanners used and energy settings (Table 1), these images as viewed in AVIZO may represent different gray values for the same material. In other words, although enamel is expected to have the same density and atomic number across each molar, the absolute gray value representing enamel will not be the same from one molar to the next. Thus, enamel may appear yellow in the *M. columbi* molars in Figure 12 but pink in the *M. primigenius* molars.

In general, the Columbian mammoth molars had a higher proportion of dense enamel comprising the occlusal surface throughout the tooth (Figure 12). The enamel plates in all *M. primigenius* molars are less well-developed than the plates in the *M. columbi* molars, as can be seen from the thin pink lophs in the former species and the prominent yellow/orange lophs of the latter species. Over the lives of these *M. primigenius* molars, enamel becomes more prominent and the lophs become more well-preserved. Figure 12 is a visual affirmation of the trend towards increasing ET and OEP in the Woolly mammoth molars (Figure 8) and the nondirectional trend in Columbian mammoth molars (Figure 7).
Discussion

Form and function of biological systems are inherently linked. Therefore, the patterns observed in enamel percentage and thickness between *M. columbi* and *M. primigenius* should be tied to the two species’ respective ecologies. Mammoth molar morphology should also be expected to be representative of the adaptive evolutionary history of both taxa. Because the collections are Late Pleistocene populations, the molars used in this study represent two lineages experiencing at least 200,000 years of directed, disparate ecological pressure. The variation in wear between the Woolly mammoth and Columbian mammoth teeth is indicative of a phenotypic divergence between the two species resulting from a divergence in ecologies. Woolly mammoth molars appear to retain a higher percentage of enamel comprising the occlusal surface of the third molar, suggesting an adaptation for a singular highly abrasive food source. The scatter in enamel percentage amongst Columbian mammoth molars, on the other hand, suggests no strong ecologically-directed pressure. Thus, *M. primigenius* was the more phenotypically stable organism, with a more highly conserved niche than *M. columbi*.

*M. columbi* remains from the Colorado Plateau and Mexico have been frequently found to have a more varied diet than *M. primigenius* from Beringia, with the former employing a composite diet of C3, C4, and CAM plants (Mead et al., 1986; Pérez-Crespo et al., 2012) and the latter adhering more strictly to a graminoid-dominated diet (Rivals et al., 2010; van Geel et al., 2008). To be an effective grazer, it would be most beneficial to retain a high enamel percentage as well as spacing between lophs. Near the base of the Woolly mammoth molar, lophs are packed tightly together with a high quantity of enamel (Figure 6) – an adaptation facilitating the increased number of lophs in this species (20-27 plates/molar; (Maglio, 1973)) and allowing them to space out later in the mammoth’s life, as the plates continue to mineralize and eventually are exposed on the occlusal surface. Because Columbian mammoths had a more varied diet, the percent enamel may track that history, and the “random” scattering of OEP values could be reminiscent of a varied diet not directed towards any preferred food source.
There appears to be no consistent separation of taxa with wear trends, thereby rejecting the null hypothesis of this study’s primary goal. Although there are disparate trends in one *M. columbi* molar and two *M. primigenius* molars, there is simply too much variation and random scatter to reliably suggest that internal molar morphology of either species is significantly different. If a molar were discovered from a location where the two populations of mammoth chronologically overlapped and a researcher were to run these same analyses, the molar would have to show either an increase or decrease in OEP with wear to be referred to *M. primigenius* or *M. columbi*, respectively. If this character were not directed, the molar could theoretically belong to either species, as half of our samples show neither a positive nor a negative correlation of OEP with wear.

On the other hand, the three *M. columbi* molars did often have a higher OEP and OEA than the three *M. primigenius* molars, as can be seen in Figures 7-10. Although there is a significant amount of scatter in the Columbian molars, the OEP is nearly always greater than 20% enamel/occlusal area. By comparison, the OEP of Woolly molars stays generally below 30% enamel/occlusal area. There are exceptions to this trend as well, as a molar from each species (*M. primigenius* - F.AM 144529; *M. columbi* - S-109-61) do not adhere to these generalizations. Still, the prevalence of this trend cannot be ignored. In these samples, the molars of the Columbian mammoth retain thicker, denser enamel plates than the plates of the Woolly mammoth molars. This may prove to be a diagnostic character useful in separating the two species if the same observation is seen in a larger sample size with temporally and spatially disparate individuals; however, where to draw the line between ET, LF, or OEP for *M. columbi* and *M. primigenius* remains ambiguous at best.

To test whether intraspecific variation is the main factor that makes separating *Mammuthus* species difficult, a future study should consider repeating this study’s methods on a collection of *Mammut americanum* molars. Unlike elephant teeth, mastodon teeth follow a vertical tooth eruption sequence much like humans. Mastodon teeth therefore develop more rapidly than elephant teeth and do not follow a heterochronic delay in mineralization. Because they develop more rapidly, the teeth are not mineralizing
while they enter occlusion and begin to wear. If a collection of mastodon teeth do not show intraspecific variation, it may suggest than the difficulty in separating *Mammuthus* species comes from the effects of dental wear rather than variation in dental eruption and mineralization patterns within species. Otherwise, if mastodon teeth also display intraspecific variation, then proboscidean teeth in general might be expected to be poor taxonomic indicators because there is simply too much variation within species to make separating different species possible.

This study supports the observation that LF will decrease with wear in *M. primigenius* molars; however, this trend is not supported in *M. columbi* molars. ET is shown to increase with wear in some, but not all, molars from both species. This quantitatively supports the findings of an earlier study (Smith and Graham, 2012) that dental wear can lead to an overlap in the characters used to separate the two species, and that relative wear stage must be taken into account in order to reliably assess the taxonomic placement of fossil molar material. The lack in consistent ET increase and LF decrease across specimens may be attributable to incomplete mineralization in the posterior portion of the molars. Because these molars are essentially locked in a particular wear stage and mineralization extent at the time of death, artificially wearing them via removal of tomographic slices does not completely mimic wear during life. A tomographic slice taken midway down the crown will include incompletely mineralized posterior lophs, neither spaced out nor encased in enamel to the degree they would be upon occlusal exposure via wear. This is a limitation inherent in the method.

Occlusal enamel area is shown to begin at a relatively high value in all *M. columbi* molars and subsequently decline to a low near the base of the crown (Figure 9). Because of the drop in total enamel comprising the occlusal surface at this point, and because the rate of wear is indirectly proportional to the amount of enamel present, it follows that the M3s for these specimens would have likely begun to wear more quickly as they progressed in age. In a similar fashion, the *M. primigenius* molars included in this study decreased in enamel near the base of the crown; however, they were also shown to begin at a lower OEA and increased the total enamel area of their occlusal surface midway through the crown (Figure 10).
This may be a consequence of *M. primigenius* adapting for a more abrasive diet. The steady decline in enamel near the end of the life of the molar is also suggestive of a more rapid wear rate as the molars neared the end of their functional use.

One possible implication of the decrease in enamel towards the base of the crown is that *M. columbi* individuals may have shifted to a less abrasive diet near the end of their life, possibly composed of increasing quantities of forbs or other less physically defended plants. To test this hypothesis, it would be beneficial to serially sample the δ¹³C composition from the enamel plates in a *Mammuthus* M3 and see whether the isotopic signature changes significantly. Because their molars continue to mineralize throughout life, elephants record a continuous record of diet via the uptake and incorporation of carbon into their dental tissue. When an enamel plate stops developing, the δ¹³C composition of that tissue is locked and can be compared to the δ¹³C composition of the elephant’s food source. The posterior adjacent enamel plate begins and ends mineralizing slightly later, so the δ¹³C signature of subsequent enamel plates will indicate paleodiet at progressive time intervals. If the above hypothesis is correct, one might expect to see a decreasing δ¹³C composition as the individual shifted from a more abrasive C4 grass diet to a less abrasive C3 forb diet. To allow for the possibility that the individual was eating abrasive C3 grasses, it would be best to combine this test with a dental microwear analysis to observe whether microwear patterns change even if the δ¹³C signature does not. Similar analyses have been conducted on proboscidean *Gomphotherium* individuals (Fox and Fisher, 2004; Calandra et al., 2010), although an ontogenetic dietary shift was not supported in either case.

One must use caution when estimating phylogenetic relationships using morphological data alone. For example, molecular, morphological and paleontological data has led to unexpected placements of major fossil Squamate clades (Reeder et al., 2015). Further, it has been demonstrated via mitogenomic sequencing that *M. columbi* nests within a subclade of endemic North American *M. primigenius*, suggesting that the two species were less divergent than previously considered (Enk et al., 2011). It is
therefore best to rely on a suite of interdisciplinary information to resolve taxonomic relationships whenever possible.

Two caveats to these conclusions must be outlined. First, my data are quite limited and represent only three individuals of each species from two temporally and spatially disparate populations. Second, there is likely to be a large amount of variation in dental characters between individuals of the same species, because there are a number of environmental (Metcalf and Longstaffe, 2012; Metcalfe et al., 2010), sexual (Averianov, 1996) and developmental (Fagan et al., 2001) factors that affect tooth growth. Therefore, some of the variation occurring between molars from the two species might be indicative of intraspecific variation rather than interspecific variation. It would be beneficial to examine samples of both species from a larger collection of populations to determine if the same trends are apparent, and to reduce the noise of physiological variation. In a similar and related case, variation in dental characters of the proboscidean Gomphotherium angustidens has been shown to infringe upon taxonomic identifications (Tassy, 2014).

During the data gathering process, the M. columbi molars were scanned on a Siemens SOMATOM 6 CT scanner, while the M. primigenius molars were scanned on a GE LightSpeed VCT CT scanner. Although these machines perform fundamentally the same task in the same manner, variation in scan quality between two machines constructed by two different companies is possible. Tomographic slices were removed in a linear fashion from the top of the crown to the base, which assumes that the phenomenon of dental wear is a linear process. This is likely to not be the case, because 1) the eruption angle in molars is known to decrease over time and 2) elephants are known to apply more occlusal force towards the anterior portion of their molars. Addressing this factor in a manner that can better approximate the heterogeneous wear rate over the occlusal surface will require a more precise method of modeling wear in a variable way. Finally, although every step was taken to ensure reproducibility of this study, there were a number of steps in data processing that were subject to the judgement of the PI, including the choice of filter, the degree to which the filter resampled the data, and the choice of threshold
value to separate enamel and dentin/cementum. These decisions would have yielded only minimal differences in area calculations, however, and the trends would likely not have changed appreciably.

Conclusions

The two Late Pleistocene mammoths, *M. columbi* and *M. primigenius*, continue to prove difficult to differentiate between on the basis of their third molar morphology alone. With internal inspection, some preliminary trends can be observed, although the small sample size of this study doesn’t preclude the possibility that these trends are just part of the natural variation of dental characters in the genus. The direct correlation between enamel thickness and occlusal enamel percentage in *M. primigenius* suggests a phenotypic adaptation towards increased grazing efficiency, while the high degree of scatter in occlusal enamel area and the inconsistency in wear trends in dental characters in *M. columbi* suggests no directed phenotypic development. A steady decline in occlusal enamel area towards the base of the crown in molars from both species suggests that, if their diet remained the same, the rate of dental wear accelerated towards the end of the molars’ functional use. This leaves open the possibility that individuals may have preferentially consumed less abrasive foods near the end of their lives in an attempt to lessen the wear rate. Serial sampling of the $\delta^{13}$C signature in adjacent enamel plates from *Mammuthus* M3s is needed to assess whether an ontogenetic shift in diet occurred in either taxa. Additionally, this study does not find support for using the internal morphology of mammoth molars to separate the two species of Late Pleistocene North American mammoths. Future studies should consider examining a larger sample size of molars from both species and from numerous populations in order to resolve whether the observed trends become more apparent. To determine whether the inability to separate species is due to intraspecific variation or the effects of dental wear, a comparison study should be run on the teeth of the American mastodon (*Mammut americanum*), a species with no heterochronic delay in tooth eruption.
The use of X-ray computed tomography in paleontology has yielded excellent results across the discipline. This study served as a preliminary examination of applying the method towards resolving some of the confusion surrounding mammoth taxonomy. Although our study did not lend support towards separating the two species, it is possible that a larger sample size may do so. Additionally, it may be interesting to examine the internal molar morphology of the two extant elephant genera to see whether they can be separated using this method. It is my hope that the continued use of interdisciplinary methods will help resolve species relationships and shed light on the complicated phylogenetics of both extinct and extant elephants.
### Table 1: Collection information and molar scan specifications for the specimens utilized in this study. Field ID is used to differentiate among the 3 Columbian mammoth specimens, which are all assigned the same Catalog # by the USNM.

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<th>Catalog #</th>
<th>Field ID</th>
<th>Institution</th>
<th>Species</th>
<th>Current (mA)</th>
<th>Voltage (kV)</th>
<th>No. of Images</th>
<th>Slice Width (mm)</th>
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Table 2: Summary of measurements taken on the surface of each specimen prior to scanning, as well as 3D volumes of molar and enamel, which were computed in AVIZO.

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<th>Width (cm)</th>
<th>Plate Number</th>
<th>LF (lophs/dm)</th>
<th>ET range (mm)</th>
<th>ET average (mm)</th>
<th>Laws (1966) Wear Stage</th>
<th>Eruption Angle</th>
<th>Tooth Volume (cm³)</th>
<th>Enamel Volume (cm³)</th>
<th>% of Enamel/Molar</th>
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<td>8</td>
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<td>XXII (22)</td>
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Figure 1: Geographic ranges of *Mammuthus columbi* (red) and *Mammuthus primigenius* (blue) based on fossil occurrences in North America throughout the Wisconsinan Glaciation (0.085 – 0.011 Ma). Map modified and images borrowed from Enk et al., 2011.
Figure 2: Lamellar frequency plotted versus enamel thickness, with the range of characters expected for each species as delineated by Maglio (1973) shown for *M. primigenius* (blue) and *M. columbi* (red). A clear overlap in characters is shown between an LF of 7 and 9 lophs/dm and an ET of 1.5 and 2.0 mm. Overlaid on these ranges are the samples used in this study – *M. primigenius* = blue triangles, *M. columbi* = red circles. A problem in consistency of characters is immediately recognizable, as only half of the molars fall within their expected ranges.
Figure 3: Diagram of a mid-sagittal section of a *Mammothus* lower third molar. Each plate is the V-shaped structure comprised of dentine and encased by enamel. The space between is filled with cementum, which may penetrate into the root. The root begins to form later in the tooth’s life; this diagram represents a tooth at a late wear stage. Scale bar is 10 cm.
Figure 4: Coronal (top) and lateral views of a *Mammuthus* lower third molar. The enamel plates appear on the occlusal surface as raised ridges, with their long axis oriented perpendicular to the long axis of the molar. The bulk of the observable molar is comprised of cementum. Dentine is less apparent on the outside of the tooth, appearing only within the exposed enamel sheaths of the lamellae. Scale bar is 10 cm. Redrawn from Maglio (1972).
Figure 5: Elephantidae phylogram, adapted from Todd, 2010. Horizontal lines are species; vertical lines are speciation events. Solid lines represent extent of fossil occurrences of species; dashed lines represent hypothesized extent of species based on last occurrence datum of assumed ancestor. Lineages are color coded by genus: black: Gomphotherium; yellow: Primelephas; brown: Stegotetrabelodon; red: Loxodonta; blue: Elephas; purple: Palaeoloxodon; green: Mammuthus. The purple triangle in Palaeoloxodon represents the variously named dwarf elephant genera of the Mediterranean Islands.
Figure 6: Evolutionary tree of the *Mammuthus* lineage, with representative species delineated by continent, time of emergence, and hypothesized descendants. *M. primigenius* is found in both Eurasia and North America and hence straddles the boundary. Adapted from Maglio (1973) and Todd (2010).
Figure 7: Columbian mammoth molar characters’ change with wear simulation. Occlusal enamel percentage (OEP; red), enamel thickness (ET; blue) and lamellar frequency (LF; orange) are plotted versus relative wear percentage, where 0% = first tomographic image of the occlusal plane and 100% = final tomographic image of the occlusal plane. OEP has a high degree of scatter for all specimens, and only one specimen (S-61-NN340) retains any sense of directionality, with OEP generally decreasing with increased wear. ET does not generally vary much with wear, although for S-61-NN340 the average ET value increases with wear. LF remains relatively stable throughout each of the molars, varying no more than 1 whole integer value. LF and ET are indirectly related. LF and OEP correlate neither directly nor indirectly. S-61-NNB40 does not have an LF value reported past 85% because of difficulty in discerning complete lophs after that point in the wear simulation.
Figure 8: Woolly mammoth molar characters’ change with wear simulation. Occlusal enamel percentage (OEP; red), enamel thickness (ET; blue) and lamellar frequency (LF; orange) are plotted versus relative wear percentage, where 0% = first tomographic image of the occlusal plane and 100% = final tomographic image of the occlusal plane. For two of the three specimens, OEP and ET increase with increasing wear percentage. Additionally, there is not much scatter for either character. One specimen, F:AM 144529, is characterized by a fairly high degree of OEP scatter and ET slowly decreases with wear. Two of the three specimens show an indirect relationship between ET and LF, as shown by an increase in ET at approximately the same wear stage that LF decreases. One specimen, F:AM 144529, has a sinusoidal trend in LF while ET slowly decreases with wear. OEP and LF are indirectly related to one another with wear, and both retain a degree of directionality.
Figure 9: OEA vs Relative Wear Percentage in Columbian mammoth molars. Except for slight variation in trends, every molar decreased in total OEA through the life of the tooth. In other words, the enamel comprising the expected chewing surface decreases in abundance over time. Different colors correspond to different Columbian mammoth molars utilized in this study.

Figure 10: OEA vs Relative Wear Percentage in Woolly mammoth molars. Although the magnitude of change varies, each molar begins at a relative low in OEA and increases as much as sevenfold, staying at a high abundance for most of the life of the tooth before dropping steadily near the bottom of the crown. Different colors correspond to different Woolly mammoth molars utilized in this study.
Figure 11: Surface views of the 6 *Mammuthus* molars utilized in this study – the *M. columbi* S-109-61 (top left), S-62-59 (center left) and S-61-NN340 (bottom left) and the *M. primigenius* F.AM 144552 (top right), F.AM 144545 (center right) and F.AM 144529 (bottom right). Relative positions of the molars is the same as shown in Figure 12. View is normal to the occlusal surface, anterior direction oriented down.
Figure 12: Comparison of the 20% interval wear stages of the 6 *Mammuthus* molars utilized in this study— the *M. columbi* S-109-61 (top left), S-62-59 (center left) and S-61-NN340 (bottom left) and the *M. primigenius* F.AM 144552 (top right), F.AM 144545 (center right) and F.AM 144529 (bottom right). Relative positions of the molars is the same as shown in Figure 11. The denser, more attenuating enamel is yellow/orange and the softer, less resistant dentine and cementum show up as purple/blue, although a difference in scanner settings may also be responsible for differences in colors between the molars (see Data and Results). Images captured in AVIZO. View is normal to the occlusal surface, anterior direction oriented down. Throughout the various wear stages, the Columbian mammoth molars show denser, thicker, and more attenuating enamel than the Woolly mammoth molars.

A) 20% relative wear. B) 40% relative wear. C) 60% relative wear. D) 80% relative wear.
References


