Dietary adaptations in the teeth of murine rodents (Muridae): a test of biomechanical predictions

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Functional dental theory predicts that tooth shape responds evolutionarily to the mechanical properties of food. Most studies of mammalian teeth have focused on qualitative measures of dental anatomy and have not formally tested how the functional components of teeth adapt in response to diet. Here we generated a series of predictions for tooth morphology based on biomechanical models of food processing. We used murine rodents (Old World rats and mice) to test these predictions for the relationship between diet and morphology and to identify a suite of functional dental characteristics that best predict diets. One hundred and five dental characteristics were extracted from images of the upper and lower tooth rows and incisors for 98 species. After accounting for phylogenetic relationships, we showed that species evolving plant-dominated diets evolved deeper incisors, longer third molars, longer molar crests, blunter posteriorly angled cusps, and more expanded laterally oriented occlusal cusps than species adapting to animal-dominated diets. Measures of incisor depth, crest length, cusp angle and sharpness, occlusal cusp orientation, and the lengths of third molars proved the best predictors of dietary adaptation. Accounting for evolutionary history in a phylogenetic discriminant function analysis notably improved the classification accuracy. Molar morphology is strongly correlated with diet and we suggest that these dental traits can be used to infer diet with good accuracy for both extinct and extant murine species. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 766–784.

KEYWORDS: dental evolution – diet – functional morphology – molecular phylogenetics – Murinae – phylogenetic comparative methods – phylogenetic discriminant function analysis – principal component analysis.

INTRODUCTION

Dietary adaptability is one of the main reasons that mammals are so successful at exploiting different ecological niches. Changes in diet are often reflected through morphology (e.g. Van Valkenburgh, 1988, 1989). Jaw and skull characteristics, musculature, stomach, and intestinal morphologies have all been shown to reflect dietary preferences (Kay & Hylander, 1978; del Valle, Manaes & Busch, 2004; Michaux, Chevret & Renaud, 2007). Dental characteristics have been determined to reflect diet best (Michaux, 1971) because teeth are the primary tools used for mechanical food processing (Lucas, 2004).

Both tooth size and tooth shape may be adapted to the physical properties of food. The probability that a food particle will be fractured depends on its roughness, stickiness, and other external characteristics and material properties (hardness, elastic modulus, etc.). These physical attributes exert direct selection on tooth size. Tooth shape is most notably adapted to the internal characteristics of the food and the resistance of food particles to fragmentation. For example, a larger tooth may be more likely to hit a food particle, whereas tooth configuration, or shape, affects the efficiency with which force is applied to the food, resulting in fragmentation (Lucas, 2004).

The mechanical properties of food were not considered until the seminal works of Rosenberger & Kinzey (1976) and Lucas (1979). Lucas & Luke (1984) later expanded on these works by considering which major tooth configurations function best on different food types. Subsequent work began to consider the relationship of tooth size and shape with both function and diet (Kay, 1975; Kay & Hylander, 1978; Sanson,

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1980; Freeman, 1981; Lucas, 1982; Rensberger, 1986; Frazzetta, 1988; Strait, 1991, 1993a, b, 2001; Freeman & Weins, 1997; Popowics & Fortelius, 1997; Dumont, Strait & Friscia, 2000; Williams & Kay, 2001; Samuels, 2009). Only a small number of studies has developed functional theory predicting tooth morphologies for specific diets, and virtually no studies have formally tested quantifiable functional associations with diet (Evans & Sanson, 1998, 2003, 2005; Yamashita, 1998; Evans, 2005; Evans et al., 2005).

Most studies remain qualitative because of the great complexity of most mammalian teeth, which makes determining homologous structures (structures with the same ancestral origin) difficult. Many different tooth conformations can reflect diet. We sought to provide the foundation for dental trait justification as well as quantify previously qualitative claims. The ability to infer a small suite of functionally relevant tooth traits will allow quick estimates of diet by systematists, paleontologists, and wildlife biologists. In contrast, direct diet determination is often difficult and time consuming, requiring lengthy observational studies or the sacrifice of numerous individuals for stomach-content analyses. This diet information could subsequently be used for paleoclimate reconstruction and examination of the response of rodents to climate changes. Furthermore, insight into which traits are under selection (that is, traits that adapt in response to diet) will help provide a more comprehensive picture of dental evolution.

Our study has three objectives:

1. Translate our understanding of the mechanics of food processing to detailed predictions about the shape and relationships of discrete features of the rodent dentition.
2. Determine whether predicted dental traits are actually correlated with diet type. In general, a consideration of the material properties of food and mammalian dentition leads to the predictions that species with plant-dominated diets have broad incisors; large, robust, highly hypsodont molars; sharp cusps in leaf and grass eaters; blunt cusps in granivores and frugivores; flattened, bladed molars; greater lophodonty (elongated cusps that form narrow ridges) or stephanodonty (developed longitudinal crests that connect transverse cusps), and large jaws (predictions are described in detail below). Species that consume animal-dominated diets are predicted to have thin, narrow, sharp incisors; small, reduced, low-crowned molars; sharp molar cusps (sharper than those of herbivores); bladed molars with well developed shearing crests; and less robust jaws. Omnivorous taxa are expected to have intermediate tooth morphologies weighted by whether plant or animal material dominates the diet.

3. Survey many aspects of tooth morphology to determine a posteriori which functional traits are the best predictors of diet and, moreover, discover potential functionally relevant traits that current biomechanical models may have overlooked.

Murine rodents (Old World rats and mice, Muridae) were chosen for several reasons. Murinae is the largest mammalian subfamily, containing more than 600 species (Musser & Carleton, 2005). Its members are found throughout Africa, Eurasia, and Australasia and in almost every terrestrial habitat from sea level to 4000 m (Carleton & Musser, 1984; Nowak, 1999). Murines have evolved a wide diversity of diets, many convergently, providing good statistical power for comparative analysis. Their diets include items as diverse as grasses, fish, seeds, earthworms, bark, and beetles. Species range from dietary specialists to generalist omnivores (Nowak, 1999). Starting with the seminal study on the functional ecology of murine teeth by Misonne (1969), there has been a recent increase in attention to morphological associations with diet in murines (e.g. Michaux et al., 2007; Lazzari et al., 2008a, b). Murines also have multiple robust, multigene phylogenies that allow us to consider the relationship between diet and dental traits in light of their evolutionary relationships (Steppan, Adkins & Anderson, 2004; Steppan et al., 2005; Jansa, Barker & Heaney, 2006; Lecompte et al., 2008; Rowe et al., 2008; Schenk, Rowe & Steppan, 2013).

Dental morphology is likewise the most variable feature of murine morphology. Incisors range from thin and narrow to broad and robust. Molars vary in size, loph and cusp arrangements and orientations, and crown heights (Misonne, 1969; Nowak, 1999). Murines have a lingual row of cusps, resulting in a triserial cusp arrangement. This derived row of cusps differs from the primitive pattern seen in cricetids. According to Lazzari et al. (2008b), the addition of a third row of cusps and the variation in murine jaw motion results in different orientations of the occlusal cutting surfaces, limiting the possibility of comparison between murines and cricetids, so our study focused on murine rodents solely.

BACKGROUND: FUNCTIONAL TOOTH AND JAW CHARACTERISTICS

Throughout the murine dental literature, qualitative assertions have been made about the morphologies expected for specific diet types (but see Williams & Kay, 2001 and Samuels, 2009, regarding other rodents). Qualitative and subjective descriptive terms such as 'highly hypsodont' (high crowned) and 'large molars' are reported with little to no mention

of the functional or biological significance of these features. We test these assertions more formally by considering functionally relevant quantitative tooth characteristics adapted to murine morphology from the available biomechanical models (Lucas, 1979, 1982, 2004; Evans & Sanson, 1998, 2005).

Both tooth size and shape greatly influence the function and occlusion of teeth. Tooth efficiency depends most strongly on tooth shape (how stresses and strains are applied to the food) and how the food responds to stress. The main function of the dentition is to break down food without itself being broken or worn beyond the point of utility, so material properties of both tooth and food must be considered (Evans, 2003). Some of the best quantitative studies focus on overall tooth size and proportions among molars (Kavanagh, Evans & Jernvall, 2007), whereas other important quantitative studies take a sophisticated approach of considering the three-dimensional structure of teeth in terms of topology (Lazzari *et al.*, 2008a).

The force required to initiate and propagate cracks through a food item is dramatically affected by the shape and orientation of the occlusal surface, namely the cusps and crests on the tooth, so our study emphasized these features. We justify the functional relevance of all characteristics and present morphological predictions for diet types. Because no formal predictions for rodent tooth morphology have been expounded in the literature, we first explain these in detail.

Tooth size and shape determine the surface area of contact between the tooth and food. The smaller the surface area of contact (crown area), the higher the stress created in the food per unit of force (Lucas, 1982). Tooth size is directly related to the ability to generate occlusal force (Lucas, 2004). All of these surface area characteristics are related to the amount of food that can be broken down per mastication cycle; reduced surface area and volume results in less food processed but increased stresses on those foods. Herbivores are expected to have relatively large molars because plant matter is composed of tough, small, sealed particles, and greater area of contact between the upper and lower molars increases grinding efficiency by increasing the chance that food particles will be hit (Renaud *et al.*, 2005). Before tough plant material can be digested, the cellulose in each cell wall must be breached so that nutrients are released. In addition, non-fruit plant material is typically low in caloric content, requiring the processing of greater volumes for a given body size, and therefore larger teeth. Samuels (2009) found herbivores show relatively higher cheek tooth surface areas than rodents with other diets. Large molars can also withstand the greater occlusal forces required for a plant-dominated diet (Stirton, 1935; Satoh, 1997; Williams & Kay, 2001) and may help reduce the wear inherent in consuming abrasive plant material (Janis & Fortelius, 1988). Likewise, carnivorous rodents (with the exception of durophagous taxa) – consumers of very hard animal matter like bones or mollusks – are expected to have reduced molars because animal matter has a high protein and caloric content and limited mastication is needed to release the food's inner contents (Janis & Fortelius, 1988; Swartz, Freeman & Stockwell, 2003).

Hypsodonty (being high crowned) allows for the mastication of hard and/or abrasive materials that leads to tooth wear. In old individuals, teeth can be worn down to the gum line, resulting in greatly reduced food intake, and even death (Satoh, 1997). Because of the abrasive nature of plants (e.g. silica in grass and exogenous grit in low-height plants), herbivores' molars are expected to be durable, robust, large, and/or hypsodont (Janis & Fortelius, 1988; Satoh, 1997; MacFadden, 2000; Lucas, 2004). Carnivorous rodents (except durophagous species) are predicted to have the opposite condition of low-crowned molars (brachydonty) because of the minimal wear inflicted by their food. Simple shearing or puncturing alone can reduce invertebrate and some vertebrate food material (Janis & Fortelius, 1988; Lucas, 2004).

Jaw-lever mechanics in mammals are related to both the size of the mandible and proportions of the skull. The mechanical properties of the jaws, mechanical advantage and velocity ratio, depend on the size of the in-force (muscle cross-sectional area) and the lengths of the in-lever and out-lever (Turnbull, 1970). Jaw length is related to the size and robustness of the mandible, which is associated with both muscle-attachment areas ('in-lever') and force transmission ('out-lever'), therefore influencing the amount of force required to occlude the teeth efficiently (Satoh, 1997; Lucas, 2004). A deep but short (robust) jaw is predicted for herbivorous taxa because of their need for increased force generation for continual comminution of tough plants. A robust jaw provides room for large masseter muscles, which provide greater muscle force and a more forceful bite (Satoh, 1997; Lucas, 2004). Animal-dominated diets do not require as much force for efficient food breakdown, so a longer, less robust jaw is expected, except in durophagous taxa. Michaux *et al.* (2007) demonstrated that herbivores have more robust jaws and a larger mandibular angle (area of insertion of the masseter), while carnivores have larger coronoid processes (area of insertion of the temporalis). Samuels (2009) found a similar result in which wider and more robust zygomatic arches were found in herbivores and larger temporal fossae were found in carnivores.

Incisors function in initial food processing and acquisition; incisor shape (depth and width) is associated with resistance to wear when abrasive material is habitually consumed. Incisors can also be used to dig, cut up food, or pierce and capture prey (Hillson, 2005). Incisor angle (prognathism) is related to the amount of pressure required for initial food processing. Large incisors are expected for most plant-dominated diets. For example, taxa with grass-dominant diets consume a high volume of food per bite, and thus require large incisors for efficient cutting of abrasive food matter (Lucas, 2004). Large, robust, procumbent incisors are expected for fruit, nut, and seed eaters, which must crack or open food items (Maier, 1984). Leaf-dominated diets are the exception; leaf eaters are predicted to have smaller incisors than other herbivores (Maier, 1984). Small, thin, sharp incisors are expected in carnivorous rodents, because this morphology is best suited for piercing prey items and aiding in animal capture (Maier, 1984; Satoh, 1997). Proodont (more anteriorly angled) incisors should be better for prey capture but develop high internal stresses, so diets requiring more cutting functions should result in opisthodont (more posteriorly oriented) incisors (proodont refers to incisors that have the cutting edge anterior to the vertical plane at the alveolus, whereas prognathism is a more general term that refers to the condition where either jaws protrudes beyond the sagittal plane of the skull). Similarly, Samuels (2009) found herbivorous rodents to have relatively broad, robust incisors and faunivores to have particularly narrow or degenerate incisors.

Molar crest length is correlated with the ability to propagate cracks in a food item and negatively correlated with the food's shear strength. Crests can also form a continuous shearing surface on which food can be broken down. Crest angle is a measure of general cusp and crest orientation, determining the way in which cusps and crests align themselves (Lucas, 2004). Crest angle may similarly be related to crack propagation but has not been formally asserted to be so, and no predictions have been made. Long molar crests are expected for diets of tough foods in which cracks do not propagate easily, like those dominated by leaves (Kay, 1975; Yamashita, 1998). Short crests are predicted for relatively hard or brittle diets, like bamboo stems or grass, as well as for fruit-dominated diets because of the relative ease with which cracks propagate through these foods; the concentration of stress on a short crest facilitates efficient crack initiation (Yamashita, 1998). Crest lengths for invertebrate-dominated diets are generally expected to be long, like those of leaf-dominated diets, because both these foods tend to be tough (Yamashita, 1998). The current consensus is that long crests are advantageous for penetrating and driving cracks through prey that are either stiff or soft, because the whole length of the crest is not in contact with all of the food at one time, lending more importance to the sharpness of the cusp tip (Evans & Sanson, 1998; but see Strait, 1993a; Evans & Sanson, 1998, for contrasting predictions).

Cusp area (a proxy for cusp volume) affects the amount of force required to propagate cracks in the food item. Crack propagation has been proposed to depend on both the volume of the cusp and the amount of food displaced. Smaller cusps require less force to propagate cracks (Evans & Sanson, 1998). Herbivores are generally expected to have sharp-bladed molars and therefore smaller cusp area. Sharp blades are advantageous for consumption of diets composed of tough foods (like leaves and grasses) because sharp blades promote crack initiation and propagation by concentrating bite forces on blade tips (Lucas, 1982; Lucas & Luke, 1984; Maier, 1984; Popowics & Fortelius, 1997). Sharp-bladed cusps, or rather cusps with a small area, are also expected in organisms consuming predominantly hard-bodied invertebrates and with leaf- and grass-dominated diets. Crest development is similar in these two diet types because of the analogous mechanical properties of chitin (invertebrates) and cellulose (plant matter) (Kay, 1975; Sanson, 1985). Cracks propagate relatively easily in soft or brittle materials, so animals whose diets are dominated by soft and brittle foods (like fruits, seeds, and nuts) should have blunt molars with larger cusp area (Rosenberger & Kinzey, 1976; Lucas, 1979; Maier, 1984; Yamashita, 1998).

Cusp height is related to the food item size that can be efficiently comminuted by the molar in one masticatory cycle. Food elasticity also interacts with cusp height and may illuminate relationships of cusp occlusion as well as general crown topology. Hard, brittle foods tend to shatter easily, but soft foods are more resistant to cracks, so cusps larger than the food may be required (Lucas, 1982). Cusp heights are predicted to be lower in herbivorous taxa, producing a generally flatter crown topology with more crests. Crests are required for diets of high toughness (like most of those dominated by plants), promoting maximal crack propagation (Yamashita, 1998; Evans, 2003). Herbivores' requirement for bulk processing requires flattened, well developed crests, which provide more surface area on which food particles can be broken down, facilitating maximal release of nutrients (Lucas, 2004). The need for these well developed crests often results in lophodonty or stephanodonty (Denys, 1994; Evans, 2003; Renaud, Auffray & Michaux, 2006). Organisms consuming a fruit-dominated diet are expected to have low cusps and basin-like molars, consisting of an efficient,

flattened enamel cutting edge with a flat mortar-and-pestle arrangement (Maier, 1984; Swartz *et al.*, 2003). Nut and seed dominated diets are expected to result in these same features because cracks are self-propagating in hard and brittle foods (Lucas & Luke, 1984; Yamashita, 1998). Organisms with animal-dominated diets are predicted to have slightly higher cusps and simplified, bladed molars.

Cusp angle is related to the application of force to the food item. Large-angled cusps (cusps directed more posteriorly, with angle measured between points BAC, Fig. 2C) distribute force differently from smaller-angled cusps (cusps directed more anteriorly). Cusp angle may also be related to the movement of the jaw. Available functional models have produced no formal predictions of cusp angles for any diet; these characteristics remain to be explored. Cusp sharpness influences the amount of force required to initiate a crack in the food item. Greater tip sharpness (a smaller radius of tip curvature) places a smaller area in contact with the food item and therefore produces a higher stress in the food for the same amount of force (following Lucas, 1982). Sharp cusp tips are predicted for animals that consume tough food materials, like leaves and grasses, because these materials resist rupture or fragmentation because of their high toughness and high strain at failure, and sharp cusp tips are required to initiate cracks in them (Popowics & Fortelius, 1997). Sharper molars are also predicted for consumers of relatively hard or brittle foods (such as seeds, unripe fruits, and nuts), because these foods have high strength, low toughness, and low strain at failure, so the highly concentrated stress created by sharp cusp tips is needed to break them apart efficiently (Lucas, 1982; Lucas & Luke, 1984). Fruit-dominated diets should produce blunt cusp tips because of the soft brittle nature of fruit and the subsequent ease of crack initiation (Maier, 1984; Yamashita, 1998). Animal-dominated diets are thought to require sharp cusp tips as well, because concentrating stress minimizes the force and energy required to initiate a crack in the food (Freeman & Weins, 1997; Evans, 2003). Arthropod cuticles are relatively 'hard', and low, sharp, cusp tips most efficiently penetrate and drive through the food item (Lucas, 2004). Generally, cusp tips adapted to animal-dominated diets are predicted to be sharper than those adapted to plant-dominated diets (Lucas, 2004).

MATERIAL AND METHODS
MORPHOLOGICAL DATA COLLECTION

Two to five individuals of each of 98 species of murine rodents were examined from wild-caught animals in museum collections. Species were selected for even distribution across a nuclear-DNA phylogeny (Rowe *et al.*, 2008) and diet type. Special attention was paid to inclusion of both dietary specialists and generalists. Dietary information for each species was extracted from the primary literature. Species were assigned to one of six diet types: herbivores, plant-dominated omnivores, animal-dominated omnivores, omnivores, insect-dominated diet, and non-insect invertebrate-dominated diet (Table 1). Method of diet determination and specific dietary findings are reported where available. Each species' diet was assigned a diet-reliability value, based on the quality and quantity of dietary information available (Table 2). Museum catalogue numbers for all specimens examined appear in Supporting Information (Table S1). Classification follows the nomenclature of Carleton & Musser (2005). A complete list of all species, number of individuals sampled, diet, and diet reliability appears in Supporting Information (Table S2).

Morphological data were taken from voucher specimens located at the American Museum of Natural History, New York, and the United States National Museum of Natural History, Washington, DC, USA. Five individuals of each species were measured whenever possible, so that intraspecific variation could be accounted for. Where possible, at least two males and females were measured. There was no

Table 1. Diet types into which the 98 study species were classified

Diet type	Definition
Herbivory	Diet composed entirely of plant material
Plant-dominated omnivory	Diet composed of primarily plant material but including some animal material
Omnivory	Diet composed of approximately equal quantities of plant and animal material
Animal-dominated omnivory	Diet composed of primarily animal material but including some plant material
Insect-dominated diet	Diet composed primarily of insects, both moderate- and hard-bodied, but possibly including some other animal material
Invertebrate-dominated diet	Diet composed primarily of non-insect invertebrates, typically soft-bodied, but possibly including some other animal material

Table 2. Diet reliability criteria, the bases on which study species were assigned to diet reliabilities

Diet reliability	Definition
1	Fair. Diet information based on that for the genus, reported without any supporting data, or based on only one field observation
2	Good. Diet information based on 2–4 field observations or on one stomach-content or faecal-pellet analysis including fewer than ten individuals
3	Very good. Diet information based on more than five field observations; two to three stomach-content or faecal-pellet analyses including fewer than ten individuals, or one stomach-content or faecal-pellet analysis including at least ten individuals
4	Excellent. Diet information based on more than four stomach-content or faecal-pellet analyses including fewer than ten individuals or on more than two such analyses with at least 20 individuals; from literature review

Table 3. Tooth-wear criteria

Wear class	Definition
1	M3 unerupted
2	M3 completely erupted and showing slight wear; all major occlusal features raised and prominent, M1 and M2 showing no to slight wear
3	M3 showing slight to moderate wear; M1 and M2 showing slight to moderate wear; major occlusal features distinct, raised, and prominent; enamel borders of laminae and cusps much higher than enclosed dentine
4	M3 showing moderate to heavy wear; M1 and M2 showing moderate to heavy wear not below widest part of the crown; pattern of laminae and major cusps present, but enamel margins low so that dentine is broadly exposed; some laminae and cusps coalesced, supplementary cusps indistinct
5	M1–M3 heavily worn, almost to the tops of roots; most occlusal features obliterated so that crowns appear nearly featureless

evidence for sexual dimorphism in dental traits. Molar wear was assessed for each individual on the basis of tooth wear criteria modified from published sources (Koh & Peterson, 1983; Voss, 1991; Musser & Heaney, 1992; Steppan, 1997; Table 3). Only specimens with slight to moderate molar wear (wear classes 2 and 3) were included. Non-molar measurements (condylobasal length, upper incisor depth and width, and jaw-lever length; Fig. 1) were taken by hand with digital calipers. Incisor width was measured across the anterior face at the occlusal edge, where they contact each other medially, and incisor depth was measured aligned to the radial axis parallel to the plane defined by the condylobasal length, following Steppan (1995). Jaw-lever length (JFL) extended from the m1–m2 boundary at the labial alveolus to the condyloid process.

Molar morphology was digitized from photographs taken with a Nikon D100 (6 megapixels, at a resolution of 3008 × 2000), in RAW format, of both the occlusal and labial views of the upper and lower right molar tooth rows as well as a lateral view of the upper incisors, for a total of five photographs per individual. Each photograph included a scale bar. For each species, 105 characteristics in all were recorded. All measurements were recorded for both the upper and lower first molar unless otherwise specified.

A short description of each of the 105 measured characteristics and a summary of functional characteristics appears in Supporting Information (Tables S9 and S10) respectively. Characteristics include jaw-lever length (Fig. 1), incisor depth, incisor width (Fig. 1), those related to molar size [e.g. lengths for each of the three molars, length of the tooth row, width of the first molar, crown area (Fig. 2A), and molar ratios], molar height (Fig. 2B), cusp area (Fig. 2C), cusp height (Fig. 2C), as well as measures of cusp angle and cusp sharpness (Fig. 2C), and incisor angle, crest length, crest angle (Fig. 2D, E). Tip or cusp sharpness relates to the ability to initiate a crack. Optimally this is the radius of curvature at the tip, but because of the error involved in fitting a circle to a feature that is small on the digital images, we used a proxy, calculated from the coordinate points of the edge of the cusp taken at 1/3 of total height of the cusp. Molars are identified using standard notation (e.g. m1 and M3 for 1st lower and 3rd upper molars, respectively). All other characteristics were extracted from the photographs with NIH ImageJ (Abramoff, Magelhaes & Ram, 2004). A more detailed description of each of these characteristics is found in Martin (2010).
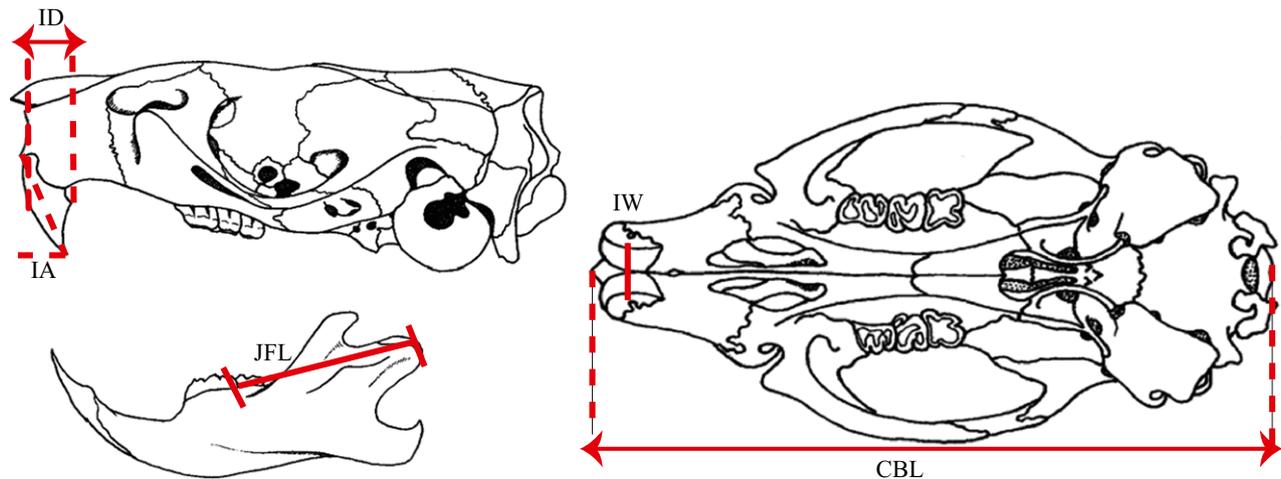
Figure 1. Incisor and skull measurements, shown on *Rattus rattus*. Reproduced with permission from Linzey (1998).

PHYLOGENETIC AND DIVERGENCE-TIME ANALYSIS

In order to apply phylogenetic correction in some analyses (see below), we constructed a chronogram of murines using published molecular sequences; most from Rowe *et al.* (2008) and Jansa *et al.* (2006). In total, 66 species were included, representing most species with morphometric data (63). Three unmeasured species were included in the phylogeny to allow for the inclusion of fossil calibrations from Schenk *et al.* (2013); the murine *Tokudaia osimensis*, and gerbil (*Gerbilliscus robustus*) and deomyine (*Uranomys ruddi*) outgroups (species with the best gene coverage were selected based on Alhajeri, Hunt & Steppan, 2015).

The final supermatrix was a concatenation of six nuclear protein-coding genes [part of intron 2 and exon 4 of acid phosphatase five (ACP5); part of exon 11 of breast cancer 1 (BRCA1); part of intron 3 and exons 3 and 4 of benzodiazepine receptor gene (BZRP); part of exon 10 of growth hormone receptor (GHR); part of exon 1 of interphotoreceptor retinoid binding protein (IRBP); part of the single exon of recombination activation gene 1 (RAG1)], and three mitochondrial protein-coding genes [part of cytochrome *c* oxidase I (COI); part of cytochrome *c* oxidase II (COII) including two tRNAs within COII; part of cytochrome *b* (CYTB)], for a total of 12 177 sites (Supporting Information, Table S11).

Divergence times were estimated simultaneously with topology and branch lengths using Bayesian inference using an uncorrelated lognormal relaxed clock model in Beast 1.8.0 (Drummond & Rambaut, 2007) on CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). ModelTest 3.1 (Posada & Crandall, 1998) was used to estimate the best-fit DNA substitution model for each data type partition separately using the Akaike information criterion (AIC;

Akaike, 1974). Our partitioning scheme included eight partitions corresponding to across-gene codon position and data type (nuclear introns, three mitochondrial codon positions, three nuclear codon positions, and tRNAs). We applied this partitioning scheme because it worked well in previous phylogenetic studies of muroids (e.g. Schenk *et al.*, 2013; Alhajeri *et al.*, 2015). Parameter values among all partitions were unlinked.

The best-fit substitution model was applied to each of the eight partitions: TrN + G for nuclear introns, GTR + I + G for the first and second mitochondrial and first and second nuclear codon positions, TVM + I + G for the third mitochondrial codon position, TVM + G for the third nuclear codon position, and HKY + I + G for tRNAs. The TVM model was generated from the GTR model in Beauti 1.8.0 (Drummond & Rambaut, 2007) by adjusting the appropriate operator settings. Clade support was determined using Bayesian posterior probabilities (PP).

Metropolis-coupled Markov chain Monte Carlo (MC^3) was run for 50 million generations, sampling every 5000 generations from the posterior distribution. Tracer 1.5 (Rambaut & Drummond, 2005) was used to determine appropriate burn-in based on convergence and stationarity leading to the exclusion of the first 10% of the of the MC^3 chain as burn-in. Out of 102 parameters; 99 had a post-burn-in effective sample size (ESS) > 200 and only three had ESS < 100 and the post-burn-in trees were summarized using TreeAnnotator 1.8.0 (Drummond & Rambaut, 2007) using the maximum-clade credibility tree criterion.

Three fossil calibrations were used to calibrate the chronogram (Supporting Information, Table S12) all of which were used previously (see Schenk *et al.*,
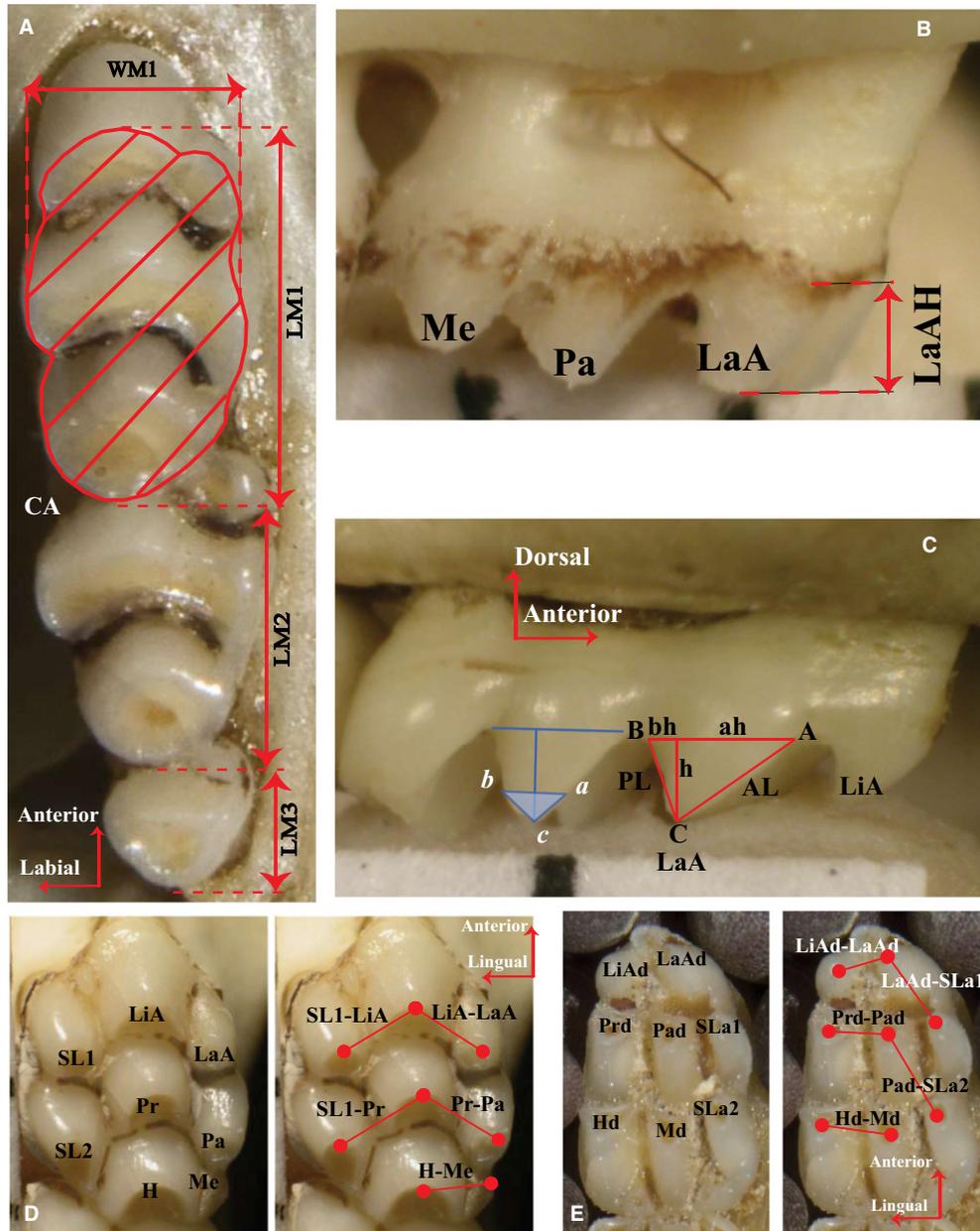
Figure 2. Tooth measurements: (A) occlusal tooth row area (CA), tooth lengths (LM1, LM2, LM3), and width M1 (WM1) shown on *Melomys capensis* (Tate, 1951); (B) lateral cusp measures, M1 *Apodemus flavicolus* (Melchior, 1834); (C) lateral cusp morphology shown on M1 of *Mus saxicola* (Elliot, 1839), named landmarks and distances are shown in red (see Supporting Information for details), whereas the corresponding landmarks (*a*, *b*, and *c*, measured at 1/3 of cusp height) used to measure cusp sharpness and cusp area are shown in blue; (D) M1 occlusal cusp and crest names and locations shown on *Golunda ellioti* (Gray, 1837). Cusp points are defined as the leading edge between the dentine and enamel boundary, lingual anterocone (LiA), labial anterocone (LaA), protocone (Pr), paracone (Pa), hypocone (H), metacone (Me), supplementary lingual cusp 1 (SL1), and supplementary lingual cusp 2 (SL2); (E) m1 shown on *Hapalomys longicaudatusi*, lingual anteroconid (LiAd), labial anteroconid (LaAd), protoconid (Prd), paraconid (Pad), hypoconid (Hd), metaconid (Md), supplementary labial cusp 1 (SLa1), and supplementary labial cusp 2 (SLa2).

2013 and references therein for justification). Lognormal prior distributions were applied to all calibrations with means and standard deviations chosen to construct 95% confidence intervals (for the origination of the taxon based on first occurrence and stratigraphic sampling) spanning 95% Marshall indices

(Marshall, 1994) as reported by the Paleobiology Database (PDB 2013). The root was calibrated using a normal prior distribution based on Schenk *et al.* (2013). The resulting chronogram was used in subsequent comparative analyses.

ORDINATION ANALYSES

Species averages were calculated for all morphological characteristics. All characteristics except angle measurements and molar ratios were size corrected by linear regressions on condylobasal length and recording of the residuals. We scaled by body size rather than tooth size, because relative tooth size is likely to be affected by diet. This approach was a more appropriate method of size correction then conducting a principal components analysis (PCA) because the first PC would likely be dominated by molar size. Although condylobasal length was chosen because it is the most-easily measured size-variable associated with dental and cranial traits, we note that it can also be affected by diet because it includes the rostrum, which tends to be short in herbivores like *Rhabdomys* and long in insectivores like *Rhynchomys*, Species-averaged size-corrected characteristics were used in all subsequent analyses. All statistical analyses were conducted in R (R Development Core Team 2013).

A separate PCA was performed on all species for all characteristics except condylobasal length in order to visualize differences in morphological variation among diets; PCA was performed on the correlation matrix because the morphological characteristics have different scales. A forward stepwise discriminant function analysis (DFA) was performed on all dental characteristics except condylobasal length, using diet categories as groups, to define canonical axes that maximize the separation among dietary groups. Stepwise DFA reviews all available characteristics at each step and determines which characteristic will contribute the most to diet-type discrimination. That characteristic is then included in the model, and the analysis continues with all remaining characteristics. Two analyses were performed, one including all species and a second including only species with a diet reliability (DR) value of two or more (59 species); some species with DR value of 2 were excluded if insufficient diet information is available (e.g. *Pseudohydromys occidentalis* Tate 1951). We then evaluated the results of each analysis using a two-sample Hotelling T^2 test to determine whether differences between diet types were statistically significant.

Geometric morphometric analyses were also conducted on a subset of taxa after digitizing coordinate data from photographs of the molars. Details are described in the Supporting Information.

PHYLOGENETIC ORDINATION ANALYSES

In addition to the traditional statistical analyses (see Supporting Information), we conducted corresponding analyses while correcting for phylogenetic relationships. All phylogenetic ordination analyses were conducted on the truncated morphological data set that only includes the 59 species with the high 'diet confidence'. We conducted a phylogenetic DFA (pDFA) using the R code published by Schmitz & Motani (2011). Because the pDFA code only allows for analyzing a maximum of 38 characteristics, we ran the analysis on the 15 characteristics that were retrieved by the traditional stepwise DFA described above (listed in Table 4, see Results) along with the 23 characteristics with the highest (absolute) traditional non-stepwise DFA1 loadings (data not shown), excluding redundant characteristics, for a total of 38 characteristics.

We also performed a phylogenetic PCA (pPCA) on the correlation matrix of all characteristics except condylobasal length (as above) in order to identify differences in morphological variation among diets in the species with the highest DR scores. We then ran a phylogenetic MANOVA (pMANOVA) on the first six phylogenetic PC axes, which together accounted for 81.3% of the variation (pMANOVA could not be run on all the characteristics due to the 'large p small n' problem encountered by our data). pPCA was conducted in the phytools library (Revell, 2012) and the pMANOVA was conducted in the Geiger library (Harmon *et al.*, 2008); both in R.

RESULTS

PHYLOGENETIC ANALYSIS

Most clades in the chronogram were well-supported and corresponded with prior molecular phylogenies that included murines (e.g. Schenk *et al.*, 2013). The chronogram (Supporting Information, Fig. S1) along with the concatenated matrix used to construct it was deposited in TreeBase under submission identification number S18779.

PRINCIPAL COMPONENT ANALYSIS

Neither the standard PCA (Supporting Information, Fig. S2) nor the pPCA (Supporting Information, Fig. S3) effectively distinguished between diet types based on a plots of the PC axes; most diet types show great variation and overlap in dental morphology. However, the results of the pMANOVA based on the

Table 4. Discriminant-function (DF) analysis coefficients for species with diet reliabilities 2–4

	DF1	DF2	DF3	DF4	DF5
Upper LaA-LiA-SL1 angle	−0.048	−0.033	0.001	0.002	0.003
Incisor depth	−2.410	−2.878	−1.777	1.178	−0.564
Lower CL-HM	0.091	4.730	5.484	−0.692	−1.337
Lower CL-PaSLa2	3.093	8.988	−1.488	−1.208	−7.006
Lower LaA height	10.166	−6.831	16.046	25.288	−20.016
Lower LaA sharpness	−2.941	−2.680	−1.924	−8.569	3.762
Ratio of m3 to m1 length	−6.816	6.227	8.446	−5.867	−1.107
Lower M ah	−2.117	−0.031	−11.016	−4.832	3.140
Upper CL-LaALiA	−2.418	−8.723	−5.811	−4.780	−6.436
Upper CL-PaPr	1.559	−7.031	2.106	4.331	7.867
Upper LaA ah	−17.271	6.483	6.466	−21.518	0.352
M3 length	−0.479	0.059	−4.233	1.669	0.116
Ratio of M3 to M1 length	3.585	4.065	1.383	0.183	1.061
Upper M ah	15.411	−9.211	6.317	12.077	−19.881
Upper Pr height	13.882	13.963	−10.253	19.196	26.149

Angle ah is illustrated in Figure 2C; other abbreviations as in Figure 2.
DF1, Discriminant Function 1; DF2, Discriminant Function 2; Lower CL-HM, lower crest length between H and M; lower CL-PaSLa2, lower crest length between Pa and SLa2; lower crest length between H and M lower LCL-HM, lower crest length between H and M; lower CL-PaSLa2, lower crest length between Pa and SLa2; upper CL-LaALiA, upper crest length between LaA and LiA; upper CL-PaPr, upper crest length between Pa and Pr.

first six PC axes indicate a significant difference in overall dental morphology based on diet (Wilk's λ = 0.35; P = 0.016).

The preliminary geometric morphometric landmark analysis separated diet types fairly well; overlap between herbivorous and omnivorous species was minimal. Deformation grids reflected the tendency of herbivorous species to have expanded crowns and more widely spaced and linearly arranged cusps, whereas those with non-insect invertebrate-dominated diets showed more chevron-shaped, compressed cusps (Supporting Information, Fig. S4).
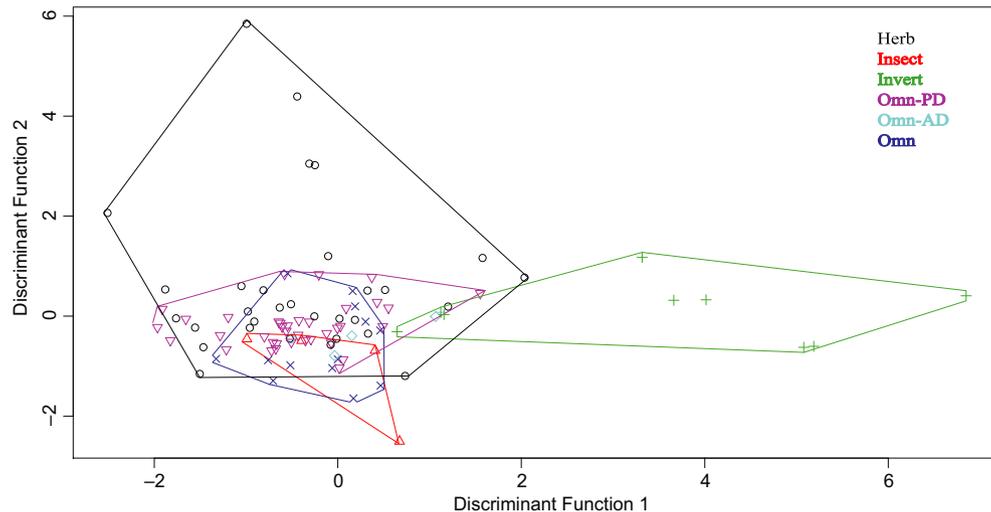
DISCRIMINANT FUNCTION ANALYSES

In the standard DFA conducted on all species, the first discriminant function, Discriminant Function 1 (DF1), was most influenced by the upper metacone posterior cusp length (Fig. 3). Species with animal-dominated diets, primarily those with non-insect invertebrate-dominated diets, had a greater upper metacone posterior cusp length, reflecting a more anteriorly angled molar cusp. Species that consume plant-dominated diets tended to have a smaller upper metacone posterior cusp length, due to a more posteriorly angled molar cusp. DF2 was most influenced by the ratio between the lengths of third upper molar and the first upper molar. Herbivores tend to have a relatively large third upper molar and species consuming any animal material a small third upper molar relative to the first upper molar.

The DFA performed on all dietary reliability classes determined five characteristics to be the best indicators of diet: incisor depth, lengths of the third lower and upper molars, ratio between the third and the first upper molars, and the upper metacone posterior cusp length. Fifty-three of 98 species (54%) were correctly assigned to their dietary categories on the basis of these five characteristics. Almost all of the correct classification resulted from classification of over 70% of species as plant-dominated omnivores. All other diet types had relatively poor classification. A table of the DFA PP and complete DF scores with diet predictions can be found in Supporting Information (Tables S4 and S5) respectively.

Species consuming non-insect invertebrate-dominated diets and plant-dominated omnivores had mean morphologies significantly different (P < 0.05) from those of species consuming all other diet types, with the exception of animal-dominated omnivores, which did not differ significantly from any other diet type (Supporting Information, Table S6).

Because imprecision in dietary reporting resulted in dietary overlap and consequent statistical noise, species with a diet classification of 1 were excluded in a 'high diet confidence' analysis, leaving 59 species. Correct classification increased to 73% (Supporting Information, Table S14). For all diet types except animal-dominated omnivores, over 50% of individuals were correctly assigned to their reported diet types. DF1 was predominantly influenced by the height of the lower metaconid and lower metaconid

Figure 3. Standard discriminant function analysis based on all molar characteristics and for all species. Herb, herbivory; Omn-PD, plant-dominated omnivory; Omn, omnivory; Omn-AD, animal-dominated omnivory; Insect, insect-dominated diet; Invert, non-insect invertebrate-dominated diet.

sharpness. DF1 separated species with animal-dominated diets from those that consumed any plant material (Supporting Information, Fig. S5). Species that consumed solely animal matter, grouped on the right side of Supporting Information (Fig. S5) with high scores on DF1, tended to have taller, sharper, metaconid cusps on the lower first molar than species consuming any plant material. DF2 was strongly influenced by the ratio between the upper molar lengths. Species consuming plant-dominated diets generally had larger molars all around, whereas species with more animal-dominated diets generally had at least some smaller molars.

Fifteen traits best distinguished dietary groups for species with diet reliabilities 2–4: the labial anterocone–lingual anterocone—supplementary lingual cusp 1 angle on M1, incisor depth, lower crest length between hypocone and metacone on m1, lower crest length between paracone and supplementary labial cusp 2 on m1, lower crest length between hypocone and metacone on m1, lower crest length between paracone and supplementary labial cusp 2 on m1, labial anterocone height on m1, labial anterocone sharpness on m1, ratio between m3 and m1 lengths, metacone height on m1, crest length between labial anterocone and lingual anterocone on M1, crest length between paracone and protocone on M1, labial anterocone anterior measure on M1 (uLaa), length of M3, ratio between M3 and M1 length, metacone anterior measure on M1, and protocone height M1. DFA coefficients appear in Table 4.

Herbivores differed significantly from omnivores ($P = 0.0007$) and differed marginally from insect and

other invertebrate consumers ($P = 0.054$ and 0.055, respectively). Species with insect-dominated diets and those with non-insect invertebrate-dominated diets differed significantly from omnivores and plant-dominated omnivores. Omnivores differed from species with all other diets except for animal-dominated and plant-dominated omnivores. Species with animal-dominated diets did not differ significantly from any other group (Supporting Information, Table S7).

The pDFA (conducted on species with high diet confidence) performed better then the traditional DFA at correctly assigning species to their dietary categories on the basis of the 38 selected dental traits (Fig. 4, Table 5 and Supporting Information, Table S14). Ninety-five percent of the herbivores were correctly assigned, as were 92% of the plant-dominated-omnivores, and 100% of the remaining diet categories (95% overall; Table 5). Significantly, the only incorrect classifications were between herbivores and plant-dominated omnivores (Table 5). The greatest separation occurred in pDF1 between species that consume invertebrates from the other diets; there was similarly a large separation between species that consume insects and omnivores from the other dietary categories in pDF2 (Fig. 4). Herbivores, plant-dominated omnivores, and animal-dominated omnivores overlapped greatly on both pDF1 and pDF2.

The phylogenetic DF1 and pDF2 were both mostly influenced by the metaconid angle proxy measure on m1 (lMdbh; Supporting Information, Table S13). The labial anteroconid angle proxy measure on m1 (lLabh) had the second strongest influence (in the opposite direction) on pDF1, whereas the alternate
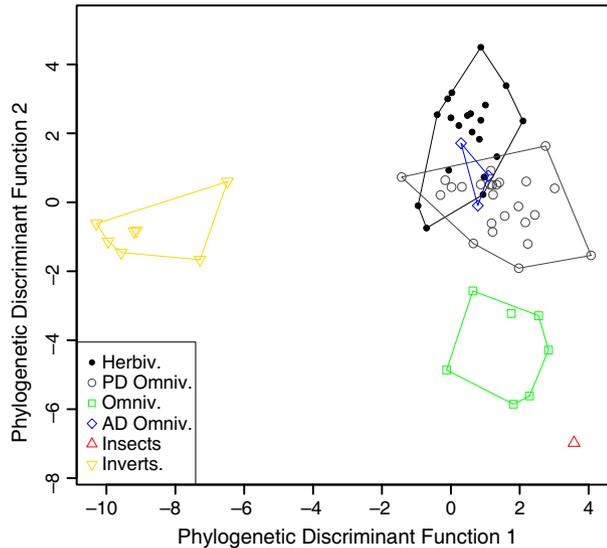
Figure 4. Phylogenetic discriminant function analysis based on all molar characteristics for species with high diet confidence (reliabilities 2–4). Herbiv., herbivory; PD Omniv., plant-dominated omnivory; Omniv., omnivory; AD Omniv., animal-dominated omnivory; Insects, insect-dominated diet; Invert., non-insect invertebrate-dominated diet.

metaconid measure on m1 (k) had the second strongest influence (in the opposite direction) on pDF2 (Supporting Information, Table S13).

DISCUSSION

PHYLOGENETIC DISCRIMINATION OF DIETS

Our results demonstrate a distinct improvement in dietary assignments using the pDFA. There have been few applications of pDFA or demonstration of improved efficacy. These results suggest that there is a stronger signal in the evolutionary transformations

(as revealed by accounting for phylogeny) than in the static morphologies. Adaptation is a dynamic process, and current morphologies may not yet be optimal for a given function due to both inheritance of ancestral phenotypes and functional tradeoffs.

TESTING PREDICTED DISCRIMINATING DENTAL TRAITS

Both DFA analyses identified incisor depth as strongly reflecting diet. Our results generally support the prediction that species consuming plant-dominated diets should have broad robust incisors whereas species consuming animal-dominated diets should have thin narrow incisors (Supporting Information, Table S8). Omnivores and animal-dominated omnivores had larger incisors than expected, possibly due to allometry and larger body sizes (Supporting Information, Table S8). Functionally, narrow incisors aid in prey capture, piercing, and cutting functions useful for animal consumption, whereas deep incisors allow for continual consumption and acquisition of abrasive plant matter (Lucas, 2004), as seen by Samuels (2009) in a broad survey of rodents. Similarly, in the very recently diverged South American mouse *Phyllotis limatus* (Thomas, 1912; Sigmodontinae), unusually narrow incisors correspond to a significant increase in animal material in its diet relative to its sister species (Steppan, 1998). Moreover, regardless of diet, rodents that experience relatively high loads on their incisors should have relatively deep incisors (antero-posteriorly) to resist encountered stresses (e.g. when piercing seeds or hard-bodied invertebrates), which may explain our observed patterns.

Our results clearly support the prediction that the third molars are relatively long in species primarily consuming plant material. Some species that consume primarily worms, such as *Chrotomys gonzalesi* (Rickart & Heaney, 1991) and *Rhynchomys isarogensis* (Musser & Freeman, 1981) that have lost their

Table 5. Phylogenetic DFA diet classifications based on species with high 'diet confidence'

	Herbiv.	PD Omniv.	Omniv.	AD Omniv.	Insects	Inverts
Herbiv.	19	2	0	0	0	0
PD Omniv.	1	23	0	0	0	0
Omniv.	0	0	7	0	0	0
AD Omniv.	0	0	0	3	0	0
Insects	0	0	0	0	1	0
Invert.	0	0	0	0	0	7
Correct classification (%)	95	92	100	100	100	100

Percentage correct classification based on this pDFA is included. *A priori* assignments are the columns.
Herbiv., herbivory; Insects, insect-dominated diet; Invert., invertebrate-dominated diet; Omniv., omnivory; AD Omniv., animal-dominated omnivory; PD Omniv., plant-dominated omnivory.

third molars and *Paucidentomys vermidax* (Esselstyn, Achmadi & Rowe, 2012) that has lost all molars; presumably because their diet does not require any significant mastication. Degenerate dentition is observed in various insectivorous or vermivorous mammals as well (Hershkovitz, 1962; Rickart, Heaney & Utzurrum, 1991; Samuels, 2009).

Our results support the prediction that crest lengths are longer in species with plant-dominated diets and are shorter in those with animal-dominated diets. The standard DFA-determined four crest length measures (crest length between the hypocone and metacone on m1, crest length between the paracone and supplementary labial cusp 2 on m1, length between the labial anterocone and supplementary lingual cusp 1 on M1, length between the paracone and protocone on M1) to distinguish among diets. The crest length between the hypocone and metacone on m1 and the length between the labial anterocone and supplementary lingual cusp 1 on M1 were longest for herbivores and shortest for invertebrate-dominated diets, as predicted by Evans & Sanson (1998).

In addition to these functional causes, crest lengths may be long because of the shift in position of the cusps and not because of selection specifically on cusp length. Cusps in a more chevron-like arrangement (as are seen in many species with animal-dominated diets; Supporting Information, Fig. S4) will naturally result in longer crests because of the absolute distance between the offset cusps. Also, consumption of different proportions of soft- and hard-bodied invertebrates may produce differences in crest length because soft-bodied invertebrates might require longer shearing crests (Strait, 1993a). This interpretation supports the predictions made by Yamashita (1998) that longer crests might be advantageous for tougher foods.

As predicted, species consuming mostly arthropods have sharp cusps. Lower labial anteroconid was sharpest in insect-dominated diets and most blunt in herbivores. Herbivores were predicted to have sharper cusps than were observed. Our results did not support the prediction that cusps are shorter in animals with plant-dominated diets and taller in those with animal-dominated diets. Our standard DFA identified three measures of cusp height: lower labial anterocone followed our prediction – the tallest cusp was in species with insect-dominated diets – but the lower metacone was tallest for omnivores and shortest in species with non-insect invertebrate-dominated diets, and the upper protocone was actually tallest for herbivores and shortest for omnivores and species with insect-dominated diets. These mixed results may be because herbivores generally have larger teeth, and therefore larger cusps, resulting in relatively taller cusps.

NOVEL DISCRIMINATING DENTAL TRAITS

In accordance with our third objective, we identified a small set of functionally relevant dental traits that had not previously been predicted directly by the available biomechanical models. The ratios of the third to first upper and lower molars clearly discriminated between herbivores (whose third molars were nearly half to two-thirds the length of their first molars) and species consuming animal dominated diets (which had smaller third molars). The expansion of the third molar provides more surface area without placing constraints of jaw size that might make expansion of the first molar more difficult. While exploring tooth development in murines, Kavanagh *et al.* (2007) discovered that herbivores generally have third molars almost as large as their first molars but that omnivores had third molars approximately half the size of first, and species with more animal-dominated diets had third molars approximately one-quarter the size of their first molars. Renvoisé *et al.* (2009) expanded sampling to arvicolines and discovered a different evolutionary pattern. The ratios of molar sizes may be more complex than previously suspected.

The labial anterocone–lingual anterocone–supplementary lingual cusp 1 angle on M1 was helpful in distinguishing diet types because it describes occlusal cusp arrangements. This angle is largest for herbivores (their cusps are more laterally aligned) and smallest for non-insect invertebrate-consuming species (which have a more chevron-shaped occlusal surface). Having laterally arranged cusps would allow for expanded crest development and thus a larger grinding surface, advantageous for a plant-dominated diet. Cusps in an offset chevron arrangement may aid in grasping and piercing functions of the molars, required for efficient consumption of animal material (Lucas, 2004). A similar pattern of cusp orientation was demonstrated in the preliminary geometric morphometric analysis (Supporting Information, Fig. S4); herbivores tended to have expanded crowns with more widely spaced cusps arranged more linearly, and consumers of non-insect invertebrates had compressed, chevron-shaped molar cusps.

Several measures reflecting lateral cusp angle (lower metacone, upper labial anterocone ah, and upper metacone ah angle proxies) were selected by our standard DFA. All characteristics consistently reflected more anteriorly angled cusps in species with animal-dominated diets and more posteriorly angled cusps in those with plant-dominated diets. Although no formal predictions have been made for cusp orientation, it may reflect the way in which force is applied to the food, aiding in crack initiation,

or in restraining moving prey. The functional consequences of cusp angle should be influenced by the direction of jaw motion, and thus might vary among muroids with different jaw actions. The pDFA retrieved slightly different cusp angle characteristics, where the most discriminating among dietary categories were the lower first molar metacone angle proxy measure (bh), the lower first molar labial anterocone angle proxy measure (bh), and the alternate lower first molar metacone measure (k).

ADDITIONAL FACTORS

Dietary overlap in morphospace may be a consequence of dietary flexibility among murines, where opportunism and seasonal variation in diet may be an adaptive strategy. Because not all taxa are highly specialized for a particular diet, precise classification may not be possible. The improvement in diet prediction that resulted from exclusion of diet-reliability class 1 suggests, however, that further improvement is possible with more thorough or accurate diet data. Some of the overlap among dietary groups (Fig. 3) we suspect to be a result of imprecision in *a priori* dietary classification. Determining the diet of a species is complicated and time consuming. For example, faecal-pellet analysis is subject to error arising from degradation of particles and constraining accurate particle identification caused by differential rates of digestion. The number of observations or individuals sampled may be inadequate for accurate determination of diet and is often not reported directly in the literature. Even when diet information is available, many reports do not quantify diet components and may classify species into only broad dietary categories. Placing foods with very different material properties (e.g. flower buds vs. bamboo) in the same dietary category can produce results in which species with highly derived and very different morphologies appear to have share the same diet.

Food availability will change with season and over geographic gradients, resulting in dietary variation within species. Seasonal changes in the level of predator competition may also affect the dietary choices of predatory species, so one observational study conducted in summer may not produce an accurate picture of the species' typical diet. Dietary uncertainty results in noisy data that presumably reduce discrimination.

Behavioral differences, like differences in method of grinding or jaw movement, are also likely to compensate for morphological deviations. In a closely related group of mice, changes in jaw movement were found to result in large differences in cusp alignment and orientation, resulting in different tooth configurations primarily with regard to

grinding and cutting surfaces despite consumption of similar foods (Lazzari *et al.*, 2008b).

Importantly, phylogenetic history also appears to influence the results. The ancestors of species that have converged on similar diets may have originally consumed different foods. The descendant species may be morphologically constrained by inheritance of a tooth shape that reflected their ancestral diet rather than their current derived diet; current morphology is a product of both adaptive changes and ancestral morphology. Some species may either not be optimized yet (too little time) or may represent a tradeoff between ancestral traits (and retained ecology) and function. Effectively, pDFA is discriminating among adaptive dietary responses (evolutionary trajectories) independent of their starting morphology, rather than among static morphologies (dietary classes), presumably accounting for its improved discrimination.

CONCLUSIONS

We developed a set of morphological predictions for murine molars that were based on biomechanical models of food processing. Furthermore, we successfully quantified functional associations of tooth shape with diet and identified a suite of functionally relevant traits that can reasonably distinguish between diet types of murine rodents. On the basis of 15 of 105 dental characteristics, 73% of species were correctly assigned to their diet types, and this improved to 95% by accounting for phylogeny and excluding the least reliable diet data.

In general, species that evolve plant-dominated diets (following Fig. 3) evolve in the direction of deep incisors; longer third upper molars; a large third to first upper molar ratio; longer crests connecting molar cusps; blunt, posteriorly angled cusps; and expanded, laterally aligned molar cusps. Species that evolve towards animal-dominated diets display the opposite trends, towards molar cusps also aligned in a distinct chevron. More specifically, the labial anterocone–lingual anterocone–supplementary lingual cusp 1 angle on the first upper molar is generally < 120° for strictly animal diets (non-insect invertebrate- and insect-dominated), between 125 and 130° for diets including animal and plant material (those of animal-dominated omnivores and omnivores), and < 131° for predominantly plant-based diets (those of herbivores and plant-dominated omnivores).

The ratios of the third to first upper molars and third to first lower molars are highly distinct in species with different diets. Herbivores and plant-dominated omnivores have third upper molars > 40% the size of the first upper molars. Omnivores and

animal-dominated omnivores have third upper molars < 40% but > 30% the size of the first upper molar. Species with non-insect invertebrate and insect-dominated diets have third upper and lower molars, < 25% the sizes of their first upper and lower molars.

Our results show that a small suite of functionally relevant dental traits can accurately determine diets for murine rodents. Furthermore, we expect that, with more precise and accurate accounts of diet or an expanded sample of species with better data, better distinctions between diet types, and therefore a clearer picture of dental evolution will be possible. Finally, there is more consistency in the evolutionary trajectories through morphospace in apparent adaptation to dietary changes (revealed by accounting for phylogenetic history) than among the static morphology of living species; the latter being the product of both adaptation and inherited shape. Methods like pDFA that account for phylogeny appear to be more powerful than their conventional applications.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Time-calibrated ultrametric chronogram from the Beast analysis. Posterior probabilities (PP) are indicated on the nodes. All other nodes (not annotated) are strongly supported (PP > 0.95). Node bars denote the 95% highest posterior densities. Nodes constrained in the analysis based on fossil calibrations are indicated with numbers inside squares that correspond with the fossils described in Supporting Information (Table S12). Relative warp deformation grid. Herb, herbivory; Invert, invertebrate-dominated diet; Omn, omnivory.

Figure S2. Principal components analysis ordination based on all species and dental characteristics. Omnivore-PD, plant-dominated omnivory; Omnivore-AD, animal-dominated omnivory.

Figure S3. Phylogenetic principal components analysis ordination for species with high 'diet confidence'. Herbiv., herbivory; PD Omniv, plant-dominated omnivory; Omniv, omnivory; AD Omniv, animal-dominated omnivory; Insects, insect-dominated diet; Invert, non-insect invertebrate-dominated diet.

Figure S4. Relative warp deformation grid. Herb, herbivory; Invert, invertebrate-dominated diet; Omn, omnivory.

Figure S5. Discriminant function analysis for all species with high 'diet confidence'. Omn-PD, plant-dominated omnivory; Omn-AD, animal-dominated omnivory; Invert, non-insect invertebrate-dominated diet.

Table S1. List of species with museum catalogue numbers. USNM, Smithsonian National Museum of National History. AMNH, American Museum of Natural History. For specimens with incomplete skulls, some tooth rows were measured from different individuals. In this case, U, upper tooth row; L, lower tooth row.

Table S2. Diet information for species included in this study. N, number of specimens examined; DR, diet reliability (see Table 2 of main text for definitions of diet reliability scores); Herbiv, herbivore; PD Omniv, plant-dominated omnivore; Omniv, omnivore; AD Omniv, animal-dominated omnivore; Inverts, non-insect invertebrate-dominated diet; Insects, insect-dominated diet; monocot, monocotyledonous plant; dicot, dicotyledonous plant.

Table S3. Principal components analysis variable loadings and eigenvalues. Abbreviations as in Figure 4 of main text.

Table S4. DFA coefficients for all species. Abbreviations as in Figure 4 of main text.

Table S5. DFA diet classifications based on discriminant variables for all species and percentage correct classification. Herbiv, herbivory; Insects, insect-dominated diet; Invert, invertebrate-dominated diet; Omniv, omnivory; AD Omniv, animal-dominated omnivory; PD Omniv, plant-dominated omnivory.

Table S6. Hotelling T^2 test P-values for all species, on only five DFA-determined characteristics.

Table S7. Hotelling T^2 test P-values for species with diet reliabilities 2–4, based on 15 DFA-determined characteristics. Abbreviations as in Table 4.

Table S8. Group means on discriminant variables for species with diet reliabilities 2–4. Abbreviations as in Table 3.

Table S9. Short descriptions of each of the 105 analyzed morphological traits. The short hand trait name matches the names in the morphological dataset deposited at Dryad. Traits with '.residuals' at the end indicate that they have been size corrected by performing linear regressions against condylobasal length and recording the residuals.

Table S10. Summary of functional characteristics, including their definition, functional significance, and the method of measure (by hand or using the program ImageJ).

Table S11. Genbank accession numbers for species with sequence data used in the Beast chronogram. *Sequences are provided from *Arvicanthis somalicus*, but morphological data are collected for *A. niloticus*. **Species with no morphological data, added in order to calibrate the chronogram (see main text).

Table S12. Calibration-point distributions including estimates for the Beast analysis. All calibrations were assigned lognormal prior distributions, except for the root calibration, which was assigned a normal prior distribution. Node numbers correspond to those in Supporting Information (Fig. S3). The ages are in million years before present. StDev, standard deviation.

Table S13. DFA coefficients for the phylogenetic analysis that incorporates species with high 'diet confidence'. Trait abbreviations as in Supporting Information (Table S9).

Table S14. Standard discriminant function analysis diet classifications based on discriminant variables for species with diet reliabilities 2–4 and correct classification percentage. *A priori* assignments are the columns. Herbiv, herbivory; PD Omniv, plant-dominated omnivory; Omniv, omnivory; AD Omniv, animal-dominated

omnivory; Insects, insect-dominated diet; Invert, non-insect invertebrate-dominated diet. Overall correct classification = 71.7%.

Table S15. Principal components analysis variable loadings and eigenvalues for the phylogenetic analysis that incorporates species with high 'diet confidence'. Trait abbreviations as in Supporting Information (Table S9).

SHARED DATA

The phylogenetic tree is available at TreeBase: https://treebase.org/treebase-web/search/study/summary.html?id=18779 (Martin *et al.*, 2016).