

RESEARCH ARTICLE

Dietary inference from dental topographic analysis of feeding tools in diverse animals

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Abstract

1. Understanding the relationship between form, function and diet in feeding structures is critical to constraining the roles of organisms in their ecosystem and adaptive responses to food resources. Yet, analysis of this relationship in invertebrates has been hampered by a reliance on descriptive and qualitative characterisation of the shapes of feeding structures. This has led to a lack of robust statistical analyses and overreliance on analogy and plausibility, especially for extinct taxa and animals that are hard to observe feeding.
2. Here we test the efficacy of dental topographic metrics in quantification of form in invertebrate mandibles and assess their relationship with diet. Specifically, we analyse the mandibles of 45 species of extant orthopterans. Orthopterans' well-constrained diets make them an ideal model system for our study.
3. We find that topographic metrics applied to Orthoptera successfully recover the same relationship between dietary intractability and dental tool morphology as they do in mammals, and that combination of individual metrics in multivariate analysis most strongly captures this relationship. Furthermore, multivariate topographic metrics calibrated to the food consumed by mammals accurately predict dietary differences between orthopterans (82% taxa correctly assigned).
4. Our results demonstrate that these metrics can be used in quantitative analysis and comparison of non-homologous mouthparts to reliably investigate the relationship between diet, form and function of feeding tools across Bilateria. We anticipate that this will facilitate more rigorous ecological analysis of fossil and historical material, providing new methods to investigate adaptive responses and community-level interactions through time.

KEYWORDS

dental morphology, dietary ecology, Ecomorphology, invertebrate, macroevolution, Orthoptera, Palaeoecology, topographic metrics

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1 | INTRODUCTION

Analysis of the relationship between form and function of feeding structures provides a powerful tool for investigating the role of organisms in their ecosystem and the nature of adaptive responses to changes in food resources. This is as true for invertebrates as it is for vertebrates, yet analysis of the relationship between form, function and diet in invertebrate feeding structures has been hampered for decades because of a general reliance on descriptive and qualitative characterisation of the shapes of feeding structures. This descriptive approach has made it difficult to undertake robust statistical testing of hypotheses, leading to an overreliance on analogy and plausibility when attempting to assess the function of feeding structures in taxa that are extinct or hard to observe feeding. Despite many calls to address this issue across a range of disparate phyla (Bernays, 1991; Blanke et al., 2017; Clemo & Dorgan, 2017; Hickman, 1980; Krings et al., 2020; Padilla, 2004; Patterson, 1984), quantitative analysis has remained largely unexplored (Patterson, 1984; but see Blanke et al., 2017 as a notable exception). The few quantitative analyses of invertebrate feeding structures performed to date offer significant improvements over qualitative classifications of morphology. These include mollusc radulae, polychaete jaws, mandibles of dragonflies and beetles, and marine arthropods, but all such studies are limited in scope. Some capture a small selection of shape variables taken to have functional significance (Clemo & Dorgan, 2017; Patterson, 1984), while the handful of recent applications of Finite Element Analyses to invertebrate feeding structures (Bicknell et al., 2018; Blanke et al., 2017; Hörnschemeyer et al., 2013; Krings et al., 2020) are focused on small numbers of taxa and individuals because of the computational demands and time-consuming nature of robust FEA.

Here we adopt a different approach, offering a potentially powerful and widely applicable solution to quantification of the morphology of feeding structures in invertebrates. Dental topographic analysis (Evans et al., 2007; Karme, 2008; M'kirera & Ungar, 2003; Pineda-Munoz et al., 2017; Ungar et al., 2018) was developed primarily for analysis of mammal teeth, but its applicability outside of gnathostome vertebrates has never been tested (apart from a preliminary proof of concept study; see Purnell & Evans, 2009). The methods are not computationally demanding and capture a number of functionally significant attributes, potentially from any kind of tooth-like structure (i.e. any anatomical component involved in the processing of food items immediately prior to ingestion). Dental topographic analysis lends itself to statistical testing and allows quantitative comparison of non-homologous structures.

Our approach addresses the long-standing call for quantification of shape in invertebrate feeding structures, exploring a range of hypotheses designed to test the relationship between dental topographic metrics and diet. Establishing this relationship will allow dental topographic measures to serve as dietary ecometrics, and extend the potential applications of this relatively simple and efficient approach to diverse homologous and non-homologous feeding structures in disparate animals.

2 | MATERIALS AND METHODS

To test whether dental topographic metrics, developed and validated on mammals, can be generalised to other groups with non-homologous dental tools, we applied them to invertebrates with well-constrained diets. Our overarching null hypothesis is that dental topographic metrics do not reflect differences between dietary categories in invertebrates. Rejection of this hypothesis leads to questions of the degree to which the dental topographic metrics track diet and the degree to which results from non-homologous feeding structures are comparable. We addressed this by testing two further hypotheses: first, that dental topographic metrics are correlated with diet in invertebrates; second, that the absolute values of dental topographic metrics are comparable between invertebrates and vertebrates that have similar diets but non-homologous food-processing structures. If this second hypothesis finds support, it follows that multivariate topographic metrics can be calibrated to the food consumed by one group of animals, and then used to infer diet in a distant group with non-homologous feeding structures. We tested this using multivariate analysis to compare feeding structures and diet in vertebrates and invertebrates.

Our analysis uses a number of topographic metrics (Figure 1; Figure S1). Orientation Patch Count Rotated (OPCR) measures the complexity of a functional food-processing surface (Evans et al., 2007; Wilson et al., 2012). Other topographic metrics—mean slope, relief index, angularity and sharpness—measure the angle, height, roughness and pointedness of the tooth surface (Karme, 2008; M'kirera & Ungar, 2003; Ungar et al., 2018). When used individually, each of these metrics captures only a single aspect of tooth morphology. Multivariate analysis, on the other hand, combines metrics to provide a more holistic evaluation of the shape of dental tools. In mammals, this approach, sometimes referred to as MPDMA (multi proxy dental morphological analysis, Pineda-Munoz et al., 2017), allows for stronger inferences of diet from tooth shape (Pineda-Munoz et al., 2017). We follow this approach here.

Our analysis is based on 45 species of extant orthopterans. Orthopterans are ideal for testing our hypotheses because diets of many taxa are well categorised on the basis of *in situ* field observations, differential feeding experiments, and crop and faecal analysis (Eiela et al., 2010; Gangwere, 1967; Gangwere et al., 1976; Gangwere et al., 1998; Gangwere & Spiller, 1995; Kang et al., 1999). Our analysis is based on the entire gnathal edge, from the pars incisor to the pars molaris, of the left mandibles removed from dry museum specimens (Figure 1), selecting the best preserved of the available specimens representing the final instar. Despite selecting the best specimens available, there was some variation in the amount of mandibular wear.

2.1 | Dietary classification of orthopteran mandibles

Species were classified as faunivorous, forbivorous, herbivorous or graminivorous based on the relative proportion of foodstuffs in their

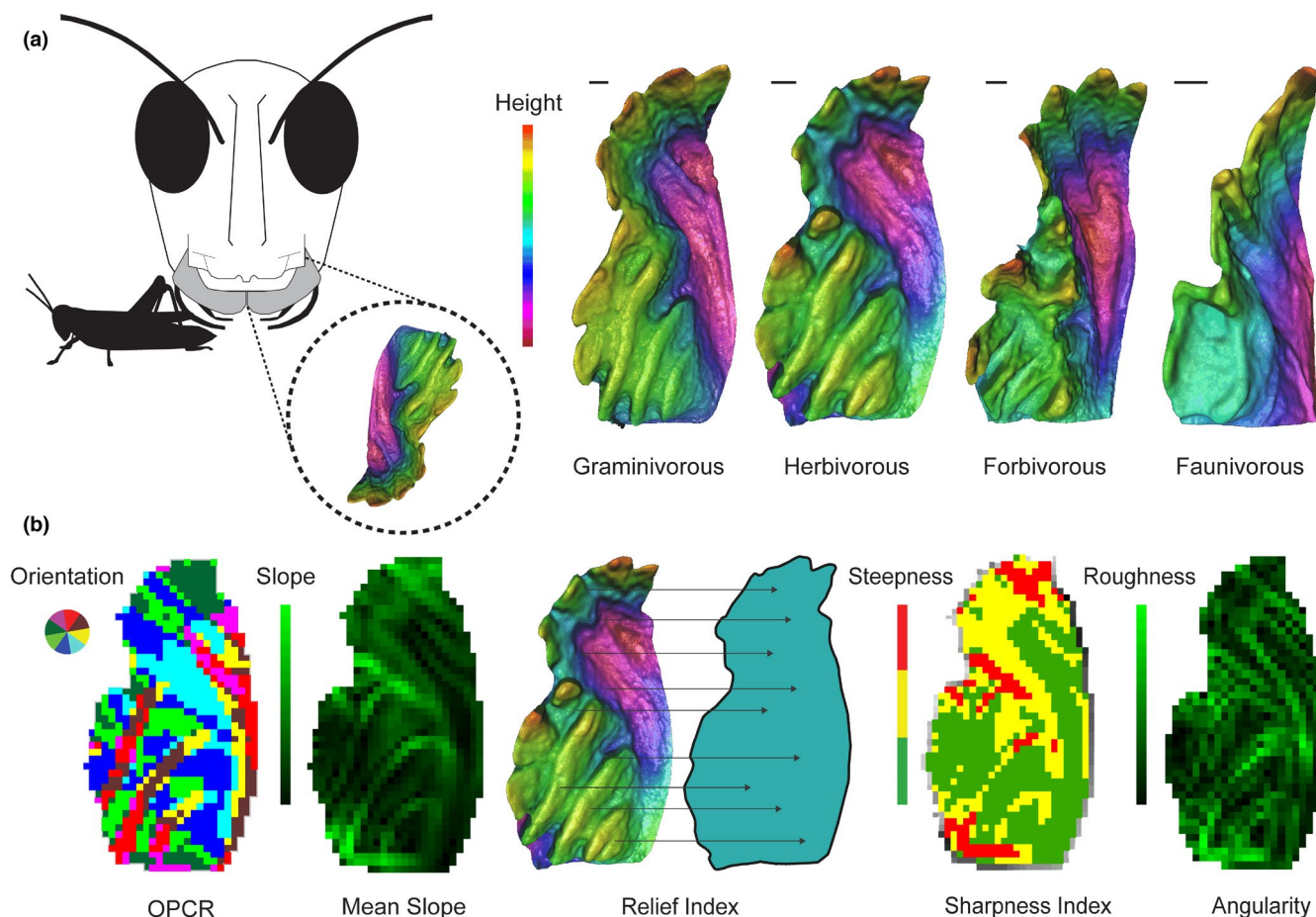


FIGURE 1 (a) The functional surfaces of orthopteran left mandibles representative of each dietary category. Scale bars = 100 μ m. Graminivorous, *Aiolopus strepens*, NHMUK 012504759. Herbivorous, *Chorthippus dubius*, NHMUK 013803298. Forbivorous, *Angaracris barabensis*, NHMUK 013803273. Faunivorous, *Velarifictorus micado*, NHMUK 012504733. (b) Topographic metrics used to analyse orthopteran mandibles. Orientation patch count rotated, OPCR: measuring complexity as the number of patches on a surface, with the number averaged after stepwise rotations (a patch being a set of contiguous points with the same orientation, as indicated in colour wheel). Mean slope: measured as the average steepness between grid points, slope as shown by colour gradient. Relief index: The ratio of the 3D surface area to its projected 2D surface area. Sharpness index: the relative proportion of the steepest section of a slope. Sections were categorised using the natural breaks classification. Angularity: the second derivative of slope, capturing surface roughness as shown in gradient. Pixelation of images in B reflects reduction of datasets to 50 rows prior to analysis (see methods). Metrics visualised for *Chorthippus dubius*, NHMUK 013803298

diet (Table S1). Diets were classified based on both quantitative and qualitative descriptions. Faunivores preferentially eat other insects and oligochaetes. Forbivores predominantly eat forbs, c. >75% (herbaceous flowering plants that are not grasses). Herbivores eat forbs and grasses in roughly equal proportions. Graminivores predominantly eat grasses, c. >75%.

For analysis, diets were ranked in order of relative intractability based on the material properties of constituent foodstuffs. In order of increasing intractability: (1) faunivory; (2) forbivory; (3) herbivory; (4) graminivory. Intractability describes the structural strength of a foodstuff and how much processing it requires (Evans & Sanson, 2005). Animal muscle and soft tissues require less processing than chitinous exoskeletons, and plant material is more intractable than both (Ennos, 2011; Evans et al., 2007). C3 photosynthesising herbaceous plants are less tough than C4 photosynthesising grasses (Bernays, 1991; Cotterell, 2010).

2.2 | Model generation and orientation of orthopteran mandibles

2.5D models of mandibles were generated using focus variation optical microscopy. To ensure metrics were generated consistently across all samples, orientation was standardised both mechanically during data acquisition and digitally after model production. Specimens were oriented to maximise data capture during model generation. For topographic metric analysis, models must be oriented with the functional surface orthogonal to the Z-axis. To orient the molar region orthogonal to the Z-axis, model orientation was standardised digitally using an 'automatic robust' method in MeasureSuite 5.1 (Alicona Imaging, GmbH). Models were cropped to their functional surface and undercuts, where models fold under themselves to produce an overhang, were removed using MeasureSuite and Rhinoceros 6 (Robert McNeel & Associates). For consistency, the

X-axis was arbitrarily set in Rhinoceros as being parallel to the oral boundary of the model. Figure 1 shows the examples of mandible surface models oriented as measured.

2.3 | Topographic metric generation and analysis of orthopteran mandibles

Models were processed using SurferManipulator (Alistair Evans; version 20110921) and Surfer® 14 (Golden Software, LLC). To permit direct comparability with previous work oriented models for single teeth were reduced to grid maps of coordinates with 50 rows of points along the Y axis (mandible length); the number of columns along the X axis of the grid was allowed to vary with mandible width (Evans et al., 2007; Pineda-Munoz et al., 2017; Rannikko et al., 2020; Smits & Evans, 2012; Winchester et al., 2014). For comparison with previous analyses of rows of teeth, point clouds were reduced to 150 rows (included in the supplement). OPCR measures the number of 'patches' on a surface, with the number averaged after stepwise rotations (a patch being a set of contiguous points with the same orientation; Figure 1). In OPCR analysis, the minimum patch size was set to 3 and models were rotated stepwise eight times (Wilson et al., 2012). Slope was measured as the steepness between grid points on the mandible surface between 0° and 90° (Figure 1). Mean slope was calculated from these measurements. Angularity, capturing the roughness of the surface, is the second derivative of slope, calculated as the mean slope of the initial slope map (Figure 1). Relief index was calculated as the ratio of the 3D surface area to its projected 2D area (Figure 1). Sharpness captures the relative proportion of the steepest section of a slope (Figure 1). Sharpness index was calculated in ArcMap™ 10.8 (Esri® Inc.) using the natural breaks method classification (Jenks' optimisation) with three classes (Karme, 2008; Ungar et al., 2018). The surface slopes were divided into three regions with the smallest possible variation within each and maximum possible variation between them. Sharpness was calculated by dividing the size of the steepest region of the slope by the combined size of the other two shallower regions. Multivariate analysis was performed through a PCA of the five individual topographic metrics. PCA was performed on the correlation matrix and factor loadings were not rotated. The resulting five principal components were saved. All statistical analyses were performed in JMP® 14 (SAS Institute) unless otherwise stated. All analyses presented here were performed on models reduced to 50 data rows. Univariate metrics of models reduced to 150 data rows are included in the supplement.

To test the discriminatory power of individual topographic metrics and multivariate analysis of orthopterans, we performed ANOVA of each topographic metric and MANOVA of the first four principal components combined. To reduce the risk of false-positive results from ANOVA due to violations in the assumption of equal variances, when variances were found to be unequal by any statistical test, Welch's ANOVA was performed. All topographic metrics and principal component values in each dietary category were normally distributed (Tables S4–S7; Shapiro–Wilk, $\alpha = 0.05$). In

Shapiro–Wilk tests, a Benjamini–Hochberg procedure with a false discovery rate of 0.05 was used to control for multiple comparisons (Benjamini & Hochberg, 1995). Tukey HSD tests were used to assess pairwise differences between dietary categories. We also tested the hypothesis that dietary categories with bigger differences in relative intractability have a larger number of significant differences across topographic metrics. This hypothesis was tested using Spearman's rank correlations to compare the difference in ranked dietary intractability values with the number of significantly different univariate and multivariate topographic metrics for each pair of dietary categories. To test the hypothesis that multivariate metrics (principal components) can be used to accurately predict diet in invertebrates an LDA of principal components 1–4 was performed. The relationship between dietary intractability and topographic metrics was tested using Spearman's Rank correlations of ordinal ranks of dietary intractability with individual metrics and with principal components. In instances where multiple comparisons were made these were controlled for using a Benjamini–Hochberg procedure with a false discovery rate set at 0.05 (Benjamini & Hochberg, 1995).

2.4 | Controlling for phylogenetic effects in orthopterans

Our comparison of metrics between orthopterans and mammals allows us to test the hypothesis that dental topographic metrics capture dietary signals that span broad phylogenetic distances. Nonetheless, we tested the hypothesis that the relationship between metric values and diet within orthopterans is more a reflection of morphological similarities corresponding to closeness of evolutionary relationships than it is a measure of ecological similarity. This requires a hypothesis of relationships within Orthoptera, but no phylogenetic tree containing all of our 45 taxa has been published and there is insufficient sequence data to produce one. We produced an informal supertree of all 45 taxa from published literature (Figure S2), constructed on the basis of well-supported nodes reflecting character state changes and genetic divergence (Chapco & Contreras, 2011; Chen et al., 2018; Fries et al., 2007; Mugleston et al., 2018; Rowell & Flook, 1998; Song et al., 2018; Zhang et al., 2013). Polytomies were used where nodes separating taxa in published literature were poorly supported (e.g. low bootstrap value).

Phylogenetic closeness was calculated as the pairwise distances of summed branch lengths between taxa, treating all individual branch lengths as equal to 1. This assumption makes the analysis tractable in the absence of a phylogeny with branch lengths, but it potentially underestimates correlations between phylogenetic closeness and topographic metrics across orthopterans because it captures topology but not differences in individual branch lengths. Mantel tests analysed correlations of phylogenetic closeness with pairwise distances of topographic metrics and principal component values, using the R package ADE4 (Dray & Dufour, 2007). Orthoptera is made up of two monophyletic suborders, Ensifera and Caelifera (Flook & Rowell, 1997). All of the faunivorous taxa in our study are members of Ensifera and make up 9 of 12 ensiferan species represented. To account for any

potential bias arising from this, correlations of phylogenetic closeness with pairwise differences in topographic metric and principal component values were tested across Orthoptera and Caelifera separately. A Benjamini–Hochberg procedure with a false discovery rate set at 0.05 was used to control for multiple comparisons.

2.5 | Comparison of non-homologous feeding structures: Orthopterans and mammals

Values for mean OPCR, mean slope and mean relief index of mollars downsampled to 50 data rows were obtained from the literature for 93 mammal taxa representative of the orders Artiodactyla, Carnivora, Diprotodontia, Rodentia and Primates (Pineda-Munoz et al., 2017; Rannikko et al., 2020).

Plant-based diets in mammals were reclassified as ‘herbivorous’ according to our dietary scheme due to a lack of published data that allows unequivocal comparisons between mammals and orthopterans at a finer scale of dietary resolution (the only exception being *Phacochoerus africanus* which was reclassified as a graminivore; Pineda-Munoz et al., 2017; Rannikko et al., 2020). Carnivorous and insectivorous mammals retained their dietary categories (Pineda-Munoz et al., 2017) for comparison with faunivorous orthopterans. Multivariate analysis was performed through a PCA combining orthopterans and mammals, based on the three individual topographic metrics available for all. PCA was performed on the correlation matrix and factor loadings were not rotated. The resulting three principal components were saved. We were unable to reject the

null hypothesis of normality for topographic metrics and principal components for each dietary category (Table S13; Shapiro–Wilk, $\alpha = 0.05$). In Shapiro–Wilk tests, a Benjamini–Hochberg procedure with a false discovery rate of 0.05 was used to control for multiple comparisons (Benjamini & Hochberg, 1995). ANOVA of principal component 1 was used to test for differences between dietary categories for orthopterans and mammal orders; post-hoc Tukey HSD tests were used to assess pairwise differences.

The most stringent test of comparability of multivariate topographic metric values across non-homologous feeding structures was provided through LDA of the first three principle components of the combined mammal and orthopteran analysis, using the resulting multivariate metric (PC1) values of mammals to predict dietary categories in orthopterans. All taxa were classified as either ‘herbivorous’ (diet made up of >50% or preferentially eat plants) or ‘carnivorous’ (diet made up of >50% or preferentially eats other animals) to avoid dietary categories unique either to mammals or orthopterans.

3 | RESULTS

Our results allow us to unequivocally reject the null hypothesis that dental topographic metrics do not differ between dietary categories in the invertebrates analysed. OPCR, mean slope, relief index and angularity all differ between dietary categories (Table 1; ANOVA, $p < 0.05$). Mean slope and OPCR each successfully differentiate between diets in four pairs of categories, more than the other univariate metrics (Table 2; Table S8; Tukey HSD, $p < 0.05$). We did not test

TABLE 1 ANOVA of topographic metrics and principal components, resulting from a multivariate analysis combining them, between dietary categories in orthopterans. Superscript W denotes Welch’s ANOVA was performed as a result of unequal variances. Significance after FDR control, shown in bold, indicates p values lower than the Benjamini–Hochberg critical value, using a false discovery rate of 0.05

Topographic metric	<i>F</i>	<i>p</i>	<i>df</i>	Significance after FDR control
OPCR	16.396	<0.001	3, 41	Yes
Mean Slope	18.986	<0.001	3, 41	Yes
Relief Index ^W	11.344	<0.001	3, 13.702	Yes
Angularity	4.307	0.010	3, 41	Yes
Sharpness	1.636	0.196	3, 41	—
Principal Component 1	23.421	<0.001	3, 41	Yes
Principal Component 2	1.517	0.224	3, 41	—
Principal Component 3	0.197	0.898	3, 41	—
Principal Component 4 ^W	3.054	0.056	3, 17.285	—
Principal Component 5 ^W	2.924	0.060	3, 19.219	—

TABLE 2 Significant pairwise differences (Tukey HSD) between dietary categories in orthopterans; lower left of table tallies the variables that differ

	Faunivorous	Forbivorous	Herbivorous	Graminivorous
Faunivorous	—	OPCR	PC1, OPCR, mean slope	PC1, OPCR, mean slope, relief index, angularity
Forbivorous	1	—	PC1, mean slope	PC1, OPCR, mean slope, relief index
Herbivorous	3	2	—	
Graminivorous	5	4	0	—

the relationship between mandibular wear and dental topographic metrics, but the results suggest the method is insensitive to a degree of variation in wear, as it is in mammals (Evans et al., 2007).

The first four principal components (PC) of the PCA capture 99% of the variance. MANOVA of PC1–4 shows significant differences between dietary categories (Figure 2a,b; MANOVA, $F = 4.056$, $p = 0.013$, $df = 3$). Taken individually, PC1 differs between dietary categories (Table 1; ANOVA, $F = 23.421$, $p < 0.05$, $df = 3, 41$) and successfully differentiates between four pairs of categories, the same as OPCR and mean slope (Table 2; Table S8; Tukey HSD, $p < 0.05$). Difference in intractability between pairs of diets correlates positively with the number of univariate topographic metrics and principal components that find significant differences between them (Spearman's rank, $\rho = 0.926$, $p = 0.008$, $df = 4$).

Linear discriminant analysis of PC1–4 classified 72% of orthopterans to the correct dietary category (Figure 2c). Taxa that were misclassified were commonly assigned to a dietary category reflecting consumption of the same food but in differing relative proportions. For example, 1 graminivorous species was misclassified as a forbivore, and two forbivorous species were misclassified as graminivores (see Table S9).

Correlation tests provide further support for the strength of the relationship between topographic metrics and dietary intractability. OPCR, mean slope, relief index and angularity all correlate with dietary intractability (OPCR and mean slope particularly strongly; Table 3). PC1 correlates more strongly with dietary intractability than any univariate topographic metric (Table 3; Spearman's rank, $\rho = -0.805$, $p < 0.001$, $df = 43$). No other principal components correlate with dietary intractability (Table 3).

Our results indicate rejection of the hypothesis that the relationship between metric values and diet in orthopterans is predominantly a reflection of phylogenetic rather than ecological similarity. The correlations of phylogenetic closeness and topographic metric values across the order Orthoptera and suborder Caelifera are significant but weaker than correlations between metric values and dietary intractability detailed above (e.g. for PC1, which is strongly correlated with diet: Mantel test across Orthoptera, $R = 0.396$, $p = 0.001$; within suborder Caelifera, $R = 0.175$, $p = 0.006$; See Table S2 for details).

Comparison of dental topographic metrics for orthopterans with mammals indicates dietary similarities, in spite of the phylogenetic distance between them. This comparability extends to the absolute values of the dietary metrics, not just their relative values in the overall range for their taxon. PCA of orthopteran and mammalian taxa reveals that PC1 primarily reflects variation between taxa with respect to diet (Figure 3). Dietary categories separate along PC1 in order of dietary intractability (Figure 3), and orthopteran dietary categories separate in the same order as in the orthopteran only analysis (Figures 2 and 3). In mammals, PC1 scores of insectivores, which have a more intractable diet, are lower than carnivores (Figure 3). Faunivorous orthopterans' PC1 scores are more similar to insectivorous mammals than they are to carnivorous mammals (Figure 3), and ANOVA of PC1 values comparing faunivorous Orthoptera

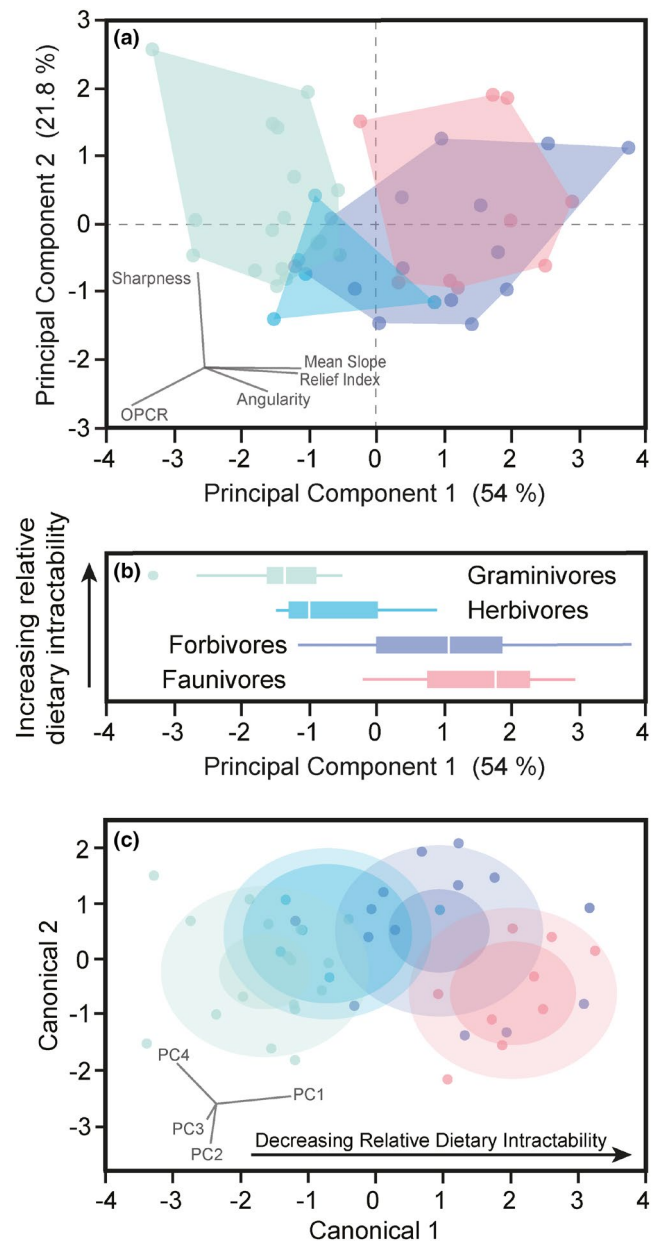


FIGURE 2 Multivariate analyses of five topographic metrics (OPCR, mean slope, relief index, angularity and sharpness) for left mandibles of 45 orthopteran species. Colour coding of dietary categories follows b. (a) Principal components analysis, showing the first two components. (b) Boxplots of principal component 1; outliers are shown as individual points. (c) Linear discriminant analysis of the first-four principal components. Outer, paler ellipses indicate the region estimated to contain 50% of the population for that group. Inner, darker ellipses indicate the 95% confidence region of the group estimated to contain the true mean of the group

and insectivorous mammal orders (Diprotodontia, Carnivora and Primates) fails to reject the null hypothesis that they are not different (Figure 3; Table 4; ANOVA, $F = 1.564$, $p = 0.222$, $df = 3, 26$). PC1 values differ between faunivorous Orthoptera and carnivorous mammals (Carnivora; Welch's T test, $t = -6.276$, $p < 0.001$, $df = 26.374$). PC1 values of herbivorous Orthoptera overlap with

TABLE 3 Results of Spearman's rank correlation tests between topographic metrics calculated for orthopteran mandibles and relative dietary intractability. Significance after FDR control, shown in bold, indicates p values lower than the Benjamini–Hochberg critical value, using a false discovery rate of 0.05

Topographic metric	Spearman ρ	p	df	Significance after FDR control
OPCR	0.701	<0.001	43	Yes
Mean Slope	−0.743	<0.001	43	Yes
Relief Index	−0.655	<0.001	43	Yes
Angularity	−0.441	0.002	43	Yes
Sharpness	0.178	0.241	43	—
Principal Component 1	−0.805	<0.001	43	Yes
Principal Component 2	0.091	0.551	43	—
Principal Component 3	0.079	0.607	43	—
Principal Component 4	0.242	0.110	43	—

herbivorous mammals (Figure 3), but ANOVA comparing herbivorous Orthoptera with herbivorous mammals (Artiodactyla, Carnivora, Diprotodontia, Rodentia and Primates) finds some significant differences (Table 4; ANOVA, $F = 7.456$, $p < 0.001$, $df = 5, 47$; pairwise differences [Table S14; Tukey HSD, $p < 0.05$], Orthoptera differ from Carnivora, Diprotodontia and Rodentia, but not from Artiodactyla and Primates). PC1 values of forbivorous orthopterans overlap more with herbivorous mammals than herbivorous orthopterans do (Figure 2), and while ANOVA finds a significant overall difference between forbivorous Orthoptera and herbivorous mammal orders, there is only one pairwise difference (Table 4; Table S15; ANOVA, $F = 2.719$, $p = 0.029$, $df = 5, 56$; Diprotodontia differ from Orthoptera, Tukey HSD, Diff = 0.729, $p < 0.05$; Artiodactyla, Carnivora, Primates and Rodentia do not, Tukey HSD, $p > 0.05$).

Our results also support the hypothesis that the relationship between multivariate topographic metrics and diet in one group can provide meaningful information on dietary differences in another group with non-homologous feeding structures. Linear discriminant analysis of the three principal components from the combined PCA, using the known diets of the mammalian taxa as a training dataset, correctly predicts orthopteran diets as herbivorous or carnivorous 82% of the time (the LDA performs better than for mammalian taxa, which were correctly classified only 62% of the time).

4 | DISCUSSION

Orthopterans and mammals shared a last common ancestor more than half a billion years ago (Dos Reis et al., 2015), and that ancestor lacked teeth, jaws or mandibles of any kind (Erwin & Davidson, 2002). However, our analysis of the essential topographic features of food-processing structures reveals a common underlying signal of morphological adaptation, driven primarily by the intractability of their diets. Unequivocal rejection of our null hypothesis and testing of the alternatives demonstrates that dental topographic measures track diet and are comparable as dietary metrics between non-homologous structures in disparate groups of organisms.

Our analysis of orthopterans indicates that dental topographic metrics capture aspects of shape of feeding structures that reflect

adaptations for food processing and intractability of foodstuffs. Complexity (OPCR), mean slope, relief index and angularity correlate with diet, and the number of pairwise differences between metrics correlates with the distance between ranked dietary categories (Table 2; Table 3; Tables S4–S7): the most different diets exhibit the greatest number of differences. Complexity correlates positively with dietary intractability (Table 3) while mean slope, relief index and angularity, each measuring a different aspect of the pointedness or height of a feeding element, correlate negatively. All these relationships are the same as those recovered in analyses of mammal teeth (Boyer, 2008; Bunn & Ungar, 2009; Evans et al., 2007; Evans & Janis, 2014; M'kirera & Ungar, 2003; Pineda-Munoz et al., 2017; Rannikko et al., 2020; Ungar et al., 2018).

Of the univariate metrics investigated, only sharpness fails to exhibit a significant pairwise difference between diets in orthopterans (Tables S4–S7). This is likely the result of the involvement of orthopteran mandibles in both food prehension and food processing (Isely, 1944). All mandibles must have sharp enough edges to cut and separate consumable fragments from larger foodstuffs, reducing variation in absolute values between dietary categories. Clearly, this is unlike the situation in organisms with functionally differentiated dentitions, such as mammals. Alternatively, the absence of significant pairwise differences in sharpness between dietary categories could be due to phylogenetically conserved developmental constraints on mandible morphology in orthopterans (Maynard Smith et al., 1985). However, as we discuss below, the relationship between topographic metric values and diet is primarily ecological.

We find that multivariate metrics more fully capture the ecological relationship between feeding element morphology and diet than any univariate metric in invertebrates, as reported in vertebrates (Pineda-Munoz et al., 2017). Multivariate metrics differentiate between and predict diet with a high degree of accuracy and precision in orthopterans, with LDA achieving 72% correct assignment. Furthermore, PC1 exhibits the strongest relationship with dietary intractability of any metric (Table 3), while being impacted less by the closeness of evolutionary relationships than similarly successful univariate metrics (Table S2). This corroborates the hypothesis that more powerful dietary inferences can be made by combining topographic variables.

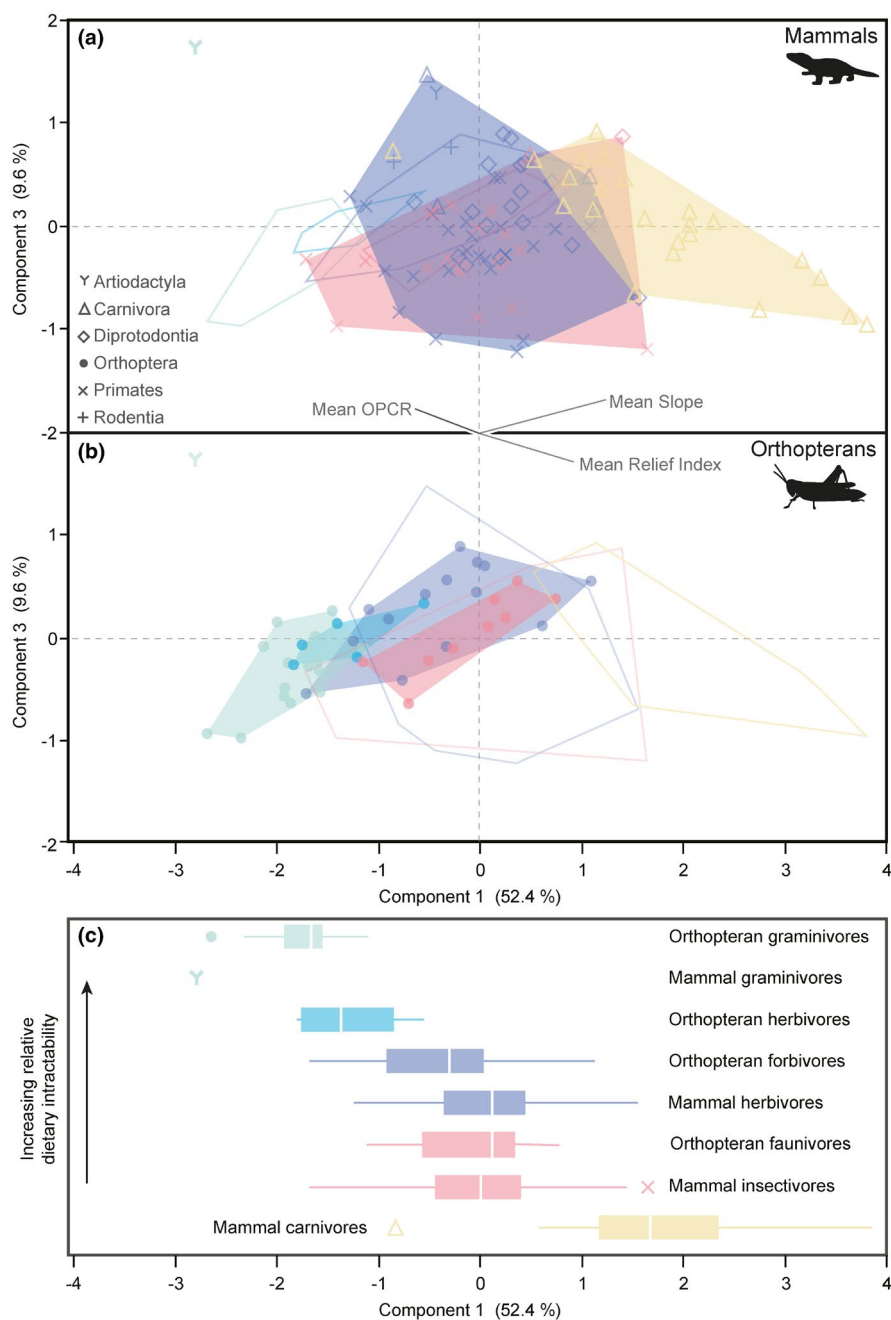


FIGURE 3 Plot of principal components 1 and 3 from analysis of three topographic metrics (mean OPCR, mean slope, mean relief index) for 45 orthopteran and 93 mammalian species with comparable diets (Pineda-Munoz et al., 2017; Rannikko et al., 2020). For clarity, the two panels focus on visualising results for mammalian (a) and orthopteran taxa (b), but the axes and convex hulls are identical. PC2, not shown, primarily reflects the separation of orthopterans from mammals. *Ursus maritimus*, a hypercarnivore, is omitted from the vertebrate carnivorous convex hull because its dentition largely reflects its ancestral herbivorous state due to its recent speciation (Evans et al., 2007). (c) Boxplots indicating the distribution of principal component 1. Outliers are shown as individual points (orthopteran graminivore, *Eucothippus angustulus*; mammal graminivore, *Phacochoerus africanus*; mammal insectivore, *Hydromys chrysogaster*; mammal carnivore, *Ursus maritimus*)

TABLE 4 ANOVA of principal component 1 values for comparable dietary categories between orthopterans and mammalian orders. Significant differences are shown in bold

Orthopteran dietary category	Mammal dietary category	F	p	df
Faunivory	Insectivory	1.564	0.222	3, 26
Herbivory	Herbivory	7.456	<0.001	5, 47
Forbivory	Herbivory	2.719	0.029	5, 56

Our analyses confirm that the relationship between topographic metric values and diet in orthopterans is predominantly a measure of ecological similarity rather than a reflection of closeness

of evolutionary relationships. The similarity of multivariate metrics between orthopterans and mammals with similar diets discussed below demonstrates this further.

Multivariate analysis provides powerful confirmation that the relationship between form and function in feeding structures holds true across phylogenetic distance: non-homologous feeding structures in distantly related taxa are comparable. Combined analysis of orthopterans and mammals finds that PC1 values are similar between comparable dietary categories in mammals and orthopterans. Even more compelling are the results of linear discriminant analysis: LDA-based entirely on the scores for mammals from the combined principle components analysis correctly differentiates between herbivorous and faunivorous orthopterans

with a level of accuracy that is marginally better than its ability to correctly categorise mammals (82% of orthopterans correctly assigned to dietary category).

Faunivorous orthopterans are indistinguishable from insectivorous mammals grouped by taxonomic order (ANOVA, $F = 1.564$, $p = 0.222$, $df = 3, 26$). This reflects similarity of diet, with orthopteran faunivores preferentially eating chitinous insects rather than softer food, such as oligochaetes (Eiela et al., 2010; Gangwere, 1967). In both invertebrates and vertebrates, graminivores have the lowest PC1 scores of any dietary category (Figure 3). Forbivorous orthopterans are more similar to herbivorous mammals than are herbivorous orthopterans (Figure 3; Table 4). This may in part reflect the coarser classification of mammal diets, which grouped browsers and grazers together as herbivores (Pineda-Munoz et al., 2017; Pineda-Munoz & Alroy, 2014), potentially masking the distinction between diets dominated by forbs, by grasses and more generalised herbivores. However, these and other significant differences between the absolute values of dental topographic metrics from orthopterans and mammals with similar diets are likely the result of functional constraints. As explained above, orthopteran mandibles must be sharp enough to function in food prehension as well as processing, unlike the functionally differentiated dentitions of mammals, in which molars are not involved in prehension. As a result, and particularly in mammals with less tractable diets, it is not uncommon for molars to have reduced or a complete absence of sharp edges (Boyer, 2008; Bunn & Ungar, 2009; Evans et al., 2007; Evans & Janis, 2014; M'kirera & Ungar, 2003; Pineda-Munoz et al., 2017; Rannikko et al., 2020; Ungar et al., 2018). Therefore, it is unsurprising that the PC1 scores of herbivorous orthopterans differ from the molars of some mammal orders (Table S14; ANOVA, $F = 7.456$, $p < 0.001$, $df = 5, 47$; Tukey HSD, $p < 0.01$). Furthermore, these differences are unlikely to be the result of phylogenetically conserved developmental constraints on dental tool morphology because topographic metrics primarily recover an ecological signal in the relationship between dental tool form and function in both invertebrates, as shown here, and vertebrates (Pineda-Munoz et al., 2017; Table 3; Table S2). This demonstrates that, while for the most part non-homologous food-processing structures from animals with similar diets are comparable, some consideration of functional constraints should be made.

By rejecting the null hypotheses that topographic metrics do not reflect dietary differences in invertebrates, our results confirm that they can provide a powerful method for quantitative analysis of feeding element morphology and its relationship to diet and function in animals outside of jawed vertebrates. Multivariate topographic metrics more fully capture the ecological relationship between feeding element form and function, convergent across non-homologous structures. By doing so, topographic metrics of feeding elements can provide robust and accurate predictions of diet in distantly related taxa. This highlights the potential of topographic metrics for investigating dietary ecology in invertebrate taxa for which in situ feeding observations are not available, including extinct taxa, for many of

which diet is poorly constrained. For example, being able to interpret the dietary significance of scolecodont morphology, which provides a fossil record of annelid jaws extending back into the Cambrian Period, would unlock not only their potential to reveal the adaptive responses of polychaetes over almost 500 million years of biosphere evolution, but also the functioning of sediment infaunal communities through time (Clemo & Dorgan, 2017). Furthermore, testing hypotheses of dietary ecology in deep time provides a framework for analysis of predator-prey arms races as a driver of trophic tiering in animal diversification (e.g. Peterson and Butterfield (2005); Sperling et al. (2013); Klug et al. (2017)).

5 | CONCLUSIONS

Dental topographic metrics provide a straightforward method for investigating the relationship between form and function of feeding structures, and our work addresses the long-standing call for quantitative approaches to analysis of feeding structures in invertebrates. Our results indicate a high degree of comparability of dental tools and dietary intractability in animals separated by vast phylogenetic distances. Further validation of the relationship between dental topographic metrics and diet would be worthwhile, adding dietary specifics for non-gnathostome groups in particular. But it is clear that multivariate dental topographic analysis can be applied with confidence to a wide range of feeding structures with tooth-like functions, enabling quantitative analysis and statistical hypothesis testing of the relationships between form, function and diet across much of bilaterian phylogeny and through half a billion years of evolution.

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CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Conceptualisation: M.A.P. and T.H.P.H.; Data Curation: C.S.; Formal Analysis: C.S. and M.A.P.; Funding Acquisition: M.A.P.; Investigation: C.S. and M.A.P.; Methodology: C.S., N.F.A., M.A.P., P.C.J.D. and Aileen O'Brien; Project Administration: C.S. and M.A.P.; Resources: C.S., M.A.P. and B. Price (UK Natural History Museum); Software: C.S. and N.F.A.; Supervision: M.A.P., T.H.P.H. and P.C.J.D.; Validation: C.S.; Visualisation: C.S. and M.A.P.; Writing—Original Draft Preparation: C.S. and M.A.P.; Writing—Review and Editing: C.S., M.A.P., P.C.J.D., N.F.A. and T.H.P.H.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Topographic metric values (orthopteran and mammalian) and 2.5D models of the functional surfaces of orthopteran mandibles are made available at <https://doi.org/10.25392/leicester.data.17840096>.

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