

# Biomechanical properties of insects in relation to insectivory: cuticle thickness as an indicator of insect ‘hardness’ and ‘intractability’

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**Abstract.** The concept of ‘hardness’ has long been used to describe the biomechanical properties of the diet of many animals. However, due to the lack of a consistent definition, and the multitude of uses to which the term has been put, the use of the term ‘intractability’ has been advocated here to represent the extent to which the structural strength, stiffness and toughness are increased in a foodstuff. The thickness of the cuticle of an insect was a good measure of the intractability of cuticle. The tremendous advantage of the use of cuticle thickness as a measure of the biomechanical properties of invertebrates means that the dietary properties of a living insectivore can be directly quantified according to the thickness of the cuticle in its faeces. The quantitative measurement of intractability obtained through this technique can be used in correlations with adaptations of the masticatory apparatus, including tooth and skull morphology, as well as more general considerations of ecology. This is a major advance on previous measures of the biomechanical properties of insectivore diets, and may represent the best technique of any dietary group in assessing the properties of its diet.

## Introduction

Invertebrates, like all organisms, are faced with many mechanical challenges throughout their lives. Locomotion (including flight), feeding and burrowing exert a diversity of stresses and strains on the organism, which is reflected in the variety of materials that have evolved to cope with these functions (such as the extreme extensibility in the locust intersegmental membrane for ovipositing: Vincent 1975). Small mammalian insectivores, such as microbats and shrews, must chew their food very finely and so must deal with the biomechanical properties of the invertebrates. It is firmly established that the biomechanical properties of foods influence the morphology and function of the feeding apparatus of an extremely wide range of organisms (e.g. primates: Lucas 1979; fish: Wainwright 1996; insects: Peeters 2002). Of particular interest to this study are the differences in skull and dental morphology that correlate with dietary ‘hardness’ found among insectivores (Freeman 1979; Strait 1993; Dumont 1995).

The major objective of this study was to establish a sound and accurate method for assessing the biomechanical properties of insects that are most relevant to insectivore feeding. Our contention is that ‘hardness’ as a description of an insectivore’s diet is not very informative and is in need of replacement. As a superior alternative to previous methods, we examine the use of cuticle thickness as a measure of an invertebrate’s ‘hardness’ or ‘intractability’. The intractability of an insectivore’s diet can be estimated from the thickness of the insect cuticle in its faeces.

Many previous studies have used the concepts of materials engineering to examine the biomechanical properties of the bodies of invertebrates. Fundamental to the understanding of materials are the concepts of stress and strain: stress is the load per unit area of the material, and strain is the amount of extension under load per unit length (Gordon 1976). Derived from these are the material properties of strength, which is the resistance of a material to crack initiation (materials with high resistance are strong, those with low resistance are weak), and toughness, the resistance to crack propagation (with tough or fragile as the extremes: Sanson *et al.* 2001). Stiffness is the resistance to deformation of a material in its elastic range where there is non-permanent deformation (stiff or pliant as the extremes), while ductility (or plasticity) is the resistance to deformation in its plastic range where there is permanent deformation (brittle or ductile as the extremes: Strait and Vincent 1998). Diversity is apparent in all of these quantities in invertebrates. On the whole, biological materials are also viscoelastic, so that their biomechanical properties depend, in part, on a temporal component, such as the speed of loading (Vincent 1990).

Components of invertebrates such as internal organs and intersegmental membranes are pliant, ductile and relatively weak, whereas sclerotised exoskeletons, including mandibles, are stiff and strong (Hepburn and Chandler 1976; Vincent 1980; Hillerton 1984). Most of the work on the material properties of insects has focussed on the cuticle.

The insect procuticle, the portion of the cuticle containing chitin, comprises the exocuticle and endocuticle. Two principal mechanical forms are stiff cuticle and pliant cuticle (Hillerton 1984). The exocuticle of stiff cuticle is sclerotised to some extent and has a lower chitin content. Pliant cuticles usually have very little or no exocuticle, and they contain greater amounts of chitin. However, most insect cuticles are more complex than these pliant and entirely sclerotised cuticles (Hepburn and Joffe 1976).

#### 'Hardness'

The use of the term 'hardness' to describe the properties of diets, including invertebrates, appears to have an inherent appeal, and it has been used for many years (Jolly 1970; Freeman 1979, 1981; Kay 1981; Strait 1993; Dumont 1995, 1999). In engineering, hardness is quantified by scratch or indentation tests (such as Mohs, Vickers, Brinell and Rockwell: Askeland and Phulé 2003). The results of hardness tests are complex, but on the whole can be considered a surface property rather than a property of the entire object.

When used in dietary studies, there is rarely, if ever, any explicit correlation between the use of the term 'hardness' and any particular physical property or component. However, there have been several efforts to estimate and correlate the hardness of the diet with various aspects of the biology of insectivores. Freeman (1981) estimated the hardness of various taxa according to an arbitrary scale from 1 to 5, e.g. Lepidoptera 2, Coleoptera 5. An estimate of the hardness of an insectivore's diet was obtained by multiplying the frequency of each taxon estimated in dietary studies by its hardness score (Freeman 1981; Rodríguez-Durán *et al.* 1993). However, these authors did not define hardness, and did not empirically derive or test the hardness scale.

Strait (1993) considered that hard foods, such as adult beetles, are those that are relatively tough, stiff, strong and brittle, and soft foods, such as moths and larvae, are relatively fragile, pliant, weak and ductile. Hardness was said to 'succinctly characterise the multitude of physical properties described above' (Strait 1993: 392). This conception of hardness does not give criteria for quantifying or classifying insects as 'hard' or 'soft' and therefore it is difficult to assess the hardness of a diet, compared with Freeman's (1981) scheme.

'Hardness' in some studies may actually be stiffness (Hillerton 1984) or apparently strength (Jolly 1970; Kay 1981). In normal conversational usage, the closest definition is most probably the resistance to deformation, regardless of whether it is elastic or plastic deformation – any material or structure that deforms easily to an applied force is 'soft', and one that does not is 'hard'. Durometer-type devices have often been used to measure 'hardness' by fracturing or 'crushing' food items using a manual or spring-loaded punch (Hill 1985; Kinzey and Norconk 1990; Fisher and Dickman 1993; Yamashita 1996; Dumont 1999; Aguirre *et al.* 2003).

From the large number of studies using the term 'hardness' and the wide variety of uses to which it is put, it is easy to agree with Boyer (1987: Preface) when he stated that 'There is probably no word in the English language for which so many definitions from so many sources have been offered as the term 'hardness''. We will address this issue by examining the factors that influence the biomechanical properties of insects.

#### Materials and structures

Biological organisms are more complex than the material properties of their separated parts. Mechanical behaviour depends on more than the strength, stiffness, toughness and Poisson's ratio values, which are all standardised for size (length, area and/or volume) and can be considered 'material properties'. What also matter are the geometrical or morphological aspects of an object, here termed 'structural properties'. These include the thickness, distribution and arrangement of one or more materials within a structure. (See Evans 2003 for a more complete analysis of the distinction between material and structure.) The ultimate mechanical performance of an object can be understood only through consideration of both the material and structural properties (see Niklas 1992 for an equivalent view). We need concepts and terms that reflect this complex interaction; 'hardness' is not sufficient.

Where strength, stiffness and toughness are parameters that are used to summarise the properties of materials, for simplicity, we can think of structural strength, stiffness and toughness as being the equivalent properties that describe structures. What we actually mean is the amount of force required to cause the structure to fail, the extent to which it deforms, and the amount of energy required to do so. This terminology differs slightly from that used in engineering, where the closest concepts are breaking load, resilience and toughness respectively, but this appears to be a useful and valid simplification.

As an example of the influence of structural properties on the biomechanical behaviour of an organism, an insectivore obtaining and trying to consume prey is not just dealing with the intrinsic strength ( $\text{N m}^{-2}$ ) and toughness ( $\text{J m}^{-2}$ ) of the materials of which the prey is constructed, but the composite of these properties with the shape, size and arrangement of the constituent materials. That is, the absolute force and energy that must be supplied by the predator to initiate and propagate cracks through the structure are important.

A prey item defended by a thin wall of strong, tough material may be equivalent to one with a thick wall of weak, fragile material with regard to a predator: the stress and work to fracture the food could be the same for the two scenarios (Sanson *et al.* 2001). In this instance, the strength and toughness (as standardised for cross-sectional area and elongation, or new surface area created respectively) may tell a different and misleading story compared with the structural strength

and toughness, and to the insectivore the final structural properties will be more important. It may be predicted that 'strength' and 'structural strength' would be correlated, because selection for increased resistance to high force may lead to a change in the material properties (strength) as well as the amount of material present (increasing the structural strength), but this is not necessarily the case.

### *Cuticle structural properties*

Foods previously considered 'hard' can really be considered 'strong' and 'stiff' in the structural sense in that a great deal of force and energy is required to deform or fracture the object. 'Hard' invertebrates such as beetles contain components composed of materials that are relatively strong, stiff and tough, but structural mechanisms (such as a thickening of the structure) may be the main cause for the large force required. The behaviour of the cuticle, and also the entire insect, is a result of the structural mechanisms operating at both the cuticle and whole organism level.

As a description of the behaviour of a structure, 'hard' is inadequate. Due to the baggage of past usage to describe materials, the term 'hard' fails to sufficiently encompass the complex behaviour of structures. Therefore, structures that require large forces for crack initiation, large amounts of energy to propagate cracks and large forces to create deformation (either elastic or plastic) and eventual fracture can be said to be 'intractable' (difficult to manage or handle, the negative of tractable, from Latin *tractare*, to handle, manage). We have introduced intractability here to describe the extent to which a structure has achieved high structural strength, stiffness and toughness.

From measurements of the properties of various insect components (Hepburn and Chandler 1976; Hepburn and Joffe 1976; Vincent 1980, 1990; Hillerton 1984), it is most likely that the main difference in the intractability of invertebrates is due to differences in the cuticle, thereby assuming that the biomechanical properties of the internal organs of intractable and tractable invertebrates are relatively similar. More specifically, we hypothesise that the stress and work to fracture are proportional to the thickness of the cuticle.

We will test this hypothesis by measuring the force and work required to fracture in punch tests for invertebrate cuticles of varying thickness. Punch tests measure the punch strength (force/area of punch;  $\text{N m}^{-2}$ ) and work to punch (force  $\times$  displacement/area of punch;  $\text{J m}^{-2}$ ). These are the stress and the work required to initiate and propagate fracture through the cuticle, and are therefore measures of the intractability ('hardness') of the cuticle. The above hypothesis can then be restated as 'punch strength and work to punch will be positively correlated with cuticle thickness', and these estimate different aspects of intractability. The objective is not to measure the intrinsic strength or fracture toughness of the insect cuticle, but the absolute stress and work required to fracture cuticle of different thickness. The

hypothesis of a correlation between cuticle thickness and measures of intractability (punch strength and work to punch) were tested by punch tests of fresh cuticle of varying thickness.

It is fortunate that the cuticle, which most probably displays the greatest diversity in intractability within invertebrates, survives the passage through the gut of a living insectivore. Although digestion of chitin has been shown in some insectivores (Jeuniaux 1961; Whitaker *et al.* 2004), a large proportion of insectivore faeces is composed of cuticular fragments from ingested insects, and so is available for analysis. Therefore, if the first hypothesis is supported, a measurement of the cuticle thickness in insectivore faeces can be used as an estimate of the quantitative intractability of the diet. Differences in cuticle thickness among samples of bats' faeces will distinguish those species whose prey is largely intractable from those with primarily tractable prey. To illustrate the practicality and value of the use of cuticle thickness to quantify the biomechanical properties, the thickness of cuticle fragments in faecal samples of several insectivorous microbat species were measured.

## **Materials and Methods**

### *Biomechanical properties of cuticle*

Adult beetles (Coleoptera) and moths (Lepidoptera) were trapped using light traps or picked from an illuminated white sheet by hand in March 2002. They were euthanased immediately before the experiment using a killing jar containing acetone and weighed ( $\pm 0.1$  mg) with a Mettler AE 166 electronic balance. The use of acetone killing jars has been found to not significantly affect the mechanical properties of insects (Evans and Sanson 1998). Sections of relatively flat cuticle at least  $2 \times 2$  mm were dissected from the insects in the following regions: thorax, abdomen (ventral and dorsal) and elytron (beetles only). All components of the insects that were of sufficient size were tested. Muscle attached to the cuticle was removed using a small paintbrush (Hepburn and Chandler 1980), taking care to avoid scratching the cuticle surface. Cuticle thickness ( $\pm 1$   $\mu\text{m}$ ) was measured with a Mitutoyo digital micrometer with hemispherical attachments to the micrometer anvils (diameter 1 mm) for measurement of thickness over a small area. Fragments were force-tested immediately after dissection to reduce the effect of dehydration.

Punch tests were carried out on 190 fragments from 17 beetles and 15 moths. Each cuticle fragment was punched using a 0.5-mm sharp flat-ended punch (area 0.196  $\text{mm}^2$ ) with clearance of 0.05 mm with the die on a Chatillon UTSE-2 Universal force tester. Force was applied to the specimen by moving the stage up towards the punch at a constant velocity ( $\sim 0.3$   $\text{mm s}^{-1}$ ), and the force was measured by a 25-kg load cell. Force and displacement of the punch were captured at 100 Hz using DT Access ver. 6.02 (Peter Fell, Monash University). The resulting force-displacement data were analysed using Leaf ver. 3.7 (Murray Logan, Monash University), which subtracted a blank run made after every 10 punches to remove the effect of friction in the machine. The software was also used to obtain the force to punch (N), punch strength ( $\text{N m}^{-2}$ ), specific punch strength ( $\text{N m}^{-2} \text{m}^{-1}$ ), work to punch ( $\text{J m}^{-2}$ ) and specific work to punch ( $\text{J m}^{-2} \text{m}^{-1}$ ), following Sanson *et al.* 2001) required to punch cuticle fragments.

Multiple fragments from the same insect were force-tested as limited samples were available in March, and so the fragments from the same insect may not strictly be considered independent from one another. However, each fragment was punched and its thickness

measured and therefore each represents a separate determination of the relationship between thickness and punch parameters. To see whether this had any effect on the conclusions, all statistical tests were carried out for random samples of one fragment per insect (giving a total of 32). No difference was found in the conclusions, and so the total sample of 190 fragments was used.

Data from the force-testing experiments (force to punch, punch strength, specific punch strength, work to punch and specific work to punch) and cuticle thickness were logged to base 10. Correlation and simple linear regression were carried out using Systat for Windows ver. 10.0 (SPSS, Inc.). Force-testing data were regressed against cuticle thickness for all cuticle samples, and separately according to insect type (beetle and moth), sclerotisation (unsclerotised or sclerotised) and both insect type and sclerotisation (e.g. unsclerotised beetle cuticle) to see whether insect type and degree of sclerotisation affected the relationship between punch parameters and cuticle thickness. For instance, we may expect that, for a given thickness, sclerotised cuticle has higher punch parameters than unsclerotised cuticle, and that beetle cuticle has higher parameters than moth cuticle. Significance of correlation and significant difference of the regression slopes from zero were tested with *t*-tests, with a *P* = 0.05 significance level. To examine which of beetles or moths tended to have the greater number of sclerotised or unsclerotised fragments per gram, the number of sclerotised or unsclerotised fragments obtained from each insect for force testing was divided by the mass of the insect.

#### Cuticle thickness in bat faeces

The microbat species *Chalinolobus gouldii*, *C. morio*, *Miniopterus schreibersii*, *Nyctophilus geoffroyi*, *Vespadelus darlingtoni*, *V. regulus* and *V. vulturinus* were trapped in harp traps at Rotamah Island, Victoria, Australia, on 5–7 February 2000. The bats were kept in a cloth bag for up to six hours after trapping, and faecal samples were collected from the bag. Trapping was carried out under *Wildlife Act 1975* and *National Parks Act 1975* Research Permit No. 10000763.

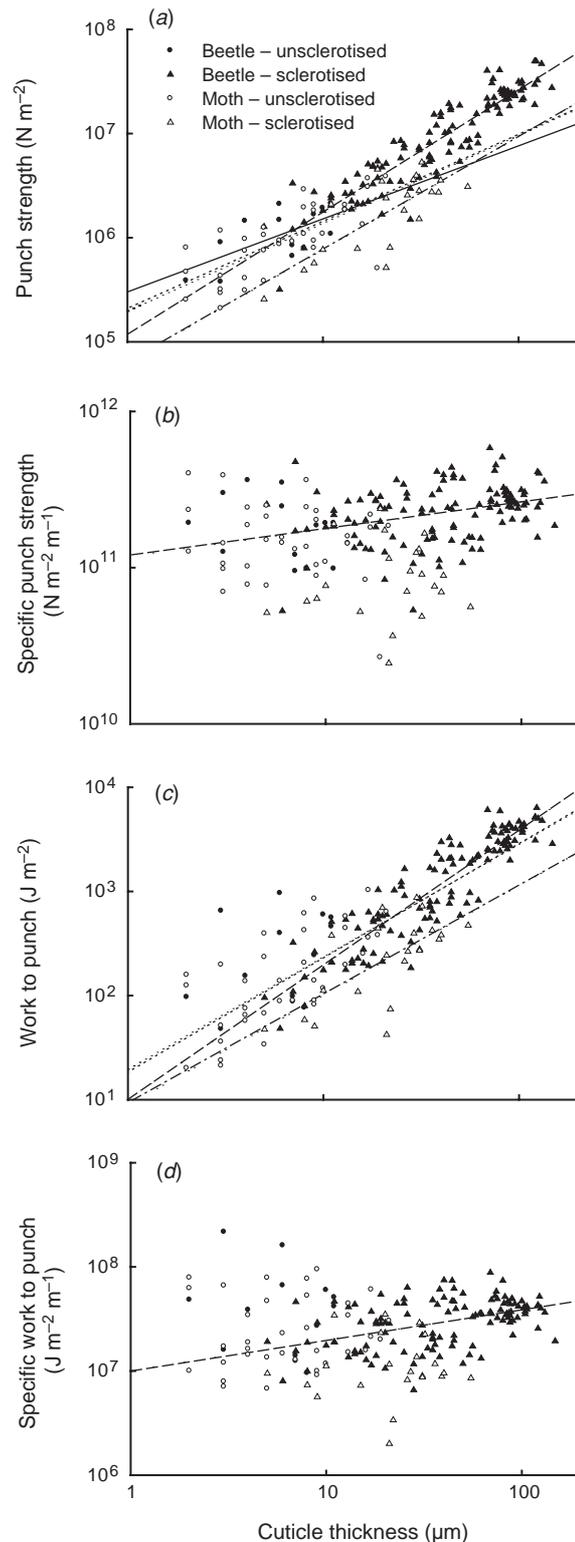
A single faecal pellet was randomly chosen from the faecal sample of each individual animal. A 1–2-mg portion of each pellet was weighed ( $\pm 0.1$  mg) with the Mettler balance, placed in distilled water and teased apart with fine forceps. All fragments of sclerotised cuticle larger than  $0.25 \times 0.25$  mm were separated. Only relatively flat cuticle fragments that were approximately of uniform thickness were used, and so limb segments, head capsules or irregularly shaped fragments were excluded. Wing segments were also excluded.

The cuticle fragments were dried and counted. The thicknesses of 20 fragments randomly selected from each portion of pellet were measured using the micrometer set-up described above. The median and maximum cuticle thicknesses were calculated.

These fragments may not be considered as strictly independent, as many are likely to be from the same insect. However, if chewing resulted in the cuticle being broken into equal-sized pieces (or if all thicknesses are broken into randomly-sized pieces) then a random selection of the fragments will represent a weighted average of the thickness according to the amount of cuticle of that thickness in the diet. In an attempt to reduce the effect of any bias due to this, the median rather than the average cuticle thickness was calculated for each individual, but this will have no effect on the maximum thickness.

## Results

Fig. 1 shows the punch strength, specific punch strength, work to punch and specific work to punch for cuticle plates of varying thickness. Punch strength and work to punch were significantly positively correlated with cuticle thickness except for unsclerotised beetle cuticle (Table 1; Fig. 1). The specific measures were significantly correlated with thick-



**Fig. 1.** Cuticle thickness ( $\mu\text{m}$ ) versus (a) punch strength, (b) specific punch strength, (c) work to punch and (d) specific work to punch from punch tests of fresh cuticle according to insect type and level of sclerotisation. Beetle unsclerotised, solid regression line; beetle sclerotised, dashed; moth unsclerotised, dotted; moth sclerotised, dot-dash.

ness for the total sample, the full sample of sclerotised cuticle and only beetle sclerotised cuticle, but not for all categories of non-sclerotised cuticle or sclerotised moth cuticle (Table 1; Fig. 1). The region of the insect that the sample was taken from is categorised with the punch parameters in Fig. 2. The median and maximum thicknesses of all cuticle fragments taken from the one insect are plotted against insect mass in Fig. 3. The average number of sclerotised fragments per weight of insect for beetles and moths was 196.08 and 17.68 fragments per gram respectively. Beetles had 20.38 unsclerotised fragments per gram, and moths 40.35.

The thicknesses of cuticle fragments found in the faeces of several microbat species are given in Table 2. Published reports of the diet of these same species are given in Table 3.

## Discussion

### *Cuticle thickness as a measure of biomechanical properties*

The strong positive correlation between both punch strength and work to punch for sclerotised cuticle thickness supports the hypothesis that these punch measures are correlated with cuticle thickness. Our contention that cuticle thickness is a good measure of invertebrate intractability is therefore supported. This was the case for the coleopterans and lepidopterans tested here, and the conclusion merits detailed confirmation with a wider range of invertebrates.

The assumption that sclerotised cuticle is a more discriminating measure of intractability than unsclerotised cuticle is reasonable as it showed a greater range of punch strength and work to punch values. In addition, most sclerotised cuticle fragments were thicker than unsclerotised fragments for both beetles and moths (lower and upper quartile ranges for unsclerotised beetle and moth: 4–9.5 and 4–9  $\mu\text{m}$ ; sclerotised beetle and moth: 20–82.25 and 16–31  $\mu\text{m}$  respectively).

The force-testing experiments reveal further details about the structural properties of cuticle. If the cuticle were a homogenous material, the force and energy required to punch the cuticle would be likely to linearly increase with thickness, or perhaps tail off with increasing thickness. Specific punch strength and specific work to punch measure this trend. Additional strengthening or toughening mechanisms in thick cuticle that are absent in thin cuticle would

increase the specific punch measures for thick cuticle. Unsclerotised cuticles of beetles and moths, and the combined sample, did not show a significant correlation of these measures with thickness (Table 1). There was a significant correlation for sclerotised beetle cuticles, however, indicating that there is some increase in structural strength and toughness above a simple additive effect of increased thickness. This was not the case for sclerotised moth cuticles, but this may be due to the smaller range of cuticle thicknesses tested.

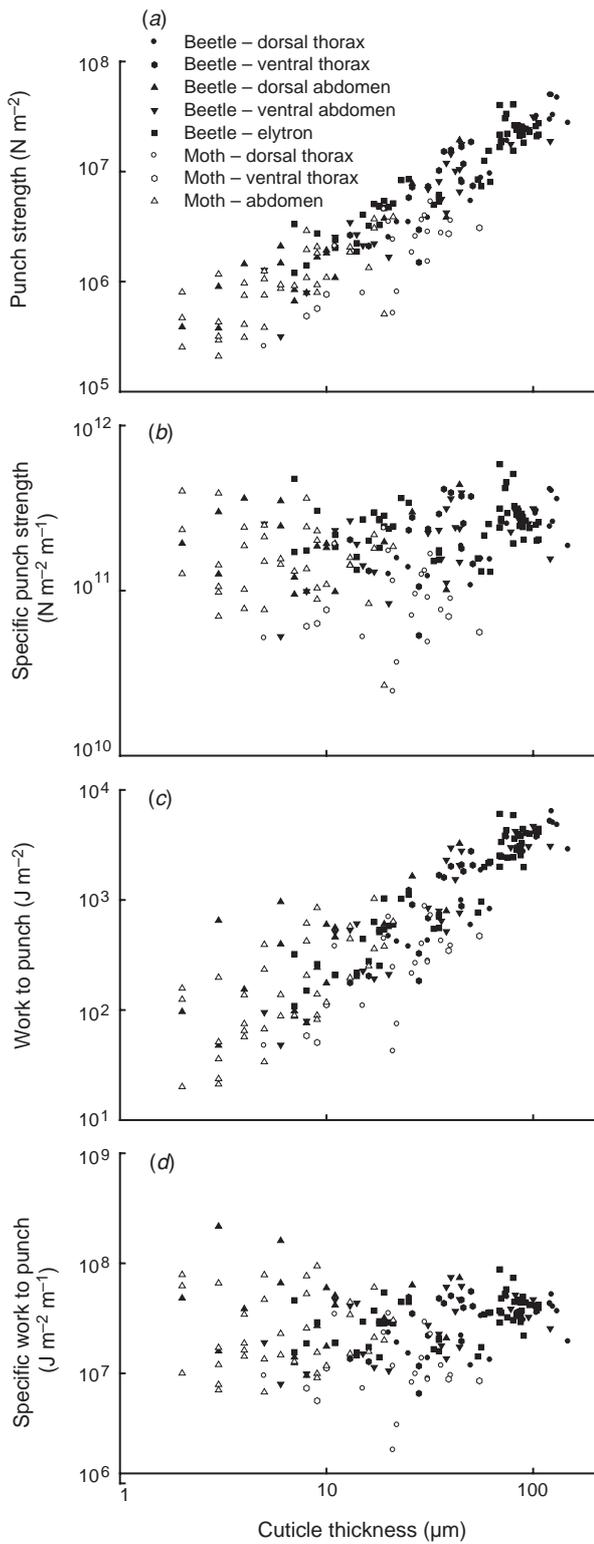
The measurement of cuticle thickness of faecal fragments is consistent with previous dietary determinations (Table 3). *C. gouldii* and *V. regulus* have been found to consume large numbers of beetles and had a high maximum cuticle thickness in the faeces (Table 2). *M. schreibersii* feeds principally on moths and had the lowest maximum cuticle thickness in the faeces. It is more difficult to show a correlation between the published diet and this study's results for the species with the second largest maximum cuticle thickness in the faeces, *V. darlingtoni* – it feeds on small insects, mainly moths, and so would not be expected to have thick cuticle in its faeces. However, the small sample sizes of this study may be the main reason for the discrepancy. The preliminary findings for the other species show promise, and in theory should give a more accurate depiction of the biomechanical properties of their diets.

### *Advantages of the cuticle thickness technique*

The correlation between cuticle thickness and intractability is not the only significant advantage in using cuticle thickness in faeces as a measure of dietary properties. The method quantitatively measures the degree of the intractability of the diet according to the mean and maximum cuticle thickness ingested within an individual or the species. Cuticle fragments that appear in faeces represent only those components of the insect that were ingested and masticated. Therefore, the results are not influenced by parts of insects that are culled and not ingested, such as head capsules and elytra. These components may be among the most intractable of the insect, but should not be taken into account when examining masticatory or dental adaptations to feeding on intractable

**Table 1. Results of correlation and regression analyses for logged values of cuticle thickness versus punch strength, specific punch strength, work to punch and specific work to punch according to insect (beetle or moth) and sclerotisation (sclerotised or unsclerotised)**  
*n*, number of samples; *r*, correlation coefficient; *t*, Student's *t*-test

	<i>n</i>	Punch strength			Specific punch strength			Work to punch			Specific work to punch		
		<i>r</i>	<i>t</i>	<i>P</i>	<i>r</i>	<i>t</i>	<i>P</i>	<i>r</i>	<i>t</i>	<i>P</i>	<i>r</i>	<i>t</i>	<i>P</i>
All samples	190	0.919	31.854	<0.001	0.305	4.399	<0.001	0.873	24.521	<0.001	0.223	3.130	0.002
Sclerotised cuticle	142	0.892	23.315	<0.001	0.393	5.056	<0.001	0.885	22.513	<0.001	0.462	6.169	<0.001
Unsclerotised cuticle	48	0.712	6.880	<0.001	0.229	-1.596	0.117	0.598	5.055	<0.001	0.012	-0.079	0.937
Beetle sclerotised	120	0.922	25.927	<0.001	0.332	3.824	<0.001	0.913	24.328	<0.001	0.451	5.494	<0.001
Moth sclerotised	22	0.760	5.231	<0.001	0.090	0.404	0.691	0.676	4.105	0.001	0.047	0.208	0.837
Beetle unsclerotised	13	0.653	2.858	0.016	0.344	-1.215	0.250	0.360	1.278	0.228	0.202	-0.684	0.508
Moth unsclerotised	35	0.732	6.180	<0.001	0.195	-1.145	0.261	0.689	5.455	<0.001	0.071	0.411	0.684



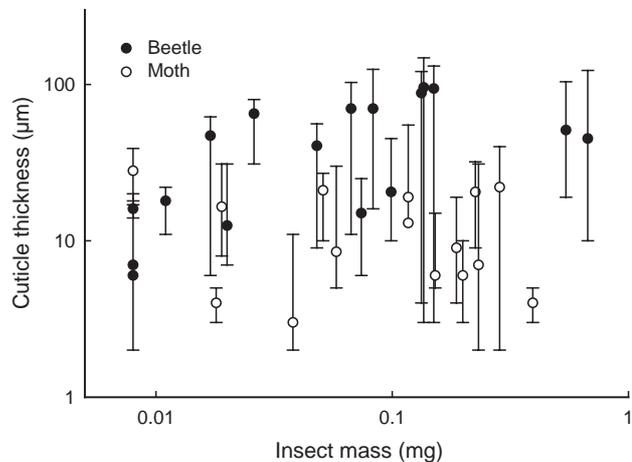
**Fig. 2.** Cuticle thickness ( $\mu\text{m}$ ) versus (a) punch strength, (b) specific punch strength, (c) work to punch and (d) specific work to punch from punch tests of fresh cuticle according to the region of the body from which the sample was taken.

foods. They may give an inflated impression of the intractability of the diet. The results will be particularly applicable to an analysis of molar function with respect to intractability, as they show the intractability of food that the molars actually processed.

Measurement of cuticle thickness is also significantly easier than conventional faecal analysis of insect diets. Dietary investigation normally requires the taxonomic identification (usually to order or family level) of the fragments. This labour-intensive and skilled activity makes use of invertebrate keys and sample collections of local invertebrates. Measuring only the thickness of the cuticle avoids many of these drawbacks, as the taxonomy of the prey is not as relevant to the masticatory adaptations as its biomechanical properties. Workers without substantial experience in identification of insect fragments can process a greater number of samples and the need for reference samples is eliminated. These advantages mainly apply where the most important factor being considered is the biomechanical properties of the diet.

Previous estimates of dietary ‘hardness’ also largely rely on estimates of the volume of invertebrate taxa in faeces, the merits of which have been debated (Kunz and Whitaker 1983; Robinson and Stebbings 1993). If there were very little digestion of cuticle, then soft, unsclerotised cuticles would still be present in the faeces (and were found in some samples examined in this study: ARE, personal observation). Given low digestion of cuticle, then, thick cuticle would still be present in the faeces of intractable feeders and absent from tractable feeders, indicating intractable and tractable diets respectively. This reduces problems with underestimation of tractable taxa, as these problems chiefly stem from the lack of identifiable parts of tractable insects.

Another significant advantage of this technique is that both the determination of cuticle thickness of fragments and



**Fig. 3.** Insect body mass (mg) versus median (circle), minimum (lower bar) and maximum (upper bar) thickness ( $\mu\text{m}$ ) of cuticle fragments used in punch testing.

**Table 2. Species means ( $\pm$ s.e.) of median and maximum thickness of cuticle fragments in the faeces of microbats, and number of fragments per milligram**  
*n*, number of individuals

Bat species	<i>n</i>	Median cuticle thickness ( $\mu\text{m}$ ) $\pm$ s.e.	Maximum cuticle thickness ( $\mu\text{m}$ ) $\pm$ s.e.	Cuticle fragments $\text{mg}^{-1}$
<i>C. gouldii</i>	4	12.25 $\pm$ 1.87	24.00 $\pm$ 3.52	— <sup>A</sup>
<i>C. morio</i>	4	5.75 $\pm$ 0.32	17.75 $\pm$ 1.26	— <sup>A</sup>
<i>M. schreibersii</i>	3	5.12 $\pm$ 0.40	11.67 $\pm$ 0.69	24.23 $\pm$ 2.99
<i>N. geoffroyi</i>	2	8.35 $\pm$ 2.62	19.50 $\pm$ 7.42	15.91 $\pm$ 2.89
<i>V. darlingtoni</i>	2	9.95 $\pm$ 0.57	26.50 $\pm$ 4.60	19.36 $\pm$ 2.38
<i>V. regulus</i>	2	8.00 $\pm$ 2.02	28.00 $\pm$ 12.73	27.48 $\pm$ 2.72

<sup>A</sup>Not measured.

any conventional quantification of frequency and volume of various taxa can be carried out on the same faecal pellet. Once any taxonomically identifiable fragments have been classified and used to estimate the proportional volume of the sample that it comprises, the thickness of the cuticle fragments can be measured, achieving both a conventional assessment of diet according to taxa present and a biomechanical measure of the intractability of the diet.

The principle of quantifying the intractability of an insect according to cuticle thickness has potential even if only published reports of the diet, rather than faecal samples, are available. The intractability of the relevant invertebrate taxa could be measured by force testing a range of body parts of a broad size and phylogenetic range to give a mean and standard deviation of intractabilities to be used as a measure of dietary mechanical properties, rather than using the qualitative scale of Freeman (1981).

#### *Cuticle thickness compared with other methods*

From the assumption that the biomechanical properties of the diet are one of the most important indicators of the

feeding decisions of the consumer, it is very important to be able to measure the properties of the foods actually eaten. For most feeding guilds, it is largely impossible to directly assess the properties of the material that an animal has consumed, and so we must find an indirect method of predicting the biomechanical properties. We will now compare the utility of the method of determining invertebrate dietary properties outlined in this paper with those of other dietary groups.

A typical method for estimating the types and proportions of items in the diet of an animal is through observations of feeding, taking note of the plants, fruits and animal components that are ingested (e.g. Van Valkenburgh 1996; Yamashita 1996). The foods must be categorised in some way that correlates with their biomechanical properties. However, this assumes that the observer can accurately determine the type of object being eaten. An alternative is to examine the stomach contents or faeces and categorise the fragments found, e.g. as monocotyledonous and dicotyledonous plants. One difficulty with this is the extensive digestion of many foods, e.g. fruits and vertebrate flesh, which limits the usefulness of faecal analysis for these groups.

**Table 3. Published reports of diets for species in Table 2 that were investigated using the cuticle thickness measurement technique**

Species	Diet	Reference
<i>C. gouldii</i>	Moths predominant food; moths and bugs in Victoria, Australia	Churchill (1998)
	Winged reproductive ants taken in large numbers; beetles heavily taken	Fullard <i>et al.</i> (1991)
	Large proportion of Coleoptera	O'Neill and Taylor (1989)
<i>C. morio</i>	Moths important dietary item	Vestjens and Hall (1977)
	Moths main dietary item	Churchill (1998)
	Particularly high incidence of Lepidoptera	Fullard <i>et al.</i> (1991)
<i>M. schreibersii</i>	Mostly Lepidoptera, also Coleoptera	O'Neill and Taylor (1989)
	Moths main dietary item	Churchill (1998)
	Mainly moths	Vestjens and Hall (1977)
<i>N. geoffroyi</i>	Mainly moths, also wingless insects	Churchill (1998)
	Winged reproductive ants taken in large numbers; beetles heavily taken	Fullard <i>et al.</i> (1991)
	Wide variety of flying and non-flying invertebrates	Maddock and Tidemann (1995)
<i>V. darlingtoni</i>	Moths most common food	Vestjens and Hall (1977)
	Mainly moths, as well as ants, bugs and beetles	Churchill (1998)
	Coleoptera, Diptera, Hemiptera and Hymenoptera approximately equal (N. Victoria, Australia)	Lumsden and Bennett (1995)
<i>V. regulus</i>	Moths, flies and beetles	Churchill (1998)
	Considerable variety in diet; high proportions of beetles and moths	Fullard <i>et al.</i> (1991)
	Favours moths	Tidemann (1995)

Even if these obstacles are overcome or not relevant, it is very likely that there is fine-level variation in the biomechanical properties of these foods within dietary categories (e.g. ripeness of fruits, age of leaves, size and type of beetles) that the animal may use to discriminate between foods. This variation may be indistinguishable either by the observer or from the examination of digestive contents. The measurement of biomechanical properties on discarded fragments of partially consumed fruit, such as by Kinzey and Norconk (1990), is almost certainly the best measure of such properties of fruits that can be made, but the level of variation within a piece of fruit is unknown, and it cannot consider fruit that is completely ingested. The use of cuticle thickness in the faeces of insectivores appears, on the basis of this limited survey, to have the potential to be the best method for the measurement of biomechanical properties of diet for any dietary type. The good correlation between thickness of cuticle and its biomechanical properties observed in this study, and the persistence of the cuticle in the faeces, largely overcomes the limitations of other methods. However, we acknowledge that a more comprehensive survey would be necessary to confirm the potential of the method.

The use of durometers, which measure the force to penetrate foods with a manual or spring-loaded punch (Kinzey and Norconk 1990; Yamashita 1996; Dumont 1999), are reasonable measures of the structural strength of the diet, and are in several respects similar to the tests carried out here. The tests used by Fisher and Dickman (1993) and Aguirre *et al.* (2003) essentially measure the structural strength of the insects and fruit. However, for all of these studies except Fisher and Dickman (1993), the speed of loading during the mechanical test is not constant, which will affect the force readings obtained due to the viscoelasticity of foods (Vincent 1990). It would need to be assessed to what extent this influences the results, as it may only be a minor effect.

Aguirre *et al.* (2003) were able to show that the structural strength of insects, particularly beetles, increased with size (mass, length and width). From estimates of the dimensions of prey in faeces, they were able to give an estimation of the 'hardness' of the diet. Substantial testing of the methods of the current study and Aguirre *et al.* (2003) would be required to establish their comparative value and significance of correlation with morphological and behavioural characteristics of the insectivores.

Punch tests have been criticised because the precise mode of failure is not known and they do not examine a specific material property (such as strength, stiffness or toughness) (Vincent 1992a, 1992b). However, this is exactly why this technique is advantageous for examining the combined effect of the material and structural properties. Aranwela *et al.* (1999: 382) compared several biomechanical tests and concluded that punching correlated well with biomechanical aspects of leaf biology, and that such tests 'may be detecting

ecologically significant variation in the fracture properties of leaves'. Punch tests have also been found to be useful in examining the biomechanical basis of sclerophylly in plants (Edwards *et al.* 2000).

#### *Biomechanical properties of beetles and moths*

Although the punch variables correlated with cuticle thickness for all insects tested (Table 1), there were significant differences between beetles and moths. First, there are large differences in the regression slopes for sclerotised and unsclerotised cuticle in the two groups (Fig. 1), indicating that, in general, beetle cuticle requires more force and energy to fracture than moth cuticle for a given thickness. In addition, the amount and thickness of cuticle differ; beetles have thicker cuticle (maximum and median; Fig. 3) and a greater number of sclerotised plate components for a given mass, signifying that, for a given mass of insect, beetles are substantially better defended by the greater amount of cuticle.

For both beetles and moths, the maximum cuticle thickness is significantly correlated with insect mass (beetle:  $r = 0.515$ ,  $t = 2.324$ ,  $P = 0.035$ ; moth:  $r = 0.524$ ,  $t = 2.219$ ,  $P = 0.045$ ), but the slope of the log-log plot for both is substantially lower than would be expected from isometry (slopes 0.002 and 0.004 for beetles and moths, respectively, compared with an expected slope of 0.333). The maximum cuticle thickness is approximately equal for beetles of 100–700 mg. Therefore, the thickness of a cuticle fragment is not a good predictor of beetle size. The same is approximately true for moths, even though the number and thickness of cuticle plates are lower. However, the taxonomy of these insects was not determined below order, and so patterns within the two orders are unknown. The finding suggests that above some threshold there is no advantage in increasing thickness with size. This could be for several reasons, not all of which relate to defence against predators, such as the cost of manufacture and transport of a thick cuticle, and could be the subject of an interesting study on the scaling of cuticle thickness with body size.

The finding that cuticle thickness is not a good predictor of beetle size at larger sizes is slightly at odds with the conclusion of Aguirre *et al.* (2003) that 'hardness' is correlated with size. Cuticle thickness may not be as good a measure of intractability of the whole beetle for large to very large sizes. This could be due to additional structural mechanisms, such as high arching of the body (Hill 1985) or structurally stronger muscles or tendons, that increase the intractability of larger beetles beyond what would be predicted by the thickness of cuticle. The lack of concordance may be because the two techniques measure intractability for different ways in which the insectivore is dealing with its prey: Aguirre *et al.* (2003) estimated the intractability for initial fracture of a whole beetle, and cuticle thickness relates to the continued breakdown of the cuticle. These will not necessarily correspond.

### *Insect biomechanical diversity*

Despite our arguing the importance of the structural features of insects, we should also examine how insects have been previously characterised in terms of material properties. We will briefly look at toughness and brittleness to see whether they are useful in describing invertebrates as foods.

Insects have been said to range from tough to fragile. However, most insect components, and whole insects, are relatively tough in that a fair amount of energy is required to fracture them and most (or all) of the parts of the body resist self-sustained crack propagation. On the whole, biomaterials are designed to resist fracture, although there are exceptions (e.g. crack generation in a probably very stiff and brittle cuticle in the process of autotomy in crickets: Vincent 1998), and we would expect catastrophic failure of components to be avoided as much as possible. This is also apparent in engineering, as the history of engineering is almost the history of attempts to prevent cracks spreading (Gordon 1976). Pliant cuticles, such as a maggot cuticle, have a toughness of  $1000 \text{ J m}^{-2}$ , which is as tough as mammalian arteries (Purslow 1980, cited in Hillerton 1984). The toughness of a whole beetle ( $2770 \text{ J m}^{-2}$ ) is greater than that of a whole caterpillar ( $390 \text{ J m}^{-2}$ ) (Strait and Vincent 1998), illustrating that toughness does vary between insects, but the relatively high toughness of insect pliant cuticles indicates that no insects can really be considered 'fragile'. Therefore, we may consider that toughness is an overriding property of insects in two respects. First, a large proportion of the remaining components of insects (e.g. internal organs, certain cuticle components) are relatively tough (which is probably largely related to their low stiffness and plasticity). Second, the fracture of whole invertebrates requires significant amounts of energy, and they do not fail in a brittle manner like some nuts, fruits, bone or shells.

Strait (1993) described beetles as brittle. This implies that either the invertebrate as a whole or each of its components separately undergo brittle fracture (see fig. 1 in Strait 1993). The assertion that 'hard' invertebrates such as beetles display brittle fracture appears to be an exaggeration. Sclerotised beetle exocuticle exhibits some brittle fracture (Hepburn and Joffe 1976), as does locust tendon (Vincent 1990), and limited brittle fracture in the fracture of a whole beetle may be responsible for rapid decreases in a force-deflection graph (Strait and Vincent 1998). Crack-stopping mechanisms, particularly the ductile endocuticle below the exocuticle, reduce the extent of free-running cracks in the cuticle. Whole insects certainly do not show brittle fracture, and require continual application of force to sequentially initiate and propagate cracks through the insect. In addition, none of the internal organs or the pliant cuticles can be considered brittle.

We can compare this characterisation of insects using materials definitions to a structural analysis of insects and

examine the wide diversity of intractability within invertebrates. An intractable organism is one that behaves intractably as a whole and/or has significant amounts of intractable (structurally strong, stiff and tough) matter. Invertebrates considered to be at the 'intractable' end of the scale would be beetles and hemipterans; 'tractable' invertebrates include ants, mosquitoes, flies, moths, spiders, caterpillars and worms. Very small insects such as ants and mosquitos, whose cuticle may be as strong, stiff and tough as larger insects, are classified as tractable because of the small absolute forces and energies required to fracture them. This is principally due to the very small amount of cuticle used in constructing their bodies.

It is apparent, then, that scale becomes an important feature of the biomechanical properties of the diet: larger invertebrates are more likely to be intractable than smaller ones. The larger an organism gets, the more intractable it tends to become, as it deals with the mechanical challenges at its own scale. Some organisms are tractable because of the general size of that organism, such as ants; others will vary in intractability according to size, which beetles and moths do, but beetles will be more intractable than moths for a given size. The findings of Aguirre *et al.* (2003) for large beetles also seem to indicate that larger size *per se* confers higher intractability.

The most significant aspect of structures compared to materials is that their behaviour can be abundantly more complex than isolated materials due to the addition of structural properties and the potential of combining more than one material. Pure materials cannot be strong, stiff and tough – there is a trade-off between these properties, as a material that is stiff and strong more easily transfers the stress onto fewer bonds and so requires little energy to fracture it (Atkins and Mai 1985). A material is therefore unable to achieve this 'Holy Trinity' of materials engineering of high strength, stiffness and toughness. However, it can be achieved in a structure as high structural strength, stiffness and toughness. Many insects as a whole, such as beetles, and even individual components such as the cuticle, are structurally stiff, strong and tough.

The definition of intractability used in this study may not be as applicable to other food categories such as fruits and bone. These foods may exhibit more brittle fracture and have lower toughness, differing from a relatively high degree of toughness in all insects. However, this supposition remains to be tested.

This paper challenges previous categorisations of insects according to the ill-defined concept of 'hardness' by erecting a new classification according to 'intractability'. The concept of 'dietary hardness' as previously used may be considered akin to measures of sclerophylly in plants – an ill-defined concept that has been imprecisely used in describing the biomechanical properties of complex biological components. The basis for a new method for assessing the bio-

mechanical properties of insects that appears better than methods currently in use is also established, achieving a more comprehensive and mechanically meaningful understanding of the diversity of the biomechanical properties of invertebrates. The thickness of cuticle fragments in the faeces of insectivores can be used as a measure of the intractability of the invertebrates in their diet. For correlations of the biomechanical properties of insects with morphological characters, this procedure is greatly preferable due to its sound biomechanical basis and quantitative nature.

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