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Local mechanical properties of the head articulation cuticle in the beetle *Pachnoda marginata* (Coleoptera, Scarabaeidae)

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Summary

Insect exoskeleton (cuticle) has a broad range of mechanical properties depending on the function of a particular structure of the skeleton. Structure and mechanical properties of the specialised cuticle of insect joints remain largely unknown to date. We used scanning (SEM) and transmission electron microscopy (TEM) to obtain information about the material structure of the gula plate, the head part of the head-to-neck articulation system in the beetle *Pachnoda marginata*. The surface of this cuticle appears rather smooth in SEM. The fibers of the exocuticle are partly oriented almost perpendicular to the surface, which is rather unusual for arthropod cuticle. Nanoindentation experiments were performed to determine the local mechanical properties (hardness and elastic modulus) of the gula material. To understand the effect of desiccation and the influence of an outer wax layer on the mechanical behavior of the material, the samples were tested in fresh, dry and chemically treated (lipid extraction in organic solvents) conditions. Nanoindentation results were found to be strongly influenced by desiccation but only slightly by lipid extraction. Decreasing water content (~15–20% of the cuticle mass) led to an increase in hardness (from 0.1 to 0.49 GPa) and elastic modulus (from 1.5 to 7.5 GPa). The lipid extraction caused a slight further hardening (to 0.52 GPa) as well as stiffening (to 7.7 GPa) of the material. The results are discussed in relation to the mechanical function of the gula plate.

Key words: desiccation, gula plate, insect cuticle, mechanical property, nanoindentation.

Introduction

The body of insects is completely covered by cuticle, which is a multifunctional interface between the animal and the environment (Hepburn and Joffe, 1976; Gorb, 2001). It serves primarily as an exoskeleton that gives the body its shape and stability. The cuticle is also a barrier against evaporation of water from the body and hence protects the insect against desiccation (Locke, 1964; De Renobales et al., 1991; Noble-Nesbitt, 1991).

Like most biological materials, cuticle is a fiber composite (Hepburn and Chandler, 1980). The fibers mainly consist of chitin, and the matrix is formed by proteins. Chitin is a natural polymer composed of 300 nm-long and 3 nm-thick nanofibrils (Vincent, 1980; Vincent, 2002). Each nanofibril contains ~19 molecular chains (Vincent, 1980; Vincent, 2002) running anti-parallel to one another (i.e. alpha chitin) (Neville, 1975; Vincent, 1980). The protein matrix stabilizes the chitin fibers. It normally contains some amount of water (in some cuticles it is ~90% of the protein matrix mass). The function of water in the cuticle is largely unknown, but presumably it is separation of the two main components of the cuticle from each other (Vincent, 1980).

Arthropod cuticle has a multilayer structure (Neville, 1975; Andersen, 1979) (Fig. 1). It typically consists of three main layers: epicuticle, exocuticle and endocuticle. The latter two layers form the procuticle. In some insects, there is a layer of mesocuticle located between the endocuticle and the exocuticle (Neville, 1975; Andersen, 1979; Noble-Nesbitt, 1991). The non-chitinous, tanned lipoproteinous epicuticle is the outer layer, which is very thin and has a relatively high tensile strength (Bennet-Clark, 1963). The surface of the epicuticle is coated with wax and lipids. The exocuticle has a dense chitin–protein structure and may become hard and stiff due to sclerotization. The endocuticle is usually the thickest region of the cuticle (Locke, 1964; Neville, 1975).

In various animal groups and on different body parts, there are different types of cuticle varying in mechanical properties from very hard and brittle to soft, ductile and also rubber-like (Jensen and Weis-Fogh, 1962). In many larvae, the cuticle is soft and colorless. During sclerotization, a hard and colored integument is formed in most adults (Fraenkel and Rudall, 1940). The variation in mechanical properties depends on

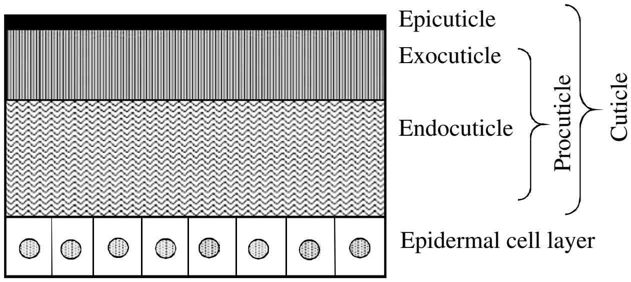

Fig. 1. Diagram of the multilayered structure of the insect cuticle. The shaded areas represent the single cuticle layers: non-chitinous epicuticle; procuticle consisting of exocuticle and endocuticle with chitin fibers usually oriented parallel to the surface. An epidermal cell layer underlies the endocuticle.

fractions of the main constituents (Hepburn and Chandler, 1976).

Mechanical properties of the insect cuticle are adapted to the function of particular body parts. Properties of the material of insect joints remain largely unknown. The present investigation focuses on the head articulation system of the beetle *Pachnoda marginata* (Fig. 2). Surfaces in this system operate in contact and must be resistant to wear and friction and at the same time have to provide high mobility of the joint. From studies on technical systems, the structure and the mechanical properties of superficial layers of the material are believed to be very important for its tribological performance (Scherge, 2003; Chakhvorostov, 2004).

All mechanical investigations were carried out on the head part of the head articulation called gula, which is normally in contact with the counter surface of the thorax during the head movement. The gula is a median ventral plate of the head, in some prognathous insects formed by a sclerotization of the neck region proximal to the posterior tentorial pits, continuous with the postmentum or submentum (Snodgrass, 1935). In the present study, the detailed information about the surface and internal structure of the gula cuticle was obtained, and mechanical properties (hardness and elasticity modulus) of its superficial cuticle layers were measured using nanoindentation. The surface morphology and ultrastructure of the cuticle were studied by means of scanning electron microscopy (SEM). The gula samples were mechanically tested in the fresh, dry and chemically treated conditions, in order to identify the influence of desiccation (dry *versus* fresh conditions) and removal of an outer wax layer (dry *versus* chemically treated conditions). Desiccation rate measurements were also carried out, in order to obtain information about the desiccation dynamics of the gula cuticle.

The aim of our study was to understand which structural features of the gula cuticle are responsible for its friction-reducing and wear-resistant properties. The following questions were asked. (1) Do the structure and local mechanical properties of the gula show any features of specialization for friction reduction and wear resistance? (2) How does the liquid content of the gula cuticle influence its mechanics? (3) How do cuticular surface waxes influence hardness and elastic modulus of the gula?

Materials and methods

Sample preparation

Samples were prepared from the gula of the beetle *Pachnoda marginata* Drury. The beetles were obtained from a commercial supplier (Insektenfarm Walta, Schkeuditz, Germany), kept in the plastic terraria and fed with various fruits. For structural and experimental studies, beetles were anesthetized with CO_2, their heads were cut off from the bodies, and the edges of the posterior openings (foramen occipitale) of the head were freed of soft tissues. To prevent specimen desiccation, fresh gula cuticles were tested immediately (within 3–5 min after dissecting from the body). Dry samples were obtained from fresh ones by drying in an oven for 24 h at 40°C. For lipid extraction (chemical treatment), the dry samples were immersed in a solution of chloroform and methanol (2:1) for 50–60 min and finally air-dried.

Scanning electron microscopy

The samples were fixed in 2.5% glutaraldehyde in a phosphate buffer (pH 7.3). The specimens were dehydrated in an ascending series of ethanol and then critical-point dried. Pieces of the dried material were fractured using a razor blade. The prepared samples were mounted on holders, sputter-coated with gold-palladium (10 nm thickness) and examined in a Hitachi S-800 scanning electron microscope (SEM) at 20 kV.

Transmission electron microscopy

For transmission electron microscopy (TEM), fresh gula was fixed for 12 h at 4°C in 2.5% glutaraldehyde (in 0.01 mol l^{-1} phosphate buffer at pH 7.3) and postfixed for 1 h in 1% osmium tetroxide in phosphate buffer at 2°C (Gorb, 1998). After washing in distilled water, the preparations were stained for 1 h at 4°C in 0.1% aqueous uranyl-acetate solution, washed, dehydrated and embedded in a low-viscosity resin (Spurr, 1969). Ultra-thin sections were picked up on

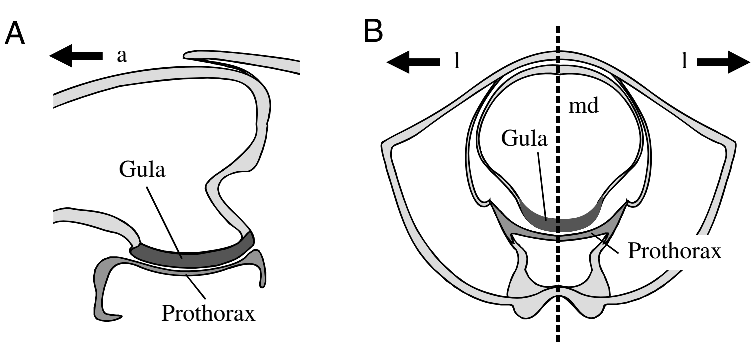

Fig. 2. Diagram of the location of the gula surface in the beetle body. Parasagittal (A) and frontal (B) virtual sections through the head–neck articulation. a, anterior direction; l, lateral direction; md, midline.

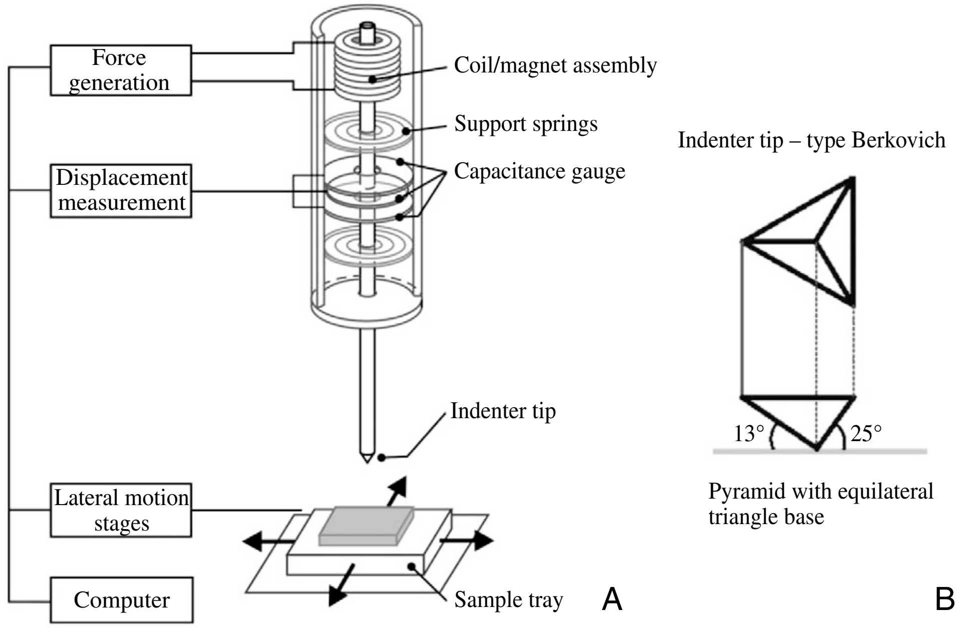

Fig. 3. (A) Diagram of the nanoindenter system used (Enders, 2000). The force imposed on the indenter is generated through a coil that sits within a circular magnet. The displacement sensing system consists of a three-plate (circular disks) capacitor. (B) Berkovich tip: a pyramid with an equilateral triangle as the base area.

copper grid slots coated with formvar film (Plano GmbH, Wetzlar, Germany), contrasted with uranyl acetate and lead citrate, and observed in TEM Philips CM10.

Desiccation dynamics measurement

To measure the desiccation rate, the head was cut from the body, freed of soft tissues and put immediately on a Mettler Toledo AG204 DeltaRange® balance (Greifensee, Switzerland), connected to a computer. The head mass was recorded for 12 h with the frequency of one registration per minute. For comparison, the same measurement was additionally performed on the cut-off gula cuticle. The experiment was repeated for three entire beetle heads and three gula preparations at a room temperature of 23.7–24.4°C and relative humidity of 41–43%.

Nanoindentation

Nanoindentation is a fast and reliable technique for evaluation of local mechanical properties, such as hardness and elastic modulus, in very small volumes of material (Oliver and Pharr, 1992; Bhushan and Li, 2003) (Fig. 3). During the past decade, this method has become an important tool in the characterization of inorganic materials. During nanoindentation, a geometrically well-defined diamond pyramid is brought into contact with the sample surface. The applied load and the displacement (indentation) into the specimen are recorded simultaneously. The load–displacement curves are used to determine the hardness and the elastic modulus of the material under consideration.

Three key parameters of the load–displacement data are necessary for determination of the hardness and elastic modulus: (1) the peak load (F_{max}), (2) the indentation depth at the peak load (h_{max}) and (3) contact stiffness (S) (Fig. 4) (Oliver and Pharr, 1992).

The hardness (H) is defined as the ratio between the maximum load and the contact area, A, generated during the indentation:

$$H = F_{max}/A \,. \quad (1)$$

A is given by:

$$A = \mathrm{k}\, h_c \,, \quad (2)$$

where k is a geometric constant of the tip (k=24.5 for the Berkovich tip), and h_c is the contact depth (Fig. 5), which can be defined as:

$$h_c = h_{max} - h_s \,. \quad (3)$$

The elastic deflection, h_s, of the surface at a particular contact perimeter depends on the indenter geometry:

$$h_s = \epsilon\,(F_{max}/S) \,, \quad (4)$$

where ϵ is a geometric constant of the indenter (Oliver and Pharr, 1992; Hay and Pharr, 2000).

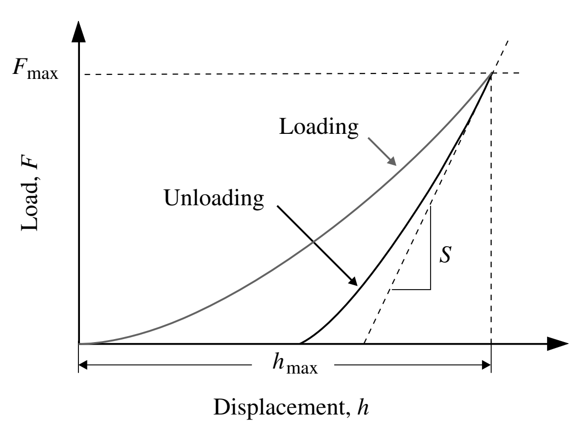

Fig. 4. Schematic representation of a load–displacement curve with the key experimental parameters. F_{max}, peak indentation load; h_{max}, indenter displacement at peak load; S, contact stiffness (after Oliver and Pharr, 1992).

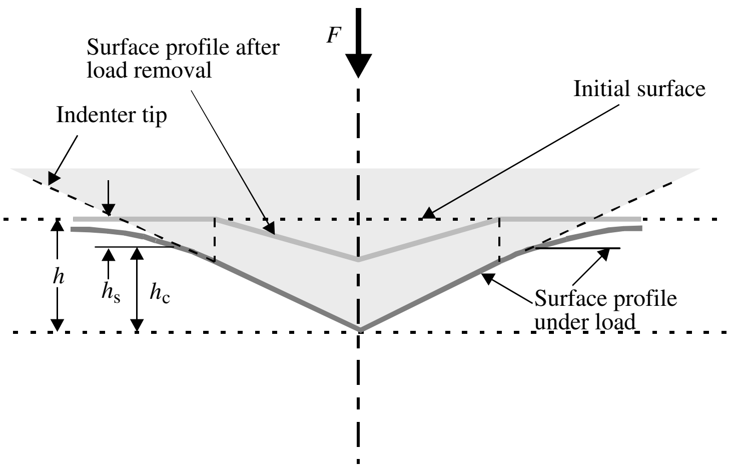

Fig. 5. Schematic representation of a section through an indentation, showing various quantities used in the analysis (Oliver and Pharr, 1992): *F*, indentation load; *h,* indenter displacement at peak load; h_c, contact depth; h_s, elastic deformation of the surface at the contact perimeter.

The reduced elastic modulus, E_r, for the specimen/indenter system can be calculated using the equation:

$$1/E_r = [(1 - v_s^2)/E_s] + [(1 - v_i^2)/E_i] , \quad (5)$$

where E_s and E_i are Young's moduli, and v_s and v_i are Poisson's ratios, for the specimen and for the indenter tip, respectively.

The relationship between the load–displacement data and the experimentally measured contact stiffness (*S*) and the contact area (*A*) is:

$$E_r = (S \sqrt{\pi})/(2\beta \sqrt{A}) , \quad (6)$$

where β is a constant depending on the tip geometry (Oliver and Pharr, 1992; Hay and Pharr, 2000) (β=1.034 for the Berkovich tip).

The mechanical properties of most layered materials and especially biological samples vary with depth. Using a special dynamic technique, the continuous stiffness measurement (CSM) (Oliver and Pharr, 1992), the contact stiffness can be measured continuously during indentation, as a function of depth. In this method, the indenter is periodically loaded and unloaded at a frequency of 75 Hz, and hardness and elastic modulus values are extracted at discrete points of the loading curve. This technique may also be applied for soft materials, such as biological materials and polymers.

Hardness and elastic modulus of the head cuticle in the beetle were measured using a Nano Indenter® SA2 system (MTS Nano Instruments, Oak Ridge, TN, USA) equipped with a Berkovich tip. The sides of the pyramid form an angle of 65.3° with the normal to the base (Fig. 5). Due to the high damping coefficient and high resonant frequency of the indenter, it was possible to perform measurements on materials with low contact stiffness and low damping coefficients.

The beetle head was mounted, using super glue, on a holder with the ventral surface of the gula facing up. We tested 10 heads with 15 indents each (*N*=150). The overall testing time for one head was 1.0–1.5 h. All indents were made on the top of the hemispherical surface of the gula and were separated from each other by 30–50 μm. The nanoindentation experiments were load-controlled. The maximum displacement was 3 μm. Since the biological samples contain liquid organic substances on the surface, the indenter tip was presumably contaminated during the test. Thus, the tip was cleaned and then recalibrated by indenting in the reference materials, such as Al and SiO_2 (fused silica) samples, between the measurements.

Atomic force microscopy

The surface profiles of the samples were investigated by atomic force microscopy (AFM) (DME, DualScope™ C-21

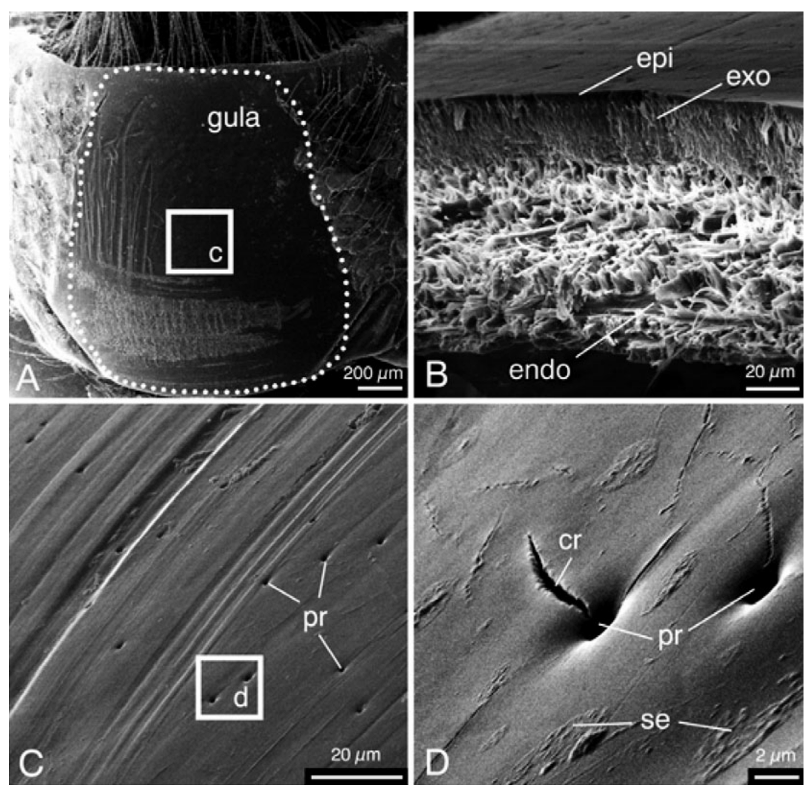

Fig. 6. SEM images of the dry gula. (A,C,D) Surface of the gula. (B) Cross fracture of the gula cuticle showing the epicuticle (epi), exocuticle (exo) and endocuticle (endo). Fibres of the outer part of the exocuticle are oriented perpendicular to the surface but are parallel in the deeper layers of the exocuticle and in the endocuticle. Pores (pr), dried organic substances (se) and cracks (cr) can be seen on the cuticle surface. c, d, rectangles indicate parts of the sample magnified in C and D, respectively.

with scanner DS 45-40 BIO; Danish Micro Engineering A/S, Herlev, Denmark) after the indentation test, in order to estimate the type of deformation behaviour (elastic, plastic, visco-elastic).

Results

Structure of the gula cuticle

The gula surface of the beetle head is rather smooth (Fig. 6A,B). Island-like structures on the surface are, presumably, dried secretory substances. They are probably delivered to the surface by large pores running deep into the material (Fig. 6C,D). Cracks found on the surface are probably the result of material desiccation (Fig. 6D). Fractures and ultra-thin cross sections of the cuticle show the layered structure of the fibre composite (Figs 6B, 7). The total thickness of the gula cuticle is ~80 μm. A very thin (1–3 μm) and dense epicuticle can be easily distinguished (Figs 6B, 7A,B). Procuticle is composed of two layers: ~22 μm-thick, dense exocuticle and a thicker endocuticle (~50 μm) that is less dense than the exocuticle. Fibres are oriented nearly perpendicular to the surface in the superficial part of the exocuticle (Figs 6B, 7B,C) and parallel to the surface in the deeper layers of the exocuticle (Fig. 7B,D) and in the endocuticle (Fig. 6B).

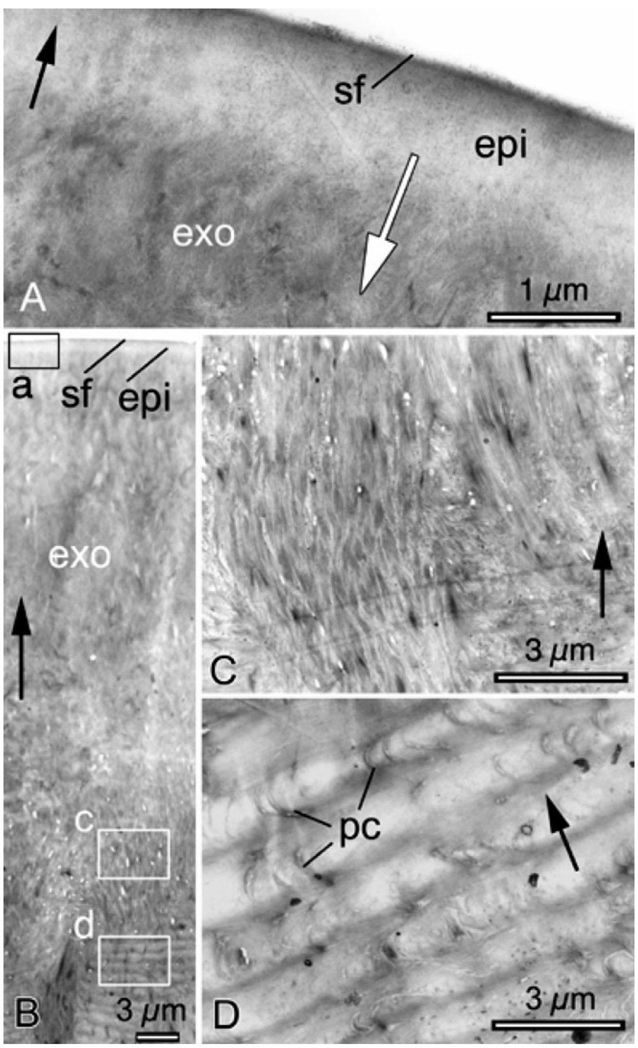

Fig. 7. TEM micrographs of the gula. (A) Detail of the epicuticle (epi). (B) Cross section of the epi- and exocuticle. (C) Detail of the upper part of the exocuticle (exo). (D) Detail of the deeper part of the exocuticle. a, c, d, rectangles indicate parts of the sample magnified in A, B, and C, respectively; black arrows indicate direction towards surface; white arrow indicates the indentation depth. Abbreviations: pc, porous channels; sf, surface.

Desiccation

Measurements of the desiccation rate were carried out to monitor the water loss in the samples during the indentation time (1.0–1.5 h). In the entire head, the loss of water in the first hour was ~20% (Fig. 8). After 10 h, the samples had lost ~60% of their initial mass. Desiccation rate of the dissected gula part was much higher than for the entire head. After approximately 40 min, the samples had dried out completely, with a mass loss of ~18%.

Mechanical properties

The nanoindentation measurements revealed a strong dependence of the mechanical behaviour on the preparation conditions (Figs 9, 10). Hardness and elastic modulus values of the fresh, dry and chemically treated samples were compared with each other at different indentation depths (250 nm, 500 nm, 1 μm and 1.5 μm). The results of statistical tests show that material properties of the gula cuticle differ significantly for the fresh, dry and chemically treated conditions (Table 1). Only elastic modulus, measured in the dry and chemically treated samples at indentation depths of 1 and 1.5 μm, did not differ significantly.

The maximum displacement for the fresh samples was 3 μm, but for the dry and chemically treated samples it was only 2 μm, because the maximum load of the instrument had

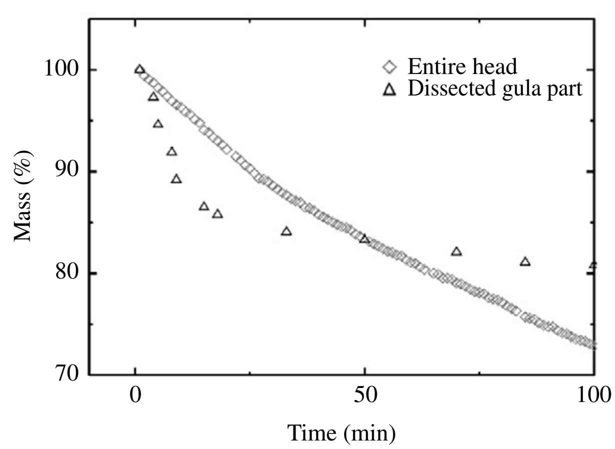

Fig. 8. Desiccation curves of the entire head in comparison with the dissected gula cuticle. Mass is shown as % of the initial head mass of the sample *versus* time of drying. The initial mass of each head was 25–30 mg. The data points are mean values of three measurements.

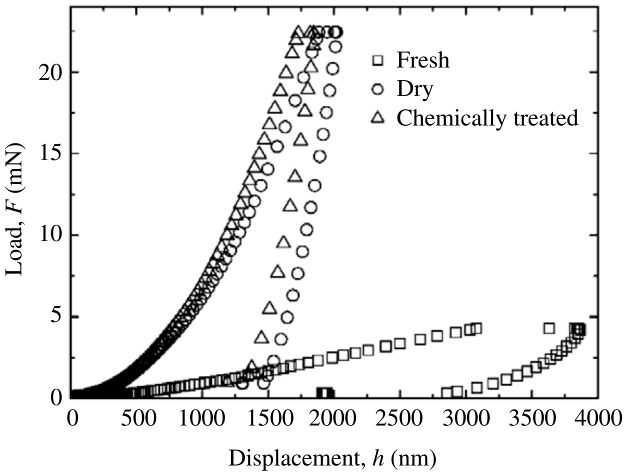

Fig. 9. Typical load–displacement curves for one sample in the fresh, dry and chemically treated conditions.

been reached. High values of hardness and elastic modulus in the first 50 nm of indentation are normally erroneous data caused by the contact formation behaviour between the indenter tip and sample due to the roughness and contamination. The data obtained at this depth were not included for further processing. For all samples, hardness and elastic modulus decreased slowly with indentation depth. However, after 1.7 μm, both parameters dropped rapidly for the dry and chemically treated specimens.

Hardness (H=0.10±0.07 GPa) of the fresh samples was significantly lower than in the dry (H=0.49±0.14 GPa) and the chemically treated (H=0.52±0.15 GPa) states (Fig. 10A)

Table 1. *Results of statistical tests (one-way ANOVA and Tukey post test) on the significance of differences between the hardness and elastic modulus for various indentation depths in fresh, dry and chemically treated conditions*

		Hardness/elastic modulus		
Depth (nm)	Condition	Fresh	Dry	Chem. treated
250	Fresh		+/+	+/+
	Dry	+/+		+/+
	Chem. treated	+/+	+/+	
500	Fresh		+/+	+/+
	Dry	+/+		+/+
	Chem. treated	+/+	+/+	
1000	Fresh		+/+	+/+
	Dry	+/+		+/–
	Chem. treated	+/+	+/–	
1500	Fresh		+/+	+/+
	Dry	+/+		+/–
	Chem. treated	+/+	+/–	

+ denotes significant difference between data; – denotes no statistically significant difference.

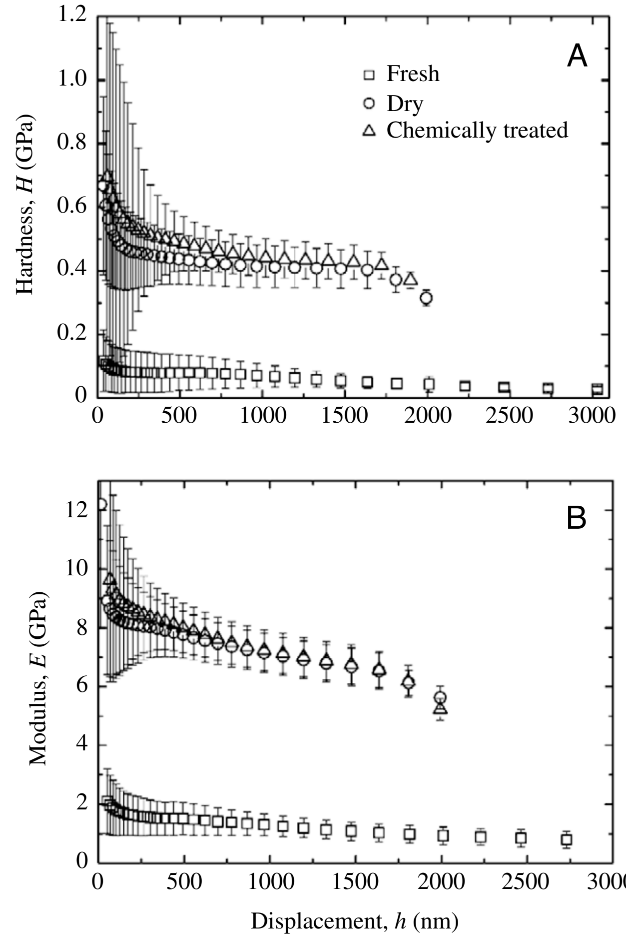

Fig. 10. Hardness (A) and elastic modulus (B) values from indentation tests (CSM technique) plotted *versus* displacement for fresh, dry and chemically treated samples. Each data point corresponds to the mean value of approximately 150 measurements ± s.d.

(ANOVA, P<0.0005; Tukey post test, P<0.0005). The difference between hardness values obtained on the dry (H=0.49±0.14 GPa) and chemically treated samples (H=0.52±0.15 GPa) was lower, but also significant (P<0.0005, ANOVA), especially in the range of depths of 200–1000 nm.

The same tendency was observed for the elastic modulus (Fig. 10B). Dry (E=7.50±1.80 GPa) and chemically treated (E=7.70±1.90 GPa) samples are significantly stiffer than fresh ones (E=1.50±0.80 GPa) (ANOVA, P<0.0005). In this case, the extraction of lipids caused a slight increase of the elastic modulus below 1 μm indentation depth. At depths higher than 1 μm, the elastic modulus values of the dry and chemically treated samples were not significantly different.

Atomic force microscopy

The surface profile of the gula cuticle was scanned using AFM after indentation tests (Fig. 11). Residual deformation of the cuticle surface after indentation is similar to an elastic-plastic contact, which is typical for most engineering materials (Bhushan and Li, 2003). The deviation of the residual imprint

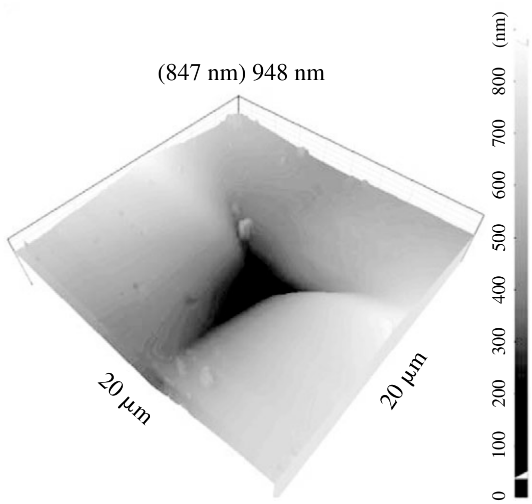

Fig. 11. AFM image of the cuticle surface after the indentation test. The image shows signs of the residual deformation and elastic recovery.

from the perfect pyramidal shape results from a visco-elastic relaxation, as is expected for soft biological materials (Wainright et al., 1976; Vincent, 1990; Kohane et al., 2003). This relaxation behaviour is especially evident on the lateral surfaces of the imprint, where the material almost goes back to its original condition ('cushion formation').

Discussion

Structure of the gula cuticle

The gula of *Pachnoda marginata* possesses an inner structure similar to that of the beetle *Geotrupes stercorarius* (Arzt et al., 2002). The epicuticle is only 1–3 μm thick and consists of numerous very thin layers running parallel to the surface. The exocuticle of the gula differs from the layered pattern of regular cuticle, where the orientation of fibres is parallel to the surface (Neville, 1975; Wainwright et al., 1976; Vincent, 1990). The fibres in the external layers of the exocuticle are oriented roughly perpendicular to the surface. A similar fibre orientation has previously been found in the contact system of attachment pads of grasshoppers (Gorb and Scherge, 2000); however, the gula and adhesive pads have very different functions.

Desiccation

As expected, the gula cuticle had a higher desiccation rate than the entire head. As the samples were ready for testing in 3–5 min, and the test duration did not exceed 1.5 h, it can be concluded that the indentations were carried out in an almost native condition of the material. The desiccation measurements showed that the water content of the dissected gula cuticle was about 18% mass. This value is comparable with literature data of approximately 12% for hard and tanned types of cuticle (Vincent and Wegst, 2004).

Mechanical behaviour of the cuticle in different conditions

In general, soft and compliant cuticle contains more water than hard and stiff cuticle (Vincent and Wegst, 2004). The gula belongs to this type of cuticle and bears a low proportion of water. In spite of this, the results show that desiccation has a great influence on the mechanical behaviour of the gula cuticle. After drying, it becomes about five times harder and stiffer (H=0.49±0.14 GPa; E=7.5±1.8 GPa) than in the fresh state (H=0.1±0.07 GPa; E=1.5±0.8 GPa). Water content seems to be a crucial factor for the mechanical properties, as previously reported by other authors (Andersen et al., 1996; Arzt et al., 2002; Enders et al., 2004; Vincent and Wegst, 2004). While structural changes have been previously studied during sclerotization (Andersen et al., 1996), the mechanical effects of drying are unknown in detail. It is obvious that water makes cuticle soft and compliant. Indentation tests on different parts of insect cuticle display also a considerable difference in the mechanical behaviour between fresh (hydrated) and dehydrated materials (Hillerton et al., 1982; Arzt et al., 2002; Enders et al., 2004).

After drying, removal of cuticle lipids caused only small additional changes in the indentation results (a slight increase in hardness and stiffness), especially at a depth below 1 μm. This confirms the hypothesis that the mechanical behaviour of the dry material is determined mainly by proteins and chitin, which could not be removed by the lipid extraction. In order to understand the exact mechanical roles of the two main components (proteins and chitin), further studies are necessary.

Hardness and elastic modulus of the gula

Our previous nanoindentation tests on the excised cuticle of the dung beetle *Geotrupes stercorarius* were performed using a Nano Indenter II (MTS Nano Instruments) without continuous measurement of hardness and elastic modulus, and therefore contained no information about gradients of the material properties in the joint cuticle (Arzt et al., 2002). In the present study, continuous stiffness mode allowed testing cuticle mechanical properties gradually at various indentation depths. SEM and TEM images were used to obtain information about the material structure in the indented region. The maximum indentation depth in our experiments was set to 3 μm, and, therefore, mechanical properties were mostly estimated for the epicuticle, whose thickness was in the range of 1–3 μm. According to models used for hardness determination in thin metal films (Jönsson and Hogmark, 1984; Burnett and Rickerby, 1987; McGurck et al., 1994; Rother and Jehn, 1996; Korsunsky et al., 1998), the results obtained for gula cuticle could also be influenced by the underlying layers of the exocuticle. Hardness measured at certain indentation depth is influenced by underlying layers of the material located at up to 10 times greater depth (Bueckle, 1965; Bhushan and Li, 2003). In the case of the gula, a 100–300 nm indentation depth can be assumed to characterize entirely the epicuticle, which is 1–3 μm. At larger indenter displacements, the data demonstrate combined properties of the epicuticle and external layers of the exocuticle. This suggestion is true only for the estimation of hardness. The elastic modulus is influenced by the entire thickness of multilayered composite material.

According to our results, the gula cuticle can be considered as a softer material coated by a harder film. At indentation depths larger than 1 μm, both the e-modulus and hardness tend to decrease. This is an indication of an increasing influence of the mechanical properties of the exocuticle. With further penetration, the exocuticle properties become more and more dominant, and, at displacements of h>2.5 μm, the data reach a certain plateau (Fig. 10). This shows clearly that the deeper cuticle layers are softer and more compliant than the outer layers. The influence of the underlying layers on the mechanical properties is even more pronounced for the dry and chemically treated samples. During the drying process, the material shrinks, and thus the indenter tip can penetrate layers that could not be reached in the fresh state because they were located considerably deeper within the material. The sharp drop in hardness values for the dry and chemically treated samples at the depth of 1.7 μm indicates the presence of even softer and more compliant layers in the exocuticle. This result might also be due to the difference in desiccation behaviour between the surface layers and the deeper ones due to a lower density of the material (Locke, 1964; Neville, 1975).

In attempting to compare the present results with the literature data, it is important to note that most experiments so far were performed on dry or rehydrated samples, and only a few indentation measurements of the fresh cuticle were reported. Previous microindentation experiments had relatively low spatial resolution and/or did not apply continuous stiffness mode to allow measurement of hardness and elastic modulus as a function of depth. The mean hardness (0.1–0.52 GPa) and elastic modulus (1.5–7.7 GPa) values obtained in our study are nevertheless similar to those of other sclerotized cuticles [Vickers hardness 0.2–0.5 GPa and Young's modulus 1–10 GPa (Vincent and Wegst, 2004)]. However, obtained hardness values (0.10±0.07 GPa) of the fresh gula samples are even below this range. This suggests that the present study may have characterised the cuticle of the insect joint in a true native condition for the first time.

Comparison with other insects

Other studies on cuticle mechanical properties using indentation have obtained comparable hardness values on dry cuticle samples of different insects. Different parts of a dehydrated locust cuticle, measured by means of a Leitz Miniload hardness tester using a Vickers diamond, had a hardness of 0.24–0.33 GPa (Hillerton et al., 1982). Dry wing membrane cuticle of the dragonfly *Aeshna cyanea* (Odonata, Anisoptera), tested with a nanohardness tester (Hysitron TriboScope), exhibited hardness of 0.2 GPa (Kreuz et al., 1999). However, dry samples of the beetle gula were even harder than any of these cuticles (H=0.49±0.14 GPa). It is also not surprising that the elastic modulus values obtained for the dry beetle gula are very high (E=7.5±1.8 GPa) when compared with those of the dry wing membrane of the dragonfly *Ae. cyanea* (E=1.5±0.5 GPa) (Kreuz et al., 1999). The reduced modulus for different dehydrated body parts of the dragonfly was determined from quasistatic nanoindentation experiments (Hysitron Inc., Minneapolis, MN, USA; http://www.hysitron.com/PDF/0501-001.pdf). The mean values were also much lower (E_r=1.5–4.7 GPa) than for dry samples in the present study.

A recent study on the integument of *Drosophila melanogaster* during various developmental stages has been recently performed using a similar method. The results showed that the thickness of the cuticle and the development stage of the insect are important factors influencing cuticle stiffness (Kohane et al., 2003). The mean E_r of 0.41 MPa, 15.43 MPa, and 4.37 MPa were determined by *in vivo* experiments for the cuticles of larvae, pupae and adult insects, respectively.

Conclusions and outlook

Our results support the assumption that the head articulation joint surface, according to its structure, consists of material adapted for friction- and wear-minimising (fibre orientation in the external layers of the exocuticle, presence of channels in the cuticle and secretory substances on the surface) and mechanical properties (hard layer of the epicuticle, with more compliant underlying layers). Surfaces of engineering bearing systems show a layered material structure with gradients in mechanical properties (Barwell, 1979; Bhushan, 1999). Technical systems demonstrate also a great difference in the mechanical properties of materials of both contacting surfaces. It is believed that such a difference is an adaptation for reduction of friction and wear. Our future investigations will concentrate on the gula counter surface located in the prothorax, in order to find a possible correlation between structure and properties of both counterparts. Friction measurements of both materials using the microtribotester are currently underway.

List of symbols

A	contact area
E_i	Young's modulus for indenter tip
E_r	reduced elastic modulus
E_s	Young's modulus for specimen
F_{max}	peak load
H	hardness
h_c	contact depth
h_{max}	indentation depth at the peak load
h_s	elastic deflection of the surface at a particular contact perimeter
k	geometric constant for the area function of the tip
S	contact stiffness
ν_i	Poisson's ratio for indenter tip
ν_s	Poisson's ratio for specimen
β	tip geometry constant
ϵ	geometric constant of the indenter

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