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Calcified cuticle in the stomatopod smashing limb

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One pair of limbs of the mantid shrimp, *Gonodactylus,* is used to smash hard-shelled prey. The composition and structural features of the cuticle allowing this were examined by microhardness testing, energy-dispersive X-ray analysis, and scanning electron microscopy. The cuticle becomes much harder toward its outer surface, and this is associated with an increased mineralization of the organic cuticle and the replacement of calcium carbonate by some form of calcium phosphate as the important mineral phase. The outer part of the limb is so hard that, although it is very brittle, it is very rarely damaged during many months of use in which it strikes thousands of highly energetic blows.

1. Introduction

The stomatopoda, mantid shrimps, are a group of tropical and subtropical marine crustaceans. They are predatory on other large animals such as snails, shrimps, crabs and fish which they subdue using their second thoracic limbs. There are 350 known species of stomatopods, ranging in length from 15 to 330 mm. They are divided into two groups: the spearers and the smashers. In the spearers the distal segment (podomere) of the second thoracic limb, the dactyl, has a sharp, harpoon-like point, which is barbed at the tip. The strike is made with the dactyl open. The action is very like that of the praying mantis, hence the name of the group. The smashing stomatopods, on the other hand, have a dactyl with few spines, or none. Instead the dactyl has a large "knee" at its base, which is used to strike the prey. The dactyl remains closed during the strike (Fig. 1). Dingle and Caldwell [1] claim that the energy of a strike of a large species is about 50 J, approaching that of a small-calibre bullet. Even a small species, 80 mm long, can break the wall of an ordinary glass aquarium. Although a smasher may make hundreds of strikes a day at hard shells the heel of the dactyl remains very smooth over many months. An account of the functional anatomy of the stomatopods is given by Kunze [2].

The arthropods are a very successful group of invertebrates, including insects, spiders, and crustaceans. The exoskeleton of arthropods is made of cuticle which may vary in its composition and properties within animals and between species, depending on the function the cuticle may have. Cuticle consists of a protein matrix bonded to chitin fibres. Chitin is a long-chain polysaccharide, poly n-acetylglucosamine. The stiffness of the cuticle depends on the amount of cross-linking, or tanning, that occurs, and also on the ratio of protein to chitin. The chitin fibrils are about 2 to 3 nm in diameter. They are arranged in a "helicoidal" fashion which makes the cuticle isotropic in the plane of the cuticle [3]. In the helicoidal cuticle the fibres are arranged in any one layer all in the same direction, but neighbouring layers are displaced by a small amount typically 5 to 10 degrees, so the preferred orientation changes cyclically through the thickness of the cuticle. There is evidence [4] that in crustacean cuticle the fibrils may join up into quite thick fibres, and that some of these fibres may pass through the thickness of the cuticle, rather than being confined to a single layer. The cuticle of an insect such as a cockroach is divided into an outer exocuticle and an inner endocuticle. The exocuticle is much more resistant to enzymatic attack than

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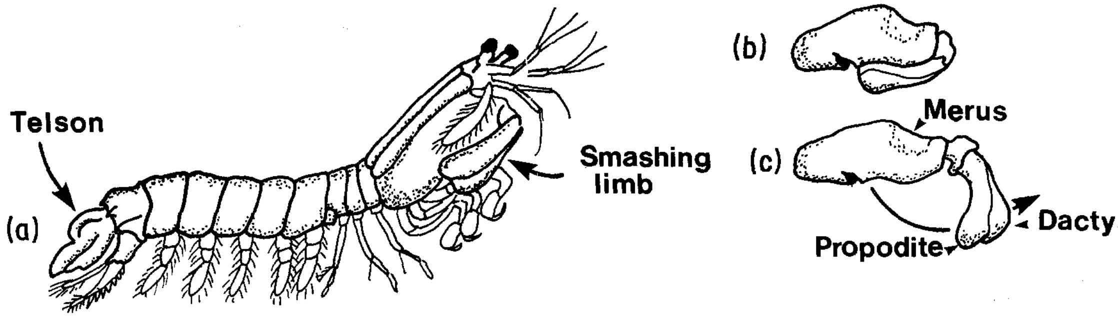

Figure 1 (a) A typical smashing stomatopod, *Gonodactylus chiragra*. This animal is about 10 cm long. The main elements of the smashing limb consists of the merus, the propodite, and the dactyl. Only the last two are considered in this paper. (b) Shows the closed position and (c) shows the smashing action.

the endocuticle, being highly tanned. It is probably also much stiffer, and may also become darkly pigmented [5].

In the stomatopods, as in most Crustacea, much of the stiffness is produced by mineralization of the cuticle with calcium carbonate and, to a lesser extent, calcium phosphate. This mineral to a large extent replaces the protein.

It appears that there must be features of the stomatopod dactyl that make it capable of dealing so many blows without becoming damaged. The objective of our investigations was to determine what these features were.

2. Specimen preparation and examination

The stomapods used in this study were *Gonodactylus* sp. which had been preserved in alcohol in the British Museum (Natural History) for many years. (The effect this preservation may have had on the mechanical properties of their cuticle is discussed below.) They had a body length of about 75 mm (Fig. 1). Reichert diamond pyramid microhardness measurements, using 20 or 50 grammes force for fifteen seconds, were taken at various points on the polished surfaces of two sections, each containing a dactyl and a propodite, sectioned at different levels. In addition, the P:Ca ratio and the distribution of various other elements with respect to Ca were determined by microprobe analysis and energy-dispersive X-ray analysis (EDAX) using JEOL JXA50 and JXA35X instruments respectively. The measured spectral peak for each element was converted to a mass ratio relative to Ca using the Colby "MAGICIV" computer program [6, 7], which, with an initial standardization, corrects for background absorption, characteristic fluorescence, backscatter losses, and ionization penetration losses. The specimens were mounted in "Specifix" (an acrylic cold-setting medium), ground on silicon carbide papers and given a final polish with 0.1 μm alumina paste.

The first section, from the right limb, cut the dactyl near its tip, and the propodite near the joint (Fig. 2). The second section cut the limb just above the joint, at the knee, where the dactyl was most enlarged. The propodite was also cut just above the joint. Each section through a dactyl or propodite showed three regions: a central cavity, (which would have been filled with soft tissue in life) a layer of rather fibrous cuticle and, on the outside, a layer of cuticle which seemed smooth. The relative thicknesses of the layers at various points are shown in Fig. 3.

A third dactyl section was prepared, from a second animal, by fracture rather than by polishing. The specimen was nicked with a scalpel in a place well away from the region finally examined, and was then split in two by forceps. The surface resulting from the catastrophic spread of the fracture was examined by scanning electron microscopy after being dried and coated with about 40 nm of gold. The region shown is roughly the same as that examined in the sections from the second section, which was polished.

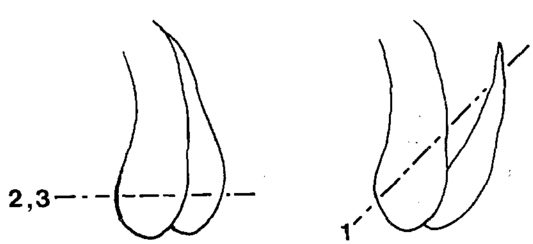

Figure 2 Diagram of propodite and dactyl showing the orientations and positions of the three sections discussed in the paper.

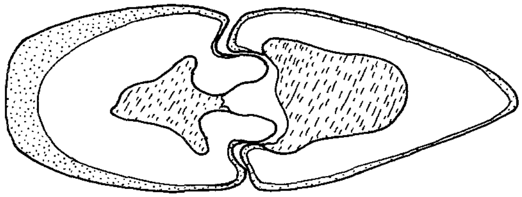

Figure 3 The second cross-section. The knee of the dactyl, to the left, has a thick, heavily calcified region, shown finely stippled. The sides of the dactyl, and the propodite (on the right) have a much thinner heavily calcified layer. The more lightly calcified, fibrous region is left blank. The soft tissue in the middle is shown hatched.

We also examined the telson of the animal. The telson is a shield protecting the animal at the rear end, and it is used in intraspecific dominance fights; each animal in turn lies on its back and receives a blow, or blows, on its telson from its opponent's dactyls.

3. Results

3.1. Morphology

High magnification photographs of the outer part of the anterior aspect of the dactyl show a fairly smooth fracture surface compared with the much more fibrous surface of the inner layer (Fig. 4a and b). In fact there is a progressive increase in roughness from the outside in. Stereo-pairs of the propodite also shows a very smooth outer fracture surface and a rougher one inside. The progressively rougher fracture surface does, however, show a clear periodic structure, difficult to make out on the harder parts, but becoming clearer within. These are the laminae produced by the helicoidal arrangement of the chitin fibrils and calcite crystals. Each lamina represents a region within which the orientation of the fibrils changes through 180°.

Fig. 5, a photograph of the telson, shows that the non-fibrous layer is very thin, only about five μm, and that the layers beneath are very fibrous indeed.

3.2. EDAX spectra

The EDAX spectra show that P, Mg, Na, K, Al, and Si were present, but that Ca was the dominant element. Following corrections introduced by the computer program [6, 7], the results were expressed as a mass ratio of each element compared with that of Ca. The absolute values of the elements, therefore, depend on the actual amount of calcium present. Al and Si, up to 0.03, 0.019, were probably caused by contamination from the alumina used during polishing. K, Na, and Mg, though present were small in amount (about 0.01, 0.008 and 0.19, respectively) and did not vary much through the specimens. The level of phosphorus, as determined by both EDAX and microprobe analysis, was often high, however, and varied in an interesting way with hardness and fracture morphology, as we show below. Its ratio relative to calcium varied from 0.027 to 0.358. Sulphur was also present in significant amounts, ranging from 0.006 to 0.066 compared with calcium. It presumably represents calcium sulphate, which is known to replace calcium carbonate to some extent in many crustacean cuticles [8]. Possibly a very small amount of this variation could be attributed to greater or lesser amounts of the amino acid cysteine, which contains one sulphur atom.

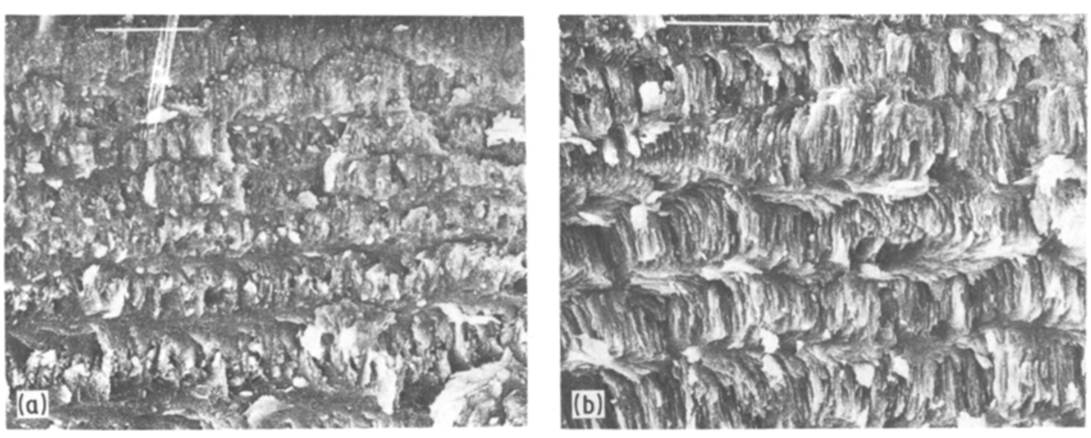

Figure 4 Fracture surface of the knee region shown in Fig. 3. In both pictures the white bar at the top left represents 25 μm. (a) The outer part of the knee, at the outer surface is at the top. (b) The inner, less highly calcified region.

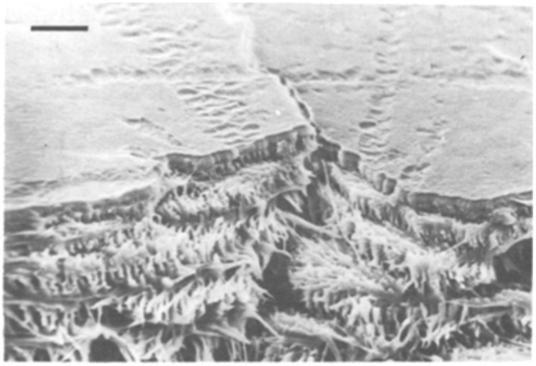

Figure 5 Fracture surface of a telson. The black bar at the top left represents 10 μm.

Fig. 6 shows the ratios of P:Ca in a dactyl. It is clear that the ratio increases from the middle of the section to the outside. These findings imply that the mineral in the inner part of the cuticle is mainly calcium carbonate, and that towards the outside there is much more calcium phosphate.

3.3. Microhardness

Measuring the microhardness presented difficulties

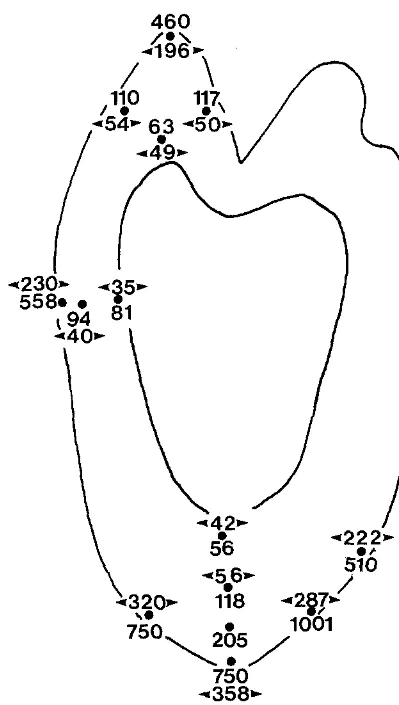

Figure 6 Section of the propodite of section 1. Values for Reichardt microhardness, and values for P:Ca (multiplied by 1000). The latter have small arrow heads. The values are taken from the area shown by the small spot. Note particularly the high values for both variables in the outermost regions.

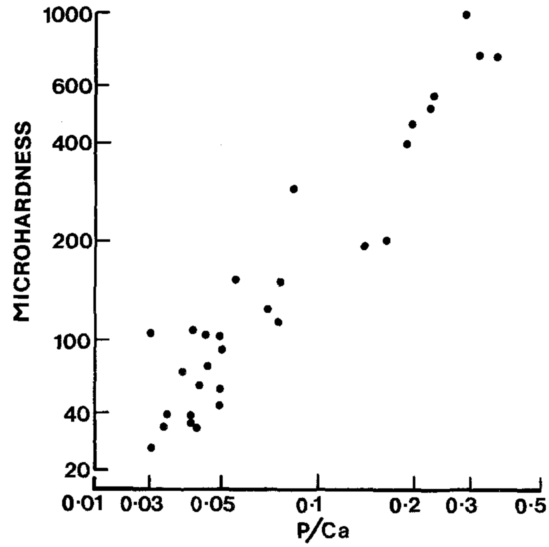

Figure 7 Relationship between microhardness and the ratio of phosphorus to calcium. The points are from both sections one and two, and from the propodite and dactyl in each section, wherever the two variables were determined nearly in the same place. Note both axes are on a logarithmic scale.

in places. The outer layer was often thin, and it was then difficult to find a spot where an indentation could be made without getting too near an edge. Furthermore, in the outer layer the diamond often did not make a measurable impression but instead shattered the surface. However, a large number of impressions were made, and the general pattern became clear.

The hardness was less in the inner, fibrous layers than in the outer layer. Fig. 6 shows some values. It was not always possible to distinguish systematic differences in hardness between layers in the fibrous region, though there was some tendency for the innermost layer to be quite soft. The variation found within the outer layer, which was always hard, may not be very meaningful, because the impressions were often difficult to make out.

Fig. 7 shows the relationship between the P:Ca ratio and the microhardness from very similar points in the dactyl and the propodite of the first section. There is obviously an extremely strong and nearly linear relationship between the two. The regression, derived from the least square fit to logged values is

$$\text{Microhardness} = 2438(\text{P/Ca})^{1.08}.$$

$r = 0.90$. (Logged values are used because the raw data have a markedly skewed distribution; logging them makes the distribution much closer to the normal distribution.)

We do not know the phosphatic mineral in the *Gonodactylus* cuticle. If it were hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, the P:Ca molar ratio would be 0.6. If it were calcium phosphate, $Ca_3(PO_4)_2$, the P:Ca molar ratio would be 0.66. The greatest P:Ca *mass* ratio in the cutical was 0.358, implying a molar ratio of 0.463. From these values, the maximum percentage of hydroxyapatite in the surface layers would be 77%, or the maximum calcium phosphate percentage 70%. Therefore, regardless of the particular form present (or of any variability in the P:Ca ratio due to non-stoichiometry) we conclude that calcium phosphate represents the *majority phase* in the surface layers. The balance of the calcium is presumably calcium carbonate [with a small percentage of calcium sulphate (~ 3%)]. It is interesting to note that a hydroxyapatite: calcium carbonate combination is also a feature of mammalian hard tissue [9], albeit with a lower calcium carbonate content (up to 5%), as a result of the possible ionic substitution of 2 OH^- and PO_4^{3-} by CO_3^{2-}.

4. Discussion

In reading this discussion it must be remembered that these specimens have been preserved for years in alcohol, and their mechanical behaviour may well have been altered somewhat by this treatment. The effect of the alcohol, if important, would presumably have been to increase the stiffness of the cuticle by irreversibly removing water, which acts as a plasticizer [10]. The effect will be relatively less in the highly mineralized region. The effect will, therefore, tend if anything to reduce the mechanical difference between the more and the less highly mineralized regions. The three different modes of investigation showed that the outer region of the cuticle of both the dactyl and the propodite was different from the inner region. The hardness was much greater, the ratio of P to Ca was much greater, and the fracture surface was much smoother. Unfortunately, the EDAX did not allow a simple measurement of the absolute amount of calcium. However, it is quite clear that the outer part of the cuticle is more heavily mineralized than the inner part. This agrees with what is known about other decapods, such as crabs and shrimps. The fracture surface is like that of various crabs that one of us (JDC) has observed with the scanning electron microscope. (The genera examined were *Carcinus, Cancer,* and *Maia.*) That is, there is a smoother outer part and a more obviously fibrous inner part. Many of the cuticles looked like the telson of *Gonodactylus* (Fig. 5). Where the dactyl of *Gonodactylus* differs from other cuticles is in the thickness of the highly calcified layer.

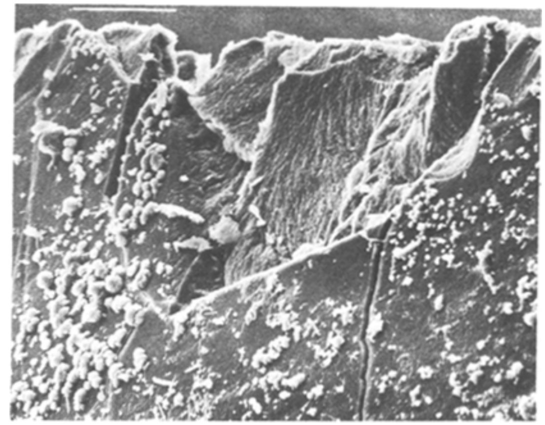

Figure 8 Outer surface of a dactyl. The dactyl broke in two along the top of the photograph. Brittle cracks run from this surface towards the bottom. The line at the top left of the photograph represents ten micrometres.

The dactyl of *Gonodactylus* is designed to break other objects. It does this by gaining a large amount of kinetic energy. If *Gonodactylus* is like the related *Hemisquilla* in this respect, it does this by the sudden release of stored strain energy [11]. This kinetic energy is then transferred to the other object. When this happens the smashing limb will be slowed down or brought to rest. The efficiency of this energy transfer will be the greater, the greater the stiffness of the dactyl. (If two bodies of equal mass and shape collide, the energy will be partitioned between them in inverse proportion to their stiffnesses.) Therefore, the fact that the knee of the dactyl is highly mineralized is advantageous to the animal. The corollary of this mineralization, however, is that the material of the outer layer is brittle. This was shown by its behaviour in the hardness tester, also, we find that if one of these dactyls is pressed hard with a metal object it will break in a brittle manner, with cracks running in all directions (Fig. 8). These brittle cracks do not, usually, pass through the fibrous inner region. The outer layer must, therefore, not be deformed much, or it will break. It is necessary to have a sufficient thickness of heavily calcified material for the structure to be stiff. The opposite tactic is shown by the telson. This has to absorb blows, and the highly calcified layer is very thin and lies on a thick bed of flexible material. The telson can deform without the strain in the highly calcified layer becoming large.

The other notable feature of the results is that the P:Ca ratio increases with the hardness. Now essentially all the calcium in the cuticle will be in the form of calcium carbonate or phosphate; the organic material having very little calcium. Therefore, the implication of this finding is that as the cuticle becomes more heavily mineralized from the inside outwards the mineral is relatively more in the form of calcium phosphate than calcium carbonate.

The smashing limb of the stomatopod is obviously well designed for its function. Although the significance of the increase in calcium phosphate at the expense of calcium carbonate is not known, it is almost certainly related to the increase in hardness that accompanies it.

We have not emphasized other points in the design of the limb, because they are not of immediate importance to materials scientists. (We could mention the power amplification in the strike, which is attained by the sudden release of strain energy stored in other parts of the cuticle, and the streamlined shape of the smashing limb, which reduces losses from drag; the Reynolds number being high enough for drag to be important.) We do, however, wish to draw the attention of materials scientists to the design of living organisms, because they are remarkable for the sophistication of their structural materials, particularly in their precise fabrication. This sophistication is achieved with often very unpromising materials, fabricated at temperatures below 40° C.

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