

## THE SPECIFIC LOCATION OF ZINC IN INSECT MANDIBLES

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The cutting edges of the mandibles in the locust have been shown to be twice as hard as the rest of the mandible (Hillerton, Reynolds & Vincent, 1982) and this was related to the cutting action employed by the insect. We have investigated the elemental composition of the mandibles of insects from several taxa to see if the hardness is due to 'mineralization' as in the 'teeth' of several other invertebrates e.g. the radular teeth of *Patella*, which contain iron and silica (Runham *et al.* 1969). For the first time we report the specific location, in the cutting edges (incisors) of insect mandibles, of large quantities of two transition elements, zinc and manganese. Metals have been found previously in only a few types of insect cuticle – in exceptional dipteran puparia and some phasmid eggs (Neville, 1975) – but their presence has not been related to any of the mechanical properties of the cuticle.

Mandibles were dissected from insects freshly killed by freezing at  $-20^{\circ}\text{C}$  and any adhering soft tissue was removed. The mandibles were rinsed briefly in distilled water and then in methanol to remove surface debris held on to the mandibles by cuticular lipids. The mandibles were then blotted and mounted on a copper or aluminium stub with silver paint. An X-ray microanalysis of the uncoated mandible was made in the scanning mode of a JEOL model 100S electron microscope fitted with a Kevex detector and a Link analyser. Analyses were made with an accelerating voltage of 40 kV, specimen tilt of  $35^{\circ}$  and a spot size of approximately 70 nm. Analyses were counted for 100s on an energy range of 0–20 k eV but spectra were recorded from 0–10 k eV only as no information is lost by using this narrower energy range. X-ray maps were made by monitoring for energy in the range 8580–8660 eV corresponding to the zinc  $K\alpha$  emission peak.

A minimum of six analyses of both left and right mandibles were made of all the species listed in Table 1. As shown for the locust (Fig. 1), all mandibles contain silicon, phosphorus, sulphur, chlorine, potassium and calcium. Such elements may be expected in a cuticular matrix normally in contact with epidermal cells. (In all spectra there were emissions corresponding to silver, copper and sometimes aluminium; these arise from the specimen stubs, the silver paint used to stick specimens on to stubs and the lenses of the microscope.) The locust mandibles also contain zinc in a sufficiently large amount to show both the  $K\alpha$  and the  $K\beta$  peaks (Fig. 1). No zinc was found in any other piece of locust cuticle examined. These were the prothorax, femur ribs, tibial shaft, tibial spines, tarsal claws, semilunar processes and the rest

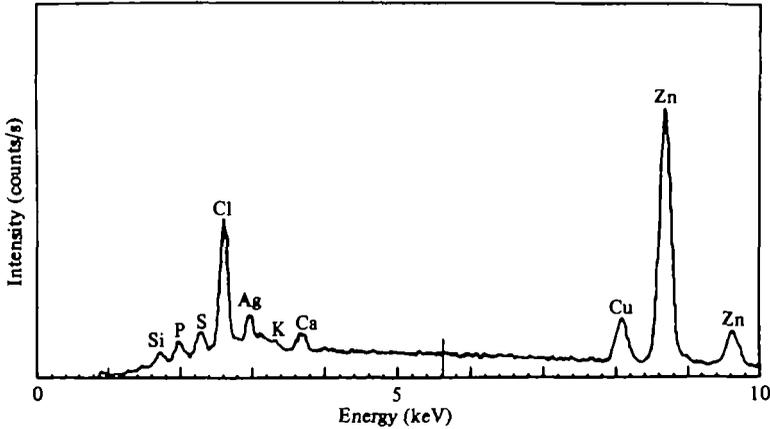


Fig. 1. X-ray microanalysis spectrum of the elemental composition (elements of atomic weight  $> 10$ ) of the incisor region of the left mandible of adult *Locusta migratoria*.

of the tibio-femoral joint, abdominal tergites and sternites and the ovipositor valves of the female. Mandibles from other species were also found to contain zinc and/or manganese (Table 1).

When present in the mandibles, zinc and/or manganese are exclusively located in the cutting edges. The location of zinc was found by X-ray mapping (Chandler, 1977) as shown for the minima caste worker of the ant, *Atta cephalotes* (Fig. 2). In all cases the occurrence of zinc is confined to the cutting edges (the teeth in the ant) and it is not found in the sheared cuticle or the supporting cuticle of the locust or any other species examined. Manganese is present in lesser quantity and therefore its location had to be found by a large number of analyses of the morphologically distinct areas of the mandibles. Again it is restricted to the cutting areas only. Most insects that were examined were herbivores and most of these contain zinc or manganese in the mandibular incisors (Table 1). The mandibles of herbivorous nereids also contain zinc (Bryan & Gibbs, 1979). Neither zinc nor manganese was found in the mandibles of the carnivorous beetle or the omnivorous cockroaches examined (Table 1).

There is some indication that the presence of zinc or manganese in the mandibles of the herbivorous insects is related to their taxonomic grouping. Thus manganese was not found in the orthopterans, phasmids or lepidopterans; and among the hymenopterans, manganese was always found together with zinc (Table 1). The picture is somewhat confused as the only coleopterans to contain zinc are wood-boring larvae. However the presence of these elements might also be related to feeding habits, the composition and mechanical properties of the foodstuff or the feeding mechanism employed.

Only ash contents of whole insects or whole cuticles have been reported previously and these are always less than 0.3% of the dry weight (Richards, 1951). The amount of zinc present in the whole mandible of the *Heliothis* larva and the locust (Table 2) shows that the zinc content, alone, of the mandible is several percent of the dry weight. In effect the actual zinc content of the cutting edges may be some 3-4 times higher still as the values in Table 2 are for the whole mandible and the zinc is highly localized.

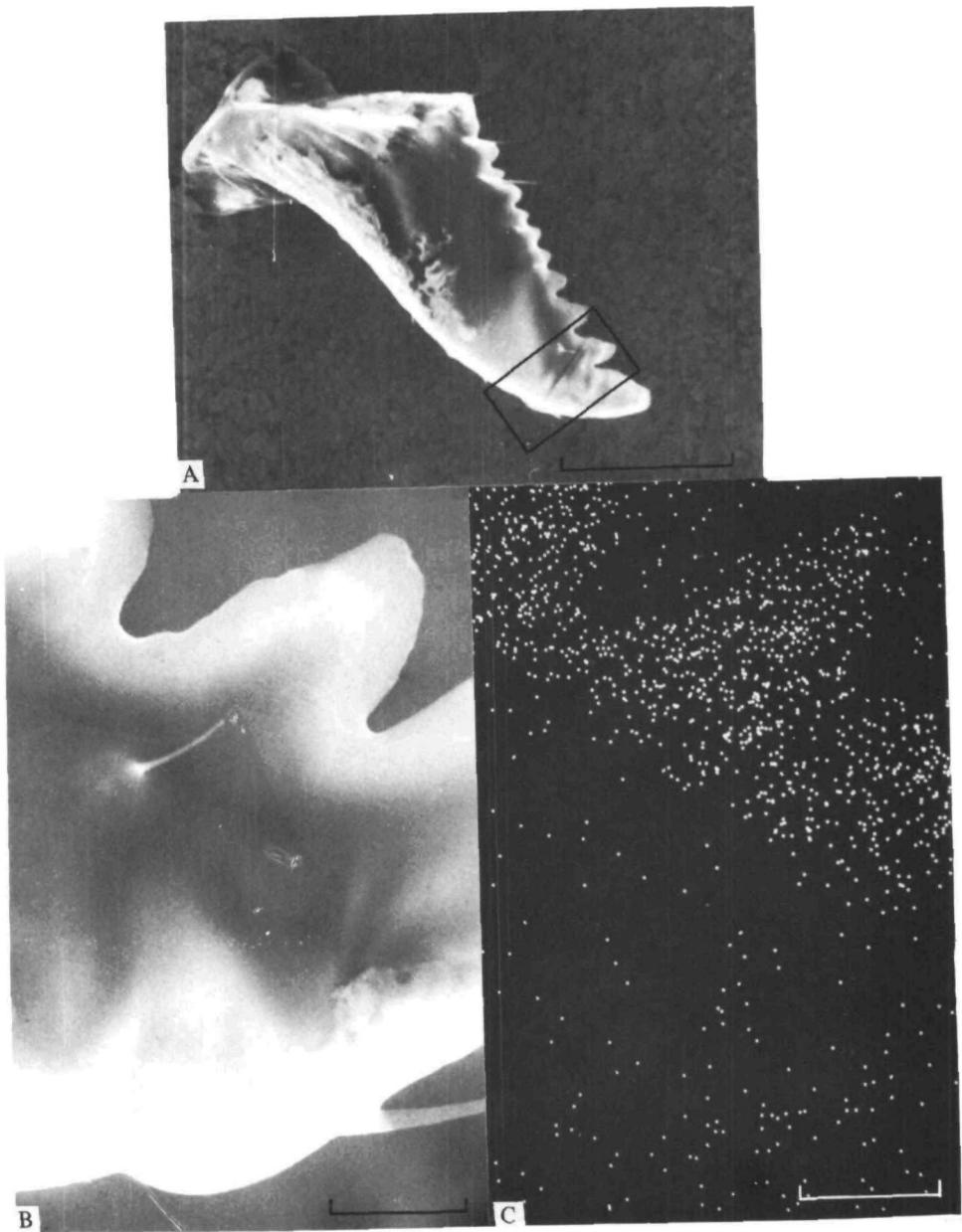


Fig. 2. (a) Scanning electron micrograph (SEM) of the right mandible of a minima caste worker of the leaf-cutting ant *Atta cephalotes*. Scale, 0.1 mm. (b) SEM of the boxed area of (a) used for X-ray mapping in (c). Scale, 5  $\mu$ m. (c) X-ray microanalysis map of the mandible area shown in (b). By comparing this map with (b) the zinc can be seen to be localized within the mandibular teeth and the immediately supporting cuticle only. Scale, 5  $\mu$ m.



Table I. The occurrence of manganese and zinc in the incisor region or the equivalent cutting surface of insect mandibles was determined by X-ray microanalysis as described for Fig. 1.

(The occurrence of manganese or zinc is shown here and denoted by their atomic symbols, absence is denoted by a dash. Except where noted Si, P, S, Cl, K and Ca were found in all instances.)

Insect	Stage of life-cycle	Occurrence or absence of manganese and zinc	Type of feeding
Orthoptera			
<i>Anacridium melanorhodon</i>	5th instar nymph	— Zn	Herbivore
<i>Locusta migratoria</i>	All stages	— Zn	Herbivore
<i>Locustana pardalina</i>	3rd instar nymph	— Zn	Herbivore
<i>Schistocerca cancellata</i>	Adult	— Zn	Herbivore
<i>S. gregaria</i>	All stages	— Zn	Herbivore
Phasmida			
<i>Carausius morosus</i>	Adult	— Zn	Herbivore
<i>Extasoma tiaratum</i>	Adult	— Zn	Herbivore
Lepidoptera			
<i>Daphnis neri</i>	Larva	— Zn	Herbivore
<i>Heliothis zea</i>	Larva	— Zn	Herbivore
<i>Hepialis</i> sp.	Larva	— Zn	Herbivore
<i>Manduca sexta</i>	Larva	— Zn	Herbivore
<i>Pieris brassicae</i>	Larva	— Zn	Herbivore
<i>Spodoptera littoralis</i>	Larva	— Zn	Herbivore
Hymenoptera			
<i>Acromyrmex octospinosus</i>	Worker	Mn Zn	Herbivore
<i>Atta cephalotes</i>	Worker	Mn Zn	Herbivore
<i>Formica lugubris</i>	Worker	Mn Zn	Herbivore
<i>F. rufa</i>	Worker	Mn Zn	Herbivore
<i>Lasius flavus</i>	Worker	Mn Zn	Herbivore
Coleoptera			
<i>Anobium punctatum</i>	Larva	Mn Zn	Wood-borer
<i>Hylotrupes bajulus</i>	Larva	Mn Zn*	Wood-borer
<i>Melolontha melolontha</i>	Larva	— —†	Herbivore
<i>M. melolontha</i>	Adult	— —	Herbivore
<i>Tenebrio molitor</i>	Larva	— —	Stored product pest
<i>T. molitor</i>	Adult	Mn —	Stored product pest
<i>Phaedon cochleariae</i>	Larva	— —	Herbivore
<i>P. cochleariae</i>	Adult	Mn —	Herbivore
<i>Leptinotarsa decemlineata</i>	Larva	— —	Herbivore
<i>L. decemlineata</i>	Adult	Mn —	Herbivore
<i>Chrysolina menthastris</i>	Adult	Mn —	Herbivore
<i>Agriotes lineatus</i>	Larva	Mn —	Herbivore
<i>Carabus violaceus</i>	Adult	— —	Carnivore
Dictyoptera			
<i>Blaberus giganteus</i>	Adult	— —‡	Omnivore
<i>Blatta orientalis</i>	Adult	— —‡	Omnivore
<i>Blattella germanica</i>	Adult	— —†	Omnivore
<i>Periplaneta americana</i>	Adult	— —	Omnivore
Dermaptera			
<i>Forficula auricularia</i>	Adult	— —†	Herbivore

\* No silicon detected. † Iron detected. ‡ Very small peaks for all elements.

Table 2. *The zinc content of the mandibles of two species of insect*

(Mandibles were dried to constant weight at 100 °C, digested with nitric acid and then perchloric acid and the zinc content determined, using a Varian model-175 atomic absorption spectrophotometer, against a zinc chloride standard.)

Species	Zinc content ( $\mu\text{g mg}^{-1}$ dry wt)		
	$\bar{X}$	S.D.	n
<i>Heliothis zea</i> Larva	13.0	$\pm 0.6$	3
<i>Locusta migratoria</i> Adult	5.4	$\pm 3.7$	4

The observation that the cutting edges of the locust mandibles are harder than the rest of the cuticle (Hillerton *et al.* 1982) may be explained, in part, by the present results as being due to the presence of large amounts of zinc. We may assume that the analogous cutting edges of other herbivorous insects that also contain zinc and/or manganese are similarly hardened, although the mandibles are often much too small to measure, and that these elements may be responsible for the hardening.

We have no evidence that zinc or manganese is involved in mineralization (e.g. Runham *et al.* 1969). Zinc is always found in the presence of a large amount of chloride, but similar amounts of chloride are present in other areas of the mandible also. The zinc may increase the density of secondary bonding within the material by forming complexes with proteins and phenols, rather like the complexes formed by zinc, protein and melanins in the vertebrate eye (Bowness & Morton, 1953), and thus increase the density and fracture toughness of the mandibular cuticle.

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## REFERENCES

- BOWNESS, J. M. & MORTON, R. A. (1953). The association of zinc and other metals with melanin and a melanin-protein complex. *Biochem. J.* **53**, 620-626.
- BRYAN, G. W. & GIBBS, P. E. (1979). Zinc - a major inorganic component of nereid polychaete jaws. *J. mar. biol. Ass. U.K.* **59**, 969-973.
- CHANDLER, J. A. (1977). X-ray microanalysis in the electron microscope. In *Practical Methods in Electron Microscopy*, vol. 5 (ed. A. M. Glauert), Amsterdam: North-Holland.
- HILLERTON, J. E., REYNOLDS, S. E. & VINCENT, J. F. V. (1982). On the indentation hardness of insect cuticle. *J. exp. Biol.* **96**, 45-52.
- NEVILLE, A. C. (1975). *Biology of the Arthropod Cuticle*. Berlin: Springer-Verlag.
- RICHARDS, A. G. (1951). *The Integument of Arthropods*. Minneapolis: University Minnesota Press.
- RUNHAM, N. W., THORNTON, P. R., SHAW, D. A. & WAYTE, R. C. (1969). The mineralisation and hardness of the radular teeth of the limpet, *Patella vulgata* L. *Zeit. Zell.* **99**, 608-626.