Zoological Journal of the Linnean Society (1998), *124*: 387–396. With 1 figure
Article ID: zj970146

Manganese and zinc in the ovipositors and mandibles of hymenopterous insects

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Received June 1997; accepted for publication October 1997

X-ray microanalysis of the ovipositor and mandibles of various hymenopterous insects has revealed the presence in many species of up to 10% wt/wt of either zinc or manganese in the cuticle. These metals appear to be involved in cuticular hardening, so helping to reduce abrasive wear. Zinc is found in the ovipositors of most Siricidae, Stephanoidea, and Chalcidoidea. In Ichneumonoidea and Cynipoidea, the metal involved is manganese. Megalyroidea are unique in the Hymenoptera in having both zinc and manganese in their ovipositors, though in different locations. Except for Bethylidae, no metals were detected in the ovipositors or stings of species that penetrate soft substrates or do not make holes at all. The cutting edge of the mandibles of many insects that chew their way through hard substrates during egress from their pupation sites almost invariably contain high concentrations of zinc, and this is present in many that lack metals in their ovipositor. The phylogenetic and ecological implications of metal occurrence are discussed.

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ADDITIONAL KEY WORDS:—Insecta – Hymenoptera – ovipositor – mandible – zinc – manganese – phylogeny – cuticle.

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INTRODUCTION

The ovipositor has been a critical organ in the evolution of the insect Order Hymenoptera (sawflies, parasitic wasps and the aculeate wasps, bees and ants), and an ability to use it to penetrate hosts, either directly or through substrates of various resistances, has enabled them to capitalize on the parasitoid way of life (Gauld & Bolton, 1988; Quicke, 1997). Indeed the majority of species of Hymenoptera are parasitoids and these represent more species than all other insect parasitoids combined (Eggleton & Belshaw, 1992 1993). Even hosts living deep within wood are not immune to attack, as, for example, the ichneumonid *Megarhyssa nortoni*, a parasitoid of woodwasps (Siricidae), can have an ovipositor up to 50 mm long though it is only 100 by 200 μm in transverse section (Vincent & King, 1996) and in other *Megarhyssa* species the ovipositor may reach a length of 140 mm (Nénon *et al.*, 1995). In using their ovipositor to drill through hard substrates such as wood, the wasps face several problems: they must be able to generate sufficient forces at the ovipositor tip to disrupt the substrate without the ovipositor buckling (Vincent & King, 1996), and their ovipositor must be able to withstand the wear and tear that hours of drilling must cause.

Ovipositors of many parasitic wasps which bore through hard wood are subject to considerable wear and tear. Taking rhyssine ichneumonids (e.g. *Megarhyssa*) as an example, if the female usually lays approximately 20 eggs during her lifetime, as can be conservatively approximated from egg counts, and takes on average one hour 'drilling' for each host (Heatwole, Davis & Wenner, 1962; Eggleton, 1989), then her ovipositor will be actively drilling for some 20 hours. This is likely to be an underestimate, however, since several authors have reported that rhyssines have relatively low ovipositional success rates and typically locate a host only on 10% of drilling attempts. Nevertheless, examination of many ovipositors of rhyssines in the collection of The Natural History Museum revealed little sign of any wear.

Ovipositors are not the only organs in parasitic wasps that are subject to considerable abrasion and need to be both hard and wear-resistant. The mandibles of those species that pupate within a hard host substrate, typically in wood, are extremely important for the successful egress of the newly eclosed wasps, and in these cases they are often very robust with chisel-like cutting teeth (Eggleton, 1989; Quicke, 1997). That these are subject to abrasion can readily be appreciated from examination of museum specimens which frequently have worn mandible edges. If the mandibles are not sufficiently hard and wear-resistant, the wasp may be unable successfully to escape from its pupation site. Here we report that these functional requirements are facilitated in many taxa by the incorporation of one of two metals, zinc or manganese, in both the mandible blade and in the ovipositor teeth, particularly those whose ovipositors have to withstand high levels of wear.

MATERIAL AND METHODS

Metal analysis

Metals were detected using electron-stimulated X-ray emission (Williams & Spratt, 1995). Only specimens that had been killed and preserved desiccated were employed to ensure that the cuticular metal ion distribution was representative of that *in vivo*.

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Either whole specimens or excised ovipositors and mandibles were carbon coated after mounting on adhesive carbon pads attached to aluminium stubs. Secondary and back-scattered electron images were recorded on a JEOL JSM 6400 or Hitachi S2500 SEM operating between 10 and 25 kV and with a working distance of 10 mm for the JEOL JSM 6400 and 35 mm for the Hitachi S2500. X-ray microanalysis was carried out on the same instruments. The JEOL JSM 6400 was fitted with a Tracor Northern Series II X-ray and image analyser and a ZMAX30 X-ray detector with a thin diamond window. The accelerating voltage was then 20 kV, working distance 39 mm, probe current 0.5 nA and live acquisition time 60 s. The Hitachi S2500 was used with a LINK AN10000 energy-dispersive analysing system. Analysis was undertaken at 15 kV accelerating voltage, 1 nA probe current and live time acquisition of 80 s. Quantitative data were extracted by reference to standards using the peak to background method (Fawke, 1996) recommended by Roomans (1988).

Materials examined

One specimen of each of the following species were investigated. The taxa investigated for this study were selected to represent a range of hymenopteran biologies. When negative findings were surprising we chose to examine closely related taxa with similar biology rather than multiple representatives of the same species. Voucher specimens are deposited at The Natural History Museum, London.

PAMPHILIOIDEA. Pamphiliidae: *Cephalcia arvensis* Panzer, Italy. TENTHREDINOIDEA. Pergidae: *Phylacteophaga* sp., Australia. CEPHOIDEA. Cephidae: *Calameuta filiformis* Eversmann, U.K. SIRICOIDEA. Siricidae: *Urocerus gigas* (L.), U.K. Xiphydriidae: *Xiphydria camelus*. (L.), U.K. ORUSSOIDEA. Orussidae: *Orussus sayii* (Westwood), U.S.A. MEGALYROIDEA. Megalyridae: *Megalyra fasciipennis* Westwood, Australia. STEPHANOIDEA. Stephanidae: *Diastephanus* sp., Singapore; gen. sp. no data. TRIGONALYOIDEA. Trigonalyidae: *Orthoganalis boliviana* (Schulz), Brazil; *Poecilogonalos costalis* (Cresson), U.S.A. EVANIOIDEA. Aulacidae: *Aulacus* sp., Canada; *Pristaulacus* sp., no data; Evaniidae: *Evania* sp., Kenya. Gasteruptiidae: *Gasteruption jaculator* (L.), U.K. PROCTOTRUPOIDEA. Diapriidae: *Trichopria verticillata* (Latreille), U.K. Monomachidae: *Monomachus* sp., Brazil. Pelecinidae: *Pelecinus polytrurator* (Drury), U.S.A. Roproniidae: *Ropronia* sp., U.S.A. PLATYGASTROIDEA. Scelionidae: gen. sp., U.K. CHALCIDOIDEA. Agaonidae: *Apocryptophagus* sp., South Africa; *Elisabethiella stueckenbergi* (Grandi), South Africa; *Philocaenus barbarus* (Grandi), South Africa; *Sycoryctes* sp. Encyrtidae: *Cerchysius subplanus* (Dalman), no data. Eurytomidae: *Sycophila biguttata* (Swederus), U.K.; gen. sp., Austria. Leucospidae: *Leucospis* sp., U.S.A. Ormyridae: *Ormyrus nitidulus* (Fabricius), U.K. Torymidae: *Torymus auratus* (Geoffroy), U.K., *Torymus* sp., U.K. CYNIPOIDEA. Cynipidae: *Andricus kollari* (Hartig), U.K.; *Cynips divisa* Hartig, U.K.; *Synergus reinhardi* Mayr, U.K. Figitidae: *Melanips opacus* (Hartig), Sweden. Ibaliidae: *Ibalia drewseni* Borries, Turkey. ICHNEUMONOIDEA. Ichneumonidae. Acaenitinae: *Coleocentrus* sp., no data; Pimplinae: *Dolichomitus* sp. U.K, *Perithous* sp., U.K.; *Schizopyga frigida* Cresson, U.K. Rhyssinae: *Rhyssa persuasoria* (L.), France. Braconidae. Braconinae: *Archibracon servillei* (Brullé), Kenya; *Coeloides sordidator* (Ratzeburg), Switzerland; *Coeloides filiformis* Ratzeburg, no data; *Euurobracon breviterebrae* Watanabe, Korea; *Rhadinobracon* sp., Kenya; *Zaglyptogastra* sp., Kenya. Doryctinae: *Zombrus* sp., Kenya. Helconinae: *Eubazus* sp., Switzerland. CHRYSIDOIDEA. Bethylidae: *Epyris niger* Westwood, U.K.

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Chrysididae: *Omalus* sp. U.K. VESPOIDEA. Mutillidae: *Mutilla europaea* L. U.K. Vespidae: *Dolichovespula arenaria* (Fabricius), U.S.A.; *Vespula germanica* (Fabricius), U.K.

RESULTS

Most of the species investigated had between 0.1 and 10.0% weight for weight (dry insects) of zinc or manganese locally within the cuticle of their ovipositors (19 out of 44) or mandibles (37 out of 42), many species including the metals in both structures (Table 1). In the ovipositors, the metals were usually restricted to the cuticle forming the serrations near the apex, but in the stephanid, high levels of zinc were present throughout the whole length, and in the doryctine braconid, *Zombus*, the manganese was most concentrated at the tip of the lower valve. Most cynipoids and ichneumonoids investigated had the ovipositor cuticle enriched with manganese, whereas the metal involved was zinc in the Siricidae, Stephanidae and Chalcidoidea. Zinc was also present in the sting of the single bethylid studied. No metals were found in the ovipositors of sawflies belonging to the families Pamphiliidae, Pergidae and Cephidae, nor in parasitic wasps of the families Trigonalyidae, Gasteruptiidae, Aulacidae, Diapriidae, Pelecinidae, Monomachidae, Scelionidae, nor in the stings of the aculeate wasps of the Mutillidae or Vespidae. *Megalyra fascipennis* (Megalyridae) was the only species in which both zinc and manganese were found in the ovipositor. However, these were not present in the same parts of the cuticle. Zinc was present at the tip of the upper valve (9th gonapophysis) whereas manganese was present in a small region just behind the tip of each lower valve.

Whilst most species had the mandible blade heavily enriched with zinc (Fig. 1A), a few also had a distinct trace of manganese either in a narrow zone just at the border between the unenriched area and the zinc zone or associated with the zinc zone. This was most evident in the cynipoid wasp *Ibalia* and the chalcidoid wasps *Ormyrus* and *Leucospis*. The sole taxon which had only manganese in the mandible was the fig wasp *Elisabethiella stueckenbergi*. No metals were present in the mandibles of the pamphiliid and pergid sawflies, nor in the vespids or pelecinid.

DISCUSSION

The presence of zinc or manganese in mandibles of some herbivorous insects has been known for some time (Hillerton & Vincent, 1982; Hillerton, Robertson & Vincent, 1984; Edwards *et al.*, 1993). These metals are associated with phenolic material and are involved in cuticular hardening. As such they reduce the abrasive wear that mandibles would suffer through chewing hard, often silica-rich, plant materials. The present results show that manganese and zinc may also play an important role in hardening the ovipositors of those hymenopterous insects that use their ovipositors to drill through hard substrates.

In the survey presented by Hillerton & Vincent (1982), Mn and Zn were present only in the mandibles of herbivorous insects with the exception of some ants which are omnivorous. The discovery of high levels of Mn and Zn in both mandibles and ovipositors of many parasitic wasps has a number of implications for their dietary physiology and interactions with their hosts. Since parasitic wasp larvae consume

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TABLE 1. Distribution of zinc and manganese in ovipositors and mandibles of various Hymenoptera. Explanation of symbols = —: not investigated; p: present but not quantified. Data for the ovipositor of the siricid woodwasp, *Sirex juvencus*, and the rhyssine ichneumonid, *Megarhyssa nortoni*, come from Vincent & King (1996). Absolute values for the metal concentrations have not been quoted since the specimens were not flat and the cuticle thickness was, in each case, ill-defined. However conditions were such as to allow a reasonable estimate within a broad range as given below; inaccuracy is unlikely to exceed 50%. Scale for Zn: 0 = <0.1%wt/wt; 1 = 0.1–1.0%wt/wt; 2 = 1.0–5.0%wt/wt; 3 = 5.0–10.0%wt/wt. Scale for Mn: 0 = <0.1%wt/wt; 1 = 0.1–1.0%wt/wt; 2 = 1.0–2.0%wt/wt; 3 = 2.0–6.0%wt/wt; 4 = 6.0–10%wt/wt

Superfamily	Species	Host or feeding situation	Egress state	Ovipositor		Mandible	
				Zn	Mn	Zn	Mn
Pamphilioidea	*Cephalcia arvensis*	phytophagous external	soft cocoon in soil	0	0	0	0
Tenthredinoidea	*Phylacteophaga* sp.	phytophagous leaf-miner miner	cocoon in leaf	0	0	0	0
Cephoidea	*Calameuta filiformis*	grass-stem borer	cocoon in grass stem	0	0	3	0
Siricoidea	*Sirex juvencus*	xylophagous	wood	p	0	–	–
	Urocerus gigas	xylophagous	wood	–	–	3	0
	Xiphidria sp.	xylophagous	wood	0	0	3	0
Orussoidea	*Orussus sayii*	wood-boring host	frass-filled wood	–	–	3	0
Megalyroidea	*Megalyra fasciipennis*	wood-boring host	wood	3	4	3	0
Stephanoidea	*Diastephanus* sp.	wood-boring host	wood	3	0	–	–
	gen. sp.	wood-boring host	wood	–	–	3	0
Trigonalyoidea	*Orthogonalis boliviana*	hyperparasitoids of exposed primary host	host remains	0	0	–	–
	Poecilogonalos costalis	hyperparasitoids of exposed primary host	host remains	–	–	3	0
Evanioidea	*Aulacus* sp.	wood boring host	thin cap of wood/bark	0	0	–	–
	Pristaulacus sp.	wood boring host	thin cap of wood/bark	–	–	3	0
	Evania sp.	oothecae of roaches	hardened egg capsule	–	–	3	0
	Gasteruption jaculator	secondary cleptoparasite on solitary bee nests	bee nests	0	0	3	0
Proctotrupoidea	*Pelecinus polyturator*	root feeding beetle larvae	deep soil, no cocoon	0	0	0	0
	Monomachus sp.	eggs of *Boreoides*	host remains	0	0	3	0
	Ropronia sp.	Symphyta	host cocoon	–	–	2	0
	Trichopria verticillata	puparia of Diptera	host puparium	0	0	3	0
Chalcidoidea	*Apocryptophagus* sp.	figs	figs	0	0	3	0
	Cerchysius subplanus	gall-forming cecidogenic insects	gall	0	0	3	0
	Elisabethiella stueckenbergi	figs	figs	0	0	0	1
	Leucospis sp.	bees, wasps	wasp nest	–	–	2	1
	Ormyrus nitidulus	gall-forming cecidogenic insects	gall	3	0	3	1
	Philocaenus barbarus	figs	figs	0	0	1	0
	Sycophila biguttata	gall-forming cecidogenic cynipid larva	oak galls	0	0	4	0
	eurytomid gen. sp.	gall former	gall	0	0	4	0
	Sycoryctes sp.	figs	figs	1	0	–	–

continued

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TABLE 1. *continued*

Superfamily	Species	Host or feeding situation	Egress state	Ovipositor		Mandible	
				Zn	Mn	Zn	Mn
Chalcidoidea	*Torymus auratus*	cynipid galls	galls	2	0	–	–
	Torymus sp.	?	?	0	0	4	0
Platygastroidea	scelionid gen. sp.	arthropod eggs	host egg wall	0	0	2	0
Cynipoidea	*Andricus kollari*	galls oak buds	galls	0	2	–	–
	Cynips divisa	galls oak leaves	galls	0	1	1	0
	Ibalia drewseni	wood-boring siricids	hard wood timber	0	2	3	1
	Melanips opacus	Diptera larva	host remains	0	0	2	0
	Synergus reinhardi	galls of *Andricus kollari* in oak	galls	0	3	3	0
Ichneumonoidea	*Archibracon servillei*	wood-boring host	wood	0	1	3	0
	Coeloides filiformis	wood-boring host	through bark	0	1	3	0
	Coeloides sordidator	wood-boring host	through bark	0	0	–	–
	Coleocentrus sp.	wood-boring host	wood	–	–	3	0
	Dolichomitus sp.	wood-boring host	wood	0	0	–	–
	Eubazus sp.	wood-boring host	wood	0	3	3	0
	Euurobracon breviterebrae	wood-boring host	(rotten) wood	0	0	–	–
	Megarhyssa nortoni	wood-boring host	wood	0	p	–	–
	Perithous sp.	sphecid wasp cells	sphecid nest	0	1	3	0
	Rhadinobracon sp.	?wood-boring host	wood	0	1	2	0
	Rhyssa persuasoria	wood-boring host	wood	0	2	3	0
	Schizopyga frigida	ectoparasitoid of spiders	none	0	0	–	–
	Zaglyptogastra sp.	twig-boring beetle larva	?host frass hole or through twig	0	0	–	–
	Zombrus sp.	wood-boring host	wood	0	3	–	–
Chrysidoidea	*Epyris niger*	dermestid beetle larva	cocoon outside more or less exposed host	3	0	3	0
	Omalus sp.	stem and wood nesting Sphecidae	wood – some chrysidids also chew hole to reach host	–	–	3	0
Vespoidea	*Dolichovespula arenaria*	–	papery cell cap	–	–	0	0
	Mutilla europaea	*Bombus* larvae or pupae	nest	0	0	3	0
	Vespula germanica	–	papery cell cap	0	0	0	0

only their hosts, the zinc and manganese they use as adults must have come from host tissues. In many of the cases considered here, the hosts of the parasitic taxa are endoxylous insect larvae which probably sequester metals for their own usage, both in their larval mandibles and often in those of the adult stage too. Once metal has been deposited in the cuticle, it cannot be resorbed, and so additional dietary metals will have to be accumulated in each instar. The taxa investigated here include both idiobiont ectoparasitoids (e.g. rhyssine ichneumonids) which rapidly consume a paralysed host, and koinobiont endoparasitoids which live for a protracted period in a more truly parasitic association with a host individual (e.g. ibaliids). In the latter case the parasitoid larva may be in direct competition with the host for uptake of metals, whereas in idiobionts, the only metals that will be available to them are those that the host has sequestered in its soft tissues, since parasitic wasp larvae do not consume hardened host cuticle such as mandibles.

There is no universally accepted phylogenetic tree for the Hymenoptera at either

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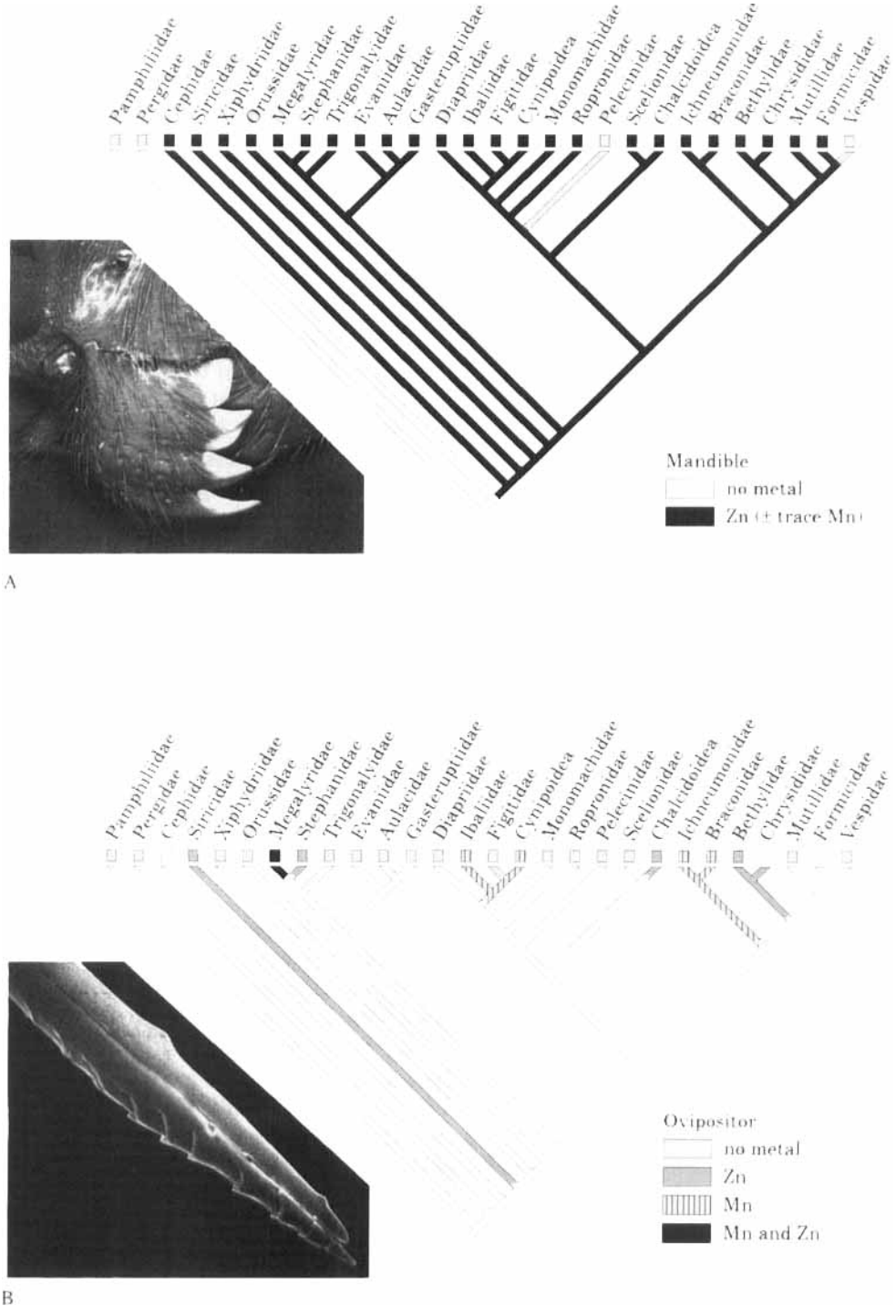


Figure 1. Distribution of heavy metals in the mandibles (A) and ovipositors (B) of various hymenopterans superimposed on a cladogram proposed by Rasnitsyn (1988) and subsequently amended by Heraty *et al.* (1994). The inserted micrographs show mandibles of *Poecilogonalos costalis* (Trigonalyidae) with (paler area) the high concentration of zinc along the cutting edge and ovipositor of *Zombrus* (Braconidae).

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family or superfamily level. The most widely used is that of Rasnitsyn (1988) which was modified by Heraty, Woolley & Darling (1994). Although, it is not totally consistent with some more recent molecular findings (Dowton & Austin, 1994) this provides the most fully resolved available. Although difficulties in obtaining suitable material for study in many groups means that our taxon sampling is not ideal, mapping the presence of metals in mandibles and ovipositors on to the best available phylogenetic tree demonstrates a clear pattern. Thus, the use of metal for cuticular hardening in adults does not appear to have been ancestral to the order, but to have evolved once at the base of the cephoid + siricoid grade coincident with, and probably as a result of, a transition to an endoxylous larval habit (Fig. 1A). The absence of either metal from pelecinid and vespid mandibles is best interpreted as reversal. The only living species of pelecinid is a parasitoid of soil-inhabiting beetle larvae. Except for the apex of its abdomen, it pupates external to the host remains without any cocoon, and so as it has no need to chew its way out of a hard substrate, it is not surprising that it no longer needs to have especially hardened mandibles. It is more surprising that the mandibles of the social vespids, *Dolichovespula* and *Vespula*, do not contain any zinc or manganese since these insects spend a considerable part of their adult life chewing prey insects and also bark and wood which they use as raw materials for nest construction. The diet of vespid larvae consists largely of chewed insect soft tissues that the workers regurgitate for them. It would be interesting to know whether this diet is deficient in metals and hence whether the absence of Mn and Zn in adult mandibles is attributable to lack of availability.

The pattern of presence of metals in the ovipositor suggests at least seven independent acquisitions within the order, counting the distinct usage of both zinc and manganese in megalyrid ovipositors as two separate evolutionary steps (Fig. 1B). Further, within at least some superfamilies (e.g. Chalcidoidea, Ichneumonoidea) metal usage in the ovipositor was by no means ubiquitous, and appears to correlate broadly with the way the ovipositor is used and the nature of the substrate which the ovipositor has to penetrate. Siricid woodwasps have zinc-enriched ovipositors whereas xiphidriids do not. This may reflect a difference in the oviposition substrate in the two families, xiphidriids ovipositing into fresh timber, often just beneath the bark, where they lay a clutch of eggs, while siricids in contrast lay single eggs in deeper drillings in the timber. Similarly no metal was found in ovipositors of taxa that do not use their ovipositors to drill through a substrate, such as the braconid *Zaglyptogastra* which has a specialized ovipositor entered through frass holes into the twig-borings of beetle larvae (Quicke, 1991). Similarly, the Gasteruptiidae and Aulacidae, putatively related families usually grouped together in the Evanioidea, have ovipositors which, despite being long and furnished with serrations, are also furnished with numerous setiform sensilla in gasteruptiids and are capable of being steered (Quicke & Fitton, 1995). Gasteruptiids, which are kleptoparasitoids of solitary bees, insert their ovipositor in the nest entrance (Malyshev, 1964) while aulacids, from what is known, pass their ovipositors down the ovipositor hole made by their woodwasp host (Skinner & Thompson, 1960). It seems likely therefore that these species do not require their ovipositor tips to be especially hardened or abrasion resistant. *Ibalia* on the other hand, also passes its slender ovipositor down a pre-drilled woodwasp oviposition hole, yet it has manganese in its ovipositor. Some taxa, for example, the pimpline wasp, *Dolichomitus*, attack hosts that are concealed in hard wood, but no metal was found in their ovipositor. However, in these, the ovipositor is threaded through pre-existing tunnels (Gauld, 1991).

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The presence of zinc in the sting of the bethylid, *Epyris*, was unexpected since these wasps sting their dermestid beetle larva hosts directly and also the adult wasp exhibits host guarding and so has to use her sting only once to subdue her victim. However, bethylids will enter into vigorous fights with intruding females and other potentially competing parasitoids. Thus the hardened sting may in this case reflect the need to penetrate an intruder's hardened cuticle rather than being associated with host stinging itself.

Apart from a minute trace (<0.1% wt./wt.) of manganese at the border between the zinc-enriched and unenriched parts of the mandibles of some taxa, manganese was found only in the ovipositors of the Ichneumonoidea, Cynipidae and Megalyroidea, and in the mandibles of one fig wasp species (*Elisabethiella*). No cuticle was found to be rich in both zinc and manganese at the same site. The results suggest that the wasps concerned have separate transport systems for the two ions. The differential incorporation of zinc and manganese in mandibles and ovipositors implies a unique functional property for each type of metal-replete cuticle.

ACKNOWLEDGEMENT

We thank support staff of the Electron Microscopy Centre, University of Southampton and at the Natural History Museum, London, for providing help and facilities. This work was funded by NERC Initiative in Taxonomy, a grant from the Leverhulme Trust and The Natural History Museum. Mark Kenis, Steve Compton, Karsten Schönrogge and David Notton provided some of the specimens investigated.

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