

SPRAY MECHANISM OF THE MOST PRIMITIVE BOMBARDIER BEETLE (*METRIUS CONTRACTUS*)

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Summary

The bombardier beetle *Metrius contractus* discharges its defensive secretion as a froth that clings to its body. When attacked from the rear, it allows the froth to build up over the gland openings near the abdominal tip; when attacked from the front, it conveys the secretion forwards along special elytral tracks. *M. contractus* has two-chambered defensive glands typical of bombardier beetles, and its secretion, like that of other bombardiers, is quinonoid and hot. Its frothing mechanism, however, is unique for

bombardiers and possibly illustrative of the ancestral glandular discharge mechanism of these beetles. *M. contractus*, thus, could be the least derived of extant bombardiers.

Key words: bombardier beetle, *Metrius contractus*, evolution, chemical defence, benzoquinone, hydrocarbon, Coleoptera, Carabidae.

Introduction

Bombardier beetles comprise two evolutionary branches within the beetle family Carabidae (Fig. 1). One branch, the brachinoid branch, includes the familiar bombardiers of the genus *Brachinus*, in which the workings of the bombarding mechanism were first elucidated. The second branch, the paussoid branch, includes beetles that also bombard, but that are of cryptic habit and more restricted distribution and, therefore, known well only to specialists. The relationship between the brachinoid and paussoid branches is subject to some dispute. Whereas some investigators, ourselves included, consider bombardiers to represent a monophyletic lineage (Aneshansley et al., 1983; Bousquet, 1986; Crowson, 1981; Deuve, 1988; Eisner et al., 1977; Erwin and Sims, 1984), in other words, that the paussoids and brachinoids are sister groups, and that the ability to bombard evolved only once in these beetles, others take the view that the bombarding mechanism evolved independently in paussoids and brachinoids (Ball and McCleve, 1990; Forsyth, 1972; Moore and Wallbank, 1968; Moore et al., 1987). Either way, there appears to be no argument that the beetles of one genus, *Metrius* (tribe Metriini), are primitive members of the paussoid branch. *Metrius*, therefore, could provide some indication of what the bombarding mechanism might have been like in ancestral paussoids. But if bombardiers are monophyletic and brachinoids are derived from paussoids, *Metrius* could also shed light on bombardier ancestry generally, including that of

brachinoids. Our purpose here is to provide some details of the defensive discharge mechanism of *Metrius contractus*, one of only three species in the single genus of the Metriini.

The most thoroughly investigated bombarding mechanism is that of the brachinines (e.g. *Brachinus*, *Stenaptinus*). As has long been known, brachinines respond promptly to assault by ejecting jets of defensive secretion (Westwood, 1839). The fluid is produced by a pair of capacious glands that lie side by side in the abdomen and open at the abdominal tip (Dierckx, 1899). The discharges are accurately aimed (Eisner, 1958; Eisner and Aneshansley, 1999) and they occur with audible detonations, hence the name 'bombardiers'. The active components of the secretion are 1,4-benzoquinones (Schildknecht, 1957), compounds known to be potently irritating (Thomson, 1971). Both arthropod and vertebrate predators are deterred by the discharges (Eisner, 1958, 1970; Aneshansley et al., 1969; Dean 1980a,b).

The most remarkable property of the brachinine spray is that it is hot (100 °C) when discharged (Aneshansley et al., 1969). This is because the quinones are generated explosively at the moment of ejection by the mixing of two sets of chemicals ordinarily stored separately in the glands. Each gland consists of two confluent compartments. One compartment (the reservoir or storage chamber) contains hydroquinones and hydrogen peroxide. The other compartment (the reaction chamber) contains a mixture of enzymes (catalases,

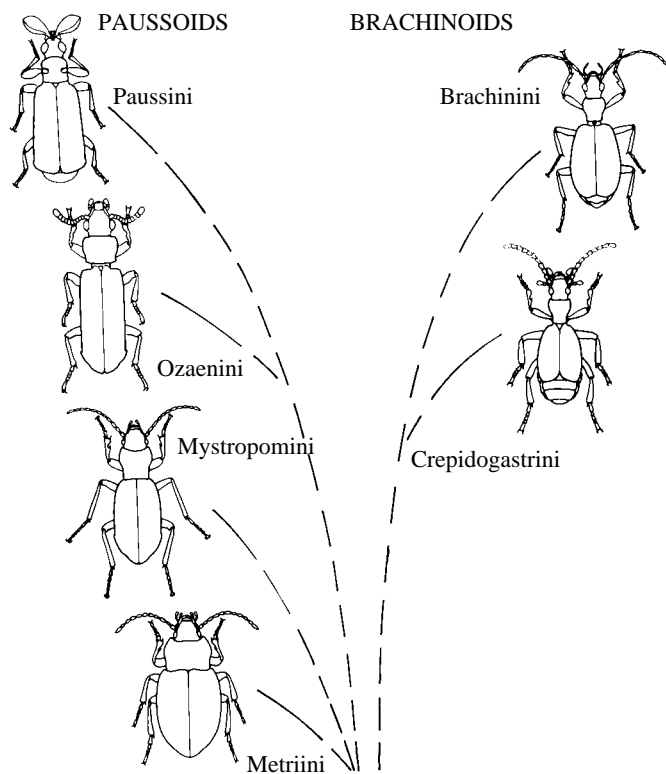


Fig. 1. Suggested phyletic relationship of bombardier beetles (family Carabidae, subfamily Paussinae). Tribal designations according to Erwin and Sims (1984).

peroxidases). To activate the spray, the beetle squeezes fluid from the reservoir into the reaction chamber. This results in the instantaneous liberation of oxygen from hydrogen peroxide, and the oxidation of the hydroquinones to quinones by the freed oxygen. The liberated oxygen also provides the propellant, causing the mixture to pop out (Dean et al., 1990; Schildknecht, 1957; Schildknecht and Holubek, 1961; Schildknecht et al., 1968, 1970). The heat that accompanies the formation of the spray is perceptible: brachinines feel hot when they discharge into one's hand (Aneshansley et al., 1969; Westwood, 1839).

We had previously come into possession of some live *M. contractus* and had reported on certain characteristics of their discharge mechanism. Thus, we had earlier shown their glands to be two-chambered and their discharged secretion, in common with that of other paussoids, to be quinonoid and hot. We had also shown the reservoirs of the glands to contain the typical precursors of the quinones, hydrogen peroxide and hydroquinones, and, as part of a separate lipoidal phase, a mixture of hydrocarbons (principally pentadecane) (Aneshansley et al., 1983; Eisner et al., 1977, 1989, 1992; Eisner and Aneshansley, 1982; Roach et al., 1979). This indicated that the discharged secretion is a two-phase system, in which the quinones are being formed in the aqueous phase, while at the same time being partitioned (in part at least) into the hydrocarbon phase. It is not known whether the beetles aimed their discharges and ejected them in the form of jets. We

had reason to believe *M. contractus* to be anomalous because those that we observed discharging in our hands seemed to emit bursts of mist rather than discrete jets. Here, we provide detailed information on the chemistry of the secretion of *M. contractus* and show that the discharge mechanism of this beetle is indeed unlike that of any other bombardier that has been studied.

Materials and methods

The beetles

Metrius contractus Escholtz were collected in California and maintained in our Cornell laboratories in small plastic cages on sand or peat moss. They were given chopped mealworms (larvae of *Tenebrio molitor*) and water (soaked cotton wads). They tended to be gregarious (Fig. 2A) and slow-moving, and were active primarily at night. Some survived for nearly 2 years.

M. contractus lack hindwings and, consequently, are flightless. Their elytra are fused mid-dorsally and form a broadly convex shield covering the back of the abdomen.

Gland morphology

For scanning electron microscopy, specimens were sonically cleaned, first in aqueous detergent solution, then in a 1:1 methanol/chloroform mixture. For study of the cuticular components of the glands, beetles were dissected following overnight immersion in dilute aqueous KOH solution.

Tethering of beetles

For experimental purposes, beetles were fitted with a small aluminum hook affixed to their elytra with a droplet of wax. The hook provided a handle by which the beetles could be picked up without being induced to discharge. The hook also provided the means by which a beetle could be attached to the end of an adjustable rod and tethered in any desired orientation.

Elicitation of discharges

Tethered beetles were induced to discharge by pinching their individual legs with fine-tipped forceps. The beetles survived the procedures uninjured. Samples of secretion for chemical analysis were obtained by holding pieces of filter paper beside the gland openings when the beetles were stimulated. The papers were promptly transferred to solvent.

Discharges on indicator paper

We had demonstrated previously that filter paper impregnated with an acidified potassium iodide/starch solution discolors instantly in the presence of 1,4-benzoquinones and can serve as an indicator paper for registering discharges of bombardier beetles (Eisner, 1958). We used this technique to depict the discharges of *M. contractus*.

Photography of discharges

Since the secretion ejected by *M. contractus* is hot, it is possible to photograph the discharges simply by causing the

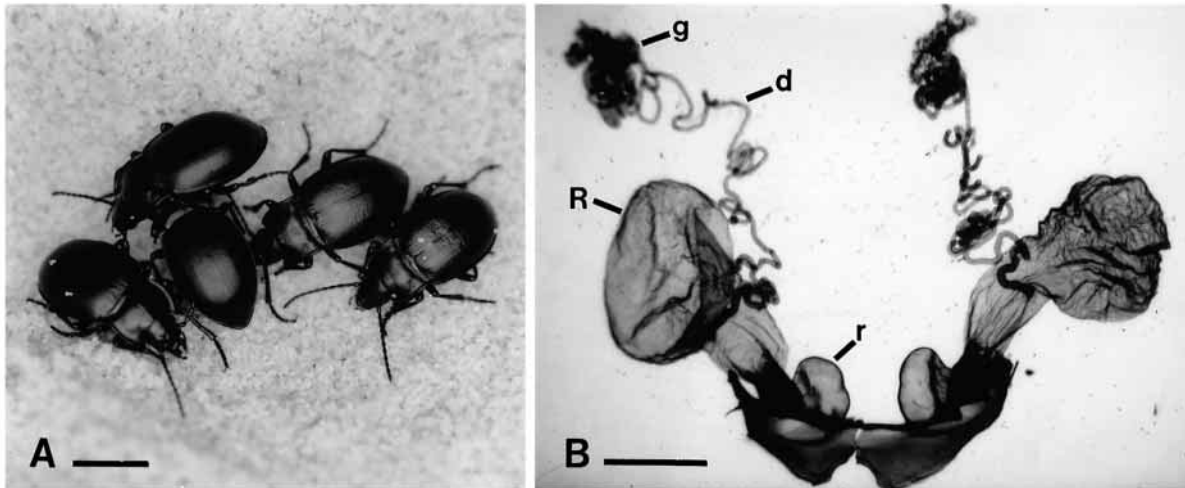


Fig. 2. (A) A group of *Metrius contractus* in the laboratory, aggregating. (B) *M. contractus* glands, treated with aqueous KOH and consisting of cuticle only. R, reservoir; r, reaction chamber; d, efferent duct; g, gland. Scale bars, A, 5 mm; B, 1 mm.

beetles to eject onto a heat-sensing device (thermocouple) that acts as a trigger to an electronic flash unit. We have provided details of this technique elsewhere (Aneshansley et al., 1969) and used it here to photograph the discharges of *M. contractus*.

Cinematography

A number of *M. contractus* discharges were filmed using a high-speed motion picture camera ($400 \text{ frames s}^{-1}$; Photo-Sonics 16mm camera, model 1PD).

Chemistry

Five samples of secretion from two beetles were collected on pieces of filter paper. Each sample was extracted with $50 \mu\text{l}$ of dichloromethane or hexane, and $0.5 \mu\text{l}$ of each sample was analyzed by gas chromatography–mass spectrometry (GC-MS) using an HP MSD linked to a gas chromatograph equipped with a $25 \text{ m} \times 0.22 \text{ mm}$ fused-silica column coated with DB-5 (J. & W. Scientific). Gas-phase infrared (GC-IR) spectra were obtained with an HP IRD linked to a gas chromatograph (using a $25 \text{ m} \times 0.32 \text{ mm}$ fused-silica column coated with DB-5). Dimethyl disulfide (DMS) and 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) derivatives were prepared as described previously (Attygalle, 1998).

Results

The glands

The location of the glands in *M. contractus* is shown in Fig. 3A. As is typical for bombardiers and evident from Figs 2B, 3B, the reservoir of the glands (R) is substantially larger than the reaction chamber (r) and, unlike the latter, is enveloped by muscles and presumably compressible. The glandular tissue (g) that secretes the reservoir contents forms a series of connected lobes lying appressed against the reservoir wall. This tissue is drained by a long, highly coiled efferent duct (d) that feeds directly into the reservoir. The glands of *M. contractus*, like the defensive glands of arthropods generally,

are integumental in origin and therefore lined throughout with cuticle. Even the efferent ducts and the microscopic tubules draining the secretory cells of the glandular tissue possess such a lining. This is readily demonstrated by treating the glands with dilute aqueous KOH, which dissolves away all tissue components and preserves cuticle only (Fig. 2B and right-hand gland in Fig. 3B). As expected, the lining of the compressible reservoir is thin and flexible, while that of the non-deformable reaction chamber is thick and rigid.

Each gland opens to the outside by way of the slit-like orifice of the reaction chamber.

Discharges on indicator paper

Pinching the legs of individual *M. contractus*, tethered and positioned in normal stance on indicator paper, revealed that

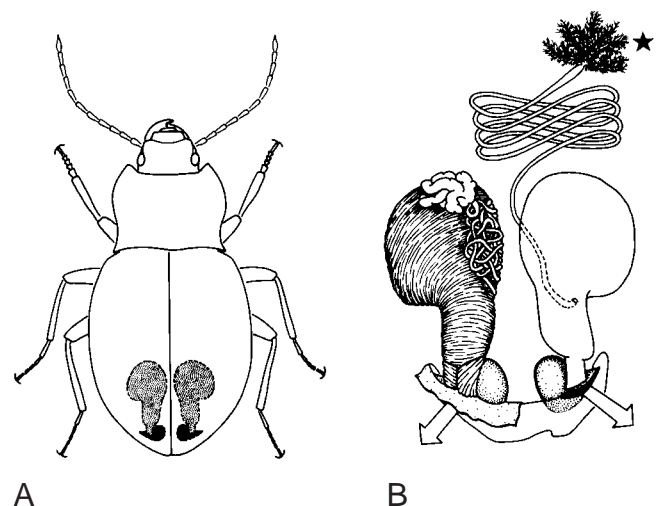


Fig. 3. (A) Diagram of *Metrius contractus*, showing the glands in place. (B) *M. contractus* glands (for labelling see Fig. 2B). The gland on the right (KOH-treated) consists of cuticle only; note that the cuticular tubules (star) of the gland tissue have survived KOH treatment.

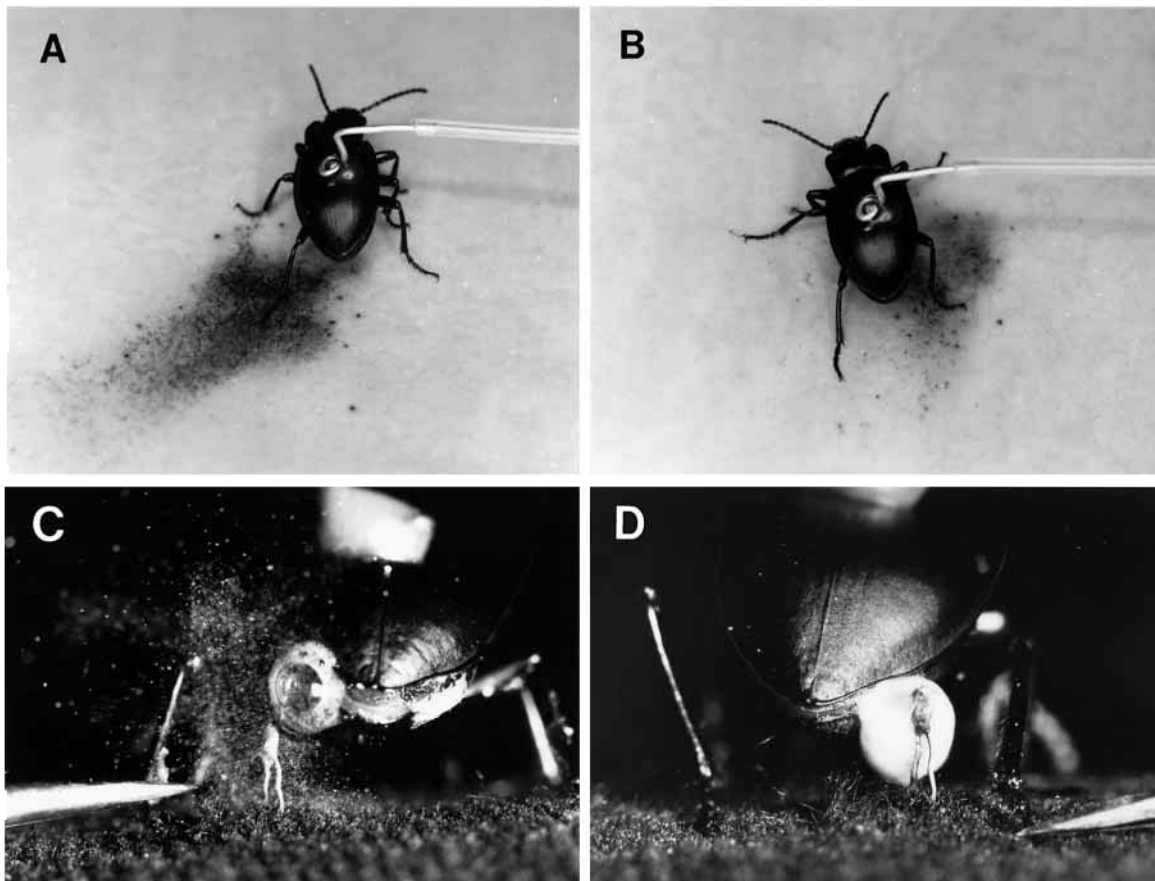


Fig. 4. (A,B) Tethered *Metrius contractus*, on indicator paper, that have discharged in response to pinching of the left hindleg (A) and the right hindleg (B). (C,D) Comparable with A and B, but showing beetles (in rear-end view) at the actual moment of secretory emission. Note, in C and D, the wire thermocouple that triggered the photographs and the pointed forceps used to pinch the hindlegs.

the beetles can exercise some control over the directionality of their emissions. Thus, stimulation of the hindleg of one side invariably resulted in a discharge of the gland of that side (Fig. 4A,B). The responses were similarly ipsilateral when individual forelegs were pinched. Moreover, stimulation of a foreleg resulted in discoloration of the indicator paper around that leg rather than the hindleg, indicating that the beetles are able somehow to direct their discharges anteriorly. As a rule, we could elicit no more than five consecutive discharges per side from any one *M. contractus*.

The evenness of the discoloration induced by the beetle's discharges on the indicator paper was notable. In other bombardiers, where the discharges occur as jets, the impact patterns on paper are characteristically spotty (Eisner, 1958; Eisner and Aneshansley, 1982; Eisner et al., 1989, 1992). *M. contractus*, it seemed, emitted its secretion diffusely, rather than as a coarse spray.

Photography of discharges

A number of photographs were obtained depicting the moment of secretory emission in *M. contractus*. Such pictures (Fig. 4C,D) confirmed that the beetle's discharges were unilateral when the stimulus was confined to one side, and

showed further that the emissions did not occur as jets. Instead, the secretion seemed to ooze from the glands as a bubbling, mist-engendering froth that clung to the beetle's body. One can easily envisage the atomized fall-out from such froth inducing the evenly discolored markings that *M. contractus* had made on indicator paper.

Cinematography

The film clarified how secretion is conveyed forwards in anteriorly directed ejections and provided data on the time course of discharges. Two film sequences from the same *M. contractus* are discussed here (Fig. 5).

In the forwardly directed discharge (upper sequence in Fig. 5), secretion made its appearance externally (4 ms) as a faint yellow streak on the margin of the elytron. As further secretion poured out, it was relayed along the elytral margin to the very base of the elytron (14 ms), where it built up as a bubbling mass (14–34 ms). The bubbling eventually ceased, but not before it had engendered considerable mist (61–184 ms).

In the posteriorly directed discharge (lower sequence in Fig. 5; note that the elytral margin bore a yellow secretory residue from an earlier anteriorly directed discharge), the

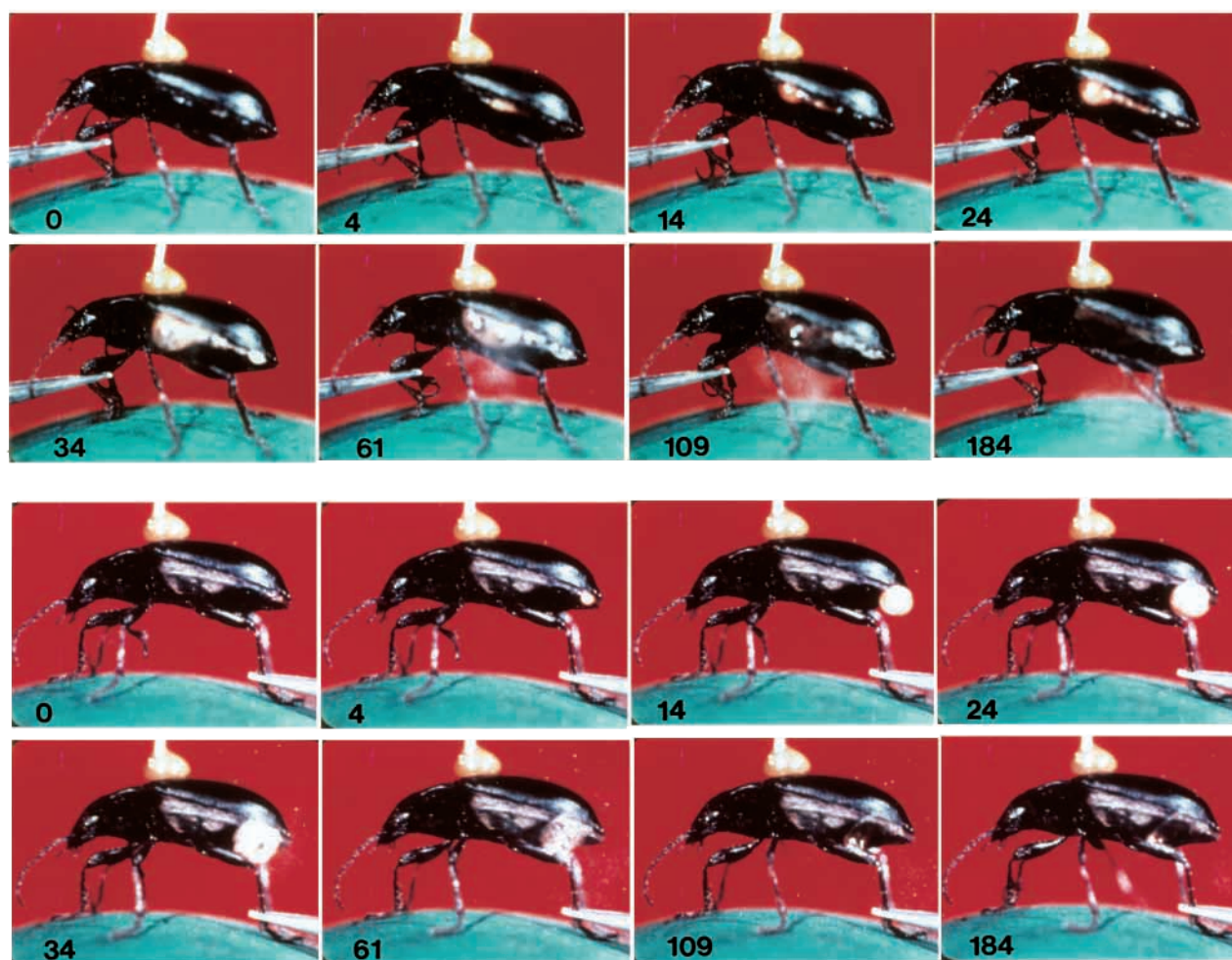


Fig. 5. Excerpts from high-speed motion picture film ($400 \text{ frames s}^{-1}$) of a *Metrius contractus*, showing a beetle discharging in response to pinching of a foreleg (upper sequence) and of a hindleg (lower sequence). Numbers give time in milliseconds from the onset of discharge.

events proceeded similarly, except that the bubbling mass built up directly over the gland opening. There was again emanation of mist (34–184 ms).

The time courses of the two discharges were similar. Secretory outflow proceeded over the first 34 ms, by which time the bubbling itself appeared to have reached its peak.

Directional control of discharges

It was clear that forwardly emitted discharges were conveyed along the elytral margin. Scanning electron microscopy showed such a margin (Fig. 6A) to take the form of a smooth continuous track (tr) extending the full length of the elytron (henceforth, we refer to the elytral margin as the elytral track). Anteriorly, where the secretion builds up when it is directed forwards, the elytral track is appropriately widened.

Scanning electron microscopy also revealed the structural relationship between the elytral track and the gland opening. This opening is ordinarily hidden from view. What is visible is the outermost portion of the channel (white arrow, Fig. 6B) by which secretion is emitted from the gland (the channel is shown magnified, in stereo view, in Fig. 7A,B).

Whether secretion is conveyed forwards after emission appears to depend on whether the abdominal tip is deflected downwards or pressed upwards against the elytra. When deflected downwards (Fig. 6B), the emission channel is disengaged from the elytral track, thereby ensuring that, if a discharge occurs, the fluid builds up at the site of emergence. When the tip is pressed upwards (Fig. 6C), the channel is lined up with the elytral track, thereby forming a continuous pathway by which secretion can be routed onto the track. We suggest that the former condition prevails when the beetle is responding to attacks from the rear and the latter when it is repelling assaults from the front. Indeed, abdominal deflective motions could be readily induced (and observed with a microscope) in *M. contractus* held by their tethering hooks and stimulated with forceps. In response to brief pinching of a hindleg, the beetles deflected the abdominal tip downwards, whereas they pressed it upwards in response to stimulation of a foreleg.

We did not succeed in preparing a *M. contractus* for scanning electron microscopy with its abdominal tip pressed upwards. The photograph in Fig. 6C was generated by taking

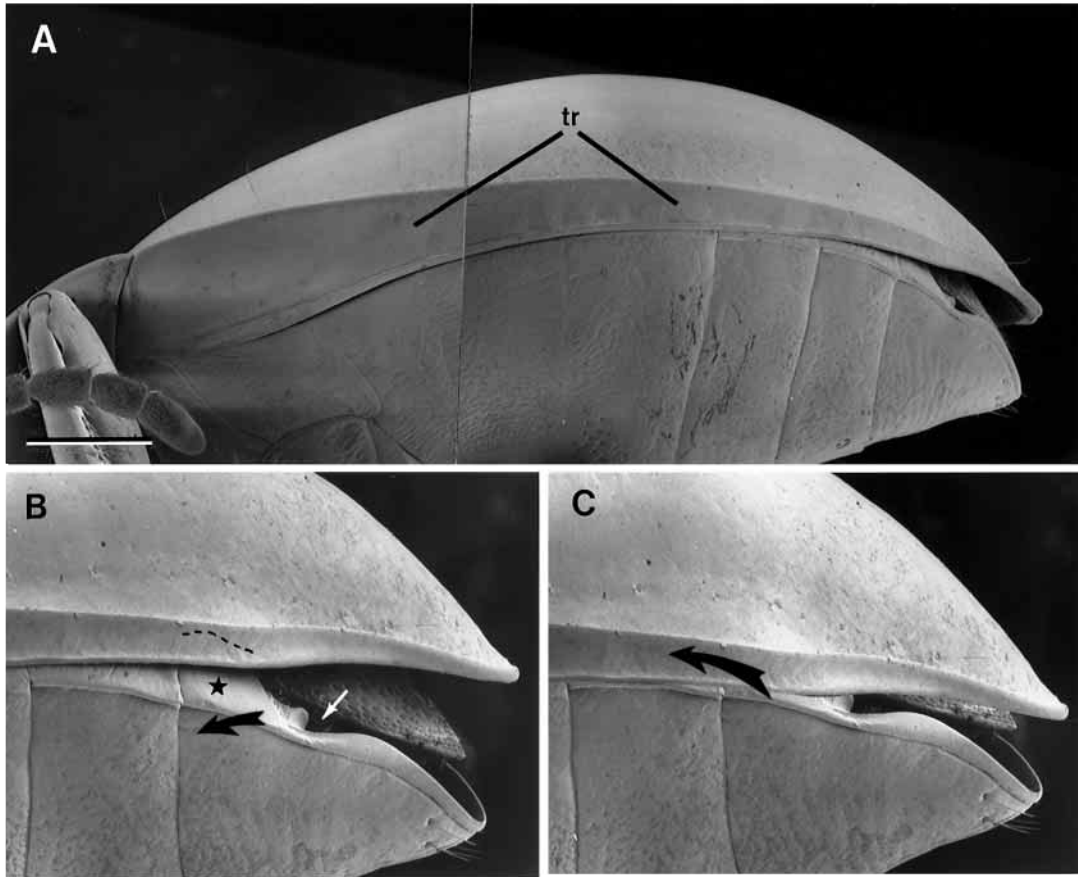


Fig. 6. Abdominal features of *Metrius contractus*. (A) Whole abdomen, left-side. tr, elytral track. (B) Abdominal tip, deflected downwards. The white arrow marks the glandular emission channel (see also Fig. 7A,B). (C) Abdominal tip, pressed upwards manually (see text for details). The black arrows in B and C show the direction that secretion would take on emission. In B, secretion would accumulate at the site of emission (posterior discharge). In C, secretion would be conveyed forwards onto the elytral track (anterior discharge). The star in B shows the tongue of the tongue-in-groove arrangement that provides for smooth operation of the abdominal deflection motions (see also the star in Fig. 7C). The broken line in B gives the position of the groove on the inside of the elytron that accommodates the tongue (see also Fig. 7D). Scale bar, 1 mm.

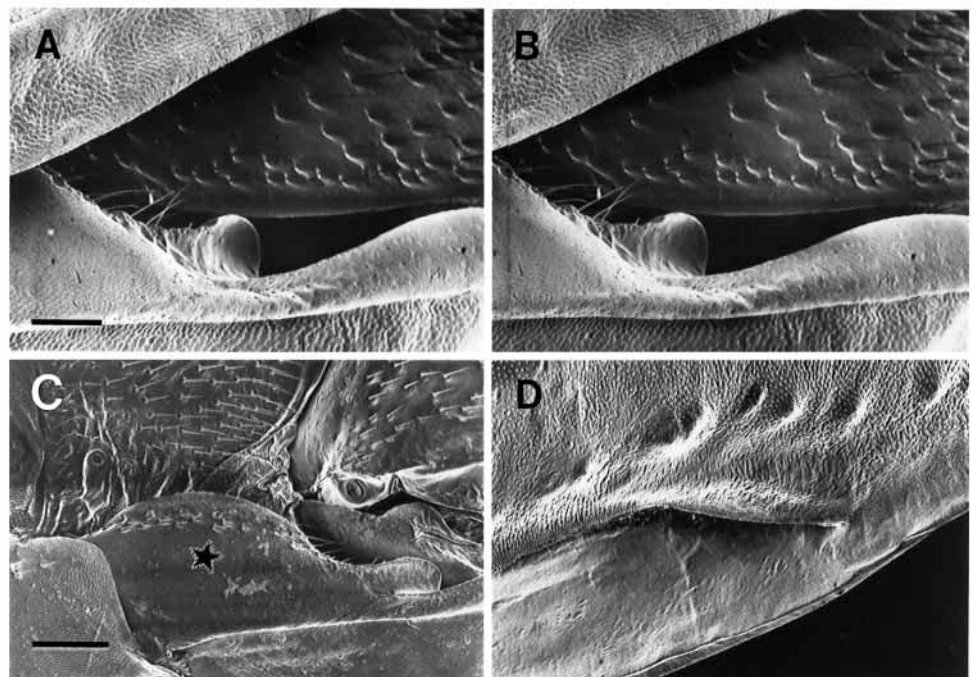


Fig. 7. (A,B) Enlarged view (stereo) of the glandular emission channel shown (white arrow) in Fig. 6B. (C) The tongue (star) of the abdominal tongue-in-groove arrangement (see also Fig. 6B). (D) The groove on the inside of the elytron that accommodates the tongue (see also the dotted line in Fig. 6B). Scale bars, A,B, 0.1 mm; C,D, 0.2 mm.

Table 1. Analytical data for components of the defensive secretion of *Metrius contractus*

No. ^a	Compound	Relative amount ^b	EI-mass spectral data (<i>m/z</i>)	GC-IR data (cm ⁻¹)
1	1,4-Benzoquinone	87.40	108	1679, 1300, 1067, 1057, 880
2	2-Methyl-1,4-benzoquinone	0.06	122	c
3	Unknown	0.11	124	c
4	2-Chloro-1,4-benzoquinone	0.97	142, 144	c
5	2-Ethyl-1,4-benzoquinone	0.18	136, 108	c
6	2-Methoxy-3-methyl-1,4-benzoquinone	0.03	152, 122	c
7	Tridecane	0.80	184	c
8	Tetradecane	7.63	198	2933, 2865, 1463
9	7-Pentadecene	1.00	210	c
10	Pentadecane	100.00	212	2933, 2865, 1463
11	6,8-Pentadecadiene ^d	0.30	208	c
12	5,7-Pentadecadiene ^d	4.20	208	c
13	5-Methylpentadecane	0.45	226, 169, 168	c
14	4-Methylpentadecane	0.24	226, 183, 182	c
15	3-Methylpentadecane	2.10	226, 197, 196	c
16	Hexadecadiene (non-conjugated) ^e	0.27	222	c
17	Hexadecene ^e	0.63	224	c
18	Hexadecane	1.68	226	c
19	6,8-Hexadecadiene ^d	0.66	222	c
20	7,9-Hexadecadiene ^d	2.04	222	c
21	(6 <i>Z</i> ,9 <i>Z</i>)-6,9-Heptadecadiene ^f	12.24	236	3017, 2933, 2868, 1460
22	(<i>Z</i>)-8-Heptadecene ^f	8.66	238	3015, 2933, 2866, 1460
23	Heptadecane	4.17	240	c
24	5,7-Heptadecadiene ^d	2.10	236	c
25	7,9-Heptadecadiene ^{d, g}	7.32	236	c
26	(7 <i>Z</i> ,9 <i>Z</i>)-7,9-Heptadecadiene ^{d, f}	37.60	236	3034, 3009, 2965, 2934, 2867, 1460
27	Nonadecane	0.46	268	c
28	7,9-Nonadecadiene ^d	0.37	264	a

^aNumbers refer to chromatographic peaks in Figure 8.

^bThere was no qualitative variation and only minor quantitative variation in the composition of the five discharge samples examined. The values listed here pertain to one sample. They give amounts of components relative to *n*-pentadecane, which is listed as 100.

^cMaterial insufficient for infrared data.

^dDouble-bond positions of these conjugated compounds were determined by the mass spectra of their MTAD adducts. The relevant *m/z* values recorded for the adducts are as follows: no. **11**, 321, 250, 236; no. **12**, 321, 264, 222; no. **19**, 335, 264, 236; no. **20**, 335, 250; no. **24**, 349, 292, 222; no. **25**, 349, 264, 250; no. **26**, 349, 264, 250; no. **28**, 377, 292, 250.

^eDouble-bond positions undetermined.

^fDouble-bond positions of these unsaturated compounds were determined by the mass spectra of their DMDS adducts. The relevant *m/z* values recorded for the adducts are as follows: no. **21**, 362, 267, 183; no. **22**, 332, 173, 159; no. **26**, 145, 159, 171, 185 (a composite spectrum of mono DMDS adducts).

^gAlthough the double-bond configurations were not determined, this diene is a geometrical isomer of no. **26**.

GC-IR, gas-phase infrared; MTAD, 4-methyl-1,2,4-triazoline-3,5-dione; DMDS, dimethyl sulfide; *m/z*, mass/charge; EI, electron ionization.

a print of Fig. 6B, cutting it in half along the pleural elytral margin, and then rejoining the two halves after the abdominal tip had been rotated upwards. Evidence for the actual occurrence of the deflections was also provided by the motion picture film. Close examination of the first frames (0 ms) of the two sequences in Fig. 5 shows that, for the forward discharge (upper sequence), the abdominal tip was pressed upwards, while for the posterior discharge (lower sequence) it was deflected downwards.

Two tongue-in-groove devices ensure that the beetle's abdominal deflections can proceed with reliable precision. The tongues of these devices are two blade-like structures

projecting upwards from the abdominal margins, just in front of the emission channels (stars, Figs 6B, 7C). The corresponding grooves are two slit-like inflections on the inside of the elytra (Fig. 7D; broken line in Fig. 6B).

Chemistry

Twenty-seven components (quinones and hydrocarbons) of the *M. contractus* secretion were characterized by GC-MS and/or GC-IR techniques, in some instances in combination with preliminary chemical transformations. These results are summarized in Fig. 8 and Table 1. There were no qualitative differences in the composition of the secretions of the two

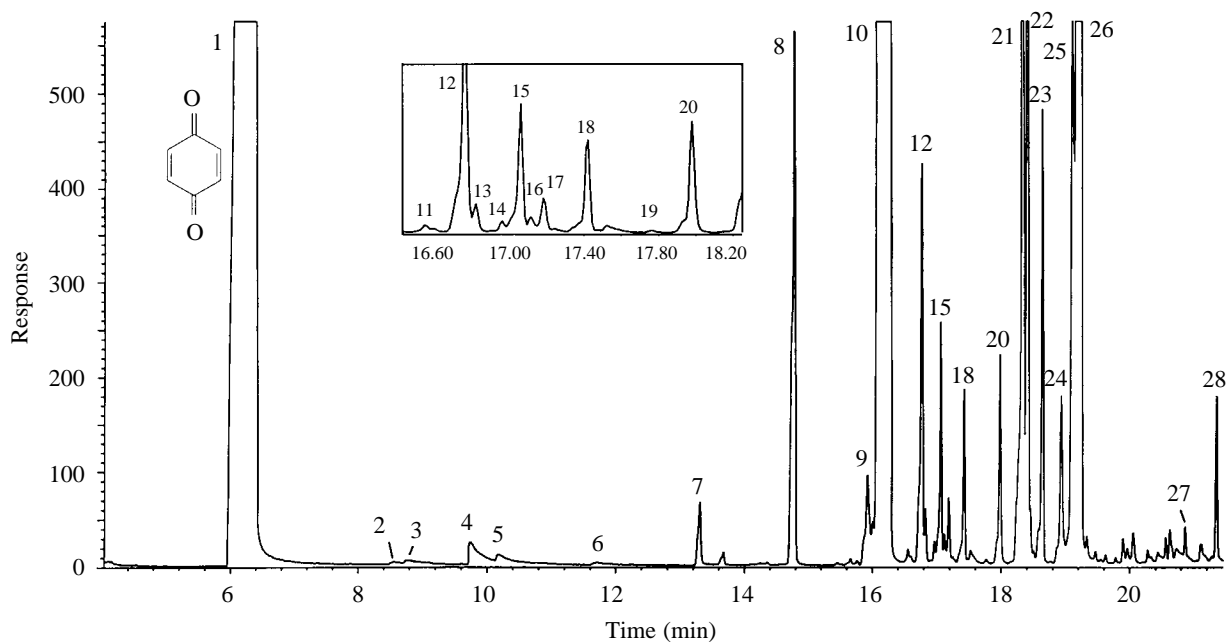


Fig. 8. A reconstructed gas chromatogram obtained from gas chromatography–mass spectrometry analysis of a dichloromethane extract of the defensive secretion of *Metrius contractus* (see Table 1 for identification of peaks). The inset is an expansion of the region from 16.40 to 18.20 min. A fused-silica column (30 m × 0.22 mm) coated with DB-5 was used. The oven temperature was kept at 60 °C for 4 min and then raised by 10 °C min⁻¹ to 160 °C.

beetles examined. Quantitative differences pertained to minor components only. 1,4-Benzoquinone, pentadecane and (7*Z*,9*Z*)-7,9-heptadecadiene were the major components in all five secretion samples examined.

The principal quinone in the secretion, as noted previously (Eisner et al., 1977), was 1,4-benzoquinone, although very small amounts of four other benzoquinones could also be detected on the basis of their mass spectra (Attygalle et al., 1993). The finding that 2-chlorobenzoquinone is the second most important quinone in the secretion (although present only at 1%) comes as a surprise, since chlorinated secondary metabolites are rare in nature (Gribble, 1998). Although we cannot rule out that chloroquinone was a contaminant, we cannot envisage how contamination at the 1% level could have occurred.

Mass spectral data served to identify *n*-pentadecane as the predominant hydrocarbon, along with eight C₁₃–C₁₉ straight-chain and branched saturated hydrocarbons.

The remaining 13 characterized constituents were three unbranched C₁₅–C₁₉ alkenes and 10 alkadienes. Dimethyl disulfide adducts (Francis and Veland, 1981) were helpful in identifying three of these unsaturated hydrocarbons. Eight of the dienes proved to be conjugated. It was therefore possible to determine their structure from the highly characteristic mass spectral fragmentation patterns of their Diels–Alder adducts with 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) (Young et al., 1990). Finally, double-bond configurations could be assigned to those components present in sufficient quantity on the basis of characteristic infrared absorption bands observed by GC-IR analysis (Attygalle et al., 1994, 1995) (see Table 1).

Discussion

In several fundamental respects *M. contractus* is typically bombardier-like. Its glands conform to the norm, being constructed of the two basic compartments, the reservoir and the reaction chamber, and its secretion is quinonoid. It ejects its secretion hot (Aneshansley et al., 1983), indicating that it is synthesizing the quinones by chemical explosion, and it discharges its secretion strictly in response to disturbance.

What is unique about *M. contractus* is its discharge mechanism (Fig. 9). No bombardiers other than *M. contractus* eject their secretion as an ooze that builds up on their bodies. Nor do any other bombardiers route emitted fluid forwards along tracks extending the length of their elytra. The bubbling undergone by the *M. contractus* secretion upon discharge may indicate that hydrogen peroxide breakdown and quinone formation, as well as the accompanying heat liberation, are still ongoing after emission of the fluid, raising the question of how *M. contractus* withstands direct exposure to such material. Notable in this respect is that *M. contractus* ejects its secretion at the relatively moderate temperature of 55 °C (recorded maximum 77 °C) (Aneshansley et al., 1983), rather than at 100 °C, as do some of the jet-ejecting species (Aneshansley et al., 1969).

Several features of the glandular apparatus bind *M. contractus* unmistakably to the paussoid lineage. The glands of *M. contractus*, in common with those of all paussoids, open laterally on the body at some distance from the abdominal tip, rather than on the tip itself as they do in brachinoids. The glands of *M. contractus* also are typically paussoid in appearance. They are, for instance, virtually indistinguishable

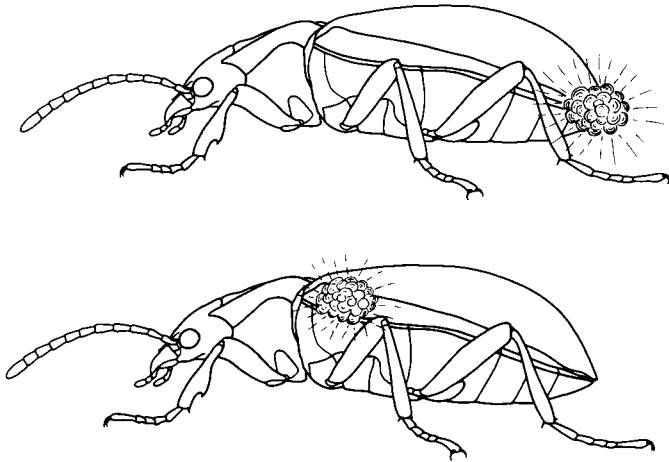


Fig. 9. Posterior (top) and anterior (bottom) secretory emission in *Metrius contractus*.

from the glands of *Mystropomus regularis* (Eisner et al., 1992) and closely similar to those of ozaenines (Eisner et al., 1989). They also share some of the features that set paussoids apart from brachinoids, such as, peculiarities of the structure of the reaction chamber, of the morphology of the wall of the efferent ducts and of the microstructure of the intracellular tubules that drain the secretory cells in the glandular tissue associated with the reservoir (T. Eisner, unpublished data).

Despite its anomaly, the discharge mechanism of *M. contractus* is basically paussoid. Both *M. contractus* and other paussoids (Eisner and Aneshansley, 1982; Eisner et al., 1992) direct their discharges by deflection of the abdominal tip. They deflect the tip downwards for posterior discharges and press it upwards for forwardly directed emissions. Both types of bombardier engage the elytra for forwardly directed ejections, but their elytra differ structurally, and they put them to use in different ways. In *M. contractus*, the elytra bear tracks along which the secretion glides forward without physically detaching from the beetle. In other paussoids, the tracks are modified in that they possess a pair of grooved protruding flanges (Fig. 10) that serve to deflect the secretory stream away from the elytral surface and forward as an aerial jet (Eisner and Aneshansley, 1982; Eisner et al., 1992). All paussoids other than *M. contractus* possess these flanges, including mystropomines, ozaenines and paussines (Eisner and

Aneshansley, 1982; Eisner et al., 1992), accounting for why all are able to eject jets of fluid. We consider the flangeless condition of *M. contractus* to be ancestral in paussoids *vis à vis* the flanged condition and illustrative, perhaps, of the earliest stage in bombardier beetle evolution. Froth emission, as exemplified by *M. contractus*, might therefore be indicative of how the bombarding mechanism originated among carabids. The evolution of flanges represented a major advance for paussoids. It enabled the beetles to aim forwards in parallel to their bodies and past their heads, and to deliver the secretory heat more fully upon the target. For *M. contractus*, retention of the ancestral discharge mechanism evidently was no handicap. For an exceptionally sluggish beetle such as *M. contractus*, unable to outrun its enemies, being left enshrouded by secretion and its vapors following discharges could clearly be helpful in keeping assailants from closing in again after an attack.

Nothing that we learned about *M. contractus* suggests that this beetle is also ancestral to brachinoids. Whether brachinoids and paussoids are sister groups or independent lineages will therefore need to be resolved on the basis of other evidence.

Some comments are in order regarding the chemistry of the secretion of *M. contractus*. First, it should be noted that both the quinones and hydrocarbons in the fluid, rather than only the quinones, can be expected to fulfill a defensive role. The quinones, as widespread components of arthropod defensive secretions, have long been known to be potently repellent to insects and vertebrates alike (Blum, 1981), but the fact that hydrocarbons, which often accompany the quinones in these secretions, might play a similar role, at least *vis à vis* insects, had mostly escaped notice. Both alkanes and alkenes have been shown to be repellent and irritant in tests with ants and cockroaches (Peschke and Eisner, 1987).

The secretion of *M. contractus* appears to be specially adapted in other ways as well. *M. contractus* is the only bombardier that emits secretion as a topical ooze, such as can be expected to confer protracted deterrence. It is also the only bombardier that produces 1,4-benzoquinone as its primary defensive quinone. Interestingly, 1,4-benzoquinone is less volatile, and therefore more persistent after ejection, than 2-methyl-1,4-benzoquinone, the primary quinone produced by other bombardiers (Eisner et al., 1977; Roach et al., 1979).



Fig. 10. Comparable with Fig. 6A, but of *Mystropomus chaudoiri*. Note the grooved flange (fl), typical of paussoids other than *Metrius contractus*, that serves as a launching guide for forwardly ejected (arrow) jets of spray. Scale bar, 1 mm.

There appear to be adaptive reasons also for the particular blend in which the hydrocarbons occur in the secretion of *M. contractus*. Aside from their defensive function, one can assume the hydrocarbons to play a double physical role. First, one would expect them to act as solvents for uptake of the quinones, as these are formed by oxidation of hydroquinones in the aqueous phase of the expelled secretory mixture. For such partitioning purposes, alkenes have been shown to be more effective than alkanes (Peschke and Eisner, 1987). Second, one would expect the hydrocarbons to act as surfactants that facilitate the spread of secretion over the body of the beetle (as in forwardly directed ejections). For such wetting purposes, a combination of alkanes and alkenes has been shown to be potentially more effective than either type of hydrocarbon alone (Peschke and Eisner, 1987). It makes sense, therefore, that the secretion of *M. contractus* should contain both alkanes and alkenes. It is, of course, conceivable that the hydrocarbons function also as frothing agents.

The secretion of *M. contractus* is unusual in that it contains conjugated dienes, of which one, (7Z,9Z)-7,9-heptadecadiene, figures prominently. This is striking not only because conjugated dienes are uncommon in nature, but because one would not expect to find dienes in coexistence with 1,4-benzoquinone, a compound usually regarded as a reactive dienophile. Why does 7,9-heptadecadiene not react with the 1,4-benzoquinone to form a Diels–Alder adduct? The answer may have a stereochemical basis. The 7,9-heptadecadiene we characterized has the Z,Z configuration, and this configuration renders it a particularly poor partner in Diels–Alder reactions, for well-understood steric reasons (Attygalle, 1998). (We suggest, therefore, that the other conjugated dienes in the secretion are likely to have Z,Z configurations as well). *M. contractus* thus appears to make use of the kind of dienes in its secretion that are least likely to have a diminishing effect on the 1,4-benzoquinone upon which it depends for protection.

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References

- Aneshansley, D. J., Eisner, T., Widom, J. M. and Widom, B. (1969). Biochemistry at 100 °C: explosive secretory discharge of bombardier beetles (*Brachinus*). *Science* **165**, 61–63.
- Aneshansley, D. J., Jones, T. H., Alsop, D., Meinwald, J. and Eisner, T. (1983). Thermal concomitants and biochemistry of the explosive discharge mechanism of some little known bombardier beetles. *Experientia* **39**, 366–368.
- Attygalle, A. B. (1998). Microchemical techniques. In *Methods in Chemical Ecology* (ed. J. G. Millar and K. F. Haynes), pp. 207–294. New York: Chapman & Hall.
- Attygalle, A. B., Svatos, A., Wilcox, C. and Voerman, S. (1994). Gas-phase infrared spectroscopy for determination of double bond configuration of monounsaturated compounds. *Analyt. Chem.* **33**, 1696–1703.
- Attygalle, A. B., Svatos, A., Wilcox, C. and Voerman, S. (1995). Gas-phase infrared spectroscopy for determination of double bond configuration of some unpolyunsaturated pheromones and related compounds. *Analyt. Chem.* **34**, 1558–1567.
- Attygalle, A. B., Xu, S.-C., Meinwald, J. and Eisner, T. (1993). Defensive secretion of the millipede *Floridobolus penneri* (Diplopoda: Spirobolida). *J. Nat. Prod.* **56**, 1700–1706.
- Ball, G. E. and McCleve, S. (1990). The middle American genera of the tribe Ozaenini, with notes about the species in southwestern United States and selected species from Mexico. *Quest. Ent.* **26**, 30–116.
- Blum, M. S. (1981). *Chemical Defenses of Arthropods*. New York: Academic Press.
- Bousquet, Y. (1986). Description of first instar larva of *Metrius contractus* Escholtz (Coleoptera: Carabidae) with remarks about phylogenetic relationships and ranking of the genus *Metrius* Escholtz. *Can. Ent.* **118**, 373–388.
- Crowson, R. A. (1981). *The Biology of the Coleoptera*. New York: Academic Press.
- Dean, J. (1980a). Encounters between bombardier beetles and two species of toads (*Bufo americanus*, *Bufo marinus*): speed of prey-capture does not determine success. *J. Comp. Physiol.* **135**, 41–50.
- Dean, J. (1980b). Effect of thermal and chemical components of bombardier beetle chemical defense: glossopharyngeal response in two species of toads (*Bufo americanus*, *Bufo marinus*). *J. Comp. Physiol.* **135**, 51–59.
- Dean, J., Aneshansley, D. J., Edgerton, H. E. and Eisner, T. (1990). Defensive spray of the bombardier beetle: a biological pulse jet. *Science* **248**, 1219–1221.
- Deuve, T. (1988). Étude phylogénétique des Coléoptères Adepaga: redefinition de la famille Harpalidae, *sensu novo*, et position systématique des Pseudomorphinae et Brachinidae. *Bull. Soc. Ent. France* **92**, 161–182.
- Dierckx, F. (1899). Étude comparée des glandes pygidiennes chez les carabides et les dytiscides avec quelques remarques sur le classement des carabides. *La Cellule* **16**, 63–176.
- Eisner, T. (1958). The protective role of the spray mechanism of the bombardier beetle, *Brachynus ballistarius* Lec. *J. Insect Physiol.* **2**, 215–220.
- Eisner, T. (1970). Chemical defense against predation in arthropods. In *Chemical Ecology* (ed. E. Sondheimer and J. B. Simeone), pp. 157–217. New York: Academic Press.
- Eisner, T. and Aneshansley, D. J. (1982). Spray aiming in bombardier beetles: jet deflection by the Coanda effect. *Science* **215**, 83–85.
- Eisner, T. and Aneshansley, D. J. (1999). Spray aiming in the bombardier beetle: photographic evidence. *Proc. Natl. Acad. Sci. USA* **96**, 9705–9709.
- Eisner, T., Attygalle, A. B., Eisner, M., Aneshansley, D. J. and Meinwald, J. (1992). Chemical defense of a primitive Australian bombardier beetle: *Mystropomus regularis*. *Chemoecology* **2**, 29–34.
- Eisner, T., Ball, G. E., Roach, B., Aneshansley, D. J., Eisner, M., Blankespoor, C. L. and Meinwald, J. (1989). Chemical defense

- of an ozaenine bombardier beetle from New Guinea. *Psyche* **96**, 153–160.
- Eisner, T., Jones, T. H., Aneshansley, D. J., Tschinkel, W. R., Silberglied, R. E. and Meinwald, J.** (1977). Chemistry of defensive secretions of bombardier beetles (Brachinini, Metriini, Ozaenini, Paussini). *J. Insect Physiol.* **23**, 1383–1386.
- Erwin, T. L. and Sims, L. L.** (1984). Carabid beetles of the West Indies (Insects: Coleoptera): a synopsis of the genera and checklists of tribes of Caraboidea and of the West Indian species. *Quest. Ent.* **20**, 351–466.
- Forsyth, D. J.** (1972). The structure of the pygidial defence glands of Carabidae (Coleoptera). *Proc. Zool. Soc. Lond.* **32**, 253–309.
- Francis, G. W. and Veland, K.** (1981). Alkylthiolation for the determination of double-bond position in linear alkenes. *J. Chromatogr.* **219**, 379–384.
- Gribble, G. W.** (1998). Naturally occurring organohalogen compounds. *Acc. Chem. Res.* **31**, 141–152.
- Moore, B. P. and Wallbank, B. E.** (1968). Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. *Proc. R. Ent. Soc. B* **37**, 62–72.
- Moore, B. P., Weir, T. A. and Pyke, J. E.** (1987). Rhysodidae and Carabidae. In *Zoological Catalogue of Australia*, vol. 4 (Coleoptera: Archostemata, Myxophaga and Adepaga) (ed. D. W. Walton), pp. 20–230. Canberra: Australian Government Publishing Service.
- Peschke, K. and Eisner, T.** (1987). Defensive secretion of a beetle (*Blaps mucronata*): physical and chemical determinants of effectiveness. *J. Comp. Physiol.* **161**, 377–388.
- Roach, B., Dodge, K. R., Aneshansley, D. J., Wiemer, D., Meinwald, J. and Eisner, T.** (1979). Chemistry of defensive secretions of ozaenine and paussine bombardier beetles (Coleoptera: Carabidae). *Coleopt. Bull.* **33**, 17–20.
- Schildknecht, H.** (1957). Zur Chemie des Bombardierkäfers. *Angew. Chem.* **69**, 62–63.
- Schildknecht, H. and Holubek, K.** (1961). Die Bombardierkäfer und ihre Explosionschemie. *Angew. Chem.* **73**, 1–7.
- Schildknecht, H., Maschwitz, E. and Maschwitz, U.** (1968). Die Explosionschemie der Bombardierkäfer (Coleoptera, Carabidae). III. Mitteilung: Isolierung und Charakterisierung der Explosionskatalisatoren. *Z. Naturforsch.* **23b**, 1213–1218.
- Schildknecht, H., Maschwitz, E. and Maschwitz, U.** (1970). Die Explosionschemie der Bombardierkäfer: Struktur und Eigenschaften der Brennkammerenzyme. *J. Insect Physiol.* **16**, 749–789.
- Thomson, R. H.** (1971). *Naturally Occurring Quinones*. New York: Academic Press.
- Westwood, J. O.** (1839). *An Introduction to the Modern Classification of Insects*, vol. 7. London: Longman.
- Young, D. C., Vouros, P. and Holich, M. F.** (1990). Gas chromatography-mass spectrometry of conjugated dienes by derivatization with 4-methyl-1,2,4-triazoline-3,5-dione. *J. Chromatogr.* **522**, 295–302.