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Chemical defense of an opilionid (*Acanthopachylus aculeatus)*

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Summary

The opilionid *Acanthopachylus aculeatus* was shown to produce a defensive secretion containing quinones (2,3-dimethyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone and 2,3,5-trimethyl-1,4-benzoquinone), confirming the findings reported nearly a half century ago in a classic study. The mechanism by which the opilionid puts the secretion to use is described. When disturbed, the animal regurgitates enteric fluid, which it conveys by intercoxal clefts to the anterolateral corners of the carapace, where the two gland openings are situated. It then injects some of its quinonoid secretion into the fluid, and conveys the mixed liquid along the length of its flanks by way of two special channels. Such a discharge mechanism may be widespread among opilionids of the family Gonyleptidae (suborder Laniatores), to which *A. aculeatus* belongs. In a bioassay based on a scratch reflex in decapitated cockroaches (*Periplaneta americana)* the liquid effluent of *A. aculeatus* was shown to be potently irritating. Use of the effluent was demonstrated to protect the opilionid against ants (*Formica exsectoides*). Wolf spiders (*Lycosa ceratiola*) were shown to be minimally affected by the effluent (they showed little response when the fluid was added to their mouthparts as they fed on mealworms, their normal laboratory prey), although they proved to be aversive to mere contact with the opiliond itself, and to reject the animal without inducing it to discharge. *A. aculeatus* may therefore contain distasteful factors besides its glandular products.

Key words: opilionid, predation, exocrine gland, 1,4-benzoquinone, repellent, Formicidae, Lycosidae.

Introduction

Naturalists have long known that chemical defenses are commonplace in arthropods. Most often these defenses take the form of dischargeable integumental sacs, laden with irritants or repellents. Knowledge of these glandular structures was initially restricted to their anatomy and histology. It was only in the past five decades that efforts focused on the function of the glands and on the chemical elucidation of their products. An early contribution to this area of research was the study of an opilionid, *Acanthopachylus aculeatus*, initiated by Clemente Estable and María Isabel Ardao in Uruguay, where the arachnid is native, and involving eventually collaboration with Louis Fieser of the Chemistry Department of Harvard. The study, which stands as a landmark in the history of arthropod exocrinology, led to the isolation of three benzoquinones: (1) 2,3-dimethyl-1,4-benzoquinone, (2) 2,5-dimethyl-1,4-benzoquinone and (3) 2,3,5-trimethyl-1,4-benzoquinone, not previously known from nature (Estable et al., 1955; Fieser and Ardao, 1956). While it was assumed all along that the secretion of *A. aculeatus* was defensive, no proof to that effect had been obtained, nor had the animal's glandular discharge mechanism been described.

We have recently had occasion to study live *A. aculeatus* in the laboratory. We were able to confirm the earlier claim that the secretion is quinonoid in nature, and in addition obtained detailed photographic documentation of the glandular discharge mechanism, and proof that the secretion has defensive potency. We also obtained evidence that the opilionid might produce additional, as yet unknown chemical factors that contribute to its unacceptability to spiders.

A. aculeatus belongs to the suborder Laniatores of the Opiliones. Quinonoid secretions may be of common occurrence in this suborder, as one might conclude from a number of chemical investigations (for references, see Acosta et al., 1993). The discharge mechanisms of the glands are not precisely the same in the different quinone-producing Laniatores. However, the mechanisms have one feature in common, namely that the quinones, upon discharge from the glands, are mixed with a diluent, in the form of regurgitated enteric fluid. Such preparative mixing of a glandular defensive product with gut fluid appears to be without parallel in other animals.

The first Laniatores species in which the glandular discharge mechanism was described is *Vonones sayi* (Eisner et al., 1971). When this animal is disturbed, it promptly emits a droplet of fluid from the mouth. The fluid does not remain in place, but seeps almost instantly to the margins of the body along two

linear clefts, formed by the closely apposed bases (coxae) of the first and second legs. The fluid is thus conveyed in about equal measure to each of the two gland openings, beside which it accumulates, forming two distinct droplets. The animal then injects a small amount of its glandular quinonoid paste into each droplet, and proceeds to administer the mixture through brushings of its forelegs. We here present photos of this discharge mechanism (Fig. 1), to provide a basis for visual comparison of the defensive emission strategies of *A. aculeatus* and *V. sayi*.

Other species of Laniatores that have been studied mix enteric fluid with glandular quinone, as does *V. sayi*, but they do not all administer the fluid with the forelegs. Rather, in some cases, they allow the fluid to spread along two specialized channels on their flanks, with the result that they become laterally coated with the fluid (for references, see Acosta et al., 1993). As we here demonstrate, *A. aculeatus* belongs to the latter category of Laniatores.

Materials and methods

Acanthopachylus aculeatus

This opilionid *Acanthopachylus aculeatus* Kirby is wide-ranging, extending from the Guayanas to Argentina (Capocasale and Trezza, 1964). Specimens were collected at various localities in the vicinity of Montevideo. Like Laniatores generally, they occur on the ground in hiding places, most commonly under rocks, sometimes in numbers rather than singly. We maintained the animals in the laboratory in groups in humidified cages, on a diet of freshly cut up mealworms (larvae of *Tenebrio molitor*) and water. We found that they fed primarily at night, and aggregated in the daytime. Only adults were available for study. Most survived for over a year.

Chemistry

Secretion was obtained by holding individual *A. aculeatus* by the body, or by some of the legs, in forceps, and squeezing them gently until droplets of the yellow fluid (mixture of enteric liquid and glandular secretion) appeared at the edge of the carapace. The fluid was then picked up in glass capillary tubing, weighed, and analyzed. Individuals were squeezed repeatedly, until no further yellowish fluid appeared on their flanks.

Qualitative analysis was effected by GC-MS and NMR spectrometry. GC-MS data were obtained with a Hewlett-

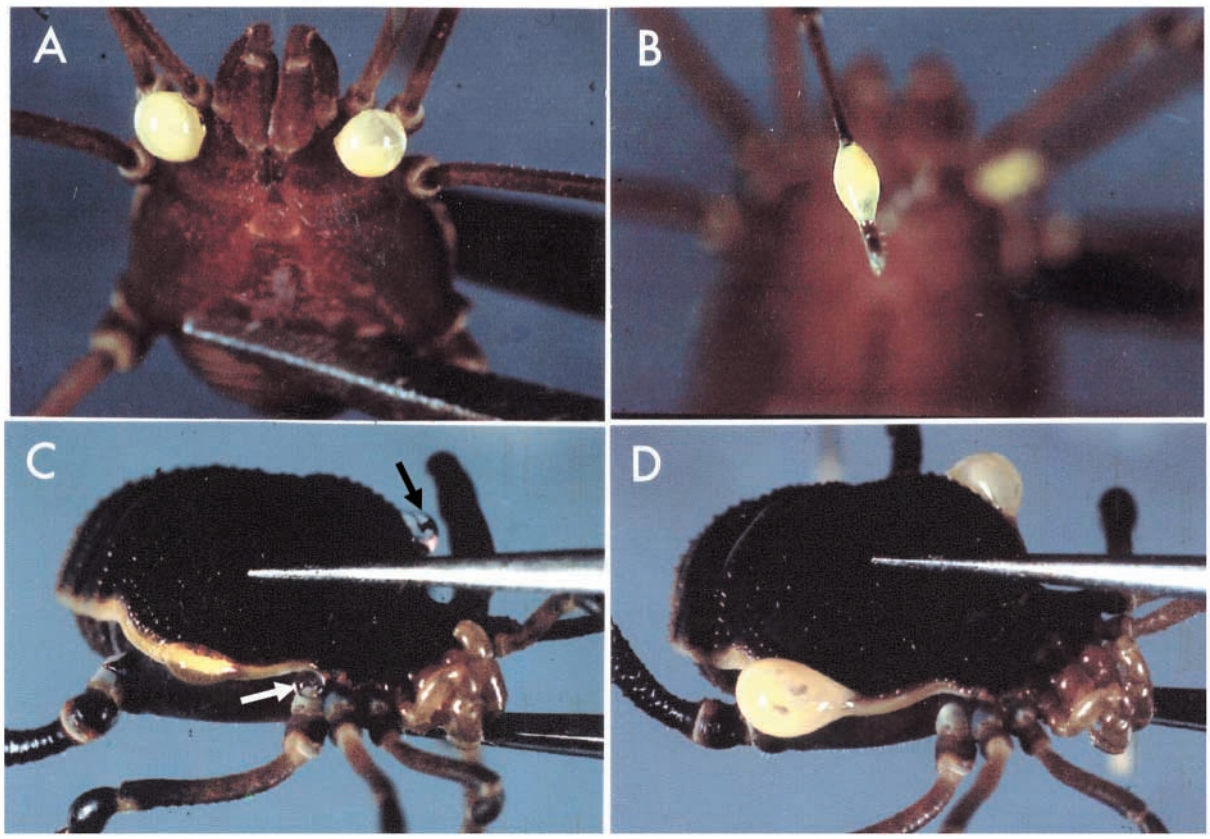

Fig. 1. (A,B) *Vonones sayi* (from Lake Placid, Florida, USA). The animal is shown in ventral view, first (A) with droplets of newly formed effluent at anterolateral corners of prosoma, then (B), moments later, after having dipped a foreleg into one of the droplets, in anticipation of using that leg to brush the liquid onto an enemy. (C,D) *Acanthopachylus aculeatus.* In the first stage of its defensive response (C), enteric fluid has accumulated to form two droplets on the lateral carapace channels (arrows). Moments later (D), yellow quinonoid secretion has been injected into the enteric fluid.

Packard (Palo Alto, CA, USA) 5890 gas chromatograph, coupled to a Hewlett-Packard 5971 mass selective detector [fused-silica capillary column coated with DB-5 stationary phase (25 m×0.25 mm; 0.25 μm film thickness)]. Oven temperature conditions were 60°C for 2 min, increased to 200°C at 5°C min^{-1}. Samples were injected with CH_2Cl_2 as solvent. NMR spectra (^{1}H-NMR, 500 MHz) were obtained in d_6-benzene, using a Varian Unity 500 spectrometer.

For quantitative determination of quinone content, fluid samples were mixed with 50 μl CH_2Cl_2, bearing 1,4-benzoquinone as internal standard, and analyzed by gas chromatography (same column and oven conditions as above) using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. The relative proportion of the three benzoquinones in the fluid samples was calculated by GC peak comparisons.

The total amount of quinone per sample was calculated as the sum of the net quantities of the three quinones present in the mixture. The quantity of each quinone was calculated from the linear regression equation (r^2=0.987) of a calibration curve constructed for 2,5-dimethyl 1,4-benzoquinone. All quinone amounts were therefore expressed as 2,5-dimethyl 1,4-benzoquinone equivalents.

Glands and discharge mechanism

Individual *A. aculeatus* were variously stimulated, by pinching either the body or individual appendages with forceps, thereby causing them to emit their defensive fluid, while the animals were under observation using a Wild M400 Photomakroscope (Heerbrugg, Switzerland), which provided the opportunity to record the events photographically.

For scanning electron microscopy, specimens were dehydrated in ethanol and gold-coated.

Irritancy of secretion

We made use of a bioassay that we had developed earlier for the assessment of irritancy of test substances (Eisner, 1961). The assay is based on the observation that when a droplet of an irritating chemical is placed on one side or the other of the fifth abdominal tergite of a decapitated nymph of the cockroach *Periplaneta americana*, the animal scratches the site with the hindleg of the side stimulated. The time interval between application of the sample and scratching provides a measure of the irritant effectiveness of the sample. Details of this assay, which we have used for assessment of irritancy of a variety of natural products, are given elsewhere (Eisner et al., 1976). Under exceptional circumstances, when a sample is especially active, the cockroach reacts to mere proximity of the test substance rather than only after contact with it. The liquid effluent of *A. aculeatus* proved to be active on near contact.

For our purposes we used last-instar *P. americana* nymphs, decapitated on the preceding day. Decapitation was effected by ligating the neck and severing the head just anterior to the ligation, thus preventing the animal from bleeding when decapitated.

Tests were effected by collecting a sample of fresh liquid effluent from an *A. aculeatus*, and bringing this liquid immediately to within close range of one side of the fifth abdominal tergite of the decapitated cockroach. The fluid sample was collected by pinching the body or a leg of the opilionid with forceps, so as to cause it to emit yellow effluent, and taking up the liquid from the animal's flanks, in a glass capillary tube (0.03 mm^2 bore). The capillary tube, filled to the brim, was then promptly pointed from a distance of 4–6 mm at the tergal surface, and the delay to onset of scratching was timed to the nearest second with a foot-operated stopwatch.

Cockroaches were used only once, each with a sample from a separate opilionid. For control purposes, to check whether the oral effluent might itself contain irritant components, samples of liquid were taken up from the edges of the carapace, immediately after an *A. aculeatus* was stimulated, but before it injected secretion into the fluid. Such fluid, was presented at 4–6 mm from the tergal surface, in capillary tubes, as were the experimental samples bearing secretion.

Predation tests (ants)

The test with ants involved introducing individual *A. aculeatus* into separate Petri dishes (9 cm diameter), each containing eight worker ants (*Formica exsectoides*) collected outdoors (Ithaca, Tompkins County, New York, USA) several hours beforehand. After 30 min exposure to the ants, the opilionid was removed from the dish and transferred to a small humidified chamber where it had access to water. After 12 h of such confinement, the opilionid was subjected to stimulation (pinching of the body and appendages with forceps), to check whether it still possessed secretory ability.

Predation tests (spiders)

Two series of tests were carried out, one to check on the deterrency of the effluent of *A. aculeatus*, the other to determine the acceptability of the opilionid itself. The tests were done with wolf spiders (*Lycosa ceratiola*), captured weeks beforehand in the environs of Lake Placid, Highland County, Florida, USA, and maintained in individual cages on a substrate of sand and a diet of mealworms.

To test for the effectiveness of the defensive fluid, individual spiders were offered a nearly full-grown mealworm, such as they had been obtaining at biweekly intervals as a matter of routine, and then, once they had killed the mealworm and had commenced feeding on it, were stimulated by the addition to the surface of the mealworm, directly at the site where the spider had inserted the chelicers, of 2 μl of *A. aculeatus* effluent (oral fluid plus secretion). The fluid had been taken up in calibrated glass capillary tubes from an opilionid immediately before application of the liquid to the mealworm. The effect of the applied fluid on the spider was noted.

To test for the acceptability of the opilionid itself, a number of *A. aculeatus* were individually released in the spider cages, and the ensuing events were noted.

Results

Chemistry

The ^{1}H-NMR and GC-MS data confirmed the presence of benzoquinone compounds 1, 2 and 3 in the effluent of *A. aculeatus*. The relative proportion (mean ± S.E.M.; 6 individual samples from 6 individuals) of the three quinones in the fluid was 49±3% (1), 11±1% (2) and 37±3% (3), roughly in line with the ratio (73:11:13) reported by Fieser and Ardao (1956). The samples also contained a small proportion (about 1%) of 2,3-dimethyl hydroquinone. The net amount of quinone (sum total of 1, 2 and 3; mean ± S.E.M.) in the same six samples was 321±66 μg (range 170–622 μg). Since the opilionids had been milked to exhaustion, we take this figure to provide an indication of the quinonoid load of the glands of the animal.

Glands and discharge mechanism

A. aculeatus respond consistently when disturbed. When merely prodded or picked up gently by the body with forceps, they may not respond at all. But if the body is squeezed, or appendages are pinched, they tend quickly to activate their defense. Onset of the response is signaled by the appearance, along the full length of the light-colored channels that demark the lateral margins of the carapace, of clear enteric fluid (Figs 1C, 2C). Almost immediately thereafter, the liquid in the channels takes on a yellow appearance, as the animal injects some of its quinonoid secretion into the fluid (Fig. 2D). Typically, the fluid builds up at the posterior end of the channels, there to form two bulging yellow drops (Fig. 1D). It is also not uncommon for the oral effluent to be delivered in pulses onto the channels (Fig. 2E), as indicated by the

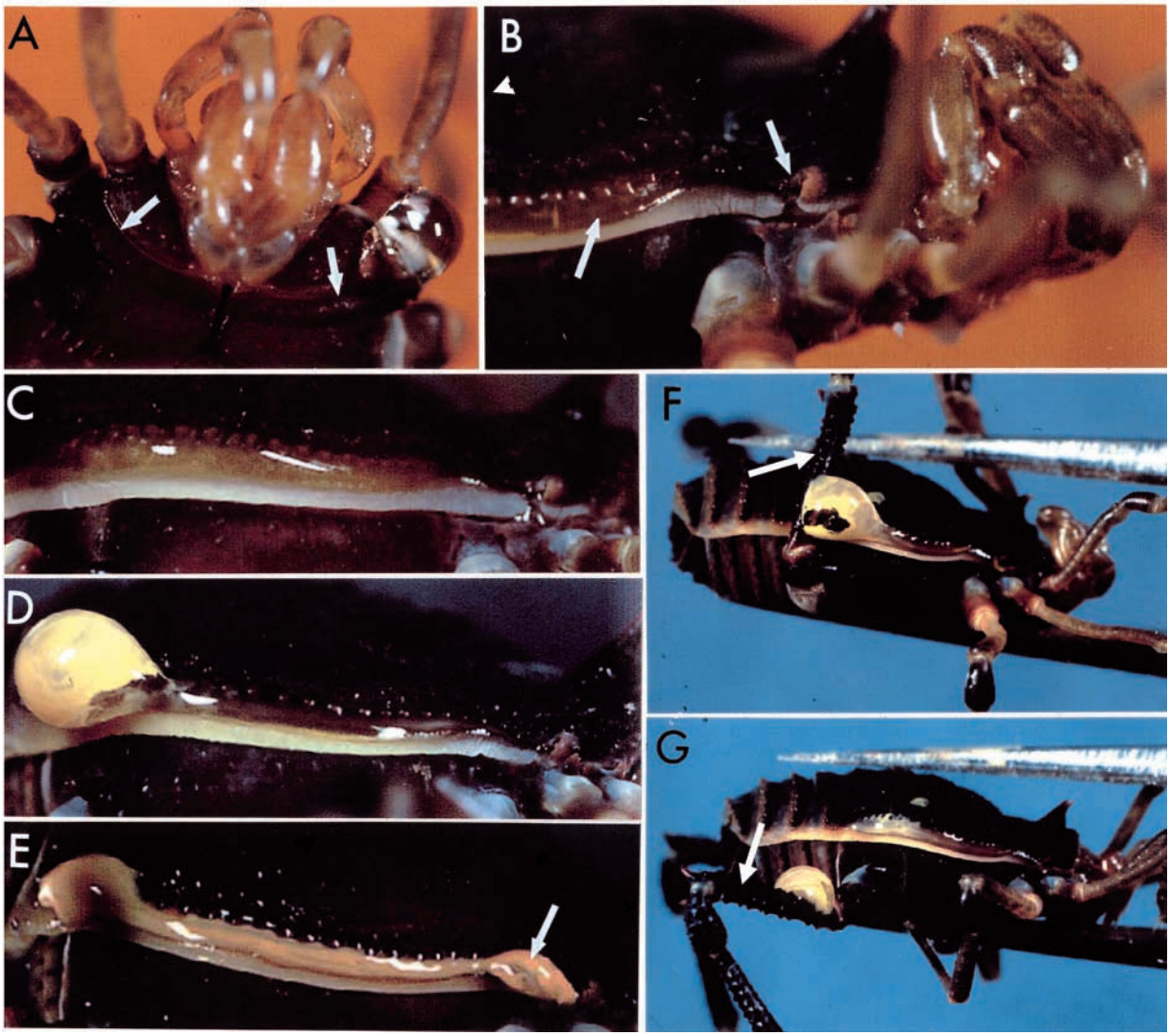

Fig. 2. *A. aculeatus*. (A) Front end, in ventral view, showing the clefts (arrows) between coxae of legs 1 and 2 that convey the oral effluent to the gland openings. (B) Front end, in right lateral view, showing the gland opening (upper arrow), and the notch under it, by which the oral effluent and secretion are routed onto the carapace channel (lower arrow) (compare with Fig. 4). (C) Right carapace channel, laden with clear oral effluent. (D) Comparable to C, after secretion has been added to effluent. (E) Comparable to C, at the moment when a second dose of secretion (arrow) is being emitted from the gland opening into the fluid in the channel. (F,G) Transfer of defensive fluid from carapace channel to femur (arrow) of hindleg.

discontinuous egress of fluid from between the bases of legs 1 and 2.

The glands consist of two sacs, situated dorsally in the prosoma, and opening at the anterolateral angles of the carapace (Fig. 3A). The gland openings are slit-like and are located directly above the junction of the first and second legs (Figs 2B, 4A). This places the gland openings directly above the point of egress of regurgitated enteric fluid, in other words, directly at the end of the clefts, between coxae of legs 1 and 2, that serves for conveyance of oral effluent to the gland openings (Figs 2A, 4B,C). Extending backwards from the gland openings, along the full length of the margins of the carapace, are the two channels along which the oral effluent spreads, after its conveyance from the mouth (Figs 2C–E, 4A). A small notch just behind each gland opening enables the liquid to flow onto the lateral channels as it emerges from between legs 1 and 2 (Fig. 2B, 4C). The notches also ensure that the oral fluid contacts the gland openings as it is routed onto the channels, thereby enabling the glands to empty some of their contents into the fluid.

Fig. 5 summarizes the sequence of events that accompany a secretory discharge in *A. aculeatus.* At the outset, enteric fluid (red) is emitted from he mouth, from where it spreads right and left along the clefts between the coxae of legs 1 and 2. The

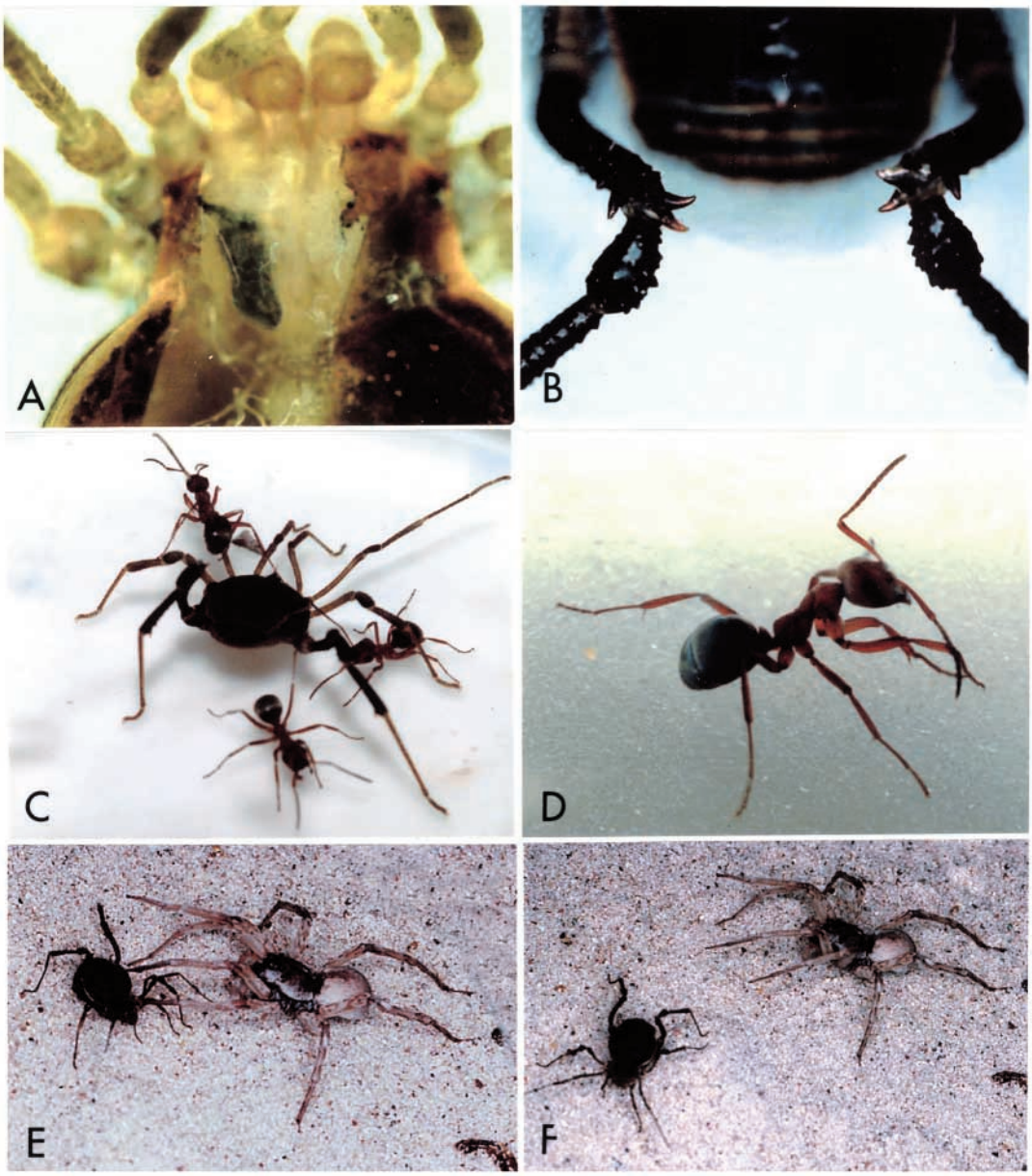

Fig. 3. *A. aculeatus.* (A) Left gland, exposed by cutting away part of the carapace. (B) Rear view of male, showing the hindlegs with their femoral spurs. (C) Individual under attack by ants (*Formica exsectoides*), before having emitted its defensive fluid. (D) Ant in the process of cleaning itself, in response to exposure to the defensive effluent of the opilionid. (E,F) Individual being inspected and spurned by a wolf spider (*Lycosa ceratiola*).

fluid then spreads upward between legs 1 and 2 onto the lateral channels, passing by the gland openings, and picking up secretion (blue) as it flows posteriorly. The mixed product then accumulates at the posterior end of the channels.

The photographs illustrate additional details. Note, for instance, that the clefts between the coxae of legs 1 and 2, which convey fluid from the mouth to the gland opening, glisten as a consequence of their wetness following an oral discharge (Fig. 2A). Note also the two dorsal apophyses on the coxa of the second leg (denoted by asterisks in Fig. 4C, and seen also in Fig. 2B). It is possible that these apophyses help guide the oral fluid onto the carapace channels as the fluid is conveyed from the mouth. Fig. 2F,G illustrate a phenomenon repeatedly noted, namely the transference, in the course of normal leg movements, of ejected defensive fluid onto the femur of the hindlegs.

Fig. 3B shows the hindlegs of a male of *A. aculeatus*, with its projecting spines. We noted repeatedly, as have others (Capocasale and Trezza, 1964), that *A. aculeatus* attempts to use its hindlegs as pinching devices when seized by hand, by pushing the femora together and exerting pressure with the spines. The spines are larger in the males than in the females (Capocasale and Trezza, 1964), raising the possibility that the structures play yet additional roles.

Irritancy of secretion

Eighteen effluent samples (regurgitant plus secretion) were tested, and in 16 cases the cockroaches responded within

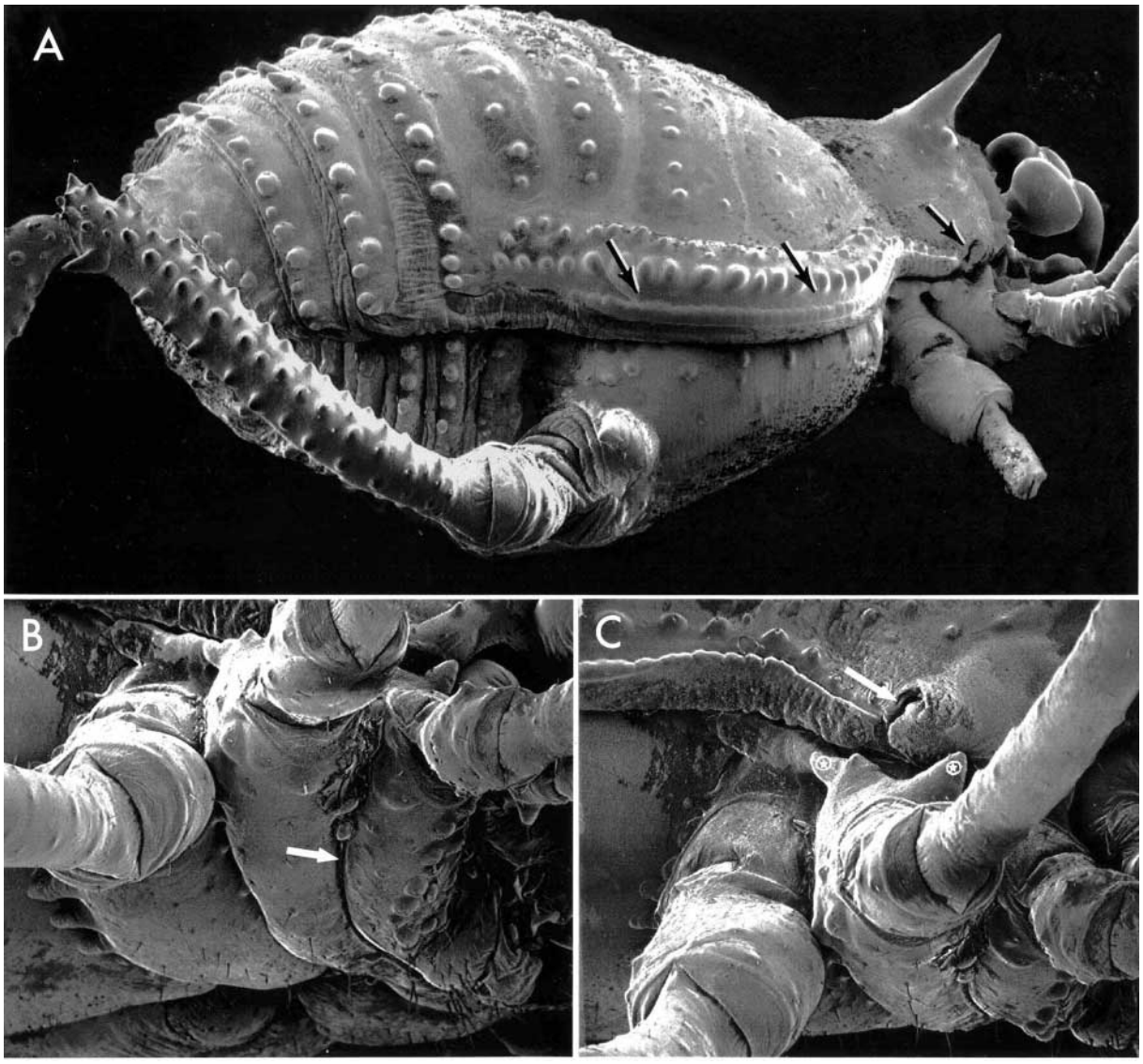

Fig. 4. *A. aculeatus*, scanning electron micrographs. (A) Lateral view, showing the gland opening (right arrow) and the carapace channel (middle and left arrows). (B,C) Close-up view of side of animal at base of legs 1 to 3. In B, the animal is tilted to expose the right-hand ventral surface of the animal, bringing into view the cleft (arrow) between the coxae of legs 1 and 2, along which the oral effluent is conveyed. In C, the gland opening (arrow) is shown in relation to the two spines (asterisks) projecting from the coxae of leg 2 that presumably help direct the oral fluid past the gland opening; the notch immediately behind the gland opening serves to convey the liquid onto the carapace channel.

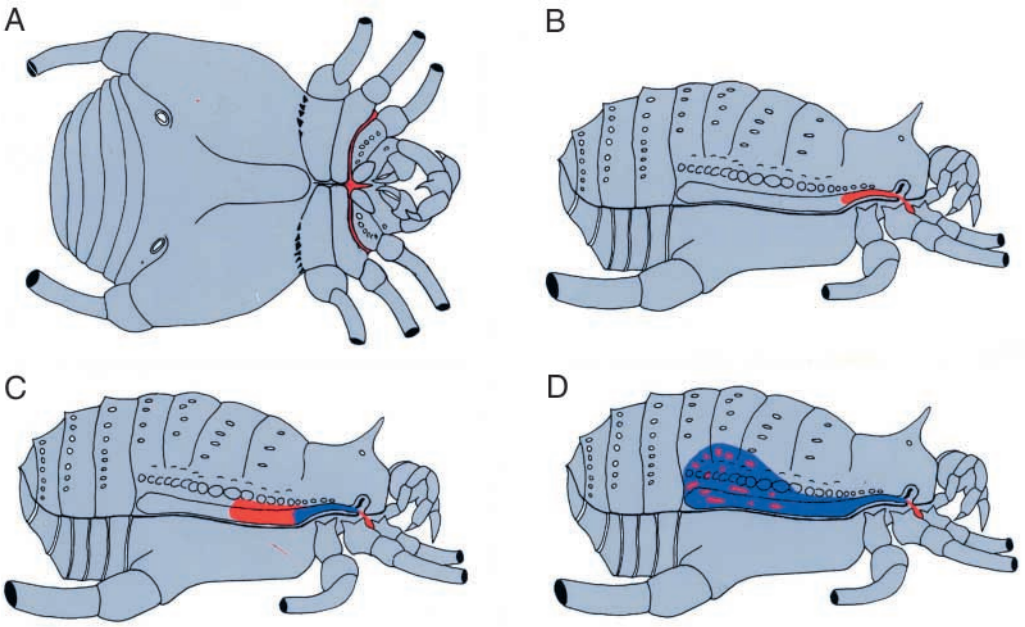

Fig. 5. Defensive fluid emission mechanism of *A. aculeatus.* (A) Ventral view; oral effluent (red) has been routed by way of the clefts between the coxae of legs 1 and 2 to the margins of the body. (B) Right-side view; the fluid is seen emerging from between legs 1 and 2 and flowing past the gland opening onto the carapace channel. (C) Quinonoid secretion (blue) has been ejected from the gland opening into the stream of oral effluent. (D) The mixture of oral effluent and secretion has accumulated on the carapace channel.

1 s of presentation of the sample. The two exceptional cockroaches responded within 2 and 4 s, respectively. Only four samples of pure regurgitant were available for testing, but they proved, without exception, to be inert (the cockroaches failed to scratch within 60 s of presentation of the sample).

Predation test (ants)

Only four *A. aculeatus* were available for testing. The results were similar with each. No sooner had the opilionid been introduced into the Petri dish, than the ants attacked (Fig. 3C). They scurried over the body of the opilionid, causing the latter to 'freeze' and to adopt a stilted stance, with its legs straightened out and the body lifted off the substrate. Events proceeded quickly, but it was clear in each case that several ants succeeded in clamping their mandibles onto legs of the opilionid. They did so only within the first minute after introduction of the opilionid, and they did not long persist in their hold. The opilionid, in each case, activated its defenses, as indicated by the visible appearance of yellowish fluid along its flanks (the times, from test onset to the appearance of the defensive fluid, were recorded as 25, 35, 35 and 50 s). Following such emission the opilionid appeared to be safe from attack. The ants, as a group, responded instantly to the emission by scurrying about quickly while showing distinct avoidance of the opilionid, and of the dabs of the opilionid's effluent that had rubbed off on the dish floor. As they dashed about, the ants paused frequently to preen, wiping antennae with the forelegs (Fig. 3D), and legs against legs.

Neither the ants nor the opilionid suffered ill-effects from the encounters. The ants, after removal of the opilionid from their midst, gradually returned to normal, resuming their ordinary ambulatory pace, and cutting back on the frequency of preening. 12 h following the encounter they seemed all to have recovered. The opilionids showed no signs of injury, even though they had been sprayed by the ants (ants that had seized the opilionid were observed in some cases to flex their abdomen forward beneath their body as they typically do when ejecting their formic acid-containing secretion) and when stimulated by pinching, 12 h after the encounter, produced yellow defensive effluent in what seemed to be normal quantity. The opilionids evidently had not expended their entire glandular reserves in the course of the ant assaults, or incurred a lasting deficit in enteric fluid. If indeed they had depleted their enteric reserves in the course of the assaults, they had evidently reacquired enough liquid by drinking to be able to produce normal amounts of diluent for their quinonoid emissions.

Predation tests (spiders)

Application of *A. aculeatus* effluent to the site where the spider had inserted its chelicers into the mealworm had surprisingly little effect. In five of 15 spiders thus tested, the spiders failed to respond altogether. They continued to feed on the mealworm, without even momentarily dislodging their chelicers. In eight of the remaining ten cases, the spiders did extricate the fangs, but they did so only briefly, for no longer than it took them to reinsert their fangs at a point adjacent to where the effluent had been applied. In two cases the spiders did release their hold altogether, and as they then backed away from the mealworm, proceeded to drag their mouthparts in the sand. But they undertook such cleansing behavior only briefly, and within 2 min in one case and 4 min in the other, returned to the mealworm to finish the meal. In all 15 cases the spiders reduced the mealworms to small packets of indigestible remains, as lycosids typically do with insect prey.

The tests with actual *A. aculeatus* also gave consistent results. Not one of the 13 opilionids offered to individual spiders sustained injury. Six were pounced upon the moment they were introduced into a spider's cage, but the spider released them instantly, by relaxing their hold and enabling the

opilionid to walk away. The other seven individuals were also spurned, but after mere palpation. They were not immediately noticed by the spider when released in the spider's cage, but when eventually detected were rejected after being touched briefly with the palps or legs (Fig. 3E,F). All 15 spiders killed and ate the mealworms they were offered as controls after the test.

Discussion

The characterization of 1,4-benzoquinones from *A. aculeatus* by Estable et al. (1955) and Fieser and Ardao (1956) represents the first definitive identification of such substances from an arthropod. There had been an earlier report of benzoquinone production by a millipede (Béhal and Phisalix, 1900), but the characterization in that instance was tenuous. By using the much simpler analytical tools available today, we were able to show that the original claims made about the quinonoid composition of the *A. aculeatus* effluent were correct.

1,4-benzoquinones are now known to be among the principal defensive substances produced by arthropods, having been isolated from, among others, millipedes, cockroaches, termites, earwigs, grasshoppers, hemipterans and carabid, tenebrionid and staphylinid beetles (Blum, 1981). Their presence in opilionids appears to be restricted to species of the families Gonyleptidae (to which *A. aculeatus* belongs) and Cosmetidae, both members of the suborder Laniatores (Eisner et al., 1978; Roach et al., 1980; Holmberg, 1986; Acosta et al., 1993). However, 1,4-benzoquinones are not the exclusive defensive compounds of the Laniatores. Some Laniatores produce phenols (Acosta et al., 1993; Eisner et al., 1977; Duffield et al., 1981), and one other, the Uruguayan *Parampheres ronae*, produces a mixture of vinyl alkyl ketones (C. Rossini, unpublished observation). The members of the second principal suborder of the opilionids, the Palpatores, produce an array of volatile branched-chain alcohols, ketones and aldehydes (Eisner et al., 1978; Ekpa et al., 1985). The single species of this suborder known to produce a quinonoid product, secretes naphthoquinones rather than 1,4-benzoquinones (Wiemer et al., 1978).

The strategy of mixing glandular secretion with oral effluent appears to be restricted to the Laniatores. When Estable, Fieser and their associates did the original work on *A. aculeatus*, they had no evidence that the quinonoid mixture they isolated from this opilionid plays a defensive role. Our demonstration that quinone emission is in fact protective in this opilionid, at least against ants, provides a functional context for the pioneering chemical discovery by these investigators.

A. aculeatus is not alone within the Gonyleptidae in spreading its defensive fluid along its flanks. The excellent description by Acosta et al. (1993) indicates that the gonyleptid *Pachyloidellus goliath* generates its protective effluent by mixing secretory product with regurgitant, much as does *A. aculeatus*, and that it also routes its effluent along carapace channels. The animal conveys its regurgitant to the gland openings by way of intercoxal clefts, as does *A. aculeatus*, and it has notches adjacent to the gland openings by which the mixed fluid is guided onto the channels. One is tempted to predict (in agreement with Acosta et al., 1993) that the Gonyleptidae all mix and route their defensive fluid in this fashion, but the mechanism could be subject to variation. Thus, while in the gonyleptid *Zygopachylus albomarginis*, which secretes a mixture of 1,4-benzoquinones and a phenol, the discharge mechanism appears to be identical to that in *A. aculeatus* and *P. goliath* (Cokendolpher, 1987), in another gonyleptid, *Goniosoma spelaeum*, the mechanism is modified in that the animal is capable both of spreading its effluent along carapace channels and of ejecting its secretion forcibly as a spray. The latter species is said to have a secondary gland opening that serves specifically for spray ejection (Gnaspini and Cavalheiro, 1998). Spray ejection has also been reported for an African Laniatores, *Larifugella natalensis* (Lawrence, 1938).

Also subject to variation may be the gonyleptids' involvement of the legs in defense. Both the incidental wetting of legs with effluent, that we noted in *A. aculeatus*, and the attempts to inflict pinches with the hind legs, occur also in some other gonyleptids (Cokendolpher, 1987; Gnaspini and Cavalheiro, 1998). Also noted in other gonyleptids (Cokendolpher, 1987) is the tendency to assume a rigid stance in response to disturbance, with legs outstretched and body raised, such as *A. aculeatus* manifested when attacked by ants.

Opilionids of the second major family within the Laniatores, the Cosmetidae, appear not to have the carapace channels present in the Gonyleptidae. Whether they all resort to leg dabbing to administer their defensive effluent, as does *V. sayi* (Eisner et al., 1971) remains unknown, although it is clear that some species at least, including the phenol-secreting *Cynorta astora*, do make use of the dabbing technique (Eisner et al., 1977).

The finding that the defensive effluent of *A. aculeatus* has irritant potency comes as no surprise. 1,4-Benzoquinones had been shown to be strongly effective in the *Periplaneta* scratch test, as might well be expected, given the widespread occurrence of these compounds in defensive secretions of arthropods. Interestingly, alkylated 1,4-benzoquinones, such as are present in opilionid secretions, had been shown to be more effective as irritants than unsubstituted 1,4-benzoquinone itself (Peschke and Eisner, 1987), explaining perhaps why the alkylated forms of these compounds should dominate in arthropod defensive secretions (Blum, 1981). The response to the *A. aculeatus* effluent in the scratch test was essentially immediate (in 13 of 15 cases), even though the samples were tested on near contact rather than actual contact, and the quinones were tested in dilute rather than pure form. Given that the regurgitant itself proved inactive in the tests, we assume the activity of the mixed effluent to have been due exclusively to the quinones.

The tests with ants showed clearly that against these predators at least, the chemical defense of *A. aculeatus* is strongly effective. While we would obviously have liked to

have had a greater number of the opilionids for testing, the results were unambiguous. They were also expected. 1,4-Benzoquinones from the secretion of a number of insects, including cockroaches, carabid beetles and tenebrionid beetles, had been shown to be highly repellent to ants (Eisner, 1958a,b; Peschke and Eisner, 1987).

Somewhat surprising were the findings with the wolf spiders. These predators seemed minimally inconvenienced by *A. aculeatus* effluent, even when the fluid was applied directly to their mouthparts. Based on these findings, we had expected the opilionid to be vulnerable to lycosid attack. However, the animals were consistently rejected by the spiders immediately on contact, before they were even prompted to emit effluent, from which we conclude that *A. aculeatus* contains additional chemical factors, repellent to spiders, but distinct from the quinones that convey protection against such other enemies as ants.

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