

DEFENSE MECHANISMS OF ARTHROPODS. XI. THE
STRUCTURE, FUNCTION, AND PHENOLIC SECRE-
TIONS OF THE GLANDS OF A CHORDEUMOID MILLI-
PEDE AND A CARABID BEETLE.¹

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In the course of exploratory field studies on arthropods with defensive glands, we came across two species which emit a strong and persistent phenolic odor when handled. One is a carabid beetle (*Chlaenius cordicollis* Kirby), the other a chordeumoid millipede [*Abacion magnum* (Loomis)]. The fact that both animals produce repellent secretions is not surprising, since many other carabids and millipedes are well known for their defensive glands. But the particular phenolic odor possessed by these two species is unlike the odor of any other arthropod secretion that has been studied (for a summary of defensive secretions of arthropods see Roth and Eisner, 1962).

The purpose of this paper is to report on the nature of the two phenols involved, and to discuss the structure and mode of operation of the glands, as well as their defensive effectiveness. Both species were collected in the environs of Ithaca, N. Y. *Abacion* was from leaf litter in deciduous woods, and *Chlaenius* from beneath rocks near a creek bed. We had available for study ten specimens of *Abacion* and about two dozen *Chlaenius*.

I. Glandular Apparatus and Discharge Mechanism.

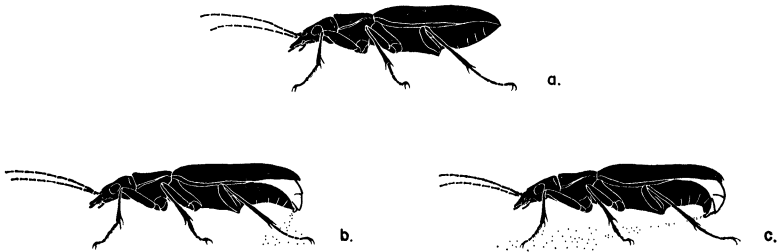
a. *Chlaenius*

Chlaenius has a pair of glands, the openings of which are visible as two tiny slits, situated submarginally on the hypopygium a short distance behind the terminal spiracles (Plate 9, fig. 1). When a live

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beetle is grasped gently by its front end, it can be induced to discharge one or more times simply by tightening the grasp intermittently or by pinching individual legs with forceps. When viewing such a beetle ventral side up with a stereomicroscope, it becomes evident that the secretion does not emerge as a liquid ooze, but is expelled from each opening as a jet of finely dispersed spray. At the moment of discharge there is seen to project from each glandular pore a short slender nozzle (Plate 9, fig. 1), from the tip of which the spray shoots forth. By



Text fig. 1. Diagrams of *Chlaenius*, showing how the beetle aims its spray by bending the tip of the abdomen. At *a*, the beetle is at rest; at *b*, the discharge is in response to stimulation of a hind leg; at *c*, the target is a stimulated middle or anterior leg.

prodding or pinching first one leg and then another, it becomes clear that the spray is not ejected in a fixed direction, but is aimed with some accuracy toward the particular appendage stimulated. Aiming is determined by the degree of flexion of the abdominal tip. When anterior legs are stimulated, the tip bends downward sharply, so that the projecting nozzles point forward almost horizontally. When middle or hind legs are stimulated, the bending is less pronounced, and the nozzles point downward at an angle (Text fig. 1). Also apparent was the fact that the discharge is not necessarily from both glands at once. When the stimulus is a unilateral one (e.g. the pinching of a leg) only one nozzle is seen to evaginate and spray, and this is invariably the one corresponding to the side of the body stimulated.

Additional experiments were designed to determine more precisely the accuracy of aiming. The technique employed was the same as used previously with other arthropods that spray (Eisner, 1958a, 1958b, 1960a; Eisner et al., 1959, 1961). Individual beetles were attached to rods and placed on sheets of indicator paper impregnated with a chemical mixture that discolors in the presence of the secretion, thus enabling a visualization of the spray through the pattern of spots

engendered on the paper. The mixture used as an indicator was an aqueous solution of ferric chloride and potassium ferricyanide. In the presence of secretion this mixture turns to an intense blue (the secretion acts as a reducing agent, causing formation of Prussian blue)².

Several beetles were subjected to traumatic stimulation, either by pinching single legs or antennae with forceps, or by touching various regions of the body with a hot probe. Any one such stimulus invariably induced a prompt aimed discharge (Plate 10, figs. 1-4). As expected, the discharge was always from one gland alone, providing the stimulus had been a unilateral one. Thus, stimulation of a leg or antenna of one side, was followed by an ejection from the gland of that side only. Similarly, when one side of the head or abdomen was touched with a hot needle, only the gland from the corresponding side discharged. But when the head was touched on both sides simultaneously, or when the abdomen was seized with broad-tipped forceps, then the discharge was a synchronous one from both glands.

Chlaenius cannot revolve its abdominal tip upward and around so as to spray upon its back. Touching the thoracic dorsum or elytra with a hot needle caused the animal to discharge forward under the abdomen in the usual fashion. Under such circumstances the traumatized region is likely to receive at best an incidental spattering of droplets.

A fair idea of the usual range of the spray can be obtained from figures 1-4 in Plate 10. Range is determined by the downward angle at which ejection occurs, hence the most anteriorly directed discharges are the farthest reaching. Maximum spray impact was within a radius of 10 cm., although occasional droplets nearly always surpassed this range, and sometimes reached as far as 50 cm. away.

The number of discharges that could be elicited from each gland of beetles that had remained undisturbed for two previous weeks ranged from two to four (five beetles tested). As a rule, the bulk of the secretion is expended with the first discharge; a much more scanty spray pattern is produced by the second discharge, and the third and fourth leave no more than a few scattered spots at close range.

Only three specimens were available for dissection, but this sufficed to establish the overall similarity of the glands to those of *Chlaenius velutinus* Duftschmid, briefly described and diagrammed by Dierckx (1899). The two glands are situated symmetrically on both sides of the midline in the posterior dorsal abdomen (Plate 11,

²We are indebted to George M. Happ for suggesting the use of this particular indicator mixture.

fig. 2). Each consists of a racemose cluster of secretory cells (A), drained by numerous fine cuticular ductules that converge to merge into a single long and slender efferent tube (B). The tube leads to a capacious and strongly muscled storage reservoir (C), from which secretion is expelled *via* a short ejaculatory duct (D) that opens on the hypopygium.

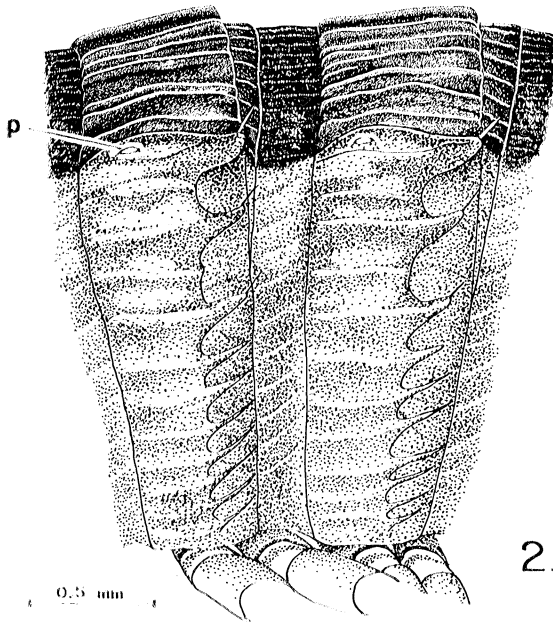
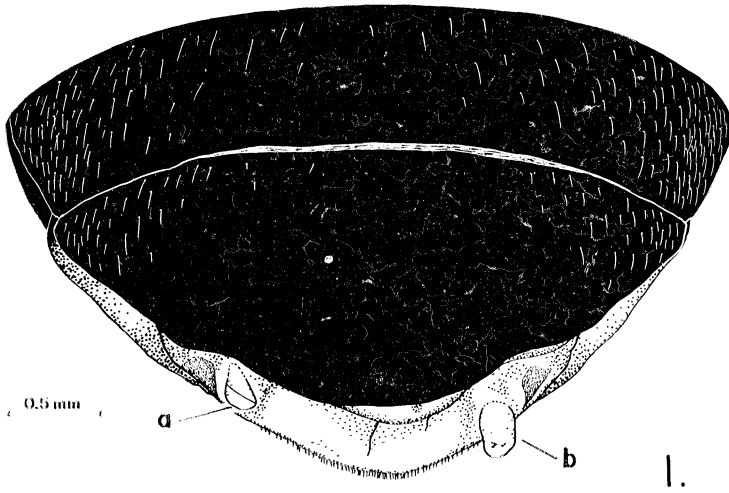
The opening itself is slit-like, the duct at this point being maintained closed under the spring-like action of an especially modified cuticular valve. Examination of a KOH-treated specimen consisting of cuticle alone confirmed the fact that closure of the orifice is maintained passively without muscle enforcement: the orifice was tightly collapsed, and could only be opened by prying with a glass needle.

The ejaculatory duct is surrounded by circular muscles (Plate 11, fig. 4, C), but these do not extend the full length of the duct. The terminal portion is naked (E), and consists of only the cuticular intima and its surrounding epithelium. It is evidently this naked section that is extruded at the moment of discharge to form the spray nozzle. Two special muscles seem to effect nozzle extrusion. One of these is a broad and short sheet of fibers (A), originating on the hypopygial cuticle near the pore, and extending obliquely to the duct to merge with the duct's intrinsic circular muscles. Contraction of this muscle pulls on the duct, forcing its evagination. The second muscle (B) inserts on a cuticular flap at the edge of the pore and, after bending around the duct, extends to attach on the hypopygial cuticle. This particular muscle serves to force apart the cuticular valve that ordinarily closes the pore, thus enabling the nozzle to be extruded at the time of discharge. Without a gaping pore orifice, extrusion would be impossible and the tube would simply buckle.

b. *Abacion*

In this millipede, as in so many others that produce defensive secretions, the glands are distributed segmentally, one pair to each of most diplosegments. Only the first postcephalic segments and a few preanal ones lack glands. The openings of the glands are tiny pores situated dorsolaterally on the anterior half of the diplosegment, each on an elongate crest that protrudes from the tergum (Plate 9, fig. 2; Plate 12, fig. 3).

The ease with which a given *Abacion* may be induced to discharge varies greatly. Some discharge at once, the moment they are first picked up, but this is the exception. More often they will tolerate considerable prodding and even prolonged handling before the dis-



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tinct phenolic odor finally becomes noticeable. But even the least responsive individual will eventually discharge when the stimulus is a more traumatic one, as for instance when legs are persistently pinched with forceps, or when the body is touched with a hot needle.

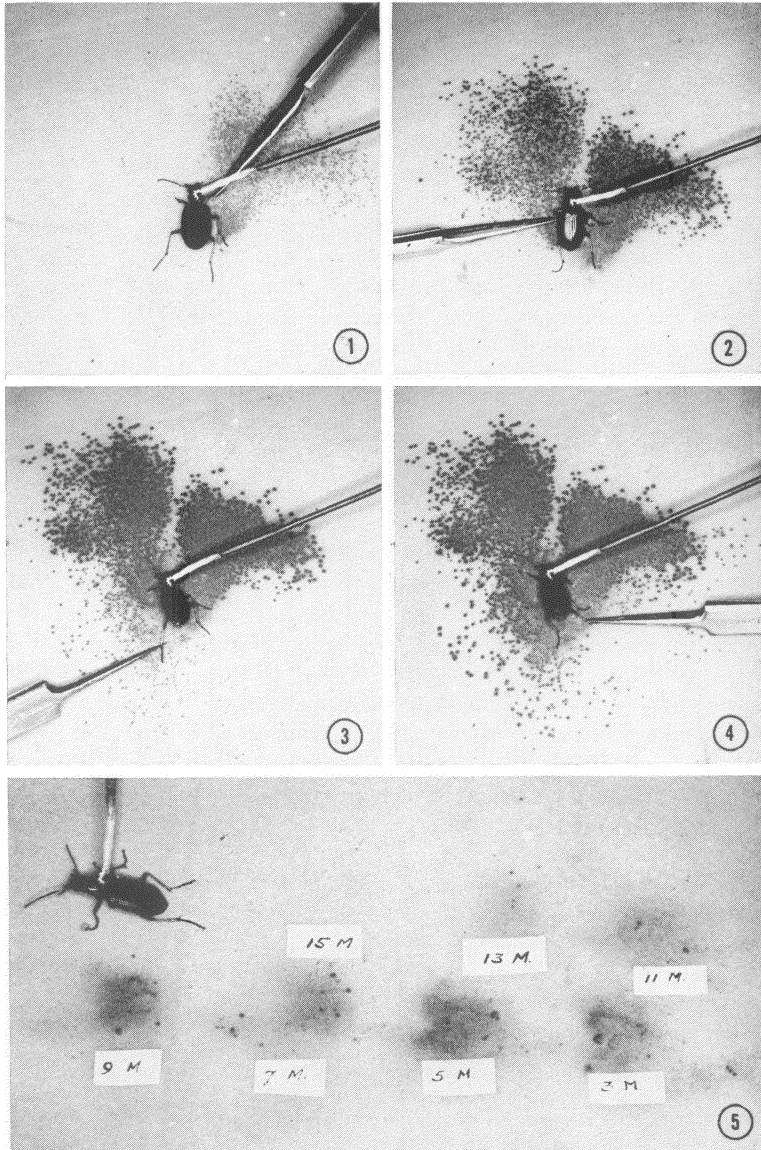
The secretion is not sprayed as in *Chlaenius*, but is discharged as discrete white droplets that ooze forth from the various glands and collect at the pore openings (Plate 12, fig. 2). The discharge is not from all glands at once, but is restricted to the glands of the region traumatized. Insufficient millipedes were available to study the precise pattern of discharge localization, or to determine in some detail the relative effectiveness of various types of traumatic stimuli. It seemed clear, however, that the first glands to discharge are those of the specific segments stimulated and that, with persistent stimulation at the same locus, the response tends to spread to adjacent segments, but never to more than a few on both sides of the area stimulated. Stimulation of the head — which lacks glands — results in an instantaneous ventral curling of the front end of the animal, so that the head is brought in close proximity to the first gland-bearing segments, which under these circumstances are ones that discharge. Once a millipede has been caused to discharge at a particular locus, subsequent discharges at other loci may usually be induced rather readily (e.g. by scratching with a cold needle), without resorting to the rather strong trauma (e.g. persistent pinching of legs, cauterization) that is ordinarily required to evoke a first discharge. In all preceding respects, *Abacion* bears close resemblance to other millipedes whose discharge mechanism has been studied in some detail (Kafatos, 1961).

Each gland of *Abacion* consists of a spherical cuticular reservoir (Plate 12, figs. 3, 4), dorsolaterally situated in the posterior half of the diplosegment, and embedded within the thick multilayered somatic musculature. As evidenced from microscopic whole mounts of stained preparations, the wall of the reservoir consists of an outer glandular epithelium and an inner cuticular intima. There is no surrounding musculature: examination in polarized light—which ordinarily reveals even the most tenuous muscle fibers (Eisner, 1962)—served to confirm their absence.

EXPLANATION OF PLATE 9

Fig. 1. Ventral view of abdominal tip of *Chlaenius*, showing the slit-like gland opening as it appears at rest (*a*), and the nozzle (*b*) that projects from the opening at the moment of discharge (the nozzle was drawn from memory after observing beetles spraying under a microscope; its general proportions are probably accurate).

Fig. 2. Left lateral view of two diplosegments of *Abacion*, showing gland openings, one of them labelled (*p*).



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The reservoir leads to the outside by way of a narrow duct, the terminal portion of which is occluded by a valvular infolding of the cuticular duct wall. A single muscle (Plate 12, fig. 4, A) inserts on this infolding, and extends to its origin on the body wall. It obviously serves to open the valve, clearing the duct lumen for the discharge.

In the absence of compressor muscles around the reservoir, there remains the question of how secretion is expelled. Perhaps compression is effected indirectly by the contraction of some of the somatic muscles that tightly surround the reservoirs. But it is also conceivable that the discharge is triggered by a rise in fluid pressure within the hemocoel, caused perhaps by a local telescoping of segments. These two possibilities need not be mutually exclusive.

II. Identification of the Phenolic Constituents of the Secretions.

a. *m*-Cresol (*m*-methylphenol) in *Chlaenius*.

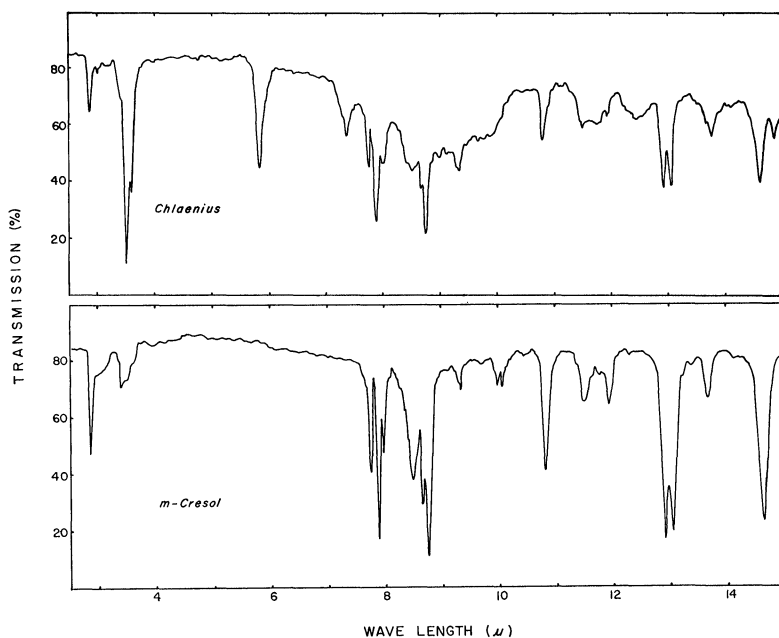
A total of ten glands were excised intact from beetles that had been freshly killed by freezing, and the secretion (a milky white emulsion) was aspirated into fine glass capillaries as it emerged from the ejaculatory ducts, following compression of the reservoirs with forceps. The entire glandular apparatus was previously blotted dry with filter paper, thus minimizing the amount of extraneous fluid taken with this secretion. The capillaries were powdered in a small mortar and extracted with carbon disulfide. The solution was then dried over anhydrous magnesium sulfate, and concentrated by evaporation of the solvent in a stream of nitrogen. The infrared spectrum of the residual solution (Model 137 Perkin Elmer Infracord Spectrophotometer; 0.5 mm. liquid cells with KBr windows) was similar in all major respects to that of an authentic sample of *m*-cresol (Text fig. 2). The discrepancy in the region of C-H stretching (*ca.* 3.5 μ) and in the carbonyl region (*ca.* 5.8 μ) suggests that *m*-cresol is not the only component of the natural product.

The presence of *m*-cresol was confirmed by vapor phase chromatography (Aerograph Model 600 "Hy-Fi", using 3% neopentyl glycol

EXPLANATION OF PLATE 10

Figs. 1-4. Four consecutive discharges of *Chlaenius*, elicited by pinching with forceps individual pro- and metathoracic legs as shown. The spray pattern is registered on filter paper impregnated with a chemical indicator (see text, part I).

Fig. 5. An individual *Chlaenius*, after having been caused to discharge, was transferred from place to place on a sheet of indicator paper. As long as residual secretion remained on its body and feet, a conspicuous discolored zone developed around it at each locus (the dark spots within each zone are footprints). Transfer was at two-minute intervals; the times given are from the moment of discharge.



Text fig. 2. Infrared spectra (in carbon disulfide) of the secretion of *Chlaenius* and of authentic *m*-cresol.

sebacate on Chromosorb W, 80/100 mesh, at 166°C, with nitrogen as carrier gas). A dried carbon disulfide solution of the secretion yielded a major peak with retention time of 6.75 min., corresponding precisely to the single peak produced by authentic *m*-cresol. A complex second peak of short retention time in the chromatograph of the natural product indicated the presence of possibly several unknown minor constituents.

It might be added that the odor of *m*-cresol is indistinguishable from that of the *Chlaenius* secretion.

b. *p*-Cresol (*p*-methylphenol) in *Abacion*

Secretion was obtained from two live millipedes by subjecting them to traumatic stimuli and taking up into capillary tubes the small droplets of secretion discharged at the gland openings. The analytical techniques employed were essentially those described above for *Chlaenius*. The infrared spectrum was found to be similar to that of an authentic sample of *p*-cresol (Text fig. 3), with the exception that the natural sample showed conspicuous bands at *ca.* 3.5 μ (C-H

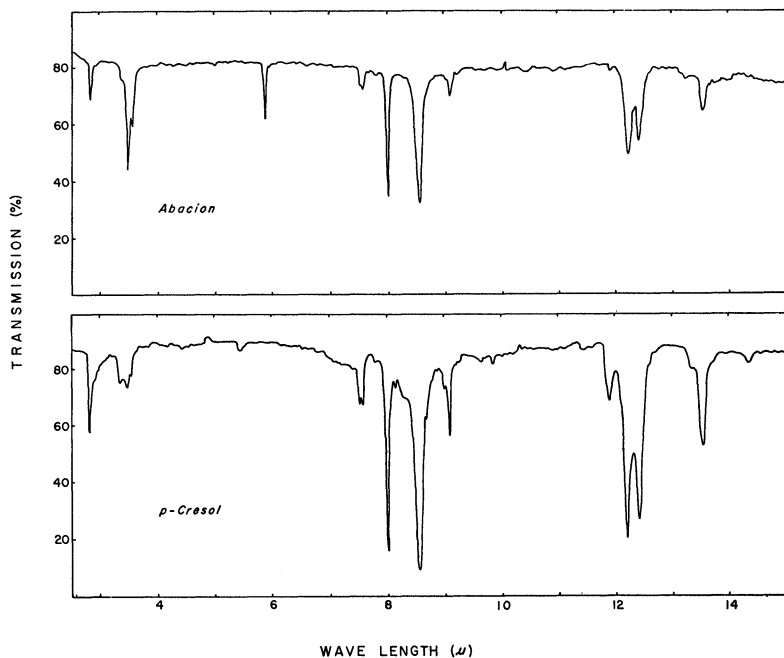
stretching) and at *ca.* 5.8 μ (carbonyl stretching), suggesting the presence of one or more minor components in the secretion.

The vapor phase chromatograph (same column as with *Chlaenius*, but run at 120°C) showed a main peak with retention time of 2.51 min., corresponding to the single peak obtained with authentic *p*-cresol. The secretion showed an additional complex peak of short retention time corresponding undoubtedly to the lesser constituents already suggested by the infrared spectrum.

The odor of *p*-cresol, which differs slightly but unmistakably from that of *m*-cresol, is identical to that of the *Abacion* secretion.

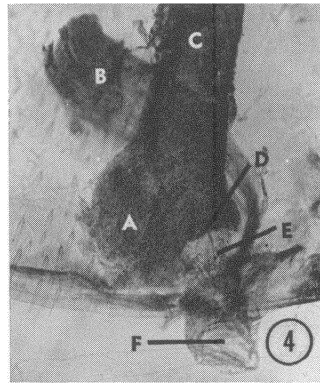
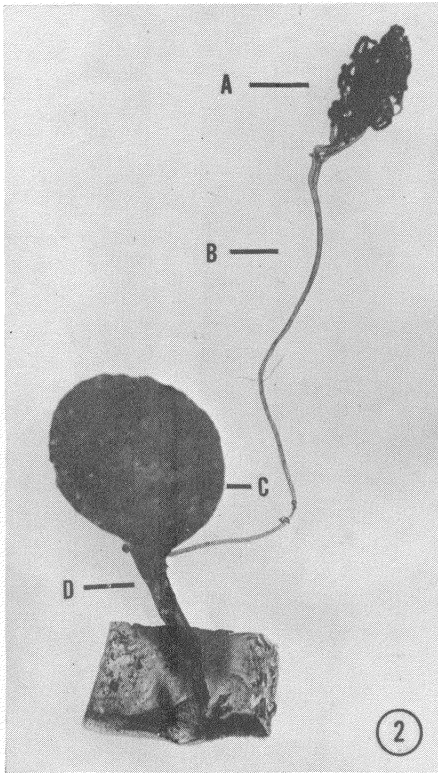
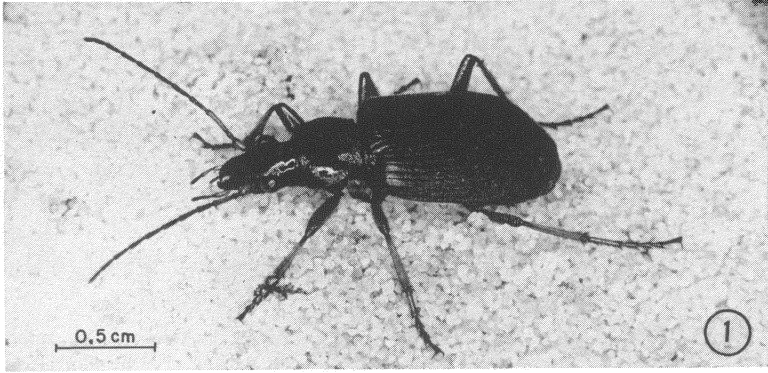
III. Repellent Effectiveness of the Secretions.

What follows are descriptions of laboratory encounters between individual *Chlaenius* or *Abacion* and a selected array of predators:



Text fig. 3. Infrared spectra (in carbon disulfide) of the secretion of *Abacion* and of authentic *p*-cresol.

ants [*Pogonomyrmex badius* (Latreille)], a collared lizard [*Crotaphytus collaris* (Say)], a blue jay [*Cyanocitta cristata* (Linnaeus)], and a grasshopper mouse [*Onychomys torridus* (Coues)]. The ants



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were from Ocean Beach Drive, S. C.; the blue jay was a laboratory-reared individual from Ithaca, N. Y., and the lizard and mouse stemmed from Arizona. Tape recordings were made of running commentaries delivered while witnessing the encounters, and these recordings provided the basis for the measurements of time intervals and other quantitative data given below. The scarcity of *Chlaenius* and *Abacion* limited the number of tests that were possible.

a. *Pogonomyrmex badius* (Latreille)

1. Versus *Chlaenius*.

The experimental conditions were similar to those that prevailed in tests with this same ant and certain other beetles, cockroaches and earwigs that also spray (Eisner 1958a, 1958b, 1960a). Individual beetles, affixed to rods, were placed one at a time just outside the nest entrance of a laboratory colony of *Pogonomyrmex*. The results were essentially the same with each beetle. The ants attacked immediately, converging upon the beetle in groups, grasping it with the mandibles while pointing their gasters forward in stinging position. Suddenly, within no more than a few seconds after initiation of the attack, the entire swarm dispersed. The ants fled aimlessly and quickly, pausing frequently for brief spells of intense cleansing activity. Their escape and cleansing behavior was identical in all major respects to that shown by this and other ants in response to arthropod secretions containing acids and quinones (Eisner, 1958a, 1958b, 1960a; Eisner et. al., 1961). Within one to several minutes after discharge, the ants seemed to have recovered completely, and had resumed their normal ambulatory pace. There were, however, no immediate new attacks. For 8 to 13 minutes after a discharge, the beetle remained invulnerable. Ants coming to within its immediate vicinity turned about abruptly and walked away, apparently repelled by residual secretion and its vapors. Some of this residual secretion must have

EXPLANATION OF PLATE 11

Fig. 1. *Chlaenius cordicollis* Kirby.

Fig. 2. Excised gland of *Chlaenius*. *A*, racemose cluster of gland cells; *B*, efferent duct; *C*, reservoir; *D*, ejaculatory duct.

Fig. 3. Grasshopper mouse eating *Chlaenius* (for details, see text, part III).

Fig. 4. Terminal portion of ejaculatory duct and associated musculature of a *Chlaenius* gland. *A*, the short flat muscle that effects nozzle extrusion; *B*, the muscle that forces apart the terminal cuticular valve of the duct, thus freeing the lumen for nozzle extrusion; *C*, ejaculatory duct, invested by circular muscles; *D*, level at which circular muscles come to an abrupt halt; *E*, naked portion of ejaculatory duct; *F*, partly extruded spray nozzle (extrusion was apparently caused by shrinkage of muscles resulting from histological fixation).

been on the substrate where the beetle sprayed, but since shifting the beetle to a new position seemed in no way to increase its vulnerability, the repellent effect must have been due, in part at least, to secretion remaining on the beetle itself. One can demonstrate visually that this is the case, simply by causing a beetle to discharge, and then transferring him onto indicator paper, moving him from place to place as the minutes go by (Plate 10, fig. 5). At each locus the paper is seen to discolor, and even as late as fifteen minutes after discharge a positive test is still obtainable. *m*-Cresol evidently dissipates rather slowly, which is to be expected in view of its low vapor pressure: extrapolation from values given in the International Critical Tables (1928) yields *ca.* 0.1 mm at 25°C.

It follows from the preceding that a given *Chlaenius*, when under attack by ants (and ants are probably important natural enemies of many carabids), is not likely to be subjected to continuous assail and forced to deplete its secretion in a rapid sequence of discharges. *Chlaenius*, like so many other carabids, walks rapidly. Actual measurements made with two individuals released on a smooth horizontal surface, showed the rate of locomotion to range from 15 to 19 cm./s. (time was measured with a stopwatch; distance was determined by chalking a line behind the beetle as it scurried along, and then laying a string along the trail and measuring its length). In the *ca.* 10 min. of invulnerability that follows a discharge, a beetle is therefore free to walk about 100 m. before it is again subject to assail. Surely, this must suffice to outdistance many an arthropod predator, and in the case of ants, even a dense swarm of them. Of course, it remains to be seen whether the repellent effectiveness of the secretion against *Pogonomyrmex* is a true indication of its defensive potential against other ants, and against arthropods in general.

There is one other observation worth mentioning, concerning the defensive use of the beetle's mandibles. It was repeatedly noticed during the early stages of an attack, before *Chlaenius* had been induced to spray, that an ant venturing to within range of the beetle's mandibles was bitten. Although such ants did not seem to receive

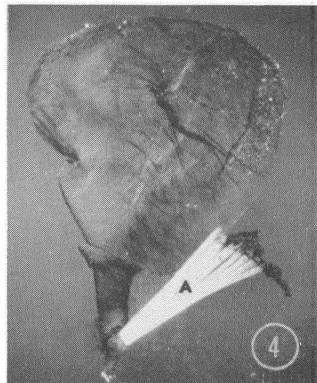
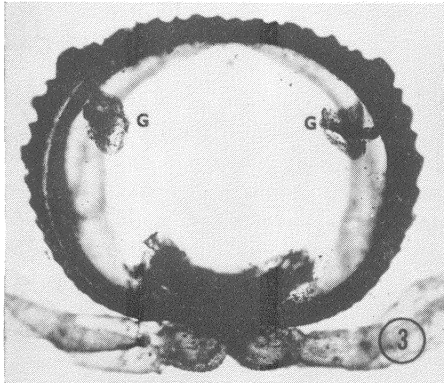
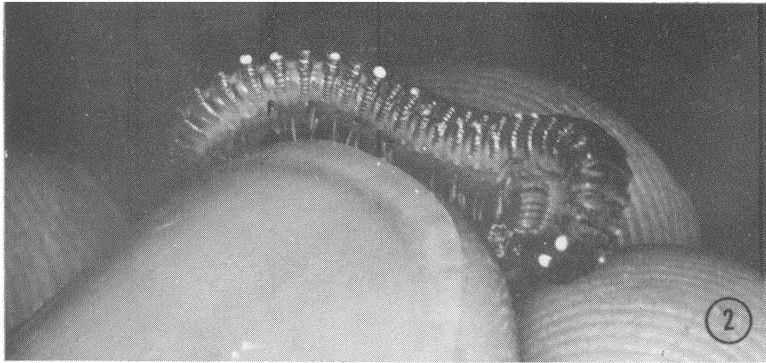
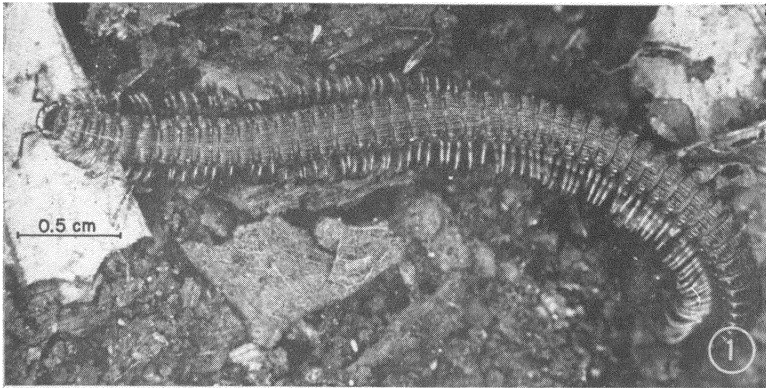
EXPLANATION OF PLATE 12

Fig. 1. *Abacion magnum* (Loomis).

Fig. 2. *Abacion* discharging white droplets of secretion in response to handling.

Fig. 3. Isolated segment of *Abacion*, treated with KOH and consisting of cuticle alone, showing the two glands (*G*).

Fig. 4. Excised gland of *Abacion*, seen in partly polarized light. Notice the strongly birefringent muscle (*A*) that controls the terminal cuticular valve of the efferent duct.



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noticeable injury, they nevertheless desisted instantly from further assault and fled. *Chlaenius* is evidently endowed with a mechanical as well as a chemical weapon, and the former might be of particular importance at times when the beetle's secretion is temporarily exhausted.

2. Versus *Abacion*.

Three millipedes (3-5 cm. in length) were released individually near the entrance of a *Pogonomyrmex* nest, at a time when the ants were highly active and aggressive, as evidenced by the readiness with which they attacked and overcame mealworms (larvae of *Tenebrio molitor*) introduced as occasional controls. The millipedes walked amidst the swarming ants, and dozens of casual encounters between ants and millipedes were seen to take place, but in not a single instance did an ant attempt to bite or sting a millipede, nor did a millipede ever discharge. The reason for this was the remarkable fact that *Abacion* responded instantly to contact with an ant by coming to an abrupt halt, and remaining motionless thereafter until the ant departed or, more usually, until seconds after the ant's departure. While "death-feigning" in this fashion, the millipede evidently fails to evoke a full-fledged aggressive response from the ants. Time and again a millipede was released near the nest entrance, but it eventually always made its way to the safe outskirts of the nest, pausing intermittently during its escape whenever single ants or groups of ants scurried over its body, but never once being induced to discharge. When an ant contacted the millipede's head, and also apparently when the glandless postcephalic segments were touched, the millipede came to a halt with its front end coiled beneath the body, a response already noticed when the animals were stimulated by hand (see Ib above). Otherwise, when contact was with the body itself, the millipede simply halted without altering its stance.

In order to test for the actual repellency of the secretion, millipedes were first subjected to artificial traumatic stimuli locally applied, and then released with the ants. Under these circumstances, the ants never even contacted the millipede, but turned around and retreated as soon as they came to within close range. As was the case with *Chlaenius*, the millipedes remained repellent for considerable periods after a discharge. Invulnerability of several minutes was the rule, even when no more than a few adjacent glands had been activated [the vapor pressure of *p*-cresol, as estimated by extrapolation from values given in the International Critical Tables (1928), is as low as that of *m*-cresol, i.e. ca. 0.1 mm at 25°C].

Abacion is slower in its locomotion than *Chlaenius*. Six specimens

(3-5 cm. in length) whose ambulatory pace was measured (same technique as with *Chlaenius*) yielded values ranging from 2.6 to 6.0 cm./s. The distance that a given *Abacion* may be expected to cover unmolested after discharging is therefore considerably less than the 100 m. one can expect from *Chlaenius*. But *Abacion*, with its two glands per most body segments, has a rather formidable secretory supply not easily depleted, particularly since its glands are likely to be called into action only a few at a time rather than all at once (at least so long as small predators are involved that inflict localized rather than generalized injury). Small arthropod predators, and ants in particular, are probably among the chief natural enemies of *Abacion*. Considering its rich glandular endowment, and the proven effectiveness of "death-feigning", *Abacion* appears indeed to be a most admirably protected animal.

b. *Grotaphytus collaris* (Say)

Two tests were made, one with *Chlaenius* and one with *Abacion*. In the case of the millipede, the lizard eyed it almost continuously as it walked about the cage, but made no effort to seize it or even to approach it. One hesitates to attribute special significance to an isolated finding of this sort, but it should be mentioned that this particular lizard was confronted with no millipedes during its one year in captivity, and that its voraciousness at the time of the experiment was demonstrated by the readiness with which it caught and ate mealworms (larval *Tenebrio molitor*).

With the single *Chlaenius* the results were dramatically different. The lizard chased the beetle and caught it in its jaws, but instantly released it again, thereafter wiping its snout for several seconds in the substrate. Some secretion had evidently hit one eye, which remained closed for more than a minute. The beetle was uninjured (it smelled conspicuously of *m*-cresol when retrieved). The lizard showed no noticeable long range ill-effects.

c. *Cyanocitta cristata* (Linnaeus)

1. Versus *Chlaenius*.

Three beetles were offered, one after the other, within a period of 30 minutes. The results were the same in each case. The bird descended from its perch, and quickly pecked the beetle to death. Except for an occasional brief but violent shaking of the head, the bird betrayed no signs of discomfort. There was no question that the beetle had sprayed: the phenolic odor was clearly apparent, even from outside the cage.

With the dead beetle in its bill, the bird returned to its perch, and while holding the beetle against the perch with a foot, proceeded to dismember it with the bill. Except for the beetle's head and perhaps a portion of the thorax, no parts were swallowed. The discarded pieces were later pecked into various crevices and other hiding places in the cage [this behavior has been noted often when captive jays are offered noxious spray, e.g. meloid, coccinellid, and lycid beetles (Linsley et al., 1961; Eisner, unpublished)].

One wonders what would have happened had the secretion hit the bird's eyes. With other arthropods that spray, this has frequently been observed, and it invariably has an instantaneous repellent effect, leaving the bird partially incapacitated for a time with one or both eyes closed, or with the nictitating membranes being drawn back and forth over the eyeballs in a quick wiping action (Eisner et al., 1961, 1962, and Eisner, unpublished). Such birds usually avoid similar prey for at least several hours after an encounter. One might argue that the jay's eyes had really been hit by the *Chlaenius* spray, but that they were unaffected by it. It is doubtful, however, that *m-cresol* is harmless. What it does to a lizard's eye has already been described. To our own eyes, even the concentrated vapors are quickly irritating. Assuming, then, that the jay's eyes had indeed been missed, this brings to light what may well be a major shortcoming of the beetle's weapon: by aiming the spray against that portion of its body directly subjected to trauma, the beetle is likely to miss the eyes of a long-billed bird such as a jay, spraying instead the relatively insensitive bill. Other arthropods that have been tested (e.g. whipscorpions, *Calosoma*) also aim toward the body regions traumatized, but since their spray is of greater range and more broadly dispersed than that of *Chlaenius*, the probability is greater that the eyes of the bird receive at least a partial dousing (Eisner et al. 1961, 1962).

2. Versus *Abacion*.

The jay promptly pecked and killed the single millipede offered, carried the corpse to its perch, and then proceeded to treat it in the same way as *Chlaenius*. No parts were eaten, although a few pieces were malaxated in the bill before being dropped. Again as with *Chlaenius*, the discarded pieces were pecked into various hiding places.

d. *Onychomys torridus* (Coues)

1. Versus *Chlaenius*.

Two beetles were offered, with identical results in each case. The mouse pursued the beetle the moment it was introduced, quickly grasped it in its front paws, and with the beetle oriented in such a way

that its rear was pushed into the sand, proceeded to eat it head first (Plate 11, fig. 3). Under these conditions most, if not all, of the secretion must have been ejected into the sand, sparing the mouse itself. The mouse betrayed no signs of discomfort and both beetles offered were devoured almost entirely. Only the legs and wings were discarded, as well as the tips of the abdomens, which presumably still contained the gland reservoirs. One might add that this same mouse, and others of the same species, have been seen to overcome and eat tenebrionid beetles of the genus *Eleodes* in exactly the same fashion (Eisner, 1960b, and unpublished). *Eleodes*, like *Chlaenius*, also sprays from the tip of its abdomen, although its secretion contains quinones rather than a phenol (Blum and Crain, 1961; Chadha et al., 1961b).

2. Versus *Abacion*.

The single millipede offered was promptly severed midway by the mouse's first bite. The mouse retreated instantly, scurrying about the cage with its muzzle intermittently pushed in the sand. Within seconds it returned to bite the millipede again, only to be repelled temporarily as before. This sequence of attack and retreat was repeated several times, until the mouse finally grasped several multisegmented portions of the millipede and ate them one by one. But most of the millipede was left uneaten. A strong odor of *p*-cresol permeated the cage. Grasshopper mice are known to pursue their prey relentlessly even in the face of repellent secretions (Eisner et al., 1961; Egoscue, 1960), and the persistent aggressiveness evidenced in this encounter was in no way unexpected.

IV. Irritant Effectiveness of the Secretions and Their Phenols.

The chemical evidence has shown (part II, above) that *m*-cresol and *p*-cresol are not the only components of the secretions. The possibility therefore exists that the unidentified lesser components contribute in an important way to the overall effectiveness of the secretion. Such possibility would not be without real precedent. For instance, the spray of the whipscorpion, *Mastigoproctus giganteus* (Lucas), contains a minor component that promotes the spread of the secretion over the cuticle of arthropod enemies, while at the same time accelerating the penetration of the poison (Eisner et al., 1961). The following experiments were aimed at determining whether the secretions of *Chlaenius* and *Abacion* are more effective as topical irritants to arthropods than pure samples of the phenols they contain. The arthropod used for the test was the cockroach *Periplaneta americana* (Linnaeus), and the technique was a slightly improved version of that employed previously for comparable purposes (Eisner et al., 1961).

In essence, the technique consists of applying the test substances topically to decapitated cockroaches, and timing the onset of the grooming reflexes that follow. The delay to onset of grooming is a criterion of the irritant effectiveness of the sample: the quicker the onset, the greater the irritability.

The droplets of sample were applied with a constant-volume micropipette (0.4 μ l) to any one of three loci: the fifth abdominal sternite (on one side of the midline), a tarsus of a metathoracic leg, and a cercus. For each locus the grooming reflex induced is unmistakably distinct (Eisner, 1961). Each roach was tested three times, once at each locus, but the sequence of loci tested was not fixed, nor was the same sample necessarily used at each locus. Recovery of 15 to 20

TABLE I

○ *m*-cresol ▽ *p*-cresol
 ● *Chlaenius* secretion ▼ *Abacion* secretion
 (n. r. = no response)

sternal reflex				tarsal reflex				cercal reflex			
○	●	▽	▼	○	●	▽	▼	○	●	▽	▼
4'22"	2'43"	2'17"	3'18"	1"	1"	1"	1"	1"	1"	1"	2"
n. r.	n. r.	n. r.	3'40"	1"		2"	2"	1"		1"	
n. r.	n. r.	n. r.	n. r.	1"		2"		2"		3"	
n. r.	n. r.	n. r.	n. r.	2"		4"		2"		4"	
n. r.	n. r.	n. r.	n. r.	6"		5"		4"		4"	

Table I. Sensitivity of *Periplaneta americana* to the secretions of *Chlaenius* and *Abacion*, and to the corresponding phenols. Sensitivity is expressed as the delay to onset of a grooming reflex (sternal, tarsal, and cercal) following topical application of a given sample. The times of delay for the three reflexes and four samples tested are given in the vertical columns. For full explanation see text, part IV.

minutes was allowed between consecutive tests on the same roach. Failure to induce a reflex within five minutes of application was considered a negative response (n. r.). Adult female roaches were used exclusively. The secretions were obtained by the same techniques as used for the chemical analyses (see part II, above).

The results are plotted in Table I. Unfortunately, with the actual secretions, the amount of material available sufficed for only relatively few tests. Still, some safe inferences can be made. First of all, as expected, the tarsi and cerci, with their relatively thin cuticle, are much more sensitive than the abdominal sternite: the reaction was always within seconds of application. By contrast, when application

was to the sternite, there was in most cases no response at all, and in the few exceptions the reflex was delayed for at least two minutes. Evidently, when the cuticular barrier is a thick one, neither the secretions nor the pure phenols are very effective irritants. Moreover, none of the samples differed detectably in their activity.

The possibility still remains that the lesser components are of more than incidental significance (they might, for instance, act as emulsion stabilizers) but the present data offer no basis for further speculation.

V. Discussion

The protective function of the glands seems established, certainly as regards ants. The special adaptive refinements of the discharge mechanism, such as *Chlaenius*' ability to aim the spray, and *Abacion*'s tendency to respond locally rather than from all glands at once, are by no means unique. Other carabids studied (*Brachinus*, *Calosoma*) also aim their spray (Eisner, 1958a; Eisner et al., 1962). In fact, aiming appears to be the rule rather than the exception among arthropods that spray (Roth and Eisner, 1962). Similarly, other millipedes studied thus far, and including juoids, spiroboloids, spirostreptoids, polydesmoids, and polyzenoids (Kafatos 1961; Eisner, unpublished), all share with *Abacion* the habit of restricting their discharge to the region stimulated.

The experiments with vertebrate predators were not nearly as conclusive as those with ants. There was only one instance of clear-cut prey rejection, involving the collared lizard and *Chlaenius*, and it is significant that the beetle survived the encounter uninjured. With the jay, the evidence clearly points to the unpalatability of *Chlaenius* and *Abacion*, but the prey was always killed before being rejected. Continued daily tests would have been desirable, since on the long run the bird might well have developed discriminatory tendencies. Similar long range experiments would have been justified with the grasshopper mouse, although with *Chlaenius* there is really no reason to anticipate eventual avoidance on the part of the mouse, since the beetle is subdued by a technique that evidently prevents effective employment of its weapon.

It may seem surprising to find a beetle and a millipede—two arthropods of widely distant phyletic positions—producing nearly identical secretions, but this situation is not without precedent. *p*-Benzoquinones, for instance, are known from the secretions of certain millipedes, phalangids, and representatives of several insectan orders (for summary, see Roth and Eisner, 1962). The aldehyde 2-hexenal

is produced by some cockroaches (Chadha et al., 1961a; Roth et al., 1956) and certain Hemiptera (Blum, 1961; Waterhouse et al., 1961).

It is also not uncommon for relatively closely related forms to produce very different secretions. Millipedes and carabid beetles do in themselves illustrate this point. Among the millipedes, non-phenolic compounds that have been identified include hydrogen cyanide, benzaldehyde (Eisner, H. E., et al., 1962), and *p*-benzoquinones (reviewed in Roth and Eisner, 1962). Of the three carabid genera other than *Chaenius* that have been studied, one (*Calosoma*) produces a phenolic compound (salicylaldehyde; Eisner et al., 1962), but the other two do not: *Pseudophonus* secretes formic acid (Schildknecht and Weis, 1961); *Brachinus* sprays *p*-benzoquinones (Schildknecht, 1957).

These chemical similarities on the one hand, and dissimilarities on the other, illustrate some striking convergent and divergent trends in the evolution of arthropod secretions — trends that will some day have to be given the proper explanation they deserve. But this will have to await an understanding of the particular biochemical pathways by which the secretions are synthesized, and of the special relationships of these pathways to the fundamental biosynthetic machinery of the cell.

Summary

1. The carabid beetle *Chaenius cordicollis* Kirby, and the chordeumoid millipede *Abacion magnum* (Loomis), produce defensive secretions which have been shown by infrared spectrophotometry and vapor phase chromatography to contain *m*-cresol (*Chaenius*) and *p*-cresol (*Abacion*)

2. *Chaenius* has two glands that open near the tip of the abdomen. It discharges its secretion as a spray, ejected from either one gland or from both, depending on whether the causative traumatic stimulus is unilaterally or bilaterally applied. The spray is not directed at random, but through bending of the abdominal tip is aimed with some accuracy toward the body region traumatized.

Abacion has a pair of glands per each of most diplosegments. The discharge is a liquid ooze, proceeding normally from only those glands close to the stimulus.

3. A brief account is given of the structure of the glands, as well as of their inferred mode of operation.

4. Experiments are described in which individual *Chaenius* and *Abacion* were exposed to attacks by ants and several vertebrates.

With the vertebrates the results were variable, but with the ants the repellent effectiveness of the secretions was clearly established.

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