

## Mammalian breath: trigger of defensive chemical response in a tenebrionid beetle (*Bolitotherus cornutus*)\*

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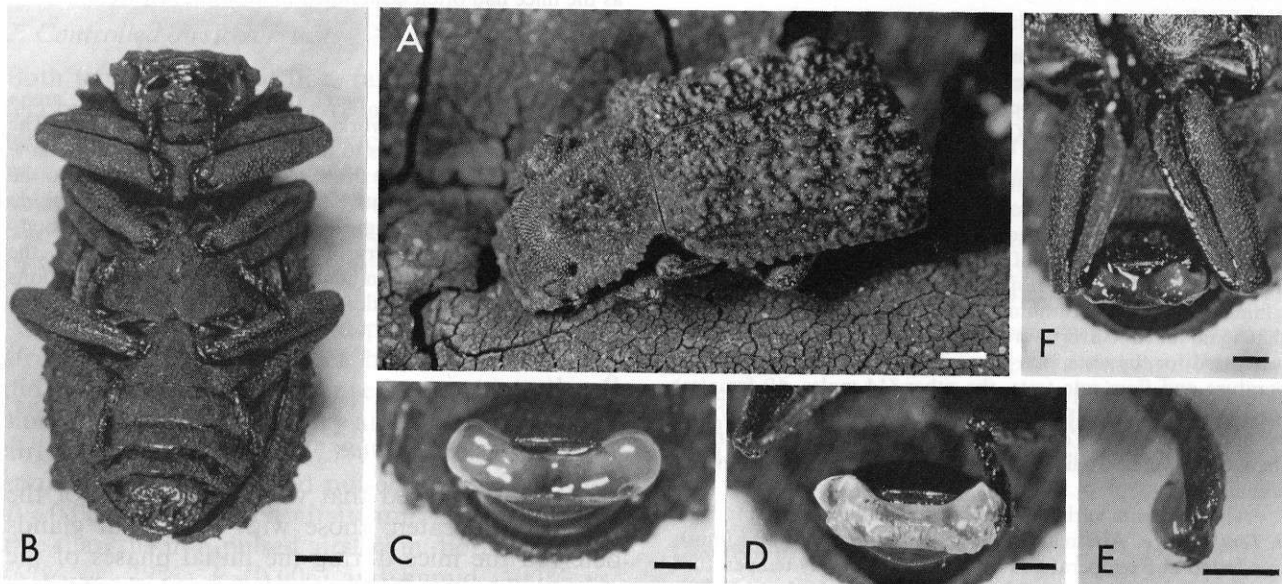
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**Summary.** The tenebrionid beetle *Bolitotherus cornutus* everts a pair of quinone-producing defensive glands in response to mammalian breath. Experiments with a controlled airstream indicate that the beetle “recognizes” breath on the basis of temperature, humidity, and airflow dynamics. Under attack by mice the beetle everts the glands immediately upon being mouthed and may secure its release as a result. Against ants the beetle is protected by its tough exoskeleton and usually refrains from everting the glands. Other arthropods also show defensive responses when breathed upon.

### Introduction

*Bolitotherus cornutus* adults have three types of anti-predator defense: (a) camouflage – a cryptic shape and color combined with a tendency to feign death when disturbed (Fig. 1 A, B); (b) physical defense – a thick and hard exoskeleton with special grooves into which legs and antennae are protectively retracted during the death feint (Fig. 1 B); and (c) chemical defense – eversible glands at the tip of the abdomen which secrete irritating benzoquinones (Tschinkel 1975a). When the beetle is disturbed, the glands are everted and stroked with a rapid movement of the hindlegs, thus spreading the secretion (Fig. 1 C–F). Earlier studies of *B. cor-*

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**Fig. 1.** **A** *Bolitotherus cornutus* (female, 10 mm body length) feigning death on its usual host fungus (*Ganoderma applanatum*); brown beetle color closely matches fungus. **B** Same, ventral view; note that the appendages are protectively retracted. **C–F** Chemical defense of *B. cornutus* in action: in response to mammalian breath, the secretion-laden glands are everted from the abdominal tip (**C**); the tarsi of the hindlegs are brushed against the glands (**D**), picking up globules of secretion (**E**); the tibiae of the hindlegs are also wetted by contact with the glands (**F**)

*nutus* suggested that human breath triggers the eversion response (Pace 1967), while mechanical stimuli (which consistently induce the death feint) elicit partial eversion at most (Weiss 1947; Tschinkel 1975b). We here report that eversion is indeed triggered by certain parameters of mammalian breath and that it contributes to the beetle's defense.

## Materials and methods

### 1. Predation tests: mice

To test for the effectiveness of the glandular defense of *Bolitotherus* vis a vis a mammalian predator, laboratory encounters were staged between individual beetles and deer mice (*Peromyscus maniculatus*). Deer mice are likely natural enemies of *Bolitotherus*: stomachs of *Peromyscus* species trapped in the Northeastern U.S. commonly contain beetle remains (Whittaker 1963), and both *Peromyscus* and *Bolitotherus* are active at night around stumps and fallen logs in eastern deciduous forests (Baker 1968; Stickel 1968).

Sixty *Bolitotherus* of both sexes were offered to 6 individually caged *P. maniculatus*. Each mouse was starved for 24 h and then presented with 5 beetles on each of 2 consecutive days; half the beetles had their gland openings blocked with Duco® cement. The mice were pretrained to feed on insects (larvae of *Tenebrio molitor*) from petri dishes; the 5 *Bolitotherus* presented in a given feeding session were offered singly in petri dishes in the following order: first and last, blocked glands; middle three, either all with unblocked glands, or including one randomly placed individual with blocked glands. The data were analyzed with  $\chi^2$  2 × 2 contingency tables, one degree of freedom, with a continuity correction.

### 2. Controlled airstream tests

To determine which parameters of mammalian breath elicit the eversion response, beetles were individually stimulated with an airstream in which temperature, flow rate, humidity, pulsation, and carbon dioxide (CO<sub>2</sub>) content were separately controlled.

Beetles were placed ventral side up on glass in death-feint posture. The airstream, from a compressed air source, was aimed at near contact range (nozzle inner diameter = 4.0 mm) onto the beetles' front half until eversion occurred or for a maximum of 10 s. Non-responding beetles were immediately stimulated with puffs of human breath; data from those beetles that again failed to evert were not tallied. Two groups of beetles were used for the tests, one (14 males, 13 females) for the temperature and flow rate tests, the other (14 males, 19 females) for the humidity, pulsation, and CO<sub>2</sub> tests. Tests at any one set of conditions were done on a single day. Beetles tested more than once were left undisturbed for at least 2 days between stimulations.

The airstream variables were controlled as follows:

*a. Temperature.* Airstream, at constant flow rate (4.0 L/min), was tested at 5 temperatures from 30–50° C [body temperature of eutherian mammals = 38 ± 2° C (Schmidt-Nielsen 1979)]. Airstream temperature was controlled (to ±1° C) by thermal jacketing of the delivery tube and measured by a thermocouple placed in the airstream outlet. Ambient temperature, measured at site of placement of beetle, was 28.3 ± 0.22° C (mean ± S.E.M., *N* = 12).

*b. Flow rate.* Airstream, at constant temperature (45° C), was tested at 5 flow rates from 1.5–5.0 L/min. Flow rate was monitored with a float flowmeter and adjusted to ±0.25 L/min. Peak expiratory flow rate of a mouse (*Mus musculus*) is approximately 0.9 L/min (Leith 1976). Since the cross-sectional area of a mouse pharynx (estimated from anatomical drawing of Cook 1965) is about 1/4 that of our nozzle opening, the latter could be expected to be delivering air at peak mouse expiration velocities when set at flow rates of 4–5 L/min.

*c. Humidity and pulsation.* The humid and dry airstreams (both at 4.0 L/min and 40° C) were generated respectively by passing air through calcium chloride crystals and through a flask containing heated distilled water (water saturation apparent from condensation on glass placed in emergent airstream). Pulsation (tested with both airstreams) was produced by rapid oscillation of the air nozzle, such that the nozzle pointed intermittently at the beetle with a frequency of 5.3 ± 0.22/s (mean ± S.E.M., *N* = 35).

*d. Carbon dioxide.* A single non-pulsed airstream was tested (4.0 L/min, 40° C) with a CO<sub>2</sub> content (5%) roughly matching that in mammalian breath; the stream was generated by controlled mixing of confluent air and CO<sub>2</sub>.

*e. Statistics.* All comparisons involving humidity, pulsation, and CO<sub>2</sub> data were self-paired; significance levels are therefore from the sign test.

### 3. Artificial jaw tests

To determine if the mechanical stimulus of a mouse bite can itself elicit glandular eversion, 33 beetles were individually "bitten" with a set of artificial jaws. The "jaws" consisted of an alligator clip (80 series, Mueller Electric Co.) modified by replacing the spring with a helical coil spring placed in the handle of the clip. With the clip opened 5.1 mm (the approximate mean distance across the folded elytra of *Bolitotherus*) the clamping force was 732 ± 10.3 grams-force (mean ± S.E.M.; *N* = 10; measurements taken with an Ohaus 8004-MO spring scale). The beetles were grasped transversely across the elytral margins, as the mice had often been noted to do with their teeth.

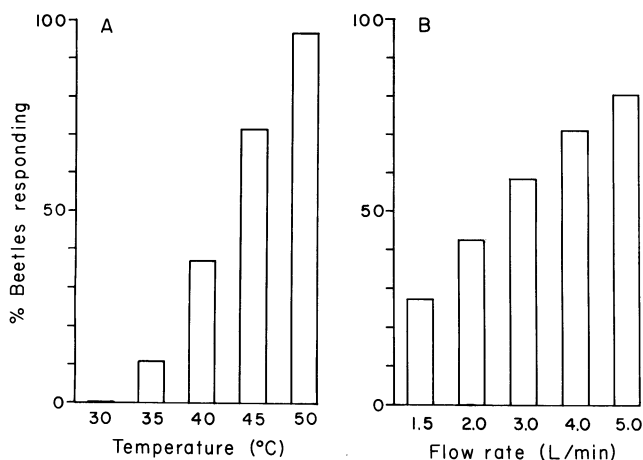
### 4. Predation tests: ants

Individual *Bolitotherus* were released into a test arena (10 × 10 cm) with 25–40 worker ants (*Formica exsectoides*). The ants were not given access to the beetles until the latter became ambulatory following the death-feint elicited by transfer to the arena. The number of ants biting the beetles and the position of the beetles' appendages (exposed, or retracted in body grooves) were noted every 30 s for 5 min, during which the beetle was subjected to uninterrupted attack; 340 observations were recorded, on 34 beetles (both sexes).

## Results

### 1. Predation tests: mice

The results showed that while all but one of the beetles were eaten, those with functional glands deterred the mice during the initial phases of the attack. Thus, while most beetles with blocked glands (97%) were killed within the first minute of the attack, only 63% of those with unblocked glands succumbed during that period ( $\chi^2 = 8.44$ , *P* < 0.005). Moreover, most (87%) beetles with un-



**Fig. 2 A, B.** Percentage of beetles (*Bolitotherus cornutus*) everting defensive glands in response to controlled airstreams [ $N=24$  to 27 (see text)]. **A** Temperature varied at constant flow rate (4.0 L/min). **B** Flow rate varied at constant temperature (45° C)

blocked glands were dropped uninjured at least once by the mice after having been taken in the mouth, while only 33% of those with blocked glands were similarly dropped ( $\chi^2=17.85$ ,  $P<0.001$ ). Gland eversion was often noted when beetles with unblocked glands were seized by the mice, and following release of such beetles the mice frequently rubbed their muzzles with the paws or in the substrate. Such cleansing behavior occurred only rarely during attacks upon beetles with blocked glands. The tip of the abdomen (where the glands are located) was often left uneaten.

## 2. Controlled airstream tests

Both temperature and flow rate had a distinct effect on eversion frequency (Fig. 2). Over the tested temperature range, which extended beyond mammalian body temperatures to a maximum of 22° above ambient level, responsiveness increased from 0 to nearly 100%. Flow rates were highly effective in the range of 4–5 L/min, the estimated expired air velocity in mouse breath.

Both humidification and pulsation of the airstream had a positive effect, but addition of CO<sub>2</sub> did not. The humid airstream elicited a higher eversion frequency than the dry airstream, both when airflow was non-pulsed (73% vs. 27% beetles responding;  $P=0.003$ ) and pulsed (100% vs. 67%;  $P=0.008$ ). The pulsed airstream was more effective than the non-pulsed airstream, both when dry (67% vs. 29%;  $P=0.006$ ) and humid (100% vs. 74%;  $P=0.016$ ). Carbon dioxide, at the concentration tested, did not significantly increase eversion frequency (42% vs. 23%;  $P>0.1$ ). The one stimulus that was 100% effective in these tests was the

humid, pulsed, 4.0 L/min airstream at 40° C. *Bolitotherus*, it seems, “recognizes” mammalian breath on the basis of its temperature, moisture, and flow dynamics.

## 3. Artificial jaw tests

Since in the tests with *Peromyscus* the beetles had not been noted to evert their glands until they were orally grasped by the mice, the eversions could have been elicited by the dental grip of the predator rather than by the breath itself. Only one beetle, however, everted the glands when “bitten” by the artificial jaws. Others withheld the glands throughout the 10 s stimulation period, even when a leg or elytron was injured by the “bite.” The stimulus was also applied (same 33 beetles) simultaneously with an airstream (4.0 L/min, 40° C, dry, steady); the response frequency was not significantly different from that to the airstream alone (23% vs. 31%;  $P>0.1$ ).

## 4. Predation tests: ants

The tests with the “jaws” led to the prediction that against non-mammalian predators *Bolitotherus* would refrain from using its glands and rely on the exoskeleton alone for protection. This was confirmed in the ant tests. The immediate response of *Bolitotherus* to ant attack was retraction of the antennae, which were kept withdrawn throughout much of the duration of the assault (70% of observations). Legs, however, were frequently exposed (96% of observations), since the beetles often attempted to escape by walking; exposed legs were often bitten by the ants. Even so, only 5 of the 34 beetles tested everted their glands, and then only after at least 2 min of persistent attack. The exoskeleton proved effectively protective: none of the beetles showed signs of injury when retrieved after the attack. The glandular secretion from the 5 beetles that did evert proved repellent to the ants: after the beetles everted their glands, the average number of ants biting the beetles was significantly lower [4.5 vs. 2.9 ants, average of all observations before and after eversion respectively; paired  $t$ -test,  $t=2.56$ , 4 d.f.,  $P<0.05$  (one-tailed)].

## Discussion

*Bolitotherus* evidently everts its glands selectively, depending on the type of predator and the severity of the attack. Its ability to cue in on mammalian breath enables it to respond preemptively to a po-

tentially lethal attack. Gland eversion can save the beetle by making it distasteful at the very moment that it is taken into the predator's mouth, before a bite is inflicted. Although in our tests even beetles with unblocked glands were almost all eventually eaten, we attribute this to their inability to drop out of sight when released. Under natural conditions, where they would fall into leaf litter, *Bolitotherus* would more readily escape re-detection.

Against ants *Bolitotherus* is able to resist passively by reliance on its armor, without calling into action the potentially more "costly" chemical defense. The fact that the quinonoid secretion, once used, proved effective against ants came as no surprise. Benzoquinones are potently repellent to vertebrates and invertebrates alike, and have been shown to be used effectively against ants by some arthropods (Eisner 1958; Eisner 1960; Eisner et al. 1971).

Personal experience tells us that the elicitation of anti-predator responses by mammalian breath may be of more general occurrence. Other tenebrionid beetles (*Blaps* spp.; *Eleodes* spp.) assume warning headstands and sometimes even discharge their defensive glands when breathed upon, while certain millipedes (*Orthoporus punctilliger*, *Glomeris marginata*) react by coiling when thus stimulated, and by emitting defensive secretion if subsequently merely touched by a warm surface. Our results with *Bolitotherus* represent the first characterization of the specific parameters of mammalian breath responsible for triggering an anti-predator response.

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