

A Comparative Study of the Chemical Defensive System of Tenebrionid Beetles. Defensive Behavior and Ancillary Features^{1,2}

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ABSTRACT

The behavior accompanying the release of the defensive secretion of 64 species of tenebrionid beetles was studied. The secretion was characteristically released by eversion of the reservoir, by exuding, or by spraying, with intermediates between these occasionally represented. Everting is considered primitive and is found among several genera of Tenebrionini, Bolitophagini, Strongyliini, and Lagriini with a few occurrences in other tribes. Spraying is restricted mostly to the subgenera *Eleodes* and *Steneleodes* of *Eleodes* but scattered occurrences are found in other subgenera of *Eleodes* as well as other genera within the family. Some species of Meracanthini and Amarygmini spray by rapidly everting the glands, a method quite distinct from all other spraying species.

Species of *Eleodes* frequently exhibit headstanding behavior when disturbed and prior to giving off secretion, but headstanding has evolved in other groups as well and is frequently associated with species which spray. Certain

species which lack defensive glands also headstand, and the evolution of headstanding is discussed in this light.

Many species in all taxa have evolved the ability to wipe the secretion via the metalegs, from the abdominal tip onto the handler; species of *Argoporis* can transfer secretion between adjacent pairs of legs. Numerous species of Diapirini, Tenebrionini, Scaurini, Ulomini and Phaleriini fold the legs and antennae flat against the ventrum and remain motionless when disturbed; this habit is also represented in a few species of other groups. In some species of Diapirini and Ulomini, the epipleural margins of the elytra are formed into "gutters" which conduct secretion forward, and in some the elytra are entirely wet by secretion. In a species of *Zadenos*, elytral sulcae conduct the secretion. All species of Platynotini possess gular stridulatory apparatus, and several stridulate when releasing secretion.

Although many arthropods possessing defensive glands release the secretion without any additional special behavior, many others have evolved a variety of postures, body movements and limb movements to increase the effectiveness of the secretion. Thus, for example, roaches of the genus *Platyzysteria* (Waterhouse and Wallbank 1967), raise the abdomen in preparation for spraying the secretion, many Hemiptera raise the side of the body from which they are about to spray in response to stimuli from that side (Remold 1962, Eisner and Meinwald 1966), and a species of phalangid has been reported to spread the secretion using the legs (Eisner et al. 1971). Among the tenebrionid beetles, certainly the most conspicuous behavior pattern associated with release of defensive secretion is the "headstanding" of certain species of *Eleodes*. This behavior was first noted in the literature by Gissler (1879) and Williston (1884), but it has surely been a matter of folk knowledge since humans first saw these conspicuous western American species. Although headstanding may have several functions for *Eleodes*, one of them is almost certainly aposematic. As a result, sympatric species not possessing defensive glands have come to mimic *Eleodes* not only in appearance but in behavior as well. Thus, the asidine beetle, *Megasida obliterata* LeConte (Eisner and Meinwald 1966), and cerambycids of the genus *Monilema* (Raske 1967), both headstand upon being disturbed.

There have been a few reports on structures and phenomena ancillary to chemical defensive systems. Thus Remold (1962) reported that the cuticle of certain geocorisid bugs has a high affinity for the bug's secretion and is rapidly wetted by it. Tschinkel (1969,

1972) described defensive behavior in *Zophobas rugipes* Kirsch and documented wiping the secretion with the metalegs in *Argoporis alutacea* Casey.

To date, there has been no systematic survey of the kinds of behavior patterns associated with chemical defensive systems in any group of tenebrionids. It is not even known how widespread the headstanding behavior is among the species of *Eleodes*. I felt, therefore, that a comparative survey of the defensive behavior of tenebrionid beetles would be of interest from the point of view of gaining broader and more complete knowledge of a single, homologous defensive system, to provide information on the evolutionary diversification of this homologous system and possibly, therefore, to shed light on the phylogeny of the Tenebrionidae. This study parallels the co-published studies on the chemical composition (Tschinkel 1975a) and gland morphology (Tschinkel 1975b) of the tenebrionid defensive system.

MATERIALS AND METHODS

The collection and maintenance of the 150 or so species have been described in the 1st paper of this series (Tschinkel 1975a). All beetles which were returned live to the laboratory were observed for defensive behavior, and a number of species were also observed in the field. In the laboratory each individual beetle was subjected to a graded series of stimuli, initially without removing it from its home box: 1) the legs were lightly pinched with forceps; 2) this pinching was intensified and made more general; 3) the beetle's body, especially the elytra, were tapped first lightly and then roughly, with forceps; and finally 4) this tapping was carried out with the fingers so that the beetle's elytra were caught roughly between the thumb and forefinger. For smaller beetles, the final stage of stimulation was to be rolled between

¹ Coleoptera: Tenebrionidae.

² This is the 2nd of 3 papers in a series entitled, "A comparative study of the chemical defensive system of Tenebrionid beetles." Received for publication July 25, 1974.

the fingers. Transfer of secretion by wiping with the hind legs was always checked by holding the beetle by the head and prothorax so that the hind legs could be moved freely. Smaller beetles were observed under the stereomicroscope.

In the laboratory, all beetles were maintained in an insectary at 28°C and 70% RH. They were housed by species and collection locality in plastic boxes with a substrate of bran and leaf litter to serve as food and hiding places, respectively. These conditions in general, and frequent handling specifically, resulted in a decrease in sensitivity to stimulation; such animals required stronger stimulation to cause release of a defensive behavior than did their counterparts in the field. Therefore, before testing in the laboratory, beetles were left completely undisturbed for at least 1 wk. Nevertheless, their sensitivity never quite matched that of newly captured animals in the field. Notes on defensive behavior upon capture in the field were thus also kept.

All available individuals (up to a limit of 20) of each species from each collection locality were tested for defensive behavior, and those patterns which could be commonly elicited from a substantial fraction of the individuals were recorded as being characteristic of that species or locality or both. For only a few species were there fewer than 5 individuals available. The tests were repeated at a later date to confirm earlier observations.

Photographs of behavior and morphological structures were made with a 35-mm single-lens reflex camera. Some structures were photographed under a Cambridge Mark II scanning electron microscope after the specimens had been gold-coated. The wiping behavior of *Argoporis* was filmed using a 16-mm Bolex Cine Camera at 64 frames/s, and drawings were made from selected frames.

RESULTS AND DISCUSSION

The defensive behavior patterns observed in this work are, in the most rigorous sense, those which can be elicited by the described stimulation of laboratory animals or by capture-trauma of field animals. The recorded behaviors therefore constitute a minimum repertoire for each species and it is possible that other situations might elicit additional behavior patterns. Most of the patterns observed were very clear and obvious, were common to most members of a species, and could be elicited repeatedly during the life of an individual, subject to habituation. It is therefore unlikely that many of the behaviors were artifacts of laboratory conditions, and this was confirmed in almost all instances in which behavior could be checked with newly captured specimens in the field.

Delivery of the Secretion.—Upon being disturbed or handled, the species release their secretion in 1 of 3 major ways: 1) the glands are everted and the small amount of secretion on the inner surface is thus aired; 2) the secretion is allowed or forced to exude from the gland and collects at the tip of the abdomen as a drop, or spreads over the posterior parts of the body; 3) the secretion is so forcibly ejected that it

is sprayed over some distance. Intermediates are also represented: thus, a number of species evert only the proximal portion of the gland reservoir (partial eversion), while other species may spray occasionally or weakly. A few species even spray by very rapidly everting the glands. (*Pyanisia tristis* Cast., *Psorodes* spp.).

It has long been assumed that the force which causes the secretion to be exuded or sprayed, or the glands to be everted, is supplied by contraction of the body musculature, especially of the membranous dorsum, and that this force is transmitted via the hemolymph. Since the reservoir walls are not muscled (except in certain Lagriini [Kendall 1968]), this mechanism seems reasonable, though it has not been supported by experimental evidence to date. Additional ejective force may derive from the elasticity of the reservoir walls themselves, and if the gland valve of certain species is opened on glands which have been dissected free, the secretion will flow out by itself (Tschinkel 1969).

Most of the muscular events in all 3 methods of delivery are probably fundamentally similar and homologous with increased power and more complex coordination required for spraying. Those species which exude or spray the secretion are supplied with a pair of valve-opener muscles, and 1 everting species (*Tenebrio molitor* L.) has been reported to possess a retractor muscle which inserts at the apex of the conical gland reservoirs (Lengerken 1925). The sequence of muscular events involved in exuding or spraying is probably as follows: 1) the last visible abdominal sternite is protracted exposing the gland orifices; 2) the intersegmental muscles of the abdominal tergites contract, constricting the body wall and raising the pressure on the body fluids (hemolymph); 3) the valve-opener muscle contracts, opening the exit channel and allowing the secretion, which is now under pressure, to leave the reservoirs. The last 2 events could also occur in reverse order.

Most species of tenebrionids are capable of several to many releases of secretion; that is, they do not give off their entire load at once. This capacity to meter repellency has obvious advantages of economy and the ability to respond in keeping with the persistence of the predator. Upon protracted disturbance, such as occurs when beetles are being "milked" for their secretion, most species do run out of deliverable secretion. Many species are able to release some secretion again by the next day or so, but one cannot assume from this that the secretion has been recharged by *de novo* synthesis, and, in fact, little is known about recharge rates in tenebrionids. Since every increment of secretion ejected is reflected in an equal decrease in body volume, it seems likely that the amount of secretion which is deliverable is limited by the degree to which muscular contraction can decrease body volume, and this is probably never equal to the entire capacity of the reservoirs, especially in large-reservoired species. Having reached the limit, body volume can then readily be made up by imbibition of water, swallowing of food and/or air, or the

Table 1.—Summary of the behavior of the taxa of Tenebrionidae. In cases where less than all members of a taxon exhibit a behavior, the fraction doing so is indicated within an open circle. A closed circle indicates that all investigated members consistently displayed the behavior, a half-closed circle that the behavior was not very distinct or was shown only occasionally by individuals. In the "Evert" column, a half-closed circle indicates partial eversion. The tribes are arranged according to Arnett (1971) with the Playtynotini and Litoborini inserted near the Opatrini. The 1st column indicates the number of species and localities used (S = species; L = localities; PDG = prothoracic defensive glands present; * = behavior determined with fewer than 5 but more than 1 individual; ** = behavior determined with one individual only).

									Ancillary		
TRIBE	GENUS	No. spp. & locals. checked	Delivery			Headstand	Fold legs	Wipe	Epipl. gutters	Affinity	Gular strigil
			Evert	Exude	Spray						
Scaurini											
	<u>Scaurus</u>	1S 1L		●							
	<u>Argoporis</u>	3S 5L	◐					●			
	<u>Apsena</u>	2S 3L	◐					●			
	<u>Eulabis</u>	?S 3L	◐					●			
	<u>Epantius</u>	1S 1L	◐					●			
Blaptini											
	<u>Blaps</u>	8S 10L			●	◐					
Amphidorini											
	<u>Amphidora</u>	1S 2L		●			◐	●			
	<u>Cratidus</u>	1S 5L			●	●		◐ ¹ / ₃			
Eleodini											
	<u>Lariversius</u>	1S 1L		●							
	<u>Trogloderus</u> *	1S 1L		●		◐					
	<u>Embaphion</u>	2S 2L		●		●		●			
	<u>Neobaphion</u>	1S 1L		●		●					
	<u>Eleodes</u>										
	(<u>Melaneleodes</u>)	7S 16L		●	◐ ² / ₃	◐ ² / ₃		◐ ² / ₃			
	(<u>Litheleodes</u>)	2S 5L		●		◐ ² / ₃		◐ ² / ₃			
	(<u>Metablapyllis</u>)	1S 3L		◐ ² / ₃	◐ ² / ₃	◐ ² / ₃		◐ ² / ₃			
	(<u>Promus</u>)	3S 4L		●		●		◐ ³ / ₄			
	(<u>Eleodes</u>)	15S 27L		◐ ¹ / ₃	◐ ² / ₃	◐ ² / ₃					
	<u>E. acuticauda</u>	5L		●	●	◐		◐ ² / ₃			

Table 1.—(Continued)

TRIBE	GENUS	No. spp. & locals. checked	Delivery			Headstand	Fold legs	Wipe	Ancillary		
			Evert	Exude	Spray				Epipl. gutters	Affinity	Gular strigil
	(<u>Steneleodes</u>)	2S 10L			●	●					
	(<u>Blapyllis</u>)	6S 6L		●		2/6		7/6	1/6		
Pedinini											
	<u>Blapstinus</u>	3S 4L		●				●			
	<u>Conibius</u>	2S 2L		●			★	★			
	<u>Notibius</u>	2S 2L		●				★			
Opatrini											
	<u>Gonocephalum</u>	3S 3L			●		●				
	<u>Parastizopus</u> *	1S 1L		●			●	●			
Platynotini											
	<u>Schelodontes</u>	1S 1L	◐	●							●
	<u>Anomalipus</u>	1S 1L		●							●
	<u>Melanopterus</u> *	2S 2L		●							●
	<u>Trigonopus</u>	1S 1L		●							●
	<u>Eurynotus</u>	1S 1L		●							●
	<u>Gonopus</u> *	1S 1L			●						●
Litoborini											
	<u>Zadenos</u>	3S 3L		●			●				
Phaleriini											
	<u>Phaleria</u>	2S 2L		●			●	●			
Bolitophagini											
	<u>Bolithotherus</u>	1S 1L	◐	◐			●	●			
	<u>Eleates</u>	1S 1L	◐	◐			●	●			

Table 1.—(Continued)

TRIBE	GENUS	No. spp. & locals. checked	Delivery			Headstand	Fold legs	Wipe	Ancillary		
			Evert	Exude	Spray				Epipl. gutters	Affinity	Gular strigil
Diapirini											
	<u>Neomida</u>	1S 3L	●				●		●		
	<u>Platydema</u>	4S 6L	●				●	3/4	●	3/4	
	<u>Diaperis</u>	1S 1L	●				●		●	●	PDG
	<u>Metaclisa</u>	1S 1L									PDG
Ulomini											
	<u>Alphitobius</u>	1S 1L	◐				◐	●			PDG
	<u>Gnathocerus</u>	1S 1L	●				●		●		
	<u>Uloma</u>	1S 1L	●				●		●		
	<u>Tribolium</u>	1S 1L	●						●		PDG
Tenebrioni											
	<u>Tenebrio</u>	2S 2L	●								
	<u>Zophobas</u>	1S 1L	●				●				PDG
	<u>Neatus</u>	1S 1L	●				◐	●			
	<u>Toxicum</u>	1S 1L	◐				●				
	<u>Alobates</u>	1S 5L	●				●	●			
	<u>Iphthimus</u>	2S 4L	●						◐		
	<u>Polopinus</u>	1S 1L	●				●				
	<u>Nyctobates</u> **	1S 1L		●			◐				
	<u>Centronopus</u>	1S 1L		●	◐						
	<u>Opatrinus</u>	1S 1L	●								
	<u>Merinus</u> **	1S 1L	●								
	<u>Cibdelis</u>	1S 2L	●				●	●	●	◐	

Table 1.—(Continued)

TRIBE	GENUS	No. spp. & locals. checked	Delivery			Headstand	Fold legs	Wipe	Ancillary		
			Evert	Exude	Spray				Epipl. gutters	Affinity	Gular strigil
	<u>Coelocnemis</u>	2S 2L	●			◐				◐	
Helopini											
	<u>Zophius</u> **	1S 1L	●								
Meracanthini											
	<u>Meracantha</u> **	1S 1L	◐		●						
	<u>Psorodes</u>	2S 1L	◐	●	◐						
Amarygmmini											
	<u>Pyanisia</u>	1S 1L	◐		●	◐	●				
Strongyliini											
	<u>Strongylium</u>	3S 3L	●								
Lagriiini											
	<u>Derolagria</u> *	1S 1L	●								

★ Do not fold legs flat against body, wipe secretion onto own ventrum.

secretion of water into the reservoirs. Any of these processes would prepare the beetle to deliver more secretion, and none would involve *de novo* synthesis. My casual observations indicate that, at least for species of *Eleodes*, repeated "milking" over a period of days results in increasing proportions of the aqueous phase in the ejected secretion. It is possible that the rate of resynthesis of the organic phase is quite low, but no experimental work exists for tenebrionids. High recharge rates are reported for the carabid, *Brachinus ballistarius* LeConte (Eisner 1958) but no efforts were made to separate the above effects from *de novo* synthesis.

Modes of Delivery by the Species.—Eversion.—Complete or almost complete eversion occurs primarily in several genera of the Tenebrionini, Ulomini (*Alphitobius*), Strongyliini and Lagriiini (Table 1). *Tenebrio* is the most familiar example of this cate-

gory, but a number of species show identical behavior. Most complete everters have small amounts of secretion.

Eversion grades through partial eversion or exuding in 2 ways—the species may never evert more than a portion of its reservoirs (partial eversion) or it may evert sometimes but not always (occasional eversion). In the latter case, eversion is often associated with having previously exhausted most of the secretion. Of course, both of these modes may occur in the same species or individual, and in fact, partial everters tend also to be occasional everters, probably because they are generally species with larger quantities of secretion than complete everters.

Partial and occasional eversion are scattered throughout a number of taxa (Table 1). Most of these are both partial and occasional, and when the reservoirs are full, they appear to exude. *Bolitotherus*

and *Eleates* are of this type, as are *Schelodontes* and *Toxicum*. *Meracantha*, *Psorodes*, and *Pyanisia* always partly evert, and in the last 2, the rapidity of the eversion causes the secretion to be sprayed, a mechanism unique to these groups. *Argoporis*, *Eulabis*, *Apsema*, and *Epantius* of the Scaurini also always partly evert, and in these species an additional structure is associated with the behavior: eversion causes a fold of membrane located dorsally between the glands and the 8th sternite to become turgid and form a transverse band with a vertical surface upon which the usually viscous secretion collects. Many of these species can recover the secretion into the reservoirs if it is not lost during exposure.

Scaurus, also of the Scaurini, is very different both in chemical composition, behavior and gland morphology (Tschinkel 1975a, b) and has recently been separated from the previously mentioned species by creating 2 new tribes for the American species, the Eulabini and Cerenopini (Berry 1973).

Exudation.—Exuding is probably the most common method of delivery and thus gives relatively little insight into taxonomic relationships except where it grades over into spraying. By definition, all species which do not spray and do not evert allow their secretion to exude forth. Most tribes show at least some species exhibiting this behavior (Table 1).

Spraying.—Spraying is much less common than exuding and partly delineates several taxa. It is most frequently encountered among the Eleodini and their allies, the Amphidorini (Adeliini) and also among the Blaptini. Isolated cases of spraying are scattered throughout the family (Table 1) and have probably evolved independently. Thus, *Nyctobates* and *Centronopus* (Tenebrionini), *Gonopus* (Platynotini) and *Gonocephalum* (Opatrini) all spray the secretion. This is also true of *Psorodes* (Meracanthini) and *Pyanisia* (Amarygmmini), but their behavior is almost certainly not homologous to that other spraying taxa, for it is accomplished by rapid eversion of the glands.

Even among the Eleodini, spraying is restricted to 3 or 4 subgenera of *Eleodes*. Most spraying species occur in the subgenera *Eleodes* and *Steneleodes*, almost all of whose species spray. Some species of the subgenera *Metablapylis* and *Melaneleodes* spray weakly and occasionally, but no species of the subgenera *Blapylis*, *Promus*, or *Litheleodes* were observed to spray. *Cratidus* of the Amphidorini sprays and generally behaves much like *Eleodes*, but the much smaller *Amphidora* of the same tribe merely exudes. All 8 available species of *Blaps* sprayed, some of them very copiously and effectively.

In Fig. 1 is plotted the frequency distribution of the sizes of species which spray and those which do not spray. It is apparent that the mean size of those species which spray is significantly larger than those which do not. Such correlation does not imply a causal relation of size to spraying, although there may be size requirements dictated by resistance to rapid flow through fine channels. The majority of the large species and those which spray belong to 2 subgenera

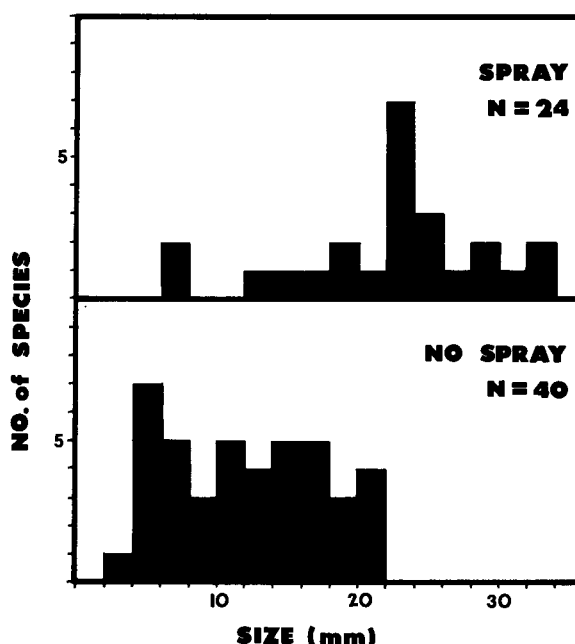


FIG. 1.—The frequency of spraying and non-spraying species related to approximate species size (length) plotted in 2-mm intervals. The difference in the means of the 2 groups is highly significant ($P < 0.001$; Student's t -test).

of *Eleodes* (*Eleodes* and *Steneleodes*) and to *Blaps*. Nevertheless, most of the species outside *Eleodes* and *Blaps* which sprayed were also relatively large. By far the smallest were 3 species of the African *Gonocephalum* (8 mm long) which are able not merely to spray backwards, but to spray within ca. 30° of directly forward as well, an ability quite rare among tenebrionids.

Postural Behavior.—Two primary types of postural defensive behavior have evolved among the tenebrionids: 1) the tendency to raise the body in a headstand (Fig. 2), first described for species of *Eleodes*, and 2) the tendency to fold the legs and antennae close to the body in a characteristic fashion (Fig. 3) and remain motionless (death-feint) (Pace 1967, Weiss 1944). Release of secretion usually accompanies this leg-folding, but it may also follow it.

Intermediates are represented in headstanding behavior. These are characterized primarily by the angle which the body makes during the headstand. Strong and persistent headstanders, such as many species of the subgenus *Eleodes*, hold their bodies almost vertically, while other species may make angles between 60° and 30° from the horizontal. It is likely that headstanding behavior has evolved several times in the tenebrionids and the headstanding of distantly related species is not necessarily homologous (see below).

Some taxa show less obvious differences in the details of headstanding. For example, the amount of flexure of each of the 3 pairs of legs varies; in species with well-developed headstanding, the relative straightening of the hind legs is much greater than

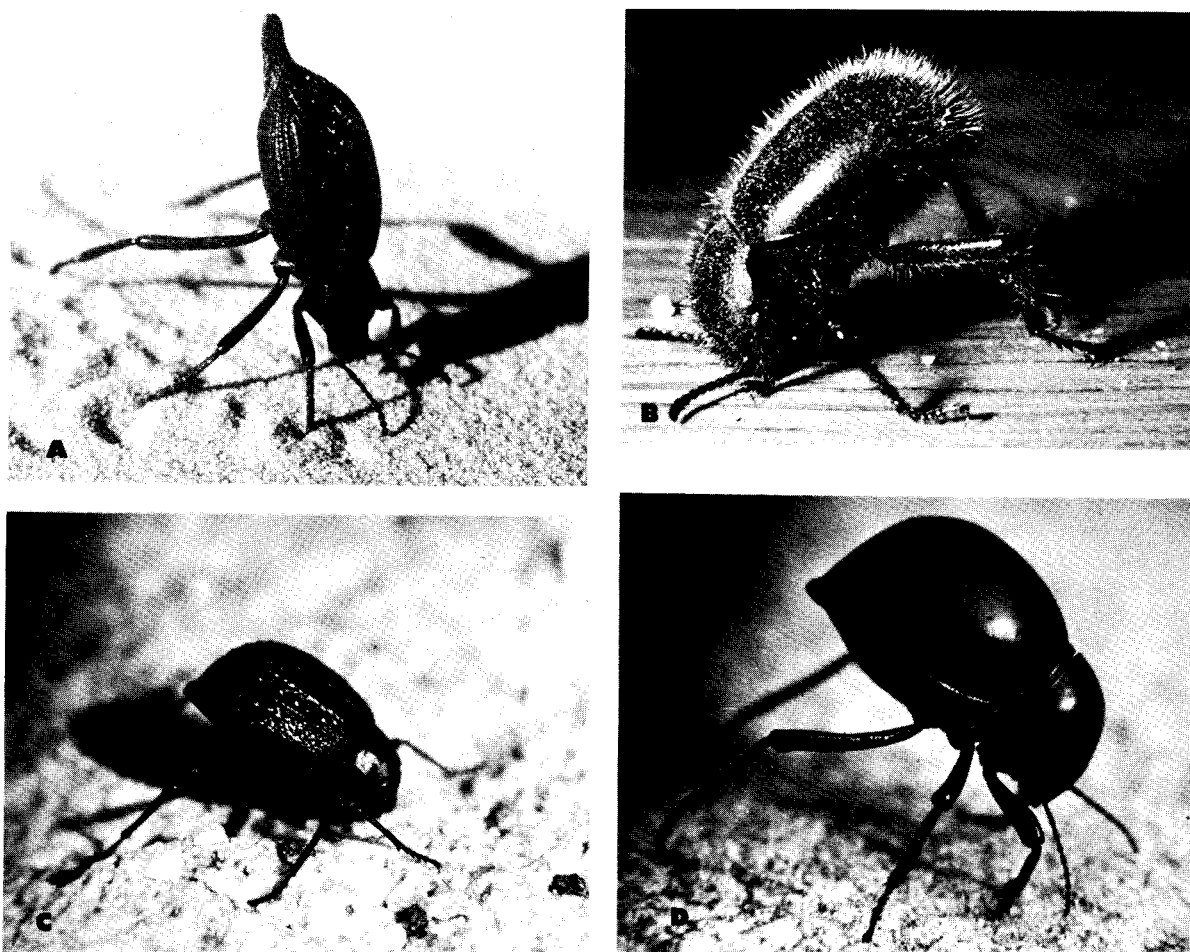


FIG. 2.—Headstanding behavior in (A) *Eleodes caudifera* Lec., (B) *Cratidus osculans* LeConte, (C) *Renatiella gebieni* Koch, and (D) *Molturodes hirtipes* Mulsant. The first 2 species possess defensive glands, the last 2 do not and belong to a related family (Tentyriidae). Note the small drop of secretion at the abdominal tip of *C. osculans* (B).

of the other 2 pairs and the prolegs may actually flex more than their resting position, while in certain species with less well-developed behavior, these differences are not so great. This causes the pivot during headstanding to lie about at the bases of the mesolegs in the former species (e.g., Fig. 2A) and at the prolegs or even anterior to these in the latter species (e.g., Fig. 2D).

Just as there was for spraying, there is a correlation between size (length) of the species and headstanding (Fig. 4). Thus, headstanding species are significantly larger ($P < 0.001$) than non-headstanding species. Again, the bulk of the large species and those which headstand belong to the genus *Eleodes*, though many of the smaller *Eleodes* headstand as well, and the difference is not as sharp as it is for spraying vs. non-spraying (Fig. 1). With only a few exceptions, distinctive headstanding is restricted to species of *Eleodes*.

Of the 64 species of tenebrionids whose behavior was observed, 45% neither sprayed nor headstood

(Table 2). While headstanding could be accompanied either by spraying or oozing, only very rarely (3%) did beetles spray which did not headstand. Thus, most species which did not headstand did not spray either. In large part, this is a reflection of the high rate of occurrence of both headstanding and spraying in species of *Eleodes*. Nevertheless, of those species outside *Eleodes* which do spray, several have evolved some tendency to raise the posterior before spraying (Table 1). Thus, *Centronopus*, *Gonopus*, *Pyranisia*, and *Blaps*, each in a separate tribe, all spray

Table 2.—The co-occurrence of spraying and headstanding behavior. The table is derived only from the 64 species which were actually observed, and the percentage falling into each behavioral category is indicated.

	Headstand	Non-headstand	Total
Spray	31%	3%	34%
Non-spray	20	46	66
Total	51	49	100

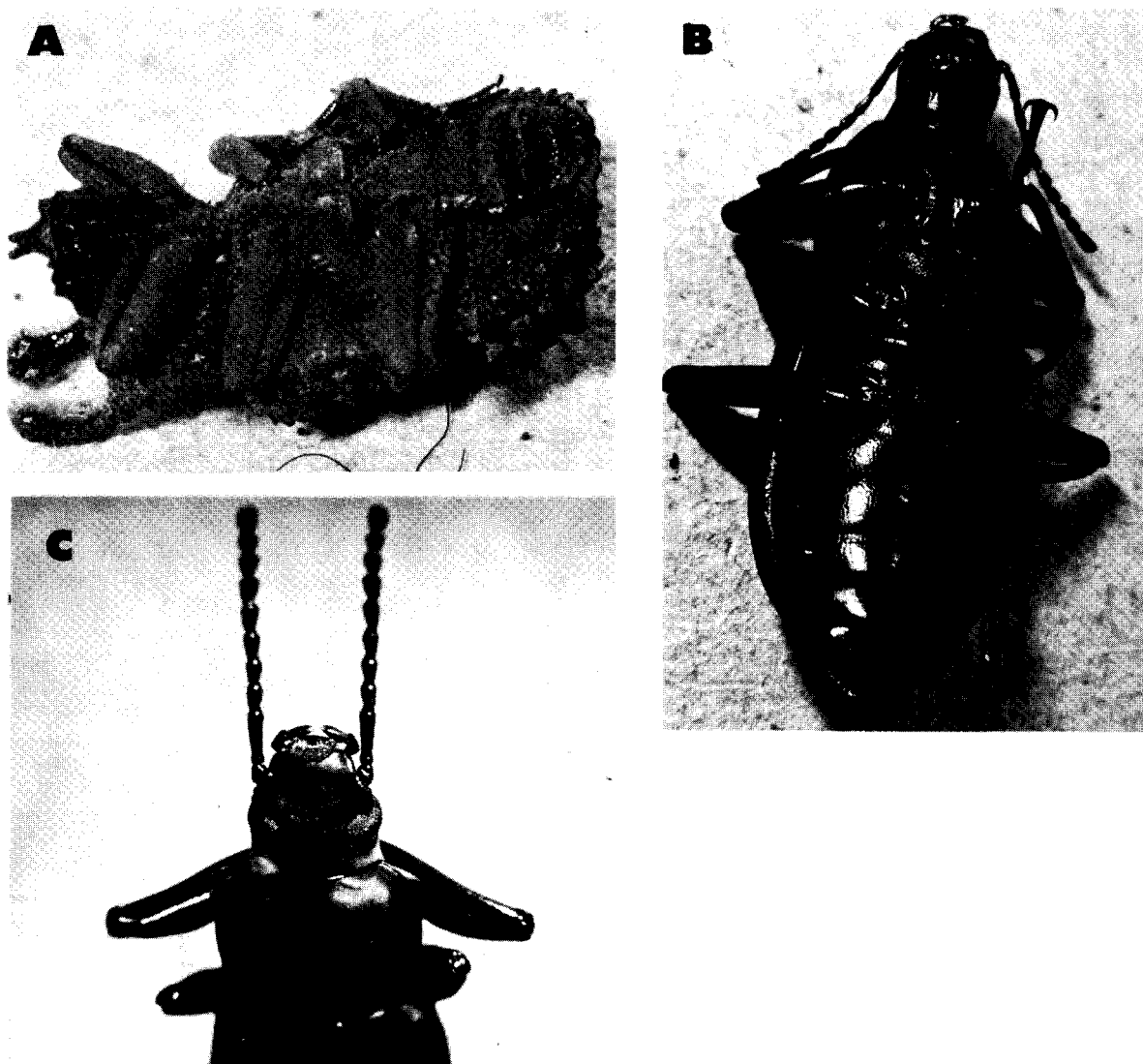


FIG. 3.—Leg-folding behavior in (A) *Bolitotherus cornutus* Panz., (B) *Polopinus nitidus* LeConte, and (C) *Zophobas rugipes* Kirsch, showing also the characteristic antennal posture during leg folding and the white, phenolic secretion released from the glands in the prothorax.

their secretion and have all evolved a low headstand. While this behavior is obviously not as distinct or well-developed as in *Eleodes*, it implies that for those species which spray, there is some advantage in raising the posterior during the act, perhaps by improving aim or range or both. The occurrence of such behavior in the spraying *Platyzosteria* roaches (Waterhouse and Wallbank 1967) further supports this notion.

Headstanding can also evolve for reasons of mimicry, either Muellierian or Batesian. Thus, species of *Megasida* (which have no defensive glands) sympatric with *E. longicollis* LeConte and *E. gracilis* LeConte, not only resemble these 2 aposematic species, but mimic their tendency to headstand when disturbed (Eisner and Meinwald 1966). Tenebrionid species with large ranges which overlap those of other

tenebrionids often show a tendency to resemble these local tenebrionids in each area of sympatry.³ Mimicry of the Batesian type may account for the headstanding of sympatric species which do not have defensive glands (e.g., *Stenomorphia angulata* LeConte and *E. acuticauda* LeConte, personal observation) and can extend across families as in the flightless black cerambycids, *Moneilema* spp. and its Batesian model, *E. longicollis* (Raske 1967). Muellierian mimicry would increase the tendency of sympatric species with defensive glands to headstand. This latter tendency is not restricted only to congeneric species; for example, *Coelocnemis magna* LeConte, where it is sympatric with *E. laticollis* LeConte, is observed to head-

³ Doyen, J. T., and R. E. Somerby. 1974. Phenetic similarity and Muellierian mimicry among darkling ground beetles (Coleoptera: Tenebrionidae). *Can. Entomol.* 106: 759-72.

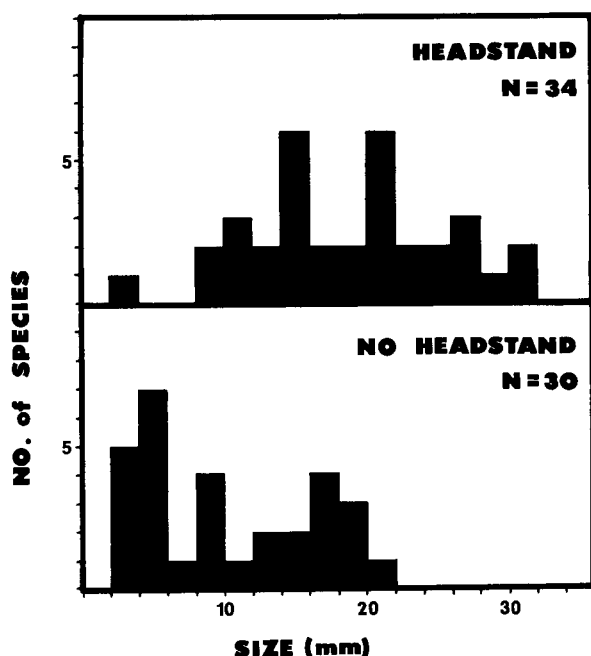


FIG. 4.—The frequency of headstanding and non-headstanding species related to the approximate species size, (length) plotted in 2-mm intervals. The difference in the means is highly significant ($P < 0.001$; Student's t -test).

stand (Table 1). Doyen (1973) reported the occasional occurrence of headstanding in other *Coelocnemis* species as well. Even *Bolitotherus cornutus* Panzer has been reported, under certain circumstances, to raise the abdomen while releasing secretion (Pace 1967).

There is, nevertheless, a residue of cases of headstanding behavior which cannot be explained as mimicry, increased efficacy of spraying, or an aposematic image. For example, several African species of the related family Tentyriidae (sometimes considered a subfamily of the Tenebrionidae) show some degree of headstanding although they are neither sympatric with aposematic, headstanding species, nor do they have glands themselves. Fig. 2 shows headstanding in *Molurodes hirtipes* Mulsant and *Renatiella gebieni* Koch. Many tenebrionids which do not headstand show a tendency to stiffen the legs upon being disturbed (e.g., *Argoporis*) and, indeed, this tendency is found in other beetle families as well. It seems likely that this stiffening of the legs is the pre-existent behavior which preadapted the tenebrionids to evolve headstanding behavior by greater extension of the hind legs with the resultant raising of the posterior. The process was probably spurred on by the advantages accruing from the distinctive aposematic image, increased exposure of the secretion and, in those species which spray, increased range and improved aim.

Another aspect of headstanding merits discussion: headstanding would probably cause a decrease in the effectiveness of secretions which contain only quinones, for the exclusively quinonoid organic phase is subnatant and the beetle would be dispensing only the

much less repellent aqueous phase (personal observations). Conversely, in those species whose secretion contains hydrocarbons in significant amounts, the repellent organic phase is supernatant, and headstanding would assure that only this phase is expelled. The effect of headstanding would thus be an increase in the potency of the ejected secretion, and would provide selective pressure toward its evolution. Perhaps it is significant that all headstanding species have secretions with substantial amounts of hydrocarbons.

The leg-folding of most species is very much alike (Fig. 3). The tarsi, tibiae and femora are closely applied to the ventral body wall, with the protibiae being anterior to the profemora, the meso- and metatibiae being posterior to their respective femora. The antennae are directed posteriad along the ventral side of the body and may or may not be in contact with the body wall. In some species, such as *Zophobas*, the antennae are held forward and parallel to one another (Fig. 3).

The leg-folding of some species of *Conibius* differs somewhat from the above in that the legs are not folded flat against the body. Rather, the tarsi project ventrally and the tibiae are not in contact with the body. Nevertheless, the legs are scissored shut and the beetle remains motionless while releasing the secretion, identifying this as a characteristic behavior pattern.

Leg-folding behavior is commonly observed among some Diaperini, Phaleriini and Bolitophagini, some of the Tenebrionini and Ulomini, and shows scattered occurrence in other tribes as well (Table 1). In species which are already quite cryptic, such as *Bolitotherus cornutus*, the effect of leg-folding is to make the beetle extremely inconspicuous (Fig. 3) for it resembles nothing so much as a small lump of the fungus in which it lives (Pace 1967). This is also true to some degree of *Polypleurus geminata*.

Spreading the Secretion with the Metalegs.—A number of species wipe the hind legs (usually the tarsi or tibiae) through the secretion which has flowed onto the posterior of the abdomen and smear this onto the animal holding them (Fig. 5). Usually, the animal was myself, and secretion was obviously being transferred from the glands to my thumb or fingers via the legs. The efficiency of this wiping behavior varies, and some species, such as *Argoporis*, are very persistent and effective wipers (Tschinkel 1972) while others seem less effective and a few (notably several species of the subgenus *Melaneleodes* of *Eleodes*) seem to transfer secretion almost fortuitously in the process of waving the legs while being held. In such cases, the behavior is still distinct and recognizable, though much less frequent. In *Argoporis* the behavior has been refined even further, and if the beetle is held in such a way that all the legs are free to move, it will transfer the sticky secretion from the hind to the middle to the prolegs, and with the prolegs it will smear the material over the head and base of the antennae (Tschinkel 1972).

Fig. 5 shows selected frames from a film sequence of the metaleg wiping behavior of *Argoporis* filmed

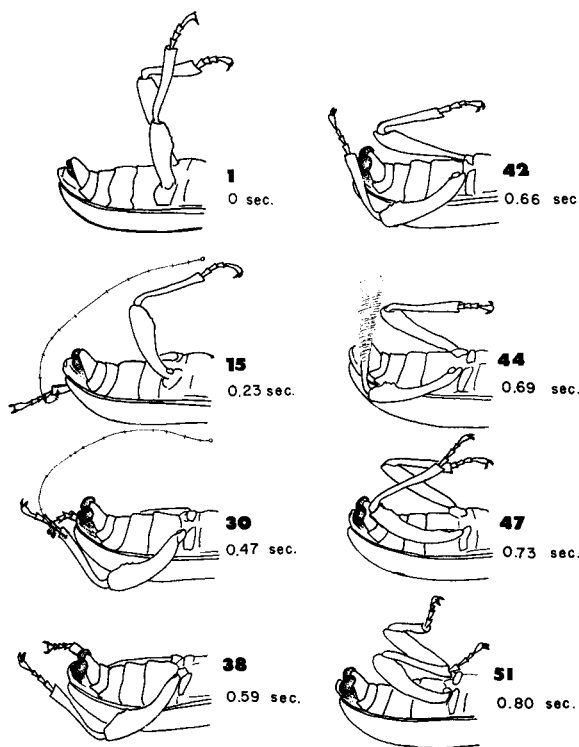


FIG. 5.—Ventral view of metaleg wiping behavior of *Argoporis alutacea* Casey, drawn from selected frames of a 51-frame movie sequence taken at 64 frames/s (shutter speed $\frac{1}{150}$ s). The frame number and approximate time elapsed from the beginning of the sequence are indicated for each drawing. Note the relatively long period during which the legs are brought backward and maintained in the ready position, and their very fast forward movement during the delivery stroke. The defensive gland reservoirs are partly protruded and become inflated early during the sequence (frames 1–30). Each leg contacts a secretion-laden gland on the forward stroke (frames 38 and 42).

at 64 frames/s. The frame just prior to the 1st detectable posteriad movement of the metalegs was denoted as the beginning of the sequence (frame 1) and all other frames are numbered in relation to it. The beetle opens the 7th sternite ventrally upon being disturbed and keeps it open. Simultaneously, the gland reservoirs are partially everted, exposing an area of more or less inflated membrane covered with the viscous secretion. As the beetle brings the legs back preparatory to wiping (frames 1–30), it also inflates the exposed gland intima causing them to project even further and to assume a turgid, glossy appearance (frames 30–51). The metalegs may be brought backward individually or simultaneously, the same individual showing both patterns. Once positioned, the legs are held for a considerable period in the ready position (frames 30–38), then brought rapidly forward, stopping briefly upon contact with the exposed glands and visibly deforming them (frames 38–42). The movement from the gland forward is so rapid that it blurred at an exposure of $\frac{1}{150}$ s (frame 44), and it seems possible that the secretion, if abundant

enough, may even be thrown. Finally, the material on the leg is wiped onto the handler, though this is not possible in the sequence shown because of the way the beetle is held. Both legs may be brought forward almost simultaneously, as in Fig. 5, but sometimes the beetle may wipe with one leg at a time.

Other species which wipe may differ from *Argoporis* in the way in which secretion is delivered, the timing of the various movements and the persistence of the behavior, but comparative quantitative information was not obtained from them. It seemed apparent, however, that in those *Eleodes* which wiped, the very rapid forward movement was not present, though this has not been verified by filming.

All observed species of the Scaurini (except *Scaurus*) similarly used their metalegs to wipe the secretion. Among other taxa occurrence of this behavior is scattered, being fairly common among part of the Tenebrionini, the Phaleriini, Bolitophagini, and being represented occasionally in a number of other tribes (Table 1). Even in *Eleodes*, this behavior is fairly common, occurring in about half of the species in a number of subgenera.

Species of *Conibius* use their hind legs to wipe the secretion over their own sterna, effectively increasing the surface area from which volatilization takes place. These species also fold their legs in a distinctive fashion (see above).

Stridulation.—In addition to releasing secretion, a number of species of Platynotini stridulate when handled. The strigil is located on the gular region of the head (Fig. 6) and is rubbed on a plectrum ventrally located in the prothoracic head socket (Fig. 6D) to produce a squeaking noise. The strigil was present in all species of the African Platynotini (Table 1) and the behavior, which is presumed to be aposematic, was found in *Melanopterus*, *Eurynotus* and *Schelodontes*. It was also found in *Opatrinus* species and, together with the gland morphology of this genus (Tschinkel 1975b), suggests that its relationship is not with the Tenebrionini where it has been placed, but with the Platynotini.

In *Eurynotus capensis* F., stridulation is also used during courtship and mating both before and after mounting (personal observation) but it is not known how or whether these sounds differ from those produced upon handling. Other cases have been reported in which stridulation is used both in a stress and a courtship situation (e.g., the Scolytid beetles) (Rudinsky and Michael 1972).

Structural Features Ancillary to Defense.—A number of structural features which increase the effectiveness of the secretion are apparent. The effect of most of these is to increase the area under influence of the secretion by causing it to spread over the elytra or sternites. There are 2 major means whereby this is accomplished. 1) The elytra have a very high affinity for the secretion and cause it to wet them rapidly, and often entirely. In species of *Diaperis* and *Platydemia* (Fig. 7b) this must be a property of the cuticular surface lipids, for no sculpturing is apparent, but in some species of *Coelocnemis* (Doyen 1973) and

some *Blapyllis* and *Cibdelis*, the reticulations of the elytral surface might provide capillary spaces which conduct the secretion (Fig. 7a, c) though there must be a strong tendency for the secretion to wet the elytra as well. 2) More commonly, the margins of the epipleura are formed into a sort of "gutter" along the lateral edge of the elytra (Fig. 8), and these gutters conduct a narrow streak of secretion forward as far as the elytral humeri. Again, this has the effect of increasing the volume of space exposed to the secretion. Conduction is very rapid, the length for most species being covered in a fraction of a second. The motive force is, presumably, a combination of capillarity and affinity, though the latter is not great enough in most species to cause it to leave the gutters and flow over the elytra.

Gutters also occur in *Gnathocerus*, *Uloma*, and *Tribolium* (Ulomini). In the last, the gutter system is elaborated even further: the pronotal margin is formed into a similar gutter, so that the abdominal gland secretion flows forward in the elytral gutters,

while that from the prothoracic glands flows around the entire margin of the pronotum as well. Thus, the secretion has been effectively spread the entire length of the body. This mechanism appears to be unique to *Tribolium* species.

In *Zadenos delandei* (Mulsant), conduction takes place in the heavy elytral sulcae (Fig. 7d), illustrating still another manifestation of this general principle.

Homology of the Behaviors.—The occurrence of similar behaviors in groups that are only distantly related and the absence of such behavior in closely related groups underlines the difficulty of identifying homology in behavior. Much of the defense-related behavior of tenebrionids seems to have evolved from preadaptation common to most species and therefore probably evolved independently in several groups. For example, headstanding in *Coelocnemis* is certainly not homologous to headstanding in *Eleodes*, and similarly with spraying in *Centronopus* and *Eleodes*. These genera are so distantly related that it is most reason-

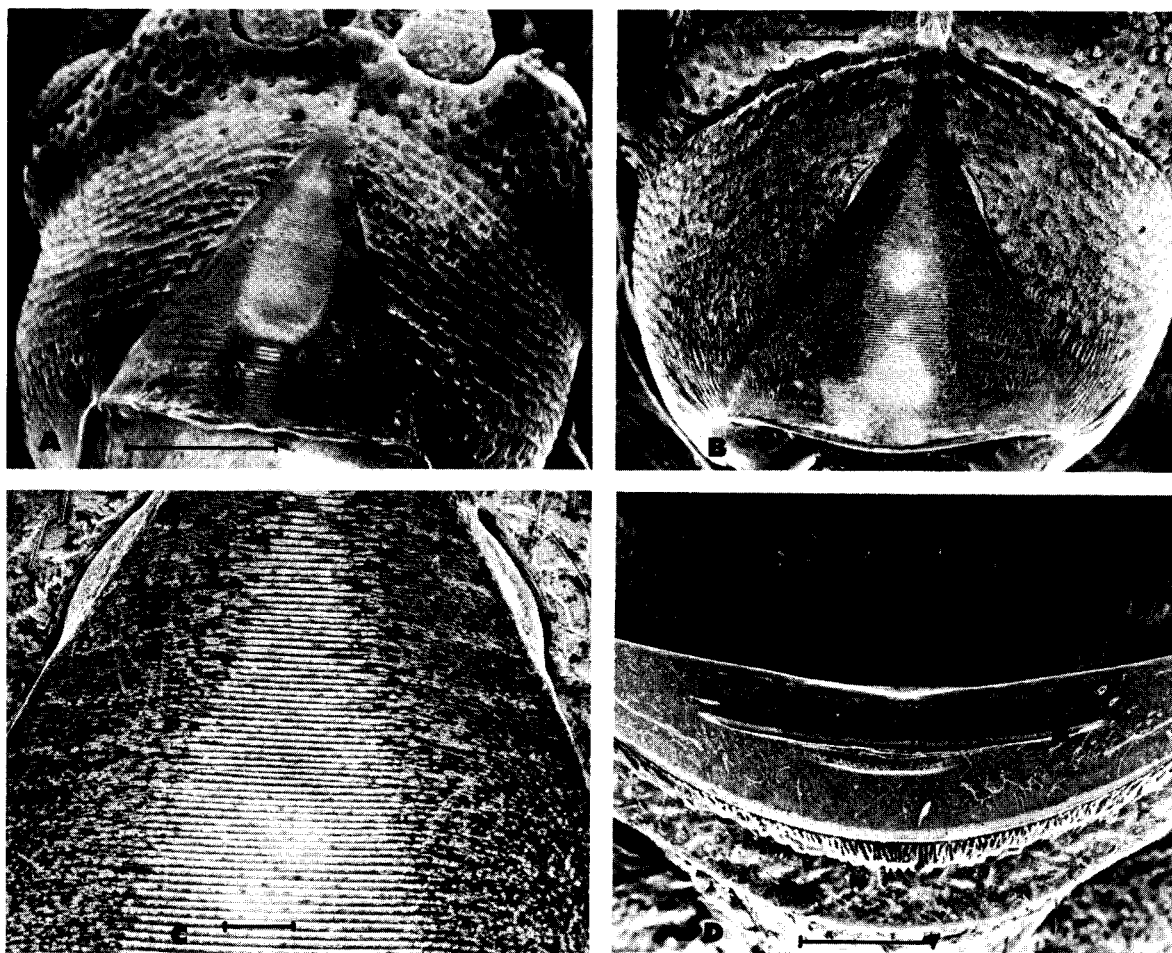


FIG. 6.—The gular stridulatory organs of (A) *Opatrinus* sp. (Tenebrionini) and (B) *Trigonopus capicola* Mulsant (Platynotini). (C) The strigil of *T. capicola* at higher magnification showing the transverse ridges. (D) The ventral plectral ridge (p) inside the prothoracic head socket of *T. capicola*. The bars denote 0.5 mm in (A) and (B), and 0.1 mm in (C) and (D).

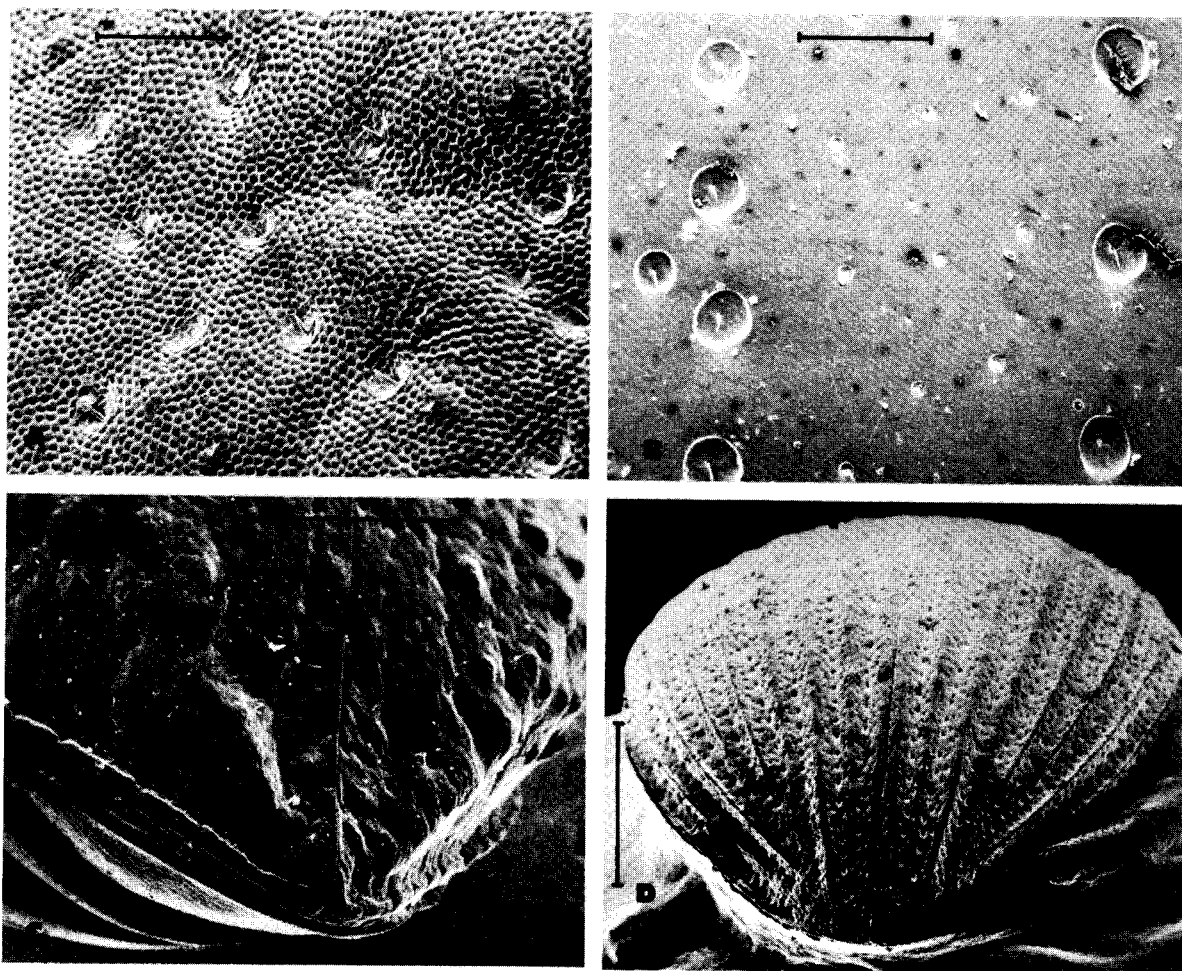


FIG. 7.—The elytral surfaces of (A) *Coelocnemis californica* Mannerheim from Mono Co., Cal., showing the roughness which gives the surface its lusterless quality and which may aid in spreading the secretion. (B) *Diaperis maculata* Oliver showing the smooth cuticle which is featureless up to ca. 5000 magnifications. Spreading of the secretion must be due to the wetting properties of the cuticle. (C) Posterior elytral tips of *Cibdelis blaschkei* Mannerheim. (D) Posterior portion of the elytra of *Zadenos delandei* showing the coarse sulcae in which secretion is conducted antieriad. The bars denote 0.1 mm in (A) and (B), and 1 mm in (C) and (D).

able to assume the behaviors to have arisen independently from the tendency to stiffen the legs upon disturbance in the 1st example and from exuding the secretion in the 2nd. In practice, the conclusion that the behaviors are homologous is made in light of knowledge of the systematic relationship of the species, and similar behaviors of closely related species are usually assumed to be homologous. Thus, the headstanding of all species in the subgenus *Eleodes* of *Eleodes* could be said to be homologous on the basis of the close relationship of the species. Yet, even such conclusions could be doubted if one assumes a strong tendency to evolve a behavior from a specific preadaptation in the appropriate circumstance. There is obviously a strong pressure to evolve headstanding in *Eleodes*. Since we do not understand the exact nature of the pressures, it is possible that even headstanding arose independently in this genus several times. The occurrence of this behavior in *Eleodes* is

mosaic, and even within species there may be heterogeneity, beetles from some localities headstanding, others not—for example, *E. extricata* (Say) (*Lith-eleodes*), *E. nigrina* LeConte (*Metablapyllis*). Heterogeneity within subgenera seems to be the rule in headstanding, spraying and wiping behavior. Few of these behaviors are represented in all of the species of any one subgenus. Furthermore, there is obvious variation in the persistence with which species and even localities carry out the behavior. Some of this heterogeneity may be artifactual, resulting from habituation of the beetles in the laboratory, but much of it is certainly real and can be observed in the field.

What is emphasized by these facts is the lability of the behaviors and our poor understanding of their evolution. All that can be said with the present knowledge is that tenebrionids have a strong tendency to evolve the behaviors under discussion, and that the preadaptations seem to be widespread in the family.

Whether any given behavior actually evolves will depend upon the ecological milieu in which the species operates, the habitat, types of predators and types and behavior of other sympatric tenebrionids. The role of mimicry in the evolution of behavior has already been discussed. Factors resident within the organism itself must also contribute: morphology of the participating parts, morphology of the defensive glands and delivery systems, other behaviors of the species, and the existence of the necessary preadaptations. Given such complexity and given the lability of relatively simple behaviors, heterogeneity, even at the species level, becomes credible. Species with wide ranges

and heterogeneous biotic and abiotic habitats would be expected to show more heterogeneity of behavior.

The effect of such heterogeneity upon the usefulness of behaviors to systematics is profound but varies according to the particular behavior in question. Thus, wiping with the hind legs seems to be a particularly labile behavior, probably having developed from the propensity to wave the legs upon loss of tarsal contact and spurred on by the advantage gained by spreading the secretion to make it more effective. Obviously, wiping behavior, due to its mosaic occurrence, cannot greatly enlighten us about the relationship of the tribes and genera. This is also true of

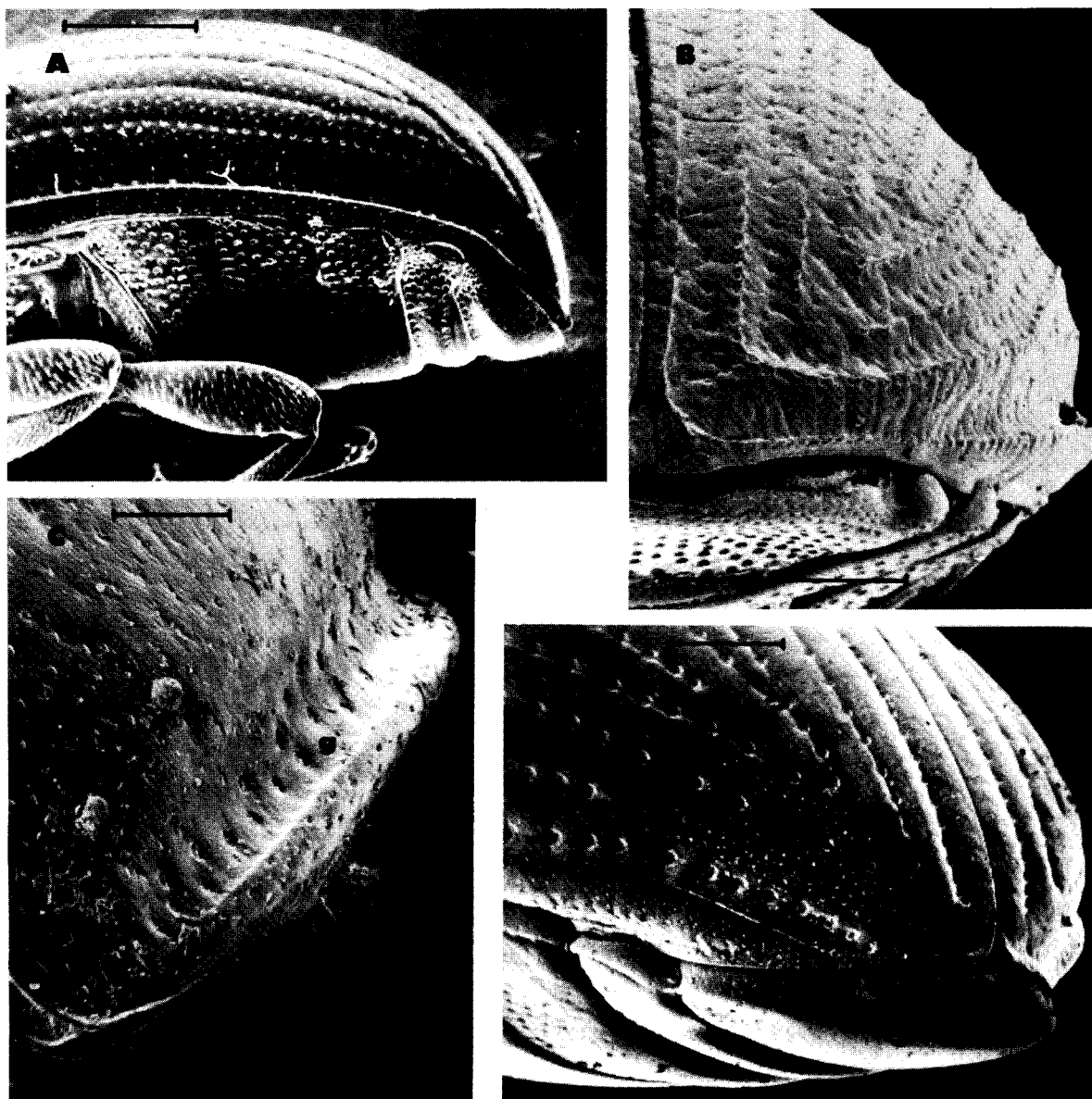


FIG. 8.—The epipleural "gutters" on the elytra of (A) *Neomida bicornis* F. (B) *Tribolium brevicornis* Uytt. (C) *Platydemus oregonense* LeConte. (D) *Uloma mentalis* Horn. The secretion, in all cases, is released just lateral to the abdominal tip and is conducted anteriorly in the "gutters" (g) at the lateral margins of the elytra. The bars denote 0.5 mm for (A), (B), and (D), and 0.1 mm for (C).

leg-folding which occurs widely in the Coleoptera. Some behaviors, such as headstanding, everting, and spraying are more consistent and thus more useful for systematic purposes. When behaviors are considered together or in groups, the trends are stronger still, and a number of groups appear moderately well delineated by their combinations of behaviors. For example, the Eleodini could be characterized as exhibiting headstanding and/or spraying, part of the Tenebrionini as everting, another part as folding the legs; most Diaperini fold the legs and have epipleural gutters and high cuticular affinity for the secretion, and most Scaurini partially evert and wipe with the metalegs. More such relationships can be seen in Table 1.

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