A Comparative Study of the Chemical Defensive System of Tenebrionid Beetles

III. MORPHOLOGY OF THE GLANDS

WALTER R. TSCHINKEL

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

ABSTRACT   The morphology of the abdominal defensive glands and associated structures of 115 species of tenebrionid beetles was studied on KOH cleared material. The glands and reservoirs of all Tenebrionidae are homologous and evolved as a pair of sacs from the intersegmental membrane between sternites VII and VIII. On the basis of reservoir morphology and secretory cell tubule termination, seven provisional gland types were established. Several of the types include species from several tribes, and several tribes contain several gland types, indicating possible incongruencies between the taxonomy and phylogeny of the family. Morphological trends in the evolution of the glands include: increase of reservoir capacity, constriction of the proximal portion of the sacs into distinct exit ducts, release of secretion by exuding or spraying rather than everting, and concentration of the secretory cell tubule terminations into restricted fields, collecting ducts or ampullae. The morphology of the glands of 58 species is illustrated and the results are discussed in light of the current taxonomy of the Tenebrionidae.

The great majority of defensive glands in arthropods are derivatives of the epidermis, and a large variety of types have been described from many orders and classes (Eisner and Meinwald, '66). In general, they consist of a cuticular storage reservoir which is often an invagination of an intersegmental membrane, and secretory cells whose large, microvilli-filled vesicles empty into the reservoir by a thin cuticular duct. Synthesis of the toxicant takes place in the secretory cells and proximal parts of the ducts (Happ, '68).

The defensive glands of the tenebrionid beetles fit this general pattern. They are epidermal in origin and consist of a pair of variously modified invaginations between the seventh and eighth abdominal sternites. Scattered reports on their morphology have appeared over a period of almost a century, beginning with that of Gilson (1889), who reported on the odoriferous glands of *Blaps mortisaga*. He noted that the cells had large, central vesicles with striate borders which drained by long tubules into the paired, sac-like reservoirs, and that the cells were arranged in lobules attached to the surface of the reservoirs by the tubules. Lengerken ('25) described

the eversible glands of *Tenebrio molitor* and noted cells of similar structure but shorter ducts distributed over the inner surface of the reservoirs. He also identified the retractor muscles with which the everted glands are retracted. Valentine ('31) also makes mention of *T. molitor's* glands as does Roth ('45) who described the abdominal defensive glands of eight other species of tenebrionids as well as the prothoracic defensive glands of *Diaperis maculata*, an additional pair of sacs lying in the prothorax and opening between the head and prothorax.

Eisner et al.'s ('64) study of the bilobed glands of *Eleodes longicollis* Lec. added the dimension of electron microscopy to previous studies and contributed greatly to the knowledge of the fine structure of all parts of the secretory cells and reservoir. They showed that the striate borders of the vesicles were actually microvilli and described two secretory units: type 1 consisted only of the secretory cell and its associated tubule-carrying cell. These were generally closely attached to the reservoir's surface. Type 2 consisted of two secretory cells in series (2a and 2b) drained by the tubule from the tubule-carrying cell. The

tubules from a cluster of type 2 cells were gathered into bundles which attached the clusters to the reservoir. In addition, Eisner et al. described the hypodermal cells responsible for the formation of the intima of the reservoir.

The studies of Tschinkel ('69, '72) on *Zophobas rugipes* Kirsch and *Argoporis alutacea* Casey have confirmed the general patterns described by Eisner and previous workers. The secretory units of *Argoporis alutacea* all resembled those of *E. longicollis'* type 2, type 1 being absent, while the units of the prothoracic glands of *Z. rugipes* seemed most like type 1. Due to the small number of ultrastructural studies, it is impossible at this point to determine any homology among the cell types.

The work of Tseng et al. ('71) on *Alphitobius diaperinus* (Panz.) added another to the list of described tenebrionid glands, and it seemed very likely that all tenebrionid abdominal defensive glands were homologous (Tschinkel, '69) and thus afforded an appropriate subject for the study of the evolutionary radiation of a single ancestral type of defensive gland in terms of gland morphology, chemical composition and defensive behavior. Furthermore, the non-comparative nature of most prior studies did not allow an estimate of the true diversity of gland structure to be made. This study was undertaken in order to assess the range of gland types found among tenebrionid beetles. It was hoped, simultaneously, that light would be shed on the phylogenetic relationships of the various groups within the family. This paper represents the third of three which encompass comparative studies of, respectively, the chemical composition of the secretions (Tschinkel, '75a), the defensive behavior (Tschinkel, '75b) and the gross morphology of the glands integrated with the data from the first two papers to delineate tentative relationships among the species.

MATERIAL AND METHODS

The collection and maintenance of the beetles have been described in a previous paper (Tschinkel, '75a). Of the 150 species collected, 115 were selected from all the major taxa within the family for study of the gland morphology. The abdomens of dried specimens were removed, placed for

a few hours in 10% KOH at room temperature, washed in water, then 70% alcohol, manually freed of the tergites, digestive tube, genital ducts and other extraneous material and stained in chlorazol black T in 95% alcohol. After washing in 70% alcohol, the sixth and seventh sternites with the attached glands were separated from the rest of the sternites. Since the seventh sternite forms the posterior edge of the abdomen and consists of a plate with dorsal and ventral exterior surfaces, and since the ventral cuticle of this plate is usually opaque, the glands and the dorsal cuticle were removed by cutting along the posterior edge of the sternite with a silver of razor blade (fig. 1). The glands thus remain attached to the more transparent dorsal portion of the seventh sternite and, after dehydration in absolute ethanol, were mounted on slides in balsam. Alternately, they were taken directly from 70% alcohol and mounted in polyvinyl lactophenol. Drawings of the glands were made on
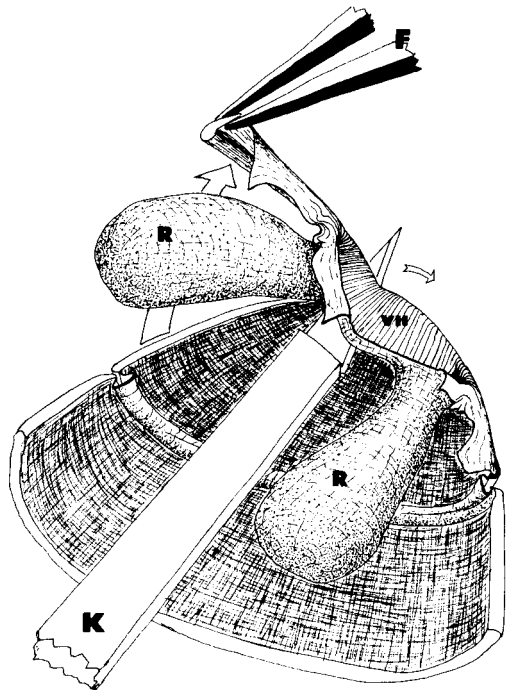

Fig. 1   Dissection of the gland reservoirs from the sternites. The dorsal portion of sternite VII with attached reservoirs (R) is cut from the remainder of the sternites with a knife (K) while being pulled upward with the forceps (F).

graph paper using a Wild M-5 Stereomicroscope with drawing tube.

RESULTS AND DISCUSSION

The abdominal glands of 58 species, mostly selected as typical of their respective taxa, are presented in figures 2 through 9. Their major features are summarized in figure 10 along with the defensive behavior and chemical composition of the secretion.

In general, the results confirm that the abdominal glands of all Tenebrionidae are homologous. All of them are in the same relative location in the body, and consist of a pair of reservoir sacs elaborated from intersegmental membrane and associated with glandular tissue. What has not pre-

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Explanation of figures 2 through 9. The dorsal plate of sternite VII is the curved structure at the top in all drawings. Its posterior margin is also the posterior margin of the intact beetle's ventrum. All structures are in dorsal view except figure 9A,B. The blind distal ends of the reservoir sacs extend anteriad (down in the drawings) in the abdominal cavity with the long axis more or less parallel to the ventral body wall. G, gland; GT, gland tubule terminations; GA, gland collecting duct ampullae; GD, gland collecting ducts; R, reservoir; GL, the limits of distribution of scattered secretory cells draining individually. The cells lie inside the curvature of the dotted line. Bars represent 1 mm.
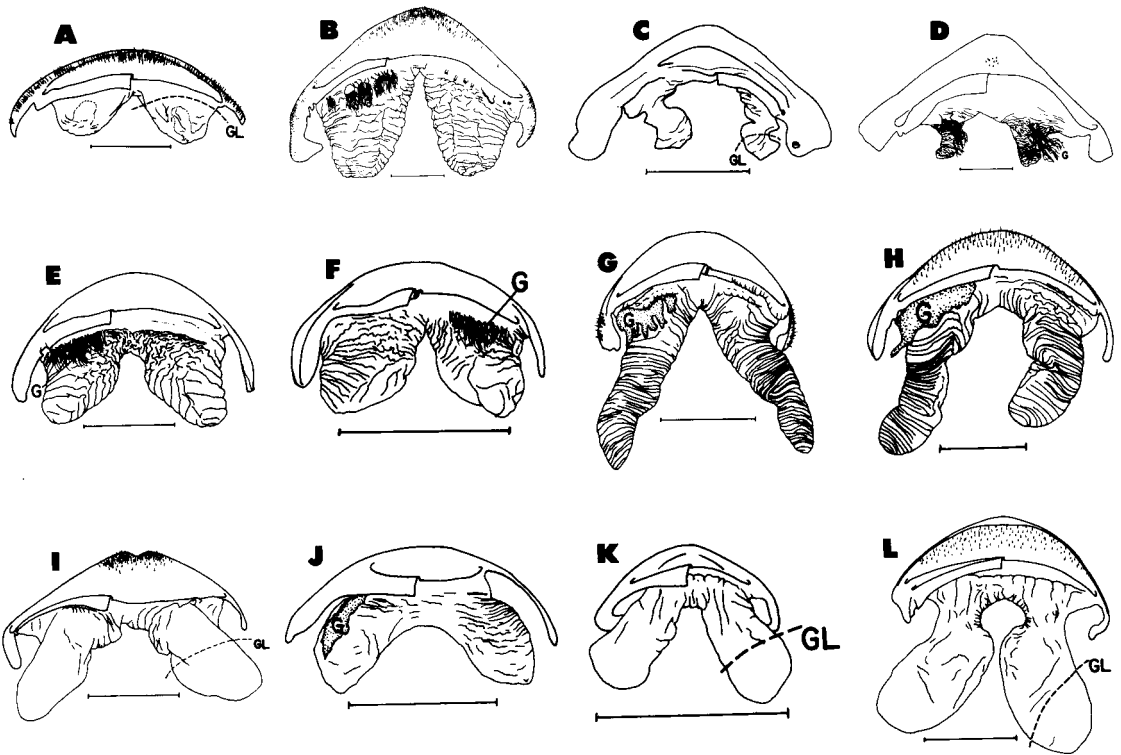

Fig. 2 The *Tenebrio* type gland and related subtypes. These are relatively unmodified from the primitive type and are everted or partly everted in use. A. *Tenebrio molitor* L. (similar: *T. obscurus* F.) (note the invaginated distal tips). B. *Zophobas rugipes* Kirsch. C. *Derolagria* sp. D. *Strongylium purpureipenne* Makl. (similar: *S. atrum* Champ., *S. Wahlbergi* Makl.). E. *Neatus tenebrioides* Beauv. (similar: *Alphitobius laevigatus* (F.), *A. diaperinus* (Panz.). F. *Apsena rufipes* Esch. (similar: *A. pubescens* LeC., *Epantius obscurus* LeC.). G. *Argoporis rufipes nitida* Csy. (similar: *A. alutacea* Csy., *A. bicolor* LeC.) note the proximolateral "ribs." H. *Cerenopus concolor* LeC. (has ribs as in *Argoporis*.). I. *Toxicum taurus* F. J. *Phaleria testacea* Say (similar: *P. rotundata* LeC.). K. *Eleates occidentales* Csy. L. *Bolitotherus cornutus* Panz.

Fig. 3   The *Blaps* type. *Blaps sulcata substriata* Sol. (similar: *B. lethifera* Marsh., *B. strancki* Rds. and *B. weidenmanni* Sol.

viously been apparent is the variety of evolutionary pathways by which the ancestral gland has diversified.

Origin of the glands.   It seems likely that the primordial gland was simply a fold of intersegmental membrane with a primitive secretory capacity of the glandular products of the hypodermis which are normally associated with the cuticle. By secretory specialization of this hypodermis, quinones originally for cuticular tanning and aliphatic hydrocarbons for waterproofing came locally (in the primordial gland) to be secreted in larger amounts due to their repellent effect on predators. The release of the secretion came about auto-
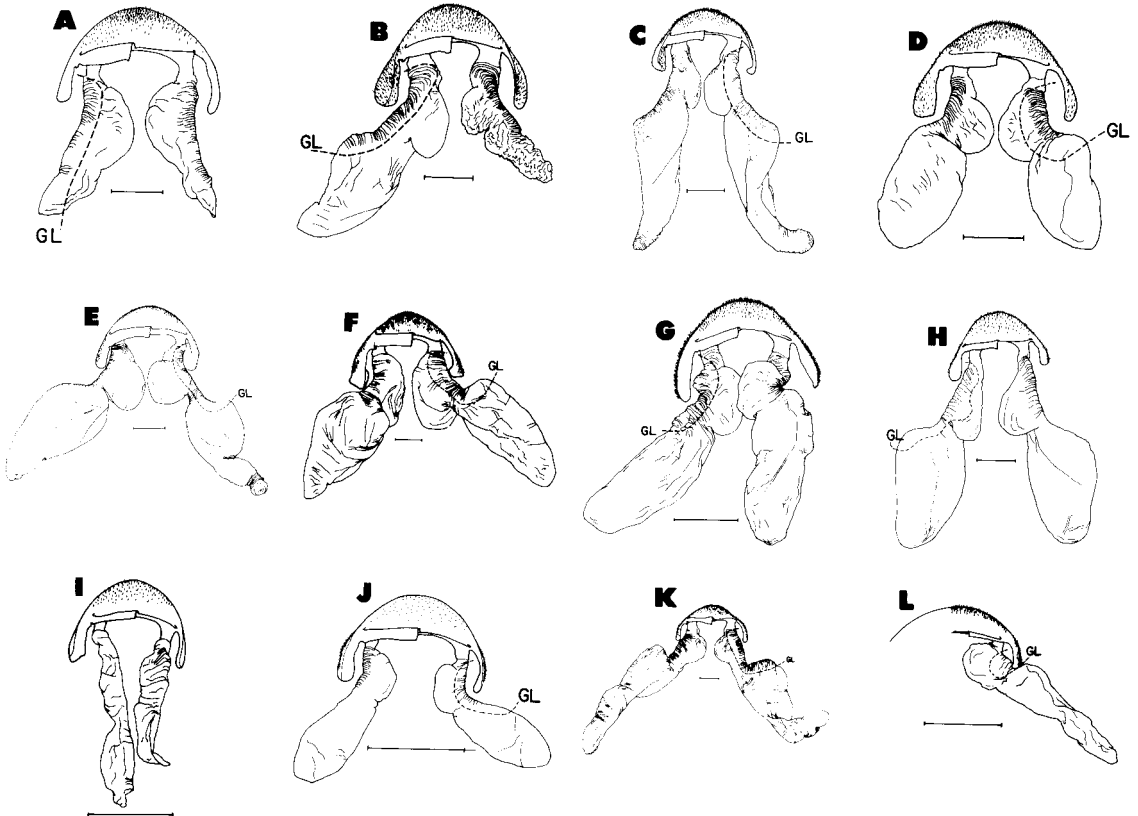

Fig. 4   The Eleodine type. The glands figured are representative of the range of forms found in the 29 species investigated. A. *Eleodes (Melaneleodes) carbonaria* (Say). B. *E. (Eleodes) gracilis* LeC. C. *E. (Metablapylis) dissimilis dissimilis* Blais. D. *E. (Blapylis) blanchardi* Blais. E. *E. (Holeleodes) beameri* Blais. F. *E. (Steneleodes) longicollis* LeC. G. *Embaphion contractum blaisdelli* Benedict. H. *Neobaphion planipennis* (LeC.). I. *Trogloderus nevadus* LaRiv. J. *Lariversius tibialis* Blais. K. *Cratidus osculans* LeC. L. *Amphidora littoralis* LeC. (left reservoir lost in dissection).

matically during the stress and struggle resulting from being taken by a predator. The membrane constituting the primordial gland, associated as it is with the other terminalia, might then be exposed simply by the increased hemolymph pressure and movements during struggle. Any repellent value of the film of secretion on the membrane would then be rapidly selected for by the partial protection from predation it afforded. Once the defensive glandular value was established, it would rapidly evolve to become more efficient. The quantity of secretion and the size of the reservoir must increase to increase the magnitude of the repellency. The delivery also becomes more efficient, resulting first in giving off the secretion without eversion and eventually in spraying it over some distance (Tschinkel, '75b). Economy of use would also have great selective value and the ability to release multiple small doses would evolve.

This hypothesis is largely supported by the actual morphology of tenebrionid glands, and an array of both primitive and advanced features is apparent, though any given gland may consist of a mosaic of primitive and advanced characters. Different features of the defensive system seem to have evolved independently of one another, perhaps in response to subtle differences in selective milieu of the species. Table 1 lists morphological, behavioral and chemical features with respect to being primitive or specialized.

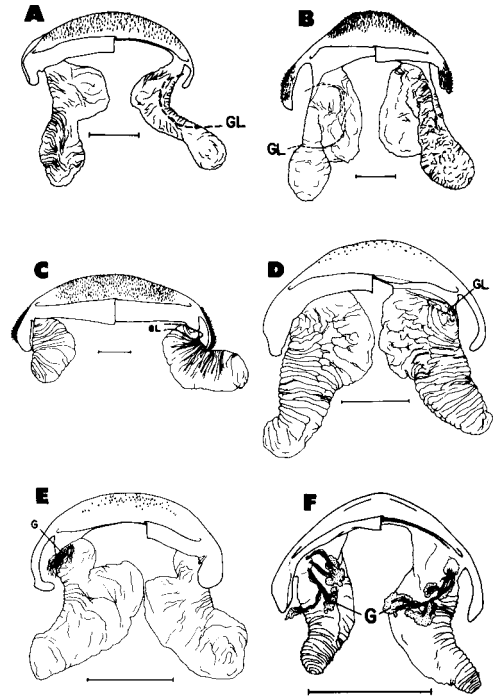*Morphological trends in the radiation*

Fig. 5   The Platynotine type. A. *Parasitizopus armaticeps simplex* Koch. B. *Gonopus agrestis* Koch. C. *Anomalipus variolosus* Sol. (similar: A. *elaphus*). D. *Melanopterus marginicollis* Muls. (similar: *M. porcus* (Muls.), *Trigonopus capicola* Muls.). E. *Eurynotus rudbecki* Koch (similar: *E. capensis* (F.). F. *Opatrinus aciculatus* LeC.

of the primitive glands.   One of the most important trends in increasing the effectiveness of the glands has probably been the increase in the capacity of the reser-
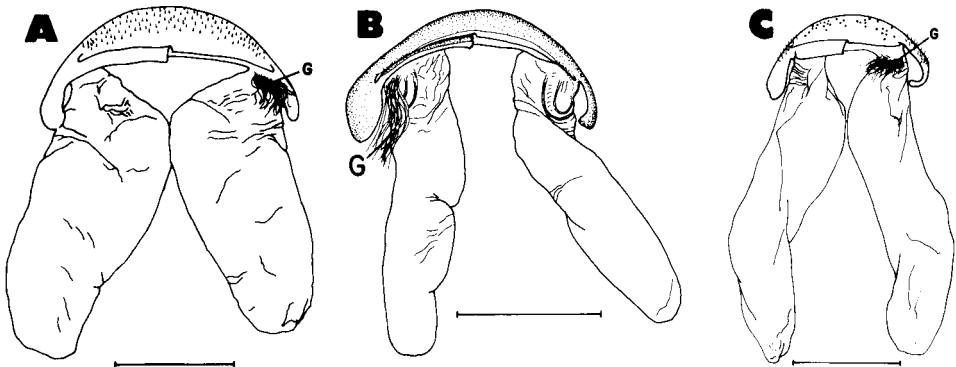

Fig. 6   The Pedinine type. A. *Gonocephalum* sp. (similar: *G. arenarium* F.) B. *Blapstinus* sp. (similar: *B. histricus* Csy., *B. sulcatus* LeC. *Conibius* sp. and *Notibius* sp.). C. *Zadenos delandei* (Muls).
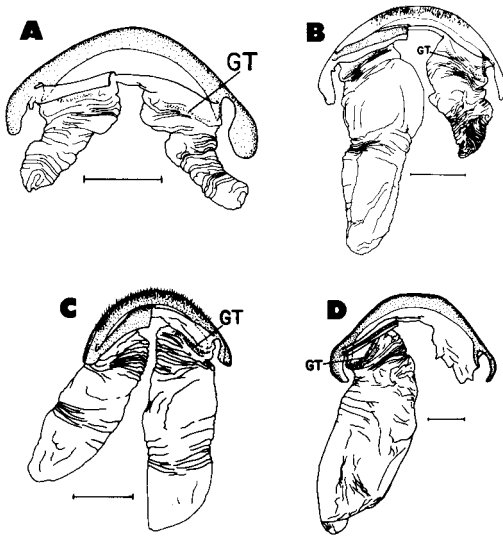
Fig. 7 The Meracanthine type. A. *Meracantha contracta* Beauv. (Similar: *Psorodes calcaratus* F.). B. *Psorodes gratilla* Herbst (note right reservoir is empty). C. *Pyanisia tristis* Lap. (similar: *P. longicollis* Champ.). D. *Eupezus natalensis* Lac. (right reservoir torn off). 7A,B are Meracanthini; 7C,D are Amargymini.

voirs. The relative repellent value must depend on the amount of secretion, though the benefit of increased amounts must be offset and eventually eliminated by the increased metabolic costs of producing large amounts of secretion and large reservoirs. This effect of quantity must be a strong selective pressure, for even species with relatively primitive glands often have large reservoirs, and indeed, in a few species (e.g. *Blaps*, fig. 3) evolution seems to have been mainly in the direction of increased size, few other morphological specializations of the glands having arisen.

To accommodate this increased volume of secretion, many species have evolved various devices to allow the reservoirs to expand and contract with the changes in the contained volume. In most cases, this adaptation is in the form of highly wrinkled or crinkled reservoir walls (apparent especially in figs. 4B, 7B, 9D), which often exert enough pressure by being stretched to cause the secretion to flow out upon opening of the exit valve (see p. 362). In some species (e.g. the *Neomida*-type, figs.

TABLE 1

Hypothetical primitive and advanced characteristics of tenebrionid chemical defensive systems and behavior

| Feature	Primitive state	Advanced state(s)
Reservoir size, quantity of secretion	Small, often only a film	Large, often copious
Reservoir shape	Conical pockets, opening at base, with common volume proximally	Sacs, emptying by distinct ducts, sometimes partly divided into chambers. No common volume
Nature of reservoir walls	Thin, smooth to moderately wrinkled	Highly crinkled, sometimes stiffened with spiral cuticular bands
Termination of secretory tubules	Scattered randomly on dorsum of reservoir	a. Tubules terminate in limited specific areas on dorsum b. All tubules empty along a line at proximal end of reservoir c. Tubules empty into large collecting ducts
Mode of delivery	Gland everted	a. Exude b. Spray
Ancillary defensive behavior	None	Wipe with hind legs, headstand, fold legs,[1] stridulate [1]
Ancillary structures	None	Conductive epipleural "gutters," conductive elytral reticulation
Chemical composition of secretion	Quinones only	Quinones and hydrocarbons, other compounds

[1] May antedate glands.
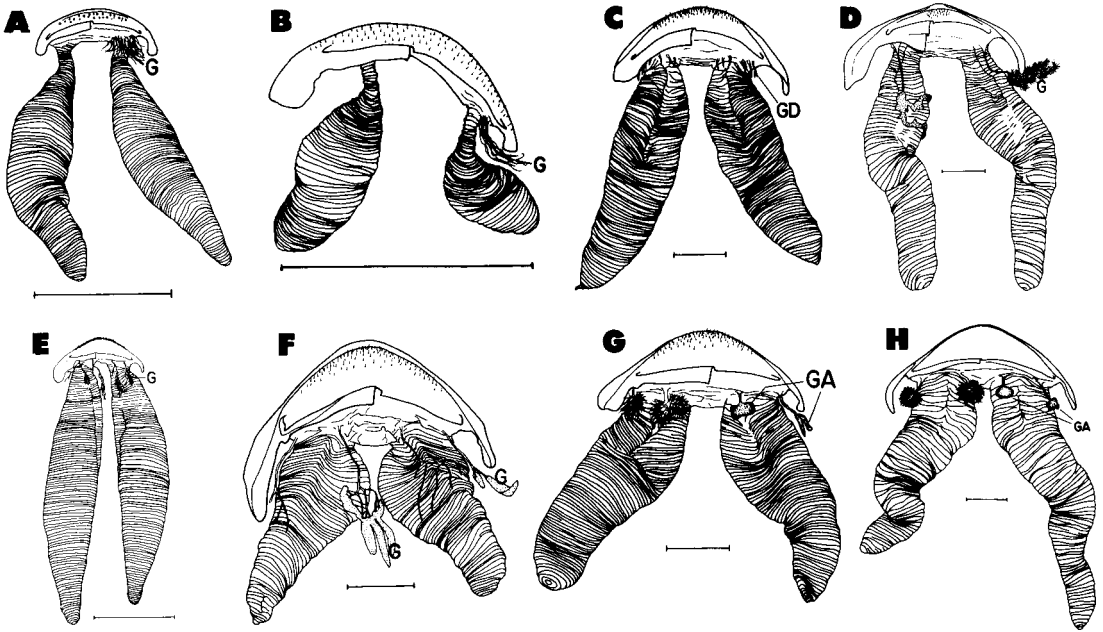
Fig. 8 The *Neomida* type. These glands all have reservoirs stiffened with thickened spiral bands. A. *Neomida bicornis* (F.). B. *Gnathocerus cornutus* F. C. *Oenopium zopheroides* (Horn) (similar: *Haplandrus fulvipes* (Hbst.)). D. *Alobates pennsylvanica* DeG. (similar: *Iphthimus serratus lewisi* Horn, *I. laevissimus* Csy., *Polopinus nitidus* LeC., *Xylopinus superdioides* Oliv.). E. *Platydema subcostatum* Lap. and Brll. (similar: *P. oregonense* LeC., *P. ellipticum* (F.), *P. americanum* Lap. and Brll.). F. *Merinus laevis* Oliv. (similar: *Nuptis caliginosus* Champ., *Upis ceramboides* L.). G. *Cibdelis bachei* Csy. (similar: *C. gibbosa* Mots., *C. blaschkei* Mann.). H. *Coelocnemis californica* Mann. (similar: *C. lucia* Doyen, *C. magna* Lec., *C. sulcata* Csy.).

8, 10) the walls have been stiffened with thickened spirals of cuticle and empty by accordioning posteriorly as the reservoir empties, preventing pockets of secretion from being caught by the collapse of the walls. After KOH treatment, such reservoirs can often be expanded to enormous volumes by simply pulling on the distal end with forceps.

A number of species in diverse groups have evolved reservoirs with more complex shapes, most commonly sock-shaped (e.g. figs. 4, 5, 10), and in extreme cases even partially two-chambered (especially fig. 4). The reasons for this are not known. Perhaps such shapes are more easily accommodated to the other internal organs. The two-chambered reservoirs may also serve a separatory function for the two-phase secretion (Tschinkel, '75a).

The shape of the reservoir reflects, to some degree, the mode of release of secretion. In the most primitive mode, eversion

(Tschinkel, '75b), the proximal end of the reservoirs must necessarily be wider than the distal end, so that the intima can be everted and retracted freely. The reservoirs of most species which evert the glands have a more or less conical shape (the *Tenebrio*-type, fig. 2 and to some degree, the Meracanthine type, figs. 7, 10) with the wide end opening proximally. As the reservoir volume increased in the course of phylogeny, everting probably gave way gradually to partial eversion and to exuding without eversion. While many exuding species may have reservoirs with wide openings proximally (figs. 5, 6, 8, 9, 10), there is a tendency for the openings to become constricted proximally into definite exit ducts (figs. 3, 4, 5, 9, 10). All intermediates can be found. Such definite exit ducts must impart certain advantages with respect to regulating the emission of secretion by valves and muscles.

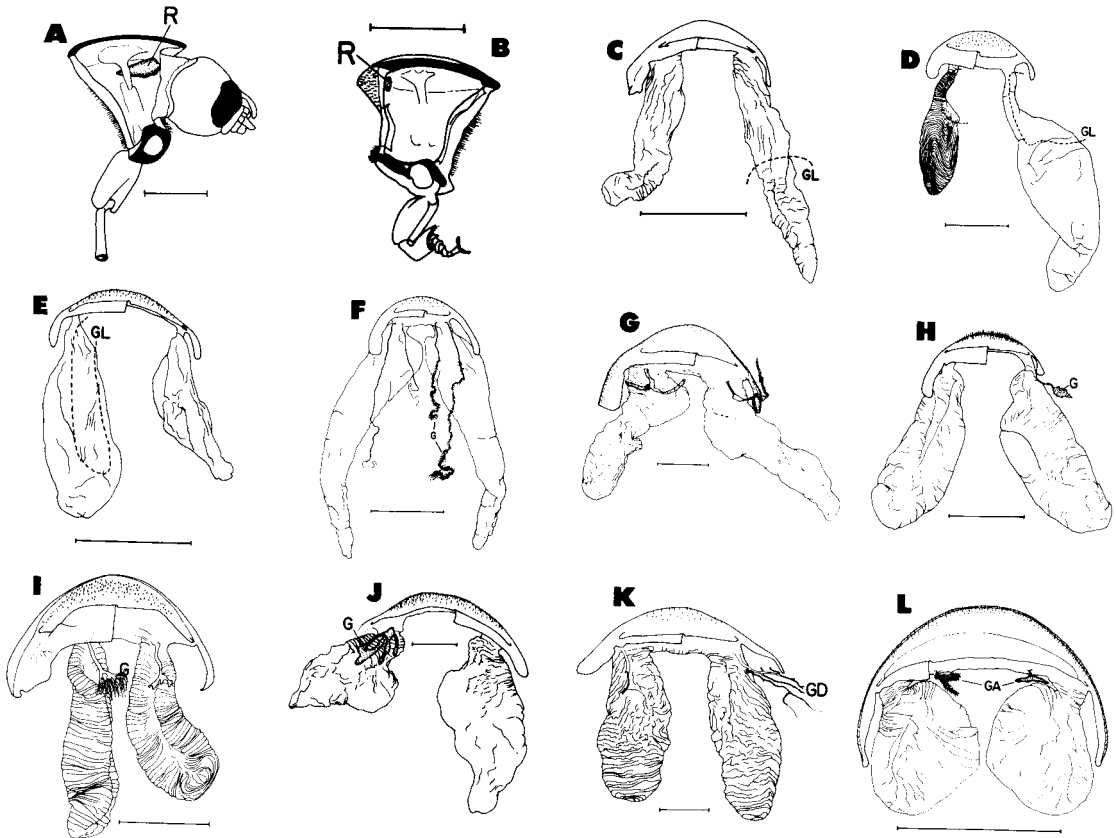Another shape character which is prob-

Fig. 9 Prothoracic glands and miscellaneous unclassified abdominal glands. A. Prothoracic defensive glands of *Diaperis maculata* Oliv. Prothorax in saggital section. B. Prothoracic defensive gland of *Metaclisa marginalis* Horn (similar: *Alphitobius laevigatus* (F.)) (prothorax in saggital section, anterior to the left. C. *Metaclisa marginalis* Horn. D. *Diaperis maculata* Oliv. (left reservoir empty and wrinkled). E. *Tribolium brevicornis* (LeC.). F. *Uloma punctulata* Lec. (similar: *U. imberbis* Lec., *U. mentalis* Lec.). G. *Scaurus aegyptiacus.* H. *Lobopoda punctulata* (Melsh.). I. *Zophius rufopictus* Wied. J. *Helops simulator* Blais. K. *Centronopus parallelus* (LeC.) (similar: *C. calcaratus* (F.)). L. *Bius estriatus* LeC. (note ampullae do not empty into reservoir proper).

ably primitive is the presence of common volume between the two reservoirs. This common volume is retained by many species with relatively advanced glands but is often lost in those which evolve definite exit ducts (fig. 10).

Valve mechanisms were not specifically investigated except for a few dissections of preserved specimens. From these and previous reports (Eisner et al., '64), the following general picture emerges: The external openings are kept closed either by hemolymph pressure or cuticular elasticity which keep the walls of the exits pressed together. A muscle attached to the dorsal surface of each external opening,

upon contraction, opens a channel through which the secretion may exit under hemolymph or elastic pressure. In those species with broad openings (e.g. *Cibdelis* fig. 8G), the muscle is very slender and opens a channel in only a small part of the entire seal the rest of which remains closed, presumably, due to the same hemolymph pressure which forces the secretion out. In species with narrow exit ducts such as *Eleodes* (fig. 4), the muscle lifts the entire dorsal side of the exit. In species which evert, it is possible that the secretion is sealed in by a similar mechanism, but it is not known whether homologous muscles exist to open the seal preparatory to ever-

sion. Perhaps this is so in species with larger amounts of secretion. In any case, the reservoir sacs are pushed partially or completely inside-out by hemolymph pressure and Lengerken ('25) reports a muscle inserting at the apex of each conical reservoir of *Tenebrio molitor* which retracts the sac after eversion. I did not search for such muscles in other everting species and they may be present.

Each secretory cell in the gland tissue drains into the reservoir through a cuticular tubule and the junctions between the tubules and reservoirs show some strong evolutionary trends. In the primitive state, the tubule terminations are scattered over the dorsal side of the reservoir sacs (fig. 10, *Tenebrio, Blaps*) but there is a strong tendency for these to become concentrated in specific areas. Several stages of increased specialization (table 1) are recognizable: (1) the tubule terminations remain scattered but lie only in restricted fields on the dorsum of the reservoirs (fig. 10, *Toxicum* (fig. 2I), *Derolagria* (fig. 2C), *Bolithotherus* (fig. 2L), *Eleates* (fig. 2K), Eleodine type (fig. 4), *Parastizopus* (fig. 5A), *Gonopus* (fig. 5B), *Metaclisa* (fig. 9C), *Diaperis* (fig. 9D), *Tribolium* (fig. 9E). (2) All tubules terminate individually along a transverse line near the proximal ends of the reservoirs (many species show this pattern, fig. 10). (3) The terminations are actually in a small number of very short, often conical collecting ducts or sites (*Zophobas* (fig. 2B), *Eupezus* (fig. 7D), *Psorodes* (fig. 7B). (4) All tubules terminate into either one or two (or a small number) of pairs of large collecting ducts (fig. 10, most *Neomida* type (fig. 8)) and several under miscellaneous types (fig. 9F–L). The ducts usually gradually increase in size as they receive more and more tubules, and are often branched as well. (5) The distal ends of the collecting ducts are expanded into ampullae and all tubules terminate directly in these. This stage is represented only by the genera *Bius* (fig. 9L), *Cibdelis* (fig. 8G) and *Coelocnemis* (fig. 8H). These five stages of increased specialization are not meant to imply a pathway of evolution.

The tendency for secretory cells to empty into the reservoir near its proximal end is not reserved to tenebrionids. A brief survey of the literature showed similar configurations in hemiptera (Remold, '62; Waterhouse and Gilby, '64), carabid beetles (Moore and Wallbank, '68; Schildknecht, '70); termites (Maschwitz et al., '72) and dytiscid beetles (Schildknecht et al., '62, '67).

The gland types

The Tenebrio type and subtypes (figs. 2, 10)

While there are no species whose glands are composed solely of advanced characters, the glands of the genus *Tenebrio* do consist exclusively of primitive ones. The reservoirs are small and eversible with only a film of secretion, the intima are thin and only moderately wrinkled, the secretory tubules are scattered over the dorsal surface of the sacs which share a common volume proximally and taper strongly distally (fig. 2A). Furthermore, *Tenebrio* has no ancillary defensive structures or behavior and its secretion consists only of quinones (Tschinkel, '75a; Schildknecht and Weiss, '60). While there are a large number of genera that share the same type of gradually tapering reservoirs (the *Tenebrio* type), all of these are advanced in at least one other character. While all release secretion by complete or partial eversion, all except *Tenebrio* show a somewhat advanced termination of the secretory tubules. Many also show some additional defensive behavior (wiping the secretion with the hind legs, or folding the legs and becoming immobile). Additional specialization within the group is also reflected in the relative reservoir size and chemical composition of the secretions. Only *Tenebrio, Zophobas* and *Epantius* secrete nothing but quinones. Other genera secrete hydrocarbons, naphthoquinones or unidentified compounds in addition.

Apparently related to the *Tenebrio* type, but diverging from it before the tubules came to empty proximally, are the Bolitophagini which could be classed as a subtype. The tubules terminate individually in a distal, dorsal field (figs. 2K,L) and the glands are partly everted in use. Further relationship to the *Tenebrio* type may be indicated by the composition of the secretion — almost exclusively benzoquinones (a trace of alkenes in *Eleates*).

Species with the *Tenebrio* type gland are bound together merely because their glands have not diverged greatly from the

Figure 10 — chart: "GENERA BY TYPE" with columns for RESERVOIR SHAPE / EXIT DUCTS / COMMON VOLUME, TRIBE, RESERVOIR MORPHOLOGY (cone NO/YES, SAC BROAD YES/NO, SPIRAL MED YES, SAC NARROW YES/NO, 2-LOBED NO), TUBULE TERMINATIONS, DELIVERY OF SECRETION, DEFENSIVE BEHAVIOR, OTHER FEATURES, and MAJ. COMPOUNDS OTHER THAN T,E.

GENERA BY TYPE	TRIBE	DELIVERY OF SECRETION	DEFENSIVE BEHAVIOR	OTHER FEATURES	MAJ. COMPOUNDS OTHER THAN T,E
TENEBRIO TYPE					
TENEBRIO	TEN		★★		NONE
STRONGYLIUM	STR		★★		--
DEROLAGRIA	LAG		★		--
ZOPHOBAS	TEN			PDG	B
ALPHITOBIUS	ULO			PDG	NONE
NEATUS	TEN	EVERT			17 B
APSENA	SCA	OR	FOLD		OT
EPANTIUS	SCA	PARTLY	LEGS		NONE
EULABIS	SCA	EVERT	AND/OR		OT
ARGOPORIS	SCA		WIPE		NQ
CERENOPUS	SCA				--
TOXICUM	TEN				11,OT
PHALERIA	PHA	★			15,17
BOLITOTHERUS	BOL				NONE
ELEATES	BOL				17
BLAPS TYPE					
BLAPS	BLA	OFTEN SPRAY	MANY HEAD-STAND		13
ELEODINE TYPE					
ELEODES	ELE	MANY SPRAY			9,11,13,M OC
EMBAPHION	ELE		MANY HEAD-STAND, FEW WIPE		9,11,13,OC
NEOBAPHION	ELE				NONE
TROGLODERUS	ELE	EXUDE			17,19
LARIVERSIUS	ELE				11,17,19
CRATIDUS	AMP	★			13,M
AMPHIDORA	AMP				
PLATYNOTINE TYPE					
PARASTIZOPUS	OPA	★			11,15
GONOPUS	PLA		MANY STRIDULATE		11,15
ANOMALIPUS	PLA		SOME FOLD LEGS	ALL HAVE GULAR STRIGIL	NONE
EURYNOTUS	PLA				NONE
TRIGONOPUS	PLA	EXUDE			NONE
MELANOPTERUS	PLA		SOME WIPE		9,15
OPATRINUS	TEN				15
SCHELODONTES	PLA				9,11,15
PEDININE TYPE					
BLAPSTINUS	PED		SOME FOLD,		P
CONIBIUS	PED		WIPE		P
NOTIBIUS	PED	EXUDE			P
GONOCEPHALUM	OPA	★			13
ZADENOS	LIT				11,13
MERACANTHINE TYPE					
MERACANTHA	MER	EVERT TO	★★		11,15,17
PSORODES	MER	SPRAY			9,11,15,17
PYANISIA	AMA	OR	FOLD		15
EUPEZUS	AMA	EXUDE			--

Figure 10

GENERA by TYPE	TRIBE	RESERVOIR MORPHOLOGY (shape / exit ducts / common volume)	TUBULE TERMINATIONS	DELIVERY OF SECRETION	DEFENSIVE BEHAVIOR	OTHER FEATURES	MAJ. COMPOUNDS OTHER THAN T,E
NEOMIDA TYPE							
NEOMIDA	DIA	● SPIRAL MED	●			GU	13
GNATHOCERUS	ULO	● SPIRAL MED	●			GU	13
PLATYDEMA	DIA	● SPIRAL MED	②			GU	13
OENOPIUM	TEN	● SPIRAL MED	Ⓜ				--
HAPLANDRUS	TEN	● SPIRAL MED	Ⓜ		MANY FOLD AND/OR WIPE		--
IPHTHIMUS	TEN	● SPIRAL MED	①	EXUDE			B
ALOBATES	TEN	● SPIRAL MED	①				B
POLOPINUS	TEN	● SPIRAL MED	①				B
XYLOPINUS	TEN	● SPIRAL MED	①				--
MERINUS	TEN	● SPIRAL MED	②				11,17
UPIS	TEN	● SPIRAL MED	②				--
NUPTIS	TEN	● SPIRAL MED	②				--
CIBDELIS	TEN	● SPIRAL MED	②			GU	11,17
COELOCNEMIS	TEN	● SPIRAL MED	②				13
UNCLASSIFIED							
HELOPS	HEL	● SAC NARROW	①				--
ZOPHIUS	HEL	● SAC BROAD	①				--
SCAURUS	SCA	● SAC BROAD	①				OT 13
METACLISA	DIA	● SAC BROAD	●			PDG	13
DIAPERIS	DIA	● SAC NARROW	●			PDG,GU	13
BIUS	TEN	● SAC NARROW		EXUDE			--
CENTRONOPUS	TEN	● SAC BROAD	①		✱		13
TRIBOLIUM	ULO	● SAC NARROW	①			PDG,GU	15
ULOMA	ULO	● SAC NARROW	②			GU	15
LOBOPODA	ALL	● SAC NARROW	①				--

Fig. 10 Summary overview of the gland morphology, chemistry and defensive behavior. The genera are arranged under gland types as defined in this paper. Closed circles indicate presence of a trait. Open circles with numbers indicate the number of pairs of collecting ducts, M indicates multiple (4–10) pairs. Stars denote exceptions to the generalizations in the same box. PDG, Prothoracic defensive gland; GU, epipleural "gutters"; abbreviations of chemicals in last column on the right are: B, benzoquinone; T, toluquinone; E, ethylquinone; P, propylquinone; M, methylmethoxyquinone; 9, l-nonene; 11, l-undecene; 13, l-tridecene; 15, l-pentadecene; 17, l-heptadecene; 19, l-nonadecene; Oc, octanoic acid; NQ, naphthoquinones; OT, other compounds. Abbreviations of tribal names are the first three letters of each: ALLeculini, AMArygmini, AMPhidorini, BLAptini, BOLitophagini, DIApirini, ELEodini, HELopini, LAGriini, LIToborini, MERacanthini, OPAtrini, PEDinini, PHAleriini, PLAtynotini, SCAurini, STRongyliini, TENebrionini, ULOmini.

ancestral type, and are not necessarily closely related.

The Blaps *type (figs. 3, 10)*

The glands of *Blaps* are morphologically somewhat advanced over the *Tenebrio* type in that the reservoirs are greatly enlarged and their proximal ends somewhat constricted into rather broad exit ducts. The tubule termination pattern is as primitive as in *Tenebrio* itself, but the mode of release is usually by spraying, often accompanied by headstanding. No other members of the Blaptini were available, so it is not possible to say how broadly this pattern occurs outside *Blaps*.

The Eleodine *type (fig. 4, 10)*

The Eleodine-type of gland is very distinctive and is represented by a group of species obviously related on other evidence as well (Blaisdell, '09). Thus, the Eleodine-type includes all species of the tribes Eleodini and Amphidorini.

The species of the subgenus *Melaneleodes* (fig. 4A) seem best to fit the fasces

of the hypothetical primitive Eleodine gland: The reservoirs are often relatively small, only moderately sock-shaped, few of the species headstand and very few spray the predominantly quinone secretion which never contains octanoic acid. In the course of evolution, the reservoirs (and hence the amount of secretion) may gradually have increased in size and became more prominently two-chambered (sock-shaped). The proportion of alkenes in the secretion increased as did the frequency and persistence of headstanding behavior. This stage is probably represented by the subgenera *Litheleodes*, *Tricheleodes* and possibly led to both *Lariversius* (fig. 4J) and *Trogloderus* (fig. 4I). The capacity to secrete 2-methyl-3-methoxybenzoquinone separated the subgenus *Blapylis* (fig. 4D) at this stage. As the relative size continued to increase, the two chambered nature was accentuated and headstanding became more pronounced, the Amphidorine glands probably arose, leading finally to the truly enormous reservoirs of *Cratidus* whose blind ends extend easily to the metacoxae. At this stage, the *Metablapylis* (fig. 4C) and *Promus* glands probably also arose. The appearance of octanoic acid in the secretion separated the genera *Embaphion* (fig. 4G) and *Neobaphion* (fig. 4H) and the subgenera *Eleodes* (fig. 4B) and *Steneleodes* (fig. 4F), but only in the first two is this acid present in large amounts. Although a few species in other subgenera may spray occasionally, it is only in the subgenera *Eleodes* and *Steneleodes* that almost all species spray their secretion, and all show well developed and persistent headstanding.

The occurrence of wiping behavior in the Eleodine-type is difficult to interpret. It occurs in a few species of almost every subgenus of *Eleodes* as well as in *Embaphion* and the Amphidorini. Perhaps this behavior has evolved independently a number of times. Upon losing tarsal contact, most beetles wave the legs, and it is probably this leg-waving that predisposes species to evolve wiping behavior.

The Platynotine-type (figs. 5, 10)

Species provisionally placed into this group show the following common features: tubules terminating along a proximal line, usually, but not always, distinct and constricted exit ducts, no common

volume (thus, completely separate reservoirs) and relatively short and wide reservoir shape often somewhat sock-shaped. Two constant features are the sclerotized lateral prong which stiffens the outer margins of the proximal reservoir ends (visible in fig. 5B,D,E) and the presence of stridulatory surfaces in the gular region of the head. In some of the species this is used to produce squeeking sounds when the beetle is disturbed, but it is most likely to have evolved for courtship (Tschinkel, '75b). With the exception of *Schelodontes* and *Gonopus* sp., Platynotini have only small amounts of hydrocarbon in their secretions. *Gonopus* (fig. 5B) and *Parastizopus* (fig. 5A) occupy a somewhat anomalous position with regard to gland morphology and secretion composition: The tubule terminations are restricted to a proximal dorsolateral field (much as in the Eleodine-type) rather than being arranged in a line as in other Platynotini. The secretion contains large proportions of alkenes, showing diversity of alkenes even within the genus. Furthermore, the reservoirs' sock-shape is accentuated to the point of being definitely bilobed. On the basis of secretion and gland morphology, *Gonopus* (Platynotini) is quite similar to *Parastizopus* (Opatrini), although the latter lacks the strigil which seems to be characteristic of the Platynotini. Whether these two genera have indeed been associated with the correct tribes must await further work.

Most of the species exude the secretion, but *Schelodontes* partially everts the glands after it has expended most of its secretion, and *Gonopus* is able to spray, which further separate it from the rest of the tribe. The tendency to stiffen the legs which can be observed in *Anomalipus* has led to the evolution of a low headstand in some *Gonopus* species, and even some *Trigonopus* species rear up into a low and crude headstand when disturbed. Folding of the legs has evolved in four out of the six genera. Thus, while the Platynotini seem to form a related group, little can yet be said about the diversification within this group and future work may result in the splitting of this gland type into several.

The Pedinine type (figs. 6, 10)

The glands of *Blapstinus*, *Conibius* and *Notibius* (fig. 6B) are practically identical

and these genera are further bound together by the secretion of propylquinone, but the inclusion of the glands of *Gonocephalum* (fig. 6A) and *Zadenos* (fig. 6C) in this type is provisional. These glands are mostly rather nondescript and may not share any real relationship. Furthermore, neither of the last two secretes propylquinone, and both lack the proximal horseshoe-shaped thickening found in the glands of *Blapstinus*, *Conibius* and *Notibius*. While most of these species exude the secretion, *Gonocephalum* sprays it and is by far the smallest tenbrionid observed to do so.

The Meracanthine type (figs. 7, 10)

On the basis of gland morphology, secretion composition and behavior, it is possible to place the Meracanthine glands (fig. 7A,B) together with the amarygmine (fig. 7C,D). The glands of both tribes share some distinctive features: the larger broadly opening proximal region which is everted during use, the lateral areas of non-stretching cuticle at the junction of the proximal and distal region, the sclerotized prongs projecting distally from the dorsal surface of the seventh sternite, the secretion of penta- and heptadecene in addition to benzoquinones, the termination of the secretory tubules along a line somewhat distal to the bases of the reservoirs and the occurrence, in both tribes, of species which spray the secretion by rapid eversion of the glands, a method not found in other taxa.

The Neomida *type (figs. 8, 10)*

The common phylogeny of the glands of the *Neomida* type is indicated by the thickened spiral bands which stiffen the reservoirs as well as by general similarities of shape, exit ducts and presence of common volume between the reservoirs. While a large fraction of the species have evolved collecting ducts through which their separate gland tissues empty into the reservoirs, a number of species are relatively primitive in this respect. *Neomida* (fig. 8A) and *Gnathocerus* (fig. 8B) lack collecting ducts and their tubules terminate individually along a proximal line. Further heterogeneity is evident in the number of collecting ducts: 6 or 8 pairs in *Oenopium* (fig. 8C) and *Haplandrus* (fig. 8C) two pairs in several genera and one pair in

several others. The collecting ducts of *Cibdelis* and *Coelocnemis* are further modified by the expansion of their distal ends into distinctive ampullae into which all secretory tubules terminate (figs. 8G,H). In *Cibdelis*, the medial ampullae differ in shape from the lateral ones.

Although all species in this type probably shared common ancestry, there has been considerable divergence in other characters of the defensive system. Some of the species *(Iphthimus, Alobates, Polopinus)* have retained the primitive character of secreting only benzoquinones, but all the others secrete 1-alkenes as well. Several species have evolved wiping behavior *(Alobates, Cibdelis, Platydema)*, some fold their legs when disturbed *(Iphthimus, Cibdelis, Platydema, Neomida)*. All species exude the secretion, although *Cibdelis gibbosa* may occasionally spray weakly. Several species have evolved epipleural gutters to conduct the secretion anteriad (Tschinkel, '75b).

Miscellaneous glands (figs. 9, 10)

A number of rare or unique glands could not be associated with any of the types or established as types themselves. There is a great deal of diversity among these. Tubule terminations range from three different types of gland fields (fig. 9C,D,E) to several types of collecting ducts (fig. 9G,H,I,K,L). The collecting ampullae of *Bius* (fig. 9L) are noteworthy because of their convergence with *Cibdelis* and *Coelocnemis* and because they do not empty into the reservoirs but into the common volume between them. Empty gland reservoirs of *Diaperis* (fig. 9D) wrinkle in a very distinctive fashion. The two pairs of collecting ducts of *Uloma* (fig. 9F) receive the tubules along most of their length rather than ramifying abruptly.

Prothoracic defensive glands

In addition to the abdominal defensive glands, a number of tenebrionids also possess a pair of defensive glands lying in the prothorax and opening on the membrane between the head and prothorax. Such glands have been previously described in *Diaperis* (fig. 9A) (Roth and Stay, '58) *Tribolium castaneum* (Roth, '43), *Gonodera murina* (L.) (Kendall, '68) and *Zophobas rugipes* Kirsch. (Tschinkel, '69). In the present study they were also found in *Al-*

phitobius fasciatus and *Metaclisa marginalis* Horn (fig. 9B). *Rhinandrus* sp. also possesses prothoracic defensive glands (Doyen, personal communication). The glands of *Gonodera, Alphitobius* and *Metaclisa* are merely small pouches, but those of *Diaperis, Tribolium* and *Zophobas* are well developed, elongate sacs which may extend more than half the length of the prothorax. All of these glands are relatively featureless when compared to the abdominal glands, and beyond their presence or absence, were not used as systematic characters. An elastic crescent of cuticle serves as a valve and is opened by a muscle attached to it (Tschinkel, '69).

Implications for the systematics of the Tenebrionidae

The present study indicates that the chemical defensive system of tenebrionids provides a valuable set of characters for the deduction of relationships and supports the familial revision of Doyen ('72). Of the three broad types of characters (chemical, behavior, gland morphology), the morphology of the gland reservoirs provides the most readily interpreted characters and was thus used to establish the gland types. The chemical composition of the secretion may provide valuable additional information, especially when distinctive compounds are secreted, but the lability of behavioral traits and, in certain cases, chemical composition, makes these less useful for systematics.

The *Tenebrio* type is almost certainly heterogeneous. Since these glands are all relatively unspecialized, there are few distinct characters on which the type may be subdivided. Further study will no doubt lead to more natural groupings of these glands. Nevertheless, it seems likely that real relationships are shared by several groups of genera. Thus, *Tenebrio, Strongylium, Derolagria, Zophobas, Alphitobius* and *Neatus* (fig. 2A–E) form one group representing four tribes, while *Apsena, Epantius, Eulabis, Argoporis* and *Cerenopus* (fig. 2F–H) form another group representing a single tribe the Scaurini (or two tribes according to Berry, '73), but this is not supported by the present work. The glands of *Scaurus* (fig. 9G) are not related to those of other Scaurini. *Toxicum* (fig.

2I), the Phaleriini (fig. 2J) and the Bolitophagini (fig. 2K,L) are each sufficiently distinct that further study will probably place each into a separate type.

Relationships based upon the chemical defensive system are completely congruent with existent classification for the genus *Blaps* and the tribe Eleodini. The glands of the Amphidorini are so similar to those of the Eleodini that these two tribes should be merged, a suggestion made by Blaisdell on other grounds.

Similar evidence suggests that the tribe Tenebrionini is not a natural group and contains at least four different types of glands (*Neomida* type (fig. 8), *Tenebrio* type (fig. 2), *Centronopus* "type" (fig. 9K) and *Bius* "type" (fig. 9L). Chemical and behavioral diversity are also strong in this tribe. The Ulomini are similarly heterogeneous and are probably not a natural group. On the other hand, chemical composition, behavior and morphology all indicate that the Meracanthini should probably be lumped with the Amarygmini, and the similarity of the larvae of both supports this, for all possess a very distinctive, strongly concave dorsum on the ninth abdominal segment (Hyslop, '15; Spilman, '66, '69).

The Platynotine type (fig. 5) includes members of three tribes (Platynotini, Opatrini, and Tenebrionini), but similarities in the gland morphology and the presence of a gular stridulatory surface indicates that *Opatrinus* (fig. 5F) should be allied with the Platynotini rather than the Tenebrionini where it is presently placed (Arnett, '71). The fact that its secretion contains little hydrocarbon at least does not contradict this rearrangement.

The *Neomida* type (fig. 8) also includes members of three tribes (Diapirini, Ulomini, Tenebrionini), and since this gland type is so distinctive, indicates a common ancestry for these species. Excluded from this group are many species of Ulomini (figs. 2E; 9E,F) and Tenebrionini (figs. 2A,B,E,I; 9K,L) as well as some Diapirini (*Diaperis, Metaclisa* (fig. 9C,D). On the basis of the presence of collecting ampullae, *Cibdelis* and *Coelocnemis* (fig. 8G,H) are closely related. Other closely related groups can be set up on the basis of collecting ducts.

The relationship of *Diaperis* (fig. 9D) to the Diapirini is anomalous. Its glands are definitely not of the *Neomida* type found in other genera of the tribe (*Platydema, Neomida* (fig. 8A,E). Some authors have placed *Diaperis* with the Bolithophagini because of the similarity of their larvae (Watt, '67). The terminations of the secretory cell tubules in the glands of both groups are scattered over a portion of the reservoir surface, but the reservoirs of *Diaperis* empty via a narrow exit duct (fig. 9D) while those of Bolitophagini have broad exit ducts and are partly everted (figs. 2K,L). The relationship of these two glands is difficult to evaluate.

Like many of the gland types, the occurrence of prothoracic glands also cuts across established tribal lines. Prothoracic defensive glands are found in Tenebrionini, Ulomini, Diapirini and Lagriini, but it is not yet clear whether these are all homologous. Many species having prothoracic glands show little relationship on the basis of the abdominal glands or other characters.

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