



New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework

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First reports of major defensive chemicals for ground beetles representing four tribes include: Morionini (formic acid), Dercylini (methacrylic and tiglic acids), Catapieseini (formic acid and decyl acetate) and Perigonini (formic acid and decyl acetate). Multiple species from Loxandriini were sampled and, shown to contain formic acid, not salicylaldehyde as previously reported. Several hexenoic acid compounds were found in the clivinine genus *Schizogenius* representing a third class of chemicals for that tribe. Salicylaldehyde was found for the first time in a species of Oodini. Additional species from Pterostichini, Patrobini and Odacanthini were sampled and the results were found to be consistent with previously published reports. The taxonomic distribution of defensive secretions is reviewed for tribes across the family Carabidae. The simultaneous occurrence of hydrocarbons and formic acid is noted in phylogenetically more derived carabids. By mapping chemical classes onto a phylogenetic hypothesis, it is shown that formic acid or other relatively strong irritants are correlated with tribes having a high species diversity in tropical regions, whereas tribes exhibiting higher diversity in temperate regions use milder saturated/unsaturated carboxylic acids. Based on this phylogenetic interpretation, the evolution and maintenance of formic acid is interpreted as the result of predation pressures and possibly the evolution of chemical mimicry.

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ADDITIONAL KEY WORDS:—phylogeny – chemical ecology – pygidial glands – formic acid.

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INTRODUCTION

We now understand that insects live in a largely chemically mediated world (Blum, 1981; Dettner, 1987). Much of that world passes by unnoticed for the average person. Subtle insect pheromones wafting on the spring breeze are bold messages to the intended receiver but are generally imperceptible to, or ignored by, the rest of the world. Potent defensive chemicals, on the other hand, are intended to be understood by all. Ground beetles (Carabidae) are well known for their bold chemical signals involving oozing, spraying and crepitating irritating mixtures of polar and nonpolar compounds (Dettner, 1987). Carabidae comprises a diverse family of about 40 000 species. No doubt defensive chemicals have been an important element in the successful diversification of ground beetles (Erwin, 1985). Over the past 40 years ground beetle defensive chemicals and the pygidial gland system have been the subject of numerous studies (Dazzini-Valcurone & Pavan, 1980; Eisner *et al.*, 1977; Kanehisa & Murase, 1977; Moore, 1979; Moore & Wallbank, 1968; Schildknecht, Maschwitz & Winkler, 1968; see Dettner, 1987 for a review of the literature). More than 350 species have been chemically investigated. However, the sampling has been taxonomically uneven. The four additional tribes we report herein make the total 41 of the 78 currently recognized tribes. We also present new information on several genera within previously studied tribes. We hypothesize that the relationship between chemical classes and diversification in certain habitats might be explained by the interaction of ground beetles with their predators and prey. Specifically ants are hypothesized to be a major influence on the course of ground beetle evolution. We discuss the intriguing, but untested hypothesis of chemical mimicry between ants and ground beetles as potential factor in the evolution and maintenance of formic acid in the Harpalinae. However, because sampling at the tribal level is incomplete and the family level phylogenetic hypothesis rather imprecise, associations regarding habitat and chemistry are presented as a means to guide further research activities rather than definitive statements.

MATERIAL AND METHODS

Specimens

Live specimens were collected from sites in the United States, Mexico and Ecuador. All specimens were collected and shipped under applicable permits (filed in Cornell University Insect Collection, CUIC). After transport to laboratory facilities at Cornell University, specimens were maintained in small plastic containers with moist sphagnum peat moss and held under a day/night and temperature regime that approximated their place of origin. Beetles were primarily fed chopped meal worm (Coleoptera: Tenebrionidae), with occasional supplement of commercial dry dog food.

The species and number of specimens are listed under each taxonomic heading below. Vouchers of most species studied are in the Cornell University Insect Collection (CUIC), Ithaca, NY. No vouchers were maintained for common, well known North American species (as noted below), however, comparative material can be found in the CUIC. In cases where specific identification was not possible because of the absence of recent taxonomic revision of the genus or because the

specimens represent an unnamed form, a unique number was assigned and this number was placed with the voucher specimens.

Preparation for morphological study

Musculature and gross structures of the glands were studied in situ in freshly killed beetles dissected under saline solution and then preserved in glycerol. Cuticular features were prepared by clearing with 10% KOH and staining with Chlorazol black and methyl cellosolve (methods used for female tracts by Liebherr & Will, 1998). Cleared and stained structures were studied in glycerol.

For electronmicroscopy, specimens were cleaned (sonicated in chloroform and ethanol, 1:1 mixture) and gold-sputter coated prior to viewing and photographing. Alcohol fixed tissues were mounted in glycerine and examined by phase microscopy and Nomarski interference-contrast microscopy. Organelles were isolated by immersing tissues in cold 10% KOH.

Observation of discharge

To determine whether secretions are discharged as a forced spray, by crepitation, or oozing, individuals were first cooled to approximately 10°C and placed on a sheet of red indicator paper (filter paper soaked in alkaline phenolphthalein solution). As the beetle warmed to room temperature individual appendages were pinched with forceps causing the beetle to discharge. Acid secretions register a white pattern on the indicator paper. By observing the release under the microscope and noting the distance and direction of the pattern on the indicator paper, the type of release was determined. In some cases a strong mist of spray was readily observed with the unaided eye in the field or under a microscope in the laboratory, therefore indicator paper was not required.

Collection of secretion for chemical analysis

Material for chemical analysis were obtained either by removal of gland reservoirs, or as secretion discharged on filter paper. For gland removal, live beetles were placed in a freezer for several minutes and dissected under distilled water. Whole gland reservoirs were placed in dry-ice cooled reactions vials. To collect discharged secretion on filter paper, beetles were held by one leg with forceps and a small strip of filter paper was held near the beetle to catch the secretion as it was sprayed. To prevent premature discharge, beetles were temporarily incapacitated by cooling them to approximately 10°C and then allowed to warm to room temperature while under observation. Once beetles became active, defensive secretion was collected on a piece of filter paper.

Chemical analysis

Defensive secretions absorbed to filter paper, or excised defensive glands were extracted with dichloromethane (100 µl), and 1 µl of the extract was injected into a GC-MS (HP 5890 gas chromatograph linked to a HP 5970 Mass Selective Detector) by splitless injection. Analyses were performed using a 25-m × 0.25-mm fused-silica capillary column coated with DB-5 (5% phenyl methylsilicone) stationary phase (0.25 µm film thickness). The oven temperature was held at 40°C for 4 min and increased to 260°C at a rate of 10°C/min and maintained at 260°C for 10 min.

For characterizing carboxylic acids their pentafluorobenzyl esters were made as described previously (Attygalle & Morgan, 1984). The mass spectra of pentafluorobenzyl esters were obtained by GC-MS using a 25-m \times 0.25-mm fused-silica capillary column coated with DB-23 stationary phase (0.25 μ m film thickness). For the localization of double bonds in unsaturated compounds their dimethyl disulfide derivatives were prepared as described previously (Attygalle & Morgan, 1986), and the mass spectra of the derivatives were recorded by GC-MS.

Terms

Gland structure terminology follows Forsyth (1972). Terms for abdominal structures follow Deuve (1993) except in the use of hemitergite to refer collectively to the median tergite and epitergite of terga VIII.

RESULTS

Results of chemical analyses are summarized in Table 1.

Newly sampled tribes

Catapiescini

Specimens examined. Both collected spray and whole glands from live specimens of *Catapiesis sulcipennis* Bates ($n=1$) (Fig. 1) from Mexico and *C. attenuata* (Chaudoir) ($n=1$) from Ecuador are the basis for the chemical analysis. Alcohol preserved *C. mexicana* (Chaudoir) ($n=1$) from Mexico was used for dissections of musculature. Dry specimens of *C. sulcipennis* ($n=4$), *C. mexicana* ($n=1$) and *C. bartyrae* Reichardt ($n=1$) were dissected to examine cuticular structures. The external structures of the abdomen were examined for additional specimens of the species above, *C. brasiliensis* (Gray), *C. columbica* Chevrolat and three unnamed forms from Ecuador and Panama.

Gland and delivery system anatomy, secretion delivery. The primary chemical components discharged by *Catapiesis sulcipennis* and *C. attenuata* are formic acid and decyl acetate (Table 1, Fig. 2). These are delivered in a directed spray and controlled by rod-like epitergites of terga VIII (Figs 3, 4) that extend and aim the gland aperture.

The defensive gland system in *Catapiesis* (Fig. 3) is typical for Harpalinae as described by Forsyth (1972). Specifically, the opening of the defensive gland efferent duct is associated with the apex of the rod-like epitergite VIII. Internally the efferent duct lies within the hemicylindrical portion of the epitergite and connects to the gland reservoir dorsally. The reservoir is elongate cordiform, and is situated in the sixth and seventh abdominal segments. The collecting canal joins the reservoir mediodorsally near the efferent duct. The collecting canal is 45 mm long and the efferent duct 3.3 mm long. The apical end of the collecting canal divides several times, ending in *c.* 125 clusters of secretory cells per side (Figs 3, 5, 6).

The abdominal configuration of *Catapiesis* species is harpalidian (Liebherr & Will, 1998; Deuve, 1993). Characteristics of this abdomen type are the produced and keeled lateral apophyses of tergite VIII and the association of the defensive gland duct openings with the apical margins of tergite VIII (Fig. 3). In catapiesines tergite



Figure 1. *Catapiesis sulcipennis* from Mexico.

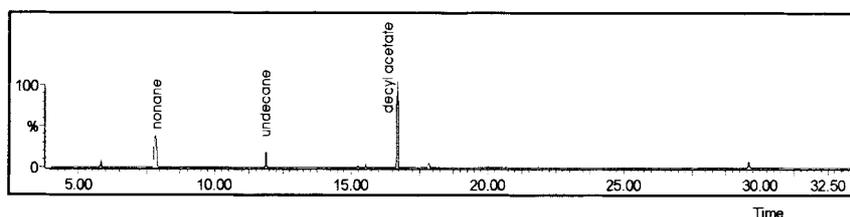


Figure 2. A reconstructed ion chromatogram obtained from the volatiles present in a dichloromethane extract of the defensive secretion of *Catapiesis sulcipennis* (sample 2). The sample was introduced by splitless injection onto a 25-m \times 0.25-mm fused-silica capillary column coated with DB-5 (0.25 μ m). The oven temperature was held at 30°C for 4 min and increased to 270°C at a rate of 10°C/min.

VIII is divided medially into hemitergites, with each hemitergite subdivided into a median tergite and an epitergite (Fig. 3). The sclerotized portion of the epitergite is hemicylindrical apically and broadly flattened basally. Muscles M2, M3 and M6 (Bils, 1976) attach to the base of the epitergite and are used to rotate the epitergite in order to aim the spray. The median tergites are separated from each other medially, and from the epitergites laterally, by a narrow band of lightly sclerotized cuticle. These flexible regions allow for a large range of motion between the subtergites. Muscles M1 and M5 (Bils, 1976) insert on the median tergites and are used to extend the tergite from its resting position telescoped within tergite VII.

Comparative aspects and phylogenetic implications. Reichardt (1973) removed *Catapiesini* from *Pterostichini* but was unable to suggest any alternative sister-group relationship.

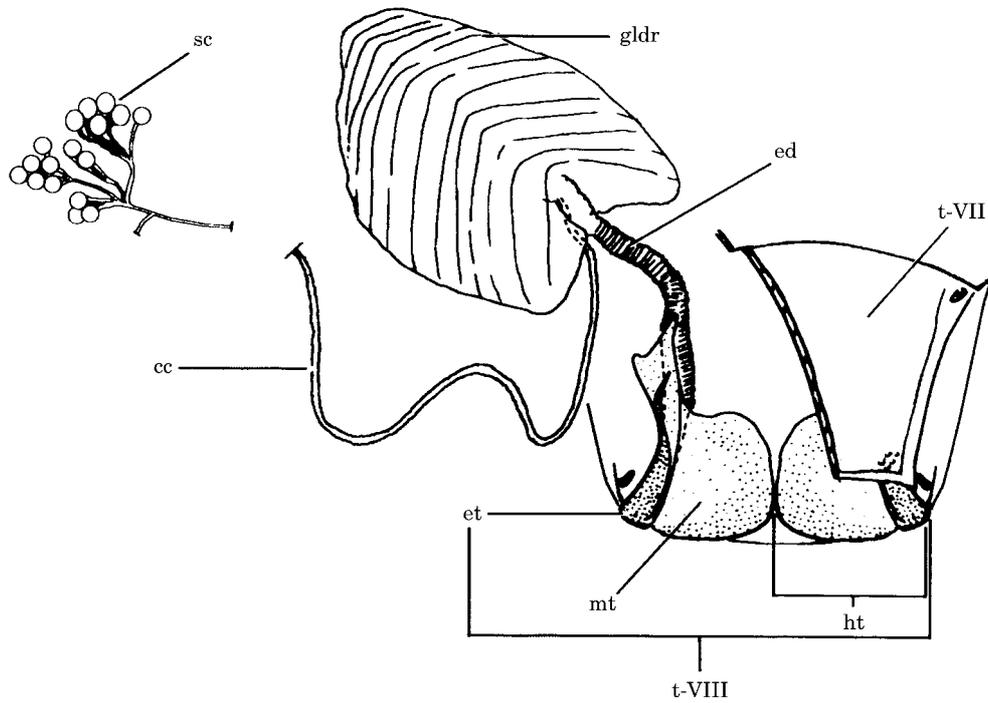


Figure 3. Cut-away diagram of *Catapiesis* abdominal tip (dorsal) showing pygidial gland system. Abbreviations: cc = signs collecting canal, ed = efferent duct, et = epitergite of tergite VIII, gldr = gland reservoir, ht = hemitergite of tergite VIII, mt = median tergite of tergite VIII, sc = secretory cell clusters, t-VII = tergite VII, t-VIII = tergite VIII.

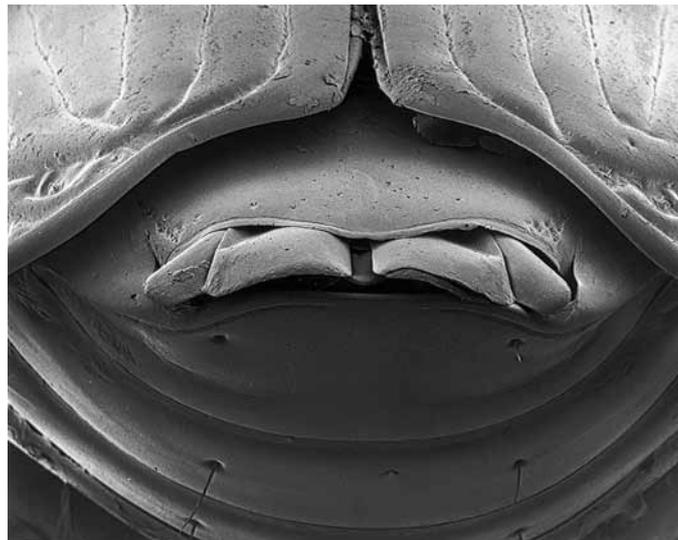


Figure 4. *Catapiesis sulcipennis*, SEM of apex of abdomen.

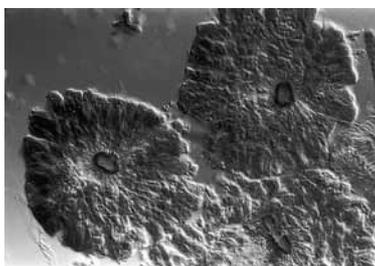


Figure 5. *Catapiesis mexicana*, secretory cells (unstained fresh mount, phase micrograph).



Figure 6. *Catapiesis bartyrae*, secretory cell cluster (treated with KOH) showing collecting tubules.

Subsequent authors have concurred with Reichard's tribal status for this group of genera (Bousquet & Larochelle, 1993; Whitehead & Ball, 1975). Erwin (1985, 1991) placed the Catapieseini in the supertribe Orthogonitae "on the basis of mandibular and elytral structures," but no explicit characters were listed. We examined the defensive chemical system in representatives of Orthogonitae to see if there were any potential synapomorphies. Orthogonitae species have variously modified terga VIII. In *Orthogonius alternans* Wiedemann (Orthogonini) tergite VIII is divided medially with keeled lateral apophyses but lacks separated epitergites. *Amorphomerus raffrayi* (Chaudoir) (Amorphomerini) has highly-developed epitergites but no medial division (see Deuve, 1993, his figs 18, 96). Additionally, *Amorphomerus* species have well-developed dorsal lobes of the reservoir not found in *Catapiesis* or *Orthogonius*.

The abdominal structures of *Catapiesis* species are quite unlike other Orthogonitae and are most similar to those of *Brachidius crassicornus* Chaudoir, *Oxyglychus laeviventris* Bates and *Cratocerus* Dejean. These genera have historically been placed in Pterostichini: Caelostomina or Morionini. Characters of the defensive chemical delivery system suggest that *Brachidius*, *Oxyglychus* and *Cratocerus* are incorrectly placed in Pterostichitae and may be better placed near catapieseines. Catapieseini and these taxa may form a complex separate from both Orthogonitae and Lebiitae of Erwin (1991) and be related to Perigonini and Dryptitae as suggested below.

Decyl acetate has been reported as a secondary compound in all members of the supertribe Dryptitae (Zuphiini, Dryptini [Kanehisa & Kawazu, 1985], and Galeritini [Rossini *et al.*, 1997]) and in Perigonini (see below). Other formic acid producers sampled (Platynini, Harpalini, Licinini, Lebiini and Odacanthini) are reported as having 2-tridecanone as the major secondary lipophilic compound (Kanehisa & Kawazu, 1985). These secondary compounds may serve as surfactants when formic

acid is present as the primary repellent. The presence of decyl acetate as a secondary compound may be a synapomorphy for the tribes of Dryptitae and its presence may be a synapomorphy of Dryptitae + Perigonini + Catapieseini. Formic acid itself is widespread in the Harpalinae, and it may prove to be a synapomorphy for a clade within the subfamily containing most of the tribes.

Morionini

Specimens examined. Whole glands were extracted from live *Morion simplex* Dejean ($n=2$) and *Moriosomus seticollis* Straneo ($n=10$) from Ecuador and analysed chemically. Dried specimens were studied for morphological characteristics. Additional dried specimens of *Morion monilicornis* (Latreille) ($n=3$) and *Morion cordatum* Chaudoir ($n=1$) were examined for internal and external cuticular structures.

Gland and delivery system anatomy, secretion delivery. The abdominal configuration of Morionini species is harpalidian; tergite VIII has well-developed lateral apophyses and is medially entire. The ovoid gland reservoirs are simple without lobes. The collecting canal joins the reservoir in the basal third. *Morion simplex* was observed spraying its defensive chemical secretions in the field and in the laboratory.

Comparative aspects and phylogenetic implications. Some larval characteristics have caused authors to place Morionini near scaritines (Lindroth, 1969), while some characteristics of the larvae and characters of adults suggest a placement near or within Pterostichini (Arndt, 1993; Bousquet & Larochelle, 1993; Straneo, 1979; Erwin, 1991) or near Cnemalobini (Liebherr & Will, 1998; Roig-Juñent, 1993).

Both morionine species sampled produce a broad mix of chemicals (Table 1). However, the primary component was formic acid. Formic acid is known only from the derived subfamily Harpalinae. Scaritines produce methacrylic and tiglic acids and other high molecular weight carboxylic acids (Dazzini-Valcurone & Pavan, 1980 and included references). Scaritines are not known to produce low molecular weight acids, such as formic or acetic acids, or hydrocarbons. Additionally, the abdominal configuration in scaritines is nebridian (Deuve, 1993; Liebherr & Will, 1998) quite unlike the abdomen of morionines. Pterostichini (*sensu* Bousquet & Larochelle, 1993) presently contains several genera that exude formic acid plus hydrocarbons, e.g. *Notonomus* Chaudoir (Moore, 1979; Moore & Wallbank, 1968) or a mix of heavier acids and formic acid as in *Abacomorphus* Chaudoir (Moore, 1979). The chemical defensive secretions of Morionini do not conflict with a placement of the tribe near or the Pterostichini.

Perigonini

Specimens examined. Whole glands were extracted from live *Diploharpus laevisimo* (Chaudoir) from Ecuador ($n=2$). Cuticular structures were studied in numerous dried specimens of *Diploharpus* species from South and Central America and *Perigona nigriceps* (Dejean) from the U.S.A.

Gland and delivery system anatomy. Gland reservoirs are simple, without lobes. Abdomen is harpalidian. Tergite VIII is medially divided and again divided laterally into small



Figure 7. *Diploharpus laevissimo*, SEM of right half of the apex of abdomen.

epitergites (Fig. 7). The aperture of the efferent duct lies within the small, appendicular epitergite.

Comparative aspects and phylogenetic implications. Perigonini has been placed in the Lebiitae (Erwin, 1991) or associated with some lebiite tribes and some ctenodactylite tribes (Jeannel, 1942). In the Lebitae tribes that have been sampled (Odacanthini, Cyclosomini, Lebiini) formic acid was always accompanied by 2-tridecanone and sometimes found with 2-pentadecanone (Moore & Wallbank, 1968; Moore, 1979; Kanehisa & Kawazu, 1985; this paper). We detected formic acid and 2-pentadecanone, but not 2-tridecanone in *D. laevissimo*.

Chemically, perigonines are also similar to members of the supertribe Dryptitae (Zuphiini, Dryptini [Kanehisa & Kawazu, 1985], and Galeritini [Rossini *et al.*, 1997]). With the notable exception of 2-pentadecanone, the primary and major secondary compounds of the Dryptitae tribes are the same as *D. laevissimo*. However, all Dryptitae have a distinct dorsal lobe on the gland reservoir not found in perigonines or in the Lebiitae. Secondary loss or absence of the dorsal lobe may be synapomorphic for the lebiite tribes, but the plesiomorphic condition is for dorsal lobes within the Harpalinae remains unclear.

The form of tergite VIII is similar to the tergite of *Catapiesis* species, however, in *catapieseines* the lateral apophysis is part of the epitergite whereas in perigonines the epitergite is a division of the hemitergite laterad of lateral apophysis. Perigonini and the tribes of the Dryptitae and *Catapieseini* are chemically similar, suggesting a close relationship among these taxa (see *Catapieseini* above).

Dercylini

Specimens examined. Whole glands were extracted from two species of *Dercylus* (*s.s.*)

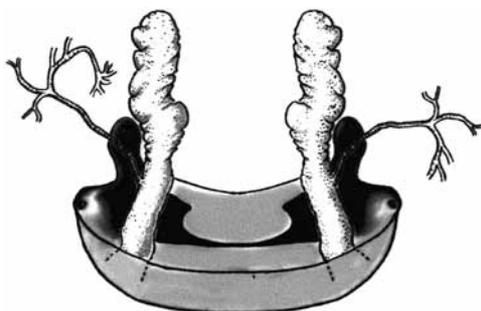


Figure 8. *Dercylus s.s.* sp. EC199801D. Ventral view of pygidial gland structures.

EC199801 ($n=2$), *Dercylus (s.s.)* EC199802 ($n=2$) from Ecuador. Cuticular structures of these same forms were studied from an equal number of specimens.

Gland and delivery system anatomy. Glands reservoirs are simple without lobes. The collecting canal enters the broad efferent duct at the duct's midpoint (Fig. 8). The abdomen type is harpalidian, but lightly sclerotized. Medially tergite VIII is membranous except for a thin band of thicker cuticle. The aperture of the efferent duct lies in a broad membrane between tergites VIII and IX.

Comparative aspects and phylogenetic implications. Both chemical and morphological characteristics are consistent either with a relationship of dercyline with oodines as suggested by Ball (1979) or with pterostichines as posited by Kryzhanovskiy (1976). Jeannel (1948) proposed a relationship with the melanchitonines, however, dercyline lacks a dorsal lobe on the reservoir, which is present in *Melanchiton kenyensis* Straneo.

Newly sampled genera and/or new chemical data for previously sampled tribes

Clivinini

Specimens examined. Whole glands were extracted from live *Schizogenius lineolatus* (Say) ($n=6$, not vouchered) and cuticular structures studied from the same species ($n=3$).

Gland and delivery system anatomy. Gland reservoirs are simple. The collecting canal joins the reservoir near the origin of the efferent duct. The abdomen is of the nebridian type (Deuve, 1993).

Comparative aspects and phylogenetic implications. This tribe is chemically heterogeneous. Quinones were identified from several *Clivina* species (Dazzini-Valcurone & Pavan, 1980), a ketone from *Dyschirius wilsoni* Sloane (Moore, 1979; Moore & Brown, 1979) and here we identified three forms of acids bearing six carbon atoms, (2-hexenoic, 3-hexenoic and 3,5-hexadienoic) from *Schizogenius lineolatus*. Similar to evidence from other character systems (Erwin, 1985; Bousquet & Larochelle, 1993; Liebherr & Will, 1998), defensive chemical compounds exclude any close relationship of clivinines to the phenetically similar Scaritini, which use aliphatic acids (Moore & Wallbank, 1968). But, defensive chemicals do not provide any clear synapomorphies for genera of Clivinini. More intensive genus-level sampling is needed to provide a clearer picture of the chemistry of this putative tribal-group.

Patrobini

Specimens examined. Whole glands were extracted from live *Patrobis longicornis* (Say) ($n=6$, not vouchered) and cuticular structures were also examined for this species.

Gland and delivery system anatomy. Structures of the glands are as described for other *Patrobis* species (Forsyth, 1972). It was noted that unlike any other species we studied, the secretion has a transparent pink colour.

Comparative aspects and phylogenetic implications. Methacrylic and tiglic acids detected in our study are consistent with results of previous studies (Kanehisa & Murase, 1977). The compounds found in Patrobini support Müller's (1975) suggestion that Patrobini is sister to a group including Trechini and Bembidiini. However, the absence of hydrocarbons from patrobines suggests a placement near the base of the Harpalinae (hydrocarbons are found in trechines but not in bembidiines) rather than within a clade containing the majority of the harpaline tribes. A relationship to the formic acid secreting *Mecyclothorax* (Psydrini) as suggested by Erwin (1985) is not supported by defensive chemical secretions.

Loxandrini

Specimens examined. Whole glands were extracted from *Loxandrus* sp EC199801L ($n=2$), EC199802L ($n=2$), EC199803L ($n=2$), EC199804L ($n=2$), EC199805L ($n=2$), *Oxycrepis* sp EC199801O ($n=2$), EC199802O ($n=2$) from Ecuador; *Loxandrus velocipes* Casey ($n=2$), *Loxandrus icarus* Will & Lieberr ($n=2$) from U.S.A. Cuticular structures of these same species were studied. Additionally, cuticular structures of numerous North American species, *Loxandrus longiformis* Sloane from Australia and several unidentified Australian species were studied.

Gland and delivery system anatomy, secretion delivery. In species of *Loxandrus* LeConte and *Oxycrepis* Reich the gland is cordiform with a distinct lobe on the efferent duct near the base of the duct (Fig. 9). The lobe of the efferent duct is variable in size and absent in some North American and Australian species of *Loxandrus*. The collecting canal joins the reservoir just above the junction of the efferent duct. Loxandrini have a typical harpalidian abdominal type with well-developed lateral apophyses.

Several *L. icarus* and *L. velocipes* were tested for mode of secretion delivery on indicator paper. A distinct spray pattern was observed. In some cases the pattern was greater than two times the length of the body.

Comparative aspects and phylogenetic implications. This tribe has only recently been recognized (Bousquet & Laroche, 1993) and in part was defined by the presence of salicylaldehyde as a major component of the defensive secretions. Salicylaldehyde was identified from two Australian species of *Loxandrus* (Moore & Wallbank, 1968; Moore, 1979). In our study we sampled two species of *Oxycrepis* and five South American and two North American species of *Loxandrus*. None contained salicylaldehyde. Formic acid was identified as the major component and acetic acid as secondary compound in all species. In addition 2-pentadecanone was found in all South American *Loxandrus* and all *Oxycrepis*. Hexenoic, seneceic and tiglic acids were also identified from several species of *Loxandrus*.

Moore (1979) reported that the species of *Loxandrus* he examined oozed their secretions (salicylaldehyde) rather than spraying, while those species we examined clearly sprayed their defensive chemicals (formic acid). Spray delivery is consistent

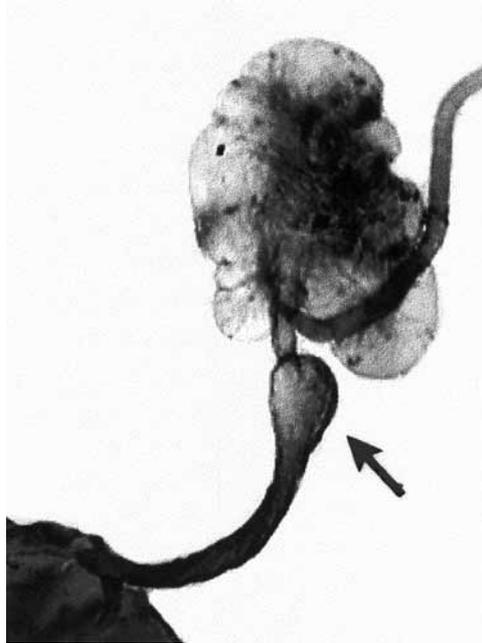


Figure 9. *Loxandrus* sp EC199802L. Cleared and stained preparation of the pygidial gland reservoir showing basal lobe of the efferent duct.

with other formic acid secreting ground beetles that have been examined for delivery mode (Moore, 1979).

The efferent duct of the pygidial glands for most previously examined formic acid spraying loxandrines have a well-defined lobe located near the base of the duct (Fig. 9). *Loxandrus longiformis* Sloane—one of the species chemically analysed by Moore & Wallbank (1968)—lacks the lobe of the efferent duct, however, the efferent duct is broadly expanded for much of its length. The efferent duct lobe is absent or quite small in some North American species of *Loxandrus*, but not expanded as in the Australian species. Whether the absence of this lobe is derived loss or primitive absence cannot be ascertained until an explicit phylogenetic study of the tribe is conducted with broader taxonomic sampling.

A similar lobe is found in *Abacetini* (*Abacetus percoides* Fairmaire examined) and may be a synapomorphy for these tribes. Also, this suggests that the lobe has been reduced or lost in both the Australian and North American lineages.

Loxandrines are chemically and/or structurally separated from other Pterostichitae tribes. The relationship of this tribe to other Pterostichitae remains ambiguous. A closer relationship with formic acid producing groups, such as Platynini, is likely.

Pterostichini

Specimens examined. Whole glands were excised from the following: *Incastichus aequidianus* Moret ($n=1$), *Abaris aenea* Dejean ($n=2$), *Pseudabarys* sp EC199801P ($n=4$), *Blennidus liodes* Bates ($n=2$) from Ecuador; *Cyclotrachelus sigillatus* (Say) ($n=1$), *Myas coracinus* (Say) ($n=1$), *Pterostichus (Pseudomaseus) luctuosus* (Dejean) ($n=1$), *P. (Monoferonia) diligendus* (Chaudoir) ($n>10$), *Gasterllarius honestus* (Say) ($n=2$) from U.S.A. Cuticular structures

were studied in an equal or greater number of all species. Remains of specimens of species from the U.S.A. were not vouchered.

Gland and delivery system anatomy. Gland reservoirs were simple without lobes and are consistent with Forsyth's (1972) description for the tribe. The abdomen is harpalidian with well-developed lateral apophyses.

Comparative aspects and phylogenetic implications. Results are consistent with previous findings for the tribe (Dazzini-Valcurone & Pavan, 1980 and included references). This tribe is poorly defined (Ball, 1979; Bousquet & Laroche, 1993; Moore, 1965), lacking any clear synapomorphies. The tribe is presently under study by the first author and defensive chemicals and delivery systems will be included in that larger analysis. Defensive chemical characters have been a factor in the exclusion of Platynini (Bousquet & Laroche, 1993) and Loxandrini (see above) from the tribe. Species sampled in this study are part of large number of pterostichine genera known to exude methacrylic and tiglic acids. Although these chemicals are widespread in the family, and are known from some basal lineages of Carabidae, it is likely that Pterostichini represents a retention of, or return to the plesiomorphic state (Fig. 10).

Oodini

Specimens examined. *Oodes amaroides* Dejean ($n=4$, [3♂ 1♀]). Cuticular structures examined in dried specimens of the same species ($n=2$). No vouchers retained.

Gland and delivery system anatomy. Gland reservoirs were simple without lobes and are consistent with Forsyth's (1972) description for the genus.

Comparative aspects and phylogenetic implications. Bousquet (1987) discussed in detail the chemical evidence for a sister group relationship of Chlaeniini and Panagaeini, a proposition that was counter to earlier hypotheses of Chlaeniini + Oodini. Our results do not conflict with Bousquet's conclusions.

Attygalle *et al.* (1991) reported 13 carboxylic acids from *O. americanus* Dejean and their study identified sexual dimorphism in the chemical compounds present. *Oodes amaroides* was found to have fewer compounds and no difference between the sexes was noticed. However, in addition to the compounds shared with *O. americanus* (Table 1), *O. amaroides* has the aromatic compound salicylaldehyde. This aromatic aldehyde is known from species in Carabini, Bembidiini, Loxandrini (Moore, 1979) and reported here in Oodini for the first time. These are widely separated lineages of carabids suggesting that use of salicylaldehyde has evolved several times in Carabidae.

Odacanthini

Specimens examined. Gland were extracted from live *Colliuris pensylvanica* (Linne) ($n=2$, not vouchered). Cuticular structures were examined from this species and *Scopodes boops* Erichson ($n=1$).

Gland and delivery system anatomy. Glands are simple without lobes (Liebherr, 1988, 1991). Tergite VIII of both species examined is divided medially and the edges of the hemitergites are reflexed at the corners just above the gland aperture.

Comparative aspects and phylogenetic implications. Recent classification efforts (Erwin, 1991; Liebherr, 1988, 1991) place Odacanthini with the Lebiitae. Specifically, Liebherr

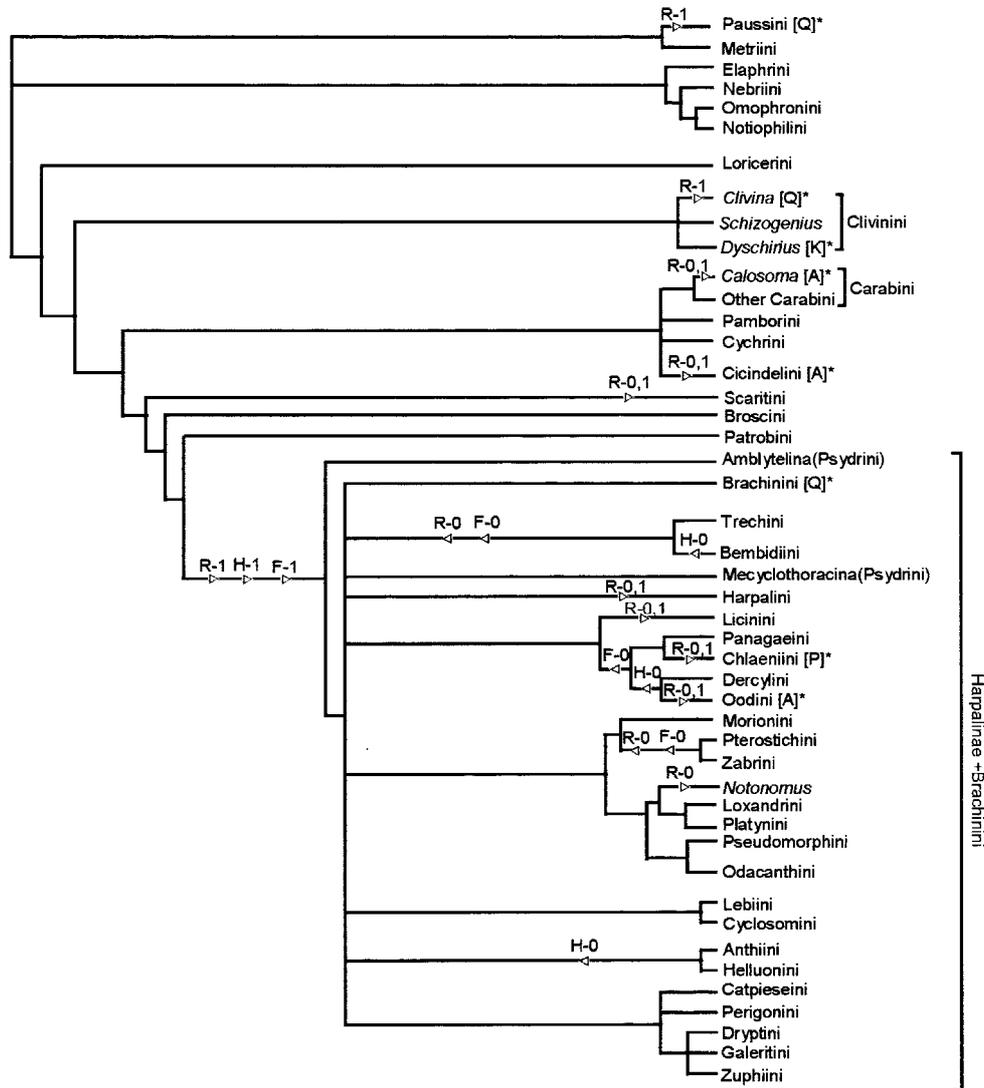


Figure 10. Phylogenetic hypothesis for carabid tribes with known defensive chemical compounds. See text for explanation. Abbreviations: R = habitat region, 0 = temperate, 1 = tropical/subtropical; H = hydrocarbons, 0 = absent, 1 = present; F = formic acid, 0 = absent, 1 = present; * [Q] = quinone, [P] = phenol, [A] = aromatic aldehyde, [K] = methyl ketone. Right pointing triangle = supposed gain, left pointing = supposed loss.

(1988, 1991) demonstrated that Odacanthini includes *Pentagonicina* (= *Pentagonicini* of Bousquet & Laroche, 1993) and this clade is sister to *Lachnophorini*. The presence of formic acid and lack of lobes on the gland reservoirs is consistent with a placement of *Colliuris* species in Odacanthini, though neither of these characteristics can be considered as synapomorphic for the tribe. Therefore, the primary defensive chemicals and gland structures are not informative regarding a tribal definition. However, other character systems, such as the female reproductive tract, are informative (Liebherr, 1988, 1991)

DISCUSSION

A phylogenetic hypothesis for the 47 carabid tribes that have been sampled for defensive chemicals (Fig. 10) can be extracted from a recently developed cladistic hypothesis for relationships of carabid tribes (Liebherr & Will, 1998). Resolution of the Harpalinae clade tribes is based on ongoing research in the Pterostichitae (KWW), and previously posited hypotheses of relationships (Erwin, 1991; Kryzhanovskiy, 1976; Bousquet & Laroche, 1993 and included references). In light of this phylogenetic hypothesis several patterns regarding chemical use emerge.

Species of basal grade carabid tribes primarily use heavier molecular weight carboxylic acids as compared to formic acid. The notable exceptions are apparently adapted to more specialized lifestyles (Erwin, 1979). These include the myrmecophilic Paussini that use quinones, and the frequently arboreal or diurnally active the groups *Calosoma* (Carabini) and Cicindelini. The chemically heterogeneous tribe Clivinini contains species that are generally fossorial in habits. However, this habit does not explain the apparent chemical shifting in clivinine genera, as scaritines are also fossorial, though species in that tribe uniformly use heavier molecular weight carboxylic acids.

Formic acid and hydrocarbons appear to have arisen in the common ancestor of the Harpalinae (Fig. 10). However, formic acid has been replaced several times by heavier molecular weight acids, quinone or phenol. Quinone, phenol and aromatic aldehydes, all ringed compounds, have arisen several times in the family.

The correlation of hydrocarbons and formic acid in the Harpalinae (Fig. 10) is nearly perfect, and was expected as lipophilic compounds have frequently been associated as surfactants (Eisner *et al.*, 1961; Rossini *et al.*, 1997, Schildknecht *et al.*, 1968) that increase the effectiveness of the primary irritant. Also, hydrocarbons themselves are potentially defensive (Peschke & Eisner, 1987). The only exception to the formic acid + hydrocarbon co-occurrence is found in the desert/steppe dwelling tribe Anthiini. What, if any, relationship the unusual life zone these beetles are adapted to has to do with their defensive chemicals is not clear.

The relationship of the habitat type in which the greatest species level diversity for a tribe or genus and the major chemical class they use can be seen in Table 2. Primarily formic acid, or in a few cases aromatic compounds, are associated with high species diversity in the tropical and subtropical regions. Conversely, nearly all tribes with their highest species diversity in the temperate regions use heavier molecular weight carboxylic acids. Within Harpalinae, shifts away from formic acid, or in some cases reversal to heavier molecular weight carboxylic acids, are also habitat associated.

The relative importance of historical and ecological explanations for the observed pattern of defensive chemical distribution in carabid beetles has been debated (Pearson *et al.*, 1988; Mooi *et al.*, 1989, Altaba, 1991). Given the hypothesis presented here, that the common ancestor of the Harpalinae utilized formic acid (Fig. 10), the presence of formic acid in descendent species can be explained simply by common ancestry. However, if one wants to hypothesize why formic acid originated in the common ancestor of Harpalinae species and why it is maintained in so many extant species and apparently lost in others, the patterns of chemical use and what habitat situations the beetles are found in must be considered.

In groups that are generally temperate but have strong representation in the tropics, e.g. *Calosoma* (Carabini) and Cicindelini, compounds other than heavier

TABLE 2. Table of primary chemical compounds and tribal or generic taxa sorted by general habitat of supposed greatest diversity. Groups may be listed in more than one column if diversity is approximately equal

Primary chemical compound	Temperate	Habitat type with greatest species diversity	
		Tropical-subtropical	Steppe
Formic acid			Anthini
			Catapiesini
			Cyclosomini
			Dryptini
			Galeritini
		Harpalini	Harpalini
			Helluonini
			Lebiini
		Licinini	Licinini
			Loxandrini
			Morionini
			Odacanthini
			Perigonini
			Platynini
			Pseudomorphini
		Psydrini (Amblytelina)	
		Psydrini (Mecyclothoracina)	
		Zuphiini	
	<i>Notonomus</i> (Pterostichini)		
High molecular weight carboxylic acids			Dercylini
		Oodini	Oodini
		Bembidiini	
		Broscini	
		Carabini	
		Cychrini	
		Elaphrini	
		Loricerini	
		Nebriini	
		Notiophilini	
		Omophronini	
		Pamborini	
		Patrobini	
		Pterostichini	
		Scaritini	Scaritini
	<i>Schizogenius</i> (Clivinini)		
	Trechini		
	Zabrini		
Phenol			
	Chlaenini	Chlaenini	Panagaeni
Quinone			Brachinini
			<i>Clivina</i> (Clivinini)
			Paussini
Aromatic aldehydes			
		Metriini	
		<i>Calosoma</i>	<i>Calosoma</i> (Carabini)
		Cicindelini	Cicindelini
		<i>Loxandrus</i> (Loxandrini, AUS)	
	<i>Bembidion</i> (Bembidiini)		
Ketone			
	<i>Dyschirius</i> (Clivinini)		

molecular weight carboxylic acids have developed. Scaritini is an anomaly in the basal grade taxa, as much of its diversity is tropical while defensive chemicals are uniformly heavier molecular weight carboxylic acids. The pedunculate body form and fossorial habit of scaritines is similar to the chemically heterogeneous clivinines. Clivinini, however, is best represented in the tropics by *Clivina* species, which are known to produce quinones.

Within Harpalinae, Bembidiini + Trechini lacks formic acid and included species have a primarily temperate distribution. It has been suggested that these tribes are closely related to Patrobini, a very different placement from what is presented here (Fig. 10). If this alternative placement proves correct, then the lack of formic acid is plesiomorphic. However, the presence of hydrocarbons in Bembidiini suggests a closer relationship of this tribe to the harpaline tribes than to Patrobini.

Saturated/unsaturated acids, quinone, phenol and aromatic aldehydes have arisen in the group ((Panagaeni + Chlaeniini) + (Dercylini + Oodini)). Oodines and chlaeniines are equally diverse in both temperate and tropical regions and, both are also chemically heterogeneous. The sex-specific chemistry found in Oodini (Attygalle *et al.*, 1991) suggest that this group and its close relatives may be producing both defensive and pheromone chemicals. Dercylini is unique among the Harpalinae as a truly tropical group that only produces methacrylic and tiglic acids.

The primarily methacrylic and tiglic acid producing tribes Pterostichini and Zabrinini are very diverse in the temperate regions. Their tropical sister group, Morionini, uses formic acid mixed with heavier molecular weight acids. *Notonomus* and Loxandrinini are placed here with Platynini and other formic acid producing tribes (Fig. 10). This arrangement and a discussion of the radiation of the formic acid producing species of *Notonomus* in warm temperate Australia and New Zealand will be the topic of a later paper on Pterostichini. In general, pterostichines are consistent with the temperate- heavier molecular weight carboxylic acids and tropical-formic acid pattern.

Erwin (1985) explained modifications of the defensive chemical delivery system, apical abdominal sclerites and elytral apex in carabids as the result of changes, or 'increase' in predator efficiency. The ground beetles are also presumed to be under similar adaptive pressure to produce effective defensive chemicals. The competition for food resources and predation pressure may have driven subtle and complex interactions between ants and ground beetles, leading to chemical mimicry. Blum (1980, 1981) speculated that the occurrence of formicid ant alarm pheromone chemicals (formic acid and undecane [summary table in Hölldobler & Wilson, 1990: 263]) in fast moving arthropods, like ground beetles, had evolved to chemically 'hide' a beetle that was in conflict with ants. Thus the discharge of this mixture of chemicals could first deter the immediate threat by its irritating effect and secondly the diffusion of the ant alarm chemical released by the ground beetle may cause a general disruption or an aggressive movement of individuals up the chemical gradient (Hölldobler & Wilson, 1990: 261). Either of these cases may give a fleeing ground beetle the opportunity to rapidly exit while the ants are busy searching for the source of the disturbance. This intriguing possibility has not yet been tested in the field or laboratory.

Also, the fact that some Harpalinae are Mullerian mimics of ants is evidence for a strong association of ants and ground beetles. Lachnophorines, dryptines and odacanthines all have taken on ant-like, narrow body forms with a constricted neck. Often the illusion is enhanced by various colour patterns and setation.

Lachnophorines have been observed running with ants and moving in an ant-like manner (J. Liebherr, pers. comm.). All ants so far investigated have alarm and defense chemicals (Hölldobler & Wilson, 1990) making them a good model pattern to follow when predators that rely on vision to locate and select prey are a major factor.

The majority of ground beetle species are in the subfamily Harpalinae. The many species sampled suggest that most of the species in the subfamily use formic acid. As these derived tribes have radiated in all biotic regions it is reasonable to conclude that formic acid is an effective deterrent for a wide range of predators. The fact that the basal lineages of Carabidae lack formic acid and are absent or poorly represented in the tropics suggest that some tropical predators, such as ants and birds, exert sufficient pressure to exclude these taxa. In fact, the most common circumstances in which more potent chemicals occur, arboreal, subcortical, diurnally active and tropical groups, are exactly the situations where one would expect the greatest predation from birds and predation and competition from ants. It is clear from the summary of evidence presented by Thiele (1977) that birds can exert a significant pressure on ground beetle populations. Ants also are recognized as predators, prey and competitors (Theile, 1977). Darlington (1971) presented distributional and diversity data for ground beetles in New Guinea that he felt was best explained by displacement of ground beetles by competing ants. Radiation of these predators/competitors of ground beetles is thought to have been coextensive with the presumed radiation of the Harpalinae in the Cretaceous (Erwin, 1985). The distribution of Formicinae ants and formic acid producing ground beetles is very similar, with Formicinae most diverse in the tropical regions, though strongly represented in the temperate regions by Formicini and Camponotini species (T. Schultz, pers. comm.).

The hypothesized radiation out of the tropical lowlands (taxon pulse or Darlingtonian waves of Erwin, 1985) of the early Harpalinae groups would have been by species equipped with formic acid. This seems only to apply to the recent radiation of carabids, as there is no evidence that older lineages ever existed in the tropical lowlands. Chemical evidence suggests that most of these older groups are of temperate origin and have in some cases reentered the tropics endowed with a derived defensive chemistry, e.g. Cicindelinae and Carabini. If the basal tribes of Carabidae do have an equatorial origin as suggested by Erwin (1985, and other works by the same), then recently evolved predators and other factors must have completely eliminated them from tropical regions.

CONCLUSIONS AND FUTURE STUDY

Caution should be used in interpreting these associations as tribes and their subordinate species ranges are not precisely known and habitat boundaries are only approximate. Tribal groups in Carabidae are reasonably well defined and are useful for discussing general trends. However, tribes, as a category, are not evolutionary equivalents except in the case of sister taxa.

The degree to which historical constraints, in terms of ancestral condition, and more recent ecological pressures play a role in the evolution and maintenance of defensive chemicals can only be teased apart with an explicit phylogenetic approach.

The phylogenetic pattern used here is too eclectic to be considered a substantive test of the agreement of ecology and chemistry. The main purpose here is to continue the task of accumulating data on defensive chemistry, now 47 of the 78 plus tribes have been covered. Continuing efforts must be made to sample the remaining taxa. Also, results across carabid tribes make it evident that chemical heterogeneity in some tribes may require more complex explanations than the chemical homogeneity of others.

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