

A COMPARATIVE STUDY OF THE CHEMICAL DEFENSIVE SYSTEM OF TENEBRIONID BEETLES: CHEMISTRY OF THE SECRETIONS

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Abstract—The chemical compositions of the defensive secretions of 147 species of tenebrionid beetles from 55 genera and 16 tribes were analysed by gas-liquid chromatography on three different stationary phases. All species contained toluquinone and ethylquinone, but benzoquinone was relatively rare. A great many species contained various 1-alkenes in addition to quinones. Species of *Pedinini* were distinguished by the secretion of *n*-propylquinone, whereas 2,3-methylmethoxyquinone set apart the subgenus *Blapyllis* (of *Eleodes*) and possibly *Amphidora*. Large amounts of octanoic acid were restricted to the genera *Embaphion* and *Neobaphion* (both *Eleodini*), and small amounts of 1-nonadecene were restricted to *Lariversius* (*Eleodini*) and *Cratidus* (*Amphidorini*). Most species of *Eleodes* had complex secretions with toluquinone, ethylquinone, 1-nonene, 1-undecene, and 1-tridecene in various ratios. 1-Pentadecene and/or 1-heptadecene occurred in substantial amounts in *Tribolium*, *Uloma*, *Phaleria*, *Pyansia*, *Melanopteris*, *Gonopus*, *Schelodontes*, *Psorodes*, *Neatus*, and *Merinus*. The genus *Argoporis* (*Scaurini*) is unique in its synthesis of 6-alkyl-1,4-naphthoquinones, and other members of the tribe also contain characteristic, as yet unidentified compounds. Many groups not distinguished by unique compounds still show characteristic combinations and ratios of compounds, and these are discussed along with problems of variation at the individual, geographic, specific, and generic levels.

INTRODUCTION

A GREAT many arthropods possess various types of glands which secrete repellent or irritating chemicals and which are used (usually, presumably) in encounters with actual or potential predators (ROTH and EISNER, 1962; EISNER and MEINWALD, 1966). The chemical constituents of a large number of these secretions have been isolated and identified (WEATHERSTON and PERCY, 1970) but only in a few cases has it actually been shown experimentally that the secretion has defensive value (EISNER and MEINWALD, 1966). Field ecological studies of the rôle of defensive glands are almost entirely lacking, but crop content records indicate that the protection afforded by the glands is not absolute (HYSLOP, 1912; McATEE, 1932; DOYEN, 1975).

While studies encompassing a number of species are common, broad comparative studies of chemical defensive systems are very few to date. MOORE and WALLBANK (1968) reported on the chemical composition and gross gland morphology of

carabid beetles, and showed a great diversity of secretions. WILSON and REGNIER (1971) recently reported a comparative study of the defence-alarm system of the ant subfamily Formicinae and postulated evolutionary lines for gland morphology, chemical composition, and function of the secretion.

Of American insects, tenebrionid beetles are probably among the most conspicuous for possessing chemical defences. They are probably equalled only by the pentatomid bugs which have flavoured many a raspberry or blackberry at picking time, and both pentatomids and, in some areas, tenebrionids have earned the common name of 'stink bugs'. In the Western states, certain species of tenebrionids have even entered the folk literature as 'circus bugs' from their conspicuous habit of standing on their heads when disturbed. The first mentions of tenebrionid defensive secretions in the scientific literature are those of GISSLER (1879) and WILLISTON (1884), who observed that seven species of *Eleodes* were able, upon being disturbed, to eject a 'pungent, vile-smelling liquid'. WILLISTON thought the source of the fluid to be anal glands, and observed that 'in individuals which have not been exhausted, it is directed backward with considerable force, as I once learned to my entire satisfaction'. WILLISTON (1884) also reports the head-standing posture of certain *Eleodes* species, though he erroneously ascribed this habit to all *Eleodes* species: 'When approached, they stand almost vertically with the abdomen directed upwards ready the moment they are touched, to eject their mephitic secretion.'

Although a number of early reports on the morphology of tenebrionid defence glands appeared, it was not until 1943 that ALEXANDER and BARTON first reported the identification of ethylquinone from the secretion of the flour beetle, *Tribolium castaneum*. LOCONTI and ROTH (1953) identified toluquinone among the compounds in *T. castaneum*'s secretion. They also identified an additional constituent as methoxyquinone, but this was later shown to be 1-pentadecene (VAN ENDT and WHEELER, 1971). Tolu- and ethylquinone were subsequently found in other tenebrionids: *Diaperis maculata* Oliver (ROTH and STAY, 1958), *Eleodes hispilabris* Say (BLUM and CRAIN, 1961), and *Eleodes longicollis* Lec. (CHADHA *et al.*, 1961). All three of these reports indicated that hydrocarbons were also present, and these were identified from *E. longicollis* by HURST *et al.* (1964) as 1-nonene, 1-undecene, and 1-tridecene. HURST *et al.* (1964) also report glucose from the aqueous phase of *E. longicollis* secretion and MEINWALD and EISNER (1964) added octanoic acid. Quinones and in some cases, 1-alkenes, have also been demonstrated in the secretions of a number of other tenebrionids: several species of *Blaps* (SCHILDKNECHT and WEIS, 1960a, b), *Tenebrio molitor* L. (SCHILDKNECHT and KRAMER, 1962), *Alphitobius diaperinus* (Panzer) (TSENG *et al.*, 1971), and *Zophobas rugipes* (TSCHINKEL, 1969). Reports by TSCHINKEL (1969, 1972) indicated that the prothoracic glands of *Zophobas rugipes* Kirsch. secreted phenols rather than the quinones of the abdominal glands, and that *Argoporis alutacea* Csy. secreted 6-alkylated naphthoquinones in addition to the benzoquinones. These two species are thus exceptions to the emerging picture of the benzoquinone-hydrocarbon nature of tenebrionid secretions.

LADISCH *et al.* (1967), using an electrochemical method, estimated the quinone content of 17 species of stored product tenebrionids from the genera *Alphitobius*, *Alphitophagus*, *Gnathocerus*, *Palorus*, *Tenebrio*, and *Tribolium*. Included was a *Tribolium confusum* Duv. mutant first reported by ENGELHARDT *et al.* (1965) which is able to secrete only about 5 per cent as much quinone as the wild type. Since cultures of this mutant (termed msg) tended to become mouldy, the authors suggest that one of the functions of the secretion may be antibiotic, a function that was first suggested by VAN WYK *et al.* (1959).

MEINWALD *et al.* (1966) reported preliminary studies which indicated that the benzoquinone nucleus is biosynthesized by a different pathway than the alkylated quinone nuclei, the first utilizing the aromatic ring of phenylalanine or tyrosine, while the latter are made from acetate units. Synthesis of quinones and hydrocarbons at the cellular level was investigated by HAPP (1968) who used chromatographic techniques to show the presence of diphenols and their glucosides in the secretory tissue of *E. longicollis* and *T. castaneum*. He suggested that the quinones are synthesized by hydrolysis and subsequent oxidation of the diphenol glycosides, and was able cytochemically to demonstrate the presence and localization of the classes of enzymes which this mechanism would require. At least the final steps take place outside the cell in cuticular organs thus avoiding damage to the cell by the toxic secretion.

It has recently become apparent that the presence of abdominal defensive glands is a character of major importance to the systematics of the Tenebrionidae and correlates almost completely with the presence of visible membranes between the terminal abdominal sternites and the non-inverted condition of the male aedeagus. On the basis of these characters, DOYEN (1972) has divided the Tenebrionidae into two families: the Tenebrionidae possessing defensive glands, and the Tentyriidae lacking them. The chemical defensive system is obviously of great phylogenetic significance to the Tenebrionidae and seemed an excellent subject for the study of the diversification of a homologous defensive system as well as possibly shedding light on the phylogenetic relationships within this confused family of beetles.

In this and two subsequent papers, I wish to report the results of a comparative investigation into the main chemical morphological and behavioural features of the chemical defensive systems of over 150 species of tenebrionid beetles.

MATERIALS AND METHODS

Collection and maintenance of the beetles

Most of the species were collected by the author during the spring of 1969, a sizeable portion were collected by Dr. John T. Doyen and a number of other collectors contributed a few specimens each. When collected in the field, the beetles were kept alive in plastic or cardboard containers with litter, soil, and bran. Sparse watering and moderate temperatures seemed to be the secrets to survival, and deaths were infrequent under these conditions.

In the laboratory, the beetles were maintained in an insectary at 28°C and 70% r.h., and were kept in plastic boxes by species. They were fed on bran or oats and were watered lightly about once a week. Species living in fungus were kept in their favourite fungus and were watered infrequently, while some species, such as *Tribolium*, were not watered at all. At long intervals, the substrate and bran were changed. Under these conditions, survival was acceptable to good.

Localities collected

Each collection locality was assigned a number and some of the localities and dates are given in Appendix A.

Identification and deposition of specimens

The specimens were separated according to species, and each species was assigned a sub-lot number consisting of the collection locality number which, if more than one species was collected at that locality, is followed by species number. Dr. Charles Triplehorn kindly carried out the bulk of the identifications while Jerry Davis, R. L. Berry, and Ron Somerby were responsible for the identification of, respectively, Pedinini, Scaurini, and *Blapyllis* of *Eleodes*. All specimens have been deposited in the museum of the Cornell University Department of Entomology under the Lot No. 1001 and are identifiable by species names and my sub-lot numbers.

Collection of the secretions

At the end of a day's collecting, the beetles were separated according to species, and the secretion was 'milked'. Small species and species which did not spray their secretion were 'milked' by roughly handling the beetles and soaking up the secretion on bits of filter paper held in forceps. The papers were then extracted and stored in carbon disulphide in 1 dram screw-cap vials with aluminium cap liners. The vials were kept dark and cool during transit, and stored in a freezer at -20°C in the laboratory. Stability, as judged by loss of quinones by polymerization and oxidation, seemed good under these conditions.

Species that were large and sprayed the secretion were 'milked' by simply handling roughly while keeping the hind end of the abdomen within the mouth of a vial. When the beetles sprayed, the secretion merely ran down the walls of the vial and collected in the bottom. For small amounts of secretion, carbon disulphide was added, but when 0.1 ml or more secretion was collected, it was stored neat.

During storage, the carbon disulphide was refilled occasionally as it evaporated, but vials which lost all solvent were discarded and not analysed.

Analysis of the secretions

All analyses were carried out by gas chromatography using a Varian Aerograph 1200 (the last few analyses were on a 1400) Gas Chromatograph with a hydrogen flame detector.

The columns used for the analysis were all made up in $\frac{1}{8}$ in. \times 5 ft aluminium tubing using Chromosorb W (acid-washed, DMCS, 80/100 mesh) as a solid support. Liquid phases were as follows: 5% SE-30, 5% NPGS, 10% FFAP (this was made up in a 10 ft long column), and 3% Carbowax 20M.

Each secretion was analysed on at least two liquid phases. The first 20 analyses were carried out on 5% SE-30, but it became apparent that after repeated runs, the column was unable to separate 1-nonene from benzoquinone, so all subsequent analyses were initially run on 5% NPGS and doubtful analyses from the first group were repeated on NPGS. This column, however, gives poor detection and resolution of octanoic acid, so all samples were run on 10% FFAP as well to check for this and other acids. In all analyses, mixtures of known standards were periodically injected and/or co-injected. Nitrogen flow for all columns was between 8 and 12 ml/min. Temperatures were as follows: 5% SE-30 initially at 85°C for about 6 min until the emergence of ethylquinone, then programmed to 185°C at 10° per min; 5% NPGS—initially at 60°C for about 2 min until the emergence of 1-nonene, then raised full power to 110°C where it was kept for 6 min until the emergence of ethylquinone, then programmed to 200°C at 20°C per min; 10% FFAP—initially at 140°C, programmed immediately upon injection to 200°C at 10° per min. The 3% Carbowax 20M column was used only for special purposes and the temperature programming varied accordingly.

The peak heights were calibrated to mass by injection of mixtures of weighed standards under standard conditions of hydrogen, nitrogen, and air flow as well as the routine temperature programme. The resulting calibration factors were used to convert each peak height in each trace to a corresponding mass of compound, and these were converted into mass percentage composition for each secretion. It is estimated that the error resulting from this method was mostly less than 10 per cent.

The aqueous phase of at least one species of every genus, and in the case of *Eleodes*, at least one species of every subgenus, was analysed for glucose and hydroquinones. Most of the aqueous phase was absorbed into the filter paper bits used to soak up the secretion, so these were extracted with distilled water; the extract was frozen and lyophilized. The residue was directly treated with hexamethyldisilazane–chlorotrimethylsilane (2 : 1) in pyridine, and the mixture analysed by GLC on 5% SE-30 ($\frac{1}{8}$ in. \times 5 ft) by programming the temperature from 140 to 200°C at 10°C per min. Under these conditions, the trimethylsilyl derivatives of glucose and the hydroquinones could be identified by comparison with authentic standards.

The majority of compounds were encountered repeatedly and were identified primarily by comparison with standard mixtures or by co-injection with these. In addition, several secretions of which I had large amounts (*Eleodes dentipes*, *E. armata*, and *E. grandicollis*) were subjected to preparative GLC, the individual compounds were isolated, and subjected to chemical identification.

When compounds of unfamiliar retention time were encountered, these secretions were subjected to preparative GLC, if quantity allowed. The isolated compounds were then subjected to chemical identification as above.

Preparative GLC was carried out with the same packing and liquid phases as the analyses, except that the columns were made up in $\frac{1}{4}$ in. \times 5 ft aluminium tubing. Nitrogen flow rate was generally about 60 ml/min and temperature programme rates were usually much lower to allow for more complete separation. The peaks were collected by passing the effluent from the column through a home-made stream-splitter (split ratio about 20 : 1) which allowed 5 per cent of the sample to be burned in the detector while 95 per cent passed to the exterior of the machine where it was condensed in 3 mm glass tubes packed with glass wool and cooled with dry ice. High boiling compounds such as quinones were collected in tubes at room temperature.

Chemical identification

Most identifications were carried out by spectroscopic techniques by comparison with model compounds. Infrared spectra of liquids were taken on capillary films with a Perkin-Elmer Infracord by evaporating carbon disulphide solutions on small salt chips. Ultraviolet spectra were taken on a Beckman DB-G Spectrophotometer. Nuclear magnetic resonance spectra were taken on either a Varian A-60 or Perkin-Elmer 60 MHz Spectrometer. Additional confirmation was often gained by co-injection of the unknown compound with the likely authentic standard on two or three different columns and in some cases this was repeated after simple derivatization of both standard and unknown.

Most authentic standards were available commercially or as gifts, but *n*-propylquinone had to be synthesized from *n*-propylphenol by oxidation with potassium dichromate. The reaction mixture was extracted with ether, dried with anhydrous magnesium sulphate, and the ether evaporated off. The residue was purified by preparative GLC on 5% NPGS.

The synthesis and purification of 6-alkyl-naphthoquinones have already been reported (TSCHINKEL, 1972).

All authentic quinone standards other than propylquinone were purified by recrystallization, but hydrocarbons and octanoic acid were of sufficient purity to be used without further treatment. Some of the hydrocarbons (1-pentadecene, 1-heptadecene, and 1-nonadecene) were used only in standard mixture of 1-alkenes available from Applied Science Laboratories.

RESULTS

Compounds previously identified from tenebrionid beetles

Among the compounds previously isolated from tenebrionid beetles are *p*-benzoquinone, *p*-toluquinone, *p*-ethylquinone, 1-nonene, 1-undecene, 1-tridecene, octanoic acid, and glucose.

Since the present work was completed, 1-pentadecene was also identified from *Tribolium* (VAN ENDT and WHEELER, 1971). The secretions of *Eleodes armata* Lec. and *E. grandicollis* Mann. were subjected to preparative GLC and five major compounds were isolated and identified by co-injection with authentic standards on

three different columns (5% SE-30, 5% NPGS, and 3% Carbowax 20M) as *p*-toluquinone, *p*-ethylquinone, 1-nonene, 1-undecene, and 1-tridecene. These compounds and octanoic acid and *p*-benzoquinone were similarly identified from the secretions of *E. longicollis* Lec. and *Embaphion muricatum*. Octanoic acid was confirmed in the latter species by reduction with LiAlH_4 and co-injection with 1-octanol on a 15% FFAP column.

GLC of trimethylsilyl derivatives confirmed in the aqueous phase of the secretions the presence of glucose and the hydroquinones corresponding to the quinones in the organic phase.

Compounds not previously identified from tenebrionids

2,3-Methylmethoxyquinone. Secretions of the species of the subgenus *Blapyllis* of *Eleodes* consistently exhibited a peak which eluted after ethylquinone. Therefore, *Eleodes (Blapyllis) blanchardi* Blais. was subjected to preparative GLC on 5% NPGS and about 2 to 3 mg of the unknown peak collected. The i.r. spectrum showed bands at (in order of decreasing intensity) 6.04, 7.66, 6.26, 8.31, 11.92, 9.25, 8.65, 9.84, 7.27, 6.91, and 3.33, and was superimposable over the spectrum of 2,3-methylmethoxyquinone. Mass spectroscopy indicated a strong molecular ion of 152 and a fragmentation pattern consistent with 2,3-methylmethoxyquinone. Co-injection with the authentic quinone failed to give separation. Both the unknown compound and the authentic quinone are unique among the simple quinones in having an intense, deep orange-yellow colour, and both had a melting point just above room temperature. The unknown compound in the secretion of *Blapyllis* species was thus identified as 2,3-methylmethoxy-1,4-benzoquinone, and this was confirmed in other species of *Blapyllis* by co-injection with the authentic quinone.

n-Propyl-1,4-benzoquinone. All analysed species of the tribe Pedinini showed a peak eluting after ethylquinone. These are mostly small species and only small amounts of secretion were available from any one locality. Thus, after co-injection on three columns had confirmed that the post-ethylquinone peaks of three collection localities of *Conibius* were identical, their secretions were combined and subjected to preparative GLC. The unknown compound had the yellow colour of a quinone, and co-injection with several quinones indicated that trimethylquinone had a similar, but not quite identical, retention time. The mass spectrum showed a parent ion of 150 suggesting that the unknown contained three additional carbons on the ring. Since tolu- and ethylquinone are also present in species of Pedinini, it seemed reasonable that the unknown was a propylquinone, the next higher homologue. *n*-Propylquinone was therefore synthesized as noted above. The mass spectral fragmentation pattern of the unknown was consistent with and similar to that of authentic *n*-propylquinone. Diagnostic peaks occurred at *m/e* of 150 (M^+), 135, 122, 107, 94, 82, and 79 and these are consistent with an unbranched side chain. The i.r. spectra were superimposable with bands at (in order of decreasing intensity) 6.00, 7.75, 11.00, 6.23, 9.46, 5.62, 3.29, 11.42, 9.08, and 6.87 μm and co-injection on three columns with authentic *n*-propylquinone failed to give any separation.

Both the unknown and the authentic quinone were oily liquids at room temperature. It was concluded that the unknown peak in the secretion of *Conibius* species was *n*-propyl-1,4-benzoquinone. This was confirmed for other species of Pedinini by co-injection with the authentic quinone.

6-Alkyl-1,4-naphthoquinones. Species of *Argoporis* all exhibited six peaks which eluted at elevated temperatures and were identified as 6-methyl-, 6-ethyl-, 6-propyl-, and 6-butyl-1,4-naphthoquinones (TSCHINKEL, 1972). Two minor peaks remain unidentified.

1-Pentadecene. The secretions of several species of tenebrionids contained a compound which characteristically eluted later than 1-tridecene on SE-30 and was incompletely separated from ethylquinone on NPGS. Two species that contained enough of this compound for preparative GLC were *Uloma punctulata* Lec., and *Pyanisia tristis* Lap. The unknown peak from each of the species was isolated and that of *U. punctulata* Lec. was subjected to mass spectroscopy and i.r. spectroscopy, whereas only i.r. was carried out on *P. tristis* Lap. Infrared of both showed bands at 10·13 and 11·20 characteristic of concerted vinyl deformation. Together with the strong band at 3·33 μm this indicated a terminally unsaturated hydrocarbon. The mass spectrum indicated a molecular ion of 210 and a fragmentation pattern characteristic of a normal 1-alkene. The compound was co-injected on three columns with authentic 1-pentadecene to confirm the identification.

1-Heptadecene. *Cratidus osculans* Lec. and *Cibdelis gibbosa* contained enough of a component eluting after pentadecene to allow preparative collection thereof. The i.r. spectrum of the *Cibdelis* compound again indicated a 1-alkene, and the mass spectral molecular ion of 238, together with the characteristic fragmentation pattern, indicated that the compounds were 1-heptadecene. This was confirmed by coinjection with authentic 1-heptadecene.

1-Nonadecene. The secretion of *C. osculans* Lec. also contained a small amount of a compound eluting later than 1-heptadecene. Subjection of the material from preparative GLC to mass spectroscopy showed a molecular ion of 266 and the characteristic 1-alkene fragmentation pattern. Co-injection of the unknown compound with authentic 1-nonadecene confirmed the identification.

Alkanals and alkenals. *Eleodes beameri* of the subgenus *Holeleodes* proved to have a unique secretion containing at least 23 different compounds. The identification of these compounds as saturated and 2,3-unsaturated aldehydes and ketones along with several 1-alkenes, and toluquinone will be reported elsewhere.

Other compounds not identified. Species of the genera *Eulabis* and *Apsena* contained small amounts of compounds with strong distinctive, phenolic-type odours, but it was not possible to identify these. *Scaurus aegyptiacus* possessed an astounding 50 or more compounds in its secretion (Fig. 1). Among them were the usual tenebrionid hydrocarbons and quinones, but most of the compounds remain unidentified. Minor components (less than 0·5%) of *C. osculans*, *Amphidora nigropilosa* Lec., and several species of *Eleodes* were also not identified. *Toxicum taurus* also contained substantial amounts of an unidentified compound.

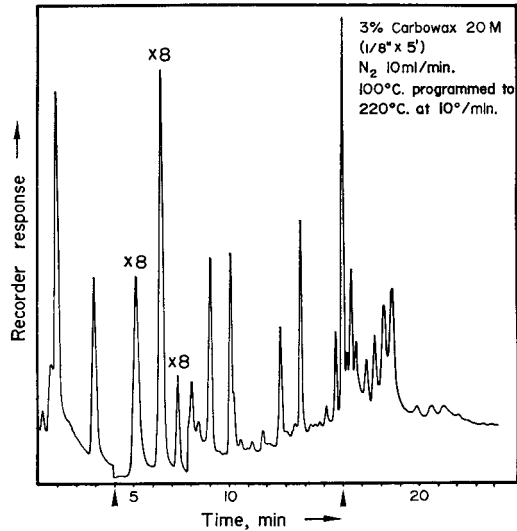


FIG. 1. Gas-liquid chromatographic analysis of the defensive secretion of *Scaurus aegyptiacus*, attenuated by a factor of eight for the three major components. The numerous minor components make up a total of about 25 per cent of the secretion.

TAXONOMY OF THE SECRETIONS

Taxa distinguished by compounds unique to them

Some of the taxa have compounds in their secretions which are strictly characteristic of those taxa. In most cases, the characteristic compounds are entirely absent from all other taxa, but in some they are present in other taxa in greatly diminished quantities. In all cases, it is possible, of course, that as yet uninvestigated species of the taxa will fail to contain the characteristic compounds.

All species of three genera (*Blapstinus*, *Conibius*, *Notibius*) of the tribe Pedinini contain *n*-propylquinone in their secretions (Fig. 26). Of the three genera, *Notibius* secretion seems to contain the most propylquinone (about 85%) and it is interesting that this is one of the very few cases where toluquinone is practically absent. In several collection localities, toluquinone was undetectable.

The subgenus *Blapyllis* of *Eleodes* is unique within the tenebrionids in that its secretion contains 2,3-methylmethoxyquinone (Figs. 10, 24). Minor amounts of a compound of similar retention time occasionally appeared in other species, and though it is reported as 2,3-methylmethoxyquinone, its identity was never confirmed because of the small quantities.

The tribe Scaurini (Figs. 1, 4, 5) contains a number of highly unusual genera. *Argoporis* is unique, even in the animal kingdom, by its secretion of 6-alkyl naphthoquinones (Tschinkel, 1972). *Apsena* has unidentified compounds not found in other tenebrionids, and the single species of *Scaurus* which was analysed contains more than 50 different compounds in its secretion (Fig. 1).

The only available species of the subgenus *Holelodes*, *E. beameri*, proved to be unique due to the presence of large amounts of saturated and unsaturated aliphatic aldehydes and the absence of ethylquinone. This secretion possesses a very distinctive odour quite different from other *Eleodes*. *E. beameri* is the northernmost species of its subgenus and it is possible that other species of the group also secrete aldehydes.

Taxa distinguished by uncommon compounds

Octanoic acid is restricted to the secretions of the tribe Eleodini and within this taxon it is found as a minor component in four subgenera of *Eleodes* (*Eleodes*, *Steneleodes*, *Tricheleodes*, and *Metablapylis*) (Fig. 10). In the genera *Embaphion* and *Neobaphion*, however, it makes up about 20% of the secretion (Fig. 9), thus distinguishing these two genera by its quantity, if not presence.

Hydrocarbons of chain length greater than 13 are not commonly found in substantial amounts, and a number of taxa are distinguished by the presence of 1-pentadecene, 1-heptadecene, and/or 1-nonadecene in more than minor amounts. Thus, the presence of large amounts of 1-pentadecene is characteristic of *Phaleria* (*Phaleriini*) (Fig. 30), *Uloma* and *Tribolium* (*Ulomini*) (Figs. 33, 34), *Pyanisia tristis* Lap. (*Amarygmini*) (Fig. 36), some species of the subgenus *Promus* of *Eleodes* (*Eleodini*) (Fig. 25), *Parastizopus balneorum* Per. (*Opatrini*) (Fig. 28), *Psorodes calcaratus* F. (*Meracanthini*) (Fig. 35), and several species of *Platynotini* (Fig. 37).

Lariversius (*Eleodini*) is characterized by 1-heptadecene (Fig. 9) as is *Neatus*, *Merinus*, and part of *Cibdelis* (*Tenebrionini*) (Fig. 40) and *Psorodes gratilla* Herbst (*Meracanthini*) (Fig. 35). *Cratidus osculans* Lec. (*Amphidorini*) (Fig. 8) characteristically secretes small amounts of the higher alkenes, 1-heptadecene and 1-nonadecene, and *Lariversius* (Fig. 9) produces both of these in larger amounts.

Although *p*-benzoquinone occurs widely throughout the family, especially in the *Eleodini*, it is only in *Eleodes longicollis* Lec. (Fig. 16) and three genera of *tenebrionini* (*Alobates pennsylvanica* DeG., *Zophobas rugipes* F., and *Ipthimus* spp.) (Figs. 38–40) that it is found in amounts greater than about 5 per cent.

Patterns of occurrence

One of the strongest generalizations is that *tenebrionid* secretions are fundamentally *quinonic* secretions. Hydrocarbons are very frequently present and may even make up the bulk of the fluid, but they are totally absent in some groups, while quinones (toluquinone, ethylquinone) are almost universally present.

The frequency of occurrence of various proportions of quinones to non-quinones underlines the fundamentally *quinonic* nature of *tenebrionid* secretions (Fig. 2). Thus, of 127 species, only 17 species have less than 50% quinones. The predominantly hydrocarbon secretions of such genera as *Platydemia*, *Gonocephalum*, and *Zadenos* are relatively rare exceptions to this rule.

Among the quinones, *p*-benzoquinone itself is relatively rare, and toluquinone and ethylquinone are practically universal. The ratio of toluquinone to ethylquinone falls within rather narrow limits (Fig. 3), most species having a slight

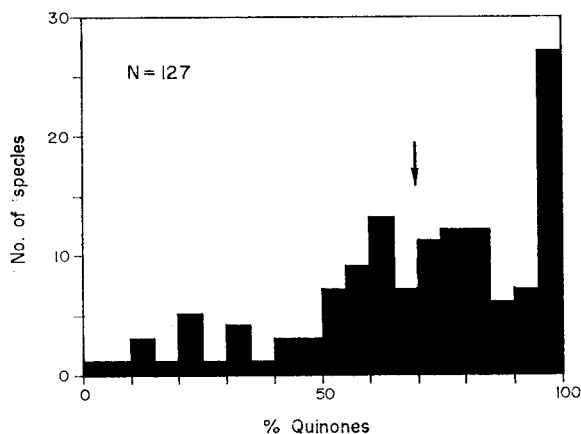


FIG. 2. The frequency among the species of various ratios of quinones to non-quinones, expressed as per cent quinones. The total percentage of all quinones is indicated in 5 per cent intervals. Non-quinones are mostly 1-alkenes, but may include other compounds as well. The arrow indicates the mean.

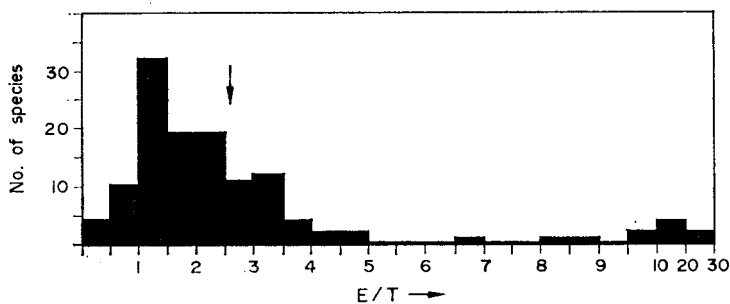


FIG. 3. The frequency among the species of the ratios of ethylquinone to toluquinone (E/T), plotted in intervals of 0.5. The mean ratio is indicated by the arrow. In only 14 out of 127 cases was toluquinone more abundant than ethylquinone (E/T less than 1.0), and the most common relationship was one in which ethylquinone predominated somewhat.

predominance of ethylquinone. In only 14 of 127 species is toluquinone predominant. Seldom does the ethylquinone predominate by more than a factor of four. Such limited distribution of ratios implies a biosynthetic link between the two compounds. Evidence for this has already been offered (MEINWALD *et al.*, 1966).

Many taxa show characteristic ratios of compounds, but the consistency of such patterns and their usefulness as a distinguishing characteristic is limited by the variability of the patterns. Variability occurs at all levels: between individuals from one locality, between localities of a species (geographic variation), between species of a genus, and so on. Fundamental to all these levels is, of course, the variation among individuals of a species, and this may be considerable.

TABLE 1—VARIABILITY OF COMPOSITION AMONG INDIVIDUALS OF A SINGLE LOCALITY AND AMONG DIFFERENT LOCALITIES OF A SPECIES

| Species | Units | Standard deviation of percentage of compounds | | | | | | |
|---------------------------------|-----------------------------|---|------|------|-----|-----|-----|----------|
| | | B | T | E | 9 | 11 | 13 | Sum S.D. |
| <i>Eleodes longicollis</i> Lec. | Localities ($n = 9$) | 7.2 | 8.4 | 7.9 | 6.2 | 8.3 | 3.4 | 41.4 |
| | Individuals ($n = 16$) | 4.8 | 8.7 | 5.3 | 8.1 | 5.7 | 3.2 | 35.8 |
| <i>Eleodes armata</i> Lec. | Localities ($n = 4$) | | 6.7 | 4.7 | 1.3 | 7.2 | 2.2 | 22.1 |
| | Individuals ($n = 15$) | | 11.0 | 4.1 | 5.2 | 5.0 | 7.0 | 32.4 |
| <i>Eleodes gracilis</i> Lec. | Localities ($n = 3$) | 0.6 | 9.0 | 12.0 | 5.5 | 7.8 | 4.4 | 39.4 |
| | Individuals ($n = 13$) | | 5.1 | 6.4 | 6.7 | 8.8 | 8.8 | 35.8 |

The standard deviation of the percentage composition of six compounds is used as an estimate of the variability. B, Benzoquinone; T, toluquinone; E, ethylquinone; 9, 1-nonene; 11, 1-undecene; 13, 1-tridecene.

Table 1 presents, for three species, the standard deviations of the mean percentage of each compound based on several localities, on the one hand, and several individuals from a single locality, on the other. In each case, the variability of the composition (as indicated by the standard deviation) is of the same order of magnitude for inter-locality and inter-individual averages though it may be slightly lower for the latter (see also Figs. 8, 11–13, 14, 18, 20, 21, and 22 for other examples of inter-locality variation). Variation is not universally large: DOYEN (1973) reports very low individual, inter-locality and inter-specific variation in *Coelocnemis*. *Eleodes acuticauda* Lec. (Fig. 12) is another example with low inter-locality variation, perhaps due to the proximity of all the localities collected. Individual variation was not investigated in this species.

FIGS. 4–42. These figures present the weight per cent composition for twelve compounds. The Y-axis represents weight per cent and the graphs are 25 per cent or multiples of 25 per cent apart. Thus, the baseline of one graph represents 25 per cent for the graph below and 50 per cent for the next lower graph. The taxon is indicated at the right along with the number of units (individuals, localities, species, etc.) encompassed in that graph. The top graph in each figure is the mean (\bar{X}) of those below. Where the figures present information for localities of a single species, the localities are denoted by their numbers in the Appendix. Generally, the minimal quantity represented in the graphs is about 0.5 per cent. *Abbreviations for Figs. 4–42*: B, *p*-Benzoquinone; T, *p*-toluquinone; E, *p*-ethylquinone; P, *p*-propylquinone; M, 2,3-methylmethoxy-*p*-quinone; 9, 1-nonene; 11, 1-undecene; 13, 1-tridecene; 15, 1-pentadecene; 17, 1-heptadecene; OC, octanoic acid; OT, other compounds. *For Fig. 4 only*: MN, 6-Methyl-1,4-naphthoquinone; EN, 6-ethyl-1,4-naphthoquinone; PN, 6-propyl-1,4-naphthoquinone; BN, 6-butyl-1,4-naphthoquinone. \bar{X} , Mean; I, individuals; L, localities; S, species; SG, subgenera; G, genera; T, tribes.

Genus *Argoporis*

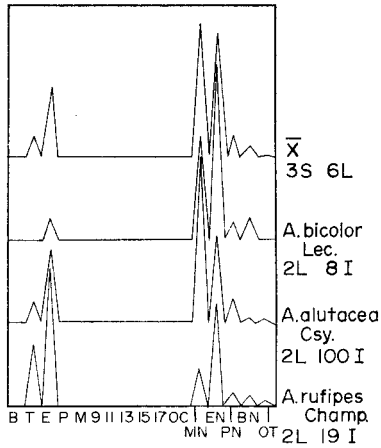


FIG. 4

Tribe Scaurini

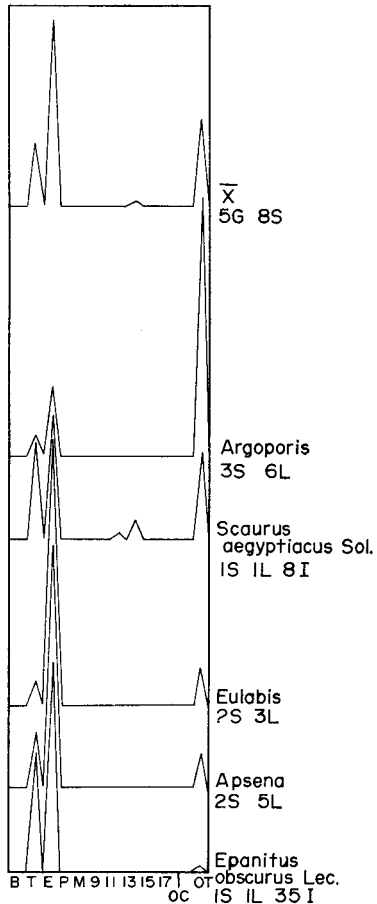


FIG. 5

Genus *Blaps*

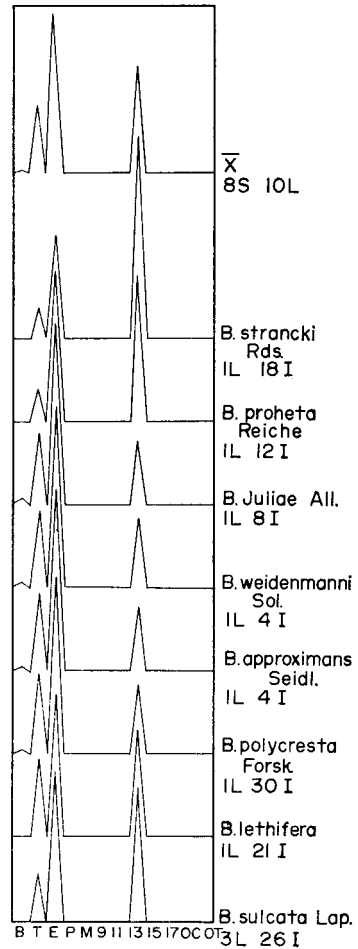


FIG. 6

Genus *Amphidora*

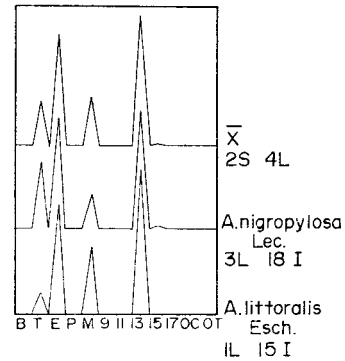


FIG. 7

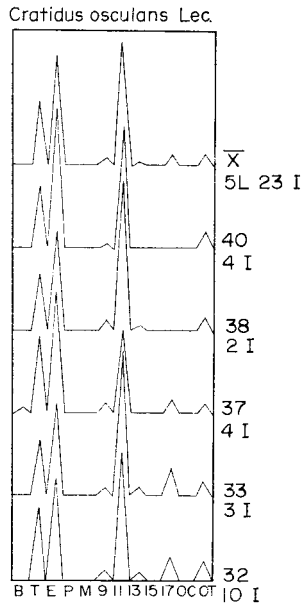


FIG. 8

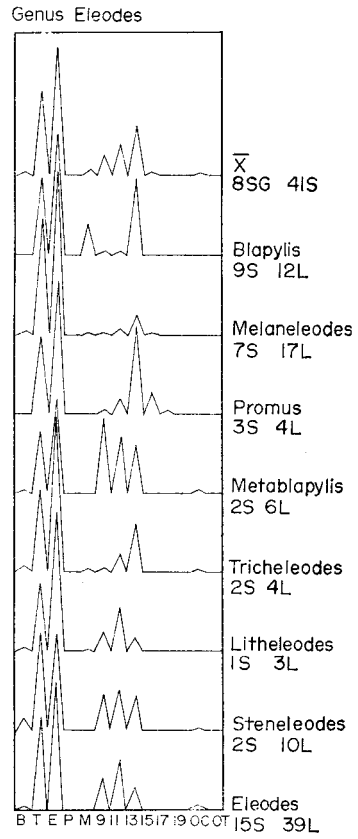


FIG. 10

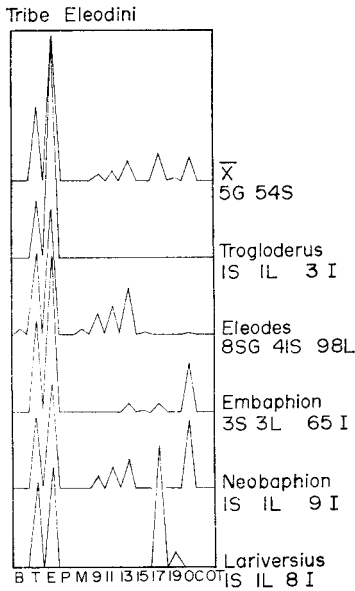


FIG. 9

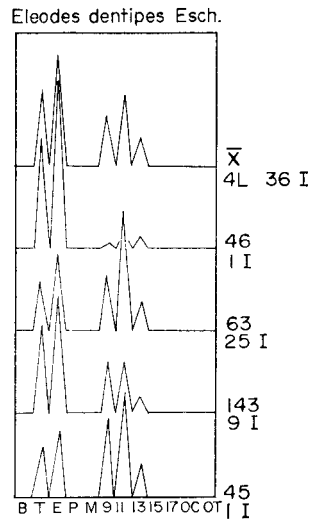


FIG. 11

Eleodes acuticauda Lec.

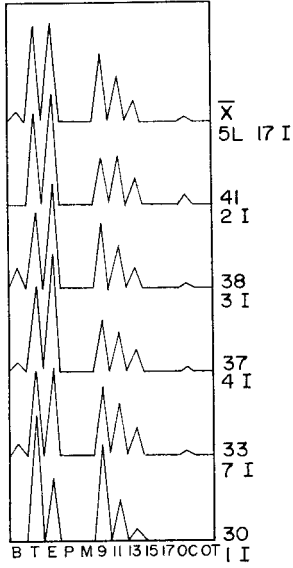


FIG. 12

Eleodes armata Lec.

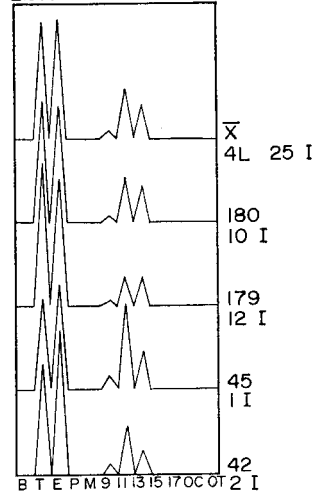


FIG. 13

Eleodes obscura (Say)

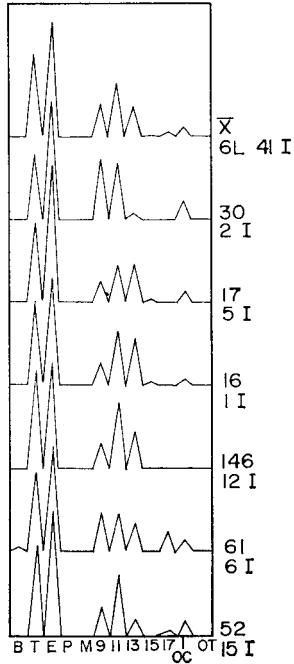


FIG. 14

Subgenus *eleodes*

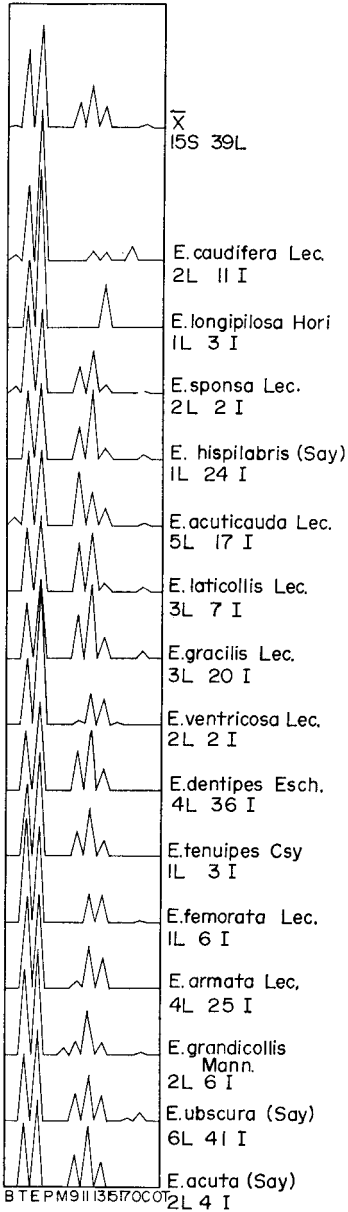


FIG. 15

Eleodes longicollis Lec.

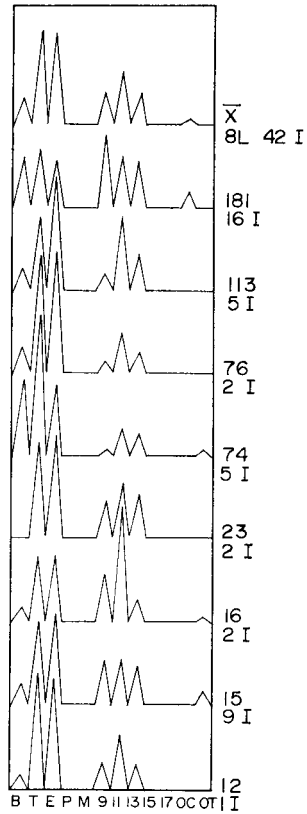


FIG. 16

Subgenus *steneleodes*

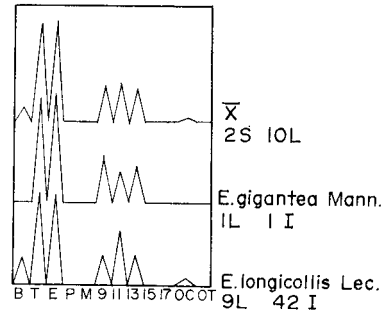


FIG. 17

Eleodes dissimilis Blais

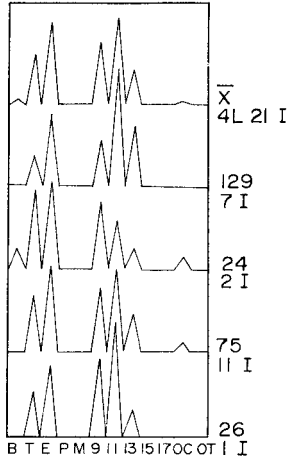


FIG. 18

Subgenus *metablapylis*

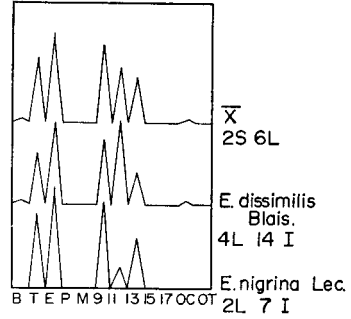


FIG. 19

Eleodes extricata (Say.)

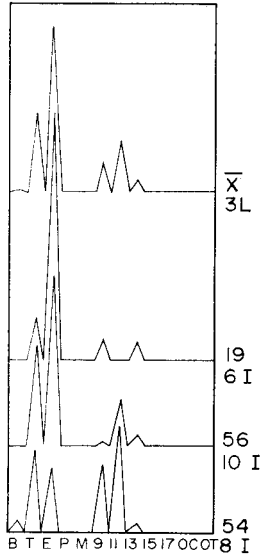


FIG. 20

Eleodes carbonaria (Say.)

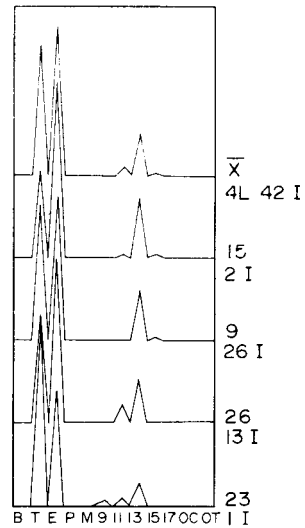


FIG. 21

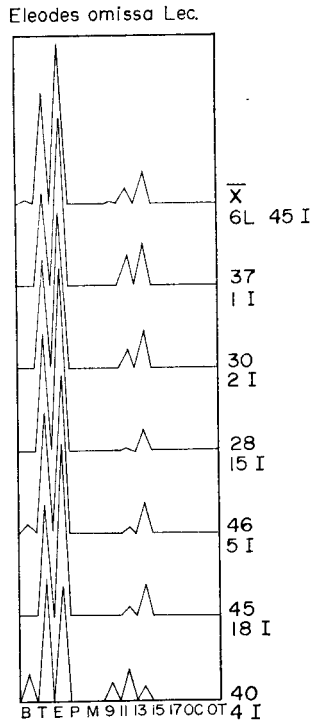


FIG. 22

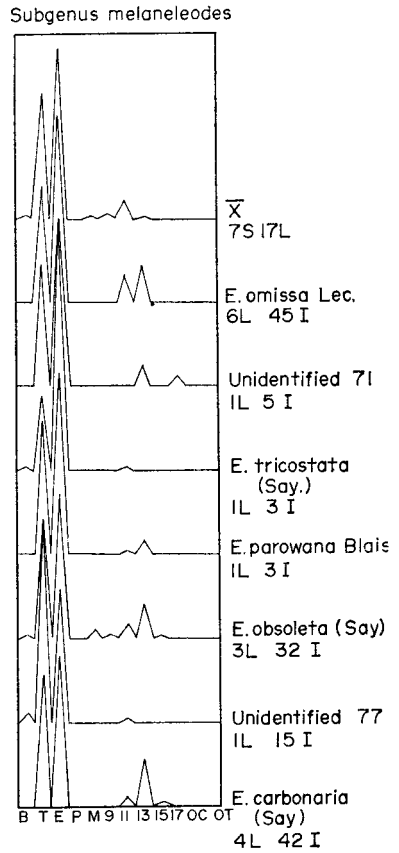


FIG. 23

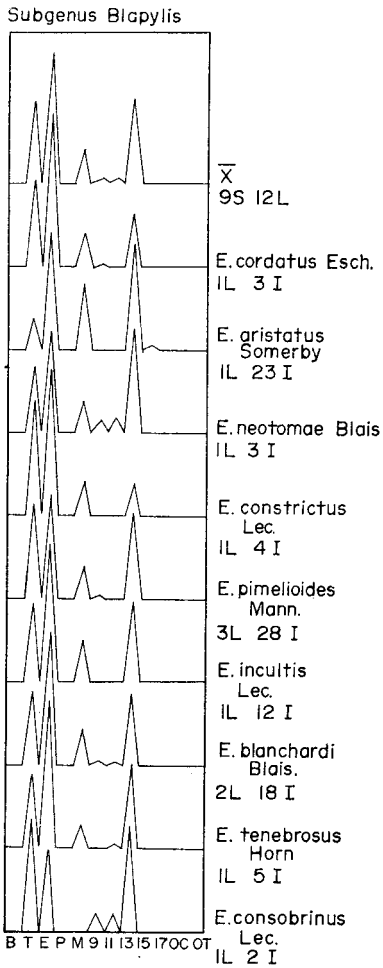


FIG. 24

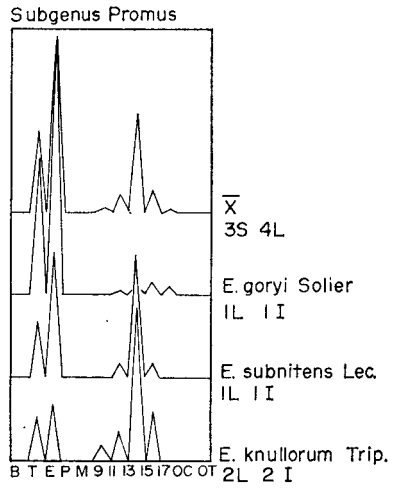


FIG. 25

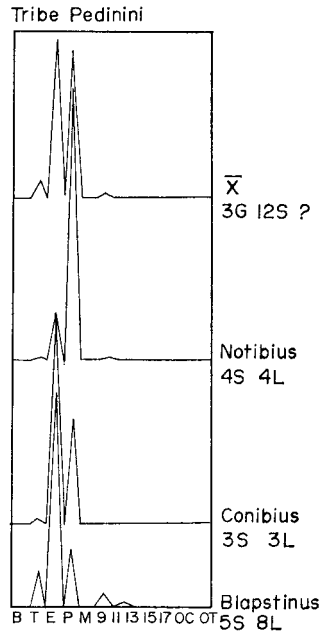


FIG. 26

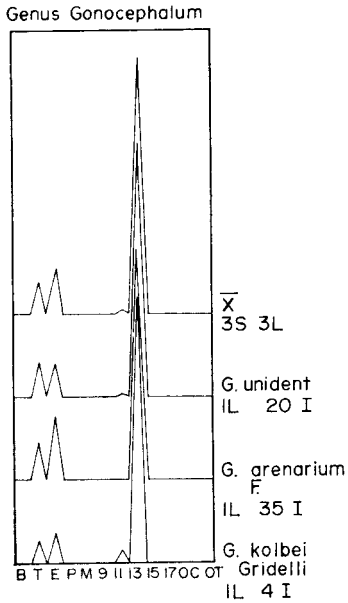


FIG. 27

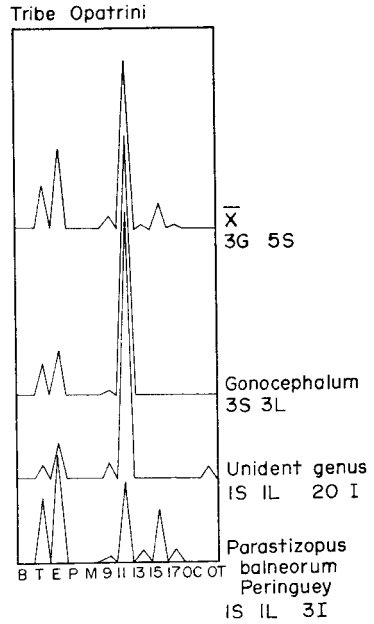


FIG. 28

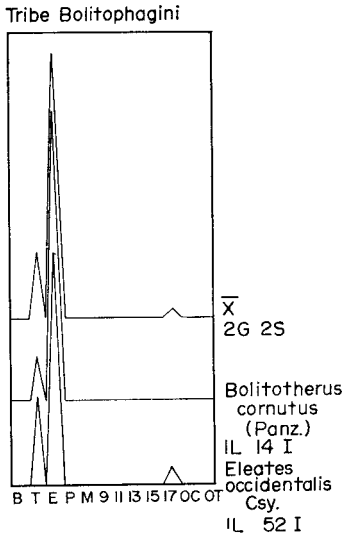


FIG. 29

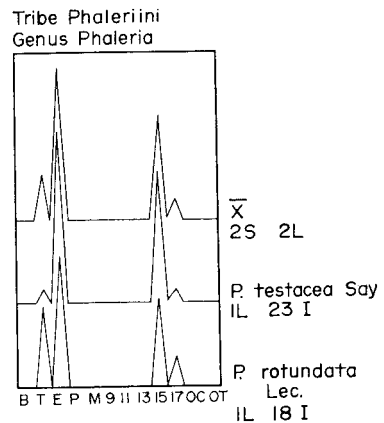


FIG. 30

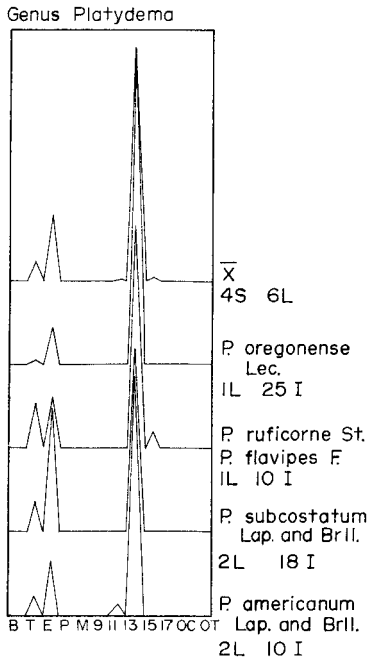


FIG. 31

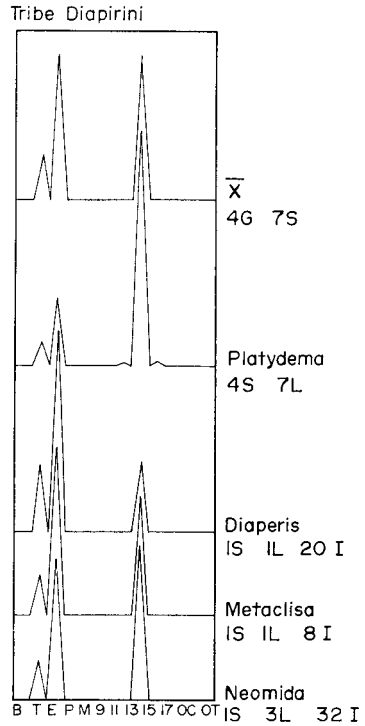


FIG. 32

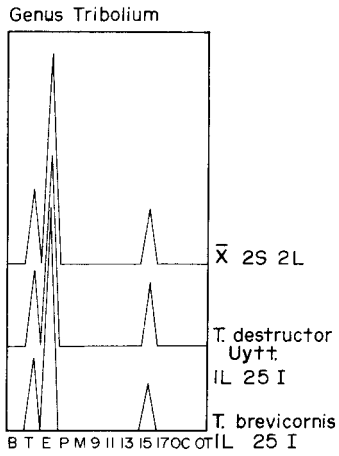


FIG. 33

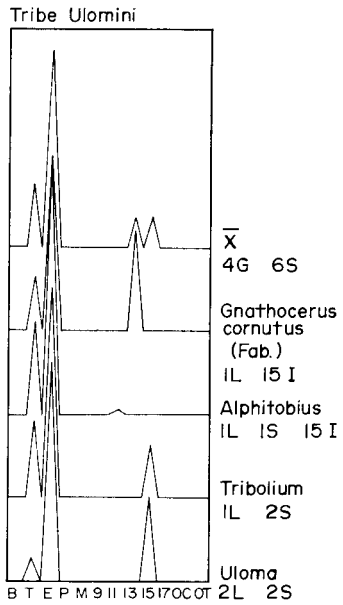


FIG. 34

Tribe Meracanthini

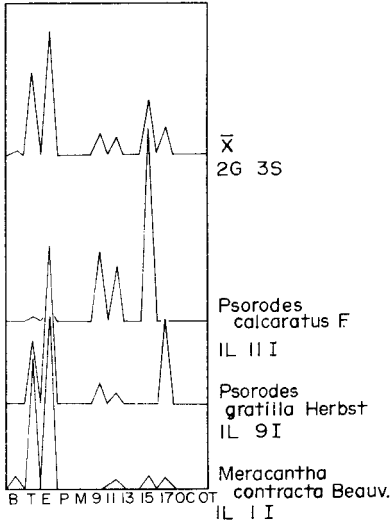


FIG. 35

Tribe amarygmini

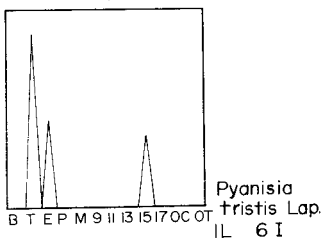


FIG. 36

Tribe Platynotini

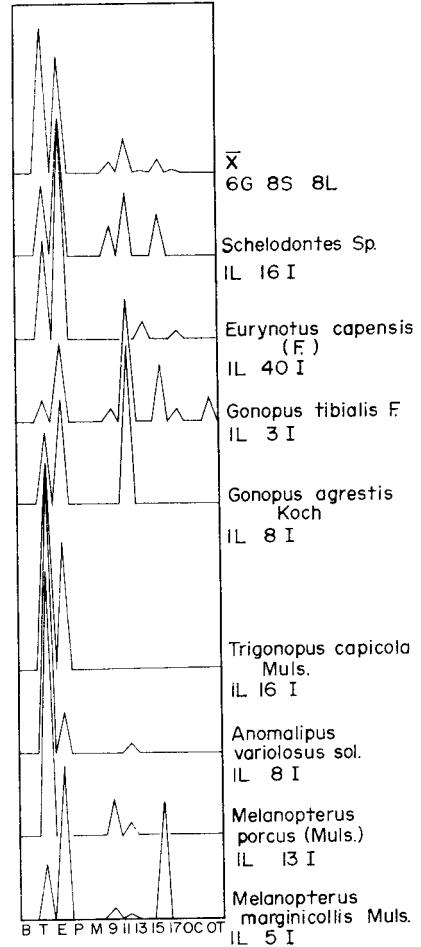


FIG. 37

Genus *Iphthimus*

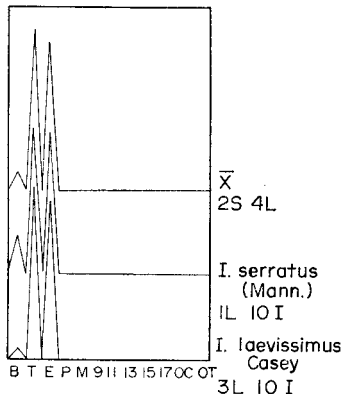


FIG. 38

Alobates Pennsylvanica Deg.

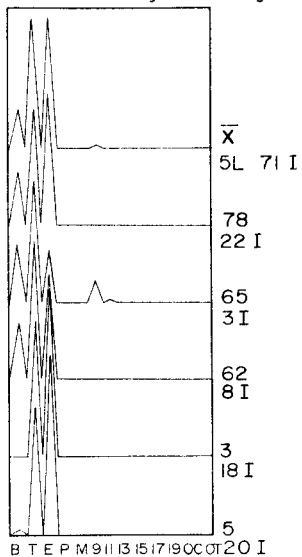


FIG. 39

Tribe Tenebrionini

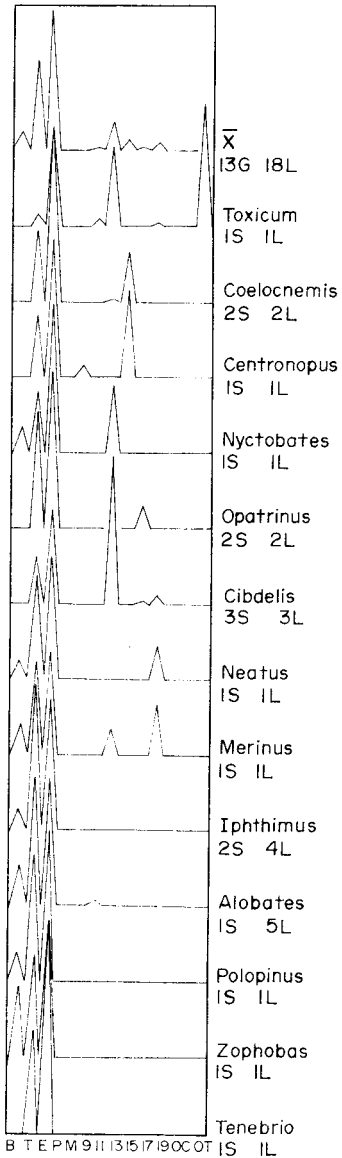


FIG. 40

Tribe Litoborini
Genus Zadenos

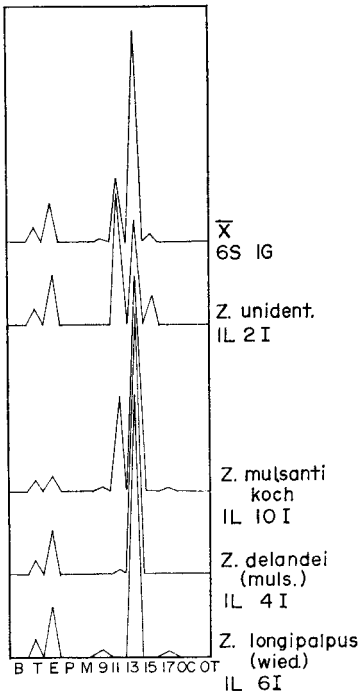


FIG. 41

Family Tenebrionidae

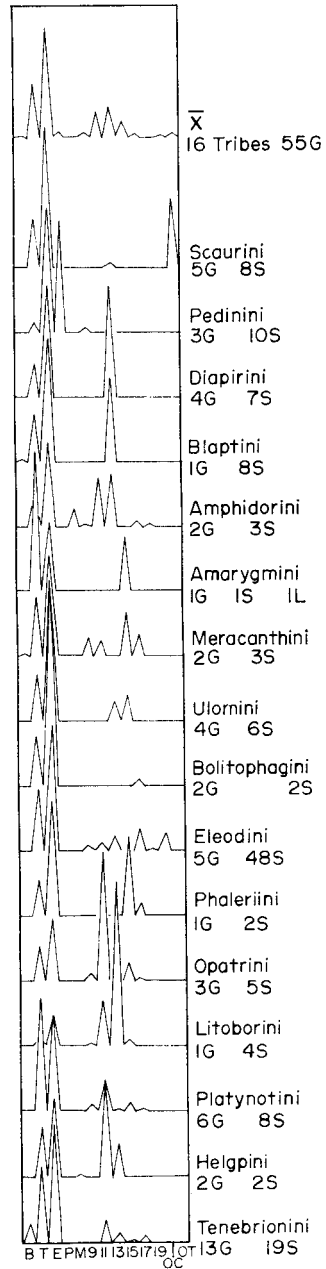


FIG. 42

Obviously then, such variation would obscure subtle differences between localities and species unless very large samples were taken. Differences of importance to the present study are therefore mostly sharp and consistent, since most samples were rather small. Easiest to use and interpret are, of course, occurrences of unique compounds, and a number of these have already been noted. Occurrence of uncommon compounds are often useful as well. Most species, however, have neither unique nor uncommon compounds and in these, the patterns of occurrence, both in terms of compounds present and their ratios, become of importance. While many taxa show non-descript patterns, many others do possess characteristic ones. Figs. 4 to 42 present the patterns of occurrence of compounds among the taxa of tenebrionids. The identity of the compound is abbreviated on the horizontal axis and the height of the triangle represents the weight per cent present. The graphs are spaced at 25 per cent intervals (or multiples thereof), and under the name of the taxon is given the number of units which make up the values presented in the graph.

Probably the simplest pattern is given by those species which secrete only quinones and entirely lack any hydrocarbons. With the exception of a few isolated occurrences among *Melaneleodes* (of *Eleodes*) (Fig. 23) which may simply be limiting cases, and two species of Platynotini (Fig. 37), this pattern is characteristic of several genera of Tenebrionini (Figs. 38-40) of Bolitophagini (Fig. 29) and of the Pedinini (Fig. 26). The last is separated from the others by the unique secretion of propylquinone in addition to the tolu- and ethylquinones. *Alphitobius diaperinus* of the Ulomini also secretes mostly quinones (Fig. 34), but other stocks have been reported to contain more alkenes (TSENG *et al.*, 1971). Likewise, among quinone-only species such as *Alobates pennsylvanica* DeG., occasional localities may exhibit small amounts of alkenes (Fig. 39, locality 65). It is interesting that most species having only quinones secrete benzoquinone, a compound relatively rare among those species which secrete hydrocarbons in addition. *Tenebrio* and the two species of Bolitophagini appear to be exceptions (Figs. 29, 40).

When quinones occur together with only a single 1-alkene, that alkene is usually 1-tridecene, and this pattern is characteristic of several groups. Species of *Blaps* (Fig. 6), *Gonocephalum* (Fig. 27), and all genera of Diapirini (Fig. 32) show this pattern. In *Platydema* (Fig. 31) tridecene actually greatly predominates over the quinones. Isolated occurrences also exist in groups of which this pattern is not generally characteristic: *Coelocnemis*, *Centronopus* (Fig. 40); *E. longipilosa* (Fig. 15); two localities of *E. carbonaria* and *E. omissa* (Figs. 21, 22), two species of *Zadenos* (Fig. 41), and *Gnathocerus* (Fig. 34).

The single alkene may also be other than tridecene. Thus, the occurrence of pentadecene as the single alkene is characteristic of *Tribolum*, *Uloma* (Figs. 33, 34), and *Pyanisia* (Fig. 36).

In the subgenus *Blapyllis* of *Eleodes*, tridecene is present as the only alkene in most species (Fig. 24), but this taxon is set apart from others by the secretion of 2,3-methylmethoxyquinone. The genus *Amphidora* seems similar in this regard, but the methylmethoxyquinone was not confirmed by other methods.

A number of groups have multiple alkenes but only one of these is present in great abundance. While *Cratidus* (Fig. 8) has many alkenes, including the rare nonadecene, only 1-undecene is present in large quantities. A somewhat similar pattern holds for *Cibdelis* whereas in *Promus* of *Eleodes* (Figs. 10, 25) tridecene predominates among the alkenes.

Phaleria is characterised by the presence of both pentadecene and heptadecene in substantial quantities (Fig. 30), and *Lariversius* is similarly characterised by heptadecene and nonadecene (Fig. 9).

Many species of the Eleodini have characteristically complex secretions (Figs. 9–25), but the genera and subgenera are not readily distinguished from one another. Many of the subgenera of *Eleodes*, with the exceptions of *Blapyllis* and *Melaneleodes*, regularly secrete nonene, undecene, and tridecene in substantial amounts (Fig. 10). Except for *Blapyllis*, *Promus*, and *Tricheleodes*, no single alkene is greatly predominant and most of the species of the subgenera *Eleodes*, *Steneodes*, *Metablapyllis*, *Litheleodes* show this pattern (Fig. 10). Both *Embaphion* and *Neobaphion* are distinguished from other Eleodine genera by the secretion of large amounts of octanoic acid (Fig. 9).

Secretions of the Meracanthini are quite variable (Fig. 35), the only common factor apparently being the consistent presence of alkenes larger than tridecene, but even these occur in greatly different amounts, even within *Psorodes*. *Psorodes calcaratus* is set apart by the presence of only small amounts of tolu- and ethyl-quinones.

Perhaps the least consistent pattern is within the African tribe, Platynotini (Fig. 37), which vary from the quinones-only secretion of *Trigonipus capicola* Muls. to the very complex mixture of *Gonopus tibialis*. Whether this is an indication of our poor taxonomic understanding of the tribe or to other factors is not known. All Platynotine species (but no other present African species) possess a gular strigil (TSCHINKEL, 1975a), indicating that the tribe may indeed be a sound one.

Similar heterogeneity is found among the species of Tenebrionini (Fig. 40). In this case, the tribe almost certainly contains a number of unrelated groups. DOYEN (1971) has separated out *Coelocnemis* and *Oenopium* into a new tribe, the Coelometopini, and TSCHINKEL (1975b) presents evidence based on defensive gland morphology that separates the tribe into at least three groups.

In addition to the abdominal defensive glands, a number of tenebrionids have a pair of glands lying in the prothorax and opening on the membrane between the head and neck. Among these are *Diaperis maculata* Oliv., *Tribolium* spp., *Zophobas rugipes* Kirsch, *Metaclisa marginalis* Horn, *Rhinandrus* spp., *Gonodera murina* (L.) (KENDALL, 1968), and *Alphitobius fasciatus* (TSCHINKEL, 1975b). Of these, the secretion of the thoracic glands of *D. maculata* (ROTH and STAY, 1958) and *Tribolium confusum* Duv. (LOCONTI and ROTH, 1953) has been shown to be identical to that of the abdominal glands, while those of *Zophobas rugipes* secrete an emulsion of phenols rather than the quinones of the abdominal glands (TSCHINKEL, 1969).

Metaclisa marginalis and three additional species of *Tribolium* were found to contain identical secretion in both pairs of glands, but, judging from odour, the thoracic glands of *Rhinandrus* produce phenols as in *Zophobas rugipes*.

DISCUSSION

Perhaps the chief obstacle to the interpretation of the chemical compositions is the almost complete absence of knowledge on the function, and therefore the selective advantage, of each of the compounds. Functions have been attributed to individual compounds in a few cases: EISNER *et al.* (1961) showed that, in the whipscorpion, octanoic acid acted as a spreading agent and lipid solvent in the very polar secretion whose chief irritant was acetic acid. In certain coreid bugs, alkanes occurring with aldehydes were thought to act as spreading agents, but no direct evidence was offered (WATERHOUSE and GILBY, 1964). Hydrocarbons in the formic acid secretion of formicine ants may act similarly in this very polar irritant (WILSON and REGNIER, 1971).

It may be erroneous in many cases to look for single functions of component compounds. Adding alkenes to a mixture of quinones changes more than its irritant quality. Physical characteristics may be very important to the functioning of the entire system. Viscous resistance to rapid flow must be critical to species which spray. To those species which head-stand, the partitioning of the two-phase secretion probably causes a greater proportion of the more irritating, organic, supernatant phase to be ejected. In those secretions consisting only of quinones, the organic phase is subnatant, while addition of alkenes makes it supernatant. One could argue that, for head-standing species, alkene secretion has selective value independent of irritant value.

In order to speculate about the functions of secretion components, it is necessary to know what type of predators have acted as selective agents in the evolution of the system. Secretions evolved against vertebrate and arthropod predators might be quite different due to different integumentary and sensory characteristics.

Unfortunately, little is known about the major predators of tenebrionids. The unpalatability of some species has been demonstrated: RASKE (1967) showed that *Eleodes* were unpalatable to skunks and some woodrats (*Neotoma*), and EISNER and MEINWALD (1966) report similar results with other potential predators. DOYEN (1975) provides circumstantial evidence from fox scats that tenebrionids with defensive secretion (*Eleodes*) are preyed upon less frequently by the Channel Island Fox than the sympatric *Eusattus* sp. (Tentyriidae) which lacks glands, though the protection afforded by the secretion is not complete. Mammalian scats and bird pellets often contain exoskeletal parts of tenebrionids, and sometimes consist exclusively of these. HYSLOP (1912) indicates that the sage hen (*Centrocercus urophasianus*) feeds largely on *Eleodes* and lists 15 other species of birds which feed on *Eleodes* more or less. BLAISDELL (1909) reports ground owls (*Speotyto cunicularia*) and skunks to feed on *Eleodes* sp. BARROWS and SCHWARZ (1895) found the remains of *Eleodes* sp. in the stomachs of crows in Kansas and Nebraska and state that "these beetles . . . fulfill most of the requirements of insect food preferred by crows; they are terrestrial, large, hard and possess a strong, offensive odor". The list of birds which WADE (1921) reports to feed on *Eleodes* overlaps largely with that of HYSLOP (1912). MCATEE (1932) reports that stomach content data indicated 175 bird species preying on tenebrionids. EISNER and MEINWALD (1966) note that the

grasshopper mouse, *Onychomys torridus*, is an effective predator on *Eleodes longicollis* in Arizona.

There have been no reports of arthropod predators of tenebrionids, but it is not possible to rule out such species as tarantulas and whipscorpions.

The literature thus indicates that, while defensive secretions do not afford tenebrionids absolute protection, they do provide some selective advantage, and it is likely that these secretions evolved in response to predation. Knowledge to date still does not allow interpretation of the composition of individual secretions. Thus, why do the Pedinini secrete three quinones, while the species of *Eleodes* mostly secrete both alkenes and quinones? Why does *Argoporis* secrete naphthoquinones in addition to benzoquinones, and why do *Embaphion* and *Neobaphion* secrete quantities of octanoic acid? Presumably, the answers lie in the sensitivities of the predators and the vagaries of the evolution of new biosynthetic capacities, but none of these questions can be answered at this time.

Judging from the convergence, coincidence and overall simplicity of the compounds used in defensive secretions (EISNER and MEINWALD, 1966; WEATHERSTON and PERCY, 1970), it seems possible that, within limits, the chemical nature of the compounds is of no great importance as long as the secretion meets certain physical requirements (lipophilicity, volatility, melting point, irritant value). BLUM (1974) has suggested that one function of rich defensive mixtures may be the "jamming" of the predator's chemosensory system. Experiments in this area are almost entirely lacking in relation to hydrophobic secretions such as those of tenebrionids.

Within classes of compounds, one can ascribe properties which may be important to detergency. Thus, to the human nose (and eyes) most benzoquinones possess a very sharp irritant property while 1-alkenes are less irritating by far. It is peculiar that some secretions consisting mostly of 1-alkenes (*Platydema*) nevertheless have the peculiar nose-biting quality of quinones rather than the heavy odour of hydrocarbons, while other secretions consisting mostly of quinones (e.g. *Zophobas*), lack this cutting property. The perceived odour, then, cannot be deduced from the GLC traces. In some cases, such as *Argoporis*, the odour quality of the whole secretion seems to be due to a very minor, as yet unidentified, component and not to the benzo- and naphthoquinones (TSCHINKEL, 1972).

Since all tenebrionids secrete alkylated benzoquinones, it seems reasonable that this synthetic capacity was an original feature of the primordial defensive gland, and that only later did other secretory abilities evolve. Some of those species with the morphologically most primitive glands, such as *Tenebrio* and *Zophobas* (TSCHINKEL, 1975b), secrete only quinones without alkenes. Beyond this, the secretion of the various common components is quite labile from taxon to taxon and it is difficult to arrive at evolutionary lineages on the basis of this evidence. The synthetic capacity of many tenebrionids is greater than the major components of the secretion indicate. If the minor or trace components are included in their synthetic repertoire, a large fraction of tenebrionids are able to synthesize most of the common compounds (three quinones and five alkenes) occurring throughout

the family. What causes some, but not others, to be secreted in large amounts is not known, but the answer must surely lie in the physical and biological agents of natural selection.

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APPENDIX

List of localities for Figs. 4-42

- (3) 5 miles NE Surgoinville on US 11W, Hawkins Co., TENN., 4 Apr. 1969; (5) 2 miles S Natural Bridge on St. Rt. 13, Winston Co., ALA., 9 Apr. 1969; (9) 6 miles E Bracketsville, Kinney Co., TEX., 13 Apr. 1969; (12) 10 miles N Park HQ on US 385 Big Bend N.P. TEX., 15 Apr. 1969; (15) 28 miles N Van Horn on Rt. 54, Culberson Co., TEX., 16 Apr. 1969; (16) 13 miles SW Whites City on US 180, Eddy Co., N.M., 16 Apr. 1969; (17) Near HQ, Carlsbad Caverns N.P., Eddy Co., N.M., 17 Apr. 1969; (19) 26 miles W Artesia on US 82, Eddy Co., N.M., 17 Apr. 1969; (23) Portal, Cochise Co., ARIZ., 21 Apr. 1969; (24) 1 m. E. Rustler's Park, Chiracahua Mts., Cochise Co., ARIZ., 21 Apr. 1969; (26) 5 miles SE Tucson on US 80, Pima Co., ARIZ., 23 Apr. 1969; (28) Jacumba, San Diego Co., CAL., 25 Apr. 1969; (30) Cameron Guard Station, on US 80 Laguna Mts., San Diego Co., CAL., 25 Apr. 1969; (32) La Jolla Farms Rd., La Jolla, San Diego Co., CAL., 28 Apr. 1969; (33) Soledad Valley, 1 mile E of Coast, San Diego Co., CAL., 28 Apr. 1969; (37) Descanso, San Diego Co., CAL., 2 May 1969; (38) Cuyamaca St. Park, San Diego Co., CAL., 2 May 1969; (40) Punta Banda, Baja Calif., MEX., 1 May 1969; (41) Playa Dorada near Ensenada, Baja Calif., MEX., 1 May 1969; (42) Scissors Crossing near Anza-Borrego St. Park, San Diego Co., CAL., 7 May 1969; (45) Queen Valley, Joshua Tree N. Mon., CAL., 10 May 1969; (46) Jumbo Rocks Campground, Joshua Tree N. Mon., CAL., 10 May 1969; (52) Zion Canyon, Zion N.P., UTAH, 16 May 1969; (54) 5 miles W. Mt. Carmel Jct., Kane Co., UTAH, 18 May 1969; (56) Bryce Canyon N. P., UTAH, 19 May 1969; (61) Coral Pink Sand Dunes, Kane Co., UTAH, 21 May 1969; (62) Fairview Terr., So. Glastonbury, Hartford Co., CONN., 14 June 1969; (63) Berkeley Marina, Berkeley, Alameda Co., CAL., 29 May 1969; (65) Archbold Biol. Sta., Lake Placid, Highlands Co., FLA., 23 June 1969; (74) 5-8 miles NNE Winslow, Navajo Co., ARIZ., 5 Sept. 1969; (75) Winslow, Navajo Co., ARIZ., 5 Sept. 1969; (76) 2 miles W. Sumalayuca, Chihuahua, MEX., 2 Sept. 1969; (78) Coddington Rd., Ithaca, Tompkins Co., N.Y., 25 Sept. 1969; (113) Kayenta, Navajo Co., ARIZ.; (146) Rawhide, NEV., June 1968; (179) Escondido Camp., St. Lucia Mts., CAL., 4 May 1970; (180) Berkeley Hills, Alameda Co., CAL., 12 June 1971; (181) S.W. Res. Station, Portal, Cochise Co., ARIZ., July 1971.