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# Chemosystematic Studies of the Triboliini (Coleoptera: Tenebrionidae): Phylogenetic Inferences from the Defensive Chemicals of Eight *Tribolium* spp., *Palorus ratzeburgi* (Wissmann), and *Latheticus oryzae* Waterhouse[1]

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Ann. Entomol. Soc. Am. 80: 398–405 (1987)

**ABSTRACT** The organic chemicals in the defensive secretions of 10 species representing both genus groups in the tenebrionid tribe Triboliini (subfamily Tenebrioninae) have been identified and quantified. All taxa contain substantial quantities of 2-methyl- and 2-ethylbenzoquinone with approximately 10-fold less of the corresponding hydroquinones. These are the only components in *Palorus ratzeburgi*, the only species examined from the *Palorus* genus group. Within the *Tribolium* genus group, the defensive secretion of *Latheticus oryzae* also contains 15-, 16-, and 17-carbon olefins. Eleven nonquinoid chemicals occur in the eight species of *Tribolium* examined. Members of the *confusum* species group (*Tribolium anaphe, Tribolium confusum, Tribolium destructor*) contain the 15-, 16-, and 17-carbon olefins. Those in the *castaneum* species group (*Tribolium castaneum, Tribolium freemani, Tribolium madens*) contain these olefins and two aromatic ketones. Species in the *brevicornis* group (*Tribolium brevicornis, Tribolium audax*) contain the olefins, the same aromatic ketones, and two aromatic esters. No taxonomically significant differences were found between the secretions of males and females. A comparison of the defensive secretions from six populations of *T. castaneum*, four of *T. confusum*, and two of *T. audax* indicated only quantitative intraspecific variation. A new species-group placement of *T. audax* is suggested based on the available morphological, biochemical, and biogeographical evidence; and chemosystematic inferences regarding the phylogeny of *Tribolium* are presented.

**KEY WORDS** *Tribolium* spp., phylogeny, biogeography, mass spectrometry, defensive secretion

THE FAMILY Tenebrionidae is large, >15,000 species (Watt 1966), and a taxonomically complex group whose higher-order classification is still poorly understood. The most recent classification treating the entire world fauna is that of Lacordaire (1859). Subsequent workers have modified portions of Lacordaire's classification and an excellent summary of these later modifications may be found in Watt (1966) and Doyen (1972).

*Tribolium* and related genera were long considered members of the tribe Ulomini (Sokoloff 1972) in the subfamily Tenebrioninae. This tribe, however, is considered by Doyen (1985) to be composed of at least four distinct lineages, and he has recently moved most of its genera to other tribes (Tschinkel & Doyen 1980, Doyen & Tschinkel 1982, Doyen 1984, 1985). A group of "ulomine" genera related to *Tribolium* and *Palorus* were grouped by Doyen into a new tribe, the Triboliini (Doyen 1985), with two genus groups: the *Palorus* group, containing almost exclusively Old World species (Halstead 1967), and the *Tribolium* group, with representatives occurring world-wide.

Within the *Tribolium* genus group, *Tribolium* has been better studied than the other genera, primarily because so many of its species are economically important. The major taxonomic work on *Tribolium* is that of Hinton (1948), with additional contributions by Doyen (1985), Good (1936), Halstead (1967, 1969), Sokoloff (1972), and Triplehorn (1978). Hinton (1948) recognized five species groups within *Tribolium*, separable on morphological and biogeographical grounds, and he offered a tentative hypothesis regarding the evolutionary relationships of these five species groups (Fig. 1A). As is the case with most systematic studies, this phylogeny was established using primarily morphological criteria.

In recent years increasing attention has been focused on the applicability of immunological, biochemical, and genetic techniques to phylogenetic

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

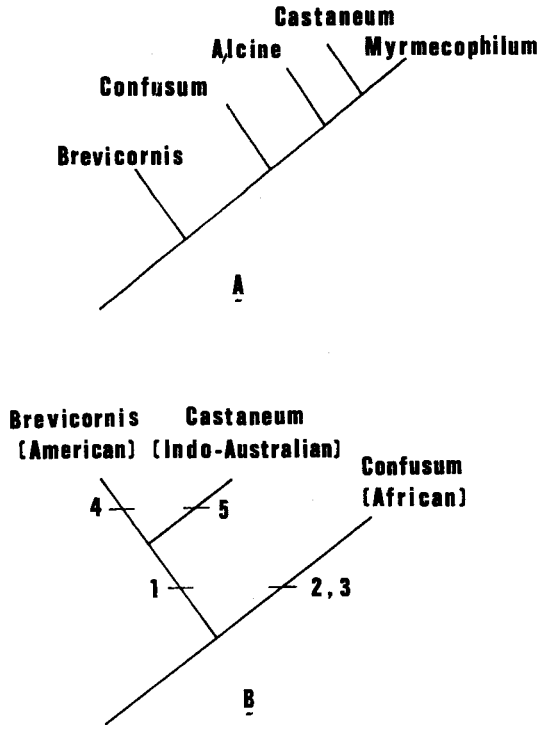


**Fig. 1.** (A) Hinton's (1948) proposed phylogeny of *Tribolium* species groups based on morphological characters and biogeographic analysis. *Alcine* species group occurs in Madagascar and the *myrmecophilum* species group occurs in Australia. (B) Inferred phylogeny of three species groups of *Tribolium* based on chemotaxonomic characters and biogeographic analysis (see Table 3 for identification of characters and their polarities).

questions (Wright 1974, Blackman et al. 1980, Oxford & Rollison 1983). Most insect phylogenetic biochemical studies have utilized primarily gel electrophoresis of enzymes (Berlocher 1984), but, as the studies of Cane (1983), Prestwich (1983), and Tschinkel (1975) show, valuable clues to phylogenetic relationships can also be inferred from the qualitative and quantitative distribution of nonmacromolecular components typical of insect defensive secretions.

All members of the Triboliini possess defensive reservoirs, which are either short and saccate with broad openings (*Palorus* genus group) or long and saccate with constricted openings (*Tribolium* genus group) (Doyen 1985). As is typical of most tenebrionids, the defensive secretions of the Triboliini appear to be dominated by quinones. Within *Tribolium*, 2-methylbenzoquinone and 2-ethylbenzoquinone and their corresponding hydroquinones are ubiquitous (Loconti & Roth 1953, Happ 1968, Sokoloff 1972, Tschinkel 1975, Markarian et al. 1978). Also reported to be present in several species of *Tribolium* are 1-pentadecene, 1,6-pentadecadiene, 1-heptadecene, 1,8-heptadecadiene, and 1,8,11-heptadecatriene (Suzuki et al. 1975a, Wirtz et al. 1978; unpublished data). Recently Suzuki et al. (1975b) reported that Japanese populations of *Tribolium castaneum* (Herbst) and *Tribolium confusum* (J. du Val) contain the aromatic ketone 2′-hydroxy-4′-methoxypropiophenone. Howard et al. (1986) found this ketone in a North American population of *T. castaneum* and identified a homolog as well (2′-hydroxy-4′-methoxyacetophenone). Howard & Mueller (1987) have recently found that the defensive secretion of the North American tenebrionid *Tribolium brevicornis* (LeConte) contains both the foregoing aromatic ketones as well as two previously unknown aromatic esters (methyl 2,5-dihydroxy-6-methylbenzoate and methyl 2,5-dihydroxy-6-ethylbenzoate).

I have conducted a detailed comparative analysis of the defensive secretions of eight species of *Tribolium* (*Tribolium* genus group) representing three of Hinton's five species groups. I have also examined the defensive secretions of *Palorus ratzeburgi* (Wissmann) and *Latheticus oryzae* Waterhouse representing, respectively, a member of the *Palorus* genus group and another member of the *Tribolium* genus group of the Triboliini. I have examined possible sex-related differences in all 10 species, and possible population-related differences in three of the species of *Tribolium*. In conjunction with morphological and biogeographical considerations, I present some chemosystematic inferences regarding the phylogeny of three of the *Tribolium* species groups.

## Materials and Methods

**Insects.** Beetles were reared at 30 ± 1°C in constant darkness on whole-wheat flour containing 3% Brewer's yeast. Adults used for chemical analysis were all 4–8 wk old. Individuals used for male/female comparisons were sexed and separated as pupae. All insects studied, including Kansas populations of *Tribolium* maintained at the U.S. Grain Marketing Research Laboratory, had been in culture for many generations. The species of *Tribolium* I studied and their sources of origin are *Tribolium anaphe* Hinton (Madison, Wis.); *Tribolium audax* Halstead (California; Idaho); *Tribolium brevicornis* (LeConte) (California); *T. castaneum* (Florida; Kansas; New York; McGill University, Québec, Canada; Veracruz, Mexico; Slough, United Kingdom); *T. confusum* (Chicago, Ill.; Kansas; McGill University, Québec, Canada; Yugoslavia); *Tribolium destructor* Uyttenboogaart (Riverside, Calif.); *T. freemani* Hinton (Japan); *Tribolium madens* (Charpentier) (Madison, Wis.). Representative voucher specimens of all species used in this study have been deposited in the Research Museum collection of the Department of Entomology, Kansas State University, Manhattan.

**Chemical Analyses.** Individual live insects were transferred to microvials constructed from Pasteur pipettes and then soaked for 30 min in 5, 10, or

15 μl of pesticide-grade 2,2,4-trimethylpentane (preliminary comparisons between "milked" defensive secretions and those obtained by soaking whole insects showed no difference between their chromatographic profiles in the region of interest). The slightly yellowish solutions obtained were then analyzed by capillary gas chromatography and mass spectrometry. Analytical analyses were conducted on a Hewlett-Packard 5730A gas chromatograph containing a HP crosslinked methyl silicone capillary column (12.5 m by 0.2 mm) and a flame ionization detector. Nitrogen was the carrier gas and injections were made using a 60-s delay in the splitless mode. Signals from the gas chromatograph (GC) were stored and peak areas were measured using a Hewlett-Packard 3380 integrator. Mass spectral analyses were conducted using a Hewlett-Packard 5790A GC containing a 30-m-by-0.2-mm Supelco DB-1 bonded phase capillary column connected to a Hewlett-Packard 5970 mass selective detector and a Hewlett-Packard 9133 data system. Retention times and mass spectra of all defensive-secretion components were compared with authentic known samples of the chemicals. Double-bond locations in all olefins were verified by mass-spectral examination of their dithiomethyl ethers (Francis & Veland 1981). Infrared-spectral examination of the olefins indicated the presence of only terminal vinyl or *cis* (Z) double bonds, or both. The structures of all components found among the defensive secretions of the species examined are shown in Fig. 2.

**Data Analysis.** Analysis of variance (ANOVA) was conducted using SAS computer programs (SAS Institute 1982) with species, sex, and source populations being the factors examined. The general linear models procedure with the least mean technique was used to separate significantly different means. Data were expressed as proportions (quinones and hydroquinones, ubiquitous among the taxa, were excluded from the calculations) and then transformed to the arcsine of the square root of proportions for ANOVA. Reported means are on untransformed data. All tests for significance were conducted at the $\alpha = 0.05$ level.

## Results

The defensive secretion of *P. ratzeburgi* contained no detectable organic constituents other than 2-methylbenzoquinone (1), 2-ethylbenzoquinone (2), and the corresponding hydroquinones (3) and (4) (Fig. 2). In contrast, the defensive secretion of *L. oryzae* contained not only the quinones and hydroquinones, but also 1-pentadecene (5), 1,6Z-pentadecadiene (6), 1-hexadecene (7), 1,7Z-hexadecadiene (8), 1-heptadecene (9), 1,8Z-heptadecadiene (10), and 1,8Z,11Z-heptadecatriene (11) (Fig. 2). In both of these species no differences between males and females were found.

All eight species of *Tribolium* contained the foregoing components 1–11. In addition, *T. audax*, *T. brevicornis*, *T. castaneum*, *T. freemani*, and *T. madens* each contained 2′-hydroxy-4′-methoxyacetophenone (12) and 2′-hydroxy-4′-methoxypropiophenone (13) (Fig. 2). *T. audax* and *T. brevicornis* further contained methyl 2,5-dihydroxy-6-methylbenzoate (14) and methyl 2,5-dihydroxy-6-ethylbenzoate (15). As with *P. ratzeburgi* and *L. oryzae*, no significant differences between males and females were found in the eight *Tribolium* species examined.

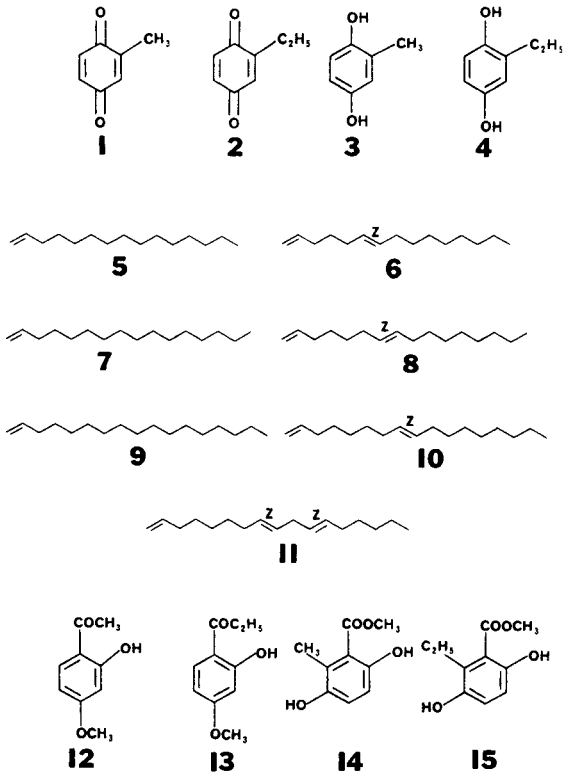


**Fig. 2.** Fifteen defensive secretion components of 10 species of tenebrionids in the tribe Triboliini. Letter Z above the internal double bonds in structures 6, 8, 10, and 11 denote that these double bonds have Z (*cis*) stereochemistry.

Because the quinones 1 and 2 and the hydroquinones 3 and 4 were common to all 10 species and showed no taxonomically useful variation, they were excluded from the analysis. The possibilities of large qualitative and quantitative intraspecific populational variation among compounds 5–15 was examined using six populations of *T. castaneum*, four of *T. confusum*, and two of *T. audax*. The results are shown in Table 1. Although modest amounts of quantitative variation occur among populations of each of the three species, no qualitative variation was found. No *T. confusum* was ever found to contain either ketones 12 or 13 or esters 14 or 15. Likewise, no *T. castaneum* was ever found not to contain 12 and 13, nor did any *T. castaneum* ever contain esters 14 and 15. Fi-

**Table 1. Mean percentage composition of the defensive secretions of different populations of *T. audax*, *T. castaneum*, and *T. confusum***

| Species | Population | Compound[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *T. audax* | California | 53.13a | 33.70a | 0.49a | 0.03a | 7.02a | 3.88a | 0.64a | 0.09a | 0.33a | 0.25a | 0.43a |
| | Idaho | 52.33a | 33.08a | 0.56a | 0.04a | 7.11a | 4.21a | 0.87a | 0.08a | 0.41a | 0.42b | 0.90b |
| *T. castaneum* | Florida | 68.52a | 4.76a | 0.57a | 0.03a | 7.06b | 14.31b | 0.81a | 0.39a | 3.57b | 0 | 0 |
| | Quebec, Can. | 54.21bc | 2.32b | 0.59a | 0.04a | 8.52b | 27.12a | 1.49a | 0.55a | 5.17ab | 0 | 0 |
| | New York | 65.52a | 2.95b | 0.46a | 0.04a | 7.42b | 18.78b | 1.12a | 0.27a | 3.44b | 0 | 0 |
| | Slough, U.K. | 63.57ab | 2.32b | 0.77a | 0.05a | 13.39a | 15.00b | 1.30a | 0.08b | 3.52b | 0 | 0 |
| | Veracruz, Mex. | 46.07c | 2.33b | 0.89a | 0.02a | 12.51a | 28.63a | 2.10a | 0.43a | 7.04a | 0 | 0 |
| | Kansas | 65.99a | 2.36b | 0.67a | 0.01a | 10.21ab | 15.84b | 0.87a | 0.54a | 3.50b | 0 | 0 |
| *T. confusum* | Chicago | 94.51b | 1.57a | 0.53a | 0.02a | 1.19a | 1.75a | 0.44ab | 0 | 0 | 0 | 0 |
| | Quebec, Can. | 93.76b | 2.49a | 0.39a | 0.02a | 0.89ab | 1.94a | 0.52a | 0 | 0 | 0 | 0 |
| | Yugoslavia | 96.59a | 1.56a | 0.42a | 0.05a | 0.54b | 0.61b | 0.24b | 0 | 0 | 0 | 0 |
| | Kansas | 96.10a | 1.86a | 0.51a | 0.05a | 0.67b | 0.80b | 0.02b | 0 | 0 | 0 | 0 |

[a] Means within a column for a given species followed by the same letter are not significantly different ($P < 0.05$; ANOVA; $n = 5$ for all means).

nally, no *T. audax* was ever found to lack either the ketones or esters.

The relative abundance values for components 5–15 for the eight *Tribolium* species and for *L. oryzae* and *P. ratzeburgi*, grouped by Doyen's (1985) genus groups and Hinton's (1948) species groups, are listed in Table 2. My placement of *T. audax* in the *brevicornis* species group rather than in the *castaneum* species group (Halstead 1969, Becker 1982) will be explained later. Apart from the qualitative species differences noted above for the ketones and esters, there are major species differences in the relative abundances of the various hydrocarbons (Table 2). These qualitative and quantitative differences were used as characters for a cladistic analysis of the eight *Tribolium* species. *L. oryzae* was chosen as the outgroup representative and character polarities were assigned based on the conditions (plesiomorphic or primitive) found in *Latheticus*. The five characters employed (Table 3) and their states in *Latheticus* and the eight *Tribolium* species, are presented in a character matrix (Table 4). Fig. 1B presents the resulting cladogram for the three species groups of *Tribolium* examined based on my chemotaxonomic character set.

## Discussion

Tschinkel's (1975) comparative study of the defensive secretions of 147 species of tenebrionids representing 55 genera and 16 tribes showed that these beetles synthesize a wide diversity of natural products. Common to all tenebrionids are 2-methyl- and 2-ethylbenzoquinone and their corresponding hydroquinones. Additional benzoquinones and naphthoquinones are present in certain taxa. The other most common chemical group in these defensive secretions is unsaturated hydrocarbons of varying chain length, and some patterns can be discerned in their distribution among taxa. Such patterns, however, frequently cross tribal lines, making it difficult to discern evolutionary relationships based solely on these chemotaxonomic characters (Tschinkel 1975).

In other cases, however, uniquely derived chemicals are present, which offer much potential for inferring phylogenetic relationships. The genus *Tribolium* contains chemicals that fall into this latter category. The ketones 12 and 13 and the esters 14 and 15 are unknown outside the genus and, within the genus, they are restricted to certain species groups. Even chemicals such as the olefins, which occur outside of *Tribolium*, still show within the *Tribolium* species groups discrete quantitative trends that permit recognition of characters and assignment of plesiomorphic or apomorphic states.

Biochemical characters, like morphological characters, may exhibit phenotypic variability among individuals. Yet both sexes of *Tribolium* possess morphologically similar prothoracic and abdominal defensive glands, and both produce essentially identical secretions from these glands. Age-related variability in the taxa that I have examined appears to reflect solely the absolute quantity of the secretions produced (Markarian et al. 1978, Wirtz et al. 1978; unpublished data[2]) and not the chemical compositions of the secretions. Hill & Tschinkel (1985), however, have reported different findings for another tenebrionid beetle. The largest source of phenotypic intraspecific variability seems to be the geographic source of the population. But, as noted earlier, that variability is only quantitative. The only apparent exception to this statement is the report of Suzuki et al. (1975b) of ketone 13 in a Japanese population of *T. confusum*. I have made a very careful examination of individual beetles from four populations of *T. confusum* from the United States, Canada,

[2] Typical absolute quantities of defensive components per insect are 50–200 μg of quinones, 5–10 μg of hydroquinones, 25–100 μg of olefins, 1–50 μg of ketones, and 1–20 μg of esters.

**Table 2. Mean percentage (±SEM) composition of defensive secretion components, excluding quinones and hydroquinones, of the Triboliini**

| Taxa | $n^a$ | Compound | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *Palorus* genus group | | | | | | | | | | | | |
| *P. ratzeburgi* | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tribolium* genus group | | | | | | | | | | | | |
| *L. oryzae* | 10 | 90.00 ± 1.01 | 1.02 ± 0.12 | 0.12 ± 0.02 | 0.01 ± 0.002 | 2.99 ± 0.45 | 4.15 ± 0.66 | 1.70 ± 0.25 | 0 | 0 | 0 | 0 |
| *Tribolium brevicornis* species group | | | | | | | | | | | | |
| *T. audax* | 30 | 52.10 ± 1.0 | 32.61 ± 0.61 | 0.52 ± 0.02 | 0.04 ± 0.01 | 7.06 ± 0.31 | 5.45 ± 1.38 | 0.77 ± 0.04 | 0.07 ± 0.01 | 0.43 ± 0.06 | 0.30 ± 0.03 | 0.64 ± 0.06 |
| *T. brevicornis* | 19 | 55.74 ± 2.18 | 9.03 ± 0.60 | 0.65 ± 0.06 | 0.01 ± 0.003 | 13.47 ± 1.24 | 1.95 ± 0.40 | 0.11 ± 0.03 | 1.07 ± 0.16 | 16.38 ± 2.22 | 0.90 ± 0.12 | 0.70 ± 0.13 |
| *castaneum* species group | | | | | | | | | | | | |
| *T. castaneum* | 47 | 62.58 ± 1.36 | 2.67 ± 0.14 | 0.66 ± 0.05 | 0.02 ± 0.004 | 9.98 ± 0.41 | 18.46 ± 1.01 | 1.13 ± 0.09 | 0.44 ± 0.03 | 4.06 ± 0.25 | 0 | 0 |
| *T. freemani* | 20 | 68.41 ± 1.30 | 5.40 ± 0.29 | 0.87 ± 0.04 | 0.04 ± 0.01 | 8.16 ± 0.51 | 9.73 ± 0.71 | 0.59 ± 0.04 | 0.65 ± 0.05 | 6.16 ± 0.39 | 0 | 0 |
| *T. madens* | 18 | 52.91 ± 2.47 | 17.42 ± 1.26 | 0.69 ± 0.03 | 0.07 ± 0.01 | 18.10 ± 0.93 | 6.86 ± 0.33 | 1.36 ± 0.07 | 0.15 ± 0.01 | 2.43 ± 0.14 | 0 | 0 |
| *confusum* species group | | | | | | | | | | | | |
| *T. anaphe* | 14 | 98.12 ± 0.16 | 0.78 ± 0.10 | 0.49 ± 0.09 | <0.01 | 0.29 ± 0.03 | 0.30 ± 0.04 | 0.02 ± 0.01 | 0 | 0 | 0 | 0 |
| *T. confusum* | 32 | 95.56 ± 0.24 | 1.87 ± 0.10 | 0.48 ± 0.03 | 0.04 ± 0.004 | 0.76 ± 0.06 | 1.09 ± 0.11 | 0.20 ± 0.04 | 0 | 0 | 0 | 0 |
| *T. destructor* | 19 | 76.71 ± 1.12 | 15.74 ± 1.20 | 0.46 ± 0.09 | 0 | 0.82 ± 0.13 | 5.70 ± 0.95 | 0.56 ± 0.08 | 0 | 0 | 0 | 0 |

[a] Number of individual beetles analyzed.

**Table 3. Characters and character states used for a cladistic analysis of eight species of *Tribolium***

| Character | Character code | Plesiomorphic state (−)[a] | Apomorphic state (+) |
|---|---|---|---|
| Ketones | 1 | Absent | Present |
| % 9 | 2 | >2% | <2% |
| Ratio % 5/% 9 | 3 | <50 | >50 |
| Esters | 4 | Absent | Present |
| Sum % 9, 10, + 11 | 5 | <15% | >15% |

[a] Based on *L. oryzae* as the outgroup.

**Table 4. Character matrix used for a cladistic analysis of eight species of *Tribolium***

| Species | Character[a] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *L. oryzae*[b] | −[c] | − | − | − | − |
| *T. anaphe* | − | +[d] | + | − | − |
| *T. audax* | + | − | − | + | − |
| *T. brevicornis* | + | − | − | + | − |
| *T. castaneum* | + | − | − | − | + |
| *T. confusum* | − | + | + | − | − |
| *T. destructor* | − | + | + | − | − |
| *T. freemani* | + | − | − | − | + |
| *T. madens* | + | − | − | − | + |

[a] See Table 3.
[b] Outgroup used to assign character polarity to *Tribolium* spp.
[c] Taxon exhibits plesiomorphic state.
[d] Taxon exhibits apomorphic state.

and Yugoslavia, using both gas chromatography and selective-ion-monitoring mass spectrometry (SIMMS), and I have never found any evidence for 13 (or 12) in my material. The limit of detectability with the SIMMS is approximately 10 pg. A similar careful examination of individual beetles representing the other members of the *confusum* species group (*T. anaphe* and *T. destructor*) likewise failed to show any evidence for the ketones 12 or 13. A resolution of the discrepancy between these results and those of Suzuki et al. (1975b) will have to await further studies.

The phylogenetic history of *Tribolium* species has been the subject of considerable discussion over the years (Sokoloff 1972), with the most comprehensive approach to the question being that of Hinton (1948). He noted that, except for those species like *T. castaneum* and *T. confusum* that are now cosmopolitan, most species can be associated with one of five distinct biogeographical regions (North and South America, Africa, Madagascar, Indo-Malaysia, and Australia), and species within each region form discrete species groups based on morphology. The American species (*brevicornis* group) were considered by Hinton to be the most primitive, based primarily on the retention of distinct marginal line on the side of the apex of the pronotum that is continuous with the lateral marginal line. Hinton considered the *confusum* species group (African) to be more closely related to the *castaneum* species group (Indo-Malaysian) than to the *brevicornis* species group. This inference was based on the fact that "primitive members" in the *confusum* and *castaneum* species groups share similar overall appearance and males possess similar secondary characters (Hinton 1948). Hinton considered the *myrmecophilum* species group (Australian) to be closely related to the *castaneum* species group and possibly even to be a subset (section) of it. The *alcine* species group (Madagascaran) seemed to be intermediate in character between those of the *castaneum* species group and the *confusum* species group. Hinton used historical biogeographic arguments to infer that the invasion of Madagascar by *Tribolium* probably involved the prototype of the *alcine* species group entering in the Upper Cretaceous from India. Hinton's phylogenetic inferences are illustrated in Fig. 1A.

More limited studies of the phylogeny of *Tribolium* have been conducted by others. Smith (1952a,b) suggested from cytological data that *T. confusum* evolved from an ancestor similar to *T. castaneum* by translocation of an autosome to the X chromosome, and that *T. castaneum* was the more primitive of these two species (Smith 1950). Wool (1982), using isoenzyme electrophoretic analysis techniques, considered the relationships of *T. brevicornis*, *T. castaneum*, *T. confusum*, and *T. destructor*. Based on genetic similarities and Nei genetic-distance estimates, Wool concluded that *T. castaneum* and *T. confusum* were much more similar to *T. brevicornis* than they were to each other, and that *T. confusum* and *T. destructor* were very dissimilar electrophoretically. It is not clear how Wool envisions the phylogeny of the four species because he did not specify which taxa were sister groups. He did, however, indicate that he too considered *T. brevicornis* to be the most primitive. Other studies that have addressed the question of the relationships of various *Tribolium* species to one another using morphological characters are summarized in Sokoloff (1972).

As yet, no cladistic analysis of *Tribolium* has been conducted. I have made a beginning in this direction, using the chemical components of the insect's defensive secretions as my character set. Unlike previous studies, summarized in Sokoloff (1972), my analysis (Fig. 1B) suggests that the *confusum* species group is not the sister group of the *castaneum* species group. Rather, based on the presence of the unique ketones shared by the *brevicornis* and *castaneum* species groups, they are sister groups to each other, and, within the defensive-secretion character set, they have no synapomorphies in common with the *confusum* species group. Using similar reasoning, the presence of the uniquely derived esters is a synapomorphy shared by *T. brevicornis* and *T. audax*, and suggests that they should be considered members of the same species group. This placement of *T. audax* is strengthened by biogeographical information. *T. audax* is an endemic American

species, as are all species currently considered to belong to the *brevicornis* species group, and neither *T. audax* nor other members of the *brevicornis* species group are known to be involved to any significant extent in dispersal via stored-grain commerce.

These conclusions of course contradict the current view that *T. audax* is a member of the *castaneum* species group (Halstead 1969). I am not arguing that the chemosystematic characters (esters 14 and 15) should be given more weight than morphological characters (primarily the shape of the pronotal margin and number of antennal club segments). However, the present chemical and biogeographical data argue at least as strongly for considering *T. audax* to belong to the *brevicornis* species group rather than the *castaneum* species group. Clearly the defensive secretions of additional members of the *brevicornis* species group need to be examined for the presence of esters before any definitive conclusions can be reached.

## Acknowledgment

I thank B. J. Harrington for her patience in introducing me to the methods of cladistic analysis and for her comments on earlier drafts of this manuscript. I also thank D. H. Blocker, J. H. Cane, J. T. Doyen, and C. A. Triplehorn for their reviews of this manuscript. C. A. Triplehorn (Department of Entomology, Ohio State University, Columbus) verified the species identification of the species of *Tribolium* used in this study. Finally, I thank C. Hampton for assistance in culturing the insects and conducting gas chromatographic analyses, and G. Milliken for assistance with statistical analyses. *L. oryzae* was obtained from Robert Mills (Kansas State University, Manhattan). *P. ratzeburgi* was obtained from Terry Arbogast (ARS–USDA, Savannah, Ga.). Non-Kansas populations of *Tribolium* were provided by Wendell Burkholder (ARS–USDA, Madison, Wis.), Richard Beeman (ARS–USDA, Manhattan, Kans.), Rudolph Strong (University of California, Riverside), and A. Sokoloff (California State University, San Bernardino).

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*Received for publication 30 July 1986; accepted 22 December 1986.*