Comparative Study of the Pygidial Defensive Systems of Carabid Beetles¹

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(Received February 16, 1977)

Investigations were made on the pygidial defensive components and gland morphologies of 132 species in 60 genera of carabid beetles. Components so far detected may be divided into six groups: (1) formic acid, (2) methacrylic, tiglic and/or ethacrylic acids, (3) other acids, such as *n*-butyric, *iso*-butyric, *iso*-valeric, *iso*-crotonic, angelic and several other acids (4) *m*-cresol, (5) benzoquinones with one chamber, (6) benzoquinones with two chambers. Gland types so far observed may be divided into three types: (1) spherical lobes which produce fatty acids, (2) elongated lobes which produce *m*-cresol, (3) long thick lobes which produce benzoquinones. There were also three different kinds of fatty-acid-producing glands at the points of entry of the collecting canals to the reservoirs. The same genera, or at least the same subgenera, shared similar components and gland types.

INTRODUCTION

Carabid beetles possess a pair of pygidial glands in the apex of the abdomen, and can secrete often by spraying a mixture of chemicals that they are known to employ in escaping from attackers such as predators. In the last decade research has progressed on the carabid defensive systems (SCHILDKNECHT et al., 1963, 1968; EISNER et al., 1963, 1970; MOORE and WALLBANK, 1968; FORSYTH, 1972; BENN et al., 1973; KANEHISA, 1973; SCOTT et al., 1975; OOSHIMA et al., 1975). They have revealed saturated and unsaturated fatty acids with short carbon chain length, cresols, benzoquinones, salicylaldehyde, and minor components such as hydrocarbons, esters, aldehydes or ketones. Some workers have also discussed the taxonomic correlation with chemical compositions.

The pygidial gland systems of carabid beetles were investigated from the chemical compositions and gland morphologies of 132 species in 60 genera collected in the Chūgoku district of Japan. This study shows some findings concerning variations of the defensive systems, and may contribute to a more comprehensive account of the defensive secretion and phylogenetic relationship within carabid beetles.

MATERIALS AND METHODS

Carabid beetles. The beetles tested were collected from crop fields, dry river beds,

¹ Supported in part by a grant from the Ministry of Education, Japan.

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mountain paths and a light trap over a year from 1971 in the Chūgoku district, (mostly Okayama prefecture). All living beetles were brought to the laboratory, immersed in ether and stored in a freezer (-20°C) until the assays.

Pygidial glands. Beetles were dissected in a small quantity of water, and gland systems were observed for variations of shape, size and number of lobes under the microscope. Some specimens were examined under a scanning electron microscope (JEOL JSM-50A); in this case the critical-point drying method was used for preparation of specimens.

Extraction of main components. Secretions were collected by causing the beetles to spray on filter papers, by pinching them at rest or walking on filter papers, or by extraction with ether. There was no significant difference of composition between ether immersion and filter paper collections, except small amounts of cuticular lipoidal substances in the immersion samples, which can be mostly removed due to their low solubility in methanol after the evaporation of ether. In addition to the immersion and filter paper collection samples, homogenized samples were also used for composition assays. When many beetles were collected, the reservoirs and glands themselves or the last four abdominal segments were cut off and homogenized with a small quantity of water and extracted with ether. When few beetles were obtained, homogenized ether extracts were combined with immersion extracts, concentrated and analyzed directly or after several fractionations.

Fractionations. Properties of chemicals were roughly discriminated by their odors and colors. Ether extracts were treated with 1% NaOH aqueous solution; the ether layer contained alcohols, aldehydes, ketones, and cuticular lipoidal substances. The aqueous layer was acidified with 1% HCl (pH indicator papers), and extracted with ether; this fraction contained fatty acids and phenolic compounds. For the identification of unsaturated fatty acids, samples were routinely subjected to catalytic hydrogenation over Adams' catalyst.

Thin-layer chromatography. Benzoquinones and cresols were clearly separated on silica gel G (Merck) used with *n*-hexane-ethylacetate (80 : 20), or benzene-ethylacetate (85 : 15). Anisaldehyde (0.5%) and sulfate (1%) in methanol solution were used for color development; characteristic colors were developed by heating at 100°C for 15 minutes. For the fatty acids, *iso*-propanol-ethylacetate-sat. NH_4OH (50 : 50 : 5), or chloroform-methanol-20% NH_4OH (100 : 50 : 3) were used, and pH indicator solution sprays were used for color development. Formic, acetic and propionic acids were separated, but other many acids overlapped.

Gas chromatography. This was performed on a Hitachi 063 equipped with TCD and stainless column (3-mm diameter $\times$ 1 m). The stationary phases and column conditions were as follows: (a) 25% Polyethylene glycol 4000 monostearate on Celite 545, 40/60 mesh at 115, 125 (mostly used) or 135°C ; (b) FFAP 15% on Neosorb NS 60/80 mesh at 125°C ; (c) Tween 80 + H_3PO_4 (9 : 1) on C-22 SK 100/120 mesh at 125°C . Helium gas was used as the carrier gas at a flow rate of 40 ml/min.

RESULTS

The results of authentic compounds are shown in Tables 1 and 2. Benzoquinones, cresols and salicylaldehyde were readily identified, based on their R_f values and characteristic colors in the thin-layer chromatography. Fatty acids were analyzed by the

Table 1. RETENTION TIMES OF ALIPHATIC ACIDS AND CRESOLS ON POLYETHYLENE GLYCOL 4000 MONOSTEARATE AT 125°C

Acid	Other name	Retention time (minutes)
Formic		5.5
Acetic		4.3
Propionic		6.7
<i>n</i> -Butyric		11.0
<i>iso</i> -Butyric		8.3
<i>n</i> -Valeric		18.8
<i>iso</i> -Valeric		14.8
2-Methylbutyric		15.2
Pivalic		9.0
<i>n</i> -Caproic		33.5
<i>iso</i> -Caproic		28.0
Acrylic	Propenoic	8.0
Methacrylic	2-Methylpropenoic	15.8
Ethacrylic	2-Ethylpropenoic	23.0
Crotonic	<i>trans</i> -2-Butenoic	22.5
<i>iso</i> -Crotonic	<i>cis</i> -2-Butenoic	20.0
3-Methylcrotonic		27.0
Tiglic	<i>trans</i> -2-Methylcrotonic	31.5
Angelic	<i>cis</i> -2-Methylcrotonic	24.5
<i>o</i> -Cresol		62
<i>m</i> -Cresol		80
<i>p</i> -Cresol		74
Salicylaldehyde		15.0

Table 2. R_F VALUES OF QUINONES AND CRESOLS

	<i>n</i> -Hexane : ethylacetate	Benzene : ethylacetate	Color (Anisaldehyde-H ₂ SO ₄)
	(80 : 20)	(85 : 15)	
<i>p</i> -Benzoquinone	0.54	0.65	Brown
2-Methylquinone	0.57	0.68	Brown
2-Ethylquinone	0.63	0.78	Brown
Hydroquinone	0.15	0.23	Brown
2-Methylhydroquinone	0.18	0.28	Brown
2-Ethylhydroquinone	0.22	0.32	Brown
<i>o</i> -Cresol	0.52	0.62	Light orange red
<i>m</i> -Cresol	0.48	0.56	Red
<i>p</i> -Cresol	0.50	0.60	Orange red
Salicylaldehyde	0.74	0.86	Orange

Fig. 1. Gas chromatograms of the pygidial gland secretions. Upper two: Secretions in ether; lower two: after hydrogenation. M: methacrylic acid; E: ethacrylic acid; T: tiglic acid; IB: *iso*-butyric acid; 2MB: 2-methylbutyric acid; A: angelic acid; IC: *iso*-crotonic acid; B: *n*-butyric acid.

Fig. 2. Schematic figures of the defensive systems. A, B and C produce fatty acids, D produces *m*-cresol, E and F produce benzoquinones.

gas chromatography, but a number were not clearly distinguishable. Methacrylic, *iso*-valeric and 2-methylbutyric acids were not separable, nor were crotonic, ethacrylic and angelic acids. After the catalytic hydrogenation, methacrylic acid changed to *iso*-butyric acid; crotonic and *iso*-crotonic acids changed to *n*-butyric acid; ethacrylic, tiglic and angelic acids changed to 2-methylbutyric acid. Two examples of ether immersions are shown in Fig. 1. Finally *iso*-valeric acid was not separable from 2-methylbutyric acid, and ethacrylic acid could not be separated from angelic acid. For the identification of these, reference was made to the results of MOORE and WALLBANK (1968) and OOSHIMA et al. (1975), who identified these compounds by mass spectrometry and nuclear magnetic resonance.

The pygidial gland system was composed of large reservoirs, synthetic lobes and efferent ducts having valves controlled with muscles. Collecting canals connected the lobes and reservoirs. The points of entry into the reservoirs were characteristic of the

Fig. 3. A: Defensive system of *Pterostichus fortis*; r : reservoir; c : collecting canal; l : lobes. B: lobes of *Drypta japonica*; C: lobes of *Anisodactylus signatus*; D: lobes of *Chlaenius pallipes*; E: lobes of *Chlaenius inops*; F: lobes of *Brachinus stenoderus*; B, D, E and F are to the same scale.

genera as shown in Fig. 2. Scaritidae, Brachinidae, Broscini and some Bembidiini had a system of connection at the middle portion of the reservoirs, while Pterostichini (Fig. 3A) and Amarini had a system of connection at the upper middle portion of the efferent ducts. The connection was observed to be at a similar but higher point in the most of the Carabidae. Harpalini, Agonini, Chlaeniini, Dryptini and Zuphini had a system connection immediately above the entry of the efferent ducts.

The shape and number of reservoirs and lobes were also characteristic as shown in Figs 2 and 3 and summarized in Table 3. Fatty-acid-secreting species had more or less spherical lobes, whereas *m*-cresol- and benzoquinone-secreting species had

Table 3. CHEMICAL COMPOSITIONS OF THE PYGIDIAL DEFENSIVE COMPONENTS OF CARABID BEETLES^a

	Components with approximate proportion (except earlier peaks) ^b	Gland types ^c
Carabidae		
<i>Campalita chinense</i> KIRBY	SA 60, M 35, T 4, UI 1 (9, 6)	Am
<i>Hemicarabus tuberculosus</i> DEJEAN et BOISDUVAL	M 90, E 9, UI 1 (")	Am
<i>Carabus procerulus</i> CHAUDOIR	M 90, T 8.9, (E) 0.1, UI 1 (")	Am
<i>C. porrecticollis</i> BATES	M 85, T 13.9, (E) 0.1, UI 1 (")	Am
<i>Apotomopterus dehaanii</i> CHAUDOIR	M 85, T 13.9, (E) 0.1, UI 1 (")	Am
" (winter)	M 85, E 13, T 1, UI 1 (")	Am
<i>A. yaconinus</i> BATES	M 80, T 18.9, (E) 0.1, UI 1 (")	Am
<i>A. japonicus</i> MOTSCHULSKY	M 85, T 13.9, (E) 0.1, UI 1 (")	Am
<i>Damaster blaptoides</i> KOLLAR	M 65, E 33.5, T 0.5, UI 1 (")	Am
" (winter)	M 65, E 34, UI 1 (")	
<i>Nebria lewisi</i> BATES	M 85, T 13.9, (E) 0.1, UI 1 (")	Ab
<i>N. macrogona</i> BATES	M 80, T 18.9, (E) 0.1, UI 1 (")	Ab
<i>N. chinensis</i> BATES	M 85, T 13.9, (E) 0.1, UI 1 (")	Ab
<i>Notiophilus impressifrons</i> MORAWITZ	M 90, T 9, UI 1 (")	Al
Scaritidae		
<i>Scarites aterrimus</i> MORAWITZ	iC 45, An 35, M 15, T 4, UI 1 (9, 6, 4)	Cb
<i>S. terricola</i> BONELLI	iC 40, An 30, M 25, T 4, UI 1 (")	Cb
<i>S. acutidens</i> CHAUDOIR	iC 40, An 35, M 20, T 4, UI 1 (")	Cb
<i>S. sulcatus</i> OLIVIER	An 35, iC 30, M 30, T 4, UI 1 (")	Cb
<i>Clivina</i> sps. (tested 2 sps.)	QM 70, QB 30	EI
Harpalidae		
Broscini		
<i>Broscosoma doenitzii</i> HAROLD	(nB) 45, (iV) 50, UI 5 (6, 4, 36)	Cb
<i>Craspedonotus tibialis</i> SCHAUM	(iB) 35, (iV) 40, (E or An) 20, UI 5 (36, 4)	Cb
Bembidiini		
<i>Trechoblemus postilenatus</i> BATES	(iB) 20, (iV) 75, UI 5 (36, 4, 28)	C 1
<i>Tachys sericans</i> BATES	(iB) 30, (iV) 65, UI 5 (")	C 1
<i>Bembidion morawitzi</i> CSIKI	M 75, T 20, UI 5 (28, 6, 4, 36)	C 1
<i>B. lissonotum</i> BATES	M 75, T 20, UI 5 (")	C 1
<i>B. semilunium</i> NETOLITZKY	M 75, T 20, UI 5 (")	C 1
<i>B. stenoderum</i> BATES	M 80, T 15, UI 5 (")	C 1
<i>Asaphidion semilucidum</i> MOTSCHULSKY	(nB) 40, (iV) 50, UI 10 (")	C 1
Patrobini		
<i>Patrobus flavipes</i> MOTSCHULSKY	M 20, (iC or C) 45, (An or E) 20, T 14, UI 1 (9, 6)	Ab
<i>Diplous caligatus</i> BATES	M 80, T 18, UI 2 (24, 9, 6)	
<i>D. depressus</i> GEBLER	M 85, T 13, UI 2	Ab
Pterostichini		
<i>Trigonognatha cuprescens</i> MOTSCHULSKY	M 95, T 4, UI 1 (24, 9, 6)	Ab
<i>Trigonotoma lewisii</i> BATES	M 95, T 4, UI 1 (")	Ab
<i>Lesticus magnus</i> MOTSCHULSKY	M 95, T 4, UI 1 (")	Ab
<i>Poecilus coerulescens</i> LINNE	M 95, T 4, UI 1 (")	Ab
<i>P. fortipes</i> CHAUDOIR	M 95, T 4, UI 1 (")	Ab
<i>Pterostichus longinquus</i> BATES	M 90, T 8.9, (E) 0.1, UI 1 (")	A 1
<i>P. fortis</i> MORAWITZ	M 95, T 3.9, (E) 0.1, UI 1 (")	Ab

	Components with approximate proportion (except earlier peaks) ^b	Gland types ^c
<i>P. rotundangulus</i> MORAWITZ	M 95, T 3.9, (E) 0.1, UI 1 (")	A 1
<i>P. prolongatus</i> MORAWITZ	M 90, T 8.9, (E) 0.1, UI 1 (")	Ab
<i>P. microcephalus</i> MOTSCHULSKY	M 95, T 3.8, (E) 0.2, UI 1 (")	A 1
<i>P. fujimurai</i> HABU	M 95, T 3.9, (E) 0.1, UI 1 (")	Ab
<i>P. daisenicus</i> ISHIDA	M 85, T 13.8, (E) 0.2, UI 1 (")	Ab
<i>P. masidai</i> ISHIDA	M 85, T 13.8, (E) 0.2, UI 1 (")	Ab
Agonini		
<i>Synuchus cycloderus</i> BATES	F 95, UI 5 (16, 45, 36, 9)	Bb
<i>S. dulcigradus</i> BATES	F 95, UI 5 (")	Bb
<i>S. callitheres</i> BATES	F 95, UI 5 (")	Bb
<i>Platynus magnus</i> BATES	F 95, UI 5 (")	Bm
<i>P. protensus</i> BATES	F 95, UI 5 (")	Bm
<i>Calathus halensis</i> SCHALLER	F 95, UI 5 (")	Bm
<i>Anchus leucopus</i> BATES	F 95, UI 5 (")	Bb
<i>Lorostemma ogurae</i> BATES	F 95, UI 5 (")	Bb
<i>Agonum daimio</i> BATES	F 95, UI 5 (")	Bb
<i>A. chalcomum</i> BATES	F 95, UI 5 (")	Bb
<i>Loxocrepis rubriola</i> BATES	F 95, UI 5 (")	Bb
<i>Colpodes atricomes</i> BATES	F 95, UI 5 (")	Bb
<i>C. japonicus</i> MOTSCHULSKY	F 95, UI 5 (")	Bb
Amarini		
<i>Amara chalcites</i> DEJEAN	M 70, T 22, (Ac) 6, (E) 1, UI 1 (9, 46)	A 1
<i>A. chalcophaea</i> BATES	M 70, T 20, (Ac) 6, (E) 3, UI 1 (")	A 1
<i>Bradytus simplicidens</i> MORAWITZ	M 70, T 20, (Ac) 6, (E) 3, UI 1 (")	A 1
<i>B. ampliatus</i> BATES	M 75, T 15, (Ac) 6, (E) 3, UI 1 (")	A 1
<i>Curtonotus giganteus</i> MOTSCHULSKY	M 75, T 15, (Ac) 6, (E) 3, UI 1 (")	Ab
Harpalini		
<i>Anisodactylus signatus</i> PANZER	F 95, UI 5 (36, 16)	Bm
<i>A. tricuspidatus</i> MORAWITZ	F 95, UI 5 (")	Bm
<i>Platymetopus flavilabris</i> FABRICIUS	F 95, UI 5 (")	Bb
<i>Trichotichnus longitarsis</i> MORAWITZ	F 95, UI 5 (")	Bm
<i>Harpalus capito</i> MORAWITZ	F 95, UI 5 (")	Bm
<i>H. griseus</i> PANZER	F 95, UI 5 (")	Bm
<i>H. sinicus</i> HOPE	F 95, UI 5 (")	Bm
<i>H. platynotus</i> BATES	F 95, UI 5 (")	Bm
<i>Anoplogenus cyaneus</i> HOPE	F 95, UI 5 (")	Bb
<i>Stenolophus agonoides</i> BATES	F 95, UI 5 (")	Bb
<i>S. iridicolor</i> REDTENBACHER	F 95, UI 5 (")	Bb
<i>S. difficilis</i> BATES	F 95, UI 5 (")	Bb
<i>Trichocellus tenuimanus</i> BATES	F 95, UI 5 (")	Bb
<i>Bradycellus inornatus</i> BATES	F 95, UI 5 (")	Bb
Licinini		
<i>Diplocheila zeelandica</i> REDTENBACHER	F 98, UI 2 (16, 36)	Bm
<i>D. elongata</i> BATES	F 98, UI 2 (")	Bm
Chlaeniini		
<i>Epomis nigricans</i> WIEDERMANN	mC 98, UI 2 (16, 36)	Db
<i>Macrochlaenites costiger</i> CHAUDOIR	mC 98, UI 2 (")	Db
<i>Chlaenius spoliatus</i> ROSSI	mC 98, UI 2 (")	Db
<i>C. circumdatus</i> BRULLE	mC 98, UI 2 (")	Db

	Components with approximate proportion (except earlier peaks) ^b	Gland types ^c
<i>C. pallipes</i> GEBLER	mC 98, UI 2 (")	Db
<i>C. virgulifer</i> CHAUDOIR	mC 98, UI 2 (")	Db
<i>C. noguchii</i> BATES	mC 98, UI 2 (")	Db
<i>C. Chlaeniellus prostenus</i> BATES	QB 55, QM 40, QE 5	Eb
<i>C. Chlaeniellus inops</i> CHAUDOIR	QB 55, QM 40, QE 5	Eb
<i>Callistoides delciolus</i> BATES	mC 98, UI 2 (16, 36)	Db
Panageini		
<i>Panagaeus japonicus</i> CHAUDOIR	mC 98, UI 2 (16, 36)	Db
<i>Dischissus mirandus</i> BATES	mC 98, UI 2 (")	Db
<i>D. japonicus</i> ANDREWES	mC 98, UI 2 (")	Db
<i>Peronomerus auripilis</i> BATES	mC 98, UI 2 (")	Db
<i>P. nigrinus</i> BATES	mC 98, UI 2 (")	Db
Lebiini		
<i>Lebia retrofasciata</i> MOTSCHULSKY	F 95, UI 5 (36, 16, 28, 9)	Bb
<i>Coptoderina japonica</i> BATES	F 95, UI 5 (")	Bb
<i>C. subapicalis</i> PUTZEYS	F 95, UI 5 (")	Bb
<i>Lebidia octoguttata</i> MORAWITZ	F 95, UI 5 (")	Bm
<i>Callida lepida</i> REDTENBACHER	F 95, UI 5 (")	Bm
<i>Dolichoctis luctuosus</i> PUTZEYS	F 95, UI 5 (")	Bb
<i>D. striata</i> SCHMIDT-GOEBEL	F 95, UI 5 (")	Bb
<i>Apristus grandis</i> ANDREWES	F 95, UI 5 (")	Bb
Dryptini		
<i>Drypta japonica</i> BATES	F 93, UI 7 (16, 14, 28, 36)	Bm
Zuphini		
<i>Planetes puncticeps</i> ANDREWES	F 95, UI 5 (16, 28, 36, 9)	Bm
<i>Galeritula japonica</i> BATES	F 95, UI 5 (")	Bm
Brachinidae		
<i>Pheropsophus jessoensis</i> MORAWITZ	QB 60, QM 40	Fb
<i>Brachinus scotomedes</i> BATES	QB 60, QM 40	Fb
<i>B. stenoderus</i> BATES	QB 60, QM 40	Fb
<i>B. chuji</i> JEDRICKA	QB 60, QM 40	Fb

^a Except solvent-like components, earlier peaks of GC and upper portion of TLC (they are about 10%).

^b Ac: Acetic acid; An: angelic acid; C: crotonic acid; E: ethacrylic acid; F: formic acid; iB: *iso*-butyric acid; iC: *iso*-crotonic acid; iV: *iso*-valeric acid; M: methacrylic acid; mC: *m*-cresol; nB: *n*-butyric acid; QB: *p*-benzoquinone; QE: 2-ethylquinone; QM: 2-methylquinone; SA: salicylaldehyde; T: tiglic acid; UI: unidentified (retention times, minutes); (): tentatively identified.

^c A, B, —: Gland types of Fig. 2; m: more than 50 lobes; b: 20—49 lobes; l: less than 19 lobes.

elongated lobes and long thick lobes respectively. The number of lobes was one of the characteristics as shown in Table 3. Carabini had more than 200 lobes, whereas some Pterostichini, Amarini, and small carabid beetles had fewer than 19 lobes.

Color and odor were also characteristic: *m*-cresol-secreting beetles had yellowish brown reservoirs, and benzoquinone-secreting beetles had brownish reservoirs. With experience even fatty acids could be discriminated by their odors.

Results are summarized in Table 3. For classification and arrangement, reference

was made to "Iconographia Insectorum Japonicorum" and HABU (1967, 1973). The following species not shown in Table 3 were also tested: *Apotomopterus japonicus daisen* NAKANE, *Trigonognatha coreana* TSHITSCHERINE, *Pterostichus yoritomus* BATES, *P. leptis* BATES, *P. noguchii* BATES, *P. nimbatidius* CHAUDOIR, *P. latemarginatus* STRANEO, *P. biexcisus subparallelus* STRANEO. and two *Pterostichus* sps., *Synuchus nitidus* MOTSCHULSKY, *S. arcuaticollis* MOTSCHULSKY, *Colpodes* sp. *Anisodactylus sadoensis* SCHAUBERGER, *A. punctatipennis* MORAWITZ, *Harpalus vicarius* CHAUDOIR, *H. pseudophonoides* SCHAUBERGER, *H. chalcatus* BATES, *Chlaenius abstersus* BATES, *C. tetragonoderus* CHAUDOIR, *C. micans* FABRICIUS, *C. naeviger* MORAWITZ, *C. posticalis* MOTSCHULSKY, *C. bioculatus* MOTSCHULSKY, *C. ocreatus* BATES, *C. variicornis* BATES. These had the same components and similar proportions to the species of the same genus shown in Table 3.

Most of the samples contained minor components, such as hydrocarbons, etc., which were found in the upper portion of the thin-layer chromatograms and with short retention time in gas chromatography. They were unidentified, but were thought to make up about 10% of the defensive secretions or reservoirs of carabid beetles. They were neglected in the proportional calculations in Table 3. Unidentified components given in Table 3 appeared clearly as peaks in gas chromatography. They were fractionated by alkali treatment, which suggests they are aldehydes, ketones, alcohols, esters or long-chain hydrocarbons. Relatively large amounts of these components were found in the Harpalini as a peak at 36 minutes of retention time, and in the Agonini at 16 minutes.

Species of the same genus had almost the same components, with the exception of the genus *Chlaenius*, in which most species secreted *m*-cresol but two species of the subgenus *Chlaeniellus* secreted benzoquinones. These properties coincided with the difference of the gland types. At the subtribe level, beetles of the same subtribe generally secreted similar components, but exceptional and indistinct cases were observed in the Broscini and Bembidiini. Higher amounts of ethacrylic acid in comparison with tiglic acid were detected in the winter collections of *Apotomopterus dehaanii* and *Damaster blaptoides*.

DISCUSSION

Carabid beetles all possess homologous pygidial defensive systems (FORSYTH, 1972), but their chemical components and gland forms are diverse. Species of the same genera, or at least subgenera, share similar components and gland forms. They are roughly divided into six groups: (1) formic acid in the Agonini, Harpalini, Licinini, Lebiini, Dryptini, and Zuphini; (2) methacrylic, tiglic and/or ethacrylic acids in the Carabidae, Pterostichini, and Amarini; (3) other acids such as *n*-butyric, *iso*-butyric, *iso*-valeric, *iso*-crotonic, angelic and several others in the Broscini, Scaritini, and several Bembidiini; (4) *m*-cresol in the Panagaeini and most Chlaeniini; (5) benzoquinones with one chamber in the Clivina and subgenus Chlaeniellus; (6) benzoquinones with two chambers only in the Brachinidae, which are known as bombardier beetles. SCHILDKNECHT et al. (1968) reported that hydroquinones and hydrogen peroxide were stored in the upper chamber and oxidized by catalase and peroxidase in the lower chamber, with the production of noise and heat. It was observed that the upper chamber was a rather opaque white and the lower chamber was brown. Other groups secrete only stored materials without noise and heat. The findings on Group (3) are

rather scattered and inconclusive, insufficient species having been tested for further grouping. Beetles of the Scaritini secreted *iso*-crotonic and angelic acids with small amounts of methacrylic and tiglic acids. This secretion contains properties of Group (2) in the different compositions. Some of the Broscini and Bembidiini secreted *n*-butyric acid with *iso*-valeric acid, or *iso*-butyric acid with *iso*-valeric acid. However, it is thought that the relationship is clear within the same genera, or at least subgenera.

Components detected in this experiment are generally identical with other results, including those for 52 Australian species in 32 genera (MOORE and WALLBANK, 1968), 38 European genera (SCHILDKNECHT et al., 1968), some Carabidae (EISNER et al., 1963, 1968; McCULLOUGH and WEINHEIMER, 1966; BENN et al., 1973).

In the Carabidae, there is a difference of properties between secreters of tiglic acid and ethacrylic acid. *Hemicarabus tuberculosus* and *Damaster blaptoides* secrete methacrylic acid with ethacrylic acid as observed in four species of *Pamborus* (MOORE and WALLBANK, 1968) and *Carabus taedatus* (BENN et al., 1973). On the contrary, two species of *Carabus* and three species of *Apotomopterus* secrete methacrylic acid with tiglic acid as observed in seven species of *Carabus* (SCHILDKNECHT et al., 1968; McCULLOUGH and WEINHEIMER, 1966) and four species of *Scaphinotus* (WHEELER et al., 1970). OOSHIMA et al. (1975) detected a small amount of ethacrylic acid with large amounts of methacrylic and tiglic acids in the hibernating *Carabus procerulus*. In this experiment, *Apotomopterus dehaanii* collected in winter were found to have more ethacrylic acid than those collected in summer. This property was also observed in other similar secreting species, such as some *Pterostichini*. It is thought to be due to a metabolic change which may replace ethacrylic acid synthesis or deposition in the hibernation period, and may also result in the difference of tiglic acid and ethacrylic acid contents among species.

Many components reported here have melting points higher than 5°C, such as methacrylic, tiglic, ethacrylic, angelic, crotonic, and *iso*-crotonic acids, *m*-cresol and benzoquinones. A daily minimum temperature of below 0°C continues for more than two months in the area of beetle collection. Minor components, such as hydrocarbons, aldehydes, etc., may have a function as solvent for major components in the reservoirs, and this property may be advantageous in the winter season. Hydrocarbons (MOORE and WALLBANK, 1968; SCHILDKNECHT et al., 1968), nonyl acetate (EISNER et al., 1968), and *iso*-valeraldehyde (SCOTT et al., 1975) were detected in some carabid beetles. Although the constituents were not revealed, the presence of hydrocarbons was postulated from the spot which occurred in the upper portion of thin-layer chromatography and with short retention time in gas chromatography. Other components, given as unidentified (UI) in Table 3, may have a similar solvent-like role. As this experiment was designed to detect major components, less attention was paid to minor components, which were partly discarded in the evaporation procedure.

All major components have some toxicity, irritant action, cytotoxicity, antifungal action and antiseptic property. The toxicity and unpleasant odor may act as a defense against attackers. Minor solvent-like components can promote, probably synergistically, the action of major components by their lipophilic property. There seems to be a close resemblance between the pygidial defensive systems and integuments, as observed in the chitinous structure of reservoirs and similar kinds of components. This chitinous structure may be effective in isolating toxic components from other tissues, such as hemolymph.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Akinobu HABU, National Institute of Agriculture, and Dr. Yutaka ISHIDA, Kobe Commercial College, for their identification of carabid beetles. Thanks are also due to Dr. Kazuyoshi KAWAZU, Department of Agriculture, Okayama University, for valuable advice on the hydrogenation technique. The authors also wish to thank to Prof. Yasunobu YASUE, Dr. Kazuo KAWADA, Messrs. Hisaaki TSUMUKI, Takashi SHIRAGA, Yukio SHIMO and Toshikazu YAMASHITA, Institute for Agricultural and Biological Sciences, Okayama University, for their valuable discussion and collection of insects.

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