Current knowledge on exocrine glands in carabid beetles: structure, function and chemical compounds

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Abstract
Many exocrine products used by ground beetles are pheromones and allomones that regulate intra- and interspecific interactions and contribute to their success in terrestrial ecosystems. This mini-review attempts to unify major themes related to the exocrine glands of carabid beetles. Here we report on both glandular structures and the role of secretions in carabid adults, and that little information is available on the ecological significance of glandular secretions in pre-imaginal stages.

Keywords
antennal glands, pygidial gland, defensive secretion, carabid beetles

Introduction
Exocrine gland secretions in insects are involved in reproductive and defensive behaviour (Pasteels et al. 1983; Blum 1996), and are important in social integration and communication among members of the same colony (as in Hymenoptera) (Hölldobler and Wilson 1990). These exocrine glands have an ectodermal origin and morphological or functional classifications have been generally used to describe them. The location and morphology of these glands are directly related to their function (Billen 1998). Many glands are common to all insects, e.g. mandibular and salivary glands, male and female accessory glands associated with reproductive organs (Dallai et al. 1999; Viscuso et al. 2001) and defensive glands (Thiele 1977), whereas some glands are char-
acteristic of a family or species (Grassé 1975; Quennedey 1998, 2000), especially in social insects (Cammaerts 1974; Bin et al. 1989; Hölldobler and Wilson 1990, also see reviews; Billen 1991; Delfino et al. 1991, 1992; Pedata et al. 1993; Bartlet et al. 1994; Isidoro and Bin 1995; Isidoro et al. 1996, 2000; Bot et al. 2001; Gobin et al. 2001, 2003; Torres et al. 2001).

Information on the chemistry of defensive secretions in many carabid species are available in Dettner (1987), Whitman et al. (1990) and Will et al. (2000). In this manuscript, carabid beetles are meant in the widest sense of the word, including the old lineage of Trachypachidae, the Rhysodidae and the Paussinae as a subfamily (as in Beutel and Leschen 2005). The nomenclature of palaearctic taxa follows Löbl and Smetana (2003).

**Adult antennal glands**

The cellular architecture of adult antennal glands has been investigated for *Platynus assimilis* (Paykull 1790) (Weis et al. 1999), *Paussus* spp Linnaeus 1775 (Di Giulio et al. 2003, 2009; Nagel 1979) and *Siagona europaea* Dejean 1826 (Giglio et al. 2005). Structural analysis shows a great number of antennal glands that have been classified into the following main categories (Noirot and Quennedey 1991, Quennedey 1998): i) unicellular gland class 2, which is not in contact with the cuticle; ii) bi- and tri-cellular gland class 3, connected to the cuticle by a cuticular duct draining the secretions outside. The first type (class 2) includes unicellular glands known as oenocytes. They are located only within the antennal lumen of *S. europaea* and are not found in other carabid species (Giglio et al. 2005). Their role in cuticular hydrocarbons secretions is suggested by Lockey (1988) and Noirot and Quennedey (1991). The second type are tri-cellular glands, composed of a secretory, an intercalary and a duct cell, and are found in *P. assimilis* (Weis et al. 1999), *Paussus favieri* Fairmaire (Di Giulio et al. 2009) and *S. europaea*. Moreover, a large number of bi-cellular glands, composed of one gland and one duct cell, are located on the antennal surface of *P. favieri*. The structural variability and distribution of the antennal glandular apparatus on Paussini, such as the myrmecophilous *P. favieri*, are closely related to their symbiotic life style (Geiselhardt et al. 2007). Predators, such as *P. assimilis* and *S. europaea*, which have free-living life habits, show a more simple glandular apparatus. Exocrine gland class 3 of the myrmecophagous *S. europaea* produces secretions that protect the surface of the antennae and sensilla from wear.

**Pygidial glands**

Ground beetles possess a pair of abdominal glands known as pygidial glands, which produce defensive secretions. Their structure consists of two sets of secretory lobes, collecting canals, collecting reservoirs and has been well described for many species
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(Benn 1973; Forsyth 1970, 1972; Scott et al. 1975; Balestrazzi et al. 1985; Rossini et al. 1997; Eisner et al. 2000; Will et al. 2000, 2010; Attygalle et al. 2004). These glands are variable in structure and in the nature of the produced substances (Thiele 1977), and discharge the secretion products by oozing, spraying or crepitation. Oozing is probably the plesiotypic mode of discharge, with active spraying and crepitation as later refinements (Moore 1979). The main function of pygidial glands is probably in the defence against predators, but also in the facilitation of the penetration of defensive compounds into the predator’s integuments, antimicrobial and antifungal activity, and in producing alarm messages (Evans and Schmidt 1990; Blum 1996).

A comparative study of the secretions of carabid pygidial glands was made by Schildknecht et al. (1968). Moore (1979) and Will et al. (2000) listed all the principal groups of secretions detected in carabid tribes: hydrocarbons, aliphatic ketones, saturated esters, formic acid, higher saturated acids, unsaturated carboxylic acids, phenols (m-cresol), aromatic aldehydes (salicylaldehyde) and quinones. Attygalle et al. (1991) showed that D8-L-valine is incorporated into methacrylic and isobutyric acids in the pygidial defensive glands of Scarites subterraneus Fabricius 1775. The pygidial glands of Helluomorphoides clairvillei (Dejean 1831) females discharge a mixture of compounds including carboxylic acid, aliphatic esters and hydrocarbons (Attygalle et al. 1992). The taxonomic distribution of defensive secretions was reviewed by Will et al. (2000) for 47 tribes. Data have shown a close relationship between chemical classes and habitat diversification. Tribes with high species diversity in tropical-subtropical and steppe habitats use formic acid as primary chemical defences, while tribes with high diversity in temperate regions use carboxylic acids, phenols, quinone, aromatic aldehydes and ketones. This can be explained by the interaction of ground beetles with their predators and prey. Specifically, ants are hypothesized to have had a major influence on the evolution of ground beetle secretions in tropical species. Bombardier beetles of the genus Brachinus Weber 1801 are able to release irritating quinones, produced by the oxidation of hydroquinones in a double-chambered apparatus (Schildknecht 1961; Eisner and Meinwald 1966; Schildknecht et al. 1968; Aneshansley et al. 1969; Eisner and Aneshansley 1999; Eisner et al. 2000); a certain amount of heat and the explosion associated with the reaction reinforce the defensive effect. Predation on these beetles appears to be rare (Juliano 1985; Bonacci et al. 2006, 2008). From the literature it is known that Anchomenus dorsalis (Pontoppidan, 1763) produces toxic methylsalycilate from its pygidial glands (Schildknecht 1970). Tiger beetle species living in moist habitats produce benzaldehyde (Altaba 1991). The carabid beetle Galerita lecontei Dejean 1831 secretes, as a spray, a mixture of formic acid, acetic acid and lipophilic components (long-chain hydrocarbons and esters) (Rossini et al. 1997). Biosynthesis of tiglic and ethacrylic acids from isoleucine via 2-methylbutyric acid was demonstrated in Pterostichus californicus (Dejean 1828) (Attygalle et al. 2007). Complex mixtures of monoterpenes are found in the defensive secretions of Ardistomis schaumii Leconte 1857 and Semiardistomis puncticollis Dejean 1831. The presence of monoterpenes in beetle secretions is well known, yet it is not very common to find the opposite enantiomers in secretions in related species (Attygalle 2009).
Exocrine glands of larval and pupal stages

Although exocrine glands and their defensive secretions are well investigated in adults, hardly any information exists for the larval and pupal stages, which are the most vulnerable stages of the beetle’s life cycle.

Glandular organs have been found in the larval stage of myrmecophilus Pseudomorpha sp. These glands are located on the head and thorax and secrete chemical compounds which repel ants (Erwin 1981). In Paussini larvae (Paussus kannegieteri Wasmann 1896) as well as in Metriini (Metrius) and Ozaenini, the modified terminal abdominal segments have glandular pores that secrete pleasant substances to attract their host ants (Arndt et al. 2005; Geiselhardt et al. 2007; Di Giulio 2008).

In the pupal stage of Carabus lefebvrei Dejean, 1826, Sturani (1962) described a “flavour humour” and suggested that this secretion has a waterproofing or an anti-predatory function. Ultrastructural analyses have shown that this exudate is secreted by an acinose abdominal complex of exocrine glandular units (Giglio et al. 2009). The independent glandular unit consists of a single secretory cell, a duct and its associated cell and belongs to gland cell class 3 according to the classification of Quennedey (1998). In the cytoplasm, the secretory cell contains abundant rough endoplasmatic reticula, glycogen granules, numerous mitochondria and many well-developed Golgi complexes producing electron-dense secretory granules. Mitochondria are large, elongated and often adjoining electronlucent vesicles. Their close association with tracheoles suggests very high aerobic metabolism. Chemical analyses of the gland secretions revealed a mixture of low molecular weight terpenes as well as ketones, aldehydes, alcohols, esters and carboxylic acids, which in adults are regarded deterrents against predators. Monoterpenes, especially linalool, were the main chemical products produced by the pupal stage of C. lefebvrei. It is suggested that this gland secretion has both a deterrent function against predators and a prophylaxis function against pathogens.

Conclusions and future studies

The present manuscript summarized the main knowledge on the exocrine glands in ground beetles. The main characteristic of glandular secretions of each life stage is its diversity and dependence on interspecific relations in the ecological niches of species. Our main future aim is to accumulate data on defensive secretions to understand, i) the mode of action of chemical compounds, and ii) species-specific variation of glandular structures and chemical secretions, paying particular attention to morphological, phylogenetic and behavioural aspects. Moreover, the need for more detailed studies on larval and pupal stages has already been stressed. Presently, the pupal stages of carabid beetles are known not to possess any physical protection, thus chemical protection provided by the abdominal glands is very important. This stage is present in environments rich in bacterial and fungal microorganisms, some of which are possible insect
pathogens. Besides, the highly lipophilic nature of monoterpenoid compounds suggests that their principal targets are bacterial and/or fungal cell membranes.

To support this hypothesis additional research is needed to evaluate the range of activity of the secretions of pupal abdominal glands towards microorganisms and fungal entomopathogens.

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References

Altaba CR (1991) The importance of ecological and historical factors in the production of benzaldehyde by tiger beetles. Systematic Zoology 40(1): 101–105. doi: 10.2307/2992226

Aneshansley D, Eisner T, Widom J, Widom B (1969) Biochemistry at 100°C: explosive secretory discharge of bombardier beetles (Brachinus). Science 165: 61–63. doi: 10.1126/science.165.3888.61

Arndt E, Beutel RG, Will K (2005) 7.8. Carabidae Latreille 1802. In: Beutel RG, Leschen RAB (Eds) Handbook of Zoology. Band/Volume IV Arthropoda: Insecta. Teilband/Part 38 Coleoptera, Beetles. Volume I: Morphology and Systematics (Archostemata, Adephaga, Myxophasa, Polyphasa partim). Walter de Gruyter: Berlin- N.Y., 1–567

Attygalle AB, Eisner T, Meinwald J (1991) Biosynthesis of methacrylic acid and isobutyric acids in a carabid beetle, Scarites subterraneus. Tetrahedron Letters 32(37): 4849–4852. doi: 10.1016/S0040-4039(00)93477-4

Attygalle AB, Meinwald J, Eisner T (1992) Defensive secretion of a carabid beetle, Helluomorphoides clairvillei. Journal of Chemical Ecology 18: 489–498. doi: 10.1007/BF00994247

Attygalle AB, Wu X, Ruzicka J, Rao S, Garcia S, Herath K, Meinwald J, Maddison DR, Will KW (2004) Defensive chemicals of two species of Trachypachus Motschulski. J Chem Ecol 30: 577–588. doi: 10.1023/B:JOEC.0000018630.79922.94

Attygalle AB, Wu X, Will KW (2007) Biosynthesis of tiglic, ethacrylic and 2-methylbutyric acids in a carabid beetle, Pterostichus (Hypherpes) californicus. Journal of Chemical Ecology 33(5): 963–70. doi: 10.1007/s10886-007-9276-3

Attygalle AB, Wu X, Maddison DR, Kipling WW (2009) Orange/lemon-scented beetles: opposite enantiomers of limonene as major constituents in the defensive secretion of related carabids. Naturwissenschaften 96: 1443–1449. doi: 10.1007/s00114-009-0596-8

Balestrazzi E, Valcurone Dazzini ML, DeBernardi M, Vidari G, Vita-Finzi P, Mellerio G (1985) Morphological and chemical studies on the pygidial defence glands of some Carabidae (Coleoptera). Naturwissenschaften 72(9): 482–484. doi: 10.1007/BF00441073

Bartlet E, Isidoro N, Williams H (1994) Antennal glands in Pyllodes chrysocephala and their possible role in reproductive behaviour. Physiological Entomology 19: 241–250. doi: 10.1111/j.1365-3032.1994.tb01048.x
Benn MH, Lencucha A, Maxie S, Telang SA (1973) The pygidial defensive secretion of Carabus taedatus. Journal of Insect Physiology 19: 2173–2176. doi: 10.1016/0022-1910(73)90132-7

Beutel RG, Leschen RAB (2005) Phylogenetic analysis of Staphyliniformia (Coleoptera) based on characters of larvae and adults. Systematic Entomology 30 (4): 510–548. doi: 10.1111/j.1365-3113.2005.00293.x

Billen J (1991) Ultrastructural organization of the exocrine glands in ants. Ethology Ecology and Evolution, Special Issue 1: 67–73.

Billen J (1998) The social insect as a glandular factory. Insect Social Life 2: 9–14.

Bin F, Colazza F, Isidoro N, Solinas M, Vinson SB (1989) Antennal chemosensilla and glands, and their possible meaning in the reproductive behaviour of Trissolcus basalis (Woll.) (Hym.: Scelionidae). Entomologica XXIV: 33–97.

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annual Review of Entomology 41(1): 353–374. doi: 10.1146/annurev.en.41.010196.002033

Bonacci T, Massolo A, Brandmayr P and Zetto Brandmayr T (2006) Predatory behaviour on ground beetles (Coleoptera: Carabidae) by Ocypus olens (Müller) (Coleoptera: Staphylinidae) under laboratory conditions. Entomological News 117(5): 545–551 doi: 10.3157/0013-872X(2006)117[545:PBOGBC]2.0.CO;2

Bonacci T, Aloise G, Brandmayr P, Zetto Brandmayr T, Capula M (2008) Testing the predatory behaviour of Podarcis sicula (Reptilia: Lacertidae) towards aposmatic and non-aposmatic preys. Amphibia-Reptilia 29: 449–453. doi: 10.1163/15685380878511986

Bot ANM, Obermayer ML, Hölldobler B, Boomsma JJ (2001) Functional morphology of the metapleural gland in the leaf-cutting ant Acromyrmex octospinosus. Insectes Sociaux 48: 63–66. doi: 10.1007/PL00001747

Cammaerts R (1974) Le système glandulaire tégumentaire du coléoptère myrmécophile Claviger testaceus Preyssler, 1790 (Pselaphidae). Zeitschrift für Morphologie der Tiere 77: 187–219. doi: 10.1007/BF00389904

Dallai R, Lupetti P, Fanciulli PP (1999) Ultrastructure of the male accessory glands of Allarma fusca (Insecta, Collembola). Tissue and Cell 31(2): 176–184. doi: 10.1054/tice.1999.0026

Delfino G, Cervo R, Calloni C, Turillazzi S (1991) Preliminary ultrastructural findings on the sternal glands of male Polistes nimpha (Christ). Ethology Ecology and Evolution, Special Issue 1: 55–58.

Delfino G, Calloni C, Turillazzi S (1992) Ultrastructure of the tegumental glands of the third gastral tergite in male Parischnogaster mellyi (Suassure) (Hymenoptera: Stenogastrine). Ethology Ecology and Evolution, Special Issue 2: 55–59.

Dettner K (1987) Chemosystematics and evolution of beetle chemical defences. Annual Review of Entomology 32(1): 17–48. doi: 10.1146/annurev.en.32.010187.000313

Di Giulio A, Fattorini S, Kaupp A, Taglianti AV, Nagel P (2003) Review of competing hypotheses of phylogenetic relationships of Paussinae (Coleoptera: Carabidae) based on larval characters. Systematic Entomology 28: 509–537. doi: 10.1046/j.1365-3113.2003.00227.x

Di Giulio A (2008) Fine morphology of the myrmecophilous larva of Paussus kannegieteri (Coleoptera: Carabidae: Paussinae: Paussini). Zootaxa 1741: 37–50.

Di Giulio A, Rossi Stacconi MV, Romani R (2009) Fine structure of the antennal glands of the ant nest beetle Paussus favieri (Coleoptera, Carabidae, Paussini). Arthropod Structure & Development 38(4): 293–302. doi: 10.1016/j.asd.2009.01.001
Eisner T and Meinwald J (1966) Defensive secretions of Arthropods. Science 153(3742):1341–1350. doi: 10.1126/science.153.3742.1341
Eisner T and Aneshansley D (1999) Spray aiming in the bombardier beetle: photographic evidence. Proc. Natl. Acad. Sci. USA 96: 9705–9709. doi: 10.1073/pnas.96.17.9705
Eisner T, Aneshansley DJ, Eisner M, Yack J, Attygalle AB, Alsop DW, Meinwald J (2000) Spray mechanism of the most primitive bombardier beetle (*Metrius contractus*). The Journal of Experimental Biology 203: 1265–1275.
Erwin TL (1981) A synopsis of the immature stages of Pseudomorphini (Coleoptera: Carabidae) with notes on tribal affinities and behaviour in relation to life with ants. The Coleopterist Bulletin 35(1): 53–68.
Evans DL, Schmidt JO (1990) Insect defenses: Adaptive mechanisms and strategies of prey and predators. University of New York Press, Albany.
Forsyth DJ (1970) The ultrastructure of the pygidial defence glands of the carabid *Pterostichus madidus* F. Journal of Morphology 131: 397–415. doi: 10.1002/jmor.1051310404
Forsyth DJ (1972) The structure of the pygidial defence glands of Carabidae (Coleoptera). Trans. Zool. Soc. Lond. 32: 249–309. doi: 10.1111/j.1096-3642.1972.tb00029.x
Geiselhardt SF, Peschke K, Nagel P (2007) A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings. Naturwissenschaften 94: 871–894. doi: 10.1007/s00114-007-0271-x
Giglio A, Ferrero EA, Zetto Brandmayr T (2005) Ultrastructural identification of the antennal gland complement in *Siagona europaea* Dejean 1826, a myrmecophagous carabid beetle. Acta Zoologica 86: 195–203. doi: 10.1111/j.1463-6395.2005.00199.x
Giglio A, Brandmayr P, Dalpozzo R, Sindona G, Tagarelli A, Talarico F, Zetto Brandmayr T, Ferrero EA (2009) The defensive secretion of *Carabus lefebvrei* Dejean 1826 pupa (Coleoptera, Carabidae): gland ultrastructure and chemical identification. Microscopy Research and Technique 72(5): 351–361. doi: 10.1002/jemt.20660
Gobin B, Rüppell O, Hartmann A, Jungnickel H, Morgan ED, Billen J (2001) A new type of exocrine gland and its function in mass recruitment in the ant *Cylindromyrmex whymperi* (Formicidae, Cerapachyidae). Naturwissenschaften 88: 395–399. doi: 10.1007/s001140100251
Gobin B, Ito F, Billen J (2003) The subepithelial gland in ants: a novel exocrine gland closely associated with the cuticle surface. Acta Zoologica 84: 285–291. doi: 10.1046/j.1463-6395.2003.00149.x
Grassé PP (1975) Traité de Zoologie. Masson, Paris, New York, Barcelone, Milan.VIII: 199–320.
Hölldobler B, Wilson EO (1990) The Ants. Springer-Verlag, Berlin, Heidelberg.
Isidoro N, Bin F (1995) Male antennal gland of *Amitus spiniferus* (Brethes) (Hymenoptera: Platygastridae), likely involved in courtship behaviour. International Journal of Insect Morphology and Embryology 24(4): 365–373. doi: 10.1016/0020-7322(95)00014-U
Isidoro N, Bin F, Colazza S, Vinson SB (1996) Morphology of antennal gustatory sensilla and glands in some parasitoid Hymenoptera with hypothesis on their role in sex and host recognition. Journal of Hymenoptera Research 5: 206–239.
Isidoro N, Romani R, Velasquez D, Renthal R, Bin F, Vinson B (2000) Antennal glands in queen and worker of the fire ant, *Solenopsis invicta* Buren: first report in female social Aculeata (Hymenoptera, Formicidae). Insectes Sociaux 47: 236–240. doi: 10.1007/PL00001709
Juliano SA (1985) Habitat associations, resources, and predators of an assemblage of *Brachinus* (Coleoptera: Carabidae) from Southeastern Arizona. Canadian Journal of Zoology 63: 1683–1691. doi: 10.1139/z85-250

Löbl I and Smetana A (2003) Catalogue of Palaearctic Coleoptera - Volume 1: Archostemata, Myxophaga, and Adephaga, Apollo Books, Stenstrup, Denmark, 819 pp.

Lockey KH (1988) Lipids of the insect cuticle: origins, composition and function. Comparative Biochemistry Physiology 89B(4): 595–645.

Moore BP (1979) Chemical defense in carabids and its bearing on phylogeny. In: Carabid Beetles: their evolution, natural history and classification. Erwin TL, Ball GE, Whitehead DR (Eds) Dr Junk W, London, 193–203.

Nagel P (1979) Aspects of the evolution of myrmecophilous adaptations in Paussinae (Coleoptera, Carabidae). In: Den Boer PJ, Thiele HU, Weber F (Eds) On the evolution of behaviour in Carabid beetles. Miscellaneous papers 18, Agricultural University Wageningen, The Netherlands, 15–34.

Noirot C, Quennedey A (1991) Glands, gland cells, glandular units: some comments on terminology and classification. Annales de la Société entomologique de France 27(2): 123–128.

Pasteels JM, Gregoire JC, Rowell-Rahier M (1983) The chemical ecology of defense in arthropods. Annual Review of Entomology 28(1): 263–289. doi: 10.1146/annurev.en.28.010183.001403

Pedata PA, Isidoro N, Viggiani G (1993) Evidence of male sex glands on the antenna of *Encarsia asterobemisiae* Viggiani et Mazzone (Hymenoptera: Aphelinidae). Bollettino del Laboratorio di Entomologia Agraria “Filippo Silvestri” 50: 271–280.

Quennedey A (1998) Insect epidermal gland cells: ultrastructure and morphogenesis. In: Harrison FW (Ed) Microscopic Anatomy of Invertebrates. Wiley-Liss, New York 11A: 177–207.

Quennedey A (2000) Perspectives on four decades of transmission-electron microscopy on insect exocrine glands. Le ghiandole esocrine degli insetti. Atti dell’Accademia Nazionale Italiana di Entomologia I: 85–116.

Rossini C, Attygalle AB, Gonza’Lez AS, Smedley SR, Eisner M, Meinwald J, Eisner T (1997) Defensive production of formic acid (80%) by a carabid beetle (*Galerita lecontei*). Proc. Natl. Acad. Sci. USA 94: 6792–6797. doi: 10.1073/pnas.94.13.6792

Schildknecht HU (1961) Die Bombardierkäfer und ihre Explosionschemie. Angewandte Chemie 73(1): 1–7. doi: 10.1002/ange.19610730102

Schildknecht H, Maschwitz U, Winkler H (1968) Zur Evolution der Carabiden-Wehrdrüsensekrete. Naturwissenschaften 55: 112–117. doi: 10.1007/BF00624238

Schildknecht HU (1970) Die Wehrchemie von Land- und Wasserkäfern. Angewandte Chemie 82(1): 16–25.

Scott PD, Hepburn HR, Crewe RM (1975) Pygidial defensive secretions of some carabid beetles. Insect Biochemistry 5: 805–811. doi: 10.1016/0020-1790(75)9024-4

Sturani M (1962) Osservazioni e ricerche biologiche sul genere *Carabus* Linneus (sensu lato). Memorie della Società Entomologica Italiana XLI: 85–202.

Thiele HU (1977) Carabid Beetles in their environments. Springer-Verlag, Berlin, Heidelberg.
Torres JA, Snelling RR, Blum MS, Flournoy RC, Jones TH, Duffield RM (2001) Mandibular gland chemistry of four Caribbean species of *Camponotus* (Hymenoptera: Formicidae). Biochemical Systematics and Ecology 29: 673–680. doi: 10.1016/S0305-1978(00)00107-1

Viscuso R, Narcisi L, Sottile L, Brundo MV (2001) Role of male accessory glands in spermatoderm reorganization in Orthoptera Tettigonioida. Tissue and Cell 33 (1): 33–39. doi: 10.1054/tice.2000.0147

Weis A, Schönitzer K, Melzer RR (1999) Exocrine glands in the antennae of carabid beetle, *Platynus assimilis* (Paykull) 1970 (Coleoptera, Carabidae, Pterostichinae). International Journal of Insect Morphology and Embryology 28: 331–335. doi: 10.1016/S0020-7322(99)00034-3

Whitman WD, Blum MS, Alsop DW (1990) Allomones: Chemicals for defense. In: Evans DL, Schmidt OJ (Eds) Insect defenses. Adaptive mechanisms and strategies of prey and predators. University of New York Press, Albany, 289–351.

Will KW, Attygalle AB, Herath K (2000) New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework. Biological Journal of the Linnean Society 71(3): 459–481.

Will KW, Gill AS, Lee H, Attygalle AB (2010) Quantification and evidence for mechanically metered release of pygidial secretions in formic acid-producing carabid beetles. 17pp. Journal of Insect Science, 10: 12.