Chapter 6
Chemical Ecology and Biochemistry of Dytiscidae

Konrad Dettner

Wenn man einen solchen Kefer [Cybister lateralimarginalis] *fängt, so lässt er insgemein zwischen dem Hals-Schild eine blaulichte Materie hervor fliessen, welche einen widerwärtigen Geruch von sich giebt und vielleicht Ursache ist, dass diese Kefer alle Zeit einen eckelhaften Gestank haben. [If such a beetle* Cybister lateralimarginalis *is caught, between the pronotum a bluish fluid appears which is characterized by a disagreeable odor that is probably responsible for the nauseous stench of the whole beetle.],*

Rösel von Rosenhof

I must tell you what happened … in my early entomological days. Under a piece of bark I found two carabi (I forget which) and caught one in each hand, when … I saw a sacred Panagæus crux major. *I could not bear to give up either of my Carabi, and to lose* Panagæus *was out of the question, so that in despair I gently sized one of the carabi between my teeth, when to my unspeakable disgust and pain the little inconsiderate beast squirted his acid down my throat and I lost both Carabi and Panagus!,*

Charles Darwin

Abstract The chapter deals with chemical mechanisms that help to control intra- and interspecific interactions with respect to predaceous diving beetles. Apart from chemical receptors and senses within Dytiscidae there are described intraspecific (pheromones) and especially interspecific interactions with respect to this water beetle family. The last group of behavioral modifying compounds includes kairomones and allomones. Allomone constituents from pygidial glands, prothoracic defensive glands, and pupal glands are completely compiled for a large group of predaceous

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diving beetles. With respect to the natural compounds, their chemistry, distribution within Hydradephaga, biological activities, and especially their significance for dytiscids are discussed. In addition, further secondary compounds from these beetles are presented, including epicuticular lipids or pigments that may be responsible for the coloration of the adult beetles and their larvae. Finally, the microorganisms and their secondary metabolites that are associated with predaceous diving beetles are presented. The described microorganisms range from culturable to non-culturable taxa.

Keywords Dytiscidae • Chemical ecology • Allomones • Glands • Secondary compounds

6.1 Chemical Ecology of Freshwater Organisms

Since 1970, after the publication of the book entitled "Chemical Ecology", edited by E. Sondheimer & J. B. Simeone, the field of chemical ecology has been recognized as a distinct interdisciplinary research area. Chemical signals are perhaps the oldest form of communication among organisms, and this discipline investigates how naturally occurring chemicals mediate ecological interactions. In most cases, chemoecological studies focus on ecological mini-systems that include few species or individuals, whereas complex biocenosis are not analyzed. Moreover, chemical ecology often starts with an observation – e.g., chemical defense of a bombardier beetle or attraction of one sex of a moth species to the other sex through sexual pheromones. Chemical ecology is concerned with the identification and synthesis of those substances (semiochemicals = ectohormones) that convey information and interact between different individuals of organisms (allelochemicals as allomones, kairomones, or pheromones). Moreover, this discipline also elucidates the exocrine gland systems, receptors, and the transduction systems that recognize and pass on these semiochemicals. In addition, the developmental, behavioral, and ecological consequences of these chemical signals also are investigated. All of these areas rely upon bioassays in the laboratory and in the field. The results of chemoecological studies may be important in plant protection, in the development of highly selective techniques for pest control, and even in integrated plant protection. Dependent on the research areas of the scientists working on chemical ecology, classification and investigation of these phenomena varies considerably. Natural product chemists and biochemists are interested in biosynthesis and chemical structures of the secondary compounds involved. In contrast, ecologists may favor research that focuses on the interactions among trophic levels. As chemical ecology studies the interactions among different individuals of the same or different species other scientists potentially are interested in knowing the senders and receivers of chemical signals, and in knowing if an ectohormone is of advantage or disadvantage for these individuals. Finally, entomologists interested in chemical ecology may focus on exocrine glands or chemical signals on the body surface or want to learn if the compounds are

biosynthesized by the insects, sequestered from plants, or produced by endosymbiontic microorganisms.

As compared with chemoecological studies in terrestrial ecosystems, which has been intensively studied the 1970s, chemical ecology of aquatic systems was initially neglected, but now there are considerable data available concerning the chemoecology of aquatic systems (e.g., Brönmark and Hansson 2012; Burks and Lodge 2002; Ferrari et al. 2010; Gross 2011). However, marine systems were often studied with the priority in identifying new biologically active natural products. In spite of the fact that freshwater chemical ecology lags behind terrestrial and marine chemical ecology, a constant increase of publications in this interesting field is recognizable (Burks and Lodge 2002). It was found that among allelochemicals kairomones mediate the majority of species interactions in freshwater systems. Fish and predaceous insects act largely as senders, zooplankton on the contrary comprise the most studied receivers. Other organisms such as predaceous insects may be both receivers of cues from larger predators as well as senders of their own cues to lower trophic levels, such as zooplankton (Burks and Lodge 2002). In freshwater systems, chemoecological investigations have especially targeted the study of predator–prey, plant-plant, and plant-herbivore interactions (including microorganisms) and the role of allelochemicals (Ferrari et al. 2010; Gross 2011).

The chapters in recent compilations on chemical ecology in aquatic systems (e.g., Brönmark and Hansson 2012) are of different significance for those who are interested in freshwater systems. Whereas information conveyed by chemical cues (Elert 2012) are highly informative, other chapters such as chemical defense (Kicklighter 2012) are only partially valuable, because marine systems are over represented and data from freshwater systems are nearly completely lacking. However taxonomically simple freshwater organisms such as Alveolata, Porifera, Cnidaria, or flatworms (Dettner 2010) are as important as chemically defended Hydrachnidia, water beetles and water bugs (Coleoptera: e.g., Dytiscidae, Noteridae, Hygrobiidae, Haliplidae; Heteroptera: Corixidae, Notonectidae, Naucoridae, Belostomatidae) or even chemically defended trichopteran larvae.

In this chapter I focus on all aspects of chemical ecology for adults and to a certain extent pupae of dytiscids. Data on glands or semiochemicals of dytiscid eggs and larvae are, unfortunately, not available, although such information would no doubt be interesting and valuable for our understanding of this family of beetles. For adult dytiscids, there exist only a few data on pheromones (Sect. 6.3) and kairomones (Sect. 6.4.1). In contrast, the Dytiscidae possess various complex glands and much is known for allomones (defensive compounds, Sect. 6.4.2). Moreover, behavior modifying chemicals may not be volatile or water soluble, but instead may cover the entire body surface as a kind of distinguishing mark, and the nature of such epicuticular lipids are examined here (Sect. 6.5.2; Dettner and Liepert 1994). Because animal coloration represent secondary compounds, natural pigments of predaceous diving beetles also are reviewed (Sect. 6.5.3). Finally, various aspects of microorganisms associated with predaceous diving beetles are presented (Sect. 6.6) and future directions in research (Sect. 6.7) are offered.

6.2 Chemical Senses

Aquatic insects evolved secondarily in aquatic environments and therefore are capable of sensing odors from a diverse range of sources (Crespo 2011). The recent review by Crespo (2011) on chemosensation and related behavior in aquatic insects is mainly focused on hemimetabolous aquatic orders including Ephemeroptera, Odonata, and Plecoptera, and the holometabolous Trichoptera and Diptera. In contrast, aquatic Coleoptera are completely omitted, however specific investigations on dytiscid beetles do exist elsewhere.

Nikolaas Tinbergen (1907–1988), a Dutch born British zoologist (Fig. 6.1a) shared the Nobel prize in 1973 with Karl von Frisch and Konrad Lorenz for research on the social behavior of animals. As early as 1936 he reported on his investigations with adults and larvae of *Dytiscus marginalis*. Although adults possess very large complex eyes (Fig. 6.1b) they do not react to living tadpoles within water filled test tubes. In contrast, adult beetles will quickly move their antennae and swim strongly within an odor plume of meat extract (Fig. 6.1c, d). Tinbergen also discusses the chemosensation of *Dytiscus* larvae. Further results concerning chemical senses of *Dytiscus* larvae are presented by Korschelt (1924).

During the next several decades the chemical senses of dytiscids were investigated by physiologists and zoologists. Schaller (1926) reported that dytiscids have very good chemical senses (odor, taste) that are especially important for detecting potential food. The receptors for these senses are located on different parts of their body. Dytiscids can taste sweet, sour, salty, and bitter with their taste receptors that are concentrated on their maxillary and labial palpi. Odor receptors (but not taste receptors) are found on the antennal surface.

Bauer (1938) showed during trainings experiments (mainly with adult *Dytiscus marginalis*) that beetles can differentiate between a variety of specific chemicals, including saccharose and hydrochloric acid. Furthermore, they can select saccharose when it is offered together with hydrochloric acid, sodium chloride, and the bitter quinine hydrochloride (bitter tasting alkaloid). Finally, they can select hydrochloric acid when it is offered together with glucose, quinine hydrochloride, and sodium chloride. However, beetles cannot differentiate between saccharose and glucose, hydrochloric and tartaric acid, quinine hydrochloride and salicin (bitter tasting alcoholic β-glucoside), or quinine hydrochloride and aloin (anthraquinone glucoside). It was found that these beetles can detect 18 different sugars and may perceive different compounds at different thresholds (e.g., saccharose 0.01 mol; sodium chloride 0.001 mol, salicin 0.0000625 mol, quinine hydrochloride 0.0000012 mol) (Bauer 1938).

Besides large species such as *Dytiscus,* chemoreception in aqueous and gas phases were studied in the a smaller species *Laccophilus maculosus* (Hodgson 1953). In this species the sensilla basiconica are located on the tips of antennae and represent chemoreceptors for gaseous and liquid stimuli. Due to inherent specialization these receptors have the lowest threshold of antennal receptors. Hodgson (1953) also reported that those sensilla basiconica that are located on the tips of the maxillary

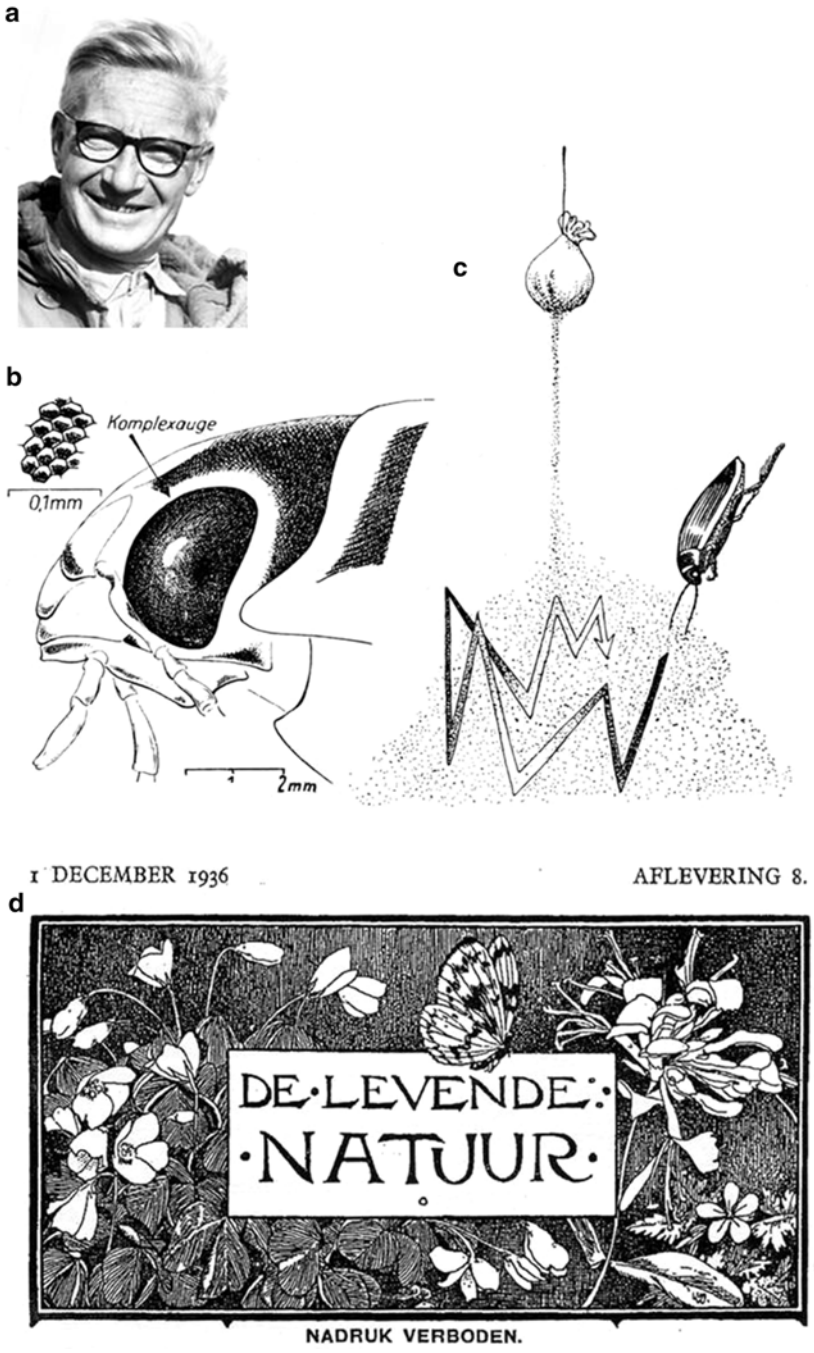


Fig. 6.1 Nobel laureate Nikolaas Tinbergen (1907–1988; **a**) and his investigations on orientation of *Dytiscus marginalis* (Tinbergen 1936). In spite of the large complex eyes of adults (**b**) during detection of prey the beetle strongly reacts to a meat broth by swimming behavior within an odor plume of a meat extract (**c** according to Tinbergen 1951). The title page of the journal "De levende Natuur" from 1936 is shown (**d**)

and labial palpi also represent chemoreceptors, although with higher thresholds. Hydrochloric acid, 1-pentanol, and sodium chloride all stimulated receptor areas on the tips of antennae and palpi. In addition, Hodgson (1951) showed that cations in uniform anion combination stimulated in the following odor of effectiveness according to the order of their ionic motilities: hydronium (=hydroxonium) >> ammonium > potassium > sodium > lithium. In contrast, anions in uniform cation combinations stimulated in the following order of effectiveness: hydroxide >> iodine$^-$ > bromine > sulfate$^-$, acetate, chloride > phosphate. With respect to low molecular organic compounds, thresholds to primary to alcohols decreased with increasing in CH_2-groups (e.g., methyl alcohol 3.6 mol, ethyl alcohol 4.3 mol, propyl alcohol 3.2 mol, butyl alcohol 0.046 mol, amyl alcohol 0.0073 mol, hexyl alcohol 0.0011 mol). This trend is apparently directly related to lipid solubility of the alcohols. Behrend (1971) analyzed the responses of single pore plate olfactory cells on odorous compounds in either air or water. The olfactory cells responded either to various organic acids and amino acids (class 1) or to nitrogenic compounds (class 2). Identical stimuli resulted in the same response in air and in water, which does not depend on the physicochemical state of the stimulating molecules within their carriers (air or water).

There exist various light microscopic and electron microscopic studies concerning the sensillae of Dytiscidae. Light microscopic details and a survey was produced by Korschelt (1923). Electron microscopic studies were performed on the fine structure of the sensilla on the distal antennal segment of *Graphoderus occidentalis* (Jensen and Zacharuk 1991), the digitiform from sensilla on the distal segment of maxillar palps of *Agabus bipustulatus* (Guse and Honomichl 1980), and antennal sensillae of *Acilius sulcatus* (Ivanov 1966). However, little recent work has been conducted on the specific microstructure of dytiscid chemical sensory structures.

6.3 Intraspecific Interactions: Sex-Pheromones

Sex pheromones are well known from Lepidoptera and other terrestrial insects, as well as a few examples from marine systems (Wyatt 2003). However observations on sex pheromones in freshwater systems are very rare in both invertebrates (e.g., *Gammarus*; Borowsky and Borowsky 1987) and vertebrates (Sorensen and Hoye 2010).

As far back as 1912 Blunck (1912b) reported that female *Dytiscus marginalis* produce a certain "Geschlechtsduft" (sexual odor) that leads males to females within an area of 20–30 cm. He also mentioned that males, excited by females, would quickly move their antennae and palpi during an increase in their swimming movements. Blunck (1912b) also found that secretions of female pygidial glands did not arouse males. Smith (1973) reported on sound production in both sexes of different species within genus *Rhantus*, which was observed in a behavioral context of emigration. During his experiments he reported that intra- and interspecific recognition is achieved through an olfactory clue, and in the laboratory interspecific location even functioned in total darkness.

Recently Herbst et al. (2011) demonstrated the presence of sex pheromones in the predaceous diving beetle *Rhantus suturalis*. Within non-permeable glass flasks,

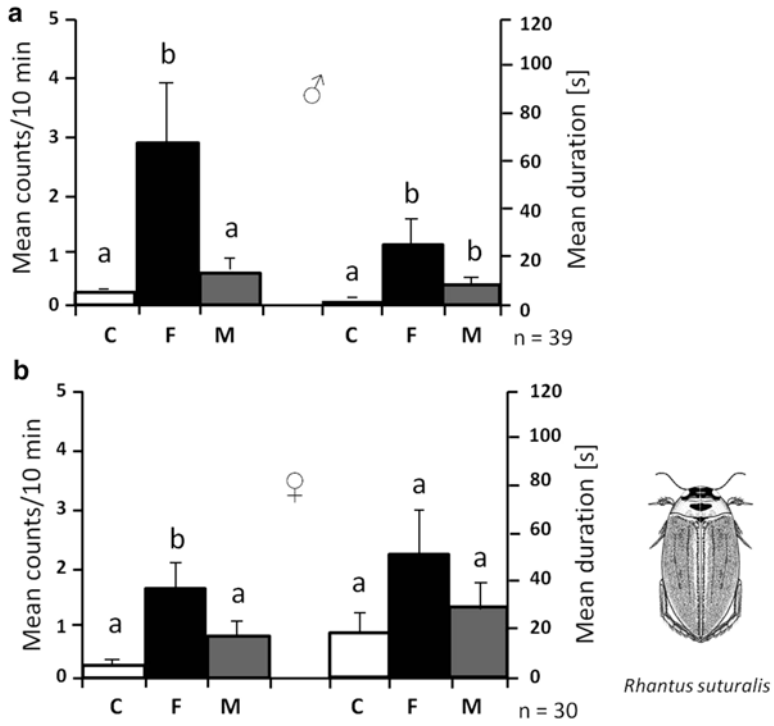


Fig. 6.2 Reaction of male (**a**, above) and female (**b**, below) *Rhantus suturalis* beetles to a vessel made of finely woven steel, containing one female (F) or one male conspecific (M) or to an empty control vessel (C). *Left*: mean counts of beetle contacts with the vessels. *Right*: mean sitting duration of beetles on the vessels. Error bars indicate standard errors. Bars with different letters are significantly different at $P \leq 0.05$. *n* number of replicates (After Herbst et al. 2011)

which did not allow the diffusion of chemicals, males and females did not stimulate any reaction by conspecifics of either sex. However in permeable vessels (e.g., made of finely woven steel) male predaceous diving beetles were significantly attracted to females. In addition, female *R. suturalis* were attracted to other females when they perceived chemical and optical cues simultaneously. Specifically, Fig. 6.2 illustrates the numbers of contacts with (left axis) and the sitting contacts with the vessel in male (a) and female (b) *R. suturalis* to a permeable steel vessel containing one female (F), one male conspecific (M) or an empty control vessel (C). Both with respect to contacts with the vessel and sitting durations on the vessels, males significantly selected females over males of controls. In addition, female *R. suturalis* had significantly more contacts with conspecifics than with males (Fig. 6.2).

With these results in mind, it would be interesting to now elucidate the chemical structure of the substances that modify female behavior in dytiscids. Some aquatic vertebrates (e.g., fishes, amphibians) unlike terrestrial insects use unusual polar compounds that serve as sex pheromones (Sorensen and Hoye 2010) such as l-kynurenine (Masu salmon of genus *Oncorhynchus*), prostaglandin $F_1\alpha$, $F_2\alpha$ (*Salmo*),

a dihydroxypregnan-20-one-3-glucuronide (African catfish *Claria*), dihydroxy-4-pregnen-3-one and prostaglandins (*Carassius auratus*), newts in the genus *Cynops* (decapeptides as sodefrin, silefrin) or the tree frog *Litoria splendida* (25-amino acid peptide splendiferin). Further data which characterize pheromones of aquatic organisms are presented by Breithaupt and Thiel (2011) and Brönmark and Hansson (2012). Remarkably both kynurenin and steroids represent important metabolites of Dytiscidae. An intriguing question for the findings of Blunck (1912b) is if the prothoracic defensive glands are important for sexual pheromone activities.

6.4 Interspecific Interactions

During evolution, predators, parasitoids, and prey have developed various methods in order to detect, to defend, or generally to interact with each other (see Peckarsky 1984; Williams and Feltmate 1992). Apart from visual communication in aquatic ecosystems with low visibility and effective superposition eyes, predaceous diving beetles seem to especially use non-visual stimuli for their interactions and rely on chemoreception, which is very efficient in both adult and larval dytiscids. Interspecific chemical interactions are generally mediated by allelochemicals, which may be further subdivided depending on whether these chemicals are advantageous for the sending (allomone, see Sect. 6.4.2) or for the receiving (kairomone, see Sect. 6.4.1) organisms.

6.4.1 Kairomones and Other Allelochemicals

Kairomones represent interspecific behavioral modifying chemicals that are of advantage for the receiver and in contrast are negative or disadvantageous for the producing organisms. They are important in most predator/prey or host/parasite-systems.

With respect to dytiscids, our knowledge of chemical ecology varies depending on if the dytiscids represent prey (Sect. 6.4.1.1) or predators (Sect. 6.4.1.2). In addition, dytiscids may perceive kairomones (Sects. 6.4.1.1 and 6.4.1.2) or may function as kairomone emitters (Sect. 6.4.1.3). In all cases, there exist many laboratory and field observations, however the mechanisms for these behavior modifying kairomones is unknown.

There is growing evidence both from laboratory and mesocosm studies that insect predators that orientate towards the water surface are often absent in the presence of fishes. However, these insects may have effects on potential prey (e.g., zooplankton) that are analogous to fish predators (Herwig and Schindler 1996). As an example, larval *Acilius semisulcatus* significantly affect the vertical distribution of *Daphnia pulex* prey (especially large specimens; Arts et al. 1981). If dytiscid predators are present, a greater percentage of *Daphnia*-prey was found near the bottom of the experimental cages. Thus it seems highly probable that chemical signals, such as kairomones produced by dytiscid beetles, are involved in this response.

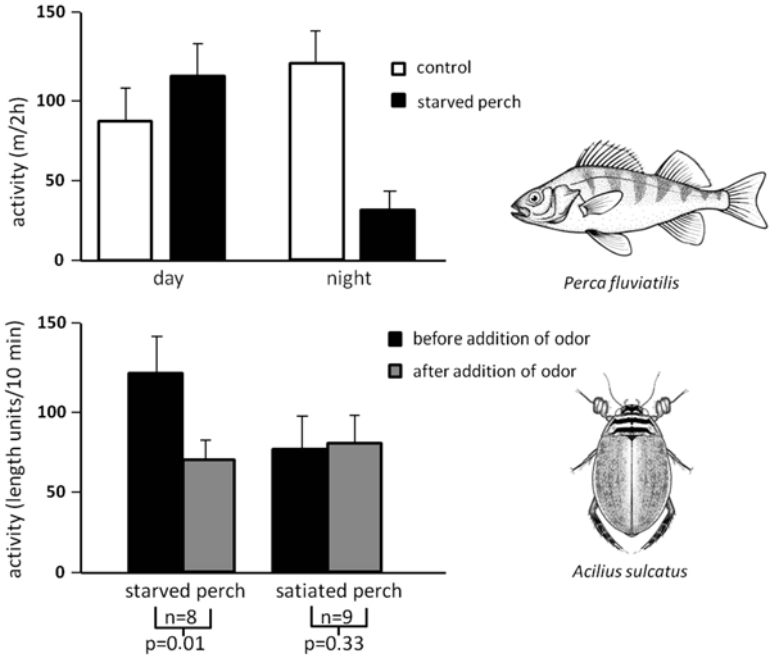


Fig. 6.3 Responses of dytiscid beetles (*Acilius sulcatus*) to chemical cues from perch *Perca fluviatilis*. *Above*: Activity (m moved within 2 h) for *Acilius sulcatus* in the different treatments in the fluviarium experiment (mean + SE). *Below*: Activity (mean + SE) of *Acilius sulcatus* counted as the number of quadrats past during 10 min before and after adding "fish-water" in the aquarium experiment. The *P*-value shows the result of the Wilcoxon-signed-rank test of the difference in activity before and after adding "fish-water". Changed according to Åbjörnsson et al. (1997)

6.4.1.1 Dytiscid Prey and Fish Predators

The importance of fish predation on aquatic insects, including some species of predaceous diving beetles, were reviewed by Healey (1984) and Sih (1987). Fish can exert strong and negative effects on dytiscid communities (Chap. 10 in this book) and may be important for food web dynamics as dytiscids can be both fish prey and predator (Chap. 8 in this book). In one example (Åbjörnsson et al. 1997) it was determined that *Acilius sulcatus* responded to chemical cues from perch (*Perca fluviatilis*). Whereas odor or visibility alone did not affect the activity of *A. sulcatus*, a significant interaction occurred when the two factors were combined (Fig. 6.3). The lowest activity of the beetles was found when *A. sulcatus* was exposed to water scented by starved perch at night (Fig. 6.3). When the activity was counted as the number of quadrats passed during 10 minutes before and after adding "fish-water", activity decreased after the addition of odor from starved perch (Åbjörnsson et al. 1997). This finding strongly suggests that beetles may alter their behavior in the presence of fish predators.

6.4.1.2 Dytiscids Predators and Vertebrate Prey

Larval dytiscids are often predators of vertebrates and may use kairomones emitted by their prey. There exist various examples where such interactions are described. In 1995, Mathis et al. reported that alarm pheromones of fathead minnows (*Pimephales promelas*) function as attractants for both predatory fish like pike (Esocidae) and adult predaceous diving beetles. When traps were supplied with skin extracts of alarm substance cells of non-breeding fishes (that had alarm pheromone cells) significantly more beetles were caught in the traps bated with alarm substances as compared with the controls (lacking alarm pheromone cells). These traps recorded seven species including *Acilius semisulcatus, Colymbetes sculptilis, Dytiscus alaskanus, D. circumcinctus, D. cordieri, Graphoderus occidentalis*, and *G. perplexus*, although only *C. sculptilis* were present in the sufficient numbers for statistical analysis. The evolutionary significance of such alarm signals that attract predators and are useful for alarm signal emitters was summarized by Chivers and Smith (1998) and Chivers et al. (1996).

Recently larvae of *Dytiscus sharpi* were recognized as being capable of detect not only prey motion but also prey scent (Inoda 2012). When larvae were exposed only to prey odors in the form of chemical signals from tadpoles they were more likely to be attracted to traps with tadpoles than to empty control traps. In contrast, *D. sharpi* larvae were not attracted to a trap containing conspecific larvae. The author suggested that the larvae are capable of recognizing prey scent (but not prey size), which may increase foraging success but decrease cannibalism.

Manteifel and Reshetnikov (2002) conducted laboratory experiments and allowed different predators to prey on noxious versus non-noxious tadpoles. Whereas predatory fishes and *Aeshna* nymphs actively consumed *Rana* tadpoles, *Bufo* tadpoles were rejected. On the contrary, larvae of *Dytiscus marginalis* attacked both tadpoles. These results are interesting from a chemically perspective, however the degrees of noxiousness of skins and interior bodies of *Bufo* and *Rana* tadpoles were not analyzed in this study. Therefore, interpretation of these results is difficult especially with respect to strategies of nutrition by different predators (i.e., sucking vs. chewing).

6.4.1.3 Dytiscid Predators and Egg-Laying Prey

In temporary pools, larvae of the mosquito *Culiseta longiareolata* are highly vulnerable to the common predatory backswimmer *Notonecta maculata* (Silberbush et al. 2010). It was recently found that adult female mosquitoes use kairomones that are released by these predators to detect the risk of predation. Specifically, oviposition of female mosquito is effectively repelled by n-heneicosane and n-tricosane, two hydrocarbon kairomones produced by *Notonecta* (Silberbush et al. 2010). The same effect was observed recently in females of the wetland mosquito *Culex tritaeniorhynchus* that strongly avoided laying eggs at oviposition sites in the presence of the predaceous diving beetle *Eretes griseus* (Ohba et al. 2012). In contrast, female

Aedes albopictus mosquitoes laid eggs in both the absence and presence of predator cues, probably because they could not detect the hitherto chemically unknown *Eretes* cues or are not sensitive to them. This was the first report to show that mosquitoes can detect the chemical cues of coleopteran beetles. In addition, Ohba et al. (2012) found that mosquito larvae near the water surface were eaten less frequently by *Eretes griseus* than those at the bottom of the containers. Therefore, filtering at the water surface appears to be an appropriate adaptive response in the presence of this predator.

Beyond the effect of dytiscids on invertebrates, Urban (2008) studied interactions between salamander larvae (*Ambystoma maculatum*) and *Dytiscus* larvae due to kairomones. It was evident that *Dytiscus* kairomones strongly reduced the daytime activity of *A. maculatum* larvae but presence of beetle larvae did not induce lower larval amphibian body masses, suggesting that perhaps feeding activity was not modified by predator presence.

6.4.2 Allomones

Allomones represent substances that are produced and released by an individual of one species that affects the behavior of an individual of another species. In contrast to kairomones (Sect. 6.4.1), allomones such as defensive compounds or antibiotics are advantageous for the sender and disadvantageous for the receiver. For both types of interactions allomones and kairomones there exist many detailed observations and bioassays in the field and the laboratory. However, compared to allomones, detailed data on the chemical character of behavior modifying kairomones are completely lacking. In contrast, hydradephagan beetles produce huge amounts of chemically identified natural products and allomones in their complex pygidial and prothoracic defensive glands. Therefore, Dytiscidae are well known to harbor elaborate biosynthetic apparatuses for manufacturing either steroids or aromatics (Blum 1981; Morgan 2004). Before reporting on the two gland systems where these natural products are produced, it is important to mention another internal structure, the rectal ampullae.

Both larvae and adult dytiscids possess rectal ampullae. If adults of larger Dytiscidae (Dytiscinae) are handled they often immediately react by depleting their rectal ampulla. This is evident by an unpleasant odor resembling hydrogen sulfide (H_2S) or ammonia (NH_3). Eisner (1970) named these defensive reflexes enteric discharges and discerns between regurgitation and defecation. After uptake of water the rectal ampulla may primarily serve as hydrostatic organ to increase the specific weight of the beetle, for example when it lands on a shining water surface (Naumann 1955; Wesenberg-Lund 1943; Hicks and Larson 1991). Moreover, a lot of valuable compounds such as ions and sugars are reabsorbed from the rectal epithelium into the hemolymph (Cochran 1975; Dettner and Peters 2010). In addition, this organ represents the first defecation-defense of adult dytiscids, before prothoracic defensive glands are depleted. Usually the rectal ampulla, which extends through the whole abdomen (Fig. 6.4a), is filled with water and very often with excrements

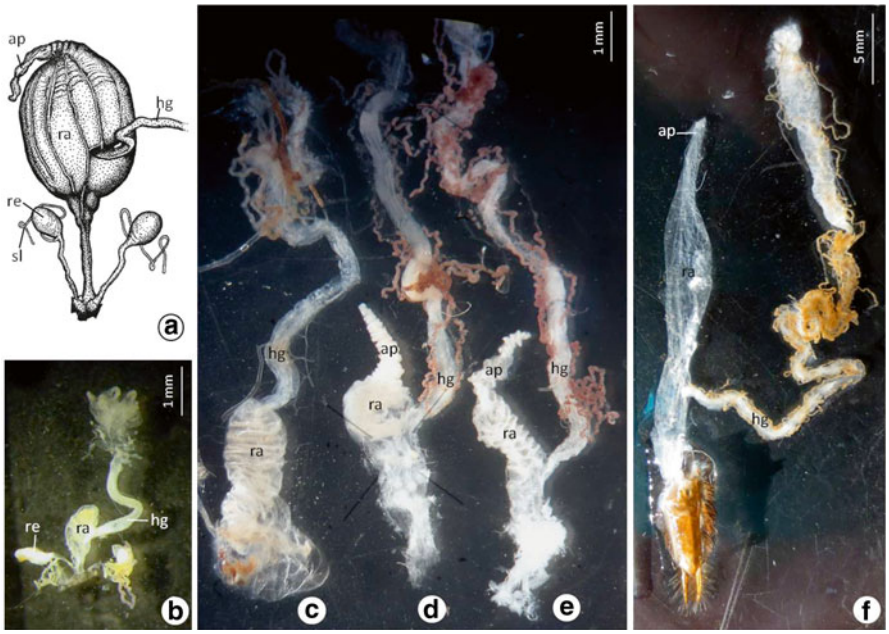


Fig. 6.4 (**a**) Filled rectal ampulla of *Dytiscus marginalis* with appendix, hind gut and paired pygidial glands. (**b**–**d**) Mid- and hind gut, rectum and rectal ampulla of *Hyphydrus ovatus* (**b**), *Ilybius crassus* (**c**), *Acilius sulcatus* (**d**), *Hydaticus seminiger* (**e**). Mid- and hind gut, rectum and rectal ampulla together with last abdominal segment and cerci of a *Dytiscus marginalis* larva (L III, **f**). (**a**) According to Naumann (1955). Abbreviations: *re* reservoir of pygidial gland, *sl* secretory lobe, *ra* rectal ampulla, *ap* appendix, *hg* hind gut

(Wesenberg-Lund 1943). Taxonomically a rectal ampulla is found in representatives of adult Dytiscinae (Fig. 6.4d, e) and Hydroporinae (Fig. 6.4b). Here the hind gut laterally meets the ampulla at its midway point (Fig. 6.4b). The same configuration was observed in *Agabus bipustulatus*. Within representatives of Colymbetinae the posterior part of the hind gut widens considerably, but otherwise the small hind gut meets the widened hind gut terminally or subapically (Fig. 6.4c). When larger and selected specimens of adult Dytiscinae are molested, odorous irritations are the only threat to humans or other vertebrate predators. It is interesting that large, full-grown larvae, especially of Dytiscinae, possess extremely lengthened rectal papillae (Fig. 6.4f). Sometimes the rectal ampulla, which also serves primarily as hydrostatic organ, is so long and extends into the larval head. It was suggested that this huge larval appendage serves to increase interior pressure in order to burst the last larval skin (Naumann 1955). Korschelt (1924) reports that the rectal ampulla of *Dytiscus*-larvae does not represent a defensive mechanism as observed in adults but is filled with water after molting. In contrast to adults, the defensive mechanisms of *Dytiscus* larvae are mechanical and are due to biting movements of sharp mandibles.

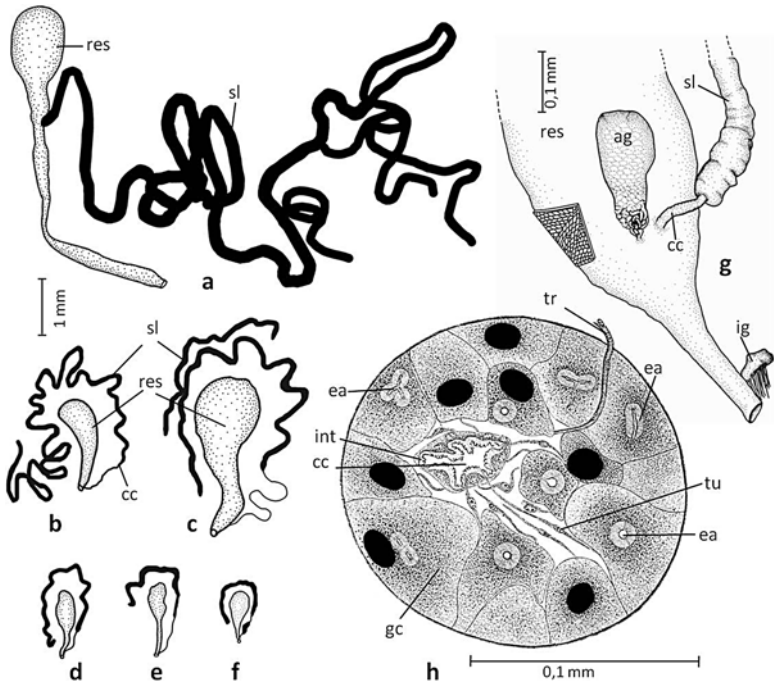


Fig. 6.5 (**a**–**f**) Structure of one pygidial gland system. (**a**) *Dytiscus marginalis*, (**b**) *Acilius sulcatus*, (**c**) *Colymbetes fuscus*, (**d**) *Copelatus (Liopterus) haemorrhoidalis*, (**e**) *Laccophilus minutus*, (**f**) *Nebrioporus depressus*. (**g**) Enlargement of posterior part of the left pygidial gland system of *Hyphydrus ovatus* (Modified after Forsyth 1968). (**h**) Section through secretory lobe of *D. marginalis* (Modified after Korschelt 1923). Abbreviations: *ag* accessory gland, *cc* collecting canal, *ea* end apparatus, *gc* gland cell, *ig* integumental gland, *in* intima, *res* gland reservoir, *sl* secretory lobe, *tr* trachea, *tu* tubule. Nuclei are *black*

6.4.2.1 Pygidial Glands

According to microtome sections, all hydradephagean families (save one) and neighboring taxa possess pygidial glands and their gland constituents (Dettner and Böhner 2009). Pygidial glands were recorded within the recently identified water beetle family Meruidae (Beutel et al. 2006), however, in Aspidytidae the histological data are absent. There exist various data concerning the anatomy and histology of the pygidial defense glands in Dytiscidae, Noteridae, Haliplidae, Gyrinidae (Forsyth 1968), Amphizoidae, and Hygrobiidae (Forsyth 1970; Figs. 6.5 and 6.6). Paired pygidial defensive glands were described for the first time in more detail in the dytiscid genera *Hyphydrus*, *Stictotarsus*, *Laccophilus,* and *Ilybius* (Forsyth 1968). Later abdominal glands from several other dytiscid species were recorded (Dettner 1985). Each gland (Figs. 6.5 and 6.6) comprises an ovoid reservoir that is covered by a muscle coat (Fig. 6.5g inlet figure; 6.6d) and leads into an efferent duct with

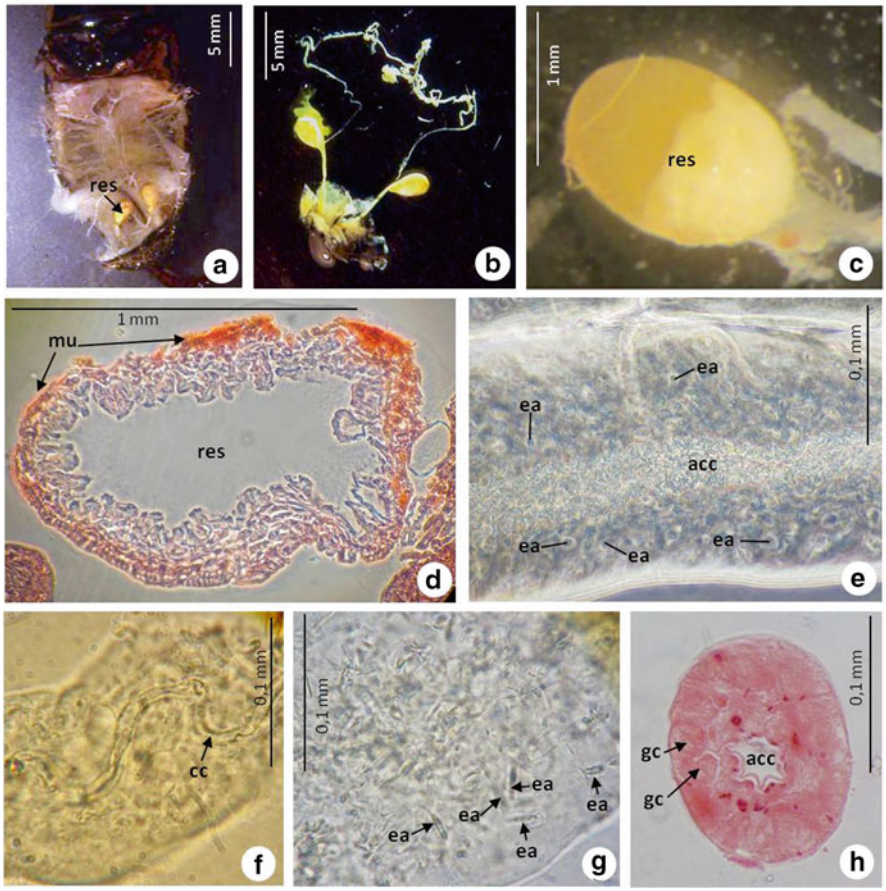


Fig. 6.6 (**a**–**h**) Structure and histology of pygidial glands of *Dytiscus marginalis*. (**a**) Dissected abdomen with paired pygidial glands. (**b**) Enlargement of two prepared pygidial gland systems. (**c**) Pygidial gland reservoir with two organic phases. (**d**) Section through gland reservoir using nuclear fast red-aluminum sulfate solution (**e**) Longitudinal view of squeezed secretory lobe. (**f**) Longitudinal view of squeezed collecting canal. g: Longitudinal view of squeezed secretory lobe with end apparatuses. (**h**) Square section through secretory lobe using nuclear fast read-aluminum sulfate solution

proximal valve. The lobular secretory tissue or secretory lobe (Figs. 6.5a–g and 6.6b, 6.6e, g) is connected to the reservoir by a collecting canal (Figs. 6.5g, h and 6.6f). The openings of the reservoirs are situated on the membranous cuticle behind the eighth abdominal tergite. According to Forsyth (1968, 1970) there exist two types of pygidial gland cells. An organelle of type I is typical for Dytiscidae but absent in Haliplidae, Gyrinidae, and Noteridae. The last three families have organelles of type II, which are also found in Laccophilinae, Hydroporinae, and some Colymbetinae and Dytiscinae. In addition, Laccophilinae and Hydroporinae possess simple unbranched type II organelles, but both simple and branched organelle-forms occur

in Dytiscinae and Colymbetinae (Forsyth 1968). Ultrastructural analyses confirmed that two types of eccrine gland cells (racemous and bulbous) exist as reported from pygidial glands of *Dytiscus marginalis* (Kuhn et al. 1972). The central cavity of the gland duct is surrounded by microvilli that are stiffened by microfibrils. Kuhn et al. (1972) reported that the Golgi-apparatus is better developed in racemous cells than in the bulbous cells. In addition, the central cavities contain fine-fluffy substances in racemous cells and osmiophilic materials in the bulbous cells. It is interesting that Forsyth (1968) could also describe accessory glands (Fig. 6.5g) in the genera *Hyphydrus* and *Stictotarsus* that open into the reservoir of the pygidial glands close to the opening of the collecting canal. According to Forsyth (1968), these accessory glands are homologous with the basal combustion chamber in bombardier beetles. Vesicle and organelle of accessory glands are similar to the gland cells of thoracic glands and of the type II cells of the pygidial gland. Moreover an integumental gland (Fig. 6.5g) with about 100 cells opens close to the external reservoir opening in *Hyphydrus* (Forsyth 1968). The secretory lobes are characterized by an axial collecting canal (Fig. 6.5e, h) that is surrounded by gland cells with type I and II organelles (Figs. 6.5h and 6.6e–h).

The secretory lobes may be lengthened (Figs. 6.5a, b and 6.6b) or even branched (Fig. 6.5a, c). The collecting canals may be lengthened as in *Colymbetes, Laccophilus* (Fig. 6.5c, e), or shorter as in *Acilius* (Fig. 6.5b), *Copelatus* (Fig. 6.5d), *Nebrioporus* (Fig. 6.5f), and *Hyphydrus* (Fig. 6.5g), or are even absent as in *Hydaticus* (not figured) and *Dytiscus* (Fig. 6.5a). In most dytiscid species studied the collecting canals unite near the reservoir opening with the efferent duct of reservoirs (Fig. 6.5b–f), however in *Hyphydrus* (Fig. 6.5g) and especially in *Hydaticus* and *Dytiscus* (Figs. 6.5a and 6.6b) the collecting canal unites more anteriorly with the gland reservoir.

According to Korschelt (1923) the pygidial gland system of *Dytiscus marginalis* is innervated by the paired second nervi that originate from the hind border of the last abdominal ganglion (ganglion VI). Obviously this large nerve (called Nervus proctodaeo-genitalis) innervates all organs from the eighth segment onwards to the abdominal tip.

6.4.2.1.1 Chemistry of the Pygidial Glands and Distribution of Pygidial Gland Constituents Within Dytiscidae and Hydradephaga

Among insects, hydradephagean beetles represent the most prominent taxa producing aromatic exocrines (Dettner and Böhner 2009). Apart from Dytiscidae, aromatic pygidial gland constituents are found in Haliplidae (Dettner and Böhner 2009), Noteridae (Dettner 1997a), Amphizoidae (Dettner and Böhner 2009), and Hygrobiidae (Dettner 1997b), however pygidial gland chemistries of Meruidae, Aspidytidae, and Rhysodidae are unknown. Unusual aromatics that are not present in dytiscid beetles are 3-hydroxyphenylacetic acid and phenyllactic acid in Haliplidae (Dettner and Böhner 2009). In closely related families pygidial glands only contain a few aromatics in usually low amounts. Gyrinidae produce phenylacetaldehyde (Dettner and

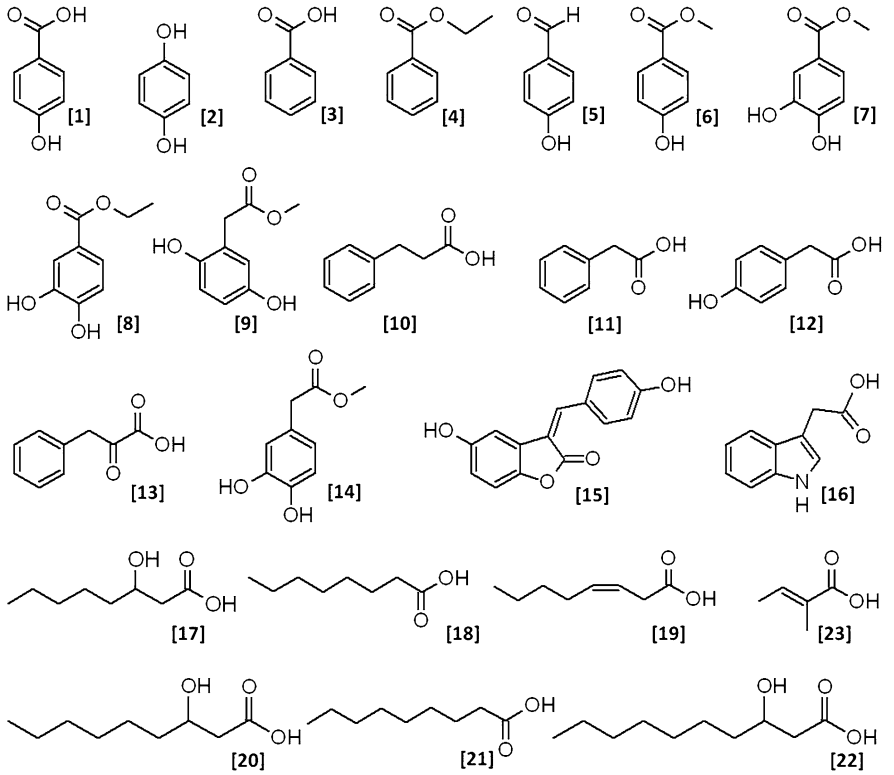


Fig. 6.7 Pygidial gland constituents **1**–**23** of predaceous diving beetles

Böhner 2009), Trachypachidae contain 2-phenylethanole and its esters (Attygalle et al. 2004) and a few carabid and cicindelid taxa contain benzoic acid, phenylacetic acid, and methylsalicylate together with salicylic aldehyde and benzaldehyde (see Francke and Dettner 2005; Dettner and Böhner 2009; Will et al. 2000).

The first results on the chemistry of the pygidial glands of dytiscids were published by Ghidini (1957). He described pygidial gland secretions of Dytiscinae as "disagreeable", whereas representatives of Hydroporinae such as *Hydroporus*, *Potamonectes*, *Deronectes*, *Stictotarsus,* and *Coelambus* were characterized as "sweet" and "agreeable" odors. I have supplied the chemical structure of many of the most common pygidial gland products in Fig. 6.7; hereafter I refer to them by number designations (bold).

Subsequently, Schildknecht et al. (1962) reported the presence of benzoic acid (**3**) (Table. 6.1, Fig. 6.7) and various other aromatics in pygidial glands of different dytiscid species. In the following years, 14 aromatic, 7 aliphatic compounds, a tryptophane-metabolite (**16**) and an unusual pigment (**15**) could be identified from this gland system (Table 6.1, Fig. 6.7; e.g., Dettner 1979, 1985; Schildknecht et al. 1983). Since then, several taxa of dytiscid beetles have been

Table 6.1 Pygidial gland constituents of predaceous diving beetles

Dytiscidae, Hydroporinae	
Hyphydrus ovatus (L.)	11, 12, 16 (Dettner 1979)
Hyphydrus aubei Ganglb.	(2),(3),11,12,13,16 (Dettner 1985)
Hydrovatus cuspidatus (Kunze)	(3)#,(11)#,12#,13#,16#,17#,(18)#,21#,22#
Hydroglyphus geminus (F.)	11,13,16 (Dettner 1979)
Geodessus besucheti Branc.	11 (Dettner 1985)
Hygrotus sanfilippoi (Fery)	11,12,13,16 (Dettner 1985)
Hygrotus inaequalis (F.)	(6)#,7#,11#,12,13,(16),17#,22 (Dettner 1979)
Deronectes aubei (Muls.)	(1)#,(3)#,7#,11#,16#
Deronectes platynotus (Germ.)	11 (Dettner 1985)
Deronectes latus (Steph.)	(3),(5),11 (Dettner 1985)
Deronectes moestus (Fairm.)	11 (Dettner 1985)
Graptodytes pictus (F.)	(3),11,12,13,16 (Dettner 1979, 1985)
Suphrodytes dorsalis (F.)	(3),11,12,(16) (Dettner 1979, 1985)
Hydroporus angustatus Strm.	11,13,16 (Dettner 1979)
Hydroporus tristis (Payk.)	11,12,16 (Dettner 1979)
Hydroporus palustris (L.)	11,12 (Dettner 1979)
Hydroporus obscurus Strm.	11,12,16 (Dettner 1979)
Hydroporus marginatus (Duft.)	11,13,16 (Dettner 1979)
Hydroporus planus (F.)	11,12,13,16 (Dettner 1979)
Hydroporus discretus Fairm. & Bris.	11,13 (Dettner 1979)
Hydroporus ferrugineus Steph.	11,13,16 (Dettner 1979)
Hydroporus obsoletus Aubé	11#,19#
Hydroporus melanarius Strm.	(5),12 (Dettner 1979)
Hydroporus pubescens (Gyll.)	(2),(3),(6),11 (Dettner 1985)
Hydroporus incognitus Shp.	11 (Dettner 1985)
Hydrotarsus lundbladi Falkenström	3#,6#,11#
Oreodytes sanmarkii (C.R.Sahlb.)	(3),(6),11,13 (Dettner 1985)
Nebrioporus depressus (F.)	11,12,(13),16 (Dettner 1979, 1985)
Nebrioporus canaliculatus (Lac.)	(2),(3),11,13,16 (Dettner 1985)
Scarodytes halensis (F.)	(3),(5),11,12,13,16 (Dettner 1979, 1985)
Stictonectes optatus (Seidl.)	(5),(6),11,(16) (Dettner 1985)
Stictotarsus duodecimpustulatus (F.)	11,12,13,16 (Dettner 1979)
Dytiscidae: Colymbetinae	
Liopterus haemorrhoidalis (F.)	(1),2,3,5,6,11,13 (Dettner 1979, 1985)
Liopterus atriceps Shp.	(1),3,(5),11,13 (Dettner 1985)
Ilybiosoma seriatum (Say.)	5,6 (Fescemyer and Mumma 1983)
Agabus binotatus Aubé	1#,3#,5#,6#,7#
Agabus guttatus (Payk.)	2,3,5,6,7,(11),(13) (Dettner 1979, 1985)
Agabus maderensis Wollaston	2#,3#,4#,5#,6#,7#
Agabus biguttatus (Oliv.)	2#,3#,5#,6#,(11) (Dettner 1979, 1985)
Agabus bipustulatus (L.)	1,2,3,5,6,(11) (Dettner 1979, 1985; Schildknecht 1970)
Agabus wollastoni Sharp	2#,3#,5#,6#,7#
Agabus melanarius Aubé	2,3,4,6,7 (Dettner 1979)
Agabus sturmii (Gyll.)	(1),2,3,5,6,(7),(11) (Dettner 1979, 1985)
Agabus nebulosus (Forst.)	2,3,5,6,7# (Dettner 1979)

(continued)

Table 6.1 (continued)

Agabus paludosus (F.)	2,3,5,6,7# (Dettner 1979)
Agabus affinis (Payk.)	2,3,5,6,7,(11) (Dettner 1979, 1985)
Agabus congener (Thunb.)	3,5,6 (Dettner 1979)
Agabus didymus (0l.)	3,5,6 (Dettner 1979)
Agabus labiatus (Brahm)	(1),2,3,5,6,(7),(11),(13),15 (Dettner 1979, 1985)
Agabus undulatus (Schrank)	(1),2,3,5,6,(7),15 (Dettner 1985)
Agabus serricornis (Payk.)	2,3,5,6,15 (Dettner 1985)
Agabus unguicularis (Thoms.)	2,3,5,6,7,(11) (Dettner 1985)
Agabus brunneus (F.)	2,3,5,6,7 (Dettner 1985)
Platambus maculatus (L.)	(1),2,3,5,6,(7),(11),18,19# (Dettner 1979, 1985)
Platambus obtusatus (Say.)	5,6 (Fescemyer and Mumma 1983)
Colymbetes fuscus (L.)	(1),2,3,5,6,7 (Dettner 1979; Schildknecht 1970)
Colymbetes schildknechti Dett.	2,3,5,6,(11) (Dettner 1985)
Ilybius chalconatus (Panz.)	2,3,5,6,7 (Dettner 1985)
Ilybius wasastjernai (C. R. Sahlb.)	5,6 (Dettner 1979)
Ilybius fuliginosus (F.)	2,3,5,6,(7),(11) (Dettner 1979, 1985)
Ilybius fenestratus (F.)	1,2,3,4#,5,6 (Dettner 1985; Schildknecht 1970)
Ilybius hozgargantae (Burm.)	1#,2#,3#,5#,6#,7# (Schaaf 1998)
Ilybius ater (Deg.)	1,2,3,5,6,(7),(11),23 (Dettner 1979, 1985)
Ilybius crassus Thoms.	2#,3#,4#,5#,6#,7, (Dettner 1979)
Ilybius quadriguttatus (Lac.)	3#,6#
Ilybius guttiger (Gyll.)	1,2,3,5,6,7#,(10)#,(23)# (Dettner 1979)
Ilybius aenescens Thoms.	(1),3,5,6,(7),(11) (Dettner 1985)
Meladema coriacea Laporte	2,3,5,6,(11),(13) (Dettner 1985)
Meladema lanio (F.)	1,2#,3#,4,5#,6#,(7)#
Rhantus suturalis (McLeay)	2,3,5,6,7,(11),(13) (Dettner 1979, 1985)
Rhantus exsoletus (Forst.)	1,2,4,5,6 (Dettner 1985; Schildknecht 1970)
Rhantus suturellus (Harr.)	2,3,5,(6),(7) (Dettner 1985)
Rhantus grapii (Gyll.)	3,5,6 (Dettner 1985)
Dytiscidae, Dytiscinae	
Eretes sticticus (L.)	(2),3,5,6,(7),(11) (Dettner 1985)
Hydaticus seminiger (Deg.)	3,5,6,10,(11)# (Dettner 1979)
Hydaticus leander (Rossi)	2,3,5,(6),10#,(11) (Dettner 1985)
Acilius sulcatus (L.)	2,3,5,6 (Dettner 1979; Schildknecht 1970)
Acilius duvergeri Gob.	1,2,3,5 (Dettner 1985)
Acilius mediatus (Say)	3,5,6 (Newhart and Mumma 1979)
Acilius semisulcatus Aubé	3,5,6 (Newhart and Mumma 1979)
Acilius sylvanus Hilsenh.	3,5,6 (Newhart and Mumma 1979)
Graphoderus cinereus (L.)	1,(2),3,5,6,7 (Dettner 1979, 1985; Schildknecht 1970)
Graphoderus liberus (Say)	3,5,6 (Miller and Mumma 1973)
Dytiscus marginalis L.	3,5,6,7,15 (Dettner 1979; Schildknecht and Weis 1962; Schildknecht et al. 1970)
Dytiscus circumflexus F.	1,2,3,5,6,7,(11) (Dettner 1985)
Dytiscus pisanus Laporte	1,3,5,6,(11),(13) (Dettner 1985)
Dytiscus latissimus L.	3,5,6 (Dettner 1985; Schildknecht 1970)

(continued)

Table 6.1 (continued)

Cybister mesomelas Guignot	3#,6#,10#,11#
Cybister lateralimarginalis (Deg.)	3,5,6,7,8 (Dettner 1985; Schildknecht 1970)
Cybister tripunctatus (Oliv.)	3,5,6 (Dettner 1985; Schildknecht 1970)
Dytiscidae, Laccophilinae	
Laccophilus minutus (L.)	(2),(3),(5),(6),14,17,19,20,22, (Dettner 1985; Schildknecht et al. 1983)
Laccophilus hyalinus (Deg.)	14,17,20,22 (Dettner 1985)

1: 4-hydroxybenzoic acid, **2**: hydroquinone, **3**: benzoic acid, **4**: benzoic acid ethylester, **5**: 4-hydroxybenzaldehyde, **6**: 4-hydroxybenzoic acid methylester, **7**: 3,4-dihydroxybenzoic acid methylester, **8**: 3,4-dihydroxybenzoic acid ethylester, **9**: 2,5-dihydroxyphenylacetic acid methylester, **10**: phenylpropionic acid, **11**: phenylacetic acid, **12**: 4-hydroxyphenylacetic acid, **13**: phenylpyruvic acid, **14**: 3,4-dihydroxyphenylacetic acid methylester, **15**: marginalin (= 4'5-dihydroxybenzalisocumaranone), **16**: 3-indoleacetic acid, **17**: 3-hydroxyoctanoic acid, **18**: octanoic acid, **19**: *Z*-3-octenoic acid, **20**: 3-hydroxynonanoic acid, **21**: nonanoic acid, **22**: 3-hydroxydecanoic acid, **23**: tiglic acid

(): minor component, without brackets = major component, # new record as compared with Dettner (1985)

checked for their pygidial gland chemistry (Blum 1981; Francke and Dettner 2005) and within insects Dytiscidae represent a valuable source for biosynthesis of various aromatic compounds (Morgan 2004) including 3-indole acetic acid (**16**, Dettner and Schwinger 1977). It is remarkable that a few aromatic main constituents from the pygidial glands (e.g., **5**, **11**) are also present in the thoracic defensive glands of the water bug genera *Ilyocoris* and *Notonecta* or the metapleural glands of various ant genera (see Blum 1981; Staddon and Thorne 1979).

Apart from benzoic acid (**3**) other chemicals (see Fig. 6.7) have been identified including 4-hydroxybenzoic acid (**1**), hydroquinone (**2**), benzoic acid ethylester (**4**), 4-hydroxybenzaldehyde (**5**), 4-hydroxybenzoic acid methylester (**6**), 3,4-dihydroxybenzoic acid methylester (**7**), 3,4-dihydroxybenzoic acid ethylester (**8**), 2,5-dihydroxyphenylacetic acid methylester (**9**), phenylpropionic acid (**10**), phenylacetic acid (**11**), 4-hydroxyphenylacetic acid (**12**), phenylpyruvic acid (**13**) and 3,4-dihydroxyphenylacetic acid methylester (**14**). Remarkably all derivatives of phenylacetic acid (**11**) such as **12** and **13** are typical for the Hydroporinae subfamily (Fig. 6.7) whose representatives share the presence of **11** as a main compound. This strong pleasant odor that is so typical for Hydroporinae is even mentioned in nomenclature. Spangler (1985) described *Hydrodessus fragrans* due to its strong pleasant fragrance during dissection. This odor is typical for **11** but not for inodorous benzoic acid (**3**). Moreover, gentle molestations of certain living Hydroporinae species, as observed in *Hydrotarsus lundbladi*, may result in liberation of small amounts of strongly smelling phenylacetic acid from their pygidial gland reservoirs.

Within Colymbetinae and Dytiscinae, phenylacetic acid (**11**) only occurs as a trace constituent (Table 6.1). However, there is one exception, as both species of *Copelatus* (*Liopterus*) investigated sequester considerable amounts of **11** in their pygidial glands (Figs. 6.7 and 6.8) and are also characterized by the sweetish odor when dissected. In contrast, the ethylester of protocatechuic acid (**8**) was only found in the genus *Cybister* (Table 6.1, Fig. 6.8).

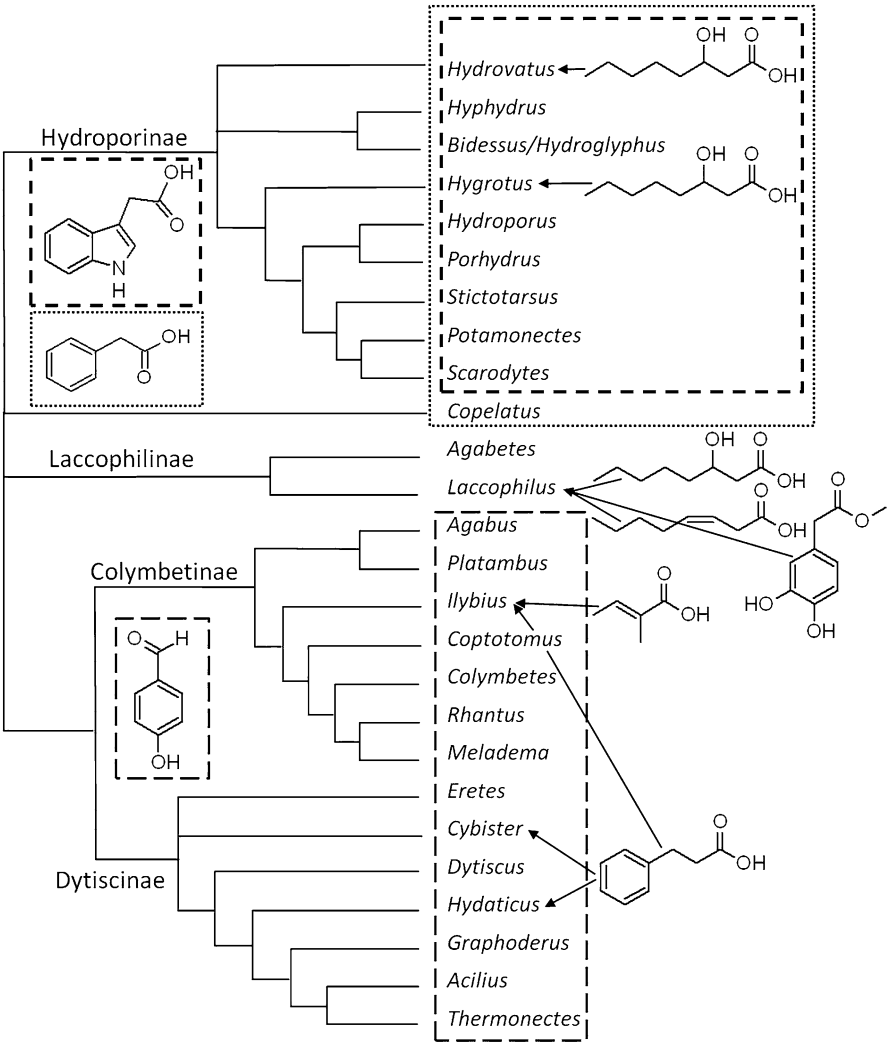


Fig. 6.8 Phylogeny of Dytiscidae genera after Burmeister (1976) and distribution of pygidial gland constituents according to Fig. 6.7. Those compounds which are present in various taxa are figured by *boxes*. Erratically found chemicals are associated with the genera by *arrows*

It was suggested that a further aromatic and extremely yellow colored substance from the pygidial glands of *Dytiscus marginalis* (**15**, marginalin, 4′,5-dihydroxybenzalisocumaranone; Schildknecht et al. 1970) was biosynthetically produced from precursors such as 2,5-dihydroxyphenylacetic acid methylester (**9**) and 4-hydroxybenzaldehyde (**5**). Principally both of these aromatics (**5** and **9**) might be produced from a precursor such as 4-hydroxyphenylpyruvic acid. Later on this compound was identified from the pygidial glands of three Agabinae (*Agabus labiatus*, *A. undulatus*, *A. serricornis*; Dettner 1985) that are closely related (Ribera et al. 2004). Moreover, it was shown that the natural marginalin from *Dytiscus* represents an

E-isomer (Barbier 1987) and may fix solidly on a variety of supports (Barbier 1990). When this compound is distributed on the beetle surface by cleaning behavior, the yellow compound is likely fixed on microorganisms and algae. Marginalin (**15**) is related to aurone, which represents a plant flavonoid that provides yellow coloration to flowers of various ornamental plants. The *Z*-configuration of most aurones represents the more stable configuration.

In addition, phenylpropionic acid (**10**) is typical for the Dytiscinae genera *Hydaticus* and *Cybister* and for one representative of Colymbetinae genus *Ilybius* (Figs. 6.7 and 6.8). In contrast, 3,4-dihydroxyphenylacetic acid methylester (**14**) are restricted to two Laccophilinae species investigated (*Laccophilus minutus, L. hyalinus;* Figs. 6.7 and 6.8). It is astonishing that most Hydroporinae not only produce the sweetish smelling compound **11** but exclusively contain considerable amounts of the tryptophane-derivative 3-indoleacetic acid (**16**), which is also present in Noteridae (Figs. 6.7 and 6.8).

Aliphatic pygidial gland constituents such as 3-hydroxy acids from octanoic (**17**), nonanoic (**20**), and decanoic (**22**) acids are typical for the Laccophilinae and more basally arranged Hydroporinae genera *Hydrovatus* and *Hygrotus* (Table 6.1, Figs. 6.7 and 6.8). These hydroxyacids are also present in pygidial glands from representatives of Haliplidae (Dettner and Böhner 2009) and in metapleural glands of certain Formicidae (see Blum 1981). Further biosynthetically related acids such as octanic (**18**), 3-octenoic (**19**; Figs. 6.7 and 6.8), and nonanoic (**21**) acids occur in the genera *Hydrovatus*, *Platambus*, and *Laccophilus*. The typical compound of many terrestrial Adephaga (see Blum 1981) that is represented by tiglic acid (**23**) is restricted to two representatives of the genus *Ilybius.*

6.4.2.1.2 Biological Activity of Pygidial Gland Secretions and Their Regeneration

The biological significance of the dytiscid pygidial gland secretions is multifunctional. At first, most compounds (apart from marginalin **15** and probably from 3-indoleacetic acid **16**) hitherto identified represent excellent preservatives that are often used in foodstuff industry. This applies especially for both aromatic compounds (**3**, **6**, and **11**) and aliphatic constituents (e.g., **17**, **19**, **20**, **22**) (Dettner 1985; Dettner and Böhner 2009). These compounds are fungicides and bactericides and show an inhibition on germination and growth of plants. Even Z-3-octenoid acid (**19**) chemically resembles the well-known preservative sorbic acid (E,E-2,4-hexadienoic acid). The role of the plant hormone 3-indoleacetic acid (**16**) in hydroporine pygidial glands remains enigmatic. One specimen of *Stictotarsus duodecimpustulatus* sequesters the same amount of compound **16** which can be isolated from 68,000 *Avena* coleoptiles, representing a rich plant source for this compound (Dettner and Schwinger 1977). This plant hormone is found in various gall-forming insects and from the metathoracic glands of few ant species (together with phyenylacetic acid **11**). However, there are no gall-forming hydroporine species known. Therefore 3-indoleacetic acid in predaceous diving beetles may represent a soft preservative especially if used together with compound **11**.

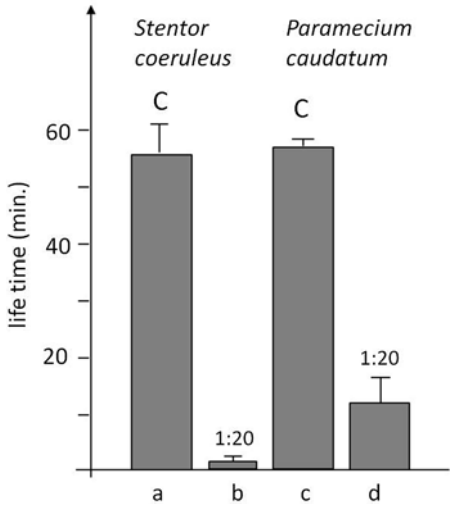


Fig. 6.9 Efficiency of water beetle pygidial gland secretions on protozoans *Stentor coeruleus* (*a–b*) and *Paramecium caudatum* (*c–d*) measured as life time (activity of cilia) at 20 °C. Columns *a*, *c*: water controls; Columns *b*, *d*: secretion of three pygidial gland reservoirs of *Acilius sulcatus* (1:20, v/v)

Finally, derivatives of tryptophan such as 3-indoleacetic acid may represent important excretional products in insects (Cochran 1975).

To distribute their pygidial gland secretions on their body surfaces, dytiscid beetles leave the water. As early as 1967 Maschwitz described this behavior and suggested that these antimicrobial secretions serve to protect the beetles from bacteria and even peritrichic ciliates. This possible protection is illustrated when aqueous dytiscid beetle pygidial gland secretions are tested against the protozoans *Stentor coeruleus* and *Paramecium caudatum* (Fig. 6.9; Cichon et al, in preparation). The behavior of both protozoans was recorded under the microscope as activity of cilia at 20 °C. In both species, diluted aqueous solutions (1:20, v/v) of gland constituents significantly reduced activity of cilia with a stronger effect in *S. coeruleus*, suggesting a negative effect of the beetle secretions on ciliates.

The pygidial gland reservoirs of dytiscids either contain fluids or solid paste-like secretions (Fig. 6.6b). Very often two organic phases, a solid and a fluid, are present within the reservoir (Fig. 6.6c). Depending on their viscosities, the pygidial gland secretions are partly depleted after molestations. Usually only small amounts of the reservoir may be depleted (~13 %,; Classen and Dettner 1983; Dettner 1985) and therefore the pygidial gland secretions of dytiscids likely do not represent defensive secretions against larger predators.

It is remarkable that the above mentioned secretion-grooming is also observed under water while the beetles clasp onto water plants or other structure. Kovac and Maschwitz (1990) described this behavior as secretion-grooming, and suggested that the secretion is used to hydrofuge sensitive body parts such as spiraculi and subelytral tergal respiratory structures. However, when contact angles of definite water droplets on elytral surfaces were carefully measured under a contact angle microscope, all secretions tested from hydradephagan beetles showed a drastic reduction of the contact angle as compared with an untreated elytron of the same beetle specimen when the corresponding second elytron was previously treated with minute amounts of

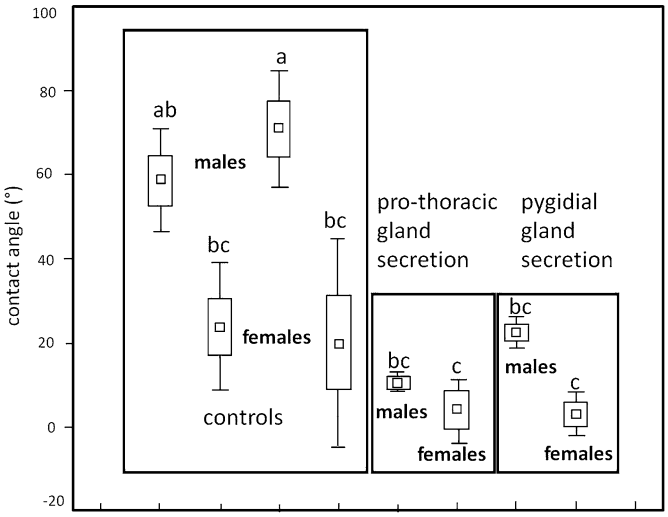


Fig. 6.10 Contact angles of water droplets placed on elytral surfaces of *Acilius sulcatus*. *Left box*: left elytron (first line) and right elytron (third line) of males; left elytron (second line) and right elytron (fourth line) of females, *central box*: effect of prothoracic gland secretion on a contact angles of male and female *Acilius* beetles, *right box*: effect on pygidial gland secretions of male and female (□: mean values; ▯: standard error of mean; and |: standard deviation; Schneider 2008)

pygidial gland secretion (Dettner 1985; Fig. 6.10). The effect of both pygidial and prothoracic gland secretions on contact angles of water droplets are evident (Fig. 6.10) (Schneider 2008). Male *Acilius sulcatus* possess smooth elytra, whereas females are characterized by grooved and hairy elytra. Therefore the contact angles of water droplets on female elytral surfaces are distinctly lower than on male elytra. When treated with prothoracic gland secretions both in males and females results a drastic reduction of contact angles that is more evident in males with their smooth elytra than in females with hairy grooved elytra (Schneider 2008).

Because the contact angle of water on solid surfaces depends both on the surface structure of the elytral epicuticle and from the degree of biofilms on these elytral surfaces, only one freshly collected beetle specimen was used per measurement (Dettner 1985). The wettability after the elytron was treated with gland substance was seen in different species and specimens independently from their pygidial gland chemistries. Even marginalin (**15**), the pigment from the pygidial and preputial glands of *Dytiscus* and few *Agabus* species may significantly lower the contact angle of a water droplet that was placed on a cleaned glass surface (Fig. 6.11). In addition, there was also a significant decrease of the contact angle of 4-hydroxybenzaldehyde (**5**), the main aldehyde of many dytiscid pygidial glands (Fig. 6.11). As many pygidial gland components are amphiphilic (i.e., have a lipophilic and hydrophilous part of the molecule) the increase of wettability of a more or less hydrophilous epicuticle after treatment with benzoic (**3**), phenylacetic (**11**), or aliphatic 3-hydroxy acids (**17**,**20**,**22**) seems plausible.

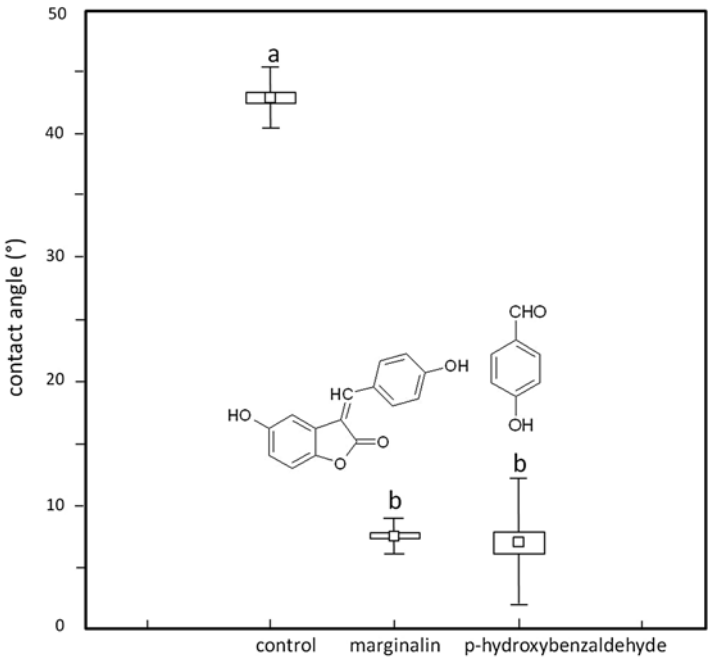


Fig. 6.11 Contact angles of water droplets placed on cleaned glass surfaces (□: mean values; ▭: standard error of mean; and |: standard deviation; Schneider 2008) which were previously treated with aqueous mixtures of marginalin (**15**) and 4-hydroxybenzaldehyde (**5**). Controls represent untreated glass surfaces

In addition to the above mentioned low molecular compounds, pygidial gland secretions of dytiscids also contain marginalin (**15**) and a glycoproteid consisting of 18 amino acids (Schildknecht and Bühner 1968). As described above, marginalin may act as a fixative. In the same way the glycoproteid forms a coherent film (see electron microscopic data in Schildknecht and Bühner 1968), when applied on a glass surface and may fix the low molecular bactericides and fungicides on the beetles surface. In addition, the 3-hydroxy acids **17**, **20**, and **22** may form polyesters that can either fix the metabolites or entangle epizoic microorganisms on the beetles surfaces (Dettner and Böhner 2009).

In general pygidial gland secretion may influence the settlement of external organisms ranging from bacteria to eukaryotic parasites such as Protozoa (Lust 1950; Matthes 1982), fungi (Laboulbeniales, Scheloske 1969), and aquatic mites (Davids et al. 2007). Prothoracic gland secretion was more effective against *Stentor* and *Paramecium* as compared with pygidial gland material (see 4.2.2.2; Fig. 6.9). Scheloske (1969) found that specimens of Hydroporinae (from 416 specimens 13.0 % were parasitized) and Laccophilinae (from 173 specimens 16.8 % were parasitized) showed increased parasitism by Laboulbeniales as compared with Colymbetinae & Dytiscinae (from 815 specimens 10.2 % were parasitized). He suggested that the significantly differing pygidial gland compounds, specifically the

missing compounds **3**, **5**, and **6** in Hydroporinae may be responsible for this effect (Scheloske 1969). However, he also mentioned that the role of prothoracic gland secretions against Laboulbeniales remains unknown.

In contrast to organisms that settle on the surface of adult dytiscids or their larvae, internal parasites such as hairworms (e.g., *Gordius* and allied genera; Blunck 1922a), trematodes (e.g., Peters 1957), or gregarines (Geus 1969; Blunck 1923b) are probably not targeted by these glandular secretions. However, it should be investigated if beetles also take up these exocrine secretions orally. In addition, it would be intriguing if maternally derived prothoracic or pygidial gland secretions have any effect on those species of proctrotrupid and chalcid Hymenoptera that parasitize submersed dytiscid eggs.

Seasonal fluctuations of pygidial gland titers were described in the genera *Acilius* (Newhart and Mumma 1979) and *Agabus* (Classen and Dettner 1983). It is unlikely that these fluctuations reflect different degrees of utilization of the gland material, but mainly reflect different age structures of the adult beetles analyzed during a season. It was shown that the secretions of young male and female beetles as determined by analysis of their internal sexual organs quantitatively and qualitatively differ from secretions of older beetle specimens (Classen and Dettner 1983; Dettner 1985), a fact that is probably due to different biosynthetic capacities of beetles of different ages. For example, freshly hatched male and female *Agabus bipustulatus* and *A. paludosus* produce very low amounts of compounds **2**, **3**, **5**, **6**, **7**, whereas older specimens of both species and sexes produce more aromatics per individual with the aldehyde **5** as a main constituent.

Activities of water beetle pygidial gland secretions on other targets are unknown. However, Lousia et al. (2010) reported that pygidial gland secretions resulted in histopathological changes in male accessory glands of *Odontopus varicornis* (Heteroptera, Pyrrhochoridae). These histological changes were described as disintegration of epithelia, disorganized tissues, swollen nuclei, vacuolized cytoplasm, pycnotic and necrotic epithelia, and enlargement of epithelial cells. The effect of these pygidial glands remain one of the largest understudied and potentially most interesting aspect of dytiscid chemical ecology.

6.4.2.2 Prothoracic Defensive Glands

Prothoracic glands (Figs. 6.12, 6.13, and 6.14) are only present within a small fraction of hydradephagean beetles (Dettner 1985). As reported by Beutel et al. (2006) prothoracic defensive glands are absent in Meruidae, Gyrinidae, and Noteridae, the latter representing the sister group of Dytiscidae. In addition, due to the absence of the prothoracic defensive glands, Aspidytidae (Ribera et al. 2002) are excluded from Dytiscidae and Hygrobiidae, which are both characterized by these peculiar thoracic complex glands (Dettner 1987; Forsyth 1968, 1970). According to the phylogeny of aquatic Adephaga (Beutel et al. 2006), Dytiscidae and Hygrobiidae represent sister groups, Amphizoidae, with no prothoracic defensive glands represent the sister group of Dytiscidae + Hygrobiidae, whereas Aspidytidae form a sister of

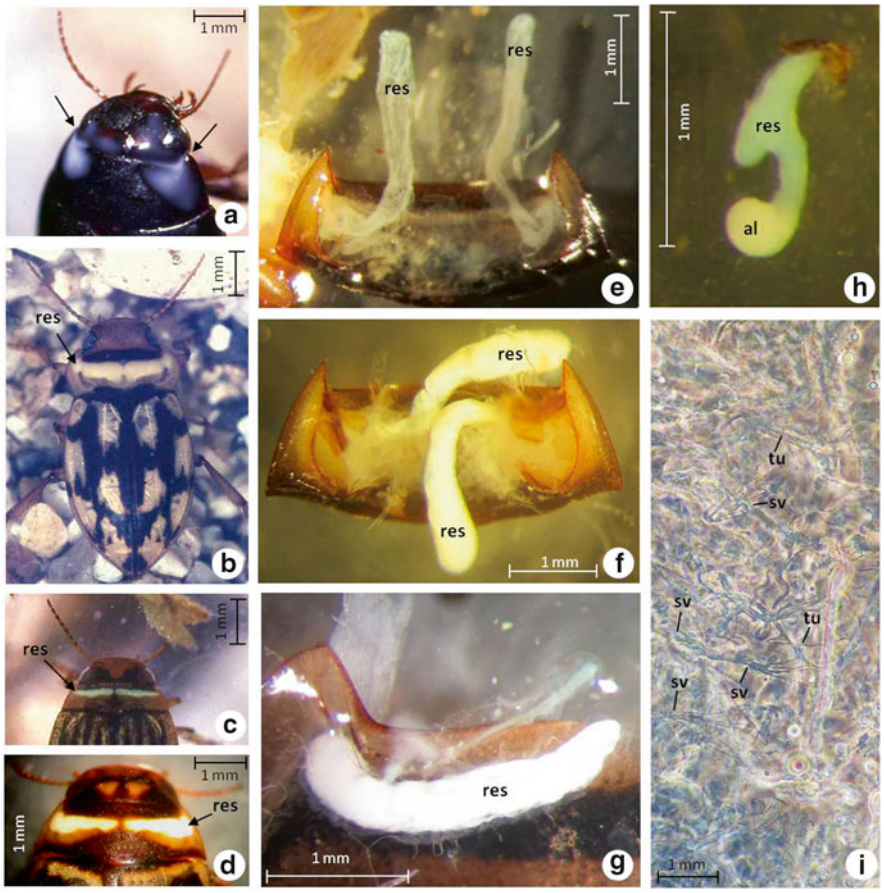


Fig. 6.12 Structure of prothoracic defensive glands of dytiscid beetles. (**a**) Molested specimen of *Ilybius* spec. depleting milky fluid from its paired prothoracic glands. (**b**–**d**) Prothoracic defensive glands of *Stictotarsus duodecimpustulatus* (**b**), *Hygrotus impressopunctaus* (**c**), *Platambus maculatus* (**d**). Prepared prothoracic defensive glands of *Ilybius fenestratus* (**h**), *Platambus maculatus* (**f**), *Acilius canaliculatus* (**g**), and *Hygrotus inaequalis* (**h**). Abbreviations: *res* reservoir of prothoracic defensive gland, *al* apical limb of reservoir, Squeeze preparation of prothoracic defensive gland tissue of *Hydaticus seminiger* with tubules (*tu*) and sieve plates (*sv*) (**i**)

(Dytiscidae + Hygrobiidae) and Amphizoidae. Forsyth (1970) suggested that the homology of the prothoracic defensive glands between Hygrobiidae and Dytiscidae (Colymbetinae, Hydroporinae, Laccophilinae, Dytiscinae) is uncertain. In Hygrobiidae the prothoracic defensive glands open near the posterolateral angle of pronotum, in contrast gland reservoirs in Dytiscidae open close to the anterolateral angle of the prothorax (Forsyth 1970).

Both the depletion and chemistry of prothoracic glands of Hygrobiidae is unknown. Therefore it is important to observe representatives of the above mentioned Colymbetinae, Hydroporinae, Laccophilinae, and Dytiscinae. When disturbed

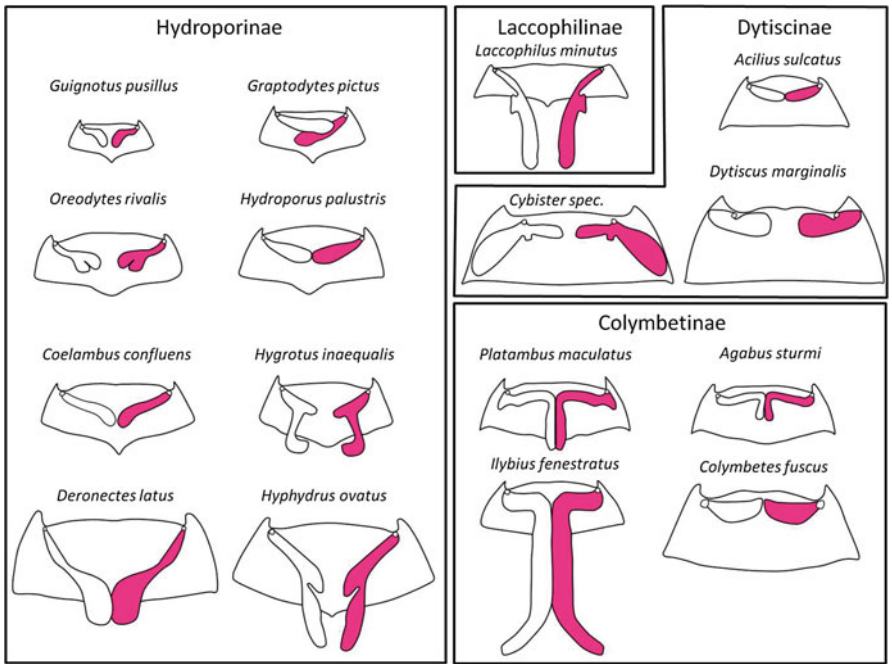


Fig. 6.13 Size and position of prothoracic defensive gland reservoirs in Hydroporinae, Laccophilinae, Dytiscinae and Colymbetinae

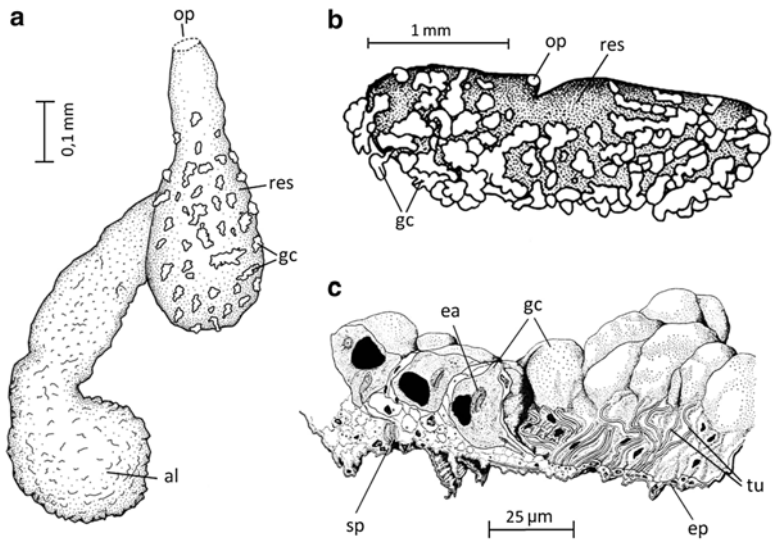


Fig. 6.14 Histology of prothoracic defensive glands. (**a**) *Hygrotus inaequalis*, (**b**) *Dytiscus marginalis* (After Korschelt 1923), (**c**) Section through prothoracic defensive gland of *Hyphydrus ovatus* (Modified after Forsyth 1968). Abbreviations: *al* apical limb, *ea* end apparatus, *ep* epidermis, *gc* gland cell, *op* opening of reservoir, *sp* sieve plate, *tu* tubule. Nuclei are *black*

these dytiscids deplete their milky secretions from their prothoracic defensive glands (see *Ilybius* species Fig. 6.12a). Predaceous diving beetles fixated in ethanol usually show adhering droplets of partly denaturated proteinaceous secretions between the posterior border of head and anterior borders of prothorax. The paired prothoracic defensive glands are sac-like structures (Figs. 6.12, 6.13, and 6.14) and are usually restricted to the anterior border of the prothorax as can be observed in *Stictotarsus* (Fig. 6.12b), *Hygrotus* (*Coelambus*) (Fig. 6.12c), *Platambus* (Fig. 6.12d), or *Acilius* (Fig. 6.12g). Openings of the reservoirs are located dorsalaterally on the cervical membrane of pronotum (Figs. 6.12, 6.13, and 6.14). In several genera such as *Oreodytes* (Fig. 6.13), *Hygrotus* s. str. (Figs. 6.12h and 6.13), *Hyphydrus* (Fig. 6.13), and partly *Laccophilus* (Fig. 6.13) reservoirs are branched. In *Cybister* (Fig. 6.13), *Dytiscus* (Fig. 6.13), and *Hydaticus* (not shown) reservoir openings are shifted more centrally and open near a tooth-like posterior projection of the anterior pronotal border. Prothoracic gland reservoirs are not covered by muscle layers as in pygidial glands (Forsyth 1968), however depletion of reservoirs is achieved by increasing of internal turgor pressure and by contraction of tergo-sternal muscles (Forsyth 1968). Discharge of secretions is finally controlled by a single muscle that has its origin on the cervical membrane.

The gland cells cover the surface of the reservoirs partly or completely depending on species. For example in *Hygrotus inaequalis* the prothoracic gland reservoir is covered by clusters of gland cells, however an apical limb of reservoir has no glandular cells (Figs. 6.12h, 6.13, and 6.14a). As already described by Forsyth (1968), secretory cells show tubuli that are connected with a typical end apparatus (Figs. 6.12i and 6.14c). Of note is that every gland cell opens individually into the prothoracic defensive gland reservoir on circular sieve plates (Figs. 6.12i and 6.14c) covering about 5–8 tubules. Sometimes pointed internal projections of the prothoracic defensive gland reservoir are present (e.g., in *Hyphydrus*, Forsyth 1968).

6.4.2.2.1 Chemistry of the Prothoracic Defensive Glands, Emphasizing Those Species with Steroidal Vertebrate Hormones

During recent years few insect taxa were shown to produce steroids that are essential for insects. These include several chrysomelid (Chrysomelidae, Laurent et al. 2005), carrion (Silphidae, Staphylinidae, Eisner et al. 2005), and lampyrid beetles (Lampyridae, Laurent et al. 2005; Gronquist et al. 2005), as well as giant water bugs (Belostomatidae, Eisner et al. 2005). In some cases, several steroids have been chemically characterized (e.g., toxic steroidal pyrones (lucibufagins) in lampyrid beetles across their developmental stages (Eisner et al. 2005)). As mentioned above, the prothoracic defensive glands of dytiscids produce an impressive array of known vertebrate steroidal hormones together with many novel steroids and these beetles are unique in manufacturing specific steroids including C_{18}, $C_{19,}$ and C_{21} skeletons (Fig. 6.15, Table 6.2). In both predaceous diving beetles and belostomatid bugs some of these molecules are assumed to be synthesized from cholesterol that is acquired from their prey (Eisner et al. 2005).

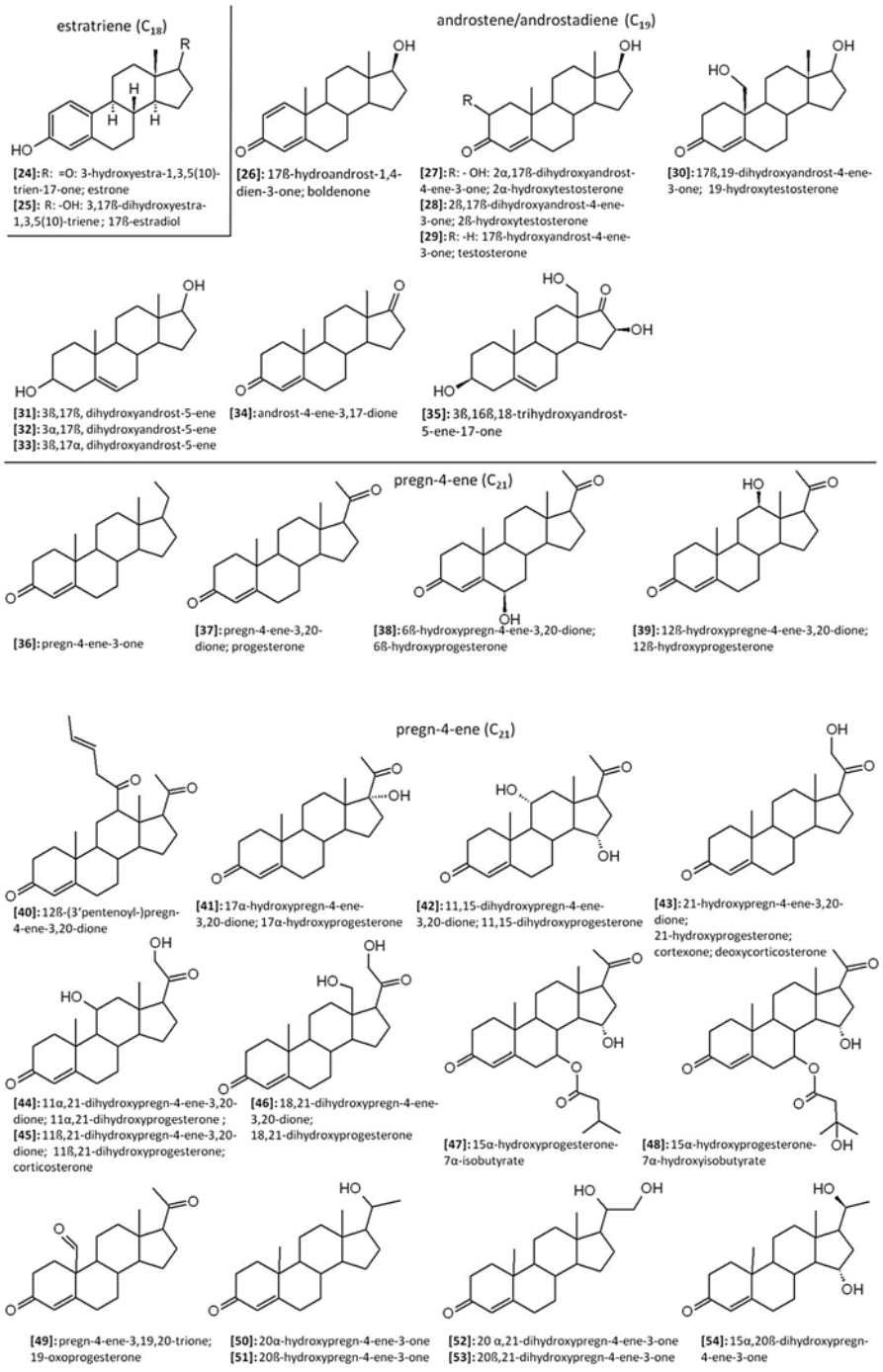


Fig. 6.15 Constituents of Prothoracic defensive glands (**24**–**108**) from dytiscid beetles with continuations

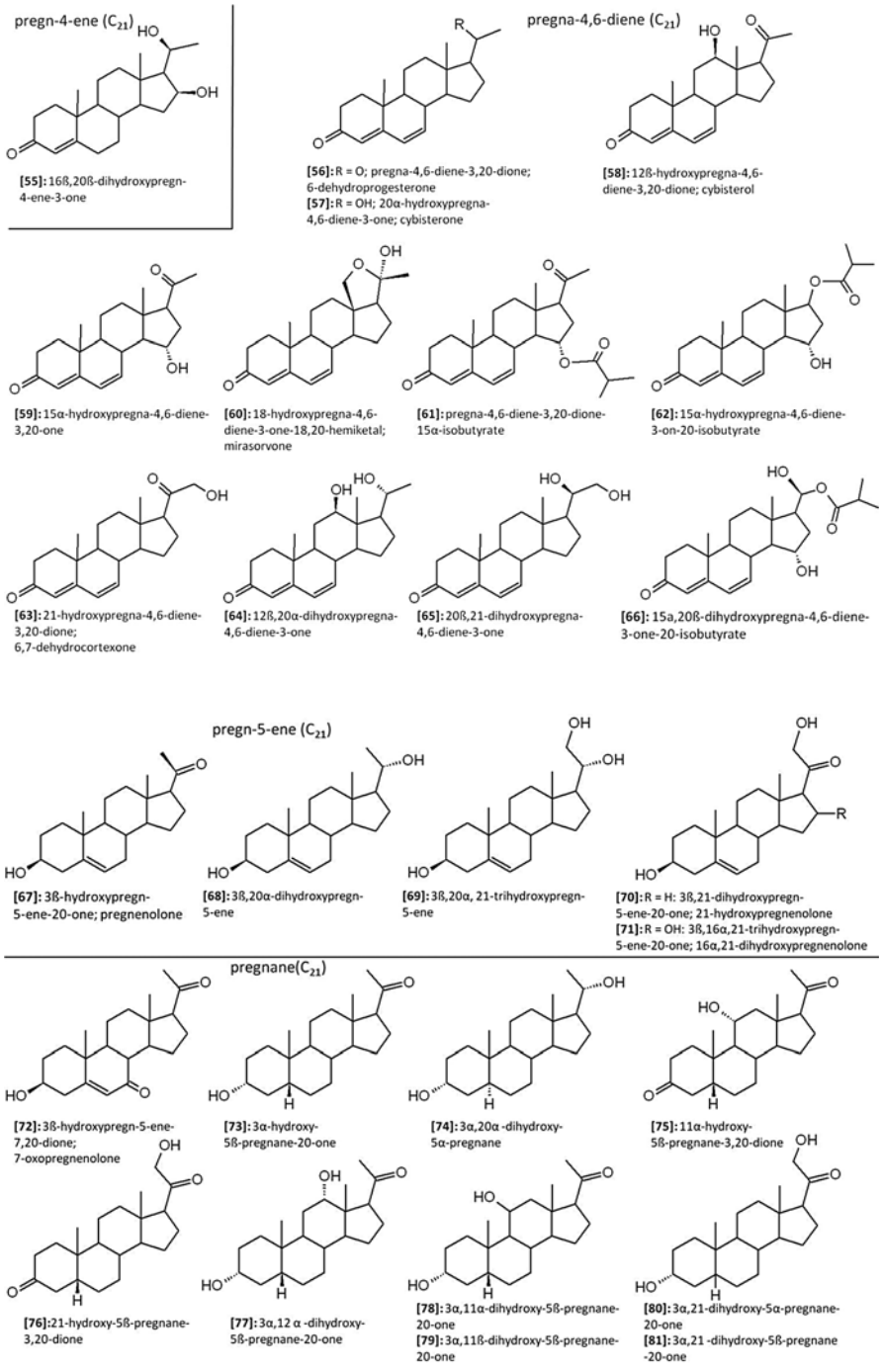
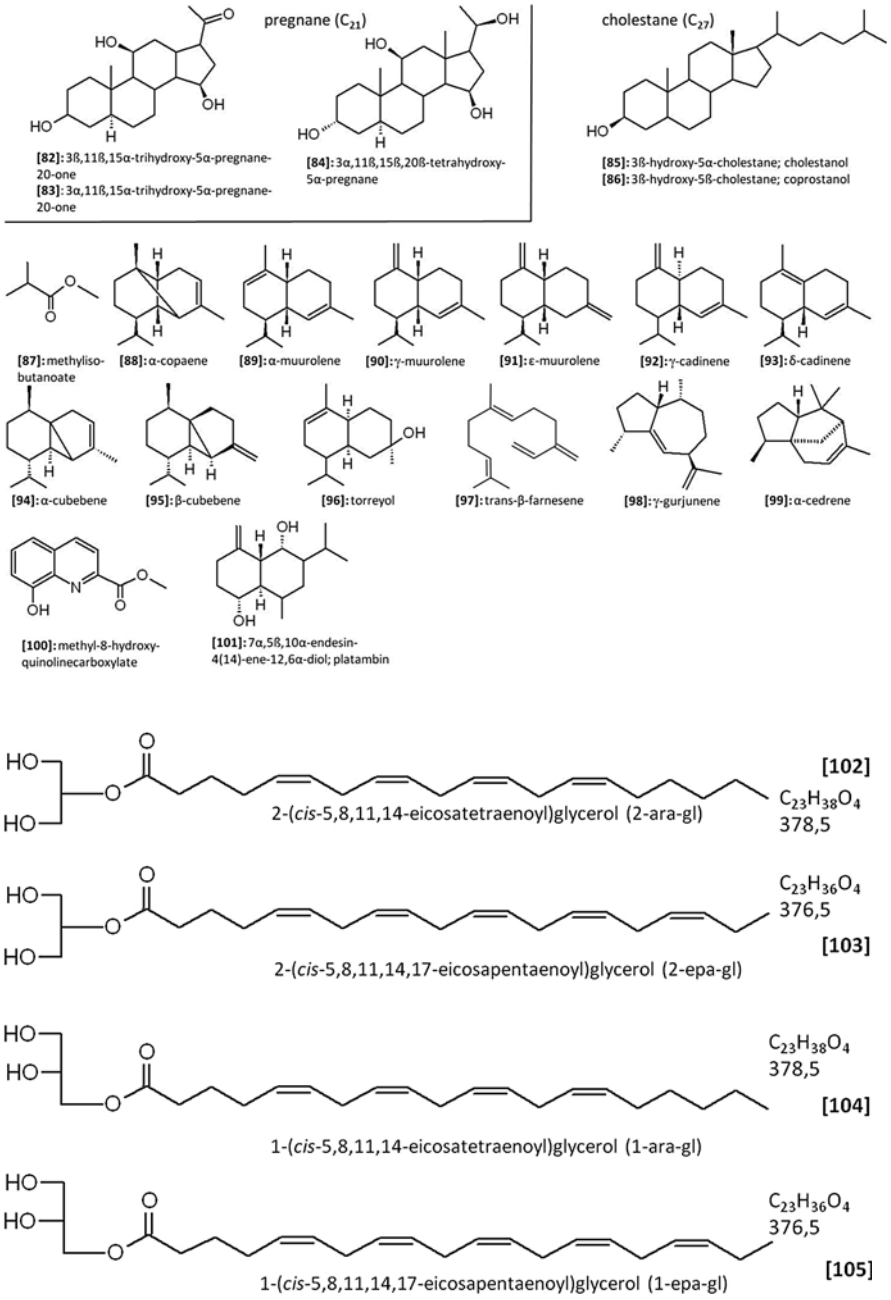


Fig. 6.15 (continued)



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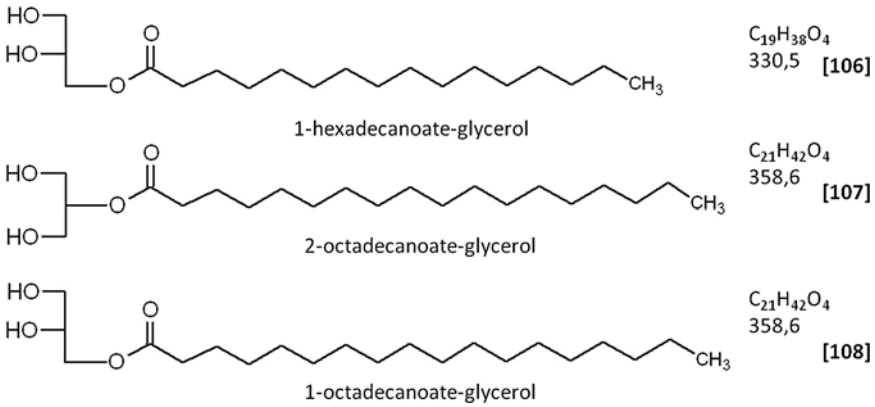

Fig. 6.15 (continued)

Table 6.2 Constituents of prothoracic defensive glands of predaceous diving beetles

Dytiscidae: Colymbetinae	
Ilybiosoma seriatum (Say.)	43 (Fescemyer and Mumma 1983; Miller and Mumma 1973, 1974; Baumgarten 1995)
Agabus guttatus (Payk.)	25,29,38,55,68,73,74,80,82,83,84 (Jungnickel and Dettner 1997)
Agabus bipustulatus (L.)	43,45,50,52,64,80,81 (Schildknecht and Hotz 1970a; Jungnickel 1998; Baumgarten 1995)
Agabus melanarius (Gyll.)	43, 34 (Jungnickel 1998)
Agabus sturmii (Gyll.)	47,48,42,59,61,62, 66 (Baumgarten 1995; Schildknecht and Hotz 1970b)
Agabus nebulosus (Forst.)	41,43,44,46,80 (Jungnickel 1998)
Agabus affinis (Paykull.)	59,102,103,104,105 (Schaaf and Dettner 2000a, b; Baumgarten et al. 1997; Schaaf 1998)
Agabus congener (Thumb.)	59,78,104,105 (Schaaf 1998)
Agabus didymus (Ol.)	70,76,81 (Schaaf 1998)
Agabus unguicularis Thoms.	25,29,43 (Jungnickel 1998)
Agabus brunneus (F.)	70,73,77,85,86 (Schaaf 1998)
Agabus undulatus (Schrank)	25,29 (Jungnickel 1998)
Platambus maculatus (L.)	54,80,101 (Schildknecht et al. 1975, 1969; Schaaf 1998)
Colymbetes fuscus (L.)	Colymbetin, (Schildknecht and Tacheci 1970, 1971)
Ilybius fuliginosus (F.)	29,30,33 (Schildknecht et al. 1967a; Jungnickel 1998)
Ilybius fenestratus (F.)	24,25,29,26,27,28,30,31,32,50,53,54,69,71,87,88,89,90,91,92 ,93,94,95,96,97,98,99,100 (Schildknecht 1977; Schildknecht et al. 1967a, 1971; Schildknecht and Birringer 1969; Jungnickel 1998; Jungnickel 1992)
Ilybius hozgargantae (Burm.)	44,51 (Schaaf 1998)
Ilybius ater (Deg.)	25,29,26,27,28,33 (Jungnickel 1998)
Ilybius crassus (Thoms.)	25,29,26,27,28,30,35,44,53,57,63,65,72,71 (Jungnickel 1998)
Ilybius guttiger (Gyll.)	25,29,26,27 (Jungnickel 1998)

(continued)

Table 6.2 (continued)

Dytiscidae, Dytiscinae	
Acilius mediatus (Say)	Unknown steroid (Newhart and Mumma 1979)
Acilius sulcatus (L.)	43,44,49,50,51,56,57,63 (Schildknecht et al. 1967b; Chapman et al. 1977; Jungnickel 1998; Baumgarten 1995)
Acilius semisulcatus Aubé	43, unknown steroid (Newhart and Mumma 1979; Miller and Mumma 1976a)
Acilius sylvanus (Hilsenhoff)	Unknown steroid (Newhart and Mumma 1979)
Graphoderus cinereus (L.)	67,75,78 (Schaaf et al. 2000)
Graphoderus liberus (Say)	43 (Miller and Mumma 1973; Baumgarten 1995)
Dytiscus marginalis L.	43,50,52,53,57,68 (Schildknecht 1966; Schildknecht et al. 1966; Schildknecht and Hotz 1967; Jungnickel 1998; Baumgarten 1995)
Dytiscus pisanus Cast.	36,37,43,56,57 (Schaaf 1998)
Cybister lateralimarginalis (Deg.)	39,40,43,57,58,63,64 (Schildknecht et al. 1967c; Baumgarten 1995)
Cybister mesomelas Guignot	Benzoic acid, pentadecanoic acid, octadecanoic acid (Dettner unpublished)
Cybister tripunctatus (Cast.)	43,51,58,63 (Chadha et al. 1970; Baumgarten 1995)
Cybister limbatus (F.)	39,43,50,57,58,63 (Chadha et al. 1970; Sipahimalani et al. 1970; Baumgarten 1995)
Cybister confusus Sharp	43 (Chadha et al. 1970; Baumgarten 1995)
Cybister spec. Mexico	58,63 (Schildknecht and Körnig 1968)
Thermonectes marmoratus (Gray)	57,60 (Meinwald et al. 1998)
Dytiscidae, Laccophilinae	
Laccophilus minutus (L.)	73,77 (Schaaf et al. 2000; Baumgarten et al. 1997)
Dytiscidae, Hydroporinae	
Hyphydrus ovatus (L.)	106,107,108, (Baumgarten et al. 1997; Baumgarten 1995)

24: 3-hydroxyestra-1,3,5(10)-trien-17-one; estrone, **25**: 3,17ß-dihydroxyestra-1,3,5(10)-triene; 17ß-estradiol, **26**: 17ß-hydroandrost-1,4-dien-3-one; boldenon, **27**: 2α,17ß-dihydroxy-androst-4-ene-3-one; 2α-hydroxytestosterone, **28**: 2ß,17ß-dihydroxyandrost-4-ene-3-one; 2ß-hydroxy-testosterone, **29**: 17ß-hydroxyandrost-4-ene-3-one; testosterone, **30**: 17ß,19-dihydroxyandrost-4-ene-3-one; 19-hydroxytestosterone, **31**: 3ß,17ß, dihydroxyandrost-5-ene, **32**: 3α,17ß, dihydroxyandrost-5-ene, **33**: 3ß,17α, dihydroxyandrost-5-ene, **34**: androst-4-ene-3,17-dione, **35**: 3ß,16ß,18-trihydroxyandrost-5-ene-17-one, **36**: pregn-4-ene-3-one, **37**: pregn-4-ene-3,20-dione; progesterone, **38**: 6ß-hydroxy-pregn-4-ene-3,20-dione; 6ß-hydroxy-progesterone, **39**: 12ß-hydroxypregne-4-ene-3,20-dione; 12ß-hydroxyprogesterone, **40**: 12ß-(3′pentenoyl-)pregn-4-ene-3,20-dione, **41**: 17α-hydroxypregn-4-ene-3,20-dione; 17α-hydroxy-progesterone, **42**: 11,15-dihydroxypregn-4-ene-3,20-dione; 11,15-di-hydroxy-progesterone, **43**: 21-hydroxypregn-4-ene-3,20-dione; 21-hydroxyprogesterone; cortexone; deoxycorticosterone, **44**: 11α,21-dihydroxypregn-4-ene-3,20-dione; 11α,21-dihydroxy-progesterone, **45**: 11ß,21-dihydroxypregn-4-ene-3,20-dione; 11ß,21-dihydroxyprogesterone; corticosterone, **46**: 18,21-dihydroxypregn-4-ene-3,20-dione; 18,21-dihydroxyprogesterone, **47**: 15α-hydroxy-progesterone-7α-isobutyrate, **48**: 15α-hydroxyprogesterone-7α-hydroxy-isobutyrate, **49**: pregn-4-ene-3,19,20-trione; 19-oxoprogesterone, **50**: 20α-hydroxypregn-4-ene-3-one, **51**: 20ß-hydroxy-pregn-4-ene-3-one, **52**: 20 α,21-dihydroxypregn-4-ene-3-one, **53**: 20ß,21-dihydroxypregn-4-ene-3-one, **54**: 15α,20ß-dihydroxypregn-4-ene-3-one, **55**: 16ß, 20ß-dihydroxypregn-4-ene-3-one, **56**: pregna-4,6-diene-3,20-dione;6-dehydro-progesterone, **57**: 20α-hydroxypregna-4,6-diene-3-one; cybisterone, **58**: 12ß-hydroxypregna-4,6-diene-3,20-dione; cybisterol, **59**: 15α-hydroxypregna-4,6-diene-3,20-one, **60**: 18-hydroxypregna-4,6-diene-3-one-18,20-hemiketal; mirasorvone,

(continued)

Table 6.2 (continued)

61: pregna-4,6-diene-3,20-dione-15α-isobutyrate, **62**: 15α-hydroxypregna-4,6-diene-3-on-20-isobutyrate, **63**: 21-hydroxypregna-4,6-diene-3,20-dione; 6,7-dehydrocortexone, **64**: 12ß,20α-dihydroxypregna-4,6-diene-3-one, **65**: 20ß,21-dihydroxypregna-4,6-diene-3-one, **66**: 15a,20ß-dihydroxypregna-4,6-diene-3-one-20-isobutyrate, **67**: 3ß-hydroxypregn-5-ene-20-one; pregnenolone, **68**: 3ß,20α-dihydroxypregn-5-ene, **69**: 3ß,20α, 21-trihydroxypregn-5-ene, **70**: 3ß,21-dihydroxypregn-5-ene-20-one; 21-hydroxypregnenolone, **71**: 3ß,16α,21-trihydroxypregn-5-ene-20-one; 16α,21-dihydroxypregnenolone, **72**: 3ß-hydroxypregn-5-ene-7,20-dione; 7-oxo-pregnenolone, **73**: 3α-hydroxy-5ß-pregnane-20-one, **74**: 3α,20α -dihydroxy-5α-pregnane, **75**: 11α-hydroxy-5ß-pregnane-3,20-dione, **76**:21-hydroxy-5ß-pregnane-3,20-dione, **77**: 3α,12 α -dihydroxy-5ß-pregnane-20-one, **78**: 3α,11α-dihydroxy-5ß-pregnane-20-one, **79**: 3α,11ß-dihydroxy-5ß-pregnane-20-one, **80**: 3α,21-dihydroxy-5α-pregnane-20-one, **81**: 3α,21 -dihydroxy-5ß-pregnane-20-one, **82**: 3ß,11ß,15α-trihydroxy-5α-pregnane-20-one, **83**: 3α,11ß,15α-trihydroxy-5α-pregnane-20-one, **84**: 3α,11ß,15ß,20ß-tetrahydroxy-5α-pregnane, **85**: 3ß-hydroxy-5α-cholestane, **86**: 3ß-hydroxy-5ß-cholestane, **87**: methylisobutanoate, **88**: α-copaene, **89**: α-muurolene, **90**: γ-muurolene, **91**: ε-muurolene, **92**: γ-cadinene, **93**: δ-cadinene, **94**: α-cubebene, **95**: β-cubebene, **96**: torreyol, **97**: trans-β-farnesene, **98**: γ-gurjunene, **99**: α-cedrene, **100**: methyl-8-hydroxy-quinolinecarboxylate, **101**: 7α,5ß,10α-endesin-4(14)-ene-12,6α-diol; platambin, **102**: 2-(*cis*-5,8,11,14-eicosatetraenoyl)glycerol (2-ara-gl), **103**: 2-(*cis*-5,8,11,14,17-eicosapentaenoyl) glycerol (2-epa-gl), **104**: 1-(*cis*-5,8,11,14-eicosatetraenoyl)glycerol (1-ara-gl), **105**: 1-(*cis*-5,8,11,14,17-eicosapentaenoyl)glycerol (1-epa-gl), **106**: 1-hexadecanoate-glycerol, **107**: 2-octa-decanoate-glycerol, **108**: 1-octa-decanoate-glycerol

What follows is an examination of the chemistry and biological significance of selected prothoracic defensive gland constituents of predaceous diving beetles that especially act as vertebrate hormones. Specifically, I describe estratrienes (**24**–**25**), androstenes/androstadienes (**26**–**35**), pregnanes (**72**–**84**) pregnenes (**36**–**55**, **67**–**71**), pregnadienes (**56**–**66**), and other major groups (Fig. 6.15, Table 6.3). In addition, the utilization of predaceous diving beetles as drugs administered to vertebrates is discussed. The significance of these gland constituents for water beetles is reported in Sect. 6.4.2.2.2. Finally, non-steroidal (**87**–**108**) prothoracic defensive gland constituents are reported.

A considerable fraction of steroids from prothoracic defensive glands in predaceous diving beetles represent well known sexual (estrogens: **24**, **25**, androgens: **26**, **29**, **34**), mineralocorticoid (**43**), or glucocorticoid (**45**) hormones in vertebrates. Table 6.3 summarizes those beetle steroids that occur within vertebrates or act as vertebrates hormones. These vertebrate hormones certainly exhibit no hormonal activities in these beetles. In addition, there exist many steroids in predaceous diving beetles whose hormonal or other activities on both vertebrates and invertebrates are unknown (**27**, **28**, **30**–**33**, **35**, **36**, **38**–**40**, **42**, **44**, **46**–**49**, **52**–**56**, **59**, **60**, **62**–**69**, **72**–**84**, **85**, **86**; see Fig. 6.15). However there exist interesting reports, where predaceous diving beetles are utilized as hormonal drugs for humans and other mammals. Therefore these data are critically discussed with respect to the distribution of prothoracic defensive gland constituents in Dytiscidae (Table 6.2).

In a rather interesting (if not perplexing) use, in East Africa predaceous diving beetles [along with whirligig beetles (Gyrinidae) and larvae of ant lions

Table 6.3 Water beetle steroids which act as vertebrate hormones (for reference numbers see Fig. 6.15)

Type of steroids	Single steroids	Occurrence within vertebrates	Function within vertebrates
C_{18}-steroid Estradienes	Estrone (**24**) 17β–estradiol (**25**)	Produced from cholesterol by mammal ovary and placenta but also by the testes in very low amounts, and possibly by the adrenal cortex (increased biological activity of **25** than **24**). Estrone (**24**) in urine of pregnant woman and mares, follicular liquor, bull-urine, palm kernel oil (Budavari et al. 1989)	Both estrogenes (**24**, **25** mainly bound to proteins), release estrus and are responsible for development and maintenance of secondary female sexual characters (e.g., breast) and further effects. Especially **24** increases proliferation in mammary epithelial cells and altered cell cycle kinetics (Rosen 2008)
C_{19}-steroids Androstenes Androstadienes	Androstenedione (**34**) Testosterone (**29**) Boldenone (**26**)	Androstenes such as **29** and **34** represent most important androgens (mainly bound to proteins) which are produced from cholesterine in interstitial cells of testes. Apart from gonads androstenedione (**34**) is also produced in adrenal glands and represents intermediate of testosterone (**29**). As compared with **34**, testosterone (**29**) is more effective and has a higher daily production in men. The androstadiene boldenone (**26**) naturally occurs in few other species (Brabander et al. 2004)	Both compounds (**29**, **34**) responsible for development and maintenance of secondary male sexual characters. Further effects in vertebrates: male behavior, anabolic activities, growth of bones. Boldenone (**26**) from *Ilybius* species (Table 6.2) represents an anabolic steroid in humans but was originally developed for veterinary use (de Brabander et al. 2004)
C_{21}-steroids Pregn-4-enes	Progesterone (**37**) 17α-hydroxy-progesteron (**41**) 20α-hydroxy-pregn-4-ene-3-one (**50**) 20β-hydroxy-pregn-4-ene-3-one (**51**) Deoxycorticosterone (**43**)	Pregn-4-enes represent active principles of mammal corpus luteum. All human gestagenes such as progesterone (**37**, produced from cholesterol, in corpus luteum during latter half of menstrual cycle), 17α-progesteron (**41**; produced during synthesis of glucocorticoids and sex steroids) and 20α-hydroxy-pregn-4-ene-3-one (**50**; represents an epimer of **51**) are	Gestagenic steroids are responsible for implantation, development, maintenance of embryos within female uteri. In case of pregnancy: continuous secretion of progesterone (**37**), which results in epithelial expansion in mammary gland and there stimulates growth of alveoli. **37** with antiovulatory effect. 17α-Hydroxy-progesteron (**41**) is a natural progestogen and in pregnancy increases in the third trimester primarily due to fetal adrenal production. In ovary,

(continued)

Table 6.3 (continued)

Type of steroids	Single steroids	Occurrence within vertebrates	Function within vertebrates
	Corticosterone (**45**)	present in predaceous diving beetles. 11-Deoxycorticosterone (**43**) is produced by adrenal glands. Corticosterone (**45**) from cortex of adrenal glands is produced in non-human mammals	placenta, adrenals **50** and **51** have properties similar to those of progesterone (**37**) and are in equilibrium with **37**. Deoxycorticosterone (**43**) with mineralocorticoid activity (these steroids such as aldosterone maintain normal blood volume, promote sodium and water retention and increase urinary excretion of potassium and hydrogen ions) and represents precursor to aldosterone. Corticosterone (**45**) represents a glucocorticoid (stimulate gluconeogenesis and increase catabolism of proteins and mobilize free fatty acids) just as cortisol
C_{21}-steroids Pregna-4,6-dienes	6-dehydroprogesterone (**56**) Cybisterone (**57**) Mirasorvone (**60**)	**56** represents a synthetic progestin (=progestogen) which prepares the uterus for implantation and pregnancy. Cybisterone (**57**) and mirasorvone (**60**) represent unique 18-oxygenated pregnan structures described for the first time for insects (Meinwald et al. 1998)	Progestins are used for hormonal contraception, prevent endometrial hypoplasia from unapposed estrogen in hormone replacement therapy. A closely related hormone with mineralocorticoid activity: 18-hydroxydeoxy-corticosterone was isolated from rat adrenals (see corticosterone **45**, Fig. 6.15; Meinwald et al. 1998)
C_{21}-steroids Pregn-5-enes	21-hydroxypregn-enolone (**70**) 16α,21-dihydroxy-pregnenolone (**71**)	21-hydroxypregnelone (**70**) was isolated from sulfate fraction of neonatal urine, together with **71**. Shackleton et al. (1987) suggest that **70** is produced in the fetal liver	

(Myrmeleontidae, Neuroptera)] are preferably collected by young girls who use them to stimulate breast development (see Chap. 1 in this book). The girls place the insects on their breasts are at first mechanically stimulated by them using the arthropods mouthparts and surfaces and they subsequently apply the secretions from prothoracic and pygidial glands. This procedure is claimed to be an efficient method to stimulate breast growth in these adolescent girls (Kutalek and Kassa 2005), however the results are anecdotal at best. As this activity is widespread in Africa among many ethnic groups it is worthwhile to search for the possible scientific base of this ethnobiologically important behavior, and I explore some of this background in more detail here.

The link between this human behavior and predaceous diving beetles is perhaps based on the biologically active chemicals produced in the prothoracic defensive glands of these insects (Table 6.2). As a girl approaches adolescence, the first outward signs of breast development begin to appear by an increase of blood gonadotropin-titres that are secreted by adenohypophysis (Rosen 2008). Later on the cyclical estrogen and progesterone secretion, and accumulation of fat in the connective tissue result in enlargement of breasts. Later as the duct systems of the milk glands (i.e., branched tubulo-alveolar modified apocrine sweat glands) grows, acquire a thickened epithelium and secretory glands at the end of the milk ducts are formed, normal female breast developmental stages can be observed. Growth hormone and glucocorticoids, insulin and progesterone contribute to the growth and differentiation of theses glands. The greatest amount of breast glandular differentiation occurs during puberty, however these processes continue for at least a decade and are enhanced by pregnancy (Rosen 2008).

Based on adult dytiscids, gyrinids, and ant lion larvae, biologically active molecules might be of interest. In Gyrinidae, which have no prothoracic glands (see Sects. 6.4.2.1 and 6.4.2.2) the pygidial glands are responsible for both defense and surface hygiene. However the typical gyrinid norsesquiterpenes gyrinidal, isogyrinidal, and gyrinidone, gyrinidione (see Dettner 1985; Meinwald et al. 1972; Schildknecht et al. 1972a) are not known to influence breast development of mammals. The same applies for the antibacterial and smelling low molecular compounds 3-methyl-1-butanol, 3-methyl-1-butanal, 2-methyl-1-propanol and 6-methyl-5-hepten-2-one from gyrinid pygidial glands (Ivarsson et al. 1996; Schildknecht et al. 1972b). On the other hand, by comparison of prothoracic gland steroids from dytiscid beetles with norsesquiterpenes from gyrinid beetles it is evident that norsesquiterpenes from gyrinids are as effective as certain prothoracic gland steroids from Dytiscidae in their penetrating ability trough gill membranes of fishes (Miller and Mumma 1976a, b).

Within predaceous diving beetles there exist a considerable number of species that contain estrone (**24**), 17β-estradiol (**25**) and testosterone (**29**) that can probably influence and stimulate breast growth in females (Table 6.2, 3). Especially various *Agabus*- and *Ilybius*-species contain these compounds (Table 6.2). In addition, progesterone (**37**), which can also influence breast growth is reported from *Dytiscus pisanus* (Table 6.2, 3). Another aspect concerns the steroid amounts per beetle. Sequestration of larger amounts of pregnane derivatives (e.g., deoxycorticosterone, **43**, in *D. marginalis*: 400 µg/beetle; cybisterol, **58**, in *Cybister* spec.: 1,000 µg/beetle) is

reported, however estrone (**24**, 2 µg/beetle *I. fenestratus*) and 17-β-estradiol (**25**, 19 µg/beetle *I. fenestratus*, Miller and Mumma 1976a; see Sect. 6.4.2.2) are only found in low quantities. Because there exist natural estrogens, synthetic estrogens (e.g., ethinylestradiol, mestranol, turisteron, moxestrol) and nonsteroid estrogens (e.g., diethylstilbestrol, dimestrol) it would be interesting to look for any of these compounds in these arthropod groups. Moreover, nonsteroid estrogens may be used therapeutically to replace natural estrogenic hormones. It should be also considered that there exist phytoestrogens and mycoestrogens that represent plant- or fungus-derived compounds, which are consumed by animals and might cause estrogenic effects. In some countries, phytoestrogenic plants have been even used in treating menstrual, menopausal, and fertility problems (Müller-Schwarze 2006). Thus, it seems possible that certain arthropod semiochemicals that simultaneously act as vertebrate hormones may bind to estrogen receptors in the mammary glands, or by possibly influencing human hormone regulation or hormone synthesis.

Schildknecht et al. (1967a) report in another paper that water beetles and especially representatives of genus *Gyrinus* were used in European alps as aphrodisiacs against cows and horses (see Ochs 1966). Because *Gyrinus* do not produce steroids, Schildknecht et al. (1967a) suggest that peoples from the alps confused *Gyrinus-specimens* with representatives of *Ilybius*.

The following non-steroid prothoracic gland constituents from adult representatives of Dytiscidae are mainly discussed in Sect. 6.4.2.2.2. Apart from methylisobutanoate (Fig. 6.15, Table 6.2, **87**, Schildknecht 1977) and the preservative benzoic acid (**3**, Fig. 6.7) pentadecanoic and octadecanoic acids have also been identified (Table 6.2). Moreover, several monoglycerides with both saturated (**106–108**, Table 6.2) and unsaturated (**102–105**, Table 6.2, Fig. 6.15) side chains have also been recorded.

Various sesquiterpenes (**88–99**) were identified by Schildknecht (1977) in *Ilybius fenestratus* (Table 6.2, Fig. 6.15). Moreover in *Platambus maculatus*, apart from steroid **55**, an additional sesquiterpene named platambin was recorded (**101**, Table 6.2, Fig. 6.15, Schildknecht 1976, 1977; Weber 1979). Up to now the biological significance of these compounds generally and especially for predacious diving beetles remains obscure.

Even the alkaloid methyl-8-hydroxyquinoline carboxylate (**100**) is abundant in prothoracic defensive gland secretions of *Ilybius fenestratus* (Schildknecht 1976). Due to the yellow color of this compound the *Ilybius* secretion shows a distinct yellow coloration. The free acid could be recently reported from the regurgitate of *Spodoptera* and *Heliothis* larvae (Pesek et al. 2009). The alkaloid derives from the tryptophan metabolism and forms complexes with bivalent metal ions. As an iron-chelator (**100**) it may generally inhibit bacterial infections in the gut. Finally methylesters of leucine and isoleucine were identified from the prothoracic defensive glands of *Ilybius fenestratus*, and in *Dytiscus marginalis*, apart from isoleucine, the valine methylester was also identified (Weber 1979).

Within the Dytiscidae (Fig. 6.16) the Hydroporinae possess well developed prothoracic defensive glands, although it remains a mystery that no constituents of the prothoracic defensive glands have been detectable by gas chromatography–mass

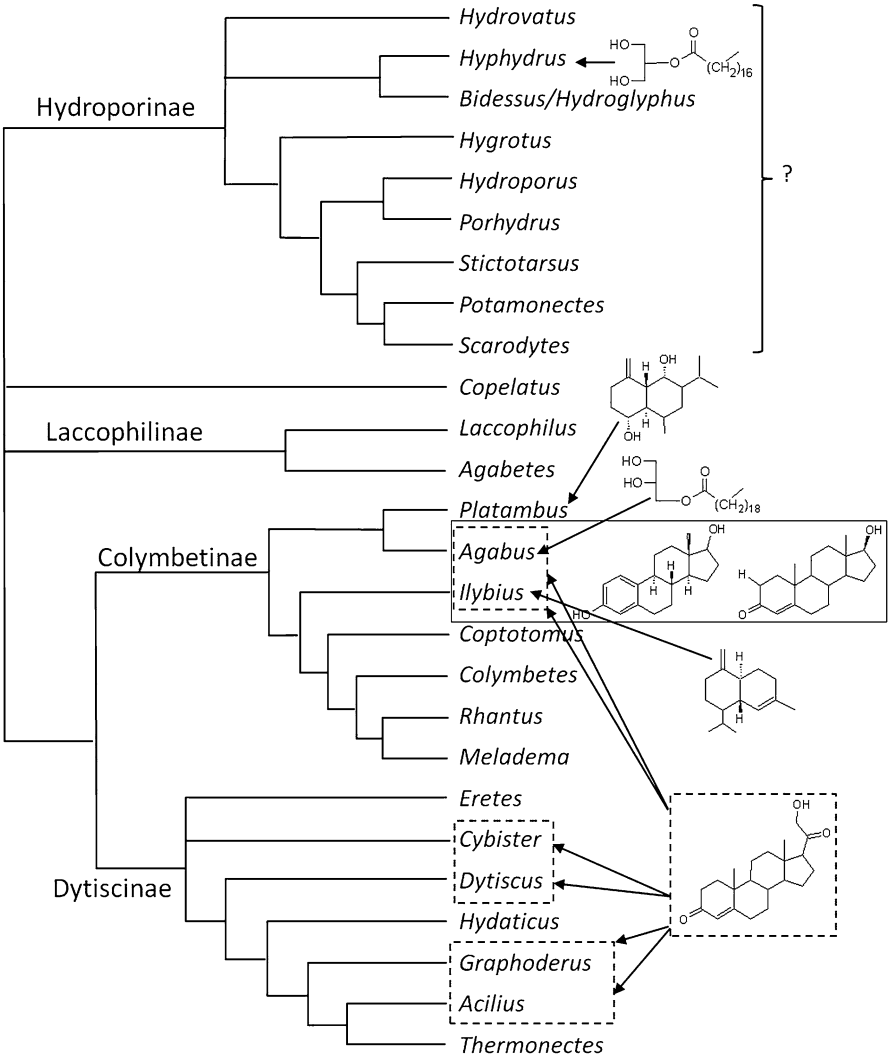

Fig. 6.16 Phylogeny of Dytiscidae genera after Burmeister (1976) and distribution of prothoracic defensive gland constituents according to Fig. 6.15. Those compounds which are present in various taxa are figured by *boxes*. Erratically found chemicals are associated with the genera by *arrows*

spectrometry. Only in *Hyphydrus* (with saturated side-chain; **106**–**108**) and 2 *Agabus*-species monoglycerides (unsaturated side chains) have been recorded (Figs. 6.15 and 6.16, Schaaf and Dettner 2000b). Within *Agabus* and *Ilybius* two estradienes ($C_{18;}$ e.g., 17ß-estradiol **25**) and ten androstenes (C_{19}; e.g., testosterone **29**) have been exclusively recorded. Other representatives from the Dytiscinae and Colymbetinae subfamily may contain up to 21 different pregn-4-enes (C_{21}; e.g.,

cortexone **43**), 11 pregna-4,6-dienes (C_{21}), 5 pregn-5-enes (C_{21}), 12 pregnanes (C_{21}) and 2 cholestanes (C_{27}). At the moment, biosynthesis of steroids in dytiscids is only partly understood. Therefore, the polarity of the chemical characters (i.e., the differentiation between plesiomorphic and apomorphic characters) is yet to be defined (see Dettner 1987). It is suggested that C_{27}-steroids might represent rather primitive characters, followed by C_{21}-pregn-5-enes and C_{21}-pregne-4-en-3-ones. If the biogenetic pathway is more advanced, C_{21}-steroids with hydroxyl, pregnanes, or other groups are more advanced. Finally, we would assume that C_{18}- and C_{19}-steroids are highly derived.

Volatile sesquiterpenoids such as platambin (**101**) or γ-cadinene (**92**) seem to be present both in Dytiscinae and Colymbetinae, however careful systematic investigations are absent. The nucleoproteid colymbetin is restricted to the genus *Colymbetes*, whereas methylisobutanol (**87**) was found in the secretion of *Ilybius fenestratus* (Table 6.2). Remarkably, *Colymbetes* does not produce steroids and instead contains the nucleoproteid colymbetin, which lowers blood pressure.

6.4.2.2.2 Biological Activity and Regeneration of Prothoracic Gland Secretions

In the past, Blunck (1911, 1912a, 1917) performed various experiments to investigate the origin, production, and function of the milky secretion that is sequestered in the prothoracic defensive glands named "Schreckdrüsen". The author characterized coloration (milky yellowish fluid), odor (very often aromatic odor) and taste (bitter) of these secretions. More recent work has concerned identification of the biological activities (e.g., feeding deterrents, toxicities, anesthetic activities, membrane absorptions) of steroids and especially defensive steroids of predaceous diving beetles and giant water bugs against both fish (Gerhart et al. 1991; Miller and Mumma 1976a, b; Schaaf et al. 2000; Selye and Heard 1943) and mammals (Selye 1941b; 1942). In addition, preliminary results have characterized pygidial and prothoracic gland secretions against epitrichic ciliates (Schneider 2008). Moreover information on feeding deterrents of polyunsaturated monoglycerides of *Agabus affinis* (Schaaf and Dettner 2000b) and amino acids of *Ilybius fenestratus* (Weber 1979) against fish have been collected. Finally the alkaloid methyl-8-hydroxy-quinolinecarboxylate (**100**) from *Ilybius fenestratus* (Schildknecht 1977) and the nucleoproteid colymbetin from *Colymbetes fuscus* were reported as active against mammal predation (Schildknecht and Tacheci 1971).

Against bluegill sunfishes (*Lepomis macrochirus*) feeding deterrents of three structurally related steroids from prothoracic glands of predaceous diving beetles were determined by using artificial food pellets (Gerhart et al. 1991). It was shown that feeding activities drastically vary depending on specific stereochemistries of the steroids involved. Deoxycorticosterone (=cortexone, **43**) showed the highest activities (94 % inhibition), followed by 20α-hydroxypregn-4-ene-3-one (**50**; 58 % inhibition), whereas its epimer 20β-hydroxypregn-4-ene-3-one (**51**) did not significantly inhibit feeding. Gerhart et al. (1991) stress that these results are in contradiction with earlier data based on toxicities and anesthetic actions by using fish

that were immersed with steroid solutions. Therefore, the authors suggest specific receptor-ligand interactions. Feeding deterrents with fully saturated pregnanes (**72**–**86**) from *Graphoderus cinereus* and *Laccophilus minutus* against the minnow *Phoxinus phoxinus* also showed that these prothoracic defensive steroids act as strong feeding deterrents against fish (Schaaf et al. 2000).

Other work has been accomplished with the effects of these steroids and mammals. Young et al. (1996) studied the behavioral and pharmacological effects of certain steroids in mice. A neurosedative behavior was found in the progesterone (**37**)-metabolite 3α-hydroxy-5α-pregnane-20-one that is chemically similar to compound **73**. An antiaggressive effect was also observed when the brain titer of the deoxycorticosterone (**43**)-metabolite 3α,21-dihydroxy-5α -pregnane-20-one (**80**) was increased. Compound **73** (3α-Hydroxy-5β-pregnane-20-one, = pregnanolon, eltanolon) was also identified as a quickly acting cardiac active hypnotic (Tassani et al. 1996). The metabolites **73** and **80** obviously interact with the γ-aminobutyric $acid_A$ ($GABA_A$) receptor/chloride canal complex in the central nervous system (Lan and Gee 1994). It is remarkable that the $GABA_A$ receptors are known to contain allosteric modulator sites for therapeutically useful drugs such as benzodiazepines and barbiturates (Lan and Gee 1994).

In detailed investigations, Miller and Mumma (1976a, b) studied toxicities, anesthetic activities. and membrane absorptions of water beetle steroids administered as solutions to immersed minnows (*Pimephales promelas*). Most active steroids in the minnow bioassay were 4-pregnen-3-ones (**36**–**55**) and related derivatives that are also present in prothoracic defensive glands. The activity of steroids was highly related to the degree of oxygenation. Those steroids oxygenated at the termini of the molecule (C_3 and C_{20} in C_{21}-steroids: **36**–**55**; C_3 and C_{17} in C_{19}-steroids: **26**–**35**) were most active; decreased or increased oxygenation of the steroid molecule resulted in a loss of activity. Remarkably, all active steroids were poorly water-soluble and 80 % of steroid absorption occurred via the gills, which are the primary site of steroid-uptake as compared with the skin (20 %).

In comparing bioassays of various structurally different steroids (only a few are also present in dytiscids) against fish (immersed minnows) and mammals (intraperitoneally injected rats), Selye and coworkers showed that those steroids are active in both (Selye 1941a, b, 1942; Selye and Heard 1943) in spite of the fact that both sets of bioassays were completely different. In fishes their activities were even augmented, with lower amounts of tested steroids necessary in fishes (as compared to mammals) to produce deep anesthesia. In mammals, pregnanes with a 3α-OH-5α-H-structure seem to be particularly effective (Purdy et al. 1990), and fast and deep narcosis (intravenous application) in mammals (Gyermek and Soyka 1975) was achieved with 3α-hydroxy-5α-pregnan-20-one and 3α-hydroxy-5β-pregnan-20-one (**73**, *Laccophilus minutus*), with both components being more effective in rats than the barbiturate thiopental (Norberg et al. 1987). Again, stereochemistry plays a central role concerning biological activities of these steroids. The presence of a 3α-OH-group is very important (Phillips 1975; Harrison et al. 1987; Purdy et al. 1990): 3α-OH-5α-H- and 3α-OH-5β-H-Steroids are effective narcotics in mammals, whereas corresponding 3β-OH-steroids are inactive.

It is highly fascinating that four pregnenes (desoxycorticosterone **45**, pregnenolone **67**, progesterone **37**, 3α-hydroxy-pregn-5-ene-20-one) were also recorded from cephalic glands of aquatic belostomatid bugs (Lokensgard et al. 1993). The authors suggest that this remarkable parallel evolution within hemi- and holometabolous fresh water taxa (i.e., belostomatids and dytiscids) may be due to specific predation pressure from fish (Lokensgard et al. 1993).

In a preliminary experiment, epitrichic ciliates in the genus *Opercularia* were isolated from procoxae of *Agabus sturmii* and mixed with droplets of either prothoracic or pygidial gland secretions of the same dytiscid species (Schneider 2008). Under the microscope the movement of the ciliae were registered at the start of the experiment. Cessation of ciliar movement was achieved after 5 min when using pygidial gland secretions, however ciliar activity halted after only 2.5 min when prothoracic gland secretions were used. This may illustrate that prothoracic gland secretions of dytiscids are also active against protozoans, which settle on the surface of many water insects and may be even more efficient as compared with pygidial gland secretion. In contrast, Lust (1950) treated several species of *Orbopercularia* and *Opercularia* with aqueous prothoracic gland secretion of *Ilybius fuliginosus* and observed that most protozoans recovered few minutes after treatment with the solution. Therefore it seems necessary to repeat such experiments by using aequimolar amounts of various prothoracic and pygidial gland constituents.

The sesquiterpene platambin (**101**) from *Platambus maculatus* (Fig. 6.15) was expected to represent a defensive substance against small mammals (Schildknecht 1977), because poikilothermic vertebrates such as amphibians and fishes should be deterred by the co-occurring steroid. Blum (1981) reports that *Cybister fimbriolatus* exudes a prothoracic defensive secretion enriched with potent odorants as sequiterpenes. He suggested either intraspecific activities of these terpenes (e.g., alarm pheromone) or activities of these terpenes as chemical alarm signals for those organisms interacting with these toxic beetles. In the laboratory, juvenile eels (*Anguilla anguilla*) are attracted to the sesquiterpene geosmine (Müller-Schwarze 2006).

The yellow colored alkaloid **100** was suggested to deter especially warm-blooded small vertebrates when the sometimes amphibious species *Ilybius fenestratus* stays on land. In contrast, the complex steroid mixture (Table 6.2) of *I. fenestratus* was expected to act against predatory fish (Schildknecht 1977). The corresponding 8-hydroxyquinoline carboxylic acid represents a strong chelator for Mg^{2+}-ions and moreover has antibiotic activities (Pesek et al. 2009). If *I. fenestratus* was fed with radioactive ^{14}COOH-marked tryptophane significant amounts were incorporated into alkaloid **100** (Schildknecht et al. 1971).

In a feeding bioassay with the two polyunsaturated monoglycerides (1-ara-gl **104**; 1-epa-gl **105**) of *Agabus affinis*, adult minnows (*Phoxinus phoxinus*) were shown to perceive these monoglycerides, and they acted as a deterrent when compared with controls. Moreover, it was shown that this deterrent effect was only achieved by administering higher amounts of both glycerides compared to those occurring in the glands of the *A. affinis* (Schaaf and Dettner 2000b). Because *A. affinis* prothoracic glands contain both four polyunsaturated monoglycerides and

the C_{21} steroid 15α-hydroxy-pregna-4,6-dien-3,20-dione (**59**) it seems probable that the monoglycerides act as emulsifiers for the prothoracic steroid of *A. affinis* that is highly water-insoluble. It is interesting to note that these monoglycerides, such as 2-ara-gl (**102**) have a cannabimimetic potential in mice, which may resemble the anesthetic effects of many steroids in vertebrates.

Amino acids that may be present as free acids or methylesters (Weber 1979) may have various effects on fishes and other predators. Adron and Mackie (1978) found that amino acids such as leucine and isoleucine may represent feeding stimulants for the rainbow trout *Salmo gairdneri*. However other data indicate that leucine and isoleucine, which are present in the secretion of *Ilybius fuliginosus*, may represent both stimulants and deterrents depending on the fish species were tested (Kasumyan and Døving 2003). A compilation from 2006 (Müller-Schwarze) indicates that various freshwater fish species can recognize various prey or plant food odors by using the chemical cues cysteine (earthworm), l-alanine, l-arginine, l-proline (invertebrates, fish, aquatic plants), tyrosine, phenylalanine, lysine (insects, plankton, crustaceans, fish), free amino acids (injured crustaceans), cysteine, asparagine, glutamic acid, threonine, alanine (plants, small animals), cysteine, and arginine (plants).

The whole water-soluble prothoracic gland secretion of *Colymbetes fuscus*, a certain fraction which was assigned as nucleoproteid colymbetin, lowered blood pressure when injected into the veins of urethane-narcotized rats (Schildknecht and Tacheci 1971). For *C. fuscus* six fractions from the prothoracic glands have been found. The two biologically active fractions had molecular masses of about 700. As compared with the alkaloid methyl-8-hydroxy-quinolinecarboxylate (**100**) from the prothoracic defensive glands of *Ilybius fenestratus* that caused clonic spasms in mice (Schildknecht 1977), the biological significance of the various sesquiterpenes from *I. fenestratus* or of platambin from *Platambus maculatus* has yet to be investigated.

Seasonal fluctuations of prothoracic defensive gland titers were described in the species *Agabus seriatus* and *A. obtusatus* (Miller and Mumma 1974; Fescemyer and Mumma 1983). In *A. seriatus* the defensive steroid titer increased from July to September, but low values were obtained during November and December. Further seasonal variations of prothoracic defensive gland constituents were recorded in *Acilius semisulcatus* (Newhardt & Mumma 1979), where the steroid titer increased from July to October, in contrast to the pygidial gland constituents that decreased from July to October. Quantization of deoxycorticosterone (**43**) was performed by means of minnow bioassay in aqueous solutions. The survival time of minnows was correlated to known concentrations of steroids (Miller and Mumma 1974). When the prothoracic gland secretions of *A. seriatus* and *A. obtusatus* were qualitatively and quantitatively analyzed by HPLC both species regenerated about 80 % of their prothoracic gland components within 2 weeks. These defensive gland secretions can be collected simultaneously by electrical shocking with five 20-mA, 90-V DC, 1-s pulses with 5 min within between each pulse (Fescemyer and Mumma 1983).

6.4.3 Other Exocrine Glands

Apart from adults, there are few data concerning exocrine glands from other dytiscid developmental stages. Brancucci and Ruhnau (1985) described parastigmatic glands in dytiscid pupae of the genera *Lancetes*, *Copelatus, Agabus*, *Eretes,* and *Dytiscus.* These glands are externally characterized by minute circular openings with a fine peritrema near each spiracle. These unusual pupal glands are described in detail morphologically (as class 3 type according to Quennedey 1998) and chemically in Carabidae (Giglio et al. 2009, 2011). Moreover, when pupal chambers of *Dytiscus* or *Copelatus* were opened special pupal aromatic odors were identified (Blunck 1923a; Naumann 1955). Blunck (1923a) used litmus paper and was successful in detecting an acid secretion near the spiracles. However, he was in doubt if these pupal secretions might deter shrews, moles, or rats that regularly feed on *Dytiscus* pupae. Casper (1913) suggested that the parastigmatic glands secrete fat-like water repellent agents that cover the pupal cuticle. The 31 low molecular weight volatiles (such as linalool, α-terpinene, β-pinene, 4,8-dimethyl-3,7-nonadien-2-ol) and especially ketones, aldehydes, alcohols, esters, and carboxylic acid from the abdominal glands of carabid pupae were suggested to have a deterrent function against predators and a prophylaxis function against pathogens (Giglio et al. 2009). Unpublished data from our lab (Jakob 2008; Dettner unpublished) showed that pupae of *Dytiscus marginalis* are characterized by a coconut-like odor, and 4-hydroxy-methylbenzoate (**6**) (Fig. 6.7) and δ-decalactone (Fig. 6.22, **109**) could be identified from the seven volatiles collected. Through GC-MS analysis of the peristimatic glands of the same species we also recorded indole (Fig. 6.22, **110**) and 1,3-dimethoxy-2-hydroxybenzene (Fig. 6.22, **111**).

Descriptions of preputial glands in males of *Dytiscus marginalis* exist (Korschelt 1923) and these glands are single layer glandular epithelia with tubules and connective tissue that are situated between the ventral borders of the paramers. By thin-layer-chromatography of the intensely yellow colored preputial gland secretion the compounds marginalin (Fig. 6.7, **15**), p-hydroxybenzaldehyde (Fig. 6.7, **5**), and 4-hydroxybenzoic acid methylester (Fig. 6.7, **6**) (Dettner unpublished) were identified.

6.5 Dermal Glands, Epicuticular Lipids, and Body Coloration by Pigments

6.5.1 Dermal Glands and Epicuticular Lipids

The cuticle of adult predaceous diving beetles is very often covered in oily materials giving the impression that they have been varnished (Fig. 6.17a, b). This appearance is obviously due to the products of dermal glands. According to Korschelt (1923) single-cell dermal glands with tubules and end-apparatuses first originate in the

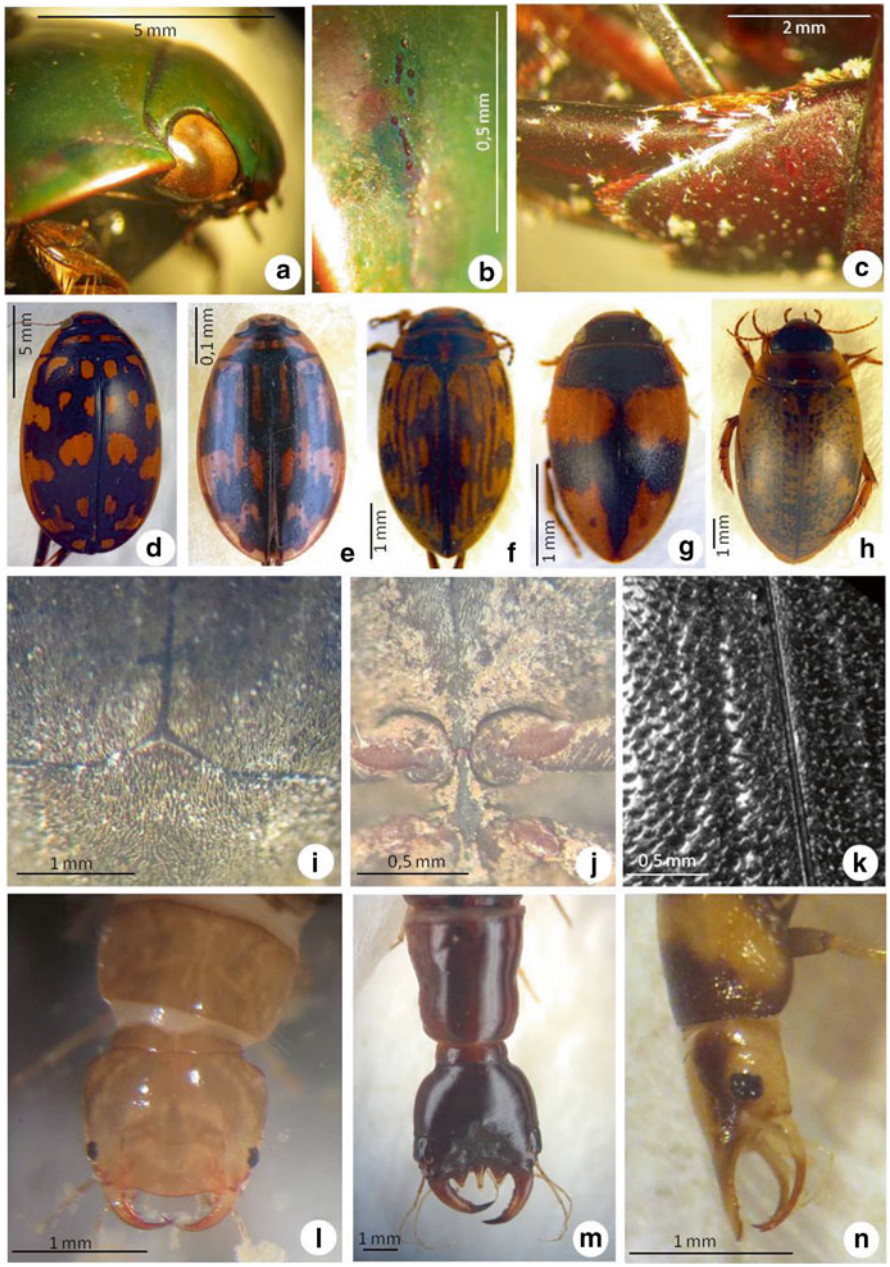

Fig. 6.17 Structural (**a**), secretional (**b**, **c**), and pigmental (**d**–**h**) coloration in Dytiscidae (adults: **a**–**k**, larvae: **l**–**n**). Head and Prothorax of *Cybister vulneratus* (**a**) with structural coloration. Groove on the right pronothal half with fluid epicuticular lipids of *C. vulneratus* (**b**). Tibia and tarsi of *C. vulneratus* with solid crystallized epicuticular lipids (**c**). *Black* and *yellow* coloration patterns in Dytiscidae: *Thermonectus* spec. (**d**), *Sandracottus vestivus* (**e**), *Scarodytes halensis* (**f**), *Graptodytes crux* (**g**) and *Agabus nebulosus* (**h**). Surface structure of *Deronectes moestus* with hairs and dark body coloration (**i**). Underside of *D. moestus* with secretions which obviously serve as adhesives for detritus particles (**j**). Black elytral surface of *Meladema coriacea* (**k**). *Dark* and *yellow* pigments in dytiscid larvae as shown by heads and thoraces of *Copelatus* (*Liopterus*) *haemorrhoidalis* (**l**), *Cybister* spec. (**m**) and *Hyphydrus ovatus* (**n**)

third larval instar, when it has left the water in order to construct a terrestrial pupal chamber; pupae also possess dermal glands. In adult beetles these glandular cells are found on the head and its appendages, the thorax, and the legs. Korschelt (1923) mentions that the density of the dermal glands is significantly larger on the dorsal side of a *Dytiscu*s adult as compared with the ventral side. He mentions about 3,000–4,000 per square mm and observed dermal cells within the abdominal tergal structures. Many authors suggest that the dermal glands represent varnish-glands, which produce oils that lower the wettability of the epicuticle. In addition, dermal glands in the area of mouth parts and near articulations of legs serve as a kind of lubricating oil (Korschelt 1923).

As far back as 1922b Blunck states that the wettability of freshly hatched beetles is lower than in older specimens. In addition, the wettability may be significantly modified by hairs (Fig. 6.17i), microsculpture of body surface (Fig. 6.17k), adhering protozoans (Fig. 6.17j), and algae and fungi. However, oily compounds (Fig. 6.17b), which are produced by dermal glands, likely aid in reducing wettability in these beetles.

Various oily materials are known from the surfaces of many dry dytiscid beetles (e.g., *Cybister, Ilybius, Agabus*), and may be recognized when fine surface structures such as microreticulations or colorations are important during determination of the beetles (Roughley 1990). To fully expose morphological features for identification it is often necessary to eliminate these materials by using diethylether, hexane, xylene, ethylacetate, limonene or 1,1,1-trichloroethane as solvents (Warner 2010; Harrison 2012). In addition, both authors generally remark that greasy beetles especially occur in long-lived species which build up considerable fat reserves that degrade and exude from the pinned specimens as an oily or varnish-like covering. Beament (1976) mentions that oily materials on aquatic insects are used for waterproofing. In addition, he found that representatives of *Agabus* and *Ilybius* are found in warm waters because they have higher transition temperatures of about 32 °C. Beament (1976) suggests that the properties of their oily secretions could limit their distribution and would be correlated with their capacity to osmoregulate. In contrast, transition temperatures in *Dytiscus* are at 24 °C, and representatives of this genus would die at 24 °C and congregate in cold water around an ice cube. Although the chemistry of these solid and oily compounds is unknown in Dytiscidae, there exist data from intersegmental glands in Ponerinae ants (Attygalle et al. 1996). These secretions contain linoleic acid, palmitic acid, methyloleate, and several long-chain hydrocarbons, and have no known behavioral-modifying or antibiotic activities but rather seem to function as lubricants.

6.5.2 Epicuticular Lipids

Within insects, lipids, and especially hydrocarbons are widespread and serve primarily as a barrier to water efflux, but also as a waterproofing epicuticular layer and may additionally or exclusively function as signals for chemical communication

(Dettner and Peters 2010). According to Blomquist (2010) cuticular hydrocarbons in insects vary from 21 to 60 carbons. As compared with hydrocarbons from plant surfaces, insect hydrocarbons possess various double bonds and methyl branches. It may be that both branching and double bonds may increase informational content of these mixtures in intra- and interspecific chemical interactions, while the waterproofing capabilities remain (Blomquist 2010; Dettner and Liepert 1994).

Concerning freshwater insects and their aquatic developmental stages, there are limited data available with respect to epicuticular hydrocarbons. For several taxa only hydrocarbons from the terrestrial adults are known but aquatic larval stages are unknown (Chrysomelidae: *Donacia*: Jacob and Hanssen 1986; Culicidae: *Anopheles*, *Aedes*, Simuliidae: *Simulium*, Psychodidae: *Phlebotomus*, *Sergentomyia*, *Psychodopygus*, Tabanidae: *Tabanus*, Glossinidae: *Glossina*: Bagnères and Wicker-Thomas 2010). So far, the only work that has identified cuticular hydrocarbons from both aquatic larvae and terrestrial adults is from the stonefly *Pteromarcys californica* (Table 6.4) (Armold et al. 1969). Specifically, adults have more surface lipids and a higher melting surface lipid than larvae, whose surface lipid is an oil at room temperature. Both stages have different surface lipid compositions with adults having a larger percentage of hydrocarbons (adult: 12 %; larva: 3 %), wax esters (adult: 4 %; larva: 1 %), free fatty acids (adult: 49 %; larva: 12 %), and sterols (adult: 18 %; larva: 1 %), while the surface lipids of larvae contain more triglycerides (adult: 7 %; larva: 78 %). With respect to hydrocarbons (Table 6.4) n-alkanes dominate in adults, however more alkenes and 3-methylalkanes are present in larvae, whereas internally branches alkanes occur in comparable titres in both stages. Among free fatty acids, octadecenoic- and octadecatrienoic acids occur in both stages, however hexadecanoic acid dominates in adults, whereas hexadecenoic acid is especially found in females. Because adult *Pteromarcys* specimens do not feed, the differences between adults and larvae cannot be attributed to nutritional effects. Also, if adult stoneflies do not drink, an efficient water conservation mechanism also would be important.

A more recent detailed compilation of epicuticular hydrocarbons from the predaceous diving beetle *Agabus anthracinus* was determined by Alarie et al. (1998). The total ion current chromatogram identified 67 different components, 64 of them could be assigned to n-alkanes (86.4 %), alkenes (27.1 %), terminally (6.1 %) and internally branched monomethylalkanes (15.1 %), or dimethylalkanes (2.7 %). Other branching points in monomethylalkanes are positions 3, 4 or 5. The main components in *A. anthracinus* were n-nonadecane (6 %), n-tricosane (12 %), n-pentacosane (6.5 %), 11-and 13-methylpentacosane (3.4 %), n-heptacosane (7.8 %), 3-methylheptacosane (4 %), 9-C_{27}: 1 (3.3 %), 7-C_{27}: 1 (3.7), 9-C_{29}: 1 (3.7 %), and 9-C_{31}: 1 (4 %).

Other data with respect to hydrocarbon patterns of Dytiscidae where recorded for *Dytiscus marginalis* (both sexes), *Agabus bipustulatus*, and *Ilybius angustior* (Jacob and Hanssen 1986). It is remarkable that several Carabidae possess internally branched monomethylalkanes between 20 and 35 %, whereas monomethylalkanes in Dytiscidae beetles possess between 3.3 and 21.2 %. Also, dimethylbranched alkanes range between 0.4 and 8.0 % in terrestrial Adephaga, whereas they are not present in three Dytiscidae species investigated (apart from *A. anthracinus*: 2.7 %).

Table 6.4 Cuticular Hydrocarbons of Stonefly *Pteronarcys* and Dytiscidae (percentual data)

		Alkanes	Alkenes alkadienes	2-Methyl-alkanes	3-Methyl-alkanes	Internally branched monomethyl alkanes	Dimethyl branched alkanes	Unidentified	
Plecoptera/Pteronartcyidae									
Pteronarcys californica	Adult	63,0	Trace	–	24	12	–	–	Armold et al. (1969)
	Larvae	42,0	12	–	31	13	–	–	“
Coleoptera/Dytiscidae									
Dytiscus marginalis	Male	36,0	59,5	1,8	0,9	1,8	–	–	Jacob and Hanssen (1986)
	Male	58,3	25,1	5,4	3,6	4,2	–	3,4	“
	Female	78,5	8,3	1,8	1,6	5,6	–	4,2	“
Agabus bipustulatus		52,7	47,3	–	–	–	–	–	“
Ilybius angustior		43,0	51,6	1,5	–	1,8	–	2,1	“
Agabus anthracinus		46,8	27,8	–	6,1	15,1	2,7	2,0	Alarie et al. (1998)

In two samples from males of the same species (*D. marginalis*) a significant variability of cuticular hydrocarbons was evident. As compared with males (alkenes 36.0–58.3 %), alkenes in female *D. marginalis* reached 78.5 %.

6.5.3 Coloration of the Integument

Coloration of the integument is important for all developmental stages of aquatic insects, including dytiscids. As predaceous diving beetle larvae and adults serve as prey for many aquatic and terrestrial predators (see Chap. 8 in this book) body coloration, including crypsis or aposematic coloration plays an important role in the aquatic and terrestrial stages of these beetles (Dettner and Peters 2010; Galewski 1971). There exist three mechanisms of coloration within dytiscids that warrant consideration: structural colors, secretion colors, and pigmentary colors.

Structural colors (Fig. 6.17a, b) result from light scattering, interference, or diffractation (Berthier 2007), and many investigations identified these colors based on beetle elytra (Sun and Bhushan 2012). Structural coloration are seldom found within adephagous water beetles but when these colors survive treatments that remove the outer waxy layer of epicuticle this type of coloration seems to be present. In addition these colors tend to vary with the direction of the incident light. In certain representatives of *Ilybius*, *Agabus*, *Cybister* (Fig. 6.17a, b), and *Dytiscus*, structural colors (including blue and green as in *Dytiscus*, Blunck 1909) can be observed. Within hydradephagen beetles diffraction grating has been described in Dytiscidae, Noteridae, and Gyrinidae (Seago et al. 2009; Hinton and Gibbs 1971). Seago et al. (2009) describe diffraction grating as a series of parallel nanoscale ridges that disperses light into ordered spectra.

Secretion colors, which are found in polyphagous water beetles such as within the genus *Helophorus*, are mainly absent in dytiscids. When cuticular surfaces are smooth (Fig. 6.17k) or hairy (Fig. 6.17i) a few species possess epidermal glands that produce a glue that allows for the adhesion of detritus particles on the beetles body surfaces (e.g., *Deronectes moestus*, Fig. 6.17j). These detritus particles may be associated with bacterial biofilms and peritrichic ciliates, which are often associated with aquatic beetles and may aid in crypsis.

The last mechanism for colors in dytiscids are pigmentary colors (Fig. 6.17) that arise from the absorption of light in the visible part of the spectrum by chemical chromophores, also called pigments (Kayser 1985). Adults and most larvae (Fig. 6.17l–n) of dytiscids are commonly dark brown, blackish, or olive in color, and therefore brightly colored (e.g., yellow, red) or marked species are the exception within some genera (Adults: *Thermonectus* Fig. 6.17d, *Sandracottus* Fig. 6.17e, *Scarodytes* Fig. 6.17f, *Graptodytes* Fig. 6.17g, *Agabus* Fig. 6.17h; Larvae: *Hyphydrus* Fig. 6.17n). In some cases, pale spots on the elytra are only visible when the elytra are lifted so that light shines through areas of reduced pigments. Vittae (with longitudinal markings) and fasciae may be either pale or dark depending on the background color. In northern latitudes lightly colored or conspicuously striped,

spotted, or mottled specimens are usually associated with streams, the margins of lakes (Young 1960a) or sand-pits (Kehl and Dettner 2003, e.g., *Agabus nebulosus*, Fig. 6.17h; *Nebrioborus canaliculatus*; *Scarodytes halensis*, Fig. 6.17f, *Hygrotus confluens*; *Hydroglyphus geminus*). Specifically, from the Nearctis Young (1960a) mentions the coloration of *Hydroporus lapponum* (edges of tundra lakes) and *Oreodytes* from streams. In contrast, Young (1960a) mentions species from peat pools or vegetated areas that are uniformly black or brown. In addition, brightly colored species of *Hydroporus* or *Nebrioporus* are found in trout ponds and streams (Galewski 1971). In addition, disruptive color patterns of predaceous diving beetles of genera *Thermonectus* (Fig. 6.17d), *Sandracottus* (Fig. 6.17e), *Hydaticus*, and *Prodaticus* in Africa, America, and Australia were reported from exposed habitats with mainly mineral substrates (Larson 1996). Within New Guinea there was found an unusually high portion of dytiscids (e.g., *Laccophilus*) with dark or melanistic forms (Balke et al. 1997). These authors suggested that the shading of the lentic habitats by the luxuriant vegetation may favor the occurrence of dark colored dytiscids.

The aforementioned pigments may be localized in different compartments. Very often all cuticle layers are translucent, and pigments within epidermal cells, within internal organs, compartments, or hemolymph are visible from the outside. In predaceous diving beetles these instances mostly occur in larval and pupal stages. In contrast, adults may possess pigments within different cuticle layers. Larvae of most dytiscid species are rather lightly colored in terms of sclerotized structures and the presence of dark dots are probably due to melanins or represent sclerotin. Some larvae possess dark or black colors (e.g., *Nartus grapei*, several *Hydroporus* species). In other cases, as in larvae of genus *Cybister*, the main total surface of the larvae is membranous and therefore lightly colored.

As in other insects, beetles and especially adephagous predaceous diving beetles may exhibit most chemical classes of biochromes, including carotenoids, chromans, flavonoids, aurones, ternary quinoids, including benzo-, naphtha-, anthra- and polycyclic quinones, tetrapyrroles, including porphyrins and bilins, indolic melanins, ommochromes, papiliochromes, purines, pterines, and isoalloxazines (Needham 1978). These pigments are either synthesized by the beetles themselves or acquired from their food. In many cases the chemical composition on these zoochromes, their distribution among Dytiscidae, and their biosynthesis is unknown.

Carotenoids represent the only tetraterpenoids found in nature that are built up from eight isoprenoid units. Absorbing visible light across 400–500 nm they display yellow to red colors (Figs. 6.17d–h and 6.18b). These pigments are lipophilic and are therefore especially found in insect eggs, and all droplets of fat in hemolymph or fatbodies are thus yellow. Carotenoids are found in most insects from all insect orders (Coleoptera: e.g., Coccinellidae, Chrysomelidae). Generally they cannot be synthesized de novo by dytiscids who may depend on exogene supply from plants, bacteria, and fungi (Kayser 1985).

Most hydradephagean beetles contain lutein, isozeaxanthin, kryptoxanthin, and β-carotene along with 1–2 unknown carotenoids (Table 6.5, Fig. 6.17; Dettner and Hopstätter 1980; Kayser and Dettner 1984). In addition, in *Gyrinus substriatus*

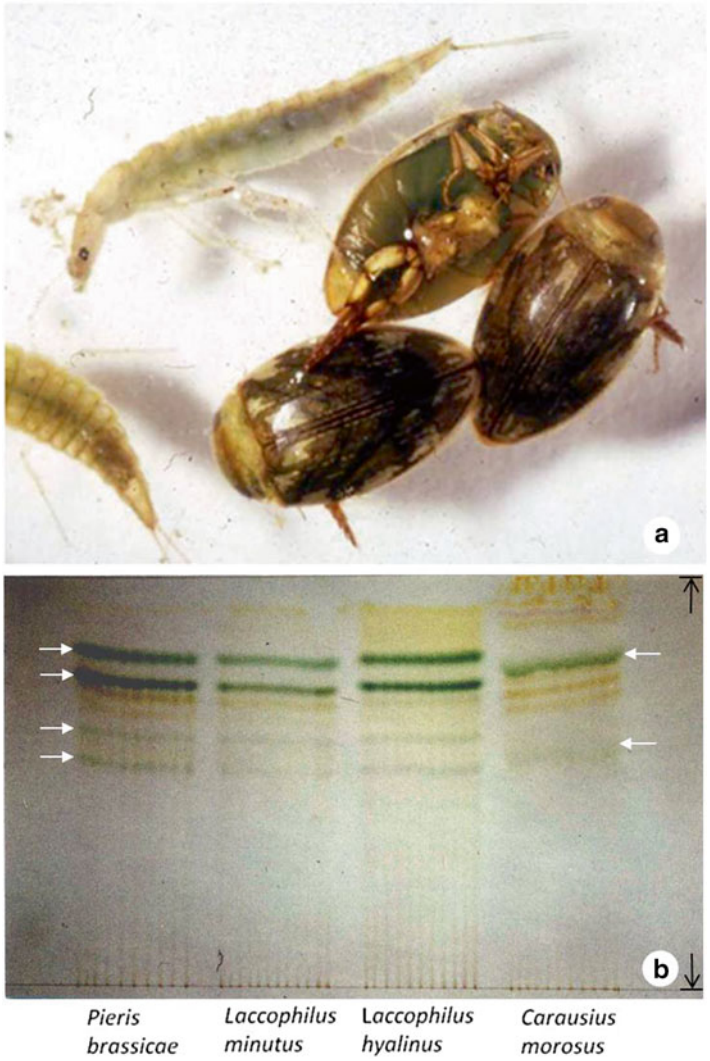

Fig. 6.18 Green coloration of *Laccophilus minutus* beetles and larvae (**a**), and TLC of extracts (**b**) from pierid butterflies *Pieris brassicae*, dytiscid water beetles *Laccophilus minutus*, *L. hyalinus* and stick insects *Carausius morosus*. In *Pieris* and *Laccophilus* there could be shown 4 pterobilin spots respectively (*white arrows*), in *Carausius* biliverdin IX**α** produces only 2 spots (*white arrows*). Animals were grinded with sodium sulfate and esterified with 8 % HCl/methanol. Chloroform extracts were used for thin-layer chromatography on silica using solvent (benzene/dioxane/glacial acetic acid: 12/2/1; v/v/v). Starting point and solvent front are marked

(Gyrinidae) isokryptoxanthin has been found, whereas *Laccophilus minutus* contains astaxanthin. Analysis of carotenoids in *Haliplus ruficolis* (Haliplidae) and *Hydroporus palustris*, as well as in some Dytiscinae (e.g., *Acilius*, *Dytiscus*) have indicated low concentrations of these yellow pigments.

Table 6.5 Carotenoids in Hydradephaga (see Sect. 6.5.3)

	Lutein (3,3′-diOH-β,ε-carotene)	Isozeaxanthin (4,4′-diOH-β,β-carotene)	Kryptoxanthin (3-OH-β,β-carotene)	Isokryptoxanthin (4-OH-β,β-carotene)	β-carotene (β,β-carotene)	Other carotenoids unidentified	Astaxanthin (3,3′-diOH-*β*,β-carotene-4,4′-dione)
Laccophilus hyalinus (de Geer)	+	+	+	−	+	1	?
Laccophilus minutus (L.)	+	+	+	−	+	1	+
Hydroporus palustris (L.)	−	−	−	−	−	−	?
Rhantus suturalis (McLeay)	+	+	+	−	−	1	?
Agabus bipustulatus (L.)	+	+	+	−	−	−	?
Acilius sulcatus (L.)	+	−	−	−	−	−	?
Dytiscus marginalis L.	+	−	−	−	−	−	?
Noterus clavicornis (de Geer)	+	+	+	−	+	−	?
Gyrinus substriatus Stephens	+	+	+	+	+	2	?
Haliplus ruficollis (de Geer)	+	−	−	−	−	−	?

Whereas chromans and flavonoids are absent in Dytiscidae, the heterocyclic aurones that represent a type of flavonoid are present as gland constituents. The yellow colored marginalin (**15**, Fig. 6.7, Table 6.1) was identified in the pygidial and preputial glands of *Dytiscus* and some *Agabus* species (see Sects. 6.4.2 and 6.4.3) (Dettner 1985).

The green color of certain *Laccophilus* species (*L. minutus*, *L. hyalinus*) is due to the mixture of carotenoids with the blue bile pigment biliverdin IXγ (=pterobiline) (Fig. 6.18). This kind of bile pigment, a tetrapyrrole, was reported for the first time for the order Coleoptera and represents the first identification of biliverdin IXγ outside the lepidopteran order (Kayser and Dettner 1984); biliverdin IXα is present in Odonata, Phasmida (Fig. 6.18b), Orthoptera, Mantodea, Planipennia, and few Lepidoptera (Kayser 1985). The four blue spots in pterobiline and the two spots in biliverdin IXα in Fig. 6.18b probably represent autoxidation products of the pure bile-pigments. Apart from the above mentioned two species, *Laccophilus complicatus* and *L. maculosus* show a green coloration (Bertrand 1928), and this color is also found in pupae of *Laccophilus maculosus*, *L. proximus*, *L. minutus*, and *L. hyalinus*. Both European *Laccophilus* species are found within dense water plants, hence their green coloration seems to provide an excellent adaptation to this environment. It may be possible that the dominant red or brown colors found in tropical Laccophilinae may be due to a morphological color change. In this respect the yellow Mediterranean *Laccophilus testaceus* only seems to represent a subspecies of *Laccophilus hyalinus* [Franciscolo 1979, *Laccophilus hyalinus testaceus* (Deg)].

Ommochromes represent the major part of coloration in insect eyes, but they are also found in the integument of many insect orders and something are responsible for the red color of internal organs (Kayser 1985). They are biosynthetically derived from trypthophan through a degradative pathway via kynurenine and 3-hydroxykynurenine, which is metabolized to xanthurenic acid, 3-hydroxyanthranilic acid, and especially into xanthommatin, acridiommantins, ommins, and ommidins (Kayser 1985). Insects, as well as some fungi and bacteria, can synthesize ommochromes, which are usually bound to protein in intracellular granules. There exist only few records for ommochromes in beetles (Linzen 1974), but dytiscids likely produce these kind of phenoxazine-pigments.

Very often a melanin-type of pigment is used to denote a black pigment without knowledge of its chemical structure. Within dytiscids dark or brown body colorations are likely due to melanins, as melanin-deposition sometimes goes along with the tanning process within the exocuticle and also represents a way of hardening the cuticle. Young (1960b) observed an increase of diffuse melanization in or on the light portions of the color-pattern of water beetles, which are likely driven by the environment in humid regions. In contrast, extension of the dark elements of the color pattern may be genetically controlled.

Dark spots are seen in many adult dytiscids (e.g., Fig. 6.17), the dark surfaces of elytra (Fig. 6.17i, k), and the dark colored sclerites (head, pronotum) in larvae (Fig. 6.17l–n). As a whole, melanins are biosynthesized by oxidation of tyrosine and comprise dark, yellow, brown and even red pigments. Their chemical structures are mostly derived from degradation products of the polymers. Melanins are classified

into eumelanins, phaeomelanins, and allomelanins (restricted to plants, fungi, and bacteria), which are based on solubilities, color, elementary composition, and type of degradation products (Kayser 1985). Degradation of eumelanins, which may be deposited in the epidermis or other tissues (about 9 % nitrogen), yields 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid or pyrrolic acids. In contrast, black allomelanins have lower amounts of nitrogen (1 %). Their degradation results in production of catechol, 1,8-dihydroxynaphtalene and protocatechuic acids.

Sclerotines are generated through sclerotization of insect proteins (arthropodins) by ortho-benzoquinones. They are widespread in insects and especially present in mechanically resistant structures such as the tips of mandibles. It seems highly probable that these pigments also occur in dytiscids, such as in the tips of larval mandibles (e.g., *Copelatus* (Fig. 6.17l), *Cybister* (Fig. 6.17m), and *Hyphydrus* (Fig. 6.17n; Young 1960b)).

The white to yellow colored pteridines or pterin pigments are biosynthesized by insects, vertebrates, and bacteria from a purin precursor (guanosine 5′-triphosphate). Lepidoptera and Hemiptera species are rich in pterin pigments (Kayser 1985). In beetles, only xanthopterin, isoxanthopterin, and leucopterin pigments are found (Kayser 1985). The presence of any of these pigments has to be confirmed in dytiscids. Other pigment types, including quinones, papiliochromes, purines, and isoalloxazines are probably absent in dytiscid beetles, however coloration chemistry of light brown or yellow structures (Fig. 6.17c–h) are unknown.

6.6 Microorganisms and Dytiscids

As in other insects, eggs, larvae, pupae, and adults of dytiscids may be associated with microorganisms. These microorganisms can be localized on the internal or external body surfaces. Internal microorganisms, although present everywhere in the host insect, are often found in mycetocytes or even mycetomes (=bacteriomes), and usually these microbial species either occur intra- or extracellularly (Dettner and Peters 2010). It is possible to isolate and to cultivate microorganisms from compartments within dytiscids, including the gut, rectum, or fat bodies. Due to the fact that certain bacteria are culturable, their biosynthetic capacities can be studied in the laboratory. The number and identity of such culturable (Sects. 6.6.1 and 6.6.2) and non-culturable (Sect. 6.6.3) microorganisms from Dytiscidae is described.

6.6.1 Taxonomically Identified Culturable Strains from the Dytiscid Beetle Gut, and Their Steroid Metabolism Under Laboratory Conditions

By using nutrient-rich and nutrient-poor media 30 eutrophic or facultatively oligotrophic bacterial strains were isolated from foregut and other compartments of *Agabus affinis* and *Hydroporus melanarius* (Schaaf and Dettner 1997).

Both tyrphophilous species were selected because they are found in waters that are characterized by low pH-values, high titers in humic acids, and low numbers of bacteria. Usually a higher fraction of bacterial species can be isolated and cultivated from the guts of invertebrates (about 5–10 %), as compared with other body compartments (König and Varma 2006). The aquatic habitats where both beetle species existed also contained a further 41 strains. All strains from both beetle crops and environments (71 isolates, + 5 reference strains) were compared. Overall the authors found autochthonous bacterial flora in the beetle foreguts, and a moderate influence of the aquatic microflora on the bacterial colonization of the beetles (Schaaf and Dettner 1997). How general this pattern is among other species in other habitats is unknown.

Because steroids are essential for insect physiology, it was suggested that the large amounts of dytiscid steroids from prothoracic defensive glands should be biosynthesized from dietary cholesterol through the help of microorganisms. As was evident in the foreguts of the two tyrphophilous dytiscid species (*Agabus affinis*, *Hydroporus melanarius*) that were analyzed microbiologically, several species of microorganisms in large amounts could be isolated and cultivated especially from this body compartment (see Fig. 6.19a). Based on classical methods of identification using shape and coloration of the colonies it was possible to isolate different microorganism strains. As an example, the foregut of *Agabus binotatus* contained at least four colonies [ABD1, ABD2 and ABD4 and ABD5 (Fig. 6.19a)]. In addition, high densities of *Actinomyces* were found (Fig. 6.19b). These bacteria are characterized by their air-myceliae (Fig. 6.19b) and their ability to produce melanin as a byproduct when secondary compounds are manufactured. The interior crop membrane of the beetles exhibited a lot of crypts, where rod-shaped or pleomorphic bacterial populations were attached to the gut wall (Fig. 6.19c, d). When these beetles take up food, crop bacteria subsequently show a drastic increase in number. In addition, after several days/weeks, the colonies become foamy, and aerobic crop-fluid changes from light to dark brown or black, which may indicate a significant increase of microbes and their co-occurring production of colored secondary metabolites.

The foregut microflora of *A. affinis* and *H. melanarius* mainly consists of Pseudomonads, Bacilli, and irregular, gram-positive rods (e.g., *Arthrobacter*, *Corynebacterium*). Of note is that these bacteria groups within the beetle crops are responsible for a multitude of various steroid transformation reactions (Schaaf and Dettner 1998). Generally, microorganisms are well known to modify the steroid skeleton in aqueous solvents through hydroxylations, reduction of carbonyl functions, dehydration, and hydrations, or are important in separating of racemates or asymmetric syntheses.

Two *Bacillus* strains were isolated from foreguts of *Agabus affinis* and were tested for their *in vitro* steroid transforming ability (Fig. 6.20 right; Schaaf and Dettner 1998). When incubated with androst-4-en-3,17-dione (Fig. 6.20, right) 13 transformation products were detected. Androst-4-en-3,17-dione was hydroxylated at C_6, C_7, C_{11} and C_{14} resulting in formation of 6β-,7α-, 11α- and 14α-hydroxyandrost-4-en-3,17-diones. One strain also produced minor amounts of 6β,14α-dihydroxyandrost-4-en-3,17-dione from androst-4-en-3,17-dione. Certain amounts of metabolites with a 6β-hydroxy-group were further oxidized

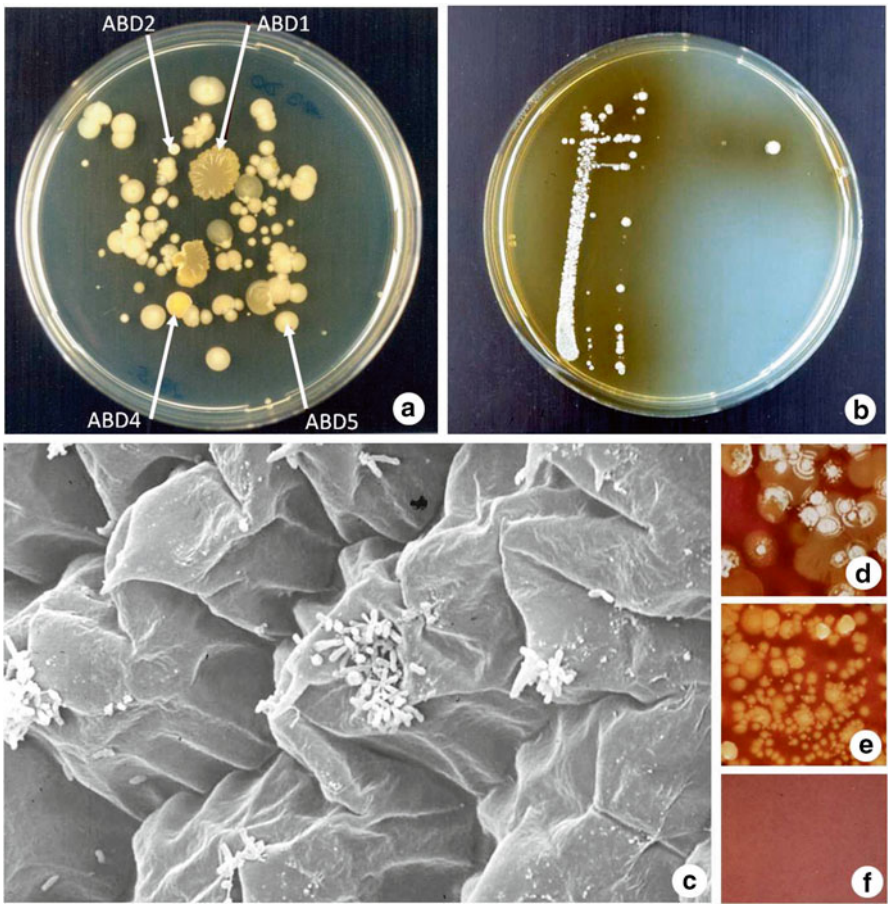

Fig. 6.19 At least four microorganism taxa (ABD1, ABD2, ABD4, ABD5) isolated from gut of *Agabus binotatus* (**a**), colony of *Actinomyces* spec. which was previously isolated from a dytiscid crop producing brown melanin within a Petri dish (**b**). REM of interior crop membrane of dytiscid beetle *Ilybius crassus* with microorganisms between the krypts (**c**). Incubated Petri dishes with isolations from the foregut (**d**), midgut (**e**) and hemolymph/fat body (**f**) of *Agabus melanarius*. There is shown at least one *Actinomyces*-species with its aerial mycelium (**d**). At least two other bacterial species are present in the midgut (**e**), whereas hemolymph/fat body host no cultivable microorganisms at all (**f**)

to corresponding 6-oxosteroids. Moreover, a specific reduction of the Δ^4-double bond resulted in production of 5α-androstane derivatives. In addition, carbonyl functions at C_3 and C_{17} were reduced leading to the formation of 3ξ-OH or 17β-OH- steroids.

If pregnenolone was used as a precursor (Fig. 6.20, left), dominating reactions were hydroxylations, with 7α-hydroxypregnenolone as major product (Fig. 6.20 left; Schaaf and Dettner 2000a). In addition both strains produced lower yields of 7β- and

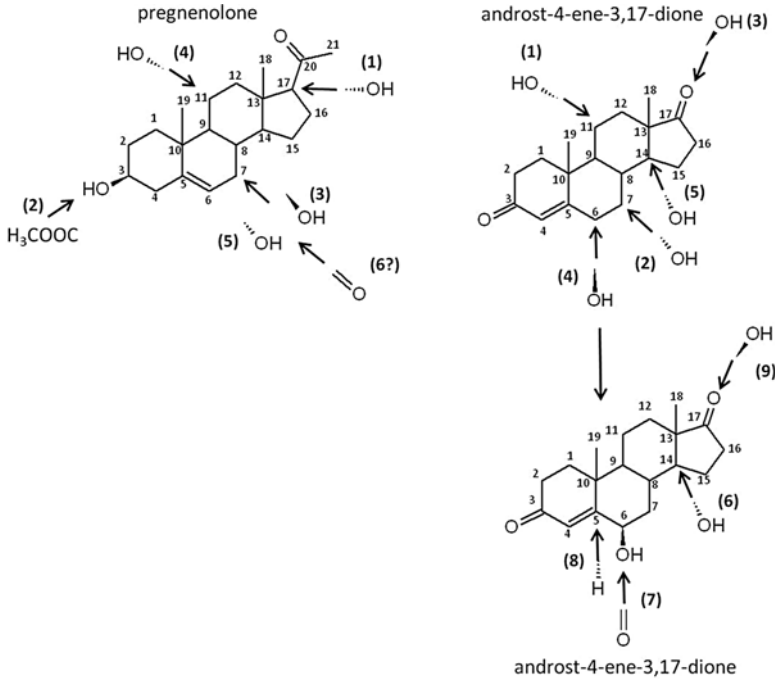

Fig. 6.20 Steroid transformation experiments with *Bacillus*-strains from guts of *Agabus affinis* water beetles. Pregnenolon (*left*) and androst-4-en-3,17-dione (*right*) were used as precursors. *Arrows* indicate those positions within steroid-skeleton where transformations occur. In addition there are indicated functional groups and the number of transformations (*brackets*)

15-hydroxypregnenolone. In contrast, 11-, 17,- and 16α –hydroxypregnenolone were only produced by strain HA-V6-3. The second strain HA-V6-11 had the capability to hydroxylate pregnenolone at C11 and C17 as well (see 7, 11α, 7β, 11α-dihydroxypregnenolone). Both strains oxidized monohydroxylated 7-OH-pregnenolones to 7-oxopregnenolone. One strain (HA-V6-3) also performed 3β-acetylation of pregnenolone in trace amounts. The major difference between the utilization of androst-4-ene-3,17-dione and pregnenolone by these *Agabus* isolates is the shift from C6 to C7, resulting in formation of 7α-hydroxypregnenolone in contrast to 6β-hydroxy-androst-4-ene-3,17-dione.

If one considers the steroidal prothoracic defensive gland compounds it seems highly probable that they are biosynthesized from cholesterol that is taken up by the beetles with their food. The above mentioned data illustrate that microorganisms in the crop may produce cholestenone and cholesteryl-3-acetate from cholesterol (Fig. 6.21). To produce defensive steroids a side chain cleavage (Fig. 6.21 scc) of cholesterol must be postulated. Pregnenolone (**67**), progesterone (**37**) and pregn-4,16-ene-3,20-dione could be present in the hemolymph (Fig. 6.21). The activity of hydroxysteroid-dehydrogenase-isomerases (Fig. 6.21, hsd), dehydrogenases (Fig. 6.21, d, h) and C_{17}-C_{20}-lyase (Fig. 6.21, ly) should be postulated. From **67**, **37**

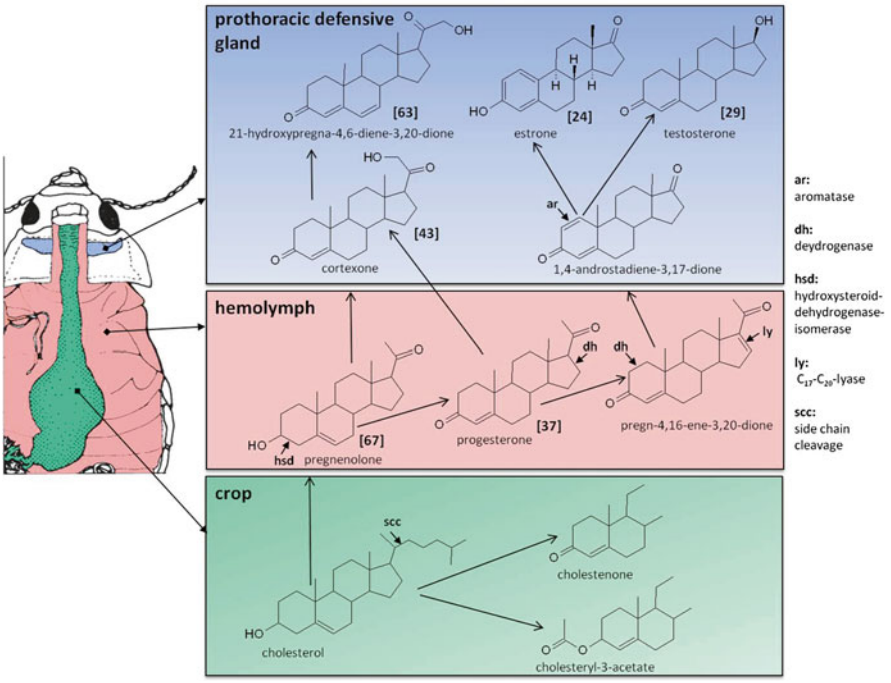

Fig. 6.21 Potential biosynthetic capabilities of microorganisms from the crop (foregut, *green*) of dytiscid water beetles to metabolize cholesterol. There are indicated further metabolites which should be present in the hemolymph (*red*) and in the prothoracic defensive glands respectively the gland reservoirs (*blue*). Numbers refer to Fig. 6.15. Important enzymes according to Swevers et al. (1991) are indicated by *abbreviations*

and pregn-4,16-ene-3,20-dione the gland cells of the prothoracic defensive glands could produce typical steroidal defensive compounds such as cortexone (**43**; biosynthesized either from **67** or **37**). 21-Hydroxypregna-4,6-diene-3,20-dione (**63**; biosynthesized from **43**), estrone (**24**; biosynthesized from 1,4-androstadiene-3,17-dione) and testosterone (**29**; biosynthesized from 1,4-androstiene-3,17-dione). To produce estrone, an aromatase (Fig. 6.21 ar) is necessary. The presence of enzymes involved in the steroid biosynthesis of vertebrate-type steroids was proven in various insect-tissues, however apart from dytiscid beetles such as *Acilius sulcatus*, the steroid concentrations are always very low (Swevers et al. 1991).

At least three investigations concerning biosynthesis of defensive steroids in Dytiscidae have been published. Schildknecht (1970) injected [4-^{14}C]-progesterone, [4-^{14}C]-cholesterol and [2-^{14}C]-mevalonolactone into *Acilius sulcatus*. In contrast to labeled mevalonolactone, cholesterol and progesterone were incorporated after 6 weeks into 6,7-dehydrocortexone (**63**), cortexone (**43**), cybisterone (**57**), 6,7-dihydrocybisterone (**50**, **51**) and 6,7-dehydroprogesterone (**56**). This indicates that these dytiscids absorb cholesterol and other steroids with their food. Biosynthetic experiments with *Agabus seriatus* and injected ^{14}C-cholesterol showed that after 3

weeks 7.5 % of incorporation occurred into deoxycorticosterone (**43**) and other prothoracic gland components (Fescemyer and Mumma 1983). In a detailed study, Chapman et al. (1977) found that pregnadiene derivatives (e.g., 6,7-dehydrocortexone (**64**)) were biosynthesized from cholesterol. The introduction of the Δ^4 and Δ^6 bonds were shown to involve the elimination of 4β and 7β hydrogens, respectively (Chapman et al. 1977). Apart from *Acilius sulcatus* and *Agabus seriatus,* a biosynthesis of vertebrate-type steroids could be only demonstrated in *Manduca sexta* (Swevers et al. 1991).

The biotechnological use of microbial steroid transformations has received increasing economical and scientific interest in the recent years. Thus, the isolation and investigation of microorganisms from 'exotic' sources associated with steroid-carrying dytiscids deserves further attention.

6.6.2 Taxonomically Identified Culturable Strains from the Dytiscid Beetle Gut and Their Secondary Metabolites Produced Under Laboratory Conditions

Nearly all insects associate with microorganisms and fungi, and sometimes these interactions are actually symbiotic. To isolate new kind of microorganisms and new natural compounds with biological activity from exotic sources various hydradephagean beetles were externally sterilized and subsequently selected compartments were analyzed for microorganisms (Gebhardt et al. 2002). Among various dytiscid hosts *Laccophilus minutus* was of interest because one bacterial strain, identified as *Bacillus pumilus*, showed remarkable activities in various bioassays. From the *L. minutus* foregut 14 bacterial strains were isolated. The *B. pumilus*-strain exhibited a pronounced herbicidal activity against both duckweed (*Lemna minor*) and a green algae (*Chlorella fusca*) (Gebhardt et al. 2002). After cultivation in a 10 L fermenter, six secondary metabolites were detected from the *B. pumilus* extract (Fig. 6.22): N-acetylphenylalanine (**112**), N-acetyltryptophane (**113**), l-isoleucine (**114**), malonic acid phenylester (**116**), 3,4-dihydroxybenzoid acid (**115**), and cyclo(propyltyrosyl) (**117**). These metabolites show some interesting biological activities. For instance, N-acetylphenylalanine (**112**) is an antidepressant and appears in large amounts in urine of individuals with phenylketonuria. Another acetylated amino acid is represented by N-acetyltryptophane (**113**), which can be used as a stabilizer of some protein solutions. l-isoleucine (**114**) represents an essential proteinogenic amino acid with various biological functions, whereas 3,4-dihydroxybenzoic acid (**115**) is antioxidant and anti-inflammatory and has tumoricidal effects. This latter compound is widely distributed in nature and occurs in various plants (Gebhardt et al. 2002; green tea), in fungi (*Agaricus, Penicillium*, *Phellinus*, Laskin and Lechevalier 1973), in bacteria (*Flavobacterium*, Kieslich 1976), as a tanning agent in the oothecas of blattid insects (Dettner and Peters 2010), and as a constituent of antimicrobial pygidial glands of dytiscids (Dettner 1985). In pygidial glands, 3,4-dihydroxybenzoic acid is found as methyl- (**7**), or ethyl- (**8**) ester.

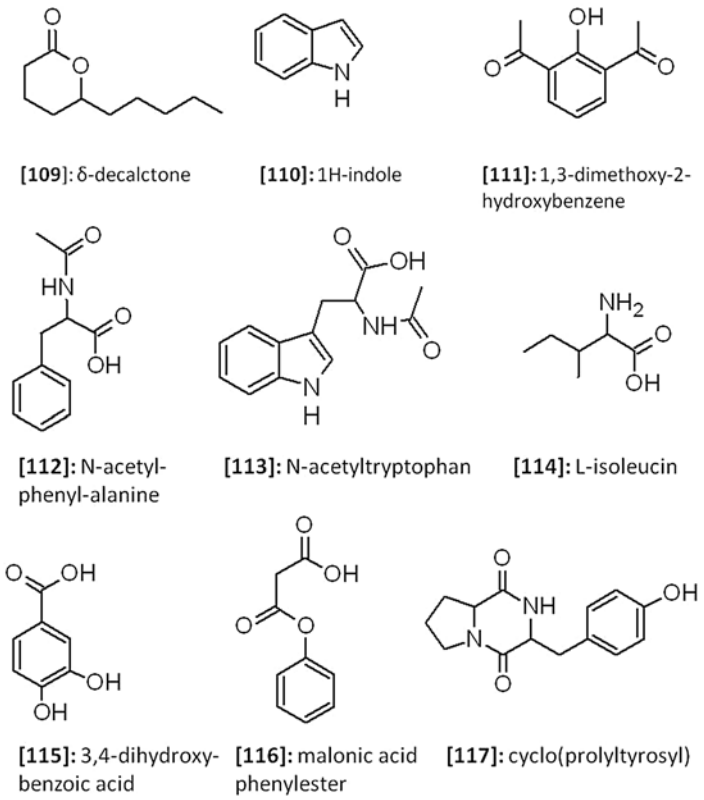

Fig. 6.22 Constituents of pupal peristigmatic glands of *Dytiscus marginalis* (constituents **109**–**111**) and secondary compounds (**112**–**117**) isolated in the laboratory from *Bacillus pumilus* which was isolated from guts of *Laccophilus minutus*

Limited data are available on malonic acid phenylester (**116**). The diketopiperazine cyclo(propyltyrosyl) (**117**) is also known as maculosin I, and was previously isolated from various other microorganisms, including the fungus *Alternaria alternata* and marine sponges (see Dettner 2011). This compound is an extremely host-specific phytotoxin from the *Alternaria-weed* pathogen, and causes black leaf blight in *Centaurea maculosa* (Strobel et al. 1990). Maculosin represents a prototype of a safe and environmentally friendly antiknapweed herbicide (Bobylev et al. 1996), which binds to cytosolic maculosin-binding proteins (Park and Strobel 1994). In addition, maculosin II (dehydrated maculosin I) and various synthetic analogues inhibit the growth of wheat coleoptiles (Bobylev et al. 2000). More recently, maculosin was found to insert into liquid crystalline phase bilayers of 1,2-palmitoyl-sn-glycero-3-phosphatidyl choline or 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl choline. Its orientation within the membranes is modulated by cholesterol (Lopes et al. 2004). Because several dytiscids produce monoglycerides (Fig. 6.15) in their prothoracic glands, maculosin could also interact with these beetle compounds.

These six above mentioned compounds are produced under laboratory conditions, and thus if they are also biosynthesized under natural conditions in the foregut of *Laccophilus minutus* it would be highly interesting to know their biological significance. When the above mentioned *Bacillus pumilus*-strain from the collection of microorganisms of BASF was investigated 5 years after isolation of the microbial material from *Laccophilus* guts the six metabolites were not produced (M. Langer, unpublished data). It seems possible then that this strain was somehow stressed when it produced the six metabolites. In contrast, a different strain (LU 2644) produced small amount of phenylacetic acid, a main pygidial gland constituent (**11**) of Hydroporinae and *Copelatus* (*Liopterus*) species (Fig. 6.7, Table 6.1). In addition, incubation of a *B. pumillus* extract with phenylalanine significantly stimulated the production of phenylacetic acid, which represented the main compound of the bacterial extract. At present it is unknown if microbial metabolites, which were isolated in the lab, are also present within the intact host insect – symbiotic/parasitic bacteria systems.

6.6.3 Non Culturable Microorganisms from Predaceous Diving Beetles

According to König and Varma (2006) only low amounts of gut microorganisms can be cultivated and therefore it is of interest if non-culturable microorganisms can be also quantified. In 2009 it was reported by Küchler et al. that specimens of *Rickettsia* were detected in four species of the genus *Deronectes* (Hydroporinae). The genus *Rickettsia* is represented by gram-negative bacteria that are present in cocci, rods, or thread-like forms. All these bacteria are obligate intracellular parasites and unlike *Chlamydia* or *Mycoplasma* they possess true cell walls.

In *Deronectes platynotus*, 100 % of all specimens investigated showed association with *Rickettsia*. In other *Deronectes* species lower numbers of investigated had some associations with *Rickettsia* (e.g., *D. aubei*, *D. delarouzei*: 40 %; *D. semirufus*: 33,3 %). All individuals of *D. latus*, *D. aubei sanfilippoi*, and *D. moestus inconspectus* were *Rickettsia* negative. Within Hydroporinae *Rickettsia* could also be identified from specimens of *Hydroporus gyllenhalii*, *H. tristis*, *H. umbrosus*, and *H. obscurus*. *Rickettsia*-positive species from Colymbetinae are *Agabus melanarius*, *A. guttatus* and *Ilybius wasastjernae*. The frequencies of *Rickettsia* infection were maintained across different seasons. *Rickettsia* was also recorded from other coleopteran families including Bruchidae (Fukatsu et al. 2000), Buprestidae (Lawson et al. 2001), Coccinellidae (von der Schulenburg et al. 2001), Curculionidae (Zchori-Fein et al. 2006), and Mordellidae (Duron et al. 2008).

Analysis of 16S rRNA gene sequences revealed a phylogenetic relationship of *Deronectes* rickettsiae with *Rickettsia limoniae*, which also was isolated from the crane fly *Limonia chorea* (Diptera, Limoniidae) and tentatively classified as members of the basal ancestral group. A similarity of *Deronectes* rickettsiae was found to *Rickettsia* of *Cerobasis guestifalica* (Psocoptera, Trogiidae) and *Lutzomyia*

apache (Diptera, Psychodinae), whereas *Rickettsia* from *D. semirufus* cluster basally with rickettsiae from leeches. Phylogenetic analysis of *gltA* (citrate synthase) gene sequences showed that *Deronectes* symbionts (from *D. platynotus*, *D. aubei*, *D. semirufus*, *D. delarouzi*) were closely related to rickettsial isolate from the spiders *Pityophantes phrygianus* and *Meta mengei*.

The distribution, transmission, and localization of *Rickettsia* in *D. platynotus* were studied using a diagnostic PCR-assay and FISH. *Rickettsia* could be identified in all compartments of *Deronectes* including the head (ommatidia), soft tissue of elytra, hemolymph, and legs. Those compartments with active metabolism, such as fat body or internal reproductive organs contain numerous Rickettsiae. Generally *Rickettsia* is more abundant in females than in males, where the bacteria dominate in accessory glands (and musculature enclosing accessory glands). When eggs of infected females of *D. platynotus* were investigated they were *Rickettsia* positive, which indicates vertical transmission. Due to the predatory lifestyle of *Deronectes*, a horizontal transmission of *Rickettsia* also seems possible, and thus aquatic prey of *Deronectes* should be analyzed in the future. The bacteria could be also found in their oocytes, follicle cells, and second and third larval stages of *Deronectes*, where the bacteria increased from earlier to later stages.

The biological role of *Rickettsia* in Coleoptera and especially in aquatic forms is largely unknown. At the moment there are no indications that *Rickettsia* infections have any effects on the fitness of the *Deronectes* host. Neither reduced body weights and fecundities (as in infected aphids) nor remarkable increases in host size as observed in leeches (Kikuchi and Fukatsu 2005) are observed. It is well known that parasitic living bacteria such as *Rickettsia*, *Spiroplasma*, *Cardinium*, and *Wolbachia* can manipulate reproduction of their hosts for their own benefit [including parthenogenesis, cytoplasmatic incompatibility, feminization, and male killing (O'Neill et al. 1997)].

6.7 Future Directions

It would be interesting if those kairomones mentioned in Sect. 6.4.1 were characterized chemically in order to perform bioassays with authentic compounds. In addition, further taxa of predaceous diving beetles should be investigated chemically in order to characterize their pygidial and prothoracic defensive gland constituents (Sect. 6.4.2). Hereby a chemotaxonomic search strategy as practiced with plants of pharmaceutical value and their biologically active natural compounds is recommended. An important question seems to be the chemical characterization of prothoracic gland constituents from Hydroporinae. In addition, both with respect to pygidial and prothoracic defensive glands several taxa of predaceous diving beetles should be investigated, including *Matus* (Matinae), *Agabetes* (Agabetinae), representatives of Methlini, Lancetinae, *Carabdytes* (Carabdytinae), *Pachydrus* (Hydroporinae), *Paroster* (Hydroporinae), *Necterosoma* (Hydroporinae) or *Laccornellus* (Hydroporinae).

Further field and laboratory bioassays are necessary to detect the effects of gland compounds on beetle relevant pathogenic bacteria, fungi and ectoparasites. In addition, the biological relevance of the plant hormone indole acetic acid from pygidial glands of Hydroporinae should be investigated. With respect to gland constituents of predaceous diving beetles biosynthetic studies, especially of aromatics and steroids, are urgently required.

Concerning microbiological data it would be worthwhile to isolate culturable microorganisms especially from the guts of other predaceous diving beetle species (see Sect. 6.6.2), in order to identify new biological active metabolites. Also, a search for cultivable microorganisms with interesting characteristics will be promising. Of great interest are those beetle species that are found in extreme habitats such as highly polluted waters or hot springs. As in bacteria from guts of larvae of *Heleomyia petrolei* (petroleum fly, Ephydridae) there might be isolated unusual microorganisms that show strong antibiotic resistance or can be grown in organic solvents (Kadavy et al. 2000).

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