# Polyketides in Insects

## Introduction

Polyketides comprise a huge class of chemicals with a broad spectrum of diverse structures. They all originate from a polyketone, the polyketide chain, giving these substances their name. In general, the polyketide chain is formed by successive addition of simple carboxylic acids like acetate, propanoate, butyrate and also more complex precursors. The polyketide formation is catalysed by special enzymes called polyketide synthases (PKS) (Hopwood and Sherman 1990; Bentley and Bennett 1999). During each extension step of one unit, the polyketide chain is elongated with two carbon atoms, where the β-carbon is a keto group. The vast diversity of polyketides is due to the stepwise reduction of a part or all of these keto groups to hydroxyls or enoyls, which are in some compounds finally completely reduced to an alkyl chain (Hopwood and Sherman 1990; Hopwood 2004). Another factor contributing to polyketide diversity are post-PKS tailoring steps further modifying PKS products (Rawlings 2001; Rix et al. 2002).

Polyketides are widespread natural products known to be present in pro- and eukaryotic organisms. Especially in bacteria like Streptomycetes and in fungi, many polyketides are described with neurotoxic activity or pharmaceutical functions, such as antibiotic, anticancer or antiparasitic activity (Teuscher and Lindequist 1994; Rein and Borrone 1999). Furthermore, various algae, dinoflagellates and plants produce polyketides (Hopwood and Sherman 1990; Teuscher and Lindequist 1994; Rein and Borrone 1999; Austin and Noel 2003). Most of the polyketides produced by dinoflagellates and plants are toxic and might function as protective device against natural enemies (Teuscher and Lindequist 1994; Rein and Borrone 1999; Manojlovic et al. 2000; Choi et al. 2004). Also in several marine invertebrate animals like sponges, tunicates and bryozoans various polyketides are known (Piel 2004; Moore 2005; Piel et al. 2005; Moore 2006; Piel 2006). These invertebrates do not produce polyketides by themselves, but associated

microorganisms are responsible for the biosynthesis. Many insects also possess components that have been suggested to be produced *via* polyketide biosynthesis. However, when these components have not been sequestered from food, it is often unclear whether the (putative) polyketides are produced by endosymbiotic microorganisms like in marine animals or whether the insects produce these components by own enzymes.

Biosynthesis of polyketides is an intensively studied field of natural product chemistry. Numerous reviews address polyketide compounds and/ or PKS in plants and especially in microorganisms (Hopwood and Sherman 1990; O'Hagan 1992; O'Hagan 1993; Staunton and Wilkinson 1998; Rawlings 1999; Schröder 2000; Rawlings 2001; Staunton and Weissman 2001; Hill 2006; Piel 2006). When compared to the knowledge of genes and enzymes responsible for polyketide biosynthesis in bacteria, fungi and plants, nothing is known on the molecular level about polyketide biosynthesis in insects, except those studies which could show that polyketides found in insects are produced by endosymbionts (Kellner 1999, 2001, 2002; Piel 2002; Piel et al. 2004a, 2005).

A problem is often to distinguish between polyketide or fatty acid origin when considering components with structures that can be deduced to e.g., acetate chains (acetogenins) or propanoate chains (propanogenins) (Francke and Schulz 1999). Numerous insect-derived natural products are supposed to be biosynthesised *via* the polyketide pathway, however, for several compounds a proof is lacking that they are indeed produced with the help of PKS. Therefore, we refer to these compounds as "putative polyketides" in this review. Only the knowledge about precursors incorporated into the growing carbon chain is in general not sufficient for classification (see below for e.g., coccinellid alkaloids or polyunsaturated fatty acids). Very similar structures might be produced *via* the fatty acid pathway with precursors also used in the polyketide pathway, but other enzymes, i.e., fatty acid synthases (FAS) instead of PKS. The elucidation of biosynthesis is even more complicated when considering that PKS and FAS may act in concert, as is known for biosynthesis of e.g., norsorolinic acid (Schweizer and Hofmann 2004). Furthermore, other enzymes like nonribosomal polypeptide synthetases (NRPS) were shown to interact with PKS in e.g., staphylinid beetles producing the polyketide pederin (Piel 2002) and NRPS also interact with FAS in e.g., *Bacillus subtilis* to produce mycosubtilin (Duitman et al. 1999). In this review, we consider compounds produced *via* the polyketide pathway only those that need PKS enzymes for their biosynthesis.

An example for a very clear-cut study on the question "polyketide or fatty acid origin" of insect natural products was conducted by Laurent et al. (2002, 2003) investigating the biosynthesis of alkaloids in ladybird beetles (Coleoptera: Coccinellidae). Several coccinellid alkaloids were supposed to be of polyketide origin (Tursch et al. 1975; Blum 1987; Daloze et al. 1994). The biosynthesis study by Laurent et al. (2002, 2003) used the fact that the fatty acid pathway needs aerobic conditions and oxygen for several oxidation steps (Luckner 1984), while the polyketide pathway does not (Ratledge 2004). The defensive alkaloid adaline in *Adalia bipunctata* is derived from seven acetate units connected during a stepwise condensation (Laurent et al. 2001), which was shown by feeding the coccinellid with [1-$^{14}$C] and [2-$^{14}$C]acetate. In vitro incubation assays with coccinellid tissue revealed a much higher $^{14}$C-acetate incorporation rate at aerobic conditions than at anaerobic ones. A fatty acid synthase inhibitor, 2-octynoic acid, reduced biosynthesis of the alkaloid. Thus, both the more effective biosynthesis at aerobic conditions and the inhibitor experiment indicate that adaline is rather of fatty acid origin than a polyketide.

The aim of this review is (1) to outline the wide range of occurrence of components in insects which might be of polyketide origin, (2) to summarise chemical studies on the origin and biosynthesis of (putative) insect polyketides, (3) to compare the polyketide and fatty acid pathway with respect to their modes of action and the different architectures of PKS and FAS. Furthermore, knowledge on PKS and FAS evolution and possible horizontal transfer of PKS genes from other organisms to insects will be addressed. The information and ideas outlined here might be useful to design molecular approaches to analyse whether insect natural products are biosynthesised *via* the polyketide pathway with the help of PKS.

Insect natural products of (putative) polyketide origin that are used for defensive purpose will be considered separately from those used as pheromones. When addressing the occurrence of (putative) insect polyketides, we will neglect those studies showing that insects are able to sequester polyketides from food (e.g., Pasteels et al. 1990; Hesbacher et al. 1995). Several excellent recent reviews of insect pheromones and their biosynthesis are available (Francke and Schulz 1999; Jurenka 2004; Francke and Dettner 2005). For example, Francke and Schulz (1999) provide detailed information on moth pheromones being frequently derived from fatty acids (acetogenins), other insect pheromones of

terpenoid origin and pheromones considered as propanogenins. Furthermore, several reviews summarise the wide range of different insect defensive compounds (e.g., Dettner 1987; Blum and Hilker 2002; Laurent et al. 2005). When considering insect pheromones and defensive components below, we focus on those for which polyketide origin is likely or has been shown. Moreover, those insect compounds will be addressed which have the same or similar chemical counterparts in bacteria, fungi or plants and have in these organisms been proven to be produced by PKS. Furthermore, we will give special emphasis to those defensive components and pheromones for which endosymbionts might be the polyketide producers.

## Defensive compounds of (putative) polyketide origin

A small number of (putative) polyketide substances are used for chemical defence in some insects. These chemical components can be either very simple in their structure, such as e.g., simple aliphatic ketones and aromatic substances like benzoquinones, or they are structurally very complex like the staphylinid toxin pederin (Fig. 1A + 1B; numbers next to the names refer to the numbers in figures). The occurrence of these compounds in insects as well as their ecological function will be addressed.

*Simple aliphatic and benzoquinoid defensive compounds*

Simple short chain ketones are used by several insects as defensive substances. For example, (*S*)-4-methyl-3-heptanone (**1**), 4,6-dimethyl-3-nonanone (**2**) and 4,6-dimethyl-3-octanone (**3**) were detected in the mutillid wasp *Dasymitilla occidentalis* (Fales et al. 1980). These ketones function as an allomone against ants which are the major predators of this mutillid wasp (Fales et al. 1980). Since (*S*)-4-methyl-3-heptanone (**1**), related ketones and alcohols often also have pheromonal activity in insects, aspects of their biosynthesis are described below in the pheromone section.

The mandibular glands of several species of some carpenter ants, *Camponotus* spec., contain polyacetate-derived aromatics like 6-methylsalicylate (**4**) and its decarboxylated derivative *m*-cresol (**5**) (Jones et al. 2004). The enzymes necessary for biosynthesis of these components have not been studied so far in insects (but see below, pheromone section). These compounds might be used as defensive compounds against other territorial

ant species since these chemical products are irritant and corrosive (Jones et al. 2004). Derivatives of 6-methylsalicylate are known in several other insects to function as pheromones (see pheromone section). In the tenebrionid beetle *Tenebrio molitor*, *m*-cresol (**5**) along with a 1,4-benzoquinone was detected in defensive glands (Attygalle et al. 1991).

Especially in tenebrionid and staphylinid beetles, 1,4-benzoquinones are very widespread (Tschinkel 1972; Blum 1981; Blum 1987; Peschke and Eisner 1987; Dettner 1993; Yezerski et al. 2004; Eisner et al. 2005). For example, the tenebrionid beetle *Eleodes longicollis* contains 1,4-benzoquinone (**6a**) together with 2-methyl-1,4-benzoquinone (**6b**) and 2-ethyl-1,4-benzoquinone (**6c**) (Meinwald et al. 1966; Eisner et al. 2005). The same quinonic compounds were detected in another tenebrionid, *Tribolium castaneum* (Blum 1987), and in the Pacific island cockroach *Diploptera punctata* (Baldwin et al. 1990). Another quinone, 2-methyl-3-methoxy-1,4-benzoquinone (**6d**), is produced in *Bolitochara*, *Leptusa* (both Staphylinidae) and *Hypophloeus* (Tenebrionidae) species (Dettner 1993). The defensive apparatus of bombardier beetles (Carabidae) contain 2-methylhydroquinone (**7**), which is chemically converted by hydrogen peroxide to the corresponding 1,4-benzoquinone and is sprayed out when the beetle is attacked by predators (Blum 1987; Dean et al. 1990; Eisner et al. 2005, and references therein). Also earwigs like *Doru taeniatum* contain two different benzoquinones, i.e., the above mentioned 2-methyl-1,4-benzoquinone (**6b**) and 2,3-dimethyl-1,4-benzoquinone (**6e**) (Eisner et al. 2000). A benzoquinone with an additional hydroxy group (2-hydroxy-5-methyl-1,4-benzoquinone (**8**)) was detected in soldiers of the termite *Odontotermes magdalenae* (Olagbemiro et al. 1988). The forest cockchafer *Melolontha hippocastani* and the European cockchafer *M. melolontha* release 1,4-benzoquinone (**6a**) and 2-methyl-1,4-benzoquinone (**6b**), respectively (Ruther et al. 2001, Reinecke et al. 2002).

Quinones are eminently reactive and toxic causing several deleterious effects like membrane damage, enzyme destruction and cell death. Moreover, they are mutagenic and carcinogenic (Öllinger and Brunmark 1991). This toxicity is due to the capacity to produce free oxygen radicals, their oxidative-reductive capacity and their strong electrophilicity enabling them to build adducts to cellular constituents (Norris 1988; Öllinger and Brunmark 1991). Thus, these characters render benzoquinones strong agents against several predators (Peschke and Eisner 1987; Olagbemiro et al. 1988; Baldwin et al. 1990; Dettner 1993; Eisner et al. 2000; Yezerski et al. 2004; Eisner et al. 2005). Additionally,

benzoquinones have antimicrobial activity against fungi and bacteria, thus protecting benzoquinone-producing insects from pathogens (Ruther et al. 2001). In cockchafers, quinones have dual functions. In addition to the antimicrobial activity which might especially protect from pathogens during life phases spent in the soil, benzoquinones act as sex pheromone attracting swarming male cockchafers (Ruther et al. 2000; Ruther et al 2001; Reinecke et al. 2002). Leal (1997) suggested that the sex pheromones in these scarab beetles might have evolved from the defensive function of these quinones.

It is unclear if these defensive and pheromonal benzoquinones are produced *via* the polyketide pathway. The biosynthesis of the quinoid nucleus (1,4-benzoquinone) of ubiquinone (coenzyme Q) in bacteria and eukaryotic microorganisms was clearly shown to be produced *via* the shikimate pathway with chorismate and 4-hydroxybenzoate as successive precursors (Meganathan 2001a, b). In animals like mammalians the precursor of ubiquinone is the aromatic amino acid tyrosine (Meganathan 2001a). Meinwald and coworkers (1966) could demonstrate that 1,4-benzoquinone (**6a**) in the beetle *Eleodes longicollis* is also derived from tyrosine (Meinwald et al. 1966; Blum 1987). However, two further benzoquinones detected in this beetle, 2-methyl-1,4-benzoquinone (**6b**) and 2-ethyl-1,4-benzoquinone (**6c**), are derived from a pathway using carboxylic acetate or propanoate as precursors, suggesting a polyketide origin for these benzoquinones (Meinwald et al. 1966; Blum 1987). These results indicate that structurally similar quinones might be synthesised in the same organism *via* different pathways. While alkylated benzoquinones in *E. longicollis* are no tyrosine derivatives, millipedes releasing the same benzoquinones use tyrosine to produce their defensive components (Blum 1987). Therefore, the structures of these quinones do not provide a hint on the type of biosynthesis.

Mellein (3,4-dihydro-8-hydroxy-3-methylisocoumarin) (**9**) often has pheromonal functions in insects (see below), however, in some species it acts as defensive compound. For example, termite species like *Cornitermes pugnax* and *C. ovatus* use mellein at low concentrations to deter ants (Blum et al. 1982a). Since mellein has antifungal activity (Cabras et al. 2006), it might protect insects from infestation with deleterious fungi. The biosynthesis of mellein will be considered below in the pheromone section.

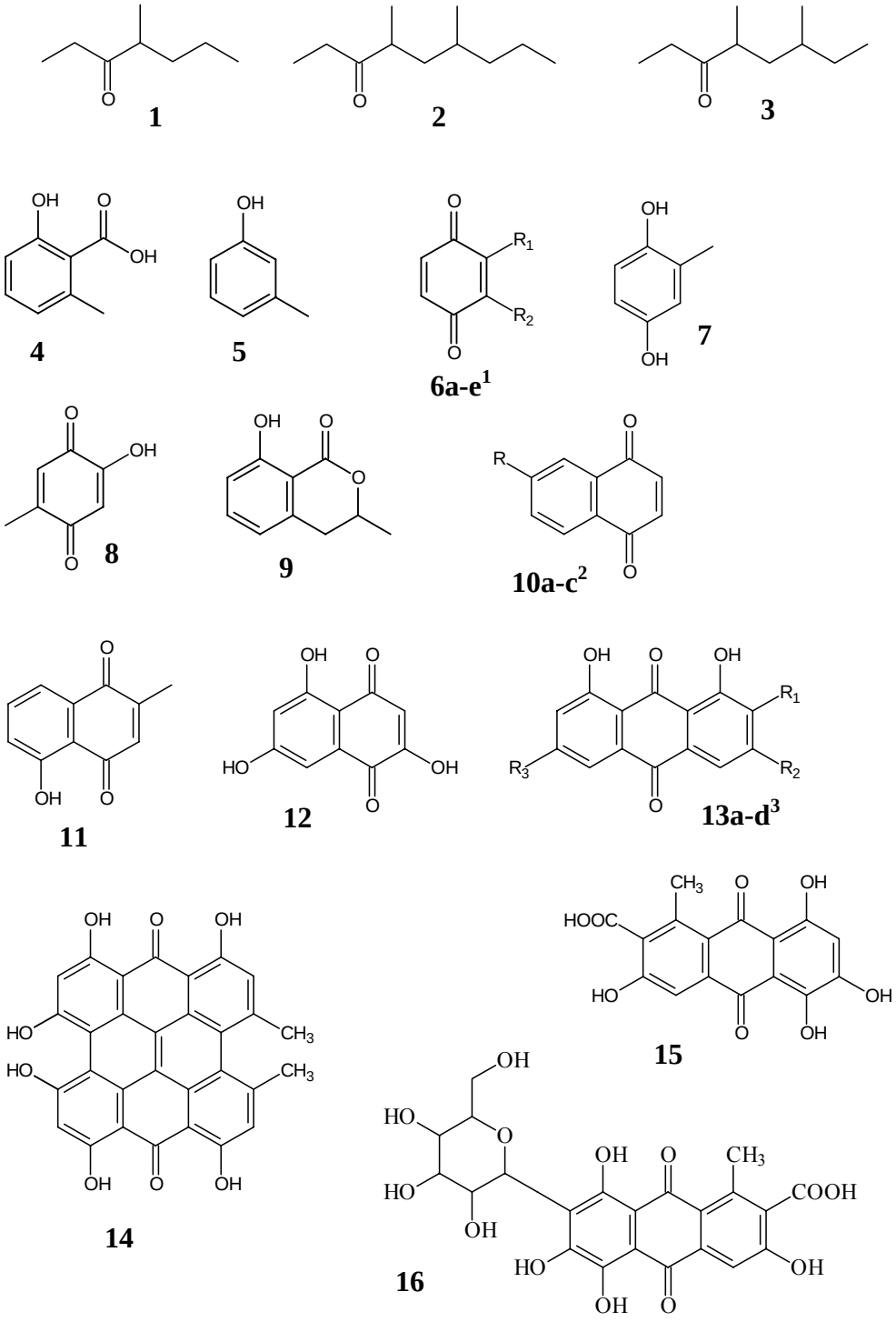


**Figure 1A.** (Putative) polyketides used as defensive compounds in insects (except **11** and **12,** see text). Numbers refer to the numbers and names given in the text; [1] **6a**: $R_1 = R_2 = H$; **6b**: $R_1 = CH_3$, $R_2 = H$; **6c**: $R_1 = C_2H_5$, $R_2 = H$; **6d**: $R_1 = CH_3$, $R_2 = OCH_3$; **6e**: $R_1 = R_2 = CH_3$; [2] **10a**: $R = CH_3$; **10b**: $R = C_2H_5$; **10c**: $R = C_3H_7$; [3] **13a**: $R_1 = H$, $R_2 = R_3 = OH$; **13b**: $R_1 = COCH_3$, $R_2 = CH_3$, $R_3 = OH$; **13c**: $R_1 = R_3 = H$, $R_2 = CH_3$; **13d**: $R_1 = R_2 = R_3 = H$.

*Naphthoquinones*

The distribution of naphthoquinones in insects is very restricted to only few tenebrionid beetles (Tschinkel 1972; Tschinkel 1975; Dettner 1993). Larvae of the genera *Hypephloeus* contain 6-methyl-1,4-naphthoquinone (**10a**), 6-ethyl-1,4-naphthoquinone (**10b**) and 6-propyl-1,4-naphthoquinone (**10c**) (Dettner 1993). Also in another tenebrionid beetle (*Argoporis alutacea*) 6-alkyl derivatives of these 1,4-naphthoquionones were detected (Tschinkel 1972). 1,4-Naphthoquinones are also known from insect relatives like daddylonglegs (*Leiobunum nigripalpi*) (Wiemer et al. 1978; Eisner et al. 2005). Analogue to other quinoid systems, also naphthoquinones are highly reactive and toxic (Norris 1988; Öllinger and Brunmark 1991). A deterrent effect of naphthoquinones against predators like ants was shown (Wiemer et al. 1978; Dettner 1993). Additionally, these quinones might protect subcortical living larvae of the genera *Hypephloeus* from deleterious fungi and bacteria present in the bark (Dettner 1993). The biosynthesis of naphthoquinones in insects is unclear, since no feeding experiments with labelled precursors were conducted so far. Studies on 1,4-naphthoquionones in some plant species showed that they are synthesised from shikimic acid *via* the shikimate pathway (Leistner 1981). However, the plant *Ancistrocladus heyneanus* produces the 1,4-naphthoquinone plumbagin (**11**) *via* acetate condensation, and plumbagin is produced *via* the polyketide pathway (Bringmann et al. 1998). Also in bacteria, the biosynthesis of 1,4-naphthoquinones like flaviolin (**12**) is catalysed by forming a pentaketide chain with polyketide synthases (Funa et al. 1999; Austin and Noel 2003). However, the shikimate pathway is used by bacteria for biosynthesis of menaquinones like the well investigated vitamin K (Young 1975; Leistner 1981; Bentley and Meganathan 1982; Meganathan 2001b). Feeding experiments with labelled precursors need to elucidate whether the naphthoquinones in tenebrionid beetles are produced via the shikimate or another pathway using precursors like in the polyketide pathway.

*Anthraquinones and derivatives*

Some scale insects are known to contain anthraquinones, anthraquinone derivatives or anthraquinone glycosides (Thomson 1971; Banks et al. 1976a, 1976b, 1976c; Eisner et al 1980; Kayser 1985; Thomson 1987, 1997). For example, the 1,8-dihydroxylated anthraquinone emodin (**13a**), well-known in different plant species (Izhaki 2002), could be detected in the mealy bugs *Nipaecoccus aurilanatus* and *Pseudococcus albizziae* (Banks et al. 1976b). In the same species, the naphthodianthrone hypericin (**14**) was detected, giving

these insects their dark colouration. This compound is synthesised from two molecules emodin by oxidative coupling. It is also present in different plant species, especially of the genus *Hypericum* (Teuscher and Lindequist 1994). However, the bugs do not feed upon plants containing anthraquinones or their precursors (Banks et al. 1976b). Emodin derivatives, 2-acetylemodin (**13b**) and its 10,10´-dimer, were detected in *Eriococcus coriaceus* and *Callococcus acaciae*, respectively (Banks et al. 1976a, 1976c; Kayser 1985). The anthraquinone kermesic acid (**15**) is produced in the scale insect *Kermes ilicis* (Kayser 1985). Scale insects studied most intensively with respect to anthraquinones are various species of the genus *Dactylopius* (Eisner et al 1980; Kayser 1985; Eisner et al. 1994; Joshi and Lambdin 1998). *Dactylopius* spec. contain the anthraquinone glycoside carminic acid (**16**) in hemolymph and muscles of both immature and adult stages. Even eggs and embryos contain carminic acid (Eisner et al 1980). The deeply red coloured carminic acid is a glycoside of the above mentioned kermesic acid (**15**). *Dactylopius confusus* lives on the prickly pear cactus (*Opuntia* spec.), a plant not containing anthraquinones or anthraquinone derivatives (Eisner et al. 1980).

In addition to the above mentioned scale insects, anthraquinones are present in some chrysomelid beetles. Leaf beetles of the tribe Galerucini contain the anthraquinones chrysophanol (**13c**) and chrysazin (**13d**) and the anthrones chrysarobin (**17a**) and dithranol (**17b**) (Howard et al. 1982; Hilker and Schulz 1991; Hilker 1992; Hilker et al. 1992; Kunze et al. 1996; Pankewitz and Hilker 2006). These anthraquinones and anthrones are present in all developmental stages like eggs, larvae and hemolymph of adult beetles (Hilker and Schulz 1991; Hilker et al. 1992). All examined host plants of these Galerucini species do not contain anthraquinones or anthrones (Howard et al. 1982; Hilker and Schulz 1991; Hilker et al. 1992; Kunze et al. 1996).

Defensive activity of anthraquinones present in Galerucini and of the anthraquinone glycoside carminic acid found in *Dactylopius* was demonstrated especially against several ant species (Eisner et al. 1980; Hilker and Schulz 1991; Hilker et al. 1992). Birds are also deterred from feeding by these anthraquinones and anthrones (Hilker and Köpf 1995; Avery et al. 1997). However, the coccinellid beetle *Hyperaspis trifurcata* and larvae of the pyralid moth *Laetilia coccidivora* preying upon *Dactylopius* are not deterred from the natural products of their prey. Instead, they take up the carminic acid from their cochineal prey for their own defence (Eisner et al. 1980; Eisner et al. 1994). Several predators of

Galerucini species of the genus *Galerucella* spec., such as e.g., *Coleomegilla maculata* (Coleoptera: Coccinellidae) or *Chrysoperla carnea* (Neuroptera: Chrysopidae), are obviously not deterred by anthraquinones of their prey (Wiebe and Obrycki 2004; Sebolt and Landis 2004; Matos and Obrycki 2006). The defensive function of anthraquinones is not restricted to antipredatory activity. These compounds are also known to have antimicrobial, antiviral and cytostatic activity (Cudlin et al. 1976; Gálvez et al. 1996; Semple et al. 2001; Kambizi et al. 2004), thereby probably protecting from attack by pathogens.

Neither Galerucini beetles nor scale insects sequester anthraquinones from their host plants. However, the biogenesis of anthraquinones has especially been studied in plants. Two major biosynthetic pathways are known to lead to plant anthraquinones. First, the shikimate or chorismate/ *o*-succinylbenzoic acid pathway is used to produce anthraquinones with only one hydroxylated ring like 1,2-dihydroxylated anthraquinones (*Rubia* type anthraquinones) (Teuscher and Lindequist 1994). Here ring A and B from the anthraquinone or anthrone originates from shikimic acid or chorismic acid and α-keto-glutarate. The third ring C is derived from mevalonic acid (Leistner 1981; Han et al. 2001). The polyketide pathway represents the second way to produce anthraquinones. Chrysophanol type anthraquinones with ring A and C being hydroxylated at least at position one and eight like in 1,8-dihydroxylated anthraquinones (e.g., emodin (**13a**), chrysophanol (**13c**)) are biosynthesised by folding of a polyketide chain (Teuscher and Lindequist 1994; Han et al. 2001). Studies in Streptomycetes revealed that also anthraquinones with another substitution pattern of the hydroxy groups, like aloesaponarin (**18**) (hydroxy groups on position 3 and 8), are derived from a polyketide chain precursor (Fu et al. 1996). In summary, anthraquinones produced *via* the polyketide pathway are mostly substituted in ring A and C, whereas anthraquinones produced *via* the chorismate/ *o*-succinylbenzoic acid pathway mostly do not have substitutions in ring A. Therefore, the anthraquinones found in scale insects and Galerucini are thought to be produced *via* the polyketide pathway.

A polyketide chain forming anthraquinones can be built up from one acyl-CoA and seven malonyl-CoA units leading to an octaketide chain by decarboxylation of each malonyl unit at every elongation step (Han et al. 2001). The chain can also be formed by eight molecules of acetate/ acyl coenzyme A (Bringmann et al. 2006). Subsequently the

elongated chain cyclises to an anthrone, which is oxidised to the anthraquinone (Leistner 1981; Hutchinson and Colombo 1999; Han et al. 2001). In Streptomycetes, this oxidation process is catalysed by an anthrone oxygenase (Chung et al. 2002). Some of these anthraquinones are subsequently bound to sugars, possibly by using post-PKS enzymes like glycosyltransferases to form glycosides (Rix et al. 2002).

Since many polyketides are produced by bacteria or fungi (see introduction for references), non-sequestered anthraquinones in insects have been suggested to be produced by endosymbiotic microorganisms (Howard et al. 1982; Kayser 1985; Hilker and Schulz 1991). However, so far microorganisms responsible for an anthraquinone production have neither been detected in the scale insects (Kayser 1985) nor in chrysomelids (Blum and Hilker 2002). Recently, the tansy leaf beetle *Galeruca tanaceti* (Galerucini) has intensively been studied with respect to the hypothesis that endosymbionts produce the beetle's anthraquinones.

First, endosymbionts were searched within *G. tanaceti* eggs, because a transmission of mutualistic microorganisms *via* the egg stage seems to be the safest way in this species since eggs overwinter. Transmission of endosymbionts to the next generation on the outside of eggs would expose endosymbionts to very diverse abiotic conditions. Furthermore, transmission of endosymbionts *via* habitat contamination is very unlikely in *G. tanaceti* since the herbaceous vegetation will be renewed when larvae hatch. However, no hints on the presence of anthraquinone-producing endosymbionts in the eggs were found by using molecular techniques for the detection of bacterial and fungal rDNA (Pankewitz et al. 2006a). Only the Gram-negative α-proteobacterium *Wolbachia pipientis* was detected in eggs of *G. tanaceti* (Pankewitz et al. 2006a, b). With respect to *Wolbachia* in *G. tanaceti*, two hypotheses were tested: (1) *Wolbachia* in *G. tanaceti* produce anthraquinones, and (2) *Wolbachia* in *G. tanaceti* belong to a specific strain tolerant for the antibiotic activity of anthraquinones. To test these hypotheses, *Wolbachia* in *G. tanaceti* were subjected to phylogenetic analyses along with *Wolbachia* in other anthraquinone-containing insects and anthraquinone-free insects. Since *Wolbachia* in *G. tanaceti* do not cluster with *Wolbachia* in other anthraquinone-producing insects, neither of the hypotheses could be proved. Moreover, while *G. tanaceti* contains *Wolbachia*, no *Wolbachia* were detected in the elm leaf beetle *Xanthogaleruca luteola*, a close relative of *G. tanaceti* that also contains the same anthraquinones. In addition to the results of the phylogenetic

analyses, this finding supported the conclusion that *Wolbachia* is no anthraquinone-producing endosymbiont in *G. tanaceti* nor are *Wolbachia* in *G. tanaceti* belonging to a specific, anthraquinone-tolerant strain.

Second, *G. tanaceti* beetles were fed with antimicrobial drugs to eliminate endosymbionts. However, beetles treated with antimicrobial agents continued to lay eggs containing anthraquinones (Pankewitz et al. 2006a). The quantities of anthraquinones of over-wintering, field-collected eggs were found to decrease (Pankewitz and Hilker 2006). These data suggested that neither the embryo nor endosymbionts are actively producing anthraquinones within the eggs. Instead, the anthraquinones present in the eggs must have been transferred from the mother to the offspring.

Third, NMR analyses of chrysophanol in *G. tanaceti* showed that this polyketide is of eukaryotic origin (Bringmann et al. 2006). Prokaryotic and eukaryotic organisms build up the same polyketide (chrysophanol) *via* different folding modes as was demonstrated by feeding experiments with $^{13}C$ labelled acetate in different organisms and subsequent NMR analyses of chrysophanol (Bringmann et al. 2006). Since the folding mode of chrysophanol in *G. tanaceti* showed the eukaryotic folding mode, a bacterial producer was excluded. These results and the above mentioned molecular studies and antibiotic treatments supported the hypothesis that the beetle itself is equipped with polyketide synthase genes (Pankewitz et al. 2006a).

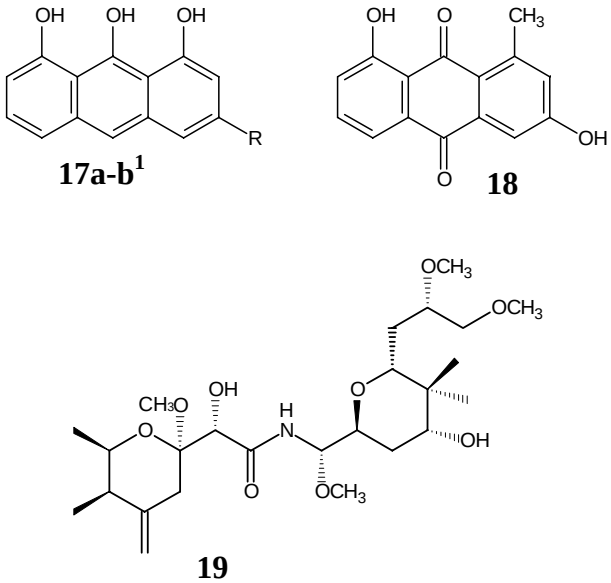


**Figure 1B.** (Putative) polyketides used as defensive compounds in insects (except **18**; see text). Numbers refer to the numbers and names given in the text; [1] **17a**: R = $CH_3$; **17b**: R = H.

*Complex polyketide of endosymbiotic origin*

The so far structurally most complex defensive polyketide in insects is pederin (**19**), which is present in several rove beetle species of the genus *Paederus* and *Paederidus* (Frank and Kanamitsu 1987; Kellner and Dettner 1995; Piel 2006). This complex polyketide deters predators like wolf spiders (Kellner and Dettner 1996). In these staphylinids, an antibiotic treatment resulted in aposymbiotic beetles not producing pederin anymore. These results clearly showed that pederin is produced in female staphylinid beetles by endosymbionts (Kellner 1999, 2001). Aposymbiotic beetles can gain their ability to produce pederin by the uptake of endosymbionts, e.g., from staphylinid eggs harbouring these microorganisms (Kellner and Dettner 1995; Kellner 2002). Analyses of bacterial rDNA from pederin-positive individuals of the rove beetle revealed the presence of a bacterium closely related to *Pseudomonas aeruginosa* (Kellner 2002). The search for polyketide synthase genes (PKS) resulted in the isolation of a PKS/ NRPS (nonribosomal peptide synthetase; for explanation see below) cluster (Piel 2002). Flanking regions of this cluster showed a prokaryotic architecture and similarity to *P. aeruginosa* genes, thus supporting the previous findings (Piel 2006). A final isolation of these bacteria from infected rove beetles could clearly demonstrate that PKS genes in staphylinid beetles are of bacterial origin (Piel et al. 2004a; Piel 2006).

## Pheromones of (putative) polyketide origin

*Simple short chained alcohols and ketones*

Pheromonal compounds found in insects are structurally highly diverse (Fig. 2; numbers next to the names refer to numbers in figure). Methyl branched ketones and alcohols are a major class of pheromones that have been suggested to be produced *via* the polyketide pathway (Morgan et al. 1992; Francke et al. 1995, Jarvis et al. 2004). For example, (3*S*,4*S*)-4-methyl-3-heptanol (**20**) is the main component present in the pheromone blend of the bark beetles *Scolytus scolytus*, *S. multistriatus* and the almond bark beetle *S. amygdali* (Blight et al. 1979; Pignatello and Grant 1983; Francke et al. 1995; Zada et al. 2004). In the ant *Leptogenys diminuta*, the (3*R*,4*S*)-isomer of the alcohol is a trail pheromone (Steghaus-Kovac et al. 1992). Alcohols with a shorter or longer carbon chain were also reported as insect pheromones, like 4-methyl-3-hexanol (**21**) and 4-methyl-3-octanol (**22**) in the ant *Tetramorium caespitum* (Morgan et al. 1992). The ketone

(*S*)-4-methyl-3-heptanone (**1**) was detected in several insect species and is most likely biosynthesised *via* the polyketide pathway from three propanoate units with a loss of one carbon atom (Jarvis et al. 2004). The ant *Aphaenogaster albisetosus* produces this compound as trail pheromone in its poison gland (Hölldobler et al. 1995). Caddies flies use this component as sex pheromone (Bergmann et al. 2001). In the leafcutter ant *Atta texana*, (*S*)-4-methyl-3-heptanone (**1**) serves as alarm pheromone (Riley et al. 1974). Similar ketones are very widespread pheromone components found in ants as trail or alarm pheromone and seem to originate from a mixed acetate/ propanoate polyketide pathway (Francke and Schulz 1999; Jarvis et al. 2004). For example, sitophilure ((4*S*,5*R*)-5-hydroxy-4-methyl-3-heptanone) (**23**) was identified as the male-produced aggregation pheromone of the rice weevil *Sitophilus oryzae*, but could also be found in other weevils (Schmuff et al. 1984; Phillips et al. 1985). However, for none of these pheromones the enzymes involved in their biosynthesis are known.

*Long chained hydrocarbons (polyenes) and similar compounds*

Several insects use long chained hydrocarbons with methyl and ethyl branches as pheromones (Fig. 2). Some nitidulid beetles produce very unique branched polyenes (Bartelt et al. 1993; Petroski et al. 1994; Bartelt and Weisleder 1996; Cossé and Bartelt 2000; Bartelt et al. 2004). These hydrocarbons are male-produced aggregation pheromones (Petroski et al. 1994; Bartelt et al. 2004). The pheromone component (2*E*,4*E*,6*E*)-5-ethyl-3-methyl-2,4,6-nonatriene (**24**) released by the nitidulid *Carpophilus freemani* is biosynthesised from one acetate, one propanoate and two butanoate units followed by a loss of one carbon unit (Petroski et al. 1994; Francke and Schulz 1999). Studies on these polyenes suggested a polyketide chain as precursor with the methyl and ethyl branches originating from propanoate or butanoate units, respectively (Petroski et al. 1994; Bartelt and Weisleder 1996). In the fatty acid pathway, the desaturation of a carbon chain is mostly due to the activity of several desaturases (Jurenka 2004) (for exceptions in some bacteria see below), but in the polyketide pathway used by nitidulids to produce their pheromones, the conjugated double bounds are due to the inactivity of the enoyl-ACP reductase (Petroski et al. 1994; Jurenka 2004).

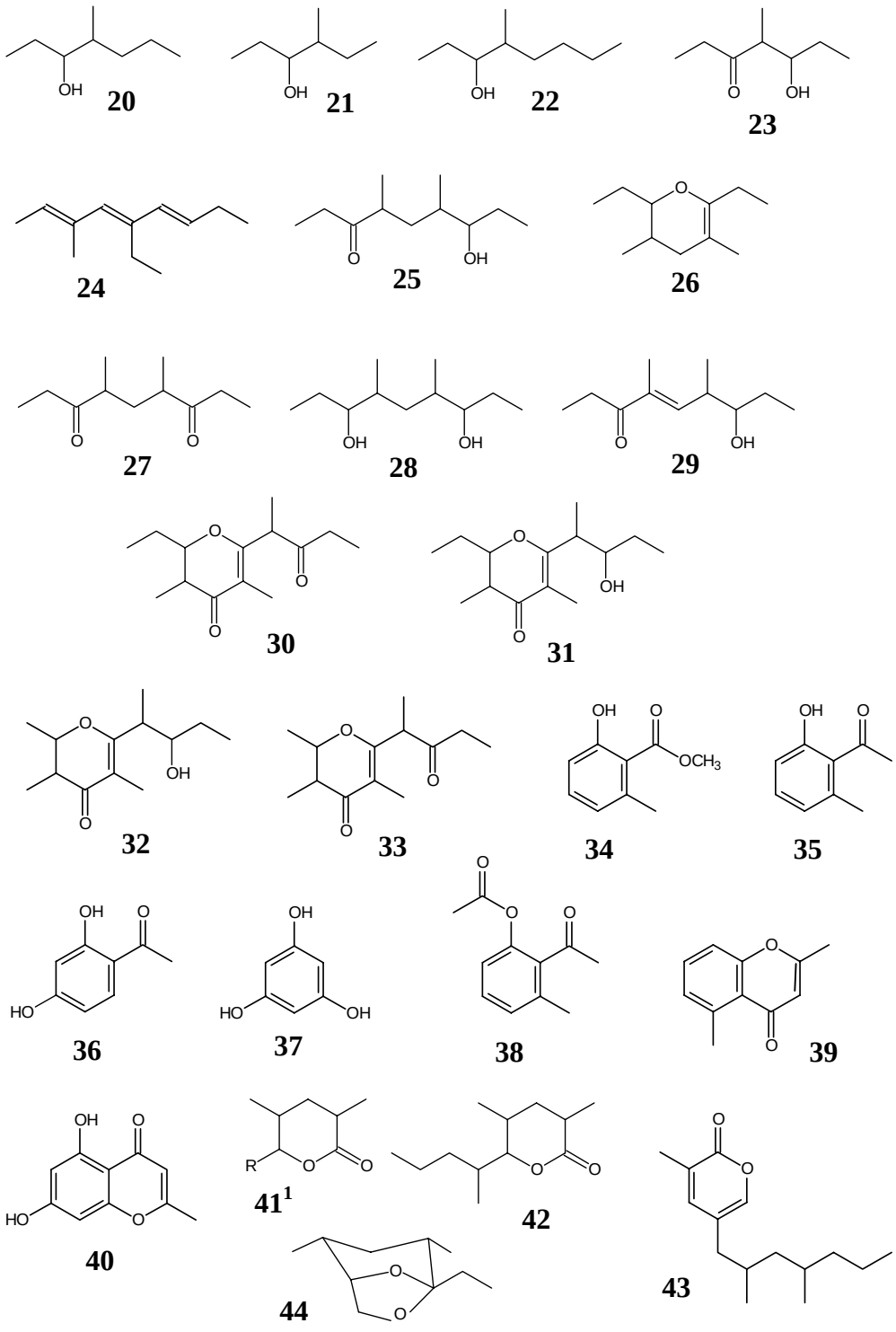


**Figure 2.** (Putative) polyketides used as pheromones in insects. Numbers refer to the numbers and names given in the text; [1] R = alkyl.

The female cigarette beetle *Lasioderma serricorne* (Anobiidae) contains several putative polyketide compounds in its sex pheromone blend (Chuman et al. 1985; Francke and Schulz 1999). Since these pheromone compounds have a carbon skeleton similar to the

above mentioned polyenes from nitidulid beetles and have the branching and oxygenation pattern as found in polyketides, they have been suggested to be biosynthesised *via* the polyketide pathway (Chuman et al. 1983; Chuman et al. 1985; Petroski et al. 1994). The polyketides found in the cigarette beetle are serricornin (= (4*S*,6*S*,7*S*)-7-hydroxy-4,6-dimethyl-3-nonanone) (**25**), anhydroserricornin (**26**), 4,6-dimethylnonan-3,7-dione (**27**), 4,6-dimethylnonan-3,7-diol (**28**), 4,6-dimethyl-7-hydroxy-4-nonen-3-one (**29**) which are potentially built up by a $C_{11}$ polyketide chain, and serricorone (**30**) and the corresponding alcohol serricorole (**31**) both cyclised from a potential $C_{14}$ polyketide chain (Chuman et al. 1983; Chuman et al. 1985). The $C_{11}$ and $C_{14}$ polyketide chain were suggested to be formed by condensation of four and five propanoate units, respectively (Chuman et al. 1985). The related polyketides stegobiol (**32**) and the ketone stegobinone (**33**) were identified as female sex pheromone of the drugstore beetle *Stegobium paniceum* (Anobiidae) (Kuwahara et al. 1978; Kodama et al. 1987). In another anobiid species, the common furniture beetle (*Anobium punctatum*), stegobiol (**32**) was also identified as female sex pheromone (White and Birch 1987).

*Methylsalicylate derivatives*

Among various aromatic compounds formed by acetate units, especially 6-methylsalicylate derivatives are present in a wide range of insect species (Birch and Donovan 1953; Tecle et al. 1986; Sun and Toia 1993). Several ant species use methyl 6-methylsalicylate (**34**) as trail pheromone, e.g., *Tetramorium impurum* (Morgan and Ollett 1987), the ponerine ants *Gnamptogenys pleurodon* (Duffield and Blum 1975), *Bothroponera soror* (Longhurst et al. 1980), several *Camponotus* species (Brand et al. 1973) and *Mayriella overbecki* (Kohl et al. 2000). This component is also used as female sex pheromone in the slave-making ant *Polyergus rufescens* (Castracani et al. 2005).

The biosynthesis of 6-methylsalicylate has especially been studied in fungi with respect to genes for PKS (Beck et al. 1990; Fujii et al. 1996; Staunton and Weissman 2001). No molecular studies are available so far on the biosynthesis of insect methylsalicylate derivatives, however, several chemical studies have been conducted. For example, feeding of the ant *Rhytidoponera aciculate* with labelled acetate showed that it produces 2-hydroxy-6-methylacetophenone (**35**) from a $C_{10}$-polyketide by subsequent de-carboxylation (Tecle et al. 1986). Studies of the biosynthesis of 2,4-dihydroxy-acetophenone (**36**) in the Australian ponerine ant *Rhytidoponera chalybaea* revealed that

also this compound is biosynthesised *via* a $C_8$-polyketide chain (Sun and Toia 1993). Further closely related components are described especially in ants. Orcinol (**37**) and 2-acetoxy-6-methylacetophenone (**38**), for example, have been reported from *Hypoclinea analis* (Blum et al. 1982b). Since these derivatives are structurally related to 6-methylsalicylic acid they might also be produced *via* polyketide formation. Chromones like 2,5-dimethylchromone (**39**) in the Australian ant *Rhytidoponera metallica* (Brophy et al. 1988) and 2-methyl-5,7-dihydroxychromone (**40**) were found in *Camponotus* species (Jones et al. 2004). 2-Methyl-5,7-dihydroxychromone (**40**) is also found in the medicinal plant *Aloe arborescens* and is synthesised by a special plant PKS called pentaketide chromone synthase (Abe et al. 2005a, b). Therefore a polyketidic origin of insect chromones might also be possible.

*3,4-Dihydroisocoumarins*

A combined synthesis using acetate and propanoate units is known for 3,4-dihydro-isocoumarins (Bestmann et al. 1997; Francke and Schulz 1999). Mellein (3,4-dihydro-8-hydroxy-3-methylisocoumarin) (**9**) is widespread in many organisms. For example, the mandibular gland secretion of carpenter ant species contain mellein (Payne et al. 1975; Jones and Fales 1983; Bestmann et al. 1997). Also in various other ant species like *Rhytidoponera metallica* (Brophy et al. 1981) and *R. chalybaea* (Sun and Toia 1993) mellein was detected. Trail pheromone activity of mellein was found in formicine ants (Bestmann et al. 1997, and references therein; Francke and Schulz 1999). The bumble-bee wax moth *Aphomia sociella* (Kunesch et al. 1987) and the oriental fruit moth *Grapholita molesta* contain mellein in male hairpencils (Baker et al. 1981). Mellein and other 3,4-dihydroisocoumarins are also well-known to be present in several fungal species and were shown to be produced from a pentaketide chain (Holker and Simpson 1981; Turner and Aldridge 1983; Cabras et al. 2006, and references therein).

Methylated derivatives of mellein are present in the trail pheromone blend of several ant species (Bestmann et al. 1997). Because of the presence of mellein in fungi it was hypothesised that ants take up mellein with their food and that the methylated derivatives are produced during digestion of mellein (Bestmann et al. 1997). However, feeding experiments with deuterated mellein did not prove this hypothesis, thus giving a first hint for *de novo* synthesis of 3,4-dihydroisocoumarins in ants (Bestmann et al. 1997). And indeed, when ants were fed with labelled acetate and propanoate, these units were

incorporated into mellein and the other 3,4-dihydroisocoumarin pheromones (Bestmann et al. 1997).

Since many endosymbionts are well-known to inhabit ants (Zientz et al. 2005), they have been suggested to synthesise the mellein-containing trail pheromones. As described above for anthraquinones, the folding mode of a fused-ring aromatic polyketide can give a hint on the organism producing this compound (Thomas 2001, Bringmann et al. 2006). The incorporation pattern of labelled precursors into mellein revealed an eukaryotic folding mode (F-mode) with two intact acetate units incorporated into the first ring (Sun and Toia 1993; Bestmann et al. 1997; Rawlings 1999). Thus, mellein is produced either by the ants themselves or by symbiotic fungi, thereby excluding bacterial symbionts as producers. Ants fed with antimycotics and antibiotics still produce mellein and other related trail pheromone components (Bestmann et al. 1997), suggesting that ant enzymes catalyse mellein biosynthesis rather than enzymes of fungal symbionts.

In contrast, endosymbionts seem to be mellein producers in several other insects than ants. For example, the gut of the mellein containing bumble-bee wax moth *Aphomia sociella* is inhabited by a mellein-producing fungus (*Aspergillus ochraceus*), thus suggesting a polyketide production by this fungus (Kunesch et al. 1987). A mellein-producing fungus (*Lasiodiplodia theobromae*) was found on the body surface of the giant white danaine *Idea leuconoe*. This fungus is also an endophyte infecting host plants of the butterfly. Mellein produced by the fungus attracts butterflies to their host plants (Matsumoto and Nago 1994; Nago and Matsumoto 1994; Schulz 1998).

*Lactones*

Several ant species are known to use δ-lactones (3,5-dimethyl-6-alkyl-tetrahydro-2*H*-pyran-2-ones) (**41**) as trail pheromone components. In formicine ants, feeding experiments with acetate and propanoate revealed that these lactones originate from a polyketide chain (Bestmann et al. 1997; Kohl et al. 2003). The lactone invictolide (**42**) formed from four propanoate units (Bestmann et al. 1997; Francke and Schulz 1999) was isolated as a part of the queen recognition pheromone in the fire ant *Solenopsis invicta* (Rocca et al. 1983a, b). Interestingly, very similar compounds were previously detected as volatile metabolites released by the fungus *Trichoderma viride* (Moss et al. 1975). Other lactones present in *Camponotus* species are synthesised from an acetate unit as starter

molecule and two or three propanoate (methylmalonyl) units (Bestmann et al. 1997). Another closely related compound, supellapyrone (5-(2,4-dimethylheptanyl)-3-methyl-2*H*-pyran-2-one) (**43**), was reported from the cockroach *Supella longipalpa* and is used as a male-attracting long range sex pheromone (Charlton et al. 1993).

*Acetals*

Multistriatin (**44**), a bicyclic acetal, was identified as pheromone component in the bark beetle *Scolytus multistriatus* (Pearce et al. 1975; Francke and Schulz 1999) and might be of polyketide orgin as suggested from the structure and the origin from propanoate (Francke et al. 1995; Phillips et al. 1985). The spiroacetal pheromone chalcogran released by the bark beetle *Pityogenes chalcographus* (Francke et al. 1977) was earlier supposed to be produced by three propanoate units and was suggested to represent a polyketide (Vanderwel and Oehlschlager 1987). But from the structure it is more likely that chalcogran is formed from five acetate units and might have a long-chain unsaturated fatty acid as a precursor (Francke et al. 1995; Francke and Kitching 2001). In similar spiroacetals from plants, the production of these components from a fatty acid was shown (Francke and Kitching 2001).

Therefore, condensation of carboxyl units to a chain does not provide a hint whether this chain is subjected to the fatty acid pathway using FAS as enzymes or to the polyketide pathway using PKS. What is known about these pathways, their enzymes and genes, where are similarities, where are differences?

## Polyketide synthases (PKS) versus fatty acid synthases (FAS)

The genes encoding PKS used to produce polyketides are known in several organisms like bacteria, fungi and plants. But, so far no PKS genes have been detected in the genome of an animal, neither in insect nor other arthropod genomes. However, the widespread distribution of PKS genes suggests that these genes might also be present in insects. Biosynthetic pathways leading to polyketides show an analogy to the well known formation of long chain fatty acids using FAS. The finding that a substance is produced *via* an acetate/ malonate pathway is not enough to separate both pathways, since these

precursors can be used by both FAS and PKS. Therefore, knowledge on FAS and their relatedness to PKS will be summarised below.

*Architecture and mode of action of FAS*

With respect to architecture of genes and enzymes, two major types of FAS are distinguished. Their classification corresponds to the one of PKS as described later. Type I FAS are multifunctional polypeptides which carry their required enzymatic activities as domains on a large multifunctional polypeptide and are therefore often called megasynthase (Hopwood and Sherman 1990; Smith 1994; Asturias et al. 2005). This type of FAS is ubiquitous in animals and fungi. In animals, FAS are organised as two polypeptide chains with each chain containing seven discrete domains with respect to their enzymatic activity (Smith 1994). Two models aim to describe the orientation of these two polypeptide chains as reported for mammalian FAS. The older one suggested a parallel head-to-tail orientation of both monomers, but recent structural analyses supported a coiled orientation of both monomers forming the protein dimer (Asturias et al. 2005; Smith 2006). There are some differences between fungal and animal type I FAS. Animal FAS are organised as homodimer, meaning that the FAS consists of two identical multifunctional polypeptide chains, whereas fungal FAS are constructed from two nonidentical polypeptides with another organisation of the different domains on both subunits (Hopwood and Sherman 1990; Smith 1994, 2006). Also recent structural analyses using X-ray crystallography with a high resolution revealed a different architecture of type I FAS in fungi and animals (mammals) (Jenni et al. 2006; Maier et al. 2006; Smith 2006). Furthermore, some biochemical differences are described between fungal and animal type I FAS. Both use different acyltransferase domains (single active site in animals and two separate transferases in fungi), different cofactors in the enoyl reductase (flavin mononucleotid in fungal FAS and no cofactor in animal FAS) and have another termination reaction after chain assemblage (termination by transfer to CoA in fungi or hydrolysis by thioesterase in animals) (McCarthy and Hardie 1983; Jenke-Kodama et al. 2005).

In contrast to the domain organisation of type I FAS, each catalytic site of type II FAS known in bacteria is on a separate, mostly monofunctional, independent polypeptide (Hopwood and Sherman 1990; Hopwood 1997; Schweizer and Hofmann 2004). Additionally, type II FAS are found in eukaryotic organelles, such as mitochondria and

chloroplasts (Schweizer and Hofmann 2004). Even though type II FAS are ubiquitous in bacteria, some bacteria of the *Actinomycetales* have additionally a type I FAS that is typical for eukaryotes (Schweizer and Hofmann 2004; Jenke-Kodama et al. 2005).

The different catalytic domains or enzymes of type I FAS in animals are similar to those in other organisms (Schweizer and Hofmann 2004). Seven catalytic domains have been described for animal type I FAS: malonyl/ acyltransferase (MAT), ketosynthase (KS), acyl carrier protein (ACP), ketoreductase (KR), dehydratase (DH), enoylreductase (ER) and thioesterase (TE) (Smith 1994; Asturias et al. 2005). The fatty acid is produced by an assembly line process. Chain building is initiated by condensation of an acetate and malonate unit. The acetate unit is attached to the ketoacyl synthase (KS) through a thioester linkage and functions as starter, the malonate unit is activated by ACP. The activating process is catalysed by MAT. During this condensation step, one carbon atom is eliminated as $CO_2$ so that the resulting carbon chain is elongated with an acetate unit in each elongation step. The β-keto group is now reduced to the hydroxy group by the activity of KR, while DH reduces the carbon-hydroxy unit to an enoyl system. Finally, the unsaturated chain is reduced to the saturated alkyl component. This cycle is done several times till the fatty acid has reached its final length. In animal FAS, a thioesterase (TE) controls the length of the growing carbon chain, and the fatty acid is released from ACP (Hopwood and Sherman 1990; Katz and Donadio 1993; Hopwood 1997, Schweizer and Hofmann 2004; Maier et al. 2006). Both FAS type I and II act iteratively; every enzymatic domain or separate protein is used in each elongation step to build the growing carbon chain (Schweizer and Hofmann 2004).

*Architecture of PKS and comparison to FAS*

As will be outlined below, PKS genes encode domains within modules or distinct enzymes catalysing biosynthetic steps that can resemble those of fatty acid synthesis, and others that differ. PKS are subdivided from a historical point of view into different classes according to the architecture of their biosynthetic enzymes resembling the classes of FAS described above (Hopwood 1997; Staunton and Weissman 2001; Shen 2003). Three major types of PKS are described (Katz 1997; Khosla 1997; Khosla et al. 1999; Moore and Piel 2000; Rawlings 2001; Staunton and Weissman 2001; Austin and Noel 2003; Hill 2005).

Type I PKS have been found in a huge number of bacteria like *Streptomyces* species or *Saccharophylospora erythraea* and fungi like *Aspergillus* species. The compounds produced by type I PKS range from relatively simple compounds like 6-methylsalicylic acid to complex substances like the macrolide polyketide rapamycin (Staunton and Weissman 2001). Type I PKS of bacteria are usually multifunctional enzymes like type I FAS. In contrast to type I FAS (acting iteratively), bacterial type I PKS are organised in modules acting noniteratively, i.e., there are multiple sets of domains, and the number of modules mostly refers to the number of acyl ($C_2$) units incorporated into the product (Staunton and Weissman 2001; Shen 2003). In contrast to bacterial type I PKS, the type I PKS usually found in fungi are monomodular and iteratively acting PKS like type I FAS. A well studied example of this monomodular fungal PKS is the 6-methylsalicylic acid synthase (6-MSAS) producing 6-methylsalicylate and in subsequent steps the derivatives like *m*-cresol (Birch et al. 1955; Beck et al. 1990; Staunton and Weissman 2001). 6-Methylsalicylate and its derivatives are frequently described as defensive substances and pheromones in insects as mentioned above. Type I and type II PKS both contain a similar set of enzymes or catalytic domains as described for the FAS, but due to the synthesised products some of the reducing enzymes (KR, DH and ER) might be missing (Kroken et al. 2003; Jenke-Kodama et al. 2005). Therefore, the so called minimal PKS for type I PKS consists of the ketosynthase (KS), the acyltransferase (AT) and the acyl carrier protein (ACP), whereas the other enzymes essential in fatty acid synthesis might be lacking (Hopwood 1997; Khosla et al. 1999). Two variations for the modular type I PKS exist, one with the AT domain in the module (*cis*-AT) and the other without the AT domain in the module. The AT domain is not incorporated into the module but distinctly associated with the module (*trans*-AT) (Piel et al. 2004b; Jenke-Kodama et al. 2005).

Type II PKS are only found in bacteria producing aromatic compounds, like e.g., actinorhodin, and have especially been studied in *Streptomycetes* (Khosla et al. 1999). Type II PKS resemble type II FAS and possess their enzymatic active sites on separate, normally monofunctional polypeptides (Shen 2003). The minimal configuration of type II PKS consists of the KS, the chain length factor (CLF) and the ACP (Hopwood 1997; Khosla et al. 1999; Rawlings 1999). The CLF from type II PKS is not found in FAS and is named after its function as enzyme controlling and determining the length of carbon chain elongation. CLF is also named KSβ because of its sequence homology to KS. The "normal" KS in PKS is therefore named KSα (Hopwood 1997; Jenke-Kodama et al. 2005).

Enzymes of PKS type III have intensively been studied especially in plants, but they have recently also been found in bacteria and fungi (Moore and Hopke 2001; Austin and Noel 2003; Saxena et al. 2003; Seshime et al. 2005). They calatyse the biogenesis of a wide range of plant secondary compounds like chalcones or flavonoids. Sometimes they are referred to as chalcone synthase (CHS) like PKS. Type III PKS enzyme complexes have a homodimeric architecture only containing the iteratively acting KS. All other enzymes present in FAS are lacking (Moore and Hopke 2001; Austin and Noel 2003). Both types of FAS and both type I and type II PKS do not use acyl CoA directly as substrate. The enzyme ACP is used in these synthases to activate acyl CoA prior to condensation steps. However, in contrast to these FAS and PKS types, type III PKS act independent of ACP, and acyl CoA is used directly as substrate (Austin and Noel 2003; Shen 2003).

Some enzymes encoded by PKS genes have no FAS counterparts. In both type I and type II PKS, enzymes like aromatases or cyclases might be necessary for polyketide production (Hopwood 1997), but such enzymes are not known for FAS complexes. Furthermore, differences in FAS and PKS domain architecture are known. For example, the TE domain in animal type I FAS is monomeric, whereas the TE domains associated with the terminal module in PKS are dimeric (Smith 2006). Such different structures might cause different interactions within the three dimensional domain complex (Smith 2006).

*Classification of PKS types and its problems*

Recent studies demonstrated that some PKS produce polyketides (aromatic and non-aromatic) which are not in accordance to the type I - III classification summarised above. Furthermore, some PKS were found to show an architecture and mode of action that do not agree with the former classification of PKS (Shen 2003; Müller 2004). The discussion on PKS classification has been addressed in several reviews and will be condensed here to 4 problems as outlined below (Staunton and Weissman 2001; Shen 2003; Austin and Noel 2003; Müller 2004):

(1) According to the current classification, bacterial type I PKS act noniteratively. However, AviM synthase, which has the characteristic domain structure of a type I PKS, was found in *Streptomyces viridochromogenes* working iteratively (Gaisser et al. 1997; Shen 2003). Moreover, stigmatellin megasynthase, which has been classified as type I PKS, uses a module twice for polyketide biosynthesis, showing that also this PKS acts

iteratively (Gaitatzis et al. 2002; Müller 2004). Therefore, the classification paradigm that bacterial type I PKS act only noniteratively, is no longer tenable.

(2) Another PKS classification paradigm, i.e., bacterial aromatic polyketides are produced *via* enzymes of type II PKS, needs to be rejected on the base of recent results. In *S. viridochromogenes*, the aromatic polyketide orsellinic acid is produced by AviM synthase that has been classified as type I PKS. Also the polyketide produced by the above mentioned type I stigmatellin synthase has an aromatic chromone structure (Shen 2003; Müller 2004). Both examples illustrate that aromatic compounds can also be produced by a type I PKS. This is interesting also with respect to insect components with polyketide structure, such as chromones in ant trail pheromones (Brophy et al. 1988; Jones et al. 2004).

(3) Furthermore, the classification paradigm that bacterial II PKS act iteratively does not hold anymore. PKS for macrotetrolide biosynthesis were found to act noniteratively and lack the ACP used to activate the acyl CoA substrate (Kwon et al. 2001; Shen 2003). Such a lack of ACP was previously only reported for type III PKS (Austin and Noel 2003).

(4) Moreover, type III PKS have been first described in plants and their presence was thought to be restricted to plants. However, as mentioned above, type III PKS were also found in bacteria and fungi. For example, 1,4-naphthoquinone flaviolin is produced by the filamentous bacterium *Streptomyces griseus* with the help of a chalcone-synthase-like-protein which belongs to type III PKS (Funa et al. 1999).

These classification problems make it difficult to hypothesise on the base of the polyketide product which type of PKS might be present in the genome of insects.

*Differences between FAS and PKS activities*

As described for the protein architecture, also the mode of action of FAS and PKS shows both similarities and differences. The immediate products of FAS are very simple in their chemical structure with only a long saturated carbon chain. In bacteria, the synthesis of unsaturated fatty acids is sometimes due to the lack of an enoyl reduction leading to a double bond (Hopwood and Sherman 1990). In animals, including insects, FAS synthesise long saturated polyketide chains where double bounds are introduced by the enzymatic activity of post-FAS desaturases (Stanley-Samuelson et al. 1988; Jurenka 2004; Choi et al. 2005).

PKS are able to produce much more complex chemical structures than FAS. This higher complexity may be due to the different molecules used for chain extension. FAS mostly use acetate as primer and malonate as extender units. In vertebrates, branched fatty acids are sometimes produced from isopropyl, isobutyryl or methylmalonate as extenders (Wakil et al. 1983; Stanley-Samuelson et al. 1988; Hopwood and Sherman 1990). However, in *Drosophila*, FAS was shown to be inactive with methyl branched starter and elongation units (Stanley-Samuelson et al. 1988). In contrast to FAS, PKS can use much more complex extenders like different branched carboxylic acids or aromatic and aliphatic rings (Katz and Donadio 1993). Different extender units can be used for each elongation step (Hopwood and Sherman 1990).

A further reason for the more complex structure of PKS products may be due to the fact that the β-keto group on the growing carbon chain has not necessarily to be reduced before the next elongation step, even though reductions of the functional groups to hydroxyl or enoyl groups or even total reduction to an alkyl chain are known (Hopwood 2004). In contrast, when FAS mediate condensation of e.g., acetate and malonate units, the growing carbon chain is usually reduced to an alkyl chain after each elongation step. Thus, PKS producing unreduced or partly reduced carbon chains lack some or all of the enzymes or domains necessary in FAS for reduction (Kroken et al. 2003).

Within a PKS complex, the activity of enzymes for reduction of a polyketide chain can alter after each elongation step (Hopwood 2004). For example, the above mentioned 6-MSAS leading to 6-methylsalicylate works repeatedly catalysing three steps of chain extension, but the reduction level of the keto group of the added acyl unit is different in every step (Staunton and Weissman 2001). Since functional groups of PKS-catalysed products are often not completely reduced in contrast to FAS products, the remaining functional groups trigger cyclisation of the polyketide chain to aromatic compounds, lactonisation or binding to glycosides catalysed by so called post-PKS tailoring enzymes (Rix et al. 2002). Side chains or other functional groups like keto or hydroxy groups often also create additional stereocenters during polyketide synthesis (Katz and Donadio 1993; Khosla et al. 1999).

*Interaction of PKS with FAS or other enzymes and fatty acid synthesis by PKS*

PKS and FAS may "cooperate" to produce a polyketidic compound. An example for this "cooperation" is provided from studies on the biosynthesis of a fungal natural product. Norsorolinic acid, a precursor of the *Aspergillus* metabolites sterigmatocystin and aflatoxin, is biosynthesised by both type I PKS and type I FAS (Schweizer and Hofmann 2004). Norsorolinic acid is composed of an anthraquinone-like polyketide with an hexanoic acid side chain. *Aspergillus* strains producing norsorolinic acid possess two types of functionally different FAS (Watanabe et al. 1996; Woloshuk and Prieto 1998). While one FAS is responsible for fatty acid production for the primary metabolism, the other so called sFAS assembles the side chain of norsorolinic acid. The regular FAS activity cannot be replaced by sFAS (Brown et al. 1996; Schweizer and Hofmann 2004). The biosynthesis of norsorolinic acids starts with the synthesis of the hexanoic acid by sFAS activity, and this fatty acid is used as primer unit for the anthraquinone-like polyketide synthesis (Schweizer and Hofmann 2004).

Also in bacteria, interactions of PKS and FAS are known. In most type II PKS, a malonyl/ acyltransferase (MAT/ AT) is absent, while present in FAS and type I PKS. In *Streptomyces*, it was shown that MAT from FAS is used by FAS and surprisingly also by bacterial type II PKS. Thus, a FAS methyltransferase provides malonyl-ACP extenders for both PKS and FAS (Revill et al. 1996; Khosla et al. 1999; Florova et al. 2002). Therefore, MAT provides a link between FAS and PKS in the same organism and shows the close relatedness between both synthases (Revill et al. 1996). MAT is the only enzyme used by both synthases. Mutations of other minimal PKS genes result in loss of polyketide production, since FAS enzymes cannot replace the missing function of other enzymes encoded by mutated PKS genes (Revill et al. 1996; Florova et al. 2002). This incompatibility protects both synthases from further "cooperations" when expressed simultaneously in the same organism. In *Streptomyces coelicolor* type II PKS ACP has been shown to catalyse the transfer of malonate to type II FAS and other PKS ACPs in the same organism (Arthur et al. 2006).

PKS also can interact with nonribosomal peptide synthetases (NRPS) which catalyse the assembly of complex polypeptides often known for their pharmaceutical activity (Schwarzer and Marahiel 2001; Mootz et al. 2002). Like PKS, NRPS are organised in modules comprising a multifunctional enzyme complex (Mootz et al. 2002). Both

synthases form mixed biosynthetic clusters (Mootz et al. 2002). This clustering can either be as distinct enzymes with separate polypeptides for PKS and NRPS associated together or with both synthases directly connected as one polypeptide chain (Du and Shen 2001, Du et al. 2001; Schwarzer and Marahiel 2001; Mootz et al. 2002). For example, the above mentioned defensive polyketide pederin from endosymbionts associated with staphylinid beetles is produced by such a NRPS/ PKS cluster (Piel 2002). The pederin cluster consists of two NRPS modules and 10 PKS modules with the NRPS modules integrated between the PKS modules (Piel 2002; Shen 2003). However, NRPS do not only interact with PKS, but also with FAS. For example, biosynthesis of the lipopeptide mycosubtilin is catalysed by clusters of NRPS and FAS enzymes (Duitman et al. 1999).

In higher eukaryotic organisms, polyunsaturated fatty acids (PUFA) are, for example, known as essential membrane components in the brain or as precursors for signal molecules like prostaglandins (Metz et al. 2001; Kaulmann and Hertweck 2002, and references therein). These PUFA are produced from saturated fatty acids synthesised by a FAS and subsequent aerobic desaturation reactions (Sprecher et al. 1995; Metz et al. 2001). In the bacterium *Shewanella*, the essential building units for PUFA are acyl-CoA and malonyl-CoA like in the normal fatty acid synthesis by FAS, but 11 regions were identified of which 8 were strongly related to PKS, and only the remaining 3 regions showed a homology to FAS proteins (Metz et al. 2001; Ratledge 2004). In general, biosynthesis of PUFA by bacteria differs from eukaryotic PUFA biosynthesis by two factors: (1) While eukaryotes use FAS for PUFA biosynthesis, bacterial PUFA biosynthesis is catalysed mainly by PKS, most likely a bacterial modular type I PKS (Metz et al. 2001). (2) Bacterial PUFA biosynthesis takes place at anaerobic conditions as they are typical for the polyketide pathway (Metz et al. 2001; Ratledge 2004), while eukaryotic PUFA biosynthesis needs oxygene. The eukaryotic marine protist *Schizochytrium* has also been shown to use PKS for PUFA production (Metz et al. 2001). Homology between prokaryotic and eukaryotic PUFA PKS suggests a horizontal gene transfer from prokyarotes to eukaryotes (Metz et al. 2001). These findings clearly point out that also unsaturated fatty acids can be produced by PKS.

Thus, different synthases can work hand in hand to produce very complex and diverse compounds. Especially the biogenesis of PUFA elucidate that knowledge of only the starter and extender units forming a substance might be misleading when concluding from

this information on the biosynthetic pathway. The studies outlined above show the need for further analyses of the enzymes involved in biosynthesis of insect defensive chemicals and pheromones in insects.

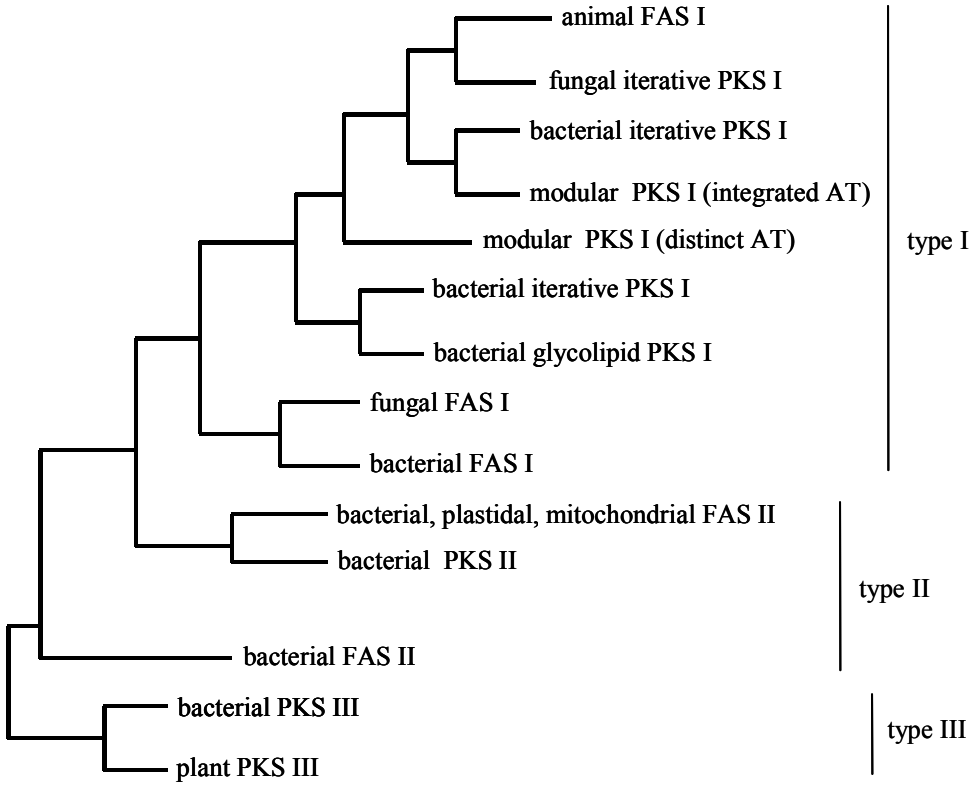


**Figure 3.** Schematic phylogenetic relationship of different types of FAS and PKS (not scaled) (summarised and modified from Jenke-Kodama et al. 2005; Moffitt and Neilan 2003).

*Evolution of FAS and PKS*

Taking into account the similar architecture of PKS and FAS complexes with the same set of enzymes or enzymatic domains and the similar biosynthetic abilities, a common evolutionary origin of these enzymes has been suggested (Hopwood and Sherman 1990, Kroken et al. 2003; Jenke-Kodama et al. 2005). First, a possible evolutionary scenario for FAS will be outlined, and second, hypothetical evolution of PKS from FAS will be discussed (compare Fig. 3).

Type I FAS show the same set of enzymatic domains like the monofunctional proteins of type II FAS, and both types seem to be evolutionary closely linked (Smith 1994). During evolutionary processes, a fusion of genes encoding the monofunctional proteins is most likely (Smith 1994; Hopwood 1997). This hypothesis was supported by findings showing

that animal type I FAS do not loose their function after proteolytic cleavage of distinct domains (Rangan and Smith 1996; Hopwood 1997). In *E. coli*, a cluster of genes encoding distinct monofunctional polypeptides similar to the arrangement in animal FAS was found, and two of these genes in this cluster, the ketoreductase (KR) and the ACP, are co-transcribed which might be a hint for the evolution of fused genes in prokaryotes (Rawlings and Cronan 1992; Smith 1994). The detection of fused prokaryotic genes encoding a multifunctional PKS with the same architecture of the enzymatic domains as in *E. coli* FAS supported the considerations that fusion of distinct FAS genes occurred first in prokaryotic organisms, and then was inherited to eukaryotes (Smith 1994). However, whether animal and fungal type I FAS evolved from ancestral prokaryotic type II FAS with distinct proteins, this is still an open question (Jenke-Kodama et al. 2005).

Phylogenetic analyses of different types of PKS and FAS demonstrated their close relatedness (Fig. 3). Type II FAS from bacteria and plants and type II PKS from bacteria are clearly distinct from type I PKS and type I FAS (Jenke-Kodama et al. 2005). As suggested by the similar architecture, type I PKS from bacteria and fungi build a major cluster. This clade forms a sister clade to animal type I FAS (Kroken et al. 2003). Type I PKS and type I FAS are grouped together, and they form a sister clade to type II PKS (Kroken et al. 2003, Jenke-Kodama et al. 2005). An independent evolution of animal and fungal FAS was suggested because of their different domain organisation and biochemistry. Therefore, fungal type I FAS are suggested to have evolved from bacterial type II FAS and to have linked proteins, while animal type I FAS and type I PKS seem to have a common ancestor (Jenke-Kodama et al. 2005).

## Conclusions: How to search for PKS in insects?

Phylogenetic analyses of PKS and FAS are usually made with sequences from the KS domain (Kroken et al. 2003; Moffitt and Neilan 2003; Jenke-Kodama et al. 2005). Therefore, all conclusions are based only on this sequence, while other findings might be obtained when analysing other domains or enzymes (Jenke-Kodama et al. 2005). However, search for PKS genes in insects are recommended to be conducted with sequences from the KS region since it is the only enzyme present in all three types of PKS (Shen 2003). Moreover, sequences of KS genes are highly conserved in PKS and FAS when comparing

to the other enzymatic domains or distinct enzymes (Hopwood 1997). This fact facilitates the design of primers binding to several possible KS genes.

The suggested common origin of animal type I PKS and type I FAS provides an argument to search in insects for PKS of type I, since animals possess type I FAS. Type II PKS are almost restricted to bacteria so far and thus, might be unlikely in insects (but see below for horizontal transfer). The only PKS type detected in higher eukaryotic organisms are type III PKS. They have especially been detected in plants (Austin and Noel 2003). One special type of plant type III PKS is, for example, the 2-pyrone synthase (2-PS) producing pyrones (Austin and Noel 2003). Similar pyrones have also been found in several ant species (see above). Thus, taking into account the similarity of polyketides in plants and insects, some insect polyketides might be produced by type III PKS.

When considering the possibility of horizontal transfer of PKS genes from bacteria, fungi or plants to polyketide containing insects, those PKS types need consideration which are present in the organisms associated with the insect species studied. A phylogenetic analysis of fungal type I PKS demonstrated that the resulting phylogenetic tree can be correlated with the chemical architecture of the produced polyketides (Kroken et al. 2003) meaning that PKS producing similar polyketides are grouped together. Therefore, when assuming a horizontal transfer of PKS genes to insects, a first possible step for PKS search in insects might be the search for PKS sequences from the associated organisms that produce the same or similar polyketides.

Horizontal transfer of PKS genes from one organism to another has been suggested in several studies. Transfer of PKS genes from bacteria to fungi has been suggested by Kroken et al. (2003) when they found that type I PKS genes encoding 6-MSAS for the production of 6-methylsalicylic acid in fungi formed a cluster within the bacterial type I PKS clade. As described above, 6-methylsalicylic acid and derivatives are also found in numerous insect species. Since insects are usually closely associated with microorganisms, horizontal gene transfer between these organisms cannot be excluded. Therefore, screening for PKS in insects releasing 6-methylsalicylic acid and derivatives should focus on the respective fungal sequences encoding 6-MSAS. The opposite situation where a PKS gene was most probably horizontally transferred from a fungus to a bacterium, was recently shown by Jenke-Kodama et al. (2005). They found a bacterial PKS in a group of fungal

PKS. Horizontal transfer of type III PKS from plants to fungi has been suggested since the majority of fungi possessing type III PKS are plant pathogens (Seshime et al. 2005).

Horizontal gene transfer both from fungi and from bacteria to insects is known for genes other than those encoding PKS. For example, the adzuki bean beetle *Callosobruchus chinensis* contains the *wsp* gene from the α-proteobacterium *Wolbachia* in its genome (Kondo et al. 2002). Treatment with antibiotics could kill the bacteria, but had no effect on the presence of the *wsp* gene as revealed from PCR. Quantitative PCR analysis could demonstrate that the genome fragment of *Wolbachia* is associated with the X chromosome (Kondo et al. 2002). Horizontal gene transfer from fungi to a beetle was shown by Shen and coworkers. The rice weevil *Sitophilus oryzae* harbours a polygalacturonase gene from a fungus in its genome (Shen et al. 2003).

When looking for genes encoding PKS that catalyse the production of 1,8-dihydroxylated anthraquinones, fungi might produce these components by fungal type I PKS, and plants might biosynthesise these polyketides by their typical type III PKS. Fungi, plants and Galerucini beetles fold the octaketide chain by the same mode to gain 1,8-dihydroxy-3-methyl-anthraquinone (= chrysophanol). Bacteria use another folding mode (Thomas 2001; Bringmann et al. 2006). These findings suggest that Galerucini leaf beetles use enzymes with similarities to plant type III or fungal type I PKS, but not to type II PKS detected only in bacteria so far. Since host plants of Galerucini do not contain anthraquinones, a horizontal transfer of plant PKS genes to the insects seems unlikely. However, it cannot be excluded that a Galerucini ancestor fed on anthraquinone containing plants and gained the respective genes from its food. Nor can horizontal gene transfer from fungi producing polyketides to Galerucini be excluded.

The detection of insect genes encoding PKS will remain a demanding task as long as the genome of the insect species studied has not been sequenced. However, the ideas and facts outlined above might help to develop a systematic search pattern which could accelerate successful detection.

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