ANNUAL REVIEWS

Annual Review of Entomology

Chemical Ecology, Biochemistry, and Molecular Biology of Insect Hydrocarbons

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Annu. Rev. Entomol. 2021. 66:45–60

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-031620-071754

Keywords

semiochemicals, stereochemistry, oenocytes, elongation, fatty acid synthase, fatty acyl-CoA reductase, alcohol oxidase/aldehyde decarbonylase

Abstract

Insect cuticular hydrocarbons (CHCs) consist of complex mixtures of straight-chain alkanes and alkenes, and methyl-branched hydrocarbons. In addition to restricting water loss through the cuticle and preventing desiccation, they have secondarily evolved to serve a variety of functions in chemical communication and play critical roles as signals mediating the life histories of insects. In this review, we describe the physical properties of CHCs that allow for both waterproofing and signaling functions, summarize their roles as inter- and intraspecific chemical signals, and discuss the influences of diet and environment on CHC profiles. We also present advances in our understanding of hydrocarbon biosynthesis. Hydrocarbons are biosynthesized in oenocytes and transported to the cuticle by lipophorin proteins. Recent work on the synthesis of fatty acids and their ultimate reductive decarbonylation to hydrocarbons has taken advantage of powerful new tools of molecular biology, including genomics and RNA interference knockdown of specific genes, to provide new insights into the biosynthesis of hydrocarbons.

INTRODUCTION

Due to their small size, insects have a larger surface area–to–body volume ratio relative to most animals and, as such, are at increased risk of desiccation (69). The insect wax layer limits water loss through the cuticle and is comprised of a complex mixture of long-chain fatty acids, methyl esters, aliphatic alcohols, aldehydes, ketones, and hydrocarbons. Although hydrocarbons are the dominant class of cuticular lipids (13), they account for less than 0.1% of the total mass of most insects, yet they reduce water permeability of the cuticle by as much as 1,300% (35, 49). Clearly, cuticular hydrocarbons (CHCs) contributed, in part, to the successful invasion of land by early arthropods. Variability in hydrocarbon profiles also led to the adaptive radiation of insects by providing an evolutionary solution to enable insects to thrive in the extreme temperature and moisture regimes characteristic of terrestrial environments. CHCs can vary in structural features including total chain length, number and positions of methyl branches and double bonds, chirality of methyl-branched compounds, and *E/Z* stereochemistry of carbon–carbon double bonds (11, 45, 77) (**Figure 1**). CHCs not only play a central role in protecting insects from environmental stress (47), but also evolved secondary functions as chemical signals (for reviews, see 53, 60). Structural diversity resulting from desaturation, methyl-branching, and chirality has allowed for the increased information content and functional significance of insect CHCs (**Figure 1**). In this review, we introduce the various behaviors mediated by CHCs, including predator–prey interactions; discuss the physical properties of hydrocarbons that make them particularly well-suited as semiochemicals; review the influence of the environment and age on CHC profiles; and discuss the role of chirality in their bioactivity. Finally, we review the biochemical aspects of CHC production.

Behavioral Roles of Cuticular Hydrocarbons

It has become increasingly clear that CHCs mediate a wide variety of behaviors in insects, particularly those that rely on a stable and minimally volatile signal. Among solitary insects, many CHCs function as short-range or contact pheromones that mediate mate recognition, courtship, and mate choice (38, 53). In addition to these functions, CHCs serve important roles in social insects as sex, nest mate, and caste recognition pheromones; as dominance, fertility, and

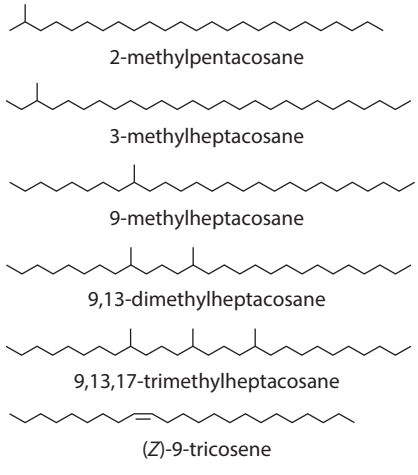

Figure 1

Structures of representative insect cuticular hydrocarbons.

task-specific signals; and as signals for maternal care of offspring (42, 70, 105). These behavioral aspects of CHCs in social insects have recently been reviewed in References 72, 85, and 101.

CHCs mediate species recognition in both solitary and social insects (100). These species-specific profiles are apparently under direct selection, and they can be used to discriminate between closely related species and may serve as chemotaxonomic characters in various social (e.g., 16, 33, 66) and solitary species (e.g., 28, 38, 88, 91). There is also a growing body of evidence that CHCs mediate predator–prey interactions, particularly among hymenopteran predators and parasitoids that use them as kairomones to locate and identify prey (8, 37, 68, 93, 97). For example, female wasps of the species *Cerceris fumipennis* provision their nests with adult buprestid beetles and seldom attack beetles belonging to other families. Female wasps recognize their beetle prey based on the presence of five classes of CHCs (97) and are deterred by dimethyl-branched hydrocarbons, a class of compounds not found in Buprestidae (97). Moreover, footprint hydrocarbons of the stink bug *Nezara viridula* act as a cue by which the egg parasitoid *Trissolcus basalis* locates suitable hosts. In fact, a blend of straight-chain CHCs induced arrestment behavior by female wasps (25). Females can also discriminate between male and female hosts by one male-specific compound—*n*-nonadecane (25). Some insects are able to detect hydrocarbons of their natural enemies and choose to oviposit elsewhere, thereby avoiding predation on their progeny. For example, two hydrocarbons (*n*-heneicosane and *n*-tricosane) associated with the common predatory backswimmer *Notonecta maculate* repel oviposition by the mosquito *Culiseta longiareolata* (98). Nevertheless, specific compounds that influence predator–prey behavior remain largely unknown. Future work focusing on identifying these behaviorally active compounds will undoubtedly provide useful insights toward understanding mechanisms mediating these ecologically important relationships.

Properties of Cuticular Hydrocarbons

The linear *n*-alkanes are ubiquitous in insects and can make up a large proportion of total hydrocarbons in the wax layer (14). For example, in tenebrionid beetles (*Eurychora* sp.), *n*-alkanes dominate total hydrocarbon profiles (75). They can exist primarily as a single component, as in *n*-pentacosane in the American cockroach, *Periplaneta americana* (63), or as a series of *n*-alkanes, such as C_{23}–C_{33} in the housefly, *Musca domestica* (83). Odd-numbered linear alkanes predominate, and the wax layer can contain long series of homologs. For example, a series of odd-numbered hydrocarbons from C_{21}–C_{37} is found in the cuticular profiles of the migratory locust *Locusta migratoria cinerascens* (46). The linear *n*-alkanes are closely packed chains of 21 to 31 or 33 carbons. This arrangement of highly hydrophobic chains makes them ideally suited for waterproofing functions, and crystals from longer chains, which have higher melting temperatures, provide better barriers against water loss.

In *n*-alkanes, increasing chain length strengthens van der Waals forces and leads to stability (47). *n*-Alkanes common to the insect cuticle have melting temperatures between 40° and 60°C (74), but introducing a *cis*- or *Z*-double bond into an alkane decreases melting temperature by approximately 50°C (52). Moreover, a methyl branch (depending on position) can reduce the melting point of an alkane by as much as 30°C because these branched compounds do not pack as tightly as *n*-alkanes (79). The introduction of double bonds and methyl branches also leads to increased structural complexity and, thereby, greater opportunities to encode diverse signal information (47, 51, 79).

Alkenes can vary not only in the stereochemistry of the double bond, but also in the length of the chains on either side of the desaturation. Many insect alkenes have *cis*-double bonds at the 9-position, as in (*Z*)-9-tricosene, the sex pheromone of the housefly, *M. domestica* (18); however, monoenes can be found with double bonds at almost any position along the chain. Alkenes with

up to four double bonds can also be found on the insect cuticle, but they are less common than monoalkenes. Methyl-branched alkanes are common constituents of the cuticular wax layer, with methyl groups on the 2-, 3-, 4-, and 5-position, and then primarily on odd-numbered carbons along the alkyl chain. The greater metabolic cost associated with producing these compounds over *n*-alkanes (82) suggests that they have adaptive value beyond waterproofing, and in fact, they have likely evolved to have functions other than waterproofing. These compounds are typically used as signals in colony and nest mate recognition, act as contact and sex pheromones, and serve as antiaphrodisiacs and kairomones for a variety of taxa (see 53).

A single insect may have a hydrocarbon profile that contains >100 individual compounds, whereas only a few may have signaling functions. The physical properties of hydrocarbons may also influence the abundance and distribution of these compounds within the wax layer of insects (50, 113). It was once assumed that the CHC layer was solid at ambient temperatures (96). It has since been suggested that simple alkane–alkene mixtures of pure compounds neither form mixed crystals nor exhibit melting point depression as expected (48), but rather form biphasic layers at ecologically relevant temperatures. Accordingly, the insect cuticle may simultaneously contain regions of a permeable melted phase of alkene and/or methyl-branched alkanes (e.g., those that serve signaling functions) above a less permeable solid phase of *n*-alkanes (52). Nevertheless, the melting dynamics of multicomponent mixtures is not well understood (48). Recently, Menzel et al. (78) used differential scanning calorimetry and a novel microrheological technique to investigate the melting behavior and viscosity of CHCs in 11 ant species. In all species tested, CHCs were solid–liquid mixtures throughout almost the entire range of temperatures that the insects would experience in their environment. Accordingly, biphasic CHC layers may be adaptive for insects by providing a uniform coating of hydrocarbons to prevent water loss through a range of environmental temperatures. This work not only provides experimental evidence for the phase separation model proposed by Gibbs (48), but also has important implications for understanding how the structure of CHCs within the wax layer influences their function as semiochemicals. In some cases, those compounds that serve as signals are more highly represented on the surface of the wax layer (see 54, 57, 62). Clearly, a better understanding of the physical properties of CHCs and the behaviors that they mediate will shed light on those evolutionary forces that led to their diversification in insects.

Influence of Environment and Diet on Cuticular Hydrocarbon Profiles

There is an overall tendency for insects in warmer climates to produce more saturated CHCs, while those in cooler climates have a higher proportion of unsaturated compounds (102), and profiles can be temporally dynamic. For example, in the harvester ant *Pogonomyrmex barbatus*, high temperatures (approximately 38°C) and low relative humidity (approximately 8%) caused an increase in *n*-alkanes in the CHC profiles of workers, suggesting that warm, dry conditions trigger changes in cuticular chemistry (107). However, dynamic changes in response to varying environmental conditions to reduce water loss may affect CHC-mediated communication (43, 78). Although longer-chain viscous alkanes would better serve the waterproofing needs of insects, they may be less effective than branched and unsaturated compounds as semiochemicals because of their ubiquity and limited information content that can only be coded by chain length. Thus, it appears that an evolutionary tradeoff exists between conflicting selection pressures imposed by the waterproofing functions of CHCs and their roles as semiochemicals at a given temperature.

In addition to their waterproofing functions, CHCs also reflect solar radiation to reduce heat loading (57) and protect insects from other environmental stressors, such as pathogens (55) and pesticides. In fact, a strain of *Anopheles gambiae* that is resistant to pyrethroids and DDT

has a thicker layer of CHCs composed of different relative amounts than do susceptible strains (3), especially on their legs (4). Moreover, silencing of *CYP4G76* and *CYP4G115* (genes that are involved in CHC biosynthesis) in the brown planthopper resulted in a decreased amount of hydrocarbons, which not only rendered the insect more susceptible to desiccation, but also increased the rate of penetration of four insecticides (108). More work is needed on this new proposed role of CHCs in insecticide resistance.

CHCs evolved to allow insects to adapt to harsh conditions, but some species may actually exploit these environmental factors to adaptively degrade CHCs into volatile chemical signals. For example, 10 (*Z*,*Z*)-9,19 dienes in the female spruce sawfly, *Pikonema alaskensis*, are photo-oxidized to the pheromone (*Z*)-10-nonadecenal, which is attractive to males (5). These dienes account for approximately 10% of the total CHCs of females but only 0.1% of those of males (6). Likewise, natural oxidation of unsaturated CHCs in the wheat stem sawfly, *Cephus cinctus*, forms the attractant 9-acetyloxynonanal (26). Photo-oxidation of CHCs has also been implicated in the formation of volatile semiochemicals in a variety of other insect taxa including *Macrocentrus grandii* (103), *Anoplophora glabripennis* (112), and *Drosophila melanogaster* (71). Recently, Hatano et al. (59) demonstrated that nymphs of the American cockroach, *P. americana*, avoided shelters exposed to volatiles originating from the breakdown of CHCs. In particular, exposing CHCs to ultraviolet radiation resulted in the release of 14 compounds detectable by the cockroach antennae, and a blend of 10 of them elicited avoidance behavior, suggesting that they serve as necromones (signals of death) or epideictic (spacing) pheromones. More work is needed to understand the extent and adaptive role of environmental factors in degrading CHCs into volatile semiochemicals.

The composition of CHCs can also be affected by diet, and in the event that these compounds serve as contact pheromones, such changes may affect mate recognition behavior and thus fitness. These diet-induced changes in CHCs may act as prezygotic mating isolation mechanisms among insects feeding on alternative host species, leading to assortative mating and behavioral isolation. For example, in the oligophagous mustard leaf beetle (*Phaedon cochleariae*), males prefer females that feed on their same natal host plant, suggesting that larval diet may influence the CHC profiles of the adults (44). Also, the use of divergent host plants between *P. cochleariae* and *Phaedon armoraciae* results in divergent CHC profiles and assortative mating, while profiles converged and mating became random when they were fed a common host plant (87). Together, these studies demonstrate a host-induced plasticity in the hydrocarbon phenotype and suggest that mate recognition systems are not under as tight a genetic control as was once thought. Components of the larval diet may supply precursors (e.g., plant lipids) for hydrocarbon synthesis or affect enzyme activity involved in de novo biosynthetic processes, and thus lead to divergence in hydrocarbon profiles (44). However, Otte et al. (86) demonstrated that rearing *P. cochleariae* on different blends of fatty acids led to quantitative differences in straight-chain and methyl-branched hydrocarbons, but these changes did not affect mating preference or result in diet-specific assortative mating. Future studies should be directed toward understanding the influence of other host constituents on qualitative and quantitative differences in CHC profiles that lead to assortative mating.

Influence of Chirality on the Bioactivity of Cuticular Hydrocarbons

With the exceptions of 2-methylalkanes or symmetrical monomethylalkanes with the branch exactly in the middle, methyl-branched alkanes that are components of many insect contact pheromones have the potential to be chiral—existing in two or more stereoisomeric forms depending on the number and positions of methyl branches. Very few studies have investigated the role of stereochemistry in contact chemoreception, and most have ignored stereochemistry altogether and instead tested the bioactivity of racemic blends. Recently, Bello et al. (7) conducted

polarimetric analysis of 36 purified monomethyl-branched hydrocarbons from 20 insect species in nine orders and found that all compounds had the (*R*)-configuration, independent of methyl branch position or chain length. This conservation in stereochemistry of methyl-branched hydrocarbons has interesting ramifications for their biosynthesis and provided strong evidence that the unsaturated intermediates during the elongation process are reduced in a stereochemically specific manner.

To date, only a few studies have investigated the role that chirality of methyl-branched hydrocarbon signals may play in courtship and mating behaviors, but it appears that at least some species can discriminate among enantiomers. For example, in the cockroach *Blattella germanica*, (3*S*,11*S*)-3,11-dimethylnonacosan-2-one stimulates courtship behaviors in males at high concentrations (36). When all four stereoisomers were tested at physiologically relevant doses, however, the naturally occurring (3*S*,11*S*)-isomer was the least effective of the four stereoisomers at eliciting courtship responses from males. Males of the brown spruce longhorned beetle, *Tetropium fuscum*, responded more strongly in bioassays to solvent-washed female carcasses treated with (*S*)-11-methylheptacosane than to the (*R*)-enantiomer (99). Males of the eastern larch beetle, *Tetropium cinnamopterum*, only displayed the full suite of mating behaviors when (*S*)-11-methylheptacosane and (*Z*)-9-heptacosene were presented together (99). However, the specific rotation of the synthetic (*S*)-11-methylheptacosane tested by Silk el al. (99) more accurately reflects the (*R*)-enantiomer (see 7), and the absolute configuration of the insect-produced compound has yet to be confirmed. In bioassays with enantiopure synthetic pheromone components, males of the parasitic wasps *Ooencytrus kuvanae* responded most strongly to the combination of (*S*)-5-methylheptacosane and (5*R*,17*S*)-dimethylheptacosane (1), while (*R*)-5-methylheptacosane inhibited attraction when paired with the dimethylalkane. Through digital polarimetry, it was recently determined that the absolute configuration of the most bioactive component of the red-headed ash borer, *Neoclytus a. acuminatus*, contact pheromone (7-MeC_{27}) is *R* (61). In behavioral bioassays, males responded more strongly to the blend of all three (*R*)-configured methyl-branched CHC pheromone components than to (*R*)-7-MeC_{27} alone. In addition, a blend of (*R*)-7-MeC_{27} with the (*S*)-minor components elicited an intermediate response, suggesting that males can discriminate among enantiomers and that unnatural (*S*)-enantiomers are neither entirely inactive nor strongly inhibitory. Together, these studies suggest that insects may biosynthesize the (*S*)-enantiomer of biologically active compounds to differentiate them from the typical (*R*)-enantiomers that dominate cuticular profiles of most insects. Nevertheless, it remains unclear whether the ability to discriminate among the stereoisomers of methyl-branched CHCs is a general phenomenon among insects.

HYDROCARBON BIOSYNTHESIS

In vivo studies with test substrates labeled at specific sites with radioactive or stable isotopes and careful analysis of metabolic products had established the biosynthetic pathways for the major hydrocarbon components in insects (10, 60) by the early 1990s. In vivo studies with microsomal preparations provided further insights into how insects produce hydrocarbons. In the past decade, the powerful tools of molecular biology have been increasingly applied to understanding hydrocarbon biosynthesis and the genes involved in producing specific hydrocarbon blends.

Oenocytes Are the Site of Insect Hydrocarbon Biosynthesis

Early work strongly implicated oenocytes as the sites of hydrocarbon biosynthesis (39, 56), which was confirmed using molecular techniques. The last step in hydrocarbon production involves the conversion of aldehydes to hydrocarbons that are one carbon shorter. The *Cyp4g1* gene in

D. melanogaster, which encodes the cytochrome P450 catalyzing the terminal steps in hydrocarbon production, is expressed predominantly in oenocytes (92). Likewise, the fatty acid synthase (FAS) gene that is involved in producing the fatty acid precursor to the 2-methylalkanes in *D. melanogaster* is also localized to the oenocytes (23). The Vontas group (3, 67) used antibodies to CYP4G16 and CYP4G17 to demonstrate that not only were these genes expressed in oenocytes in *A. gambiae*, but, surprisingly, they were localized to the cell membranes of oenocytes.

The location of hydrocarbon synthesis on the cell membrane of oenocytes makes for efficient transfer of the final products to lipophorin transport proteins, without the need for transport proteins in the oenocytes to translocate the apolar hydrocarbons to the surface of the cell. Lipophorins then transport the CHCs to the cuticle and other sites in the insect. It is not known how hydrocarbons are transported across the cuticle, but pore canals undoubtedly play a role. It also appears that traffic can be bidirectional because apolar pesticides can move across the cuticle (2). Furthermore, transport and unloading of CHC cargos can be site specific because, in lepidopterans from families that produce unsaturated hydrocarbon pheromones, the C_{17} to C_{25} hydrocarbon pheromones or pheromone precursors are transported to the pheromone gland, whereas longer-chain hydrocarbons are transported to the surface of the insect to form the cuticular wax layer (65).

Biosynthetic Pathways for Hydrocarbons

The biosynthesis of hydrocarbons can be divided into four distinct steps (**Figure 2**): (*a*) formation of the saturated or unsaturated straight-chain and methyl-branched fatty acid precursors; (*b*) elongation of these fatty acids to very long chain fatty acyl-CoAs; (*c*) conversion of the fatty acyl-CoAs to alcohols; and (*d*) oxidation of alcohols to aldehydes, which are then oxidatively decarbonylated to hydrocarbons by CYP4G enzymes. **Figure 2** shows the pathway for the methyl-branched alkanes. The *n*-alkanes are synthesized without the incorporation of methylmalonyl-CoA, and alkenes are formed by the desaturation of an 18-carbon fatty acid, which is then elongated, reduced to an alcohol, oxidized to an aldehyde, and decarbonylated.

Production of Fatty Acid Precursors

2-Methylalkanes arise from the elongation of the carbon skeleton of either valine (even number of carbons in the chain) or leucine (odd number of carbons in the chain) (9). The FAS gene that produces the *n*-2 methyl-branched fatty acid precursor to the 2-methylalkanes has been identified in *D. melanogaster*. Knockdown of the FAS gene ($FASN^{CG3524}$), one of three FASs in *D. melanogaster*, markedly decreased production of 2-methylalkanes (23) but not of *n*-alkanes or alkenes, providing strong evidence that this FAS is required for 2-methylalkane synthesis. An alternate FAS, $FASN^{CG3523}$, is expressed in the fat body but not oenocytes, suggesting that all the enzymes necessary for hydrocarbon production are localized to the oenocytes but that some fatty acids may be imported to oenocytes for *n*-alkane production (111).

The 3-methyl- and internally methyl-branched hydrocarbons all arise from the incorporation of propionate as a methylmalonyl-CoA derivative during chain elongation (for a review, see 10). In vivo studies in *P. americana* showed that the labeled carbon from [1–^{13}C]propionate was found exclusively in the 4-position of 3-methylpentacosane, as demonstrated by carbon-13 nuclear magnetic resonance (34), showing that it was incorporated as the second group added to the growing chain. If the propionate was added toward the end of synthesis by elongases, then the labeled carbon would have appeared in the 2-position. Similar results tracing the incorporation of [1–^{13}C]propionate into mono- and dimethylalkanes in *M. domestica* (31) and *B. germanica* (19) by mass spectrometry indicated that the methyl group was put on early in chain synthesis, rather

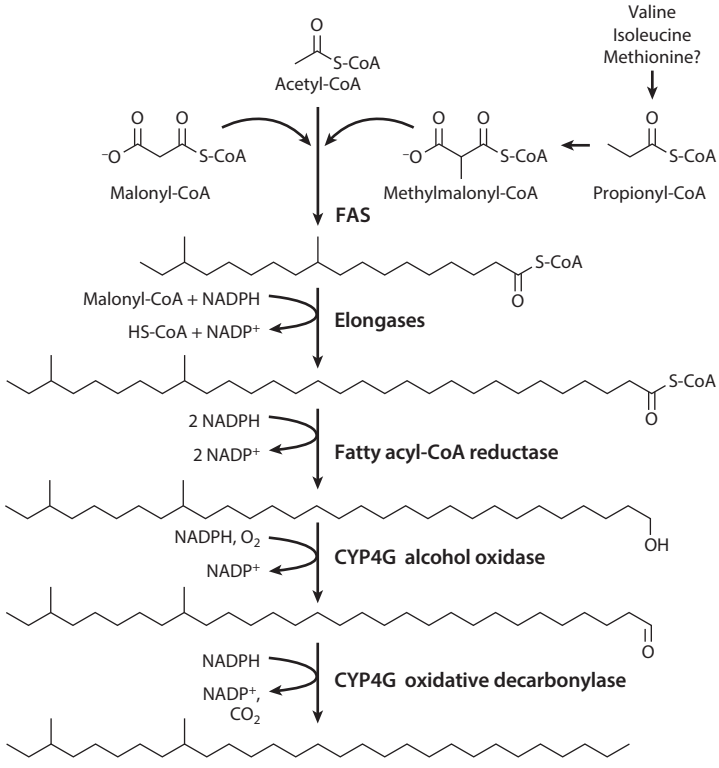

Figure 2

Biosynthetic pathways for internally methyl-branched hydrocarbons in insects. Abbreviation: FAS, fatty acid synthase.

than toward the end of the process, thus implicating a FAS rather than an elongase in the methyl group's synthesis. The propionate group for methylalkanes, inserted as a methylmalonyl-CoA unit, is derived from valine, isoleucine, or methionine in the housefly (31) in place of a malonyl-CoA at specific points during chain elongation (**Figure 2**). Small amounts of methyl-branched fatty acids with the appropriate methyl branch positions have been found in *B. germanica* (64) and *M. domestica* (15), supporting this biosynthetic route.

A comparison of a soluble and a microsomal FAS in *B. germanica* (64) and *M. domestica* (56) showed that the microsomal FAS was more effective than the soluble FAS in incorporating methylmalonyl-CoA into the growing fatty acid chain. This result suggested that there may be a specific FAS that inserts methylmalonyl-CoA at specific points during the formation of the methyl-branched fatty acid precursors to the internally branched hydrocarbons. Very recent work by Pei et al. (90) identified three functional fatty acid synthase genes from *B. germanica* and, using RNA interference (RNAi) knockdown for each one, showed that knockdown of *BgFas1* decreased methyl-branched hydrocarbon production. Similarly, *Rhodnius prolixus* has three FASs, and knockdown of FASN3 decreased methyl-branched hydrocarbon production compared to controls and decreased desiccation resistance (80). Work is needed to determine the subcellular location of the proteins from these FAS genes to determine whether they are membrane bound. It has been proposed that the evolution of the oenocyte-specific fatty acid synthases may have led to different

isomers of methyl-branched hydrocarbons, with the methyl branch position shown to differ by two carbons in two sympatric grasshopper species (41).

The desaturases that are involved in alkene production have been studied in *D. melanogaster*. Desaturase 1 (*desat1*) in *D. melanogaster* uses both palmitic acid and stearic acid to form palmitoleic ($\Delta 9$ $C_{16:1}$) and oleic ($\Delta 9$ $C_{18:1}$) acids. This gene is expressed in both the fat body and oenocytes and appears to play a role in general lipid metabolism and in hydrocarbon production (110). A *desat2* converts myristic ($C_{14:0}$) to myristoleic ($\Delta 9$ $C_{14:1}$, an *n*-5 double bond) acid in flies that produce a 5,9-alkadiene (27). A second desaturation is required for females that produce 5,9- and 7,11-dienes. RNAi was used to study this gene (22), which was found only in females and thus was named *desatF* (for a review, see 110).

Elongation

The regulation of chain length to produce the specific blends of hydrocarbons that are used for chemical communication in individual species appears to reside in the microsomal fatty acyl-CoA elongase reactions, although the fatty acyl-CoA reductases (FARs) might also play a role (104, 106). Fatty acyl-CoA elongases catalyze the condensation of malonyl-CoA and a fatty acyl-CoA, and three additional enzymatic steps are required to reduce the ketone to an alcohol, followed by dehydrogenation and reduction. In other taxa, the first step controls the chain length specificity (58), and it is assumed that the same holds true for insects. The *D. melanogaster* genome contains genes for 19 elongases, with only a few of them characterized to date (84, 110). When the elongase *eloF* from *D. melanogaster* (22) was expressed in yeast, it was able to elongate both saturated and unsaturated fatty acids up to C_{30}. Silencing of *eloF* resulted in a dramatic decrease in C_{29} dienes and an increase in C_{25} dienes, along with a concomitant decrease in courtship and mating activities of male flies. Expression of *eloF* was much higher in females than males. The brown planthopper, *Nilaparvata lugens*, has 20 elongase genes, nine of which are essential to survival (73). None of the elongases involved in hydrocarbon production have been expressed and assayed, and we do not know the chain length specificity of any elongase.

Acyl-CoA Reductases

FAR activity was demonstrated in microsomes of integument-enriched tissue from *M. domestica*, and the aldehyde intermediate was trapped with hydroxylamine (95), suggesting that the aldehyde was derived directly from the fatty acyl-CoA. However, this function has been reassessed in light of the finding that all insect FARs examined to date reduce the acyl-CoA to a primary alcohol, and CYP4G proteins both oxidize alcohols to aldehydes and then oxidatively decarbonylate aldehydes (76). It now appears that FARs reduce the acyl-CoA to alcohols. An expressed FarO from *D. melanogaster* reduces 24:0-CoA to the corresponding C_{24} alcohol (24), but its chain length specificity was not examined.

CYP4Gs Oxidize Alcohols to Aldehydes, Which Are Then Decarbonylated to Hydrocarbons

Insects use an aerobic mechanism involving cytochromes P450 for the oxidative decarbonylation of aldehydes to hydrocarbons, in contrast to plants and algae, which use an anaerobic mechanism (30). This difference is consistent with plants appearing on land much earlier than insects. During that time, plants had to contend with a relatively low-oxygen environment (89), whereas insects developed an aerobic mechanism for hydrocarbon production consistent with higher oxygen levels when their ancestors adapted to terrestrial habitats. It is now clear that a cytochrome P450 is

involved in the alcohol to aldehyde conversion, and the subsequent conversion to hydrocarbon and carbon dioxide in a process that requires molecular oxygen and NADPH (81, 94, 95).

Full confirmation of the oxidative decarbonylation system proposed in insects awaited the cloning, expression, and characterization of the enzymes involved. Integument-enriched cytochrome P450 cDNAs from *M. domestica* were isolated (92). One of these, CYP4G2, has 71.7% amino acid identity and 81.8% similarity to its ortholog, CYP4G1, from *D. melanogaster*. The relatively high ratio of cytochrome P450 reductase (CPR) to CYP4G1 in *D. melanogaster* oenocytes (92) suggested the possibility that CPR was needed for CYP4G2 to properly fold. Expression of the CYP4G2–CPR fusion protein yielded an enzyme that, in the presence of carbon monoxide, absorbed light energy at 450 nm and converted long-chain aldehydes to alkanes (92). Silencing *CYP4G1* in oenocyte cells of *D. melanogaster* resulted in a dramatic decrease in hydrocarbon production. In addition, the expressed CYP4G2, as a fusion protein with CPR, converted both tritium- and deuterium-labeled octadecanal to heptadecane (92). Thus, there is strong evidence that CYP4G2 and CYP4G1 are the cytochromes P450 involved in hydrocarbon biosynthesis. To date, CYP4G2 (housefly; 92), CYP4G16 and CYP4G17 (*A. gambiae*; 3, 67), CYP4G11 (*Apis mellifera*; 17), and CYP4G55 and CYP4G56 (*Dendroctonus ponderosae*; 76) have been expressed as fusion proteins with housefly CPR and shown to be oxidative decarbonylases. All insects whose genomes have been sequenced have one or more CYP4Gs that are involved in catalyzing the last step in hydrocarbon production (40). The occurrence of only one or two CYP4Gs in most insects suggests that they have a broad chain-length specificity, as does the observation that CYP4G55 and CYP4G56 can use C_{10} and C_{18} alcohols and aldehydes as substrates (76).

MacLean et al. (76) demonstrated that CYP4Gs 55 and 56 from the bark beetle *D. ponderosae* both oxidized alcohols to aldehydes and decarbonylated aldehydes to hydrocarbons that were one carbon shorter. This ties together the activity of FARs (which produce alcohols) and the CYP4Gs. The CYP4Gs should now be referred to as alcohol oxidases/aldehyde decarbonylases. This same enzyme apparently can oxidize alcohols to aldehydes and then break a carbon–carbon bond to remove the carbonyl group as carbon dioxide. CYP4Gs are the only known cytochromes P450 that cleave carbon–carbon bonds to yield a saturated alkane (40).

Earlier studies using microsomal fractions from the housefly assumed that the oxidative decarbonylase activity was associated with the endoplasmic reticulum (95). The Vontas laboratory (3, 67) showed that CYP4G16 from *A. gambiae* is present on the cell membrane by microscopy using antibodies to CYP4G16. CYP4G16 is attached to the cell membrane at all developmental stages, whereas CYP4G17 is attached to the cell membrane in larval stages but is found throughout the cell in adults. This work should be extended by exploring the subcellular location of these enzymes in other insect species.

RNAi knockdown of CYP4G genes in a variety of insects demonstrated that CYP4G genes are involved in hydrocarbon production and yielded definitive information regarding their role. RNAi knockdown of CYP4G1 in *D. melanogaster* resulted in almost complete inhibition of hydrocarbon production, less resistance to desiccation, and reduction in courting and mating success, thus verifying the dual roles of hydrocarbons in preventing water loss and serving in chemical communication (92). Injection of dsCYP4G122 and dsCYP4G123 in *Tenebrio molitor* resulted in increased susceptibility to desiccation. Moreover, adults of the parasitoid *Scleroderma guani* emerging from *T. molitor* hosts with silenced CYP4G lost the searching behavior that was characteristic of those emerging from control pupae (109). Similar experiments have shown that CYP4G51 in the pea aphid, *Acyrthosiphon pisum* (20); CYP4G102 in the migratory locust, *L. migratoria* (114); CYP4G19 in the German cockroach, *B. germanica* (21); and CYP4G122 and 123 in *T. molitor* (109) all modulate hydrocarbon production in their respective species.

The origin and evolution of the CYP4G subfamily in insects were examined by Feyereisen (40) by comparing 368 sequences from 24 insect orders and 167 species. The active site has been highly conserved, and all sequences have an approximately 44–amino acid chain inserted between the G and H helices. As a result, he concluded that the "detailed evolutionary history of CYP4G genes does not support the 'stability' of these essential genes, but rather a 'revolving door' pattern where their essential function is maintained despite an apparently random birth and death process."

FUTURE DIRECTIONS

Based on the explosion of information on the role of CHCs in chemical communication that has occurred in the past four decades, we expect that new roles for insect hydrocarbons will continue to be found where a relatively nonvolatile signal is needed. Given the wide range of behaviors that hydrocarbons are known to mediate, we expect that other, novel functions have yet to be discovered. The importance of chirality in chemical communication with methyl-branched hydrocarbons is just now being explored, and future work will undoubtedly address this issue more fully. Wicker-Thomas et al. (111) and Dembeck et al. (29) used gene silencing in *D. melanogaster* to provide convincing evidence of the importance of a large number of suspected genes in hydrocarbon production, and future work will almost certainly involve expressing and characterizing many of these genes. As more and more insect genomes are sequenced and techniques such as the use of RNAi, along with expression and cloning of key genes involved in hydrocarbon biosynthesis, are employed, our understanding of these critical processes will undoubtedly increase. Moreover, it appears that CYP4G16 is localized to the cell membrane—a phenomenon that may be ubiquitous among insects and needs to be further explored (3). If the CYP4Gs are attached to the cell membrane, then the subcellular location of the FARs, elongases, and FASs involved in hydrocarbon production also needs to be examined. Exploiting this new knowledge of hydrocarbon production and regulation to advance the management of insect pests is a major challenge facing insect scientists.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Kelsey Tobin, Scott Gula, and Matthew Ethington for their thoughtful comments and thorough reviews of earlier drafts of this review, and two anonymous reviewers for improving the article. Special thanks to Kelsey Tobin for also helping to compile the list of references.

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