

ECOLOGICAL, BEHAVIORAL, AND BIOCHEMICAL ASPECTS OF INSECT HYDROCARBONS*

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■ **Abstract** This review covers selected literature from 1982 to the present on some of the ecological, behavioral, and biochemical aspects of hydrocarbon use by insects and other arthropods. Major ecological and behavioral topics are species- and gender-recognition, nestmate recognition, task-specific cues, dominance and fertility cues, chemical mimicry, and primer pheromones. Major biochemical topics include chain length regulation, mechanism of hydrocarbon formation, timing of hydrocarbon synthesis and transport, and biosynthesis of volatile hydrocarbon pheromones of Lepidoptera and Coleoptera. In addition, a section is devoted to future research needs in this rapidly growing area of science.

INTRODUCTION

In the first major review of the biochemical, ecological, and behavioral importance of hydrocarbons to insects (58) we were able to find 130 papers on the subject. Were we to attempt a complete survey of the literature today, the papers cited would number several thousand. Indeed, the field continues to grow so rapidly that comprehensive coverage is impossible. Although hydrocarbons may be simple in chemical terms, the ways in which insects and other arthropods have evolved to use them for prevention of desiccation, as a barrier to microorganisms, and numerous other biochemical, physiological, and semiochemical functions are far from simple. All the areas covered in the 1982 article (sex pheromones, species and caste recognition cues, epideictic and territorial pheromones, alarm, recruitment and chemical defense, thermoregulatory pheromones, kairomonal cues for parasites, and the biochemistry of hydrocarbon production) have experienced subsequent steady attention. Many of these topics (epideictic and territorial pheromones and

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thermoregulatory pheromones) are not included in this paper, nor are numerous additional studies on the use of hydrocarbons for chemical taxonomy and systematics, physical properties of various hydrocarbon classes, identification methods and analytical techniques, syntheses of novel and enantiomerically pure hydrocarbons, and environmental interactions affecting hydrocarbon production and properties. Discussion of these areas can be found in several articles (40, 41, 54, 76, 77, 82). This review instead focuses on the enormous advances made since 1982 on understanding hydrocarbons as ecological, behavioral, and physiological signals, with particular attention to the social insects, the biochemistry and physiology of hydrocarbon production, and methodological problems in clarifying these issues.

ECOLOGICAL AND BEHAVIORAL ASPECTS

It is becoming increasingly clear that a major function of cuticular hydrocarbons in arthropods is to serve as recognition signals between two or more individuals. One or more components of the complex mixture of hydrocarbons found on the cuticle of almost all arthropods is often the primary chemical cue that answers questions such as: Are you a member of my species? Are you the same gender as me? For social insects, are you a member of my colony? Are you a member of my nest? To which caste do you belong? Are you a queen or perhaps brood? Are you a worker trying to convey to me the need to accomplish a certain task? Are you closely related kin? And for many arthropods that exist as inquilines in the nests of social insects, can you recognize that I am alien?

Species and Gender Recognition

Analysis of many solitary and social insect species have shown that, in general, hydrocarbon profiles tend to be species specific (54). Most of these studies, however, are based on simple comparisons of either qualitative or quantitative differences among taxa (often determined by various multivariate statistical techniques) and not on bioassays that provide a definite link between the hydrocarbons and the behavioral responses by the insects. Equally important as species recognition is gender recognition. Here, too, cuticular hydrocarbons play important roles in many species. Examples are known in which either males or females contain distinctive hydrocarbons not produced by the other gender (tsetse flies: 83, 84, 105; cerambycid beetles: 43). In other cases, the same hydrocarbons are present, but in gender-specific relative abundances (*Drosophila* spp.: 6, 22, 65, 66, 109; parasitoids: 53, 55, 64). Numerous examples are also known, however, in which both genders have the same hydrocarbons and appear to have the same relative abundances of all components (*Drosophila* spp.: 59; hymenopterous parasitoids: 53, 56, 60, 63). Most studies have assessed gender differences empirically rather than through bioassays. In some cases, however, bioassays have been developed that showed unequivocally that the hydrocarbons in question are used by the insects for gender recognition (15, 43, 55).

Nestmate Recognition

Considerable efforts have been made toward understanding the mechanisms of nestmate recognition, and the findings are as complex as the insect societies themselves. Recent major reviews of nestmate recognition considered ants (112), semisocial and social wasps (100), bees (11), and termites (21). Critical to the study of such recognition chemicals (frequently described as Gestalt odor, nest odor, or colony odor) are a large number of methodological issues, which have been reviewed by Vander Meer & Morel (112). A major issue for many studies is the nature of the chemical extracts reputed to contain the nestmate recognition cues, which are in many (but not all) cases assumed to be cuticular hydrocarbons. Nonpolar solvents (e.g., hexane, pentane, and methylene chloride) are not specific for hydrocarbons and readily dissolve other compounds that have a significant portion of their structure as an alkane chain (112). Commonly, solvent extracts are obtained by suspending insects in the solvent from several minutes to an hour or more, enough time to extract most of the cuticular lipids (usually primarily hydrocarbons), most glandular lipids (frequently not hydrocarbons), and internal lipids including those in the hemolymph (large quantities of hydrocarbons and other bound lipids). Furthermore, addition of solvent extracts (whether crude extracts or purified isolated chemicals) back onto a live insect results not only in deposition of extracted lipid but also in the disruption of the native lipids on the test insect and likely initiates physiological processes to replace or metabolize components of this disturbed lipid layer. This problem can be avoided by using inanimate dummies as test vehicles for the chemical extracts, and several researchers have indeed adopted this approach (112). A major disadvantage of this approach, however, is that movement is also an important cue for many social insects, and unless special methods are developed to provide movement to the inert dummy (62), the signal modality perceived by the test organisms is somewhat artificial.

A different type of chemical delivery system was developed by Takahashi & Gassa (106) and offers considerable promise for a variety of recognition bioassays. They extracted two species of Japanese termites (*Reticulitermes speratus* and *Coptotermes formosanus*) with hexane, isolated and purified the hydrocarbons, and suspended the hydrocarbons by ultrasonic agitation in a 2% Triton X-100 solution that was used to topically coat the dorsal surface of a live termite with one worker-equivalent of suspended hydrocarbons. After 15 minutes, either a conspecific or heterospecific worker or soldier was introduced with the treated live termite and the resulting behavior recorded. Control experiments were run with termites treated only with the Triton X-100. Apparently these aqueous suspensions disturb the cuticular surface of treated insects much less than organic solvents do, thus allowing a clean test of the effect of added hydrocarbons in the absence of physiological responses by treated insects. Further work is needed to determine the value of this technique in other species.

Determining whether a chemical cue is perceived as a signal of nestmate recognition is almost always accomplished by laboratory bioassays that measure agonistic responses. These bioassays are critical to testing alternative hypotheses

regarding the nuances of nestmate recognition systems. A variety of bioassays have been developed, yielding variable results (94, 112). Researchers seldom justify their choice of a particular bioassay and rarely compare results using different bioassays. A recent study of the Argentine ant, *Linepithema humile*, compared four bioassays for consistency between replicates, the similarity of results between assays, and the ability to predict whole-colony interactions from bioassay results (94). The six major findings of this study were that (a) scoring methods within all assays were correlated, but some assays were less consistent than others; (b) all assays were not equally likely to reveal aggressive acts during individual trials, but assays that generated the highest aggression scores were ones in which the most ants were involved; (c) ants were most likely to show aggression when tested in bioassays that mimic critical ecological contexts such as competition for food or defense of nest; (d) assays using isolated pairs of ants did not give clear predictions concerning whole-colony interactions over time; indeed, some colonies that fought in bioassays merged when the entire colonies were allowed to interact; (e) there was a weaker correlation between trials within an assay than between assays, suggesting substantial heterogeneity in either chemical cues, perceptive abilities, or aggressiveness of individual colony members; and (f) adequate replication is essential irrespective of the bioassay used. Similar conclusions are likely for aggression bioassays using wasps, bees, or termites.

Despite these caveats, numerous studies have yielded critical insights into the mechanistic aspects of nestmate recognition in social insects. One important parameter is colony size: When colonies are small, individual insects can learn the semiochemical profile of nestmates, even if they are rather individualistic. In contrast, members of large colonies are thought to require the sharing of individual chemical profiles to produce an average colony profile, which is the parameter that must be learned (25, 26). In ants, the source of the shared chemicals is either the postpharyngeal gland (PPG) for species that engage in trophallaxis, or the cuticle for species that engage only in colony grooming behaviors (112). For wasps, surface lipids are shared either by grooming behaviors or possibly by transfer from the Dufour's gland (29), and the same is possibly true for bees. In termites, no glands are known that contain cuticular lipids, which may be shared solely by grooming activities (21).

Although few authors (11, 112) have argued that semiochemicals other than cuticular hydrocarbons are important nestmate recognition cues, the vast preponderance of authors have concluded that cuticular hydrocarbons are the most important cues. For ants, two basic models have been set forth to explain the origin and maintenance of colony-specific nestmate recognition cues. The queen-centered model states that queens biosynthesize the cues *de novo* and these are then subsequently distributed among the workers. This model predicts that the queen must produce substantial quantities of these chemicals, that she is the source of the colony Gestalt, and that workers separated from contact with their queen will lose these cues compared to queenright nestmates. Alternatively, the worker-centered model states that nestmate recognition cues are made and distributed primarily

by workers. This model predicts that no aggression results when nestmates from split colonies are reunited (72). In several species of *Camponotus*, cross-fostering experiments indicated that the queen odor masks any innate odor that the workers might possess (12–14). Provost (89) showed that *Leptothorax lichtensteini* workers separated for three months will not accept each other unless the queen is transferred back and forth between the groups during separation. In addition, comparative cuticular hydrocarbon analyses by Provost et al. (90) on artificial colonies of *Messor barbarus* with differing numbers of queens suggested that the queen-produced hydrocarbons were the important nestmate cue. These studies all support the queen-centered model. Other studies, however, have supported the worker-centered model. Crosland (24) showed that workers of *Rhytidoponera confusa* separated from their own colony and housed with an alien conspecific queen were still readily accepted by their former nestmates upon return to their colony, whereas non-nestmate unrelated workers were immediately attacked. Similar results were found for *Leptothorax curvispinosus* workers (104).

An elegant test of these two models is provided by the behavioral and biochemical experiments of Lahav et al. (71, 72) using the polygynous species *Cataglyphis niger*. Aggression bioassays indicated that encounters between nestmates from split colonies or nonsplit colonies were peaceable, that the presence or absence of a queen had no effect on aggression, and that all workers readily attacked *C. niger* workers from an alien colony. These results are all in better agreement with a worker-centered model than a queen-centered model. Analysis of PPG contents and cuticular extracts by gas chromatography (GC)-mass spectrometry indicated that queens and workers had similar overall hydrocarbon composition, but that queens had three times more hydrocarbons in their PPG than workers did (their heads are the same size), whereas the amount of hydrocarbon on the thoracic surface was the same for queens and workers. Tests to determine comparative de novo biosynthetic rates of newly synthesized hydrocarbons from ^{14}C -acetate in queens and workers indicated that hydrocarbon production in the queen PPG was 20 times less than that in the worker PPG of either queenright or queenless colonies. Queens did not generate substantial quantities of hydrocarbons in other tissues such as in the ovaries, nor did queen age affect production. This biochemical evidence again supports the worker-centered model more than the queen-centered model. Additional studies using radiolabeled donors measured the flow of hydrocarbons between workers and queens. Queens received more PPG hydrocarbon in trophallactic exchange than they gave, but transfers to the cuticle by grooming were low and the same for both workers and queens, again in accordance with a worker-centered model. In group encounters workers transferred slightly more hydrocarbons to a queen than to a worker. Lahav et al. (72) suggest that this slight preference allows the queen to accumulate copious PPG content, thus acting as a mechanism to maintain the queen's individual odor as similar as possible to the average colony odor.

Dani et al. (30) conducted several experiments on the paper wasp *Polistes dominulus* to test the importance of individual hydrocarbons as nestmate recognition cues.

The cuticular lipid profile of this species is dominated by a series of *n*-alkanes, monomethyl alkanes, and monoenes (10, 29). A series of synthetic hydrocarbons were obtained and applied either singly or as mixtures at 40 μg (approximately one eighth of normal cuticular hydrocarbons). In additional experiments, 200- μg treatments of single synthetic hydrocarbons were used. Application of *n*-alkanes, either singly or as a mixture, did not cause the treated wasp to be attacked. In contrast, wasps treated with methyl alkanes or with monoenes were attacked by colony members, and the intensity of the aggressive response was greater for 200- μg treatments than for 40- μg treatments. These experiments indicate that the wasps can detect the increased levels of the hydrocarbons (12% to 60%) and that they use them for nestmate recognition. Earlier studies (10, 29, 39, 73, 78) implicating methyl-branched hydrocarbons as the important semiochemical cues in nestmate recognition were all conducted using univariate and multivariate statistical analyses of composition data rather than direct experimental manipulations. Ruther et al. (95) also tested the aggressive responses of foraging hornets to dead dichloromethane-extracted European hornet, *Vespa crabo*, workers treated topically with low levels of synthetic hydrocarbons. The authors found that forager aggressiveness to hornets with added hydrocarbons was much greater in foragers about to leave the nest than in foragers who had left the nest before being used in the bioassay. These results can possibly be explained by the leaving forager being in an ecological context in which nest defense is paramount, whereas the returning forager is not likely in most cases to be met at her nest by an alien hornet. Presentation of frozen nestmates without added chemicals elicited no aggressive responses, whereas presentation of a dead non-nestmate elicited strong aggressive responses. The conclusions of the authors must be tempered somewhat, however, as they did not test responses of hornets to extracted hornets without added chemical, and it is likely that their extraction procedure failed to extract each dead hornet completely and reproducibly. In addition, because their quantitative analysis of the hornet lipids relied on GC-mass spectrometry, their use of the internal standard 1-eicosene is problematic, as the total ion current for any given class of chemicals is strongly dependent on the functional nature of the molecules, i.e., alkenes are not good standards for *n*-alkanes or methyl-branched alkanes, and 1-alkenes might not give the same ion response as internal alkenes. In addition, the dichloromethane extracts contained several terpenes and terpene esters (likely from glands rather than from cuticular surface lipids), and the 1-eicosene is definitely a poor model for the ion current properties of these compounds. Despite these caveats, Ruther et al. (95) showed that the hornets perceived and responded to low levels of added individual hydrocarbons.

Task-Specific Cues

Gordon and her colleagues have conducted an interesting series of experiments on the semiochemical roles of cuticular lipids, especially hydrocarbons, of the red harvester ant, *Pogonomyrmex barbatus*, a species that engages very little in

trophallactic exchanges. These studies have addressed nestmate recognition (116), caste-specific differences in hydrocarbon composition in relation to task-related behaviors (115, 117), task decision control by cuticular hydrocarbons (45), and a detailed analysis of the chemical composition of the ant cuticular lipids (85). This species is a good model for semiochemical studies because it consists of colonies of only moderate size (10,000 to 12,000 workers) headed by only a single queen and a modest repertoire of caste-specific behaviors (tasks). These ants have two groups of cuticular lipids: the hydrocarbons, which are the predominant compounds in terms of actual amounts (111 of them, but 30 of them make up about 90% of the total), and the wax esters, which are present in a much lower total abundance than the hydrocarbons despite there being about the same number of individual wax esters (112) as there are individual hydrocarbons, i.e., many small wax ester peaks compared with many, much bigger hydrocarbon peaks (85). About 30 of the most abundant hydrocarbons were used by the authors in all behavioral analyses. For most of their multivariate statistical studies, the authors used only the five most-abundant hydrocarbons, because with numerous independent variables statistical artifacts are highly likely when making multiple comparisons (116), unless very large sample sizes are used. They have used novel bioassays in these studies, using small glass blocks as "ants" presenting various test mixtures of chemicals. As have other investigations conducting recognition bioassays, they used mandible openings as a measure of aggression. Nestmate recognition studies were done with the extracts of midden workers, individuals that work primarily outside the nest, moving debris (mostly small stones) from one place to another (44). Either total extracts or hydrocarbons alone were sufficient cues to enable workers (of unknown caste composition) emerging from a laboratory nest to distinguish nestmates from non-nestmates (116). Inquiry into whether different castes possess different task-related cuticular hydrocarbon profiles (115) utilized three field-collected, relatively easily discernable groups of workers: patrollers, workers whose role is to locate and inform the colony of seed sources; foragers, workers that leave the nest and retrieve the seeds located by the patrollers; and nest maintenance workers, which drag refuse out of the nest. Each of these groups differed in the relative proportions of *n*-alkanes, monomethyl alkanes, dimethyl alkanes and alkenes, as well as in some individual hydrocarbons when analyzed by multivariate techniques. Enough variability exists, however, that discriminant analysis failed to assign various individuals to the correct task about 25% of the time. Subsequent studies (117) indicated that the cuticular hydrocarbon composition of the various task-related castes were correlated with environmental variables, in particular temperature and relative humidity.

It is well known that ants, organized without any central control, must respond to changing environmental and ecological conditions to adjust the number of workers performing any given task in relation to the needs of the colony. The question is, do the ants use differences in hydrocarbon profiles to recognize the task being performed at that time by the ants that they encounter? If they do, then this information could allow each ant to make a decision on whether to perform a

particular task. Green & Gordon (45) designed a clever set of experiments to answer this question. They picked two task groups: (a) foragers, which spend a great deal of time outside the nest, thus being exposed to warmer and drier conditions, and (b) nest maintenance workers, who stay mostly inside, where it is cooler and more moist. Foragers have higher ratios of *n*-alkanes to *n*-alkenes and branched hydrocarbons than nest maintenance workers do (117). In a field bioassay, these investigations inhibited foraging by removing patrollers returning to the nest. After 30 minutes without patrollers entering the nest, Green & Gordon mimicked the flow of returning patrollers by dropping 3-mm-diameter glass beads coated with one insect-equivalent of extracted cuticular lipids into the nest opening at the rate of 1 every 10 seconds until the number of beads approximated the number of ants previously collected. The extract was either total lipids of patrollers, patroller hydrocarbons, nest maintenance worker hydrocarbons (to test for task specificity control), or pentane (solvent blank). In addition, live patrollers were captured and returned to the nest as a positive control for forager activity. Foraging activity was monitored by counting foragers within one meter of the nest entrance for one hour. The authors found that, when one ant-equivalent of patroller hydrocarbons on the glass beads was presented just inside the nest entrance at intervals corresponding to normal patroller activity and at the time of day that foraging would normally occur, the glass bead with hydrocarbon extract triggered a full and normal foraging response by the colony. Beads with nest maintenance worker hydrocarbons did not elicit foraging responses. These results strongly suggest that an ant can assess the task status of another nestmate from its cuticular hydrocarbon profile and can adjust its own behavior accordingly.

Dominance and Fertility Cues

A number of social insects show female dominance hierarchies within a colony, frequently closely related to the ovarian status of the alpha-individual. A long-standing problem has been to identify the cues by which individuals both maintain their status and recognize the status of their nestmates. Several recent studies strongly implicate cuticular hydrocarbons as the primary cue. Monnin et al. (80) showed that, in the queenless ponerine ant *Dinoponera quadriceps*, in which only one dominant alpha-female mates and reproduces in each colony, the dominant female contained significantly greater quantities of 9-hentriacontene (presumably of *Z*-stereochemistry) than did her sterile nestmates. The remaining 80 hydrocarbons found on the cuticle of this species did not vary as a function of dominance status, and the 9-C_{31:1} did not occur in the Dufour's gland of this species. Peeters et al. (87) further showed that if the alpha-female is removed from the colony, then one of the beta-workers will increase its level of the 9-C_{31:1} and acquire alpha-status. Liebig et al. (74) studied the ponerine ant *Harpegnathos saltator*, in which queen-worker dimorphism is very limited, and only a few mated workers reproduce once the founding queen senesces. Furthermore, worker oviposition is regulated by highly specific aggressive interactions among nestmates that can recognize different levels

of ovarian activity. Variations in cuticular hydrocarbons correlated with oogenesis for both queens and workers. 13,23-Dimethylheptatriacontane was present in egg-layers but not in infertile workers and senescent queens. Other hydrocarbon components also varied, with egg-layers also characterized by an elongation of the chain lengths of the hydrocarbons compared with non-egg-layers. In this species, cuticular hydrocarbons also serve as a fertility signal and indicator of dominance relationships to nonreproductive members of the colony. Similar findings were made by Cuvillier-Hot et al. (27) for the queenless ponerine ant *Diacamma ceylonese* and for the neotropical ponerine *Pachycondyla cf. inversa* (52, 107).

In addition to ponerine ants, the social wasp *Polistes dominulus* uses cuticular hydrocarbons as tokens of dominance and reproductive status (10, 101). In this species, nests are frequently established by several females and dominance hierarchies are established with only one alpha-female becoming an egg-layer. Immediately after nest establishment, the future alpha- and subordinate females have identical cuticular hydrocarbon profiles. However, as soon as new workers begin to emerge, alpha-females are easily chemically distinguished from their subordinate foundresses and new workers. Workers and subordinates were characterized by *n*-alkanes and monomethyl alkanes, whereas the alpha-female was characterized by alkenes and dimethyl alkanes. After experimental removal of the alpha-female, the new dominant female took on the hydrocarbon profile of the alpha-female. Sledge et al. (101) discussed these changes in terms of a switch from behavioral dominance to chemical signaling, which involves less risk to the dominant female.

Chemical Mimicry

Since the initial postulation by Howard et al. (61) that a staphylinid beetle achieves complete integration as a termitophile by mimicking precisely the cuticular hydrocarbons of its termite host, numerous other studies (recently reviewed in Reference 33) support the concept that inquilines of social insects use this adaptive strategy. The methods by which inquilines mimic host-specific cuticular hydrocarbons cover a spectrum from total de novo biosynthesis (recorded so far only for highly integrated inquilines with a long history of coevolution with the hosts) to behavioral acquisition by various physical contacts with the hosts, and every possibility in between (33). Recent examples of chemical mimicry by inquilines of social insects include myrmecophilous crickets (2) and the eucharitid parasitoid *Kapala sulcifacies* with the ponerine ant *Ectatomma ruidum* (64), the myrmecophilous beetles *Zyras comes* (Staphylinidae) and *Diaritiger fossulatus* (Pselaphidae) with the black shining ant *Lasius fuliginosus* (1), and the myrmecophilous salticid spider *Cosmophasis bitaeniata*, a predator of the tree ant *Oecophylla smaragdina* (3).

Not all examples of chemical mimicry of social insects involve inquilines. Liepert & Dettner (75) describe how the predominant aphidiid parasitoid *Lysiphlebus cardui* forages among black bean aphid colonies tended by the ant *Lasius niger* without being attacked by the ants. This parasitoid qualitatively mimics the

hydrocarbon profile of the aphids, and the ants behave toward the parasitoids just as if they are aphids; other species of aphid-tending ants show the same behavior toward this parasitoid (114). Another aphidiid parasitoid that attacks the black bean aphid, *Trioxya angelicae*, is vigorously attacked by honeydew-collecting ants when encountered in aphid colonies. This species does not have a cuticular hydrocarbon profile that mimicks that of the aphids.

Obligate social parasites of a number of social insect species have lost the worker caste and are unable to found independent nests. They must therefore infiltrate a host nest and “fool” the hosts into accepting them and rearing their brood. Bagnères et al. (4) showed that the wasp *Polistes atrimandibularis*, an obligate social parasite of *Polistes biglumis bimaculatus*, uses a complex sequence of chemical mimicry of the cuticular hydrocarbons of its host to achieve this end. In early June, the parasite queens, fertilized the previous summer, begin searching for a host nest containing only the host queen and developing brood. At this time, the parasite’s hydrocarbon profile is dominated by alkenes, whereas host hydrocarbon profiles contain only saturated hydrocarbons. Soon after nest invasion, the parasite loses all of its alkenes and its profile now partly matches the saturated hydrocarbon profile of the host. By the time the host workers emerge, host and parasite queens have indistinguishable hydrocarbon profiles. By autumn, the parasite offspring begin to emerge as adults and outnumber host offspring adults. The newly emerged *P. atrimandibularis* have cuticular hydrocarbon profiles intermediate between their host and their mother before she invaded the nest. Host workers have no unsaturated hydrocarbons. At the end of the summer descendants of both species leave the nests to mate, and after hibernation females begin a new cycle. Although no radiolabeling experiments were conducted, it appears that the parasite can modulate its alkene production to mimic the host hydrocarbon profile at critical points in the colonial cycle.

Polistes sulcifer, an obligate social parasite of *Polistes dominulus* (111), has evolved a different approach. Overwintering *P. sulcifer* females begin searching for host nests during the middle of May, aggressively attack the host colony, and often kill the alpha-host female (110). The parasite female then becomes the new dominant female of the colony. Before usurpation, the two species have different cuticular hydrocarbon profiles. Within 90 minutes of usurpation, the parasite hydrocarbon profile begins to match that of its host and by day 3 the profiles are indistinguishable. It is not known whether the parasite utilizes host hydrocarbons from the nest surface to effect this transformation or whether she changes her own biosynthesis to produce them. Later, the parasite queen changes the hydrocarbon composition of the nest by the addition of a parasite-specific hydrocarbon (9,15-dimethylnonacosane). Turillazzi et al. (111) propose that if the parasite queen limits attack by host workers by chemical mimicry, she may also manipulate the hydrocarbon profile of the nest on which host nestmate recognition template is based. As they note, to ensure her reproductive success, the parasite queen must not only be accepted as the alpha-individual, but must also ensure that the host workers raise her immature brood by failing to distinguish them from host brood.

A different system of chemical mimicry is found in the tropical ant *Cardiocondyla obscurior* (23). Males of this species are either wingless and aggressive, or winged and docile, and both compete for access to virgin queens in the nest. The wingless males attack each other but do not attack the winged males. Cremer et al. (23) showed that the winged males closely mimic the cuticular hydrocarbon profile of the virgin queens (and the winged males are accordingly courted by the wingless males), whereas the cuticular hydrocarbons of the wingless males are substantially different from those of the queens. This situation is unusual and suggests a new evolutionary context for chemical deception whereby chemical female mimicry enables two alternative reproductive strategies by males to coexist.

Primer Pheromones

Primer pheromones have long been postulated for insects, but the nature of delayed response between stimulus and final physiological or behavioral result has been a major impediment to studies in this area. A long-standing problem of great interest is ascertaining what stimulates and controls the shift from solitary to gregarious behavior in locusts (42). Heifetz et al. (51) reported that cuticular lipids from *Schistocerca gregaria* nymphs induce gregarious behavior in solitary nymphs and are likely detected via the antennae. In further studies, Heifetz et al. (50) reported that it was only the hydrocarbon fraction of the cuticular lipids, and not the wax esters, that were the active components. They further showed that antennal preparations of crowded *S. gregaria* nymphs and adults exposed to their hydrocarbons generate rapid, short-lived increases of inositol triphosphate (IP₃) but not cAMP, that this generation is dose dependent and species specific, and that it is significantly impaired in the presence of a specific inhibitor of trimeric G protein function, GDP- β -S. These data strongly suggest that the cuticular hydrocarbons are specifically perceived by antennal cells and function as contact primer pheromones that trigger behavioral phase transitions in these locusts.

HYDROCARBON BIOSYNTHESIS

In addition to the numerous advances made since 1982 in understanding the functional roles of hydrocarbons in arthropods, similar advances have been made in understanding the biochemistry and physiology of hydrocarbon production and regulation. The biosynthesis of hydrocarbons has been extensively studied in the dipterans *Musca domestica* (7) and *Drosophila melanogaster* (67), and considerable work has also been done on the cockroaches *Periplaneta americana* and *Blattella germanica*, the termite *Zootermopsis angusticollis*, and several other insects (82). The timing of hydrocarbon production during development has been investigated in the cabbage looper, *Trichoplusia ni*, and the armyworm, *Spodoptera eridania*, and the transport of hydrocarbons by lipophorin in a number of species has been examined (20, 97 and references therein, 99). In *M. domestica*, the chain

lengths of cuticular hydrocarbons are modified by ovary-produced ecdysteroids to function as a sex pheromone, and work on this insect has shed light on the steps that regulate the chain length of insect-produced hydrocarbons (7). A combination of *in vivo* and *in vitro* studies using both radio- and stable isotope techniques established the biosynthetic pathways for the major types of hydrocarbons. Little is known of either the enzymology or the molecular biology of hydrocarbon production, although studies in these areas are in progress.

Methyl-Branched Hydrocarbons

Figure 1 summarizes our current understanding of hydrocarbon formation. *n*-Alkanes and *n*-alkenes are formed by the elongation of appropriate fatty acyl-CoAs

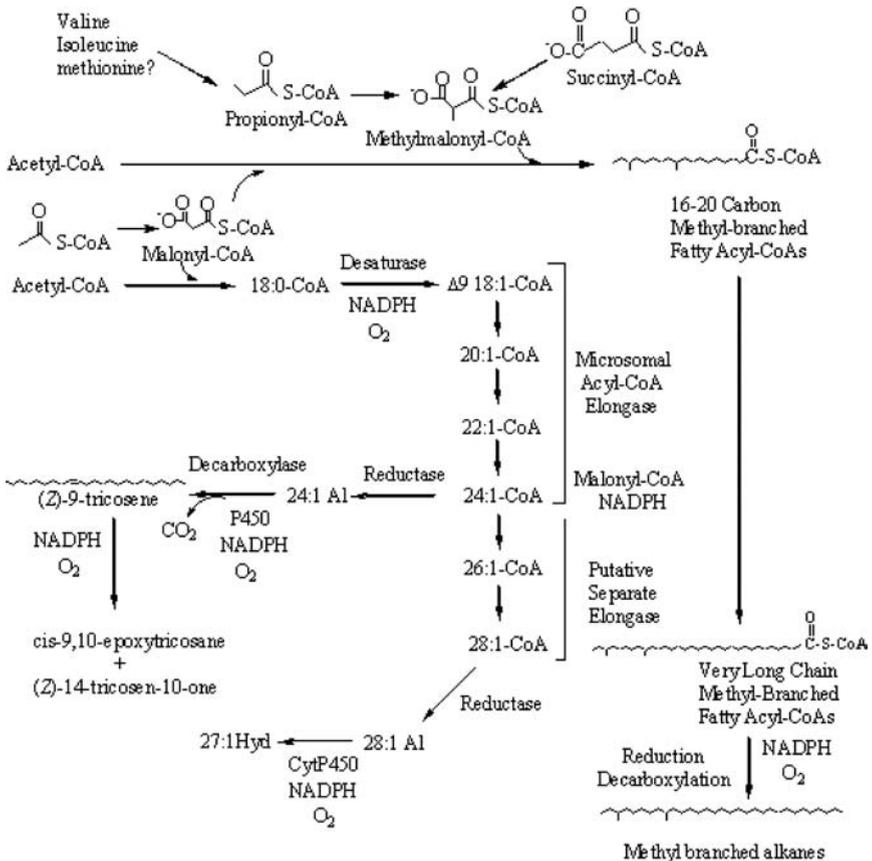


Figure 1 Proposed biosynthetic pathways for unsaturated and methyl-branched hydrocarbons in insects. Shorthand abbreviation for fatty acids: xy:z stands for the number of carbons:number of double bonds; Al, aldehyde; Hyd, hydrocarbon.

followed by conversion to the hydrocarbon one carbon shorter. The methyl group of the methyl-branched hydrocarbons arises from the substitution of methylmalonyl-CoA in place of malonyl-CoA at specific points during chain elongation (82). In some insects, including *M. domestica* (34) and *B. germanica* (16), the methylmalonyl-CoA unit arises from the branched amino acids valine, isoleucine, and perhaps methionine, whereas in the termite *Z. angusticollis* (49), succinate is converted to methylmalonyl-CoA, apparently by alimentary canal microorganisms. Consistent with these findings, both *M. domestica* and *B. germanica* have low or undetectable levels of vitamin B₁₂ (necessary for the interconversion of succinyl-CoA and methylmalonyl-CoA), whereas the termite *Z. angusticollis* has high levels of vitamin B₁₂ (118). Although nothing is known about the regulation of the number and positions of the methyl-branching units, evidence has been obtained from studies in both *M. domestica* (47) and *B. germanica* (46, 68) that a microsomal fatty acid synthase (FAS), as opposed to the soluble FAS, is involved in producing methyl-branched fatty acid precursors (9). In the American cockroach, *P. americana*, which produces 3-methylpentacosane as its only major methyl-branched hydrocarbon, carbon-13 NMR studies with labeled propionates showed that the branching methyl group is inserted early in chain elongation and not as the penultimate unit (35). Likewise, in the house fly, carbon-13 studies using mass spectrometry demonstrated that the methyl-branching unit, in both 3-methyl and internally branched methylalkanes, was inserted early in the elongation process (34).

Almost nothing is known about the absolute stereochemistry of methyl branches in insect hydrocarbons. In *B. germanica*, 3,11-dimethylnonacosane is converted to 3,11-dimethylnonacos-2-one (17), which has the 3S, 11S configuration (86, 95). This stereochemistry suggests that the enzyme responsible for oxidizing the 2-position selectively interacts with only one of four possible stereoisomeric alkane precursors or that other enzymes selectively produce only one chiral hydrocarbon for the oxidase reaction.

Alkenes

The positions of the double bonds in unsaturated hydrocarbons are determined by fatty acyl-CoA desaturases. Thus, many insect hydrocarbons have double bonds at the 9-position, arising from the ubiquitous Δ^9 -desaturase acting on stearoyl-CoA. The Δ^9 -desaturase has been cloned from *M. domestica* (38) and *D. melanogaster* (119). The protein *desat1* in *D. melanogaster* is a Δ^9 -desaturase that preferentially acts upon palmitoyl-CoA, leading to hydrocarbons with 7,11-double bonds (28). An unusual gene, *desat2* (28, 67), located close to the gene *desat1* in the *Drosophila* genome appears to be responsible for the 5,9-dienes present in African Tai females. The protein *desat2* is also a Δ^9 -desaturase but preferentially uses myristoyl-CoA, leading to hydrocarbons with double bonds at the 5,9-positions (28). The American cockroach synthesizes linoleic acid (18:2, $\Delta^{9,12}$) de novo (8), which it elongates and decarboxylates to form the 27:2 hydrocarbon, with double bonds at the 9,12-positions (36).

Chain Length Regulation

Regulation of chain length in hydrocarbons appears to reside in the fatty acyl-CoA elongase reactions and not in the reductive conversion of acyl-CoAs to hydrocarbon. The American cockroach produces three major hydrocarbons, *n*-pentacosane, 3-methylpentacosane, and (*Z,Z*)-9,12-heptacosadiene. Studies on microsomes from integument tissue showed that stearoyl-CoA was elongated only to 26 carbons (to serve as the precursor to *n*-pentacosane), whereas linoleoyl-CoA was elongated to 28 carbons (to serve as the precursor to the C27 diene) (113). The female house fly produces monoenes of 27 carbons and longer for the first two days after adult eclosion, and then switches to producing (*Z*)-9-tricosene under the influence of 20-hydroxyecdysone (20-HE) at three days posteclosion (7, 108). Microsomes from day-1 house flies readily elongated both 18:1-CoA and 24:1-CoA up to 28 carbons, whereas microsomes from day-4 females elongated 18:1-CoA to 24:1-CoA and did not effectively elongate 24:1-CoA. In contrast, males, which produce 27 carbon and longer alkenes, readily elongated both 18:1-CoA and 24:1-CoA to fatty acids of 28 carbons. It is speculated that 20-HE represses the production of the specific elongase(s) (condensing enzyme) that converts 24:1-CoA to 28:1-CoA, and the build-up of 24:1-CoA leads to the production (*Z*)-9-tricosene (7).

Mechanism of Hydrocarbon Formation

The mechanism of hydrocarbon formation from very-long-chain acyl-CoA is controversial. Work in plants (18), an alga (32), and a vertebrate (19) indicated that the acyl-CoA is converted to an aldehyde, which is then decarbonylated to the hydrocarbon one carbon shorter in a reaction that does not require oxygen or any cofactors, with the carbonyl carbon released as carbon monoxide. In contrast, evidence in insects shows the aldehyde is decarboxylated to hydrocarbon and carbon dioxide by a cytochrome P450 enzyme that required NADPH and molecular oxygen (91, 92). The resolution of this controversy awaits the cloning and characterization of the enzymes involved.

Timing of Hydrocarbon Synthesis and Transport

In the cabbage looper, *Trichoplusia ni*, and the armyworm, *Spodoptera eridania* (both Lepidoptera: Noctuidae), at eclosion (all molts) all the hydrocarbon present on the surface of the insect remains on the larval or pupal exuvia (31, 37, 48). Thus, insects must synthesize a new complement of hydrocarbon at each molt. Studies on the timing of hydrocarbon production showed that hydrocarbon present on newly molted insects is synthesized during the previous instar, stored internally, and then becomes the hydrocarbon that provides the full complement of hydrocarbon for the newly molted insect. The critical role of hydrocarbon in preventing a lethal rate of desiccation (40, 41) has dictated the evolution of a system that provides for a full complement of hydrocarbon for the newly molted insect.

The storage and transport of hydrocarbons in insects have received scant attention. In a number of species, hemolymph hydrocarbons are carried by lipophorin (20, 97 and references therein), and there has been a tacit assumption that most internal hydrocarbons are associated with the hemolymph. However, in *M. domestica*, Schal et al. (97) showed that less than 20% of the internal hydrocarbons are in the hemolymph, and large amounts are found in the ovary. The recognition that newly synthesized hydrocarbon first appears in the hemolymph associated with lipophorin has required a paradigm shift in understanding hydrocarbon deposition on the surface of the insect. The old model, in which hydrocarbon is transported from the site of synthesis in epidermal cells via "pore canals," must be re-examined. Indeed, how hydrocarbon is transported from hemolymph lipophorin to the surface of the insect is not known. Recent work in the termite *Z. angusticollis* (99) suggests that specificity in the deposition of hydrocarbons can occur, and work is needed to explore this possibility.

A number of insects have glands or organs that contain substantial quantities of hydrocarbons, not all of which have the same composition as that found on the cuticle. In the desert ant, *Cataglyphis niger*, both the hemolymph and crop contain the same hydrocarbons that are found in the postpharyngeal gland (102). It is well known (93) that in insects hydrocarbons are produced by oenocytes associated with either fat body or epidermal tissue and not by specialized organs such as the postpharyngeal gland. Therefore these gland- and organ-specific hydrocarbons are likely transported from the site of synthesis by hemolymph (presumably bound to lipophorin) and then selectively transported into the target tissue (102). Similar findings have been made for the front basitarsal brush of the ponerine ant *Pachycondyla apicalis* (103) and for a number of parasitoid Dufour's glands that contain large quantities of hydrocarbon, not always identical to cuticular profiles (57). A remarkable specificity occurs in several lepidopterans, in which shorter chain pheromone or pheromone precursor hydrocarbons (69, 70, 98) are synthesized by oenocytes and then cotransported by lipophorin along with the very-long-chain cuticular hydrocarbons. The short-chain hydrocarbon pheromones or pheromone precursors are selectively transported to the pheromone gland, whereas the cuticular hydrocarbons become distributed over the cuticle (70). How this remarkable targeting specificity occurs is unknown.

Biosynthesis of Volatile Hydrocarbon Pheromones of Lepidoptera and Coleoptera

A number of moths in the families Geometridae, Arctiidae, and Noctuidae utilize hydrocarbons, or their epoxide derivatives, as volatile sex pheromones (79). These hydrocarbons often have double bonds at the 6,9- or 3,6,9-positions, consistent with deriving from linoleic or linolenic acid. The 19- and 21-carbon components arise after chain elongation of linoleic and linolenic acid, whereas the 17-carbon components could arise from the direct decarboxylation of the parent fatty acids (69). A few even-chain hydrocarbons with the 3,6,9- and 6,9-double bond positions

have been identified, and Miller (79) suggested that they could be formed by direct reduction of the carboxyl group. A more likely possibility is α -oxidation followed by decarboxylation. However, no data are available, and both routes are possible.

Schal et al. (98) showed that the arctiid moths *Holomelina aurantiaca* and *H. laeae*, both of which use 2-methylheptadecane as pheromones, synthesized the pheromone in the oenocytes. 2-Methylalkanes can arise from the carbon skeleton of either leucine (odd-chain-length hydrocarbon) or valine (even-chain length) (82). 2-Methylheptadecane in *Holomelina* is transported in the hemolymph bound to lipophorin along with longer chain cuticular hydrocarbons, and the short-chain pheromone hydrocarbon is selectively taken up by the pheromone gland. A similar situation exists in the gypsy moth, *Lymantria dispar*, in which the pheromone precursor, 2-methyloctadec-(Z)-7-ene, is synthesized by oenocytes, transported by lipophorin to the pheromone gland, and selectively taken up and epoxidized to the active pheromone component (69, 70).

A series of methyl- and ethyl-branched, conjugated triene and tetraene hydrocarbons with 12 to 17 carbons were identified as the male sex pheromone components of six *Carpophilus* fungus beetle species (5). These unusual hydrocarbons are synthesized by male-specific tissue on the posterior portion of the abdomen (81). Carbon-13 studies using NMR and mass spectrometry demonstrated that biosynthesis involved the incorporation of acetate (straight-chain portion of the molecule), propionate (methyl branches), and butyrate (ethyl branches), presumably by a fatty acid-type pathway in which the unsaturated intermediate in each elongation step is retained, followed by decarboxylation (5, 88).

FUTURE DIRECTIONS

Continued studies will undoubtedly reveal many new physiological and semiochemical functions for hydrocarbons in arthropods. Bioassays that test these putative functions in natural systems in ways that do not cause the animals to biosynthesize additional lipids of their own are needed to assign function unambiguously. Studies of receptor interactions with hydrocarbons are needed as well as studies that identify the minimal hydrocarbon moieties needed to convey a particular semiochemical signal. Given the diversity of known arthropods and their multitudes of life histories, an array of combinations of specific hydrocarbons/hydrocarbon classes may regulate the behavior of particular arthropods. The roles of chirality in hydrocarbon biochemistry, physiology, and semiochemistry are virtually unknown. Some progress is being made in understanding the genetics and evolution of arthropod hydrocarbons, but much remains to be learned. Future work on hydrocarbon biosynthesis and transport should address chain length specificity, determination of the number and positions of both double bonds and methyl branches, the stereochemical mechanisms of hydrocarbon formation, and the means by which hydrocarbons are partitioned between the cuticle and specialized organs or glands. Research is needed to isolate and characterize the key enzymes involved in hydrocarbon formation, including the fatty acid synthases that form the methyl-branched fatty acids, the elongases, the reductase that converts

very-long-chain acyl-CoAs to aldehyde, and the enzymes that convert aldehyde to hydrocarbon.

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