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Hydrocarbons as contact pheromones of longhorned beetles (Coleoptera: Cerambycidae)

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The cuticular wax layer of insects is comprised of a complex mixture of long-chain fatty acids, alcohols, esters, aldehydes, ketones, and hydrocarbons that protect insects from desiccation (Gibbs, 1998). The components of the wax layer, particularly the hydrocarbons, also act as contact pheromones that mediate mate recognition (Howard and Blomquist, 2005). In fact, there is a growing body of evidence that contact pheromones play important roles in the mating systems of beetles in the family Cerambycidae, the longhorned beetles (e.g., Kim *et al*., 1993; Fukaya *et al*., 1996, 1997, 2000; Wang, 1998; Ginzel *et al*., 2003a, 2003b, 2006; Ginzel and Hanks, 2003, 2005; Lacey *et al*., 2008). These chemical signals elicit mating responses from males and have been confirmed in a number of cerambycid species, including those in the primitive subfamily Prioninae (Barbour *et al*., 2007), and more derived subfamilies of Cerambycinae (e.g., Ginzel and Hanks, 2003; Ginzel *et al*., 2003a, 2003b, 2006; Lacey *et al*., 2008) and Lamiinae (e.g., Wang, 1998; Fukaya *et al*., 2000; Ginzel and Hanks, 2003; Yasui *et al*., 2003; Zhang *et al*., 2003; see Table 17.1).

Cerambycid beetles are collectively known as longhorned beetles because many species have elongate filiform antennae (Linsley, 1964), and this diverse family includes more than 35,000 species in ~4,000 genera (Lawrence, 1982). Our understanding of chemical communication in cerambycids pales in comparison to what is known about the geographical distribution and taxonomy of this family (Hanks, 1999). Nevertheless, longhorned beetles are among the most important pests of woody plants in natural and managed systems worldwide, and many attack domestic broadleaf trees and shrubs (Solomon, 1995). The larvae are phytophagous, and colonize hosts that vary greatly in quality and may be healthy, moribund, recently killed or even decomposing (Hanks, 1999). Stressed and dying host trees become available sporadically and unpredictably when they are damaged or weakened by environmental stresses (reviewed by Hanks, 1999), and their nutritional quality declines rapidly as subcortical tissues are degraded by xylophagous competitors, including buprestid and scolytid beetles, as well as by other cerambycid species and saprophytes. Thus, early colonizers of moribund trees are afforded better larval nutrition, and selection favors behaviors in adults that expedite mate location and recognition and rapid mating and oviposition. These behaviors include the use of contact pheromones in mate recognition. This chapter first discusses the use of contact pheromones in the mating systems of cerambycids, then details the copulatory behavior of longhorned beetles, and explores the

Table 17.1 *Summary of published research on contact pheromones of longhorned beetles and bioassays used to confirm activity.*

Subfamily (Tribe)	Species	Stimulus	Biological activity	References
Cerambycinae (Callidiini)	*Semanotus japonicus* (Lacordaire)	Ether extracts of ♀	Stimulates mating behavior in ♂	Kim *et al*, 1993
Cerambycinae (Cerambycini)	*Nadezhdiella cantori* (Hope)	Hexane extracts of ♀	Freshly killed ♀ and ♀ extracts are bioactive	Wang *et al*., 2002
Cerambycinae (Clytini)	*Megacyllene caryae* (Gahan)	Z9-C_{29}	Stimulates mating behavior in ♂	Ginzel and Hanks, 2003 Ginzel *et al*., 2006
Cerambycinae (Clytini)	*Megacyllene robiniae* (Förster)	Filter paper exposed to ♀	♂remain longer with filter paper exposed to females than control	Galford, 1977
		Z9-C_{25}	Stimulates mating behavior	Ginzel and Hanks, 2003 Ginzel *et al*., 2003
Cerambycinae (Clytini)	*Neoclytus a. acuminatus* (F.)	7-MeC_{27}, 7-MeC_{25}, 9-MeC_{25}	7Me-C27 is the major component of the contact sex pheromone and 7Me-C25 and 9Me-C27 act as synergists.	Lacey *et al*., 2008
Cerambycinae (Clytini)	*Neoclytus m. mucronatus* (F.)	Hexane extracts of ♀	♂ attempt to mate with reconstituted ♀	Ginzel and Hanks, 2003
Cerambycinae (Clytini)	*Xylotrechus colonus* (F.)	*n*C_{25}, 9-MeC_{25}, 3-MeC_{25}	♂ attempt to mate with hexane-washed ♀ treated with all three compounds	Ginzel *et al*., 2003
Lamiinae (Acanthocinini)	*Dectes texanus* LeConte	Ether extracts of ♀	♂ attempt to mate with reconstituted ♀	Crook *et al*., 2004
Lamiinae (Gleneini)	*Paraglenea fortunei* Saunders	Hexane extracts of ♀	♂ attempt to mate with reconstituted ♀	Wang *et al*., 1991
Lamiinae (Lamiini)	*Anoplophora chinensis* (Förster)	Dead ♀ and hexane extracts of ♀	~50% of ♂ attempt to mate with recently killed ♀ and dummies treat with extract	Wang, 1998

Lamiinae (Lamiini)	*Anoplophora malasiaca* Thompson	Hexane and ether fractions of crude ether extracts of ♀ elytra Solvent extracts of ♀ and ♂ 10-heptacosanone 12-heptacosanone (*Z*)-18-heptacosen-10-one, (18*Z*, 21*Z*)-heptacosa-18,21-dien-10-one, (18*Z*, 21*Z*, 24*Z*)-heptacosa- 18,21,24-trien-10-one	♀ attempt to mate with glass rods treated with both saturated subfraction of hexane fraction and ether fraction. ♂ attempt to mate with glass dummies treated with ♀ extract, addition of ♂ reduces bioactivity ♂ attempt to mate with glass rod treated with all five compounds. Synthetic blend of all five compounds except 12-heptacosanone ~ twice as bioactive	Fukaya *et al*., 2000 Akino *et al*., 2001 Yasui *et al.* 2003
Lamiinae (Lamiini)	*Anoplophora glabripennsis* (Motschulsky)	*Z*9-C_{23}, *Z*9-C_{25}, *Z*7-C_{25}, *Z*9-C_{27}, *Z*7-C_{27}	Mixture of all five compounds applied to glass dummies elicited abdominal bending in ♂	Zhang *et al*., 2003
Lamiinae (Lamiini)	*Plectrodera scalator* (F.)	Hexane extracts of ♀	♂ attempt to mate with reconstituted ♀	Ginzel and Hanks, 2003
Lamiinae (Lamiini)	*Psacothea hilaris* (Pascoe)	Ether extracts of ♀ *Z*8-$_{21}$Me-C_{35}	♂ attempt to mate with treated dummies ♂ display a dose-dependant response to treated gelatin capsules, but weaker than that elicited by crude extract	Fukaya and Honda, 1995 Fukaya *et al*., 1996
Lamiinae (Monochamini)	*Acalolepta luxuriosa* Bates	Solvent extracts of ♀ elytra	♂ attempt to mate with dummies treated with extracts	Kubok *et al*., 1985
Lamiinae (Monochamini)	*Monochamus alternatus* Hope	Hexane extracts of ♀	♂ attempt to mate with dummies treat with 3-5 female equivalents of extract	Kim *et al*., 1992
Prioninae (Prionini)	*Prionus californicus* Motschulsky	Hexane extracts of ♀	56% of ♂ display abdominal bending toward reconstituted ♀ (extracts applied to solvent-washed ♀)	Barbour *et al*., 2007

Adapted from Allison *et al*., 2004.

use of bioassays to demonstrate the role of hydrocarbons in mate recognition. Two methods of sampling cuticular components – traditional whole-body solvent extraction and solid phase microextraction (SPME) wipe sampling – are detailed. Quantitative and qualitative differences in the hydrocarbon profiles of males and females are then discussed, as well as the biological relevance of cuticular hydrocarbons specific to males. The focus then shifts towards the use of single components and blends of compounds as contact pheromones of cerambycids.

Contact pheromones in the mating systems of longhorned beetles

Volatile male-produced sex and aggregation pheromones have been identified for a number of species, including eleven species in three tribes of the subfamily Cerambycinae alone (see Lacey *et al*., 2004, 2007; Hanks *et al*., 2007). Most of these pheromones share a common structural motif: six-, eight-, or ten-carbon chains with hydroxyl or carbonyl groups on C_2 or C_3. Some of these compounds (e.g., (2, 3) hexanediol and (*R*)-3-hydroxy-2-hexanone) may even serve as a widespread aggregation pheromone for this diverse and speciose subfamily. In fact, live traps baited with generic blends of racemic 2-hydroxy-3-hexanone and 3-hydroxy-2-hexanone captured both males and females of three sympatric cerambycine species (Hanks *et al*., 2007). The structure of these compounds is unique to the Cerambycidae; among the beetles, semiochemicals used by members of even closely-related families are quite different. Mate location and recognition in beetles in the subfamily Cerambycinae appear to involve three sequential behavioral stages: (1) both sexes are independently attracted to volatiles emanating from the weakened larval host plant; (2) males attract females over shorter distances with aggregation pheromones; and (3) males recognize females by contact pheromones (Ginzel and Hanks, 2005).

In cerambycids of diverse subfamilies, males orient to females only after contacting them with their antennae, and it appears that mate recognition is mediated by contact chemoreception alone (see Hanks, 1999). Males may even come within millimeters of females and not recognize them if antennal contact is not made. The reliance on chemical rather than visual cues for mate recognition may allow males to more readily detect females on the adult food source, larval host or in the dark. In some species there is marked sexual dimorphism in antennal length; the antennae of males are often more than double the length of those of females (e.g., see volumes indexed by Linsley and Chemsak, 1997). These elongate antennae may aid in locating mates. For example, male *Phoracantha semipuncatata* walk along the larval host trees with their antennae outstretched before them searching for females. Males recognize females by antennal contact alone and longer antennae contribute to a wider antennal spread, increasing the probability of encountering a mate (Hanks *et al*., 1996). The antennae of male *P. semipunctata* contain *sensilla trichodea*, which may serve as contact chemoreceptors (Lopes *et al*., 2005). After initial antennal contact with a female, male cerambycids of different subfamilies display a similar progression of behaviors that lead to copulation (see Ginzel *et al*., 2003a). The male stops walking immediately after touching a female with his antennae, and then aligns his body with the female,

grasping the pronotum or elytra with his forelegs. The male then bends his abdomen to couple the genitalia and mates with the female.

The use of bioassays to study contact chemoreception of cerambycids

The first empirical evidence that cerambycids use contact pheromones for mate recognition came from Heintz (1925), who reported that males of flower-visiting lepterines recognized females solely by antennal contact (see Linsley, 1959). Interestingly, male beetles whose antennae were removed were unable to recognize conspecific females. Our understanding of contact chemoreception in this economically important family has subsequently increased greatly through the use of bioassays. In fact, most recent studies (see Table 17.1) have relied on a common bioassay to demonstrate that contact pheromones mediate mate recognition in cerambycids. In the assay, a female beetle is freeze-killed, and after being allowed to warm to room temperature, is presented to a male in a Petri dish arena. If the male attempts to mate with the female, it demonstrates that recognition cues on the cuticle are intact and behavior is not involved in mate recognition. The hydrocarbons are then stripped from the cuticle of the female by immersing her in successive aliquots of hexane or some other nonpolar solvent. These washes presumably contain all of the hydrocarbon components of the cuticle. The female is then allowed to air dry before she is reintroduced to the male to test whether he will respond to her in any way. If the male does not respond, it confirms that solvent washing removed chemical cues that mediate mate recognition. To test whether the extract resulting from the solvent washes contains the pheromone, the extract is pipetted back onto the female's body and the reconstituted female is presented again to the same male. The bioactivity of these extracts is often tested at 0.1 female-equivalent (FE) increments, where one FE is the total amount of hydrocarbons extracted from an individual female. This assay is useful for testing not only the bioactivity of the crude extract, but also that of fractions of the crude extract and synthetic compounds.

Interestingly, males of some species respond more readily to freeze-killed females than to solvent-washed females that have been reconstituted with crude cuticular extracts. For example, only 40% of male *Megacyllene caryae* tested display the full progression of mating behavior ending with abdominal bending toward reconstituted females, suggesting that solvent extraction may scramble the profile of hydrocarbons present in the stratified wax layer (Ginzel *et al*., 2006). Additionally, males of *Neocyltus acuminatus acuminatus* do not respond as strongly to freeze-killed females as to live ones. Freeze-killed females elicited abdominal bending from only 35% of males, suggesting that freezing may also alter the chemical cue or perhaps behavioral or physiological cues are necessary for mate recognition (Lacey *et al*., 2008).

Models or dummies have also been used to demonstrate the use of contact pheromones in the Cerambycidae (see Table 17.1). For example, male *Acalolepta luxuriosa* display abdominal bending toward a model made from solvent-washed elytra of females to which crude hexane and ether extracts of females have been applied. Kim *et al*. (1992) immersed female Japanese pine sawyers, *Monochamus alternatus*, in hexane and tested the bioactivity

of the resulting extract in a bioassay using a dummy made of a glass rod. A dummy coated with 3 to 5 FEs of extract elicited male mating behavior. Similarly, male *Psacothea hilaris* attempt to mate with gelatin capsules treated with female extract; the primary component of the pheromone was later identified as (*Z*)-21-methyl-8-pentatriacontene ((*Z*)-21Me$C_{35:1}$; Fukaya *et al*., 1996, 1997). More recently, it was also demonstrated that male *Prionus californicus*, a member of the primitive subfamily Prioninae, attempt to mate with a ground glass stopper coated with one FE of crude cuticular extract (Barbour *et al*., 2007). These assays support the notion that neither vision nor the behavior of females is important for mate recognition in many longhorned beetles.

Sampling cuticular hydrocarbons

Solvent extraction: Contact pheromones have usually been identified by comparing the hydrocarbon profiles of whole-body solvent extracts of male and female beetles (see references in Table 17.1). As males seldom attempt to mate with conspecific males (Ginzel *et al*., 2006), the compounds that mediate mate recognition are often unique to the cuticular extracts of females. To identify female-specific compounds that may serve as contact pheromones, solvent extracts of males and females are analyzed by coupled gas chromatography–mass spectrometry (GC-MS). In some cases, such as the contact pheromones of *Xylotrechus colonus,* only a few compounds are unique to the female cuticle. For example, there are three early-eluting compounds (i.e., *n*-pentacosane, 9-methylpentacosane, and 3-methylpentacosane) in the extracts of females that are either absent or present in very small amounts in extracts of males (see Figure 17.1; Table 17.2; Ginzel *et al*., 2003a). Interestingly, when the bioactivity of these compounds was tested in assays like those described above, all three were necessary to elicit the full sequence of mating behavior in males.

Solid phase microextraction: Solid phase microextraction (SPME) has recently been used as an alternative to solvent extraction for studying the cuticular hydrocarbons of insects, including cerambycids (e.g., Turillazzi *et al*., 1998; Peeters *et al*., 1999; Liebig *et al*., 2000; Sledge *et al*., 2000; Roux *et al*., 2002; Ginzel *et al*., 2003b, 2006; Lacey *et al*., 2008). SPME is a solvent-less sampling technique and yields samples that are qualitatively and quantitatively similar to those obtained by solvent extraction (Moneti *et al*., 1997; Monnin *et al*., 1998; Bland *et al*., 2001; Tentschert *et al*., 2002). Moreover, wiping the SPME fiber over the cuticle primarily samples the outer surface of the wax layer, making extracts free from internal body lipids and exocrine gland secretions that may contaminate solvent extracts. An SPME apparatus resembles a syringe and contains a retractable fused-silica fiber, coated with a thin polymer film (Millar and Sims, 1998). The most common SPME coating used in sampling cuticular hydrocarbons of longhorned beetles is 100 μm polydimethylsiloxane (Ginzel *et al*., 2003b; Ginzel *et al*., 2006; Lacey *et al*., 2008). During sampling, the fiber is wiped across the cuticular surface with the polymer essentially acting as a sponge. The fiber is then retracted back into the protective sheath and extended again inside a heated GC inlet where the analytes are thermally desorbed.

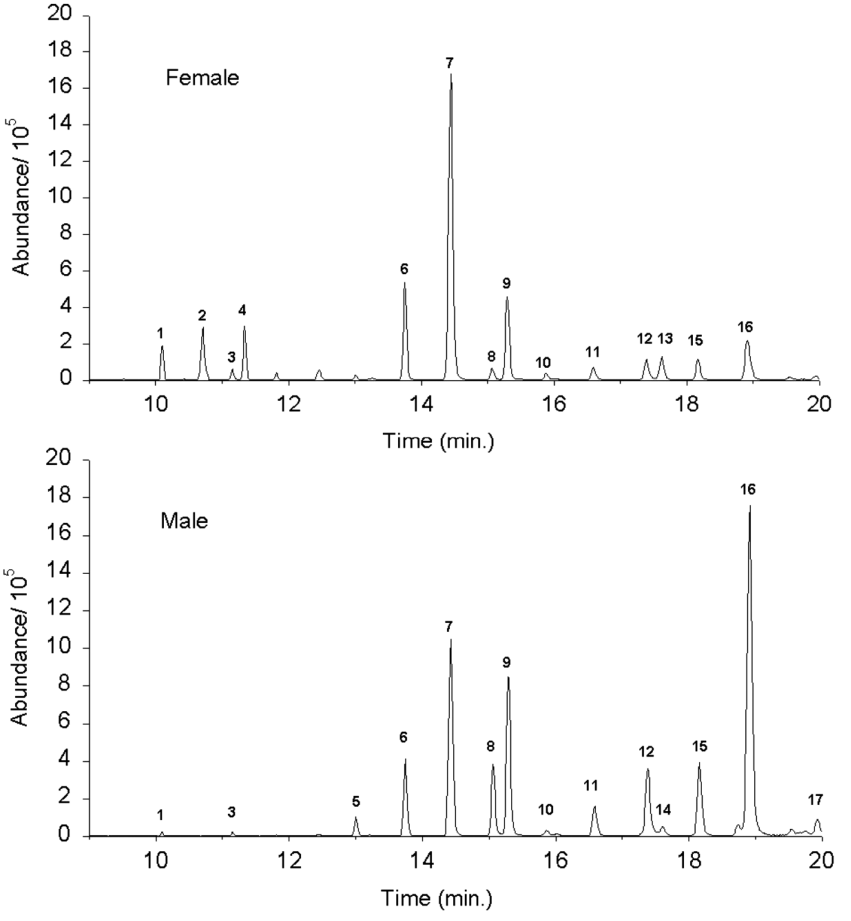

Figure 17.1 Representative gas chromatograms of hexame extracts of a *Xylotrechus colonus* female (top) and male (bottom). Reproduced from Ginzel *et al*., 2003a with permission of Springer Science and Business Media.

SPME has been used in conjunction with solvent extraction to identify the contact pheromones of *Megacyllene robiniae* (Ginzel *et al*., 2003b)*, M. caryae* (Ginzel *et al*., 2006), and more recently *N. a. acuminatus* (Lacey *et al*., 2008). It appears that wipe sampling by SPME may yield a more representative profile of cuticular components than solvent extraction (Ginzel *et al*., 2003b). For example, the contact pheromone of *M. robiniae,* (*Z*)-9-pentacosene (*Z*9-$C_{25:1}$), comprised ~16% of the total hydrocarbons in hexane extracts of females and was co-dominant with two other hydrocarbons that were not biologically active. In contrast, *Z*9-$C_{25:1}$ was dominant in the SPME wipe samples of female elytra, thoracic tergites and abdominal sternites and represented ~34% to 36% of the sampled hydrocarbons, suggesting that hydrocarbons that cue mate recognition are more abundant on the surface of the wax layer of females where they are readily accessible to the antennae of males (Ginzel *et al*., 2003b). Interestingly, *Z*9-$C_{25:1}$ was also present in the hexane extract

Table 17.2 *Cuticular hydrocarbons of female and male* Xylotrechus colonus.[1]

Peak Number	Hydrocarbon	Female	Male	Diagnostic ions
1	*n*-C_{25}	+	+	352 (M+)
2	9-MeC_{25}	+	–	140, 252/253, 366 (M+)
2	11-MeC_{25} (trace)	+	–	168/169, 224/225
3	2-MeC_{25}	+	+	323, 351, 366 (M+)
4	3-MeC_{25}	+	–	309, 337, 366 (M+)
5	2-MeC_{26}	–	+	337, 365, 380 (M+)
6	*n*C_{27}	+	+	380 (M+)
7	11,13-MeC_{27}	+	+	168/169, 196/197, 224/225, 252/253, 394 (M+)
8	2-MeC_{27}	+	+	351, 379, 394 (M+)
9	3-MeC_{27}	+	+	337, 365, 394 (M+)
10	*n*-C_{28}	+	+	394 (M+)
11	13-MeC_{28}	+	+	196/197, 238/239
11	12, 11-MeC_{28} (trace)	+	+	168/169, 182/183, 252/253, 266/267
12	C_{29}:1	+	+	406 (M+)
13	C_{29}:1	+	–	406 (M+)
14	3-MeC_{28}	–	+	351, 379, 408 (M+)
15	*n*-C_{29}	+	+	408 (M+)
16	11, 13, 15-MeC_{29}	+	+	168/169, 196/197, 224/225, 252/253, 280/281, 422 (M+)
17	C_{31}:1	–	+	434 (M+)

[1] Peak numbers correspond with Figure 17.1; "+" indicates compound is present and "–" indicates it is absent. 11-MeC_{25} and 12,11-MeC_{28} coeluted in trace amounts with other compounds. Peaks 12 and 13 represent isomers of the same alkene. Reproduced from Ginzel *et al*., 2003a with permission of Springer Science and Business Media.

of males but represented a negligible proportion of the SPME samples of the male cuticle, suggesting that this compound is not on the surface of the cuticle of males where it would be detected by the antennae, but rather sequestered deeper in the wax layer. Moreover, in the congener *M. caryae* an important component of the contact pheromone, *Z*9-$C_{29:1}$, was the only compound among the dominant hydrocarbons that was present in higher abundance in SPME than in solvent extracts (Ginzel *et al*., 2006). SPME was also recently used to identify minor components of the *N. a. acuminatus* contact (Lacey *et al*., 2008). There are a number of advantages to SPME over solvent extraction. Namely, SPME may provide

a clearer representation of the semiochemicals present in the wax layer, and it is a non-destructive sampling technique. Living insects can be repeatedly sampled by SPME and later used in bioassays – an important consideration given the difficulty in rearing the large numbers of insects required for meaningful statistically-based laboratory experiments.

These sampling techniques are not without limitations. For example, high-molecular weight compounds that are beyond the analytical range of GC and electron-ionization MS (EI-MS) may be present in the wax layer and serve as synergists or minor components of contact pheromones. Recently, matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry was used to demonstrate that the wax layer of 12 insect species of diverse taxa contains high-molecular weight hydrocarbons as long as C_{70} (Cvačka *et al*., 2006). These long-chain length compounds would likely decompose even in high-temperature GC columns, and identification by EI-MS would be very difficult as the molecular ion would likely not survive.

Qualitative and quantitative differences in hydrocarbon profiles of the sexes

Cuticular hydrocarbon profiles of male and female beetles of some cerambycid species are qualitatively quite similar, with most cuticular components occurring in both sexes. For example, in *X. colonus* there are only four compounds that are unique to the female cuticle and another three that are male specific (Ginzel *et al*., 2003a). Moreover, cuticular profiles of male and female *Anoplophora glabripennis* share a series of saturated, branched, and unsaturated compounds, but five alkenes are far more prevalent in the extracts of females. Interestingly, all five of these compounds are necessary to elicit abdominal bending in males (Zhang *et al*., 2003). In *M. caryae,* however, cuticular profiles of females contain a number of aliphatic hydrocarbons that are not present in the wax layer of males. In fact, extracts of males and females were qualitatively very different and sex-specific compounds represented almost half of the hydrocarbons of females and a third of the hydrocarbons of males (Ginzel *et al*., 2006). In cases where there are many qualitative differences in the profiles of males and females, it is often most expedient to first test the biological activity of these compounds by functional group. For example, the bioactivity of the most abundant synthetic straight-chain alkanes, branched alkanes and monoenes present in the extract of female *M. robiniae* were tested by functional group, and only the synthetic monoenes elicited a mating response in males similar to that of crude extact. Moreover, of the monoenes, males responded most strongly to *Z*9-C_{29}:1 alone – the contact pheromone. Cuticular extracts of males also contained a greater proportion of longer-chain hydrocarbons, which also appears to be true for *M. caryae* and *X. colonus* (Ginzel *et al*., 2003a, 2006). On the other hand, solvent extracts of female *Anoplophora malasiaca* contain longer-chained hydrocarbons which are lacking in the hydrocarbon profiles of males (Akino *et al*., 2001). Sex-based differences in alkyl chain lengths of cuticular hydrocarbons have also been reported in other insects such as the tsetse fly (Nelson and Carlson, 1986) and the bark beetle *Ips lecontei* (Page *et al*., 1997).

Males of many cerambycid species compete aggressively for mates and after copulation a male often guards a female by grasping her elytra with his forelegs and accompanying

her as she seeks out oviposition sites (see Hanks, 1999). Males may even remain paired with the female for long periods of time, repeatedly copulating with her, while defending her from challenging males. Rival males deploy a variety of tactics to displace paired males, including antennal lashing, biting, and head butting. There is some evidence that male-specific cuticular components mediate aggressive behavior and male–male competition. For example, male *Nadezhdiella cantori* lash with their antennae and front legs and also violently bite dead conspecific males and even paper rolls treated with solvent extracts of males (Wang *et al*., 2002). There is also evidence to suggest these male-specific hydrocarbons may be acting as chemical deterents to mating or abstinons. For example, hexane extracts of female *Semanotus japonicus* elicit abdominal bending in males but, when combined with extracts of males, their bioactivity is greatly reduced (Kim *et al*., 1993).

Contact pheromones as single compounds or blends

Mate recognition in cerambycids can be mediated by either a single component or blends of several compounds (Table 17.1). In the subfamily Cerambycinae, for example, contact pheromones of the congeners *M. caryae* and *M. robiniae* are single alkenes and also chain-length analogs (Ginzel *et al*., 2003b, 2006). Although the contact pheromones of these two closely related species are quite similar, it appears that the composition of cerambycid contact pheromones is not phylogenetically conserved. In fact, the contact pheromone of *M. robiniae*, *Z*9-$C_{25:1}$, is also one of five alkenes that mediate mate recognition in the lamiine *A. glabripennis* (Zhang *et al*., 2003). Even other members of the tribe containing the *Megacyllene* species use contact pheromones that are blends of saturated *n*- and methyl-branched compounds. The contact pheromone of *X. colonus*, for example, is a mixture of *n*-C_{25} and two branched alkanes (Ginzel *et al*., 2003a). Moreover, a branched alkane, 7-methylheptacosane (7Me-C_{27}), mediates mate recognition in *N. a. acuminatus*, but two other branched compounds, 9Me-C_{27} and 7Me-C_{25}, act as synergists (Lacey *et al*., 2008). Interestingly, 9Me-C_{27} is part of the cuticular profile of female *M. caryae* (Ginzel *et al*., 2006) and apparently a component of the contact pheromone of *A. malasiaca*, a member of the subfamily Lamiinae (Fukaya *et al*., 2000), further suggesting that some hydrocarbons may be common to cerambycids. In addition to 9Me-C_{27}, the contact pheromone of *A. malasiaca* consists of a blend of seven other hydrocarbons and five ketones, and it also appears that three gomadalactones (oxabicyclo[3.3.0]octane compounds with an aliphatic chain) serve as synergists (Fukaya *et al*., 2000; Yasui *et al.*, 2003, 2007). The structure of this pheromone is unique among the cerambycids because it is composed of two classes of compound. To date, no other polar compounds have been identified as contact pheromones of longhorned beetles.

A number of cerambycid contact pheromones are methyl-branched alkanes that have chiral carbons. In the case of *N. a. acuminatus*, for example, the three bioactive methyl-branched alkanes are chiral. However, these compounds are available in vanishingly small

amounts from each insect, and with current analytical limitations it is nearly impossible to determine their natural enantiomeric ratio in the wax layer. Moreover, there are currently no chiral stationary phase GC or LC columns capable of resolving enantiomers of long-chain methy-branched hydrocarbons (Lacey *et al*., 2008). Irrespective of the naturally occurring enantiomeric ratios, male *N. a. acuminatus* respond to racemic standards, suggesting that even if females produce only one enantiomer its activity is not influenced by the presence of the other. In *P. hilaris*, the major component of the contact pheromone (*Z*8–21Me-$C_{35:1}$) is a long-chain methyl-branched alkene, and accounts for approximately 60% of the hydrocarbons extracted from the female elytra. Nevertheless, the bioactivity of the synthetic compound is considerably less than that of the crude solvent extracts of females, suggesting that enantiomeric composition may influence its activity. Although only the (*Z*)-configuration of 8–21Me-$C_{35:1}$ was found in extracts of females, Fukaya *et al*. (1997) evaluated the bioactivities of synthetic (*R*)- and (*S*)-enantiomers of both (*Z*)- and (*E*)-isomers of the synthetic compound. Males displayed a greater response to the (*Z,R*)- and (*Z,S*)-isomers but the response to the different optical enantiomers of this compound were similar, suggesting that males can distinguish between the two geometric isomers, but optical rotation has little influence on the bioactivity of the pheromone. It is likely that males respond less readily to the synthetic pheromone because it lacks minor components that are present in the crude extract that may act as synergists.

Conclusions

Contact pheromones play an essential role in the mating systems of many longhorned beetles and may also be important in the divergence of this speciose group by acting as prezygotic mating isolation mechanisms. Our understanding of the chemically-mediated mate location strategies of cerambycids suggests that volatile sex pheromones are highly conserved and share a common structural motif. In fact, even species that share the same host plant may be attracted to one another's volatile aggregation pheromones (see Hanks *et al*., 2007). Furthermore, the niches of closely related cerambycid species often overlap and species-specific contact pheromones may play a vital role in maintaining reproductive isolation.

To date, research on the chemical ecology and contact chemoreception of cerambycids remains largely descriptive in nature. Although our understanding of mate recognition in this group has improved considerably in recent years, most work has focused on the more advanced subfamilies. Further research on the primitive subfamilies, including the Parandrinae, Prioninae and Aseminae, will shed light on the evolution of mating systems in this economically important family. Finally, the biosynthesis, regulation and transport of these semiochemicals remain virtually unexplored in the longhorned beetles. By applying powerful tools of molecular biology and physiology to understanding these processes, effective pest-management tactics targeting the chemically-mediated mating behavior of the beetles are likely on the horizon.

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