CHAPTER TWENTY-ONE

# Pheromones of Heliothine Moths

N. KIRK HILLIER and THOMAS C. BAKER



## Introduction

Within noctuid moths, species in the subfamily Heliothinae (also known as owlet moths) represent an excellent model system for examining divergence of traits associated with pheromone production, detection, and processing in closely related species. Species of Heliothinae are ubiquitous, with many species distributed globally. Estimates of diversity have included 25–28 genera and approximately 365 species (Cho et 2008). Most species are seed and bud feeders, with many having the common names of "budworms" or "bollworms" depending on their host association (Hardwick 1965, 1970;

TABLE 21.1

Heliothine spp. with documented pheromones and/or odor-mediated behavior to pheromone components (i.e., attraction to synthetic lures in the field trapping)

| Species | Common name | Known distribution | Pest status | Citation(s) |
|---|---|---|---|---|
| | | | *Heliothis* spp. | |
| *H. belladonna* | None | Western North America | Host unknown | Landolt et al. (2006) |
| *H. maritima* | Fulvous clover | Eurasia | Minor polyphagous pest | Szöcs et al. (1993) |
| *H. maritima adaucta* | Flax budworm | Japan (subspecies) | Minor polyphagous pest | Kakizaki and Sugie (1993) |
| *H. ononis* | Flax bollworm | Eurasia and western North America | Minor pest on *Linum* spp. | Steck et al. (1982) |
| *H. peltigera* | Bordered straw | Eurasia, Africa | Major polyphagous pest | Dunkelblum and Kehat (1989) |
| *H. phloxiphaga* | Darker spotted straw moth | North America | Minor polyphagous pest | Raina et al. (1986); Kaae et al. (1973) |
| *H. subflexa* | Physalis bud moth | North America, South America | Minor pest–host specialist | Vickers (2002); Heath et al. (1990); Teal et al. (1981a) |
| *H. virescens* | Tobacco budworm | Americas, Caribbean, Hawaii | Major polyphagous pest | Hendricks et al. (1989); Shaver et al. (1989); Teal and Tumlinson (1989); Ramaswamy and Roush (1986); Teal et al. (1986); Ramaswamy et al. (1985); Vetter and Baker (1983); Pope et al. (1982); Klun et al. (1980a); Mitchell et al. (1978); Roelofs et al. (1974) |
| | | | *Helicoverpa* spp. | |
| *H. armigera* | African bollworm | Eurasia, Africa, Australia, Oceania | Major polyphagous pest | Zhang et al. (2012); Kvedaras et al. (2007); Dong et al. (2005); Kehat and Dunkelblum (1990); Nesbitt et al. (1979); Dunkelblum et al. (1980); Kehat et al. (1980); Gothilf et al. (1979); Rothschild (1978); Piccardi et al. (1977) |
| *H. assulta* | Oriental tobacco budworm | Africa, Asia, Australia, Oceania | Major polyphagous pest | Park et al. (1996); Park et al. (1994); Cork et al. (1992); Sugie et al. (1991) |
| *H. gelotopoeon* | South American bollworm | Southern South America | Major polyphagous pest | Cork and Lobos (2003) |
| *H. punctigera* | Australian bollworm | Australia | Major polyphagous pest | Rothschild (1978); Rothschild et al. (1982) |
| *H. zea* | Corn earworm | Americas | Major polyphagous pest | Descoins et al. (1988); Pope et al. (1984); Teal et al. (1984); Vetter and Baker (1984); Klun et al. (1980b) |
| | | | Other genera | |
| *Schinia bina* | Bina flower moth | North America | Nonpest, feeds on selected Asteraceae | Underhill et al. (1977) |
| *Schinia meadi* | Mead's flower moth | Western North America | Host unknown | Steck et al. (1982) |
| *Schinia mitis* | Matutinal flower moth | Southeastern United States | Nonpest, specialist on *Pyrrhopappus* spp. | Mitchell (1982) |
| *Schinia suetus* | None | Western North America | Nonpest, specialist on *Lupinus* spp. | Byers and Struble (1987) |

Mitter et al. 1993; Matthews 1999) (Table 21.1). In addition, the degree of host association is variable, ranging from species that are highly host specific to those that exhibit wide polyphagy, feeding on >100 plant species, or families.

Host associations are known for approximately one quarter of heliothines, of which 70% are considered largely oligophagous, with the remaining 30% being polyphagous (Cho et al. 2008) (Table 21.1). For example, *Heliothis subflexa* larvae feed exclusively on fruits of *Physalis* spp. (Solanaceae; e.g., groundcherries, tomatillos, cape gooseberries), whereas primrose moth, *Schinia florida*, larvae are exclusive feeders on buds of evening primrose (Onagraceae: *Oenothera* spp.) (Hardwick 1958). Species in other genera, such as *Pyrrhia* and *Asidura*, maintain strict host-specialist relationships. Several heliothine species are important agricultural pests, particularly those in the genera *Heliothis* and *Helicoverpa* (Table 21.1). Based on a molecular phylogeny by Cho et al. (2008), early divergent lineages are almost exclusively host specialists, whereas species that fall into the "*Heliothis* group" represent the most extreme polyphages. This latter group includes what are more commonly referred clades of the "corn earworm complex" and "tobacco budworm group" (Cho et al. 2008).

The *Heliothis* group includes some of the most important agricultural pests worldwide, causing massive annual crop losses, particularly in the developing world. Estimates range between US$3 billion and US$7 billion in control costs per annum for the most prevalent agricultural pest species: *Helicoverpa armigera*, *H. zea*, *H. assulta*, *H. punctigera*, and *Heliothis virescens* (Fitt 1989). These species are important pests on a range of forage, oilseed, and food crops, including cotton, corn, sorghum, soybean, flax, tobacco, and tomato.

## Worldwide Diversity of Heliothine Pheromone Composition

Divergence in olfactory communication is evident among heliothine species, based on shifts in the use of key components within species' pheromone blends that function to optimize attraction of conspecific males and reduce attraction of males to females of closely related species. Multicomponent pheromones have been described in all heliothine species studied to date, and their function has been supported by studies investigating pheromone gland composition, behavior, electrophysiology, field trap attraction, and odorant receptor (OR) gene expression.

Avoidance of mating mistakes with members of a wrong species during mate finding and courtship is of paramount importance for sympatric moth species, including heliothines. Male moths respond with remarkable sensitivity and selectivity to very precise multicomponent mixtures of female-emitted sex pheromone components. Deviations in blend ratios involving key components of pheromone mixtures will significantly affect the degree to which males are attracted to conspecific females and deterred from attraction to heterospecifics. Isolation is further augmented by structural specificity in genitalia, with some reports documenting irreversible genitalia "locking" between *Heliothis virescens* and *Helicoverpa zea* in the field and laboratory (Hardwick 1965; Shorey et al. 1965; Teal et al. 1981b; Stadelbacher et al. 1983). In instances where prezygotic isolation is incomplete, such as in mating of *H. virescens* and *H. subflexa*, there are distinct fitness costs: hybrid male progeny of these two species are sterile, producing largely apyrene sperm (Proshold and LaChance 1974). Attraction and subsequent successful copulation are therefore tightly linked to an individual's ability to produce (usually female), detect, and respond with appropriate behavior (male) to a conspecific versus a heterospecific signal.

With the exception of one species, all heliothines studied to date use (*Z*)-11-hexadecenal (Z11-16Ald) as a component of the female-produced sex pheromone (figure 21.1; Table 21.2). In most cases, Z11-16Ald is the predominant component in female effluvia. Two aldehydes used as minor components, (*Z*)-9-tetradecenal (Z9-14Ald) and (*Z*)-9-hexadecenal (Z9-16Ald), appear to be key variable components whose interchangeability dictates the species specificity of many blends. Throughout the Heliothinae, shifts in male preference for blends that include either Z9-14Ald or Z9-16Ald have apparently occurred, contributing to reduction in mating mistakes and hence to reproductive isolation and possibly speciation. Evidence also suggests isolation between *H. virescens* and *H. subflexa* is modulated by pheromone preference controlled by a single locus containing four OR genes that modulate pheromone behavioral response specificity via OR ligand selectivity (Gould et al. 2010; Vásquez et al. 2011; Wang et al. 2011).

In addition to the sex pheromone "components" in emissions produced by the pheromone glands of many moth species, there are other volatile compounds in effluvia that may decrease attraction of heterospecific males due to additional olfactory pathway antagonism that unbalances an otherwise balanced blend (Domingue et al. 2007; Baker 2008). In many cases, the effects of a heliothine species' pheromone components themselves on creating attraction depend on their relative ratio in the mixture. Sometimes an excess proportion of a minor component may decrease attraction of conspecific males by creating an excessive olfactory pathway antagonism that unbalances what should have been a balanced pheromone blend. In heliothines, such interactions have been supported by studies investigating combinatorial coding of pheromone components and pheromone-related compounds at the sensillar and antennal lobe (AL) levels (Christensen et al. 1990, 1995; Vickers et al. 1998; Vickers and Christensen 1998, 2003; Vickers 2006a,b; Hillier and Vickers 2011a). This interaction has been further proposed as "balanced" olfactory antagonism, wherein a continuum of negative and positive male behavior is modulated (Baker 2008).

## Biosynthesis of Pheromone Blends in Genera *Heliothis* and *Helicoverpa*

### Sequence of Action of Δ11-Desaturase and β-Oxidation Determine Composition of Many Pheromone Blends

The pheromone communication systems of heliothine moths all include various behaviorally active pheromone-component end products that are produced along pheromone biosynthetic pathways outlined by Jurenka (2003) for *Heliothis virescens* and *H. subflexa* (figure 21.2). As shown in Table 21.2, nearly all heliothine species use Z11-16Ald as the major (most abundant) sex pheromone component in their blends. The only exceptions thus far are *Helicoverpa assulta* in Asia in which Z9-16Ald is the major component, present at 10 times the abundance of Z11-16Ald (Cork et al. 1992; Park et al. 1994), and the South American species *H. gelotopoeon* in which Z11-16Ald is absent and replaced instead by saturated hexadecanal (16Ald) as the major component, with a nearly

TABLE 21.2

Heliothine female-emitted sex pheromone blends.

Pheromone components confirmed as both being produced by females and required for male attraction in laboratory and field studies are shown in headers in plain black non-italicized text. Compounds found to be produced by females but have no demonstrated behavioral effects on males or else have effects only in certain local populations are shown in headers in italicized black text. Black numbers indicate the percentages of compounds produced by females relative to Z11-16Ald (or relative to Z9-16Ald, *Heliothis assulta; or* to 16Ald, *H. gelotopoeon*). Check marks indicate trace amounts found. Green numbers indicate ranges of component percentages that have been shown to optimally evoke male attraction. Red numbers indicate heterospecific pheromone components shown to reduce attraction (behaviorally antagonistic) at the indicated percentages

| | Compounds evidenced in chemical communication | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | *14Ald* | Z9-14Ald | *Z9-14OH* | *Z9-14Ac* | 16Ald | *Z7-16Ald* | *Z7-16Ac* | Z9-16Ald | Z9-16OH | Z9-16Ac | Z11-16Ald | Z11-16OH | Z11-16Ac |
| *North and South America* | | | | | | | | | | | | | |
| *H. phloxiphaga* GLAND | | | | | ✓ | | | 0.5 | | | 100 | 7.2 | |
| *H. phloxiphaga* RESPONSE | | | | | | | | 0.6 | | | 100 | 2–3, 4 | |
| *H. subflexa* GLAND | ✓ | ✓ | | | 2.4 | | 3.6 | 42.9 | | 9.9 | 100 | 5.8 | 22.7 |
| *H. subflexa* RESPONSE | | | | | | | | 10–50 | | | 100 | 1–50> | 15–20 |
| *H. virescens* GLAND | ✓ | 0.4–6 | | | 25.6 | ✓ | | ✓ | | | 100 | | |
| *H. virescens* RESPONSE | | 5–50 | | | | | | | | | 100 | 3–30 | 0.1> |
| *H. gelotopoeon* GLAND | ✓ | | | | 100 | | | 84 | | | | | |
| *H. gelotopoeon* RESPONSE | | | | | 10> | | | 100 | | | 1> | | |
| *H. zea* GLAND | | 0 | | | ✓ | ✓ | | 1.8 | | | 100 | | |
| *H. zea* RESPONSE | | 1–3, 3> | | | | | | 1–15 | | | 100 | 0.1–10 | 0.1–10 |
| **H. armigera* GLAND | | 0.3 | | | | ✓ | | 2.5 | | | 100 | | |
| **H. armigera* RESPONSE | | 0.3–5, 25> | | | | | | 3–3000 | | | 100 | | |
| *Europe and Africa* | | | | | | | | | | | | | |
| *H. armigera* GLAND | | 0.3 | | | | ✓ | | 2.5 | | | 100 | | |
| *H. armigera* RESPONSE | | 0.3–5, 25> | | | | | | 3–3000 | | | 100 | | |
| *H. assulta* GLAND | | | | | ✓ | | | 100 | ✓ | 3051 | 6.5 | ✓ | ✓ |
| *H. assulta* RESPONSE | | 1> | | | ✓ | | | 100 | 2> | 30 | 5.0 | | 1.5 |
| *H. maritima* GLAND | | | | | 5.5 | | | ✓ | | | 100 | 8.1 | |
| *H. maritima* RESPONSE | | | | | 3–6 | | | | | | 100 | 6–20 | |
| *H. peltigera* GLAND | ✓ | 14.6 | ✓ | ✓ | ✓ | ✓ | | ✓ | | | 100 | 24.3 | ✓ |
| *H. peltigera* RESPONSE | | 5–50 | | | | | | | | | 100 | | |
| *Asia and Australia* | | | | | | | | | | | | | |
| *H. maritima adaucta* GLAND | | | | | ✓ | | | | | | 100 | 24.5 | |
| *H. maritima adaucta* RESPONSE | | | | | | | | | | | 100 | 1–3 | |
| *H. armigera* GLAND | | 0.3 | | | | ✓ | | 2.5 | | | 100 | | |
| *H. armigera* RESPONSE | | 0.3–5, 25> | | | | | | 3–3000 | | | 100 | | |
| *H. assulta* GLAND | | | | | ✓ | | | 100 | ✓ | 3051 | 6.5 | ✓ | ✓ |
| *H. assulta* RESPONSE | | 1> | | | ✓ | | | 100 | 2> | 30 | 5.0 | | 1.5 |
| *H. punctigera* GLAND | | <0.5 | | | | | | | | | 100 | 25 | 41.7 |
| *H. punctigera* RESPONSE | | 5 | | | | | | | | | 100 | 100> | 1–10 |

**H. armigera* is a recent introduction to Brazil (Tay et al. 2013).

SOURCES: *Heliothis maritima* (Szöcs et al. 1993); *Heliothis maritima adaucta* (Kakizaki and Sugie 2003); *Heliothis peltigera* (Dunkelblum and Kehat 1989); *Heliothis phloxiphaga* (Raina et al. 1986); *Heliothis subflexa* (Teal et al. 1981a; Klun et al. 1982; Heath et al. 1990 showed that OH is needed; Vickers 2002; Groot et al. 2007); *Heliothis virescens* (Roelofs et al. 1974; Pope et al. 1982; Vetter and Baker 1983; Teal et al. 1986; Vickers et al. 1991); Helicoverpa armigera (Piccardi et al. 1977; Nesbitt et al. 1979; Kehat and Dunkleblum 1990; Zhao et al. 2006; Zhang et al. 2012); *Helicoverpa assulta* (Sugie et al. 1991; Cork et al. 1992; Park et al. 1994; Zhao et al. 2006); *Helicoverpa gelotopoeon* (Cork and Lobos 2003); *Helicoverpa punctigera* (Rothschild et al. 1982); *Helicoverpa zea* (Klun et al. 1980b; Pope et al. 1984; Vetter and Baker 1984; Teal et al. 1984; Vickers et al. 1991; Fadamiro and Baker 1997; Quero and Baker 1999).

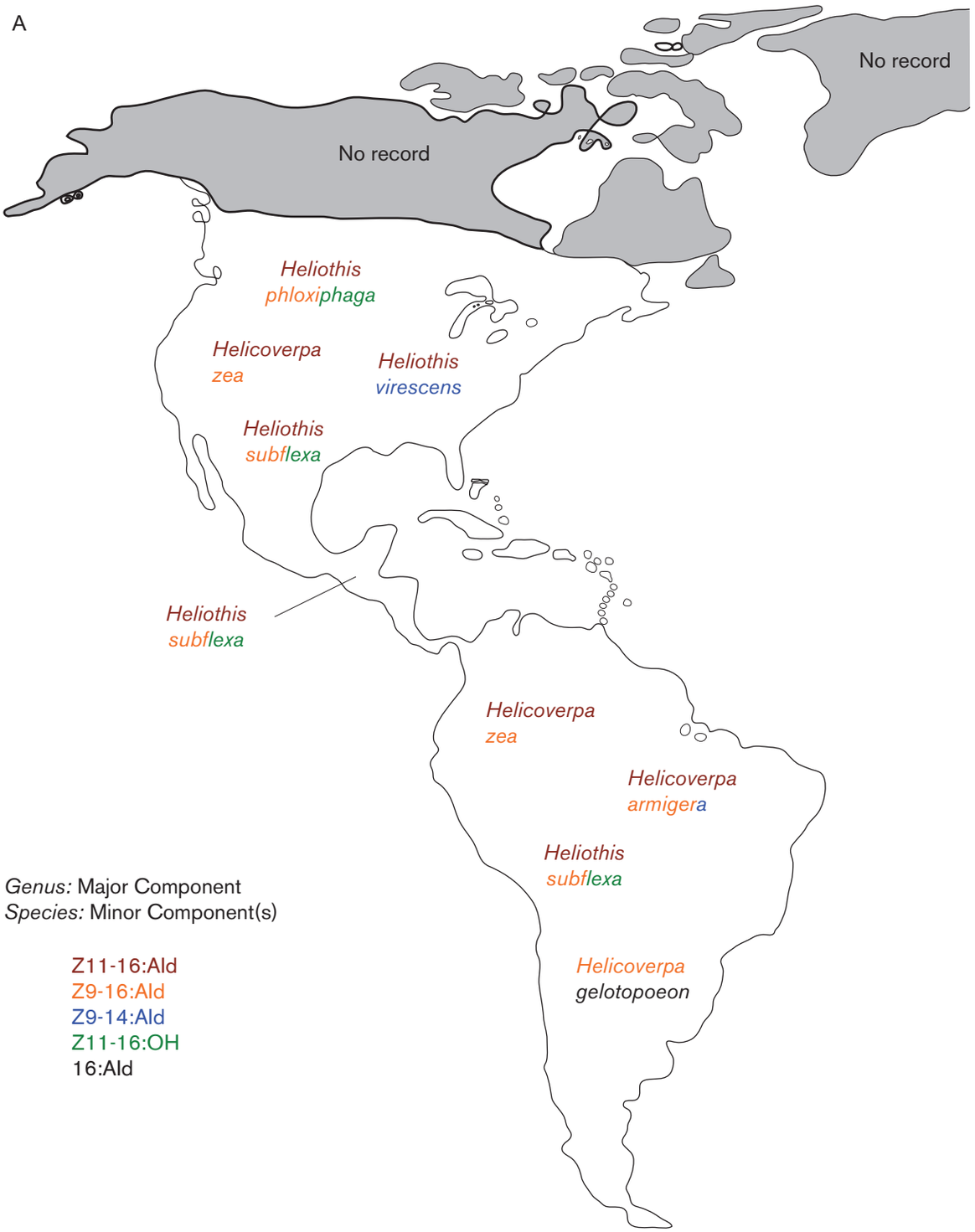



FIGURE 21.1 Worldwide distribution of *Helicoverpa* and *Heliothis* spp. and differences in their pheromone blends involving behaviorally important two-component mixtures. Genus names are color-coded according to the most abundant (major) component in the pheromone blend, as indicated in the key at the bottom of the figure. The colors depicted in each species' name denote experimentally demonstrated behaviorally important secondary components corresponding to the compounds in the color-coded key at the bottom of the figure. Some specific names are split into two colors because for those species, two secondary components are both important contributors to male behavioral response. (A) The Americas.

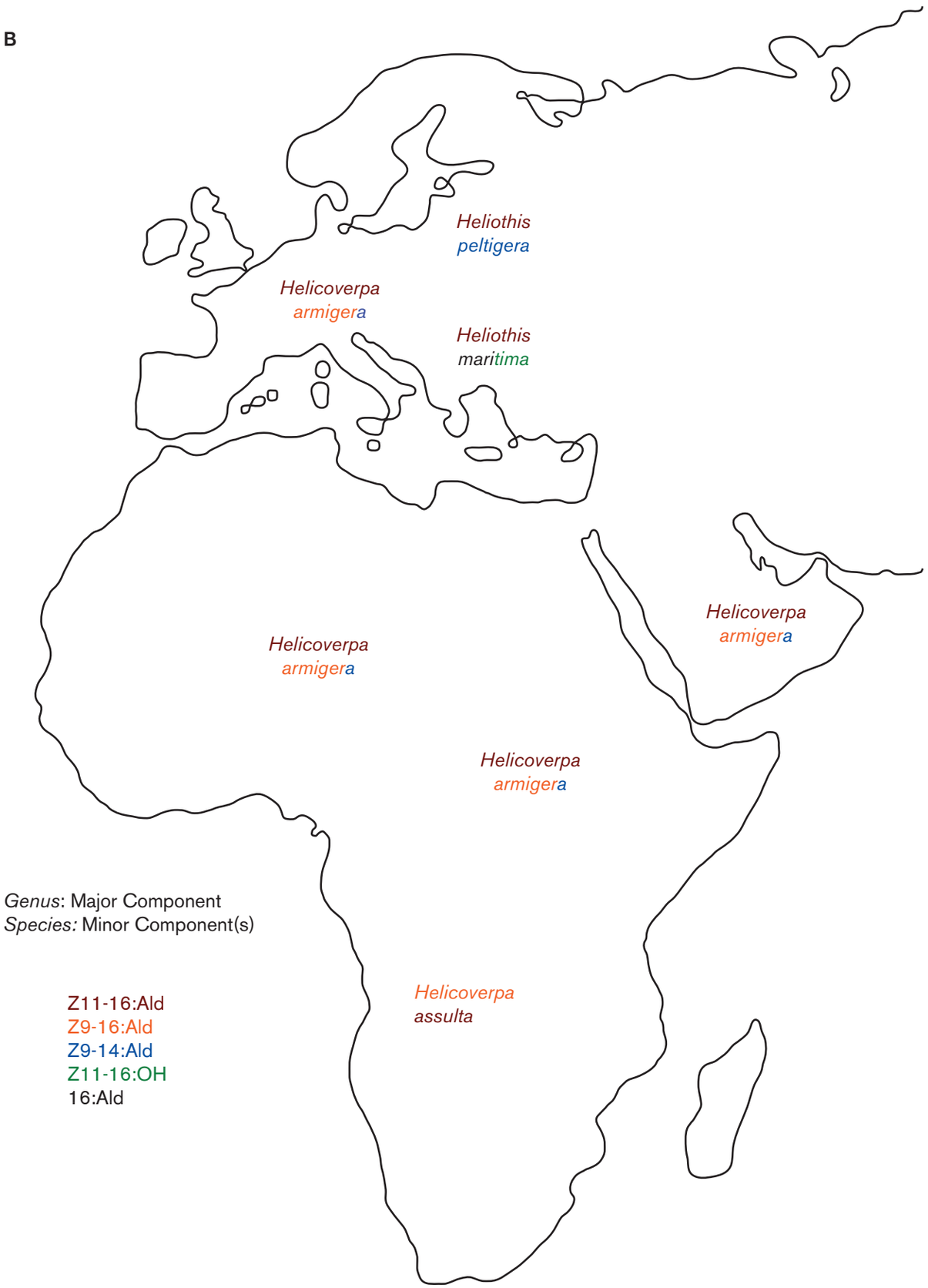



FIGURE 21.1 *(continued)* (B) Europe and Africa.

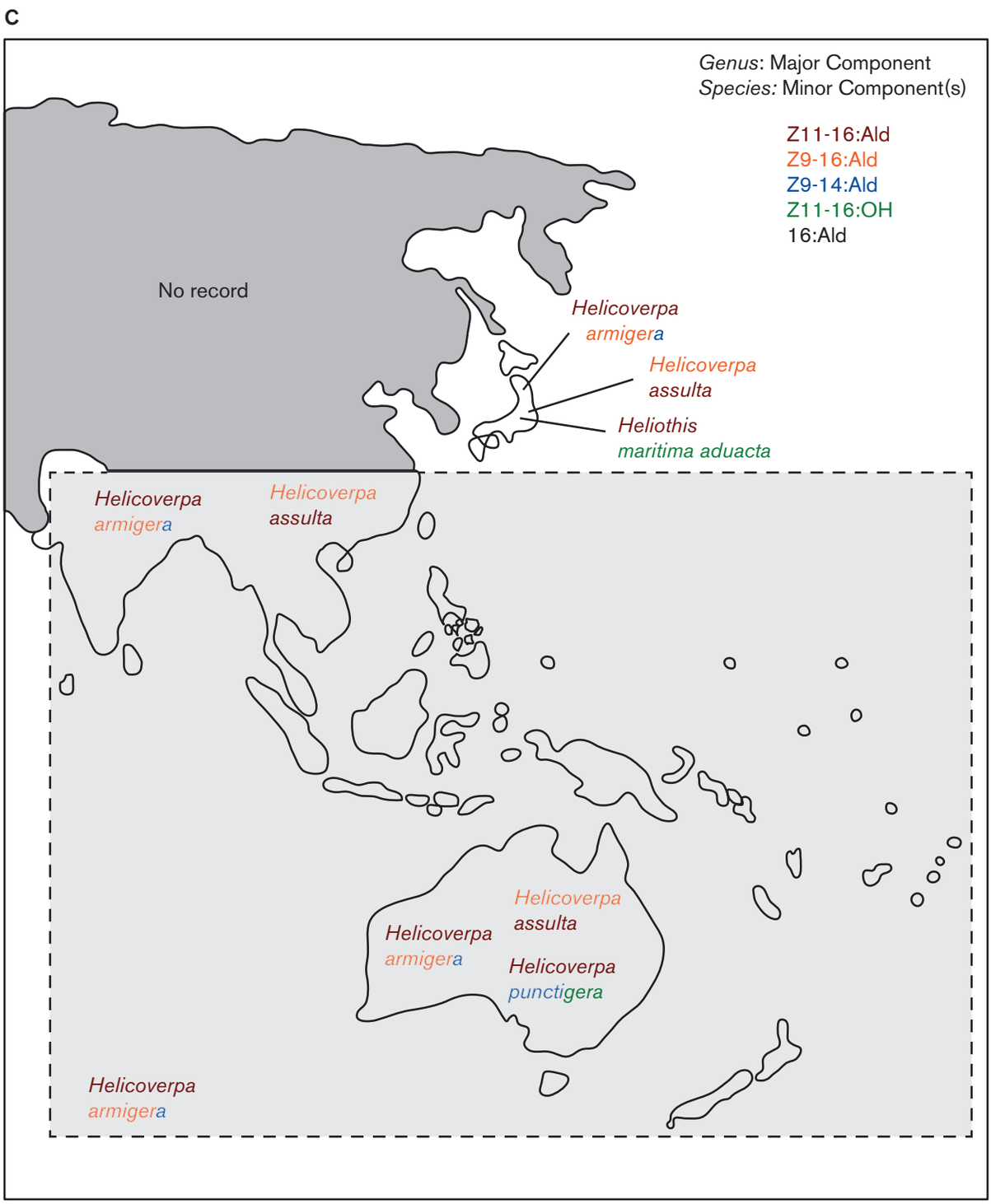



FIGURE 21.1 *(continued)* (C) Australasia. Gray boxed area denotes the putative distribution of *H. armigera* throughout the island regions of Australasia.

equal amount of Z9-16Ald to comprise an unusual two-component blend (Cork and Lobos 2003).

Considering all known pheromone blends of *Helicoverpa* and *Heliothis* spp. (Table 21.2), it is clear that nearly all make use of Δ11-desaturase acting on the C16 fatty acyl substrate (16CoA) rather than on a C18 fatty acyl substrate (18CoA) (figure 21.2), to produce Z11-16Ald and the related compounds (*Z*)-11-hexadecen-1-ol acetate (Z11-16Ac) and (*Z*)-11-hexadecen-1-ol (Z11-16OH) (figure 21.2). A second route that results in Z9-16Ald as either a major or a minor component involves Δ11-desaturase acting on the 18CoA substrate, followed by a chain-shortening, β-oxidation step to produce the Z9-16Ald and related alcohol and acetate. This route in which chain shortening follows desaturation of the long fatty acyl group (18CoA) has been

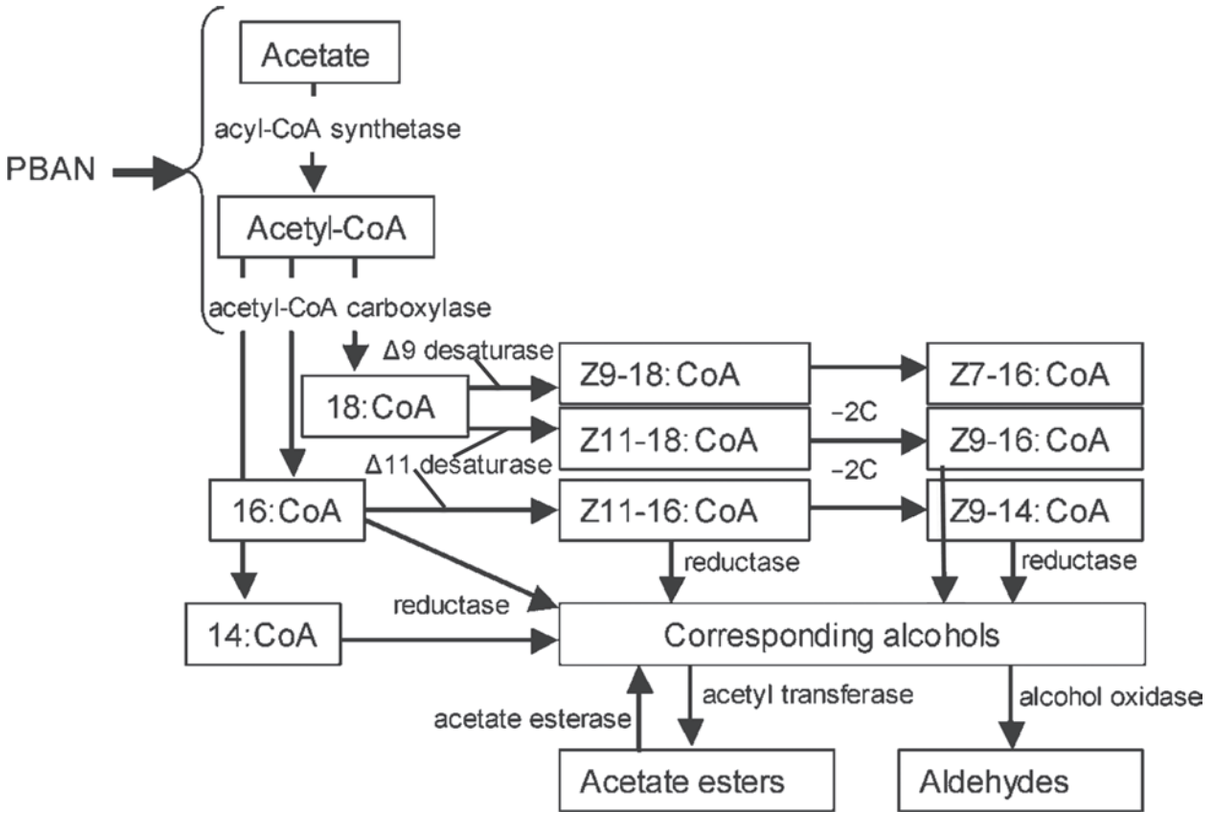



FIGURE 21.2 Pathways for biosynthesis of heliothine moth sex pheromone components. Two key enzymes explain most of the variation in blends: a Δ11-desaturase that places a (Z) double bond in the 11 position on either the C18-fatty acyl chain or the C16-fatty acyl chain, and a β-oxidation enzyme that shortens the fatty acyl chain by removing the first two carbons after the acyl group on the chain. When the Δ11-desaturase acts on the C16-fatty acyl chain, it produces Z11-16CoA that eventually gets reduced to Z11-16OH and then oxidized to Z11-16Ald. A β-oxidation step acting on Z11-16CoA ("-2C" at middle-right of figure) produces Z9-14CoA that eventually gets reduced to Z9-14OH and oxidized to Z9-14Ald, a key secondary component of *Heliothis virescens*, *H. peltigera*, and *Helicoverpa punctigera* (see figure 21.1). When the Δ11-desaturase acts on the C18-fatty acyl chain, it produces Z11-18CoA which gets chain-shortened via a β-oxidation step to Z9-16CoA ("-2C" at middle-right of figure), which is then reduced to Z9-16OH and oxidized to Z9-16Ald, the major component of *H. assulta* and *H. gelotopoeon*. Z9-16Ald is also a key minor component for *H. zea* and *H. armigera* (see figure 21.1).

SOURCE: Adapted from Jurenka (2003) and Groot et al. (2004).

favored by species that use large percentages of Z9-16Ald as a minor component (e.g., *H. subflexa)*, or predominant percentages of Z9-16Ald as a major component, (e.g., *H. assulta*, *H. gelotopoeon*) in their blends. Other species produce only small amounts of Z9-16Ald as pheromone components, and thus not as much 18CoA is used as the substrate for the Δ11-desaturase compared to the much larger amounts of 16CoA substrate used by these species for their blends that have Z11-16Ald as the major component and Z9-16Ald as a minor component (e.g., *H. phloxiphaga*, *H. armigera*, and *H. zea*). Another route involved in species using Z9-14Ald as a minor component requires that a β-oxidation chain-shortening step occur after desaturation. But here the β-oxidation step acts on the shorter, Δ11-16CoA substrate, thereby shortening Z11-16CoA down to Z9-14CoA, which is then reduced to (*Z*)-9-tetradecen-1-ol (Z9-14OH) and oxidized finally to Z9-14Ald (figure 21.2). The use of this post-Z11-16Ald chain-shortening step for producing Z9-14Ald is seen in *H. peltigera*, *H. virescens*, *H. punctigera*, and *H. armigera*. Rarely, some species use the saturated 16Ald as a major or minor pheromone component (e.g., *H. gelotopoeon* and *H. maritima*), and in these cases the 16CoA is reduced to the saturated hexadecanol (16OH) without the intervening step of Δ11-desaturase acting on it first, with the 16OH then being oxidized to 16Ald.

## Fatty Acyl Reductase and Alcohol Oxidase Determine the Amount of Alcohol versus Aldehyde Sex Pheromone Composition of Many Blends

The terminal enzymatic steps, especially the reduction of the CoAs to the alcohols, oxidation of the alcohols to the aldehydes, as well as acetylation of the alcohols to the corresponding acetates, all can contribute significantly to the final pheromone blend composition. Fatty acyl reductases (FARs) have now been isolated from *Helicoverpa armigera*, *H. assulta*, *Heliothis virescens*, and *H. subflexa* and are shown to have the same substrate preferences across species (Hagström et al. 2012). The FARs of all four species prefer to reduce (*Z*)-9-tetradecenoate over (*Z*)-11-hexadecenoate, and the latter is reduced preferentially over (*Z*)-9-hexadecenoate. Thus, for all four species, the final differing species-specific blend ratios of the C14 and C16 aldehydes depend not on the differential selective activity of each species' FARs, but rather on the differing amounts of C16- and C14-acyl substrates that are available for the FARs to reduce (Hagström et al. 2012).

For all species, analyses of the amounts of Z11-16OH found in pheromone glands or shown to be emitted by female heliothines as pheromone "components" have been quite variable

and a source of confusion in the literature for heliothine sex pheromones. It seems that significant amounts of Z11-16OH can always be solvent extracted from heliothine female glands, but the volatilization of Z11-16OH from the glands may be negligible. For instance, two groups found no Z11-16OH in the airborne collections from calling female *H. virescens* (Pope et al. 1982; Teal et al. 1986), but they were able to find significant percentages of it in the blends extracted from female glands. These findings of Z11-16OH in the solvent extracts were in agreement with those of several other groups (e.g., Ramaswamy et al. 1985; Ramaswamy and Roush 1986) that did not conduct airborne collections. Insight into why alcohols were not found in airborne emissions came from Teal and Tumlinson (1986), who demonstrated by applying synthetic Z9-14OH to the glands of *H. virescens* females that this compound is enzymatically converted to Z9-14Ald as it travels through the glandular epithelium to the gland's surface cuticle, suggesting the activity of a cuticular-bound oxidase. Thus, amounts of alcohols such as Z11-16OH found in gland extracts of many species likely represent only glandular biosynthetic precursors of aldehydes that are actually emitted from the gland surface after being oxidized to corresponding aldehydes by the glandular tissues.

Studies with *H. phloxiphaga* (Raina et al. 1986) further support the idea that the pheromone blend emitted can also differ quantitatively from the gland contents. When very short extraction times (<3 min) of female glands were used that probably extracted mainly the compounds present on the gland surface, the amounts of Z11-16OH in the blend were low (2.9%) and behaviorally optimal for male attraction in a wind tunnel. When extraction times of 10–15 min were used, however, that were more likely to extract pheromone-component precursors from inside the gland, the amounts of Z11-16OH increased to >4.5%, and in a wind-tunnel bioassay, this larger proportion reduced male attraction significantly (Raina et al. 1986).

As much as possible, extraction procedures should be developed that accurately estimate the actual emitted signal that has been selected for inducing male behavior, rather than the composition of the pool of precursors residing in the interior of the pheromone gland tissues. Cork et al. (1992) showed that in *H. assulta* very late in the scotophase, after the optimal calling period, the amount of the Z9-16Ald major component in the gland had diminished by more than two-thirds from its amount at the peak, whereas the amount of Z9-16OH had increased by nearly 50-fold. This finding again indicates that alcohols that can be extracted from heliothine female pheromone glands are most often biosynthetic precursors waiting to be oxidized to the aldehyde pheromone components.

In a few species, corresponding acetates to the aldehydes and alcohols, such as Z11-16Ac, have been found to be produced by females, and to cause slight but significantly elevated amounts of attraction of male conspecifics. In *H. assulta*, Z11-16Ac seems to vary geographically in its production by females (amounts found in the glands) (Cork et al. 1992; Park et al. 1994), as well as in its effect in blends containing the two main components, where it increases male *H. assulta* attraction (trap capture) in regions corresponding to its production by females (Cork et al. 1992; Park et al. 1994).

Although its effect on increasing attraction of conspecific males is small and varies according to geographic regions for both *H. assulta* in Asia and *H. subflexa* in North America, the effect of the addition of Z11-16Ac in reducing or eliminating attraction of heterospecific males in North America is pronounced. Males of species sympatric to *H. subflexa*, such as *H. zea* and *H. virescens*, are not attracted at all to calling *H. subflexa* females (Lelito et al. 2008), an effect that is undoubtedly due to Z11-16Ac in the *H. subflexa* blend. Trace amounts of 0.1% or 1% Z11-16Ac added to the otherwise highly attractive blends of *H. virescens* or *H. zea* can substantially reduce attraction of the males of these two species (Fadamiro and Baker 1997; Vickers and Baker 1994, 1997; Baker et al. 1998a, 1998b). These three species are sympatric in parts of North America, and they are not temporally distinct in generational phenology or calling periodicity. It is evident that blends containing this acetate function as a means of prezygotic mating isolation, keeping heterospecific males from wasting time in long-distance orientation to females that will result in no viable progeny. At the same time, emitted blends containing this compound will for females minimize the chances that they will be harassed, with persistent courtship attempts, by heterospecific males and thus be subjected to fruitless courtships and couplings with such males. Z11-16Ac in blends thereby works at long distance to reduce male and female pre-copulatory and copulation mistakes of all types that will reduce reproductive fitness in both sexes.

Heliothine moth sex pheromone systems thus are exemplars of moth pheromone evolution via *adaptive* responses to heterospecific blends in which different species-specific blends have diverged during a reproductive character displacement process in zones of sympatry of two established species (Butlin 1987), or during a speciation event that includes reinforcement (Butlin 1987). A "species recognition" mechanism (Paterson 1985; Lambert et al. 1987) orchestrating the evolution of these pheromone blend ratios solely through normalizing (stabilizing) selection lacking adaptive responses to heterospecific pressures is not well supported. Not only is there strong behavioral aversion of heliothine males of some species to blends containing trace amounts of heterospecific components such as Z11-16Ac in areas of sympatry, but also there are olfactory sensory neurons (OSNs) on their antennae that are specifically tuned to such heterospecific components. It is hard to reconcile the presence of heterospecific-component-tuned OSNs expressed on male antenna as being anything but an adaptive natural selective response to mate finding and mating mistakes. These OSNs represent the neuronal hardwiring of males that helps them classify, at a distance, pheromone blends as being from heterospecific females and thus avoid wasting time advancing upwind in their pheromone plumes. It would be difficult to find more definitive proof for pheromones being sculpted by adaptive responses to erroneous heterospecific cross-communication than in the types of heliothine males' OSNs that are dedicated only to the detection of heterospecific pheromone components. This is not to say that blends have not also been shaped over time by the effects of normalizing selection orchestrated by females emitting very low rates of a population-mean optimal blend. Such females should attract (select for) only males that are equipped with an optimally functioning olfactory system having the greatest sensitivity and selectivity for the correct blend ratio. It appears though from abundant experimentation that very few heliothine moth species have males exhibiting such sensitivity and selectivity for only a very limited range of blends.

## Hormonal Control of Pheromone Biosynthesis and Emission

Most heliothines studied to date share similar activity patterns for oviposition, feeding, and mating. In terms of calling

behavior, peak pheromone production and mating receptivity by females occur approximately 2–6 h after the onset of scotophase (Shorey and Gaston 1965; Mbata and Ramaswamy 1990; Ramaswamy 1990). Furthermore, age influences pheromone production in heliothines, with peak production approximately 48 h postemergence. Peak pheromone production is modulated by several factors, including titer of juvenile hormone, octopamine (Christensen et al. 1992; Rafaeli 2009), and pheromone biosynthesis activating neuropeptide (PBAN; Raina and Klun 1984; Raina et al. 1986, 1987; Raina and Kempe 1990, 1992; for review, see Raina 1993). Finally, examination of pheromone production in *Heliothis virescens* has demonstrated that males transfer a pheromonostatic compound that inhibits the release of pheromonotropin and PBAN (Ramaswamy et al. 1995).

### PBAN Activity

A thorough investigation of pheromone biosynthesis is beyond the focus of this chapter. However, a significant body of knowledge has been generated from heliothines to elucidate control of pheromone production and biosynthetic pathways. Indeed, species such as *Heliothis virescens* and *Helicoverpa zea* have been used as model systems in initial and continuing studies on such phenomena. The first evidence for neuroendocrine (peptidergic) control of female pheromone production in moths was demonstrated in *H. zea*, ultimately leading to the discovery and characterization of PBAN (Raina and Klun 1984; Raina et al. 1986, 1987; Raina and Kempe 1990, 1992; for review, see Raina 1993). Subsequent work identified the gene encoding PBAN and related peptides by using *H. zea* as a model (Jurenka et al. 1991; Davis et al. 1992; Ma et al. 1996, 1998; Choi et al. 2005). Such peptides are produced in localized regions of the brain (subesophageal ganglion, corpora cardiaca), and ganglia of the ventral nerve cord (Kingan et al. 1992, 1993; Jurenka and Rafaeli 2011). Neural and endocrine factors have been identified that may regulate PBAN production and modulate pheromone biosynthesis (Teal et al. 1989; Christensen et al. 1991; Rafaeli and Gileadi 1995). Groot et al. (2005) also demonstrated that PBAN injections in female *H. virescens* and *H. subflexa* could be used to reduce variation in production between virgin and mated females and in photophase compared to scotophase.

Several studies (again within Heliothinae) subsequently have demonstrated PBAN acts directly on the pheromone gland when the gland is stimulated in vivo or in vitro with peptide extracts (Rafaeli and Jurenka 2003). Binding occurs with epidermal cells of the pheromone gland, and G protein-coupled receptors for PBAN have been localized from *H. zea* female pheromone glands (Choi et al. 2003; Kim et al. 2008). Rate-limiting enzymatic studies in *H. armigera* suggest that PBAN specifically influences early incorporation of acetate and the activity of CoA carboxylase (Tsfadia et al. 2008; Jurenka and Rafaeli 2011). In female *H. armigera* the product malonyl-CoA is modified in series by fatty acid synthetase, Δ11-desaturase steps, and differential activity of reductases or oxidases to produce aldehydes, alcohols, and acetates (Jurenka and Rafaeli 2011). In males, the Δ11-desaturase appears to be absent, and similar steps of reduction and oxidation lead to the production of 16–18 chain-length alcohols and acetates.

Early work by Teal and Tumlinson (1989) documented enzymatic activity within pheromone glands of *H. subflexa*, *H. virescens*, and *Hydraecia micacea*. By applying primary acetates and alcohols to the surface of pheromone glands, the corresponding alcohols and aldehydes were produced by female *Heliothis* spp. For heliothines, which rely on aldehydes as primary components, this step is critical: *H. micacea* did not produce aldehydes in response to alcohol application. This study provided the first evidence for oxidative steps in pheromone biosynthesis in these species.

In other moth species, PBAN activity appears more complex, affecting the activity of reductases and possibly other receptor types (Tillman et al. 1999). In *H. virescens*, PBAN influences two different stages in pheromone production, as pheromone synthesis from exogenously applied lipids is also increased (Eltahlawy et al. 2007). It remains unclear which secondary enzymatic step is influenced later in pheromone production, but it has been proposed to influence reduction of fatty acids to alcohols and aldehydes, as has been shown in other Lepidoptera (Tillman et al. 1999). In addition, heliothine species express a chain-shortening mutation that influences the production of many minor components. For example, in *H. subflexa*, Z9-16Ald is generated via Δ11 desaturation of octadecanoic acid (18OOH) followed by chain shortening, and *H. virescens* similarly uses hexadecanoic acid (16OOH) as the substrate for Δ11-desaturase followed by chain shortening to generate Z9-14Ald plus the non-chain-shortened Z11-16Ald (Choi et al. 2005).

Studies have examined the kinetics of biosynthesis in *H. virescens* to further quantify metabolites and determine flux in acetate products in pheromone production, both in photophase and scotophase (Foster and Anderson 2012). Finally, Vogel et al. (2010) have documented the transcriptome of female *H. virescens*, identifying a series of candidate genes that may influence pheromone biosynthesis. This information will provide novel strategies to examine the biosynthetic pathway within *H. virescens* and serve as a basis for interspecific variation noted in the Heliothinae.

PBAN has also been implicated in courtship pheromone biosynthesis in males, with evidence of immunoreactivity in the central nervous system of *H. armigera* (Rafaeli et al. 1991; Jurenka and Rafaeli 2011). Gene transcripts for PBAN receptors have also been documented in *H. armigera*, and RNA interference (RNAi) studies have demonstrated that production of male hairpencil compounds is influenced by PBAN (Bober and Rafaeli 2010). Given the similarity in derivative compounds used for biosynthesis in both male and female heliothines, it is perhaps not surprising to see homology in the mode of action of PBAN.

## Specificity of Response by Males to Female-Emitted Compounds

The differential attraction or deterrence of males to the various combinations of pheromone gland volatiles outlined above and in Table 21.2 is integral to our understanding of how pheromone blend compositions have been shaped over evolutionary time. It is therefore best to try to understand the published data concerning male behavioral responses to different blends in the context of geographical areas of sympatry and seasonal synchrony of adults occupying the same habitats, such as in agricultural crops or surrounding vegetation.

Despite wide geographic distributions of many of these heliothine species, there is no evidence of the existence of mul-

tiple cryptic species across their ranges. For instance, an intense sampling (mitochondrial DNA from 249 individuals) of populations of *Helicoverpa armigera* around the world supports this insect's status as a single species across Africa, Asia, and Australia (Behere et al. 2007). A similar examination of *H. zea* from both North America and South America supported its status as a single species that had diverged from *H. armigera* 1.5 million years ago (Behere et al. 2007). Even though there may appear to be differences in pheromone blends and male behavioral response profiles over heliothines' wide geographic areas, these must be considered to be pheromone polymorphisms or "dialects" such as have been found in different parts of the world for the noctuid moth *Agrotis segetum* (Löfstedt et al. 1986) or for the saturniid moths in the genus *Hemileuca* (McElfresh and Millar 1999, 2001; see Allison and Cardé, Chapter 2, this volume).

For the above-mentioned *Helicoverpa* and *Heliothis* spp., there are no instances of sexual activity occurring during any hours of the diel except during scotophase. Moreover, no studies have documented significant partitioning of scotophase into species-specific sexual activity periods that might have been selected for to avoid or minimize heterospecific mate location, courtship, and copulation mistakes. Therefore, below we compare the pheromone blends of two or more species, pair by pair, for species having overlapping geographical ranges (sympatry) and overlapping adult seasonal flight periods (seasonal synchrony). Discussing such pairings may elucidate how adaptive responses to each others' pheromone blends might have helped shape their sex pheromone communication systems via divergence during a speciation event followed by reinforcement of two newly discrete communication channels, or by reproductive character displacement-related shifts in two already established species in their zones of sympatry (Butlin 1987).

## Blend Specificity in North and Central America

### OPTIMIZATION OF INTRASPECIFIC COMPONENT RATIOS

Species in the Americas exhibit differences in their sex pheromone blends that involve selective elimination or modulation of the use of two different enzymes, Δ11-desaturase and an enzyme that performs β-oxidation (chain shortening). The order of action of these two enzymes can also be reversed. A major selective force determining which compounds get biosynthesized for the species specificity of these pheromone blends is male preference. Although males have been selected to have a broad response spectrum to enable them to "track" (respond to) the blends of any and all conspecific females in their environment, they will be penalized by responding too broadly, that is, to heterospecific pheromone blends resulting in no offspring or hybrid progeny having reduced inclusive fitness. The degree to which such mating "mistakes" can evolutionarily sculpt male discrimination for blends having a certain composition of components at particular ratios explains why behavioral experiments are so illuminating. Such tests examine the sensitivity and specificity of male response to blends comprised of various potential synthetic pheromone components emitted in different blend ratios, and thus probe the olfactory blend integration system of a given species that has been selected for over time. We can thereby begin to understand, by determining the ranges of responsiveness that males exhibit to possible pheromone blends, the limits on blend composition that mate finding, courtship, and mating mistakes have placed on these pheromone systems.

*Heliothis virescens* males require the presence of Z9-14Ald and Z11-16Ald to fly upwind to and locate a source of sex pheromone. Of the known pheromone blends of North American heliothines, *H. virescens* is the only species that uses Z9-14Ald, and this component involves the chain shortening of Z11-16CoA to create the Z9-14CoA needed for Z9-14Ald. *H. virescens* males respond very well in wind-tunnel assays to percentages of Z9-14Ald in blends loaded on dispensers that ranged from 5% to 50% Z9-14Ald (Vickers et al. 1991). In field-trapping studies, Groot et al. (2010a) found that *H. virescens* males were trapped in equivalently optimal numbers when 1–10% Z9-14Ald was loaded onto dispensers with Z11-16Ald, with significant numbers of males still captured to lures loaded with 25% Z9-14Ald. The airborne percentages of Z9-14Ald relative to Z11-16Ald will of course be much higher due to the 14-carbon aldehyde's higher volatility. Pope et al. (1982) measured percentages of Z9-14Ald emitted by individual females and found that only 3 of 40 females emitted over 10% Z9-14Ald in Z11-16Ald (10%, 11%, and 14%). The rest of the females' emitted percentages varied between 7% and 2% with a mean of 5.04% Z9-14Ald. Thus, it is the females that have a narrow variance in emission of Z9-14Ald in their blends, and the experimentally demonstrated male acceptance of quite broad ratios of Z9-14Ald in Z11-16Ald seems to indicate that neither males nor females have imposed any kind of strong stabilizing selection on this two-component blend.

In addition to Z11-16Ald, *H. subflexa* males require the emission of large proportions of both Z11-16OH and Z9-16Ald in the blend to be attracted (Vickers 2002). Z11-16Ac is a fourth component, increasing male attraction when added to the Z11-16Ald, Z11-16OH, and Z9-16Ald three-component blend (Groot et al. 2009a). Emission of three pheromone-gland-constituent acetates, including Z11-16Ac, along with the above-mentioned three requisite components (Vickers 2002), had been reported to be more important for male attraction in the eastern (North Carolina) compared to the western (Mexico) part of this species' range (Groot et al. 2007). However, these field-trapping tests showed that there was a significant increase in trap catch in *both* regions due to the addition of just Z11-16Ac to this three-component blend, with no further increase when the two remaining acetates were also added (Groot et al. 2007). Therefore, this suggests not only is there no significant geographic variation in response to this four-component blend, but also that Z11-16Ac must now be considered to be a fourth pheromone component of this species' pheromone. Its effect on increasing trap catch was consistent in all tests in all regions. Groot et al. (2007) then compared differences in the ratios of increase in trap catch in both regions due to the addition of Z11-16Ac (or the acetates together) to the three main components compared to the three main components alone. When this trap catch ratio analysis was performed, the increase in trap catch in North Carolina compared to the blend lacking Z11-16Ac was declared to be significantly greater (approximately fourfold) than the increase in Mexico (approximately twofold). However, attaching statistical significance to such a ratio difference in magnitude of trap catch between two regions is problematic because in moth field-trapping pheromone blend experiments, the magnitude of increase in trap catch, even from the same locale but from different fields, in response to

equally extra-component-fortified blends can vary greatly for many different reasons (cf. Baker and Cardé 1979). The shape of the trap capture response profile may vary (i.e., ratio of captures of suboptimal blends relative to optimal blends) according to test location and population density. A further consideration is that the lure compositions that were tested were based on gland extracts (Groot et al. 2007), which in heliothine moths may vary considerably from the gland volatiles' actual emission ratios (Pope et al. 1982, 1984).

Z11-16Ac is always present in female pheromone gland extracts whenever (*Z*)-7-hexadecen-1-ol acetate (Z7-16Ac) and (*Z*)-9-hexadecen-1-ol acetate (Z9-16Ac) are found there, and thus Z11-16Ac should always be present in females' volatile emissions whenever either of these other two acetates are emitted. Hence, there is no known natural situation in which Z7-16Ac can affect male behavior on its own as a female-emitted pheromone component, and it does not matter that Z7-16Ac was shown in one of the three field tests to increase trap catch (as did Z11-16Ac in that same test) when added, as the only acetate, to the three-component requisite blend (Groot et al. 2007). Furthermore, regarding Z9-16Ac, in no case was this acetate shown to have any effect on behavior when added by itself to the three-component blend lacking the other acetates. Therefore, again, evidence suggests that Z11-16Ac must be considered to be the only proven fourth component of the *H. subflexa* blend.

The range of ratios of both Z11-16OH and Z9-16Ald relative to the Z11-16Ald major component that results in optimal *H. subflexa* male behavioral responses is quite broad. Ratios of Z11-16OH that result in optimal male attraction in wind-tunnel assays ranged from 1% to 50% of the Z11-16Ald amount, with no diminution of attractiveness (Vickers 2002). In field-trapping studies using just these three components, the ratio requirements for Z11-16OH seemed much narrower but that is because greater than 10% Z11-16OH was not tried (Groot et al. 2007). Similar to the wind-tunnel results of Vickers (2002), either 1% or 10% Z11-16OH significantly increased trap catch compared to treatments lacking Z11-16OH (Groot et al. 2007). Interestingly, when the fourth component, Z11-16Ac, was present in the blend in these field trials, a slightly broader range of percentages of Z11-16OH, from 1% to 25%, resulted in equivalently high increased levels of trap catch compared to blends lacking Z11-16OH (Groot et al. 2007). In wind-tunnel assays, the percentage of Z9-16Ald in the blend that resulted in optimal attraction ranged from 10% to 50% (Vickers 2002).

*Helicoverpa zea* males require only a small amount of Z9-16Ald in the blend with Z11-16Ald to evoke optimal attraction, although a small amount of Z9-14Ald can substitute for the Z9-16Ald (Table 21.2; Vickers et al. 1991), despite the fact that *H. zea* females do not synthesize or emit detectable amounts of Z9-14Ald (Pope et al. 1984). The range of percentages of Z9-16Ald that evoke optimal attraction when blended with Z11-16Ald is from 1% to 15% (Klun et al. 1980b; Vetter and Baker 1984; Vickers et al. 1991), even though Z9-16Ald makes up only approximately 1–2% of *H. zea* female effluvia (Pope et al. 1984). Males do not require emission of any other compounds, including Z7-16Ald or 16Ald (Vetter and Baker 1984), even though both of these compounds are emitted by *H. zea* females (Pope et al. 1984) and are found in gland extracts (Klun et al. 1980b).

*H. phloxiphaga* males have a critical requirement for optimal male attraction: a very small amount of Z9-16Ald (no more than 0.6%) for optimal male attraction as well as only a small amount of Z11-16OH (2–3%), both admixed with Z11-16Ald (Table 21.2). When Z11-16OH is deleted from the blend, or when it exceeds 4%, male attraction is severely reduced (Raina et al. 1986). The requirement for a precise percentage of Z11-16OH (2–3%) to be blended with Z11-16Ald seen in *H. phloxiphaga* is unique among the heliothines (Raina et al. 1986).

## BEHAVIORAL ANTAGONISM TO HETEROSPECIFIC BLENDS AND BLEND COMPONENTS

Infield-trapping experiments using large-diameter Hartstack wire-screen traps (Hartstack et al. 1979), female *Heliothis subflexa* and *H. virescens* were both able to attract significant numbers of *Helicoverpa zea* males (Groot et al. 2009a), and also to attract small numbers of each other's males (Groot et al. 2006, 2009a). In these experiments, males did not have to orient all the way to the calling female pheromone source; they only needed to approach within approximately 25 cm of the female to be captured. Wind-tunnel experiments testing cross-attraction of male *H. virescens*, *H. subflexa*, and *H. zea* all the way to the calling females themselves showed that males of none of the three species were able to lock onto the plumes and fly all the way upwind to arrive at heterospecific females (Lelito et al. 2008). The reasons for this inability in males of all three species originates with blends emitted by heterospecific females that contain behaviorally antagonistic components or else are comprised of unbalanced, behaviorally antagonistic blend ratios deficient in one or more essential components. *H. virescens* males are not attracted to *H. subflexa* females because although these females do emit a small amount of Z9-14Ald, the large percentages of Z11-16Ac and Z11-16OH emitted by *H. subflexa* females are antagonistic to *H. virescens* male attraction. The addition of just 0.1% Z11-16Ac or ≥3% Z11-16OH to the optimal *H. virescens* blend is sufficient to reduce male *H. virescens* attraction significantly (Vetter and Baker 1983; Vickers and Baker 1997). *H. virescens* males are not attracted to *H. zea* females because they do not emit any Z9-14Ald, and for *H. virescens* Z9-16Ald cannot substitute behaviorally for the absence of Z9-14Ald (Vickers et al. 1991). Similarly, *H. virescens* males would not be attracted to *H. phloxiphaga* females for the same reason as for *H. zea* above. In addition, the 3% Z11-16OH emitted by the *H. phloxiphaga* females would be behaviorally antagonistic to *H. virescens* males (Vetter and Baker 1983).

Female *H. virescens* probably do not attract *H. subflexa* males because they do not emit the large percentage of Z9-16Ald or any Z11-16OH, both of which are required for response by *H. subflexa* males (Vickers 2002). Male *H. subflexa* likewise will not be attracted to *H. zea* females due to the lack of emission of Z11-16OH (Pope et al. 1984), as well as the tiny amount of Z9-16Ald (1–2%) emitted by *H. zea* females. These low levels of Z9-16Ald would be inadequate to attract *H. subflexa* males (Vickers 2002). Similarly, *H. subflexa* males would likely not be attracted to *H. phloxiphaga* females because the percentages of Z9-16Ald and Z11-16OH (0.4% and 6.7%, respectively) they produce in their glands (Raina et al. 1986) are too small to evoke upwind flight to the source in *H. subflexa* males (Vickers 2002).

*H. zea* males are not attracted to *H. virescens* females because even though a small percentage of Z9-14Ald can substitute for the Z9-16Ald of *H. zea* females, the amount emitted by *H. virescens* females will exceed that amount and be behaviorally antagonistic (Table 21.2; Vickers et al. 1991). Also, the addition of small percentages of Z9-16Ald to the Z9-14Ald that *H. virescens* females typically emit has been shown to antagonize flight to nearly complete suppression of male attraction (Shaver

et al. 1982). *H. zea* males are not attracted to *H. subflexa* females because the percentage of Z11-16OH produced by *H. subflexa* females is antagonistic to their upwind flight (Quero and Baker 1999; Quero et al. 2001). Finally, *H. zea* males should not be attracted to *H. phloxiphaga* females because the percentage of Z11-16OH they produce (2–3%) will be antagonistic to *H. zea* male upwind flight (Quero and Baker 1999; Quero et al. 2001). However, a small amount (5%) of cross-attraction of *H. zea* males in the field (cross-trapping in large cone traps) was found using both *H. phloxiphaga* calling females (Kaae et al. 1973; Raina et al. 1986) and the *H. phloxiphaga* synthetic blend (Raina et al. 1986). The percentage of Z11-16OH in the blend emitted by *H. phloxiphaga* females does not appear to be enough to completely prevent cross-attraction of *H. zea* males, although the amount of cross-attraction cannot be assessed without knowing how many *H. zea* males would have been trapped in response to *H. zea* females if these females had been used as the proper control treatments in these same experiments. Note that it is unlikely that *H. zea* males would occupy the same habitat at the same time of the year as *H. phloxiphaga* females.

For *H. phloxiphaga*, the requirement for a precise percentage of Z11-16OH (2–3%) in the blend may explain why cross-attraction of male *H. phloxiphaga* to females of other sympatric heliothine moths does not occur (Raina et al. 1986). Thus, *H. phloxiphaga* males will not be attracted to *H. virescens* (Pope et al. 1982; Teal et al. 1986) or to *H. zea* (Pope et al. 1984) females due to their lack of emission of Z11-16OH, or to *H. subflexa* females because the large percentages of Z11-16OH emitted by *H. subflexa* females will be behaviorally antagonistic to *H. phloxiphaga* male attraction (Raina et al. 1986).

## HETEROSPECIFIC FEMALE PHEROMONE PLUME INTERFERENCE VIA OVERLAPPING PLUMES?

The effect of communication interference on mate attraction by calling females has been measured by placing two calling heterospecific females on either side of a calling conspecific female (Lelito et al. 2008). Male orientation behavior was measured to quantify the impact of potentially antagonistic pheromone plume strands of heterospecific females interleaved with conspecific plume strands. In five out of six possible combinations, the close positioning of two heterospecific interfering females next to one conspecific female generating overlapping plumes caused a significant, 50–80%, reduction of attraction of males to within 10 cm of their female. The one exception was of *Helicoverpa zea* males. Attraction of *H. zea* males to a calling *H. zea* female, although significantly reduced by the presence of two calling *Heliothis subflexa* females, was not at all reduced by the presence of two calling *H. virescens* females (Lelito et al. 2008). The degree of coincident, completely mixed heterospecific and conspecific plume strands in this experimental setup was measured via a four-channel EAG as being approximately 50% mixed.

## GEOGRAPHIC VARIATION IN THE PHEROMONE BLENDS OF *HELIOTHIS SUBFLEXA* AND *H. VIRESCENS*

The female-produced pheromone blends in the *Heliothis virescens* and *H. subflexa* pheromone communication systems have been reported to vary significantly across a geographical range in North America (Groot et al. 2009a). Unfortunately, Groot et al. (2009a) did not actually measure any variation in the *pheromone* of this species, because measurements were not reported for variation in the major component, Z11-16Ald, to determine how it may have covaried with any of the minor sex pheromone components (nor were the compositions of the emitted mixtures reported). In all the gland extractions for both species, only the large number of behaviorally inert minor gland extract constituents, plus the few actual minor sex pheromone components known to be emitted by females of each species, were analyzed. The actual quantities of these compounds were not reported, but rather, only their ratios of relative abundance were calculated. Again, to quantify geographic variation in the behaviorally active pheromone blends of these two species, the amount of covariance of Z11-16Ald abundance with the abundances of minor pheromone components needed to be reported, but it was not. We thus do not know how, or whether, these pheromone blends varied geographically. Moreover, the degree of ratio variation of even the behaviorally active actual minor pheromone components will have been influenced by the amounts of some of the more abundant, behaviorally inert constituents in the gland extracts. Because all results were reported as percentages of all minor/inert components, fluctuations in the amounts of these compounds, such as 16Ald for *H. virescens* and Z9-16Ac, 16Ald, and Z9-16OH for *H. subflexa*, will have confounded even these gland-constituent ratio results in unknown ways, and the actual *emitted* blend ratios in still other indiscernible ways.

## SEASONAL "TEMPORAL VARIATION" IN FEMALE PHEROMONE BLENDS

In addition to varying geographically, the sex pheromone blends of *Heliothis subflexa* and *H. virescens* have been reported to vary temporally (i.e., among years) (Groot et al. 2009a). Again, unfortunately, variation in "chemical communication" that was mentioned in the title of Groot et al. (2009a) was not measured, because chemical communication in these species involves the complete sex pheromone blend, with Z11-16Ald being the essential component in both species. The abundance of Z11-16Ald was not reported, and thus it is difficult to determine how it may have covaried with the abundances of behaviorally active minor components. Minor gland constituents, including minor pheromone components, are all behaviorally inert in any blends without the inclusion of Z11-16Ald; thus, the seasonal variation in behaviorally inert compounds was all that was analyzed (Groot et al. 2009a). Finally, because ratios and not amounts were reported, fluctuations in the amounts of non-pheromonal component in gland extracts will have confounded the ratios of even the known, behaviorally active minor pheromone components in the glands in unknown ways.

The "positive assortative" attraction of North Carolina *H. subflexa* and *H. virescens* males in the field to females collected from North Carolina rather than from Texas was implicated as being due to differences in the pheromone blends of the females that had been collected from these two regions (Groot et al. 2009a). However, the lower trap captures of North Carolina male *H. subflexa* and *H. virescens* in response to Texas females than to North Carolina females could be due to many other factors than the quality of these females' sex pheromone blends. For example, Texas females may inherently call less frequently than North Carolina females and thus have a smaller total attraction period during the night compared to North Carolina females. Unfortunately, the reciprocal field

tests were not conducted in Texas to see whether this is true, and that perhaps North Carolina females would attract more males in Texas as well as in North Carolina.

#### "PHENOTYPIC PLASTICITY" IN FEMALE BLEND COMPOSITION FOLLOWING EXPOSURE TO HETEROSPECIFIC PHEROMONE PLUMES?

Groot et al. (2010b) exposed female *Heliothis subflexa* to the synthetic pheromone blends of *H. virescens* and *H. subflexa* over three continuous days and reported that the gland compositions of female *H. subflexa* seemed to change after they had been exposed to the *H. virescens* heterospecific pheromone blend, but not after they were exposed to the conspecific *H. subflexa* blend. Females that exhibited a blend shift showed a slight, but significantly, elevated percentage of Z11-16Ac and the two other acetates in their gland extracts. Groot et al. (2010b) proposed this phenotypic plasticity as a mechanism for females to increase their mating success in the presence of higher density populations of *H. virescens* that might otherwise have increased the chances of *H. subflexa* females cross-attracting *H. virescens* males and incurring reduced fitness.

There are several difficulties in trying to assess the validity of these results, not the least of which is that all gland-extracted compounds were reported as "normalized" values to Z9-16OH (Groot et al. 2010b). First, the titers of Z9-16OH extracted, and their variation from gland to gland, were never shown, nor was the calculation method for normalizing the abundances. If Z9-16OH was present in very small amounts and used as a denominator in calculations, then very slight variations in the titers of Z9-16OH could create large variations in the normalized amounts of other compounds. Regardless, it is not possible from the information given to calculate back to find the actual titers of pheromone components that were extracted or to determine whether *the pheromone*, i.e., the *blend ratios of behaviorally active components*, did actually vary significantly between the differently exposed groups of females. Some of the increases in gland constituents deemed "significant," such as those of Z7-16Ac and Z9-16Ac after heterospecific pheromone exposure, appear to be vanishingly small and no larger than those from the females exposed to the conspecific blend (Groot et al. 2010b).

Second, the exposure of a group of females to the heterospecific *H. virescens* blend versus their blank control was done as a single lengthy cohort over several weeks during an earlier time period than was the exposure of the second several-weeks-long cohort of females to the *H. subflexa* blend versus their blank control. The difference in the apparent increase of "acetates" in the former group to that of the latter group might have been due to some unknown differences in the quality of the *H. subflexa* females being tested during these earlier weeks versus those tested during the later sets of weeks.

Third, it should be noted that following *H. subflexa* females' preexposure to the heterospecific *H. virescens* blend, the normalized amount of Z11-16Ald major component extracted from these females also was seen to increase to seemingly as large a degree as that of Z11-16Ac. This calls into question whether the *pheromone blend* (e.g., the ratios of behaviorally active components including Z11-16Ald) in these females actually changed significantly, even though the amount of increase in the acetates was the authors' focus. Unfortunately, we are not given enough information to be able to calculate whether the actual abundances of the *pheromone blend* components changed (or not) because we were not shown how these normalized data were obtained.

Fourth, the Groot et al. (2010b) experiments were performed in the laboratory by using prolonged, 3-day exposures of females located in close proximity (in the upwind portion of the aeration cylinder) to sources of synthetic pheromone blends. These were extremely long-duration, chronic exposure levels that would never occur in nature (3-day exposure as adults and 1–7 days as pupae). Calling female moths' pheromone plumes do not persist for more than a few tens of seconds in the field. Pheromone researchers over decades have found it virtually impossible to locate a calling female of any species under natural conditions in the field because they usually become mated so quickly that only moths already *in copula* are found. In addition, such brief natural occurrences of heterospecific female plume emission in nature will be made even more fleeting due to the plumes' meanderings caused by shifts in wind direction.

Finally, the percentages of minor components in heliothine moth gland extracts have been known to vary considerably due to extracts being prepared over periods differing by only a few hours during scotophase (Pope et al. 1984; Park et al. 1996), or due to the glands being extracted for different durations, even if only by a few minutes (Raina et al. 1986). Also, the compounds present in a solvent extract from a pheromone gland, which may include biosynthetic precursors, are not necessarily reflective of the blend volatilizing into the air from the gland surface.

## Blend Specificity in South America

#### BEHAVIORAL ANTAGONISM TO HETEROSPECIFIC BLENDS AND BLEND COMPONENTS

In South America, the same three types of non-cross-attractive heterospecific interactions between *Helicoverpa zea*, *Heliothis virescens*, and *H. subflexa* as in North America (see previous sections above) should be occurring. In Uruguay, Argentina, and Chile, however, there is another species, *H. gelotopoeon*, a pest of local field crops, that is sympatric and synchronic with these first three species (Cork and Lobos 2003). Females of *H. gelotopoeon* emit an approximately 1:1 ratio of 16Ald and Z9-16Ald, and they do not emit any Z11-16Ald (Table 21.2). In fact, addition of ≥1% Z11-16Ald to the 16Ald-plus-Z9-16Ald blend significantly antagonizes conspecific male attraction (Cork and Lobos 2003). The optimal conspecific blend involves only Z9-16Ald, with 10–100% 16Ald admixed with it. Females will be completely unattractive to males of the other three species, all of which require Z11-16Ald in their blends. Also, male *H. gelotopoeon* will be deterred from being attracted to females of the other species due to the predominance of Z11-16Ald as the major component in their blends. Thus, both females and males of *H. gelotopoeon* should be prevented from making mating mistakes with these other three species in their environment.

## Blend Specificity in Europe and Asia

#### OPTIMIZATION OF INTRASPECIFIC COMPONENT RATIOS

Male *Helicoverpa armigera* require the presence of Z9-16Ald as a secondary component in their female sex pheromone blends

(Kehat et al. 1980; Kehat and Dunkelblum 1990). At least 3% Z9-16Ald relative to Z11-16Ald must be present in a two-component blend to be optimally attractive to *H. armigera* males, with ≥24% causing a reduction in male attraction (Table 21.2; Kehat et al. 1980). In addition, whereas it had previously been thought that *H. armigera* was a species that did not use Z9-14Ald as part of its blend, Zhang et al. (2012) found that very small percentages (0.3–5.0%) of Z9-14Ald can contribute to doubling the trap capture of males when it is added to the Z11-16Ald-plus-Z9-16Ald blend. The component Z9-14Ald is found at a level of approximately 0.3% in female pheromone gland volatiles (Zhang et al. 2012), so it must be considered to be a pheromone component that contributes to increased male attraction in this species (Zhang et al. 2012). Indeed, further support for this compound as a sex pheromone component for *H. armigera* comes from the studies of Rothschild (1978) who showed that the addition of 2–5% Z9-14Ald to Z11-16Ald in Australia increased male trap catch significantly.

*H. assulta* is only one of two known *Helicoverpa* or *Heliothis* species in which Z11-16Ald is not the major pheromone component. Here, Z9-16Ald is the major component, with Z11-16Ald contributing to optimal male attraction when present at only 5–10% of the Z9-16Ald (Table 21.2). It has been suggested that *H. assulta* is a polymorphic pheromone species with geographical variation in the most effective blends in different regions. Males in Thailand were trapped optimally when the percentage of Z11-16Ald in a blend of Z11-16Ald plus Z9-16Ald was 13% of the amount of Z9-16Ald, whereas males in China and Korea were trapped optimally when Z11-16Ald was 2–5% (Cork et al. 1992). Too much Z11-16Ald in the blend reduces *H. assulta* male response significantly; no males were found to fly upwind to and locate sources emitting the *H. armigera* blend ratio of 3% Z9-16Ald/97% Z11-16Ald. With the optimal *H. assulta* blend ratio of Z9-16Ald to Z11-16Ald, addition of the Z11-16Ac and Z9-16Ac female gland constituents in Korea significantly increased male response (trap catch), indicating activity as two minor sex pheromone components in the *H. assulta* pheromone communication system in Korea (Cork et al. 1992). However, in China the addition of acetates to the blend reduced male trap catch and the addition of the acetates to the Thailand aldehyde blend had no effect on trap catch of males there (Cork et al. 1992).

Males of *Heliothis peltigera* are attracted optimally in field tests to a two-component blend of Z11-16Ald plus Z9-14Ald, with anywhere from 20% to 50% Z9-14Ald added as the minor component. The percentage of Z9-14Ald relative to Z11-16Ald extracted from female pheromone glands was observed to be 13–17% (Table 21.2; Dunkelblum and Kehat 1989). In the wind tunnel, Z9-14Ald blended with Z11-16Ald at both 5% and 50% of the amount of Z11-16Ald elicited equivalently high levels of upwind flight, source contact, and copulatory attempts. Addition of 30% Z11-16OH to the binary blend, mirroring the 24% found in female glands, evoked no significant changes in these categories of behavior (Dunkelblum and Kehat 1989).

*H. maritima* has been designated as having many subspecies, and among the seven such subspecies are *H. m. hungarica* in Hungary and *H. m. aduacta* in Japan. Males of *H. m. hungarica* in Hungary have been shown to respond optimally to the only two ratios that were tested that approximate the 100:8.1:5.5 ratio of Z11-16Ald/Z11-16OH/16Ald gland constituents shown to be produced by Hungarian females (Szõcs et al. 1993). Two blend ratios, 100:20:6 and 100:6:3, of these three compounds were shown to have an equivalently high level of male attraction in a wind tunnel (Table 21.2). A ratio similar to these was also used successfully in field-trapping tests. Binary blends were not tested against either in the field or in the wind tunnel against the ternary blends, so it is not clear whether both Z11-16OH and 16Ald are pheromone components or only one of them is. Addition of a fourth compound, Z9-16Ald, that had been isolated in trace amounts from *H. m. hungarica* female glands, helped increase attraction in the wind tunnel when added at 0.1% Z11-16Ald, but in field-trapping experiments this compound had no effect (Szõcs et al. 1993). It may be tentatively labeled also as being a pheromone component, based on the wind-tunnel results.

Females of *H. m. aduacta* in Japan produce ratios of Z11-16Ald/Z11-16OH/16Ald (100:24.5:2.4) that are quite similar to those produced by the Hungarian (subspecies) females (Kakizaki and Sugie 2003). Field-trapping tests showed that binary blends of 1% Z11-16OH relative to Z11-16Ald attracted and captured males, whereas each compound alone did not. In several experiments, trap capture was highest when the percentage of Z11-16OH was in the range of 1–5%. The addition of either 16Ald or Z9-16Ald to the binary blend had no effect on male trap capture (Kakizaki and Sugie 2003).

It may be concluded from work on Japanese *H. maritima* that *H. maritima* in Japan definitely, and in Hungary most likely, use Z11-16Ald and Z11-16OH as sex pheromone components. There is no strong evidence for 16Ald or Z9-16Ald being behaviorally active in Hungary and no evidence at all that they are active in Japan. No ranges of Z11-16OH were tried in binary blends in Hungary, and so we do not know whether the 1–5% Z11-16OH used in Japan is a different optimal ratio than the 6–20% tested in Hungary. Therefore, there may be no strong differences in Japan and Hungry in the binary blends used by these two "subspecies."

## BEHAVIORAL ANTAGONISM TO HETEROSPECIFIC BLENDS AND BLEND COMPONENTS

The most geographically widespread species, *Helicoverpa armigera*, might be expected to exert the biggest influence on heterospecific pheromone blend specificity across Europe and Asia. In both Hungary and Japan, *Heliothis maritima* does not use Z9-16Ald as a minor component, using Z11-16OH instead; therefore, its blend does not attract *H. armigera* males, which require Z9-16Ald. *H. maritima*, conversely, would not be attracted to *H. armigera* females because they do not emit significant percentages of Z11-16OH that *H. maritima* males need to be attracted.

In the Mediterranean region, *H. peltigera* females produce a large percentage (15%) of Z9-14Ald as their secondary pheromone component, a percentage that is antagonistic to *H. armigera* male attraction (Dunkelblum and Kehat 1989). Percentages of Z9-14Ald that are this high, coupled with the large percentage of Z11-16OH produced by *H. peltigera* females, which is not a pheromone component of this species, were shown to effectively eliminate attraction of *H. armigera* males (Dunkelblum and Kehat 1989). Conversely, *H. peltigera* males will not be attracted to *H. armigera* females because they emit too little Z9-14Ald (0.3%; Zhang et al. 2012) to be effective for *H. peltigera* attraction, as shown by the wind-tunnel studies of Dunkelblum and Kehat (1989) in which 1% Z9-14Ald was ineffective in causing upwind flight of *H. peltigera* males.

Male *H. peltigera* should not be attracted to female *H. maritima* because they do not produce detectable amounts of Z9-14Ald. It

is not clear whether *H. maritima* males would be attracted to *H. peltigera* females because the latter produce significant amounts of Z11-16OH, a secondary pheromone component used by *H. maritima*; however, *H. peltigera* females also produce Z9-14Ald that might be antagonistic to *H. maritima* male attraction. This hypothesis would need to be confirmed experimentally, as would the amounts actually emitted by both *H. peltigera* and *H. maritima* females, not just the amounts extracted from glands.

The species *H. assulta* and *H. armigera* are sympatric over large areas of Asia and may present an interesting study in the evolution of blend specificity as a consequence of selection to reduce mating mistakes. In wind-tunnel cross-attraction studies, Ming et al. (2007) showed that calling *H. assulta* and *H. armigera* females were completely unattractive to *H. armigera* and *H. assulta* males, respectively. It had previously been shown that *H. armigera* males could be cross-attracted to the synthetic two-component *H. assulta* blend of 97% Z9-16Ald plus 3% Z11-16Ald but that *H. assulta* males were not attracted to the *H. armigera* blend of 97% Z11-16Ald plus 3% Z9-16Ald (Zhao et al. 2006). Therefore, Ming et al. (2007) conjectured that because *H. armigera* males were not attracted to calling *H. assulta* females, other compounds emitted by *H. assulta* females might be responsible for antagonizing *H. armigera* attraction. It is possible that these additional antagonistic compounds might be the corresponding acetates, such as Z11-16Ac that were found to be produced by *H. assulta* females from populations in China (Cork et al. 1992).

However, Wu et al. (2013) later demonstrated in wind-tunnel experiments with two-component synthetic blends of Z9-16Ald plus Z11-16Ald that ratio differences alone could explain the lack of cross-attraction of *H. armigera* males to *H. assulta* females. It may still be possible that the emission of other compounds such as Z9-16Ac and Z11-16Ac in the *H. assulta* gland volatiles (Cork et al. 1992) might explain the lack of *H. armigera* attraction to calling *H. assulta* females. This possibility needs to be investigated in experiments using synthetic mixtures and moths of carefully chosen geographic origin.

No additional compounds need to be involved to explain the absence of cross-attraction of *H. assulta* males to *H. armigera* females. Differences in the ratio of *Z*9-16Ald to *Z*11-16Ald between the species are enough to prevent cross-attraction (Zhao et al. 2006; Ming et al. 2007). However, *H. armigera* females also have been shown to produce small amounts (0.3%) of Z9-14Ald as part of their sex pheromone blend (Zhang et al. 2012), and between 0.1% and 1% Z9-14Ald added to the *H. assulta* two-component blend was shown to be behaviorally antagonistic to male *H. assulta* attraction in the field (Boo et al. 1995). Thus, Z9-14Ald might contribute to further reducing the chance of male *H. assulta* attraction to *H. armigera* females.

It should be expected that *H. assulta* males will, across their geographic range, not be attracted to any of the other heliothine species whose pheromone blends are thus far known, because of their preference alone for the skewed ratio of predominantly Z9-16Ald compared to Z11-16Ald. The same should be true in the other direction: males of no other species should be attracted to female *H. assulta* due to this unique Z9-16Ald-to-Z11-16Ald ratio. In addition, the requirement by *H. peltigera* for Z9-14Ald as a secondary component would prevent these males from being attracted to *H. assulta* females. *H. maritima* does not use Z9-16Ald as a secondary component; it uses Z11-16OH instead. Because *H. assulta* females do not emit Z11-16OH, this is yet a further reason (in addition to the atypical Z9-16Ald-to-Z11-16Ald ratio) why *H. maritima* males should not be cross-attracted to *H. assulta* females.

### Blend Specificity in Australia

It was reported that the "native" budworm of Australia, *Helicoverpa punctigera*, uses a sex pheromone blend ratio of 100:100:5 of Z11-16Ald/Z11-16Ac/Z9-14Ald, respectively (Table 21.2; Rothschild et al. 1982). In exhaustive field trials, this ratio resulted in optimal trap capture of males. In addition to the above-mentioned three components, Z11-16OH was found to be a constituent of female pheromone glands at levels of ≥15% relative to Z11-16Ald, but its inclusion in synthetic blends did not affect trap capture levels.

It is highly unlikely that *H. punctigera* males would be attracted to *H. assulta* females due to the predominance of Z9-16Ald in that species' blend, plus the lack of emission of Z9-14Ald and low level of emission of Z11-16Ac (Cork et al. 1992). Attraction of *H. assulta* males to *H. punctigera* females would be low due to the need for Z9-16Ald to predominate in a blend (Zhao et al. 2006; Wu et al. 2013). It should be expected that *H. punctigera* males will not be attracted to *H. armigera* females due to the latter females' lack of emission of Z11-16Ac. *H. armigera* males likewise will not be attracted to *H. punctigera* females due to the negligible amounts of Z9-16Ald in *H. punctigera* female glands, and their excessive amounts of Z11-16Ac and Z11-16OH, the latter of which has been shown to reduce upwind flight and source location by *H. armigera* males when present at ≥5% relative to Z11-16Ald in the *H. armigera* two-component blend of Z11-16Ald plus Z9-16Ald (Kehat and Dunkelblum 1990).

## Hybridization Studies Reveal Heritable Features of Pheromone Production and Attraction

### Heliothine Hybrids in Nature and in the Laboratory

Despite evidence of optimized sex pheromone blends for individual species, there is some evidence that heterospecific matings might occasionally occur among heliothines in the field. Cross-attraction among sympatric populations of several heliothine species have been documented in field-trapping studies (Hardwick 1965; Klun et al. 1980a; Cork et al. 1992; Cork and Lobos 2003; Wang et al. 2005; Groot et al. 2006, 2009a). Any cross-attraction among heliothines resulting in heterospecific copulations has high negative fitness consequences. For instance, *Helicoverpa zea* males have been shown to be attracted to female *Heliothis virescens* and *H. subflexa* in the field (Groot et al. 2006, 2009a), but without the prospect for viable progeny. In the laboratory, *H. zea* males paired with *H. virescens* females experience irreversible locking of genitalia (Shorey et al. 1965). Likewise, *H. virescens* and *H. subflexa* are able to hybridize, but there is a fitness cost due to the sterility of male hybrids, and also possibly from the emission of less attractive pheromone blends by hybrid female offspring (Laster 1972; Proshold and LaChance 1974; Laster et al. 1976; Teal and Oostendorp 1995). In these two species and in *H. zea*, the presence of either Z9-14Ald or Z9-16Ald in the blend is critical to modulating attraction in possible zones of sympatry where such heterospecific mating mistakes might occur (figure 21.1). Male attraction to blends containing either of these components appears to be dictated by how broadly or narrowly tuned OSNs are in "B-type" *H. subflexa* and *H. virescens* trichoid sensilla (Cossé et al. 1998) on their antennae (Baker et al. 2004, 2006; Gould et al. 2010; see sections below on olfac-

tory architectures of hybrids and parental species). With regard to other components that can vary, the addition of Z11-16OH to a mixture is critical for *H. subflexa* male attraction; conversely, the presence of Z11-16Ac will typically suppress approach by heterospecific males such as *H. virescens* or *H. zea* (Vickers and Baker 1997; Fadamiro et al. 1999; Vickers 2002).

The existence of these mechanisms for pre-mating isolation suggests that hybridization of species such as *H. virescens* and *H. subflexa* in nature is rare. However, hybridization in the field cannot be dismissed because these two species can be readily hybridized in the laboratory (Laster 1972; Teal and Oostendorp 1995; Groot et al. 2004). The presence of hairpencil organs in male heliothines, their active display during courtship, and the behavioral activity of courtship pheromones in these species provide evidence that there have likely been significant levels of heterospecific cross-attraction in the past to long-distance female-emitted sex pheromone. The cross-attraction will have resulted in selection for choosy females that require males to identify themselves as conspecific via olfactory cues emitted during the males' courtship displays (Birch et al. 1990; Hillier and Vickers 2011b). Such displays, if heritable, may additionally have contributed to the process of runaway sexual selection, in which increasingly discriminating females select for the best, most reproductively "fit" males that can produce the optimal quality and quantities of olfactory signals (Birch et al. 1990; Hillier and Vickers 2004; Hillier and Vickers 2011b).

## Heritable Features Affecting Pheromone Blend Biosynthesis

Phenotypic variation in female sex pheromone mixture production and male attraction is modulated in the Heliothinae through autosomal inheritance (i.e., not on sex chromosomes). Wang and colleagues (Wang et al. 2005, 2008; Zhao et al. 2005, 2006; Ming et al. 2007; Wang 2007; Zhang et al. 2010) have investigated species isolation, fitness costs, and heritable features of hybridization in *Helicoverpa armigera* and *H. assulta*. These species produce different ratios of Z11-16Ald and Z9-16Ald as primary and secondary sex pheromone components (100:2.1 for *H. armigera* and 1739:100 for *H. assulta*; Wang and Dong 2001; Wang et al. 2005). Hybrid females produce these components in a ratio similar to, but slightly higher than, *H. armigera* (100:4). This difference is likely due to increased use of both palmitic (C16) and stearic (C18) acids as substrates for Δ9-desaturase and Δ11-desaturase, respectively, for producing Z9-16Ald, instead of only stearic acid for Δ11-desaturase in *H. armigera* to get to Z9-16Ald. Of course, Δ11-desaturase would use palmitic acid as the substrate in both *H. armigera* and the hybrids for them to create the Z11-16Ald end product. Biosynthesis of these components suggests polygenic determinism in these hybrids (Wang et al. 2005), similar to what has been found in studies of hybrid and backcross progeny of *Heliothis virescens* and *H. subflexa* (see next paragraph).

*H. virescens* and *H. subflexa* hybrids were studied initially to test the feasibility of the sterile insect technique in controlling *H. virescens* populations (Proshold and LaChance 1974). Variation in sex pheromone biosynthesis has been well researched in hybrid and backcross studies of *H. virescens* and *H. subflexa* (Groot et al. 2006, 2009b). Studies using amplified fragment length polymorphism marker mapping and backcross families of these species isolated a series of quantitative trait loci (QTLs) that are closely linked with either *H. virescens* or *H. subflexa* pheromone blend phenotypes. Six QTLs that influence pheromone biosynthesis have been identified in these species (Sheck et al. 2006; Groot et al. 2009b, 2013).

In comparison to *H. subflexa* females, *H. virescens* pheromone glands contain more 16Ald, which is *not* a pheromone component of either species; concentrations of this compound are higher in backcross progeny possessing at least one copy of chromosome 24 from *H. virescens* (Groot et al. 2009b). Presence of *H. virescens* chromosome 13 likewise increases the production of the Z9-14Ald pheromone component of *H. virescens*.

Finally, relative acetate production in backcross progeny is also dependent on the presence, absence, and interaction of chromosomes 4 and 22 from *H. virescens*. Presence of either chromosome from *H. virescens* significantly decreases acetate production and is also linked with increased production of the *H. subflexa* pheromone component Z11-16OH (Groot et al. 2006). This finding suggested the presence of a gene encoding acetyl transferase on this QTL. However, candidate genes for acetyl- and acyl-transferases have not been located on this QTL, suggesting rather that activity is modulated by a transcription factor on this chromosome that underlies this variation (Groot et al. 2009b). Subsequent work by Groot et al. (2009b) experimentally confirmed that there was intense interspecific selection against the *H. subflexa* females exhibiting phenotypes that they had created that had shown enhanced acetate production. In cage trials using female *H. subflexa* with introgressed QTLs from *H. virescens* to produce low amounts of acetates, male *H. virescens* mated significantly more frequently with these females than those with normal acetate production (Groot et al. 2006).

These studies confirm that production of multicomponent pheromone blends in these species are autosomally inherited, that they can be proportionally influenced by the chromosomal copies present, that QTLs from single chromosomes may influence multiple components, and that epistatic interactions may occur between QTLs on different chromosomes. Such traits directly influence the degree to which conspecific males are attracted, the mating success of individuals, and the fitness of progeny.

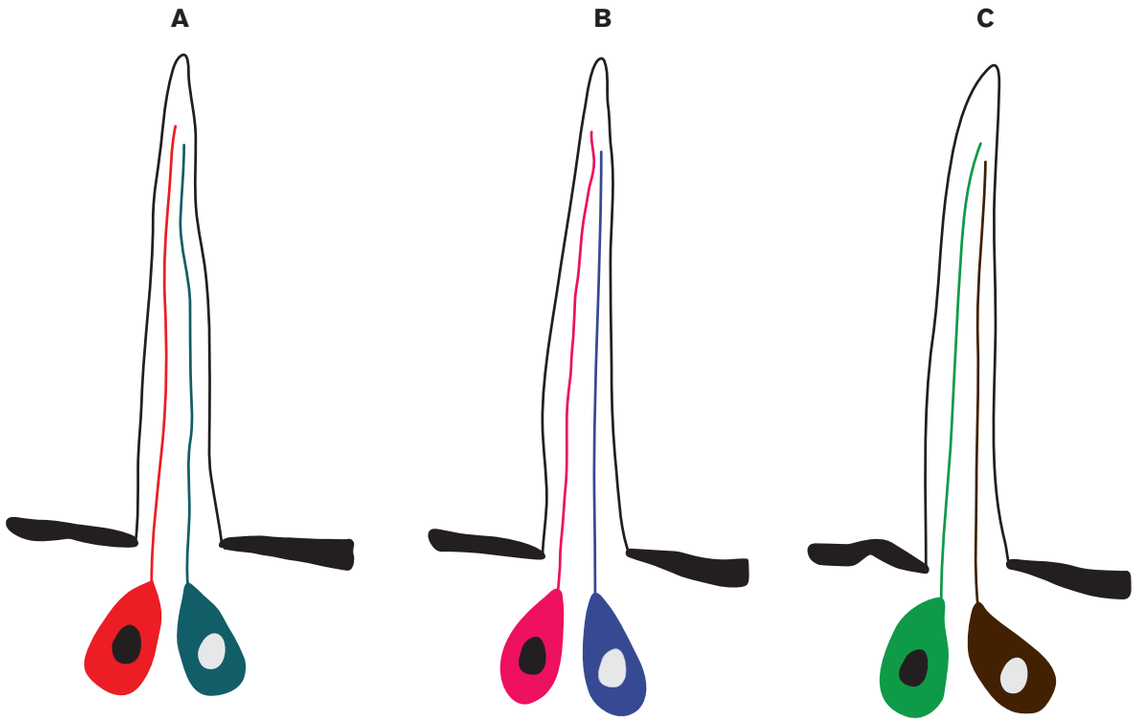
## Heritable Features Affecting Male Response Specificity to Sex Pheromone Blends

Hybrids of *Heliothis virescens* and *H. subflexa* have been used to explore how the specificity of sex pheromone behavioral response might be orchestrated by their peripheral and central olfactory pathways (Baker et al. 2006; Vickers 2006a,b). A key determinant for response specificity of male heliothines and hybrids is the type of OR that is expressed on OSNs that are housed in trichoid sensilla on male heliothine moth antennae and are sensitive to pheromone components (figure 21.3).

### COUPLED QTL AND MALE PHEROMONE RECEPTION STUDIES

As with biosynthetic variation in the females, ORs of heliothine moths represent heritable features that are known to modulate attraction of male progeny to selected pheromone blends. Heritability of pheromone blend preference has been investigated in *Heliothis subflexa* and *H. virescens* by performing behavioral preference tests on hybrids combined with single-cell recordings from hybrid and parental type males

*Heliothis virescens sensilla trichodea*




| | HvOR13 | HvOR11 | HvOR6 | HvOR15 | HvOR14 | HvOR16 |
|---|---|---|---|---|---|---|
| Z11-16:Ald | ● | | • | | | • |
| Z9-14:Ald | ≡ | | ● | | ▥ | ▥ |
| Z11-16:OAc | | | | | ▦ | • |
| Z11-16:OH | | | • | | | ● |
| Z9-16:Ald | | | • | | | |

FIGURE 21.3 Relationship between OSNs co-compartmentalized within A-, B-, and C-type sensilla of *Heliothis virescens* and the ligand sensitivities of the ORs (Wang et al. 2011) that have been shown via in situ hybridization studies to be expressed on them (Große-Wilde et al. 2007; Baker 2009; Krieger et al. 2009). Adapted from Wang et al. (2011). (Top) Optimal sensitivities of OSNs that reside in different sensilla to the pheromone-related compounds Z11-16Ald, Z9-14Ald, Z11-16OAc, and Z11-16OH, as shown in single-cell recording studies (Berg et al. 1995; Baker et al. 2004). On each of these OSNs, the expression of the ORs Hv13, Hv11, Hv6, Hv14, and Hv16, along with Orco (Hv2) co-expressed with all the ORs, is shown, as was demonstrated using in situ hybridization (Große-Wilde et al. 2007; Baker 2009; Krieger et al. 2009). The expression of Hv15 in B-type sensilla on the companion OSN that expresses Hv6 has not been resolved, hence the question mark. (Bottom) Response spectra of the ORs to possible pheromone component ligands, summarized from the work of Krieger et al. (2004, 2009) and Große-Wilde et al. (2007). The size of the circles represents the magnitude of the response of the ORs to compounds presented at a concentration of 10–4 M. Filled circles represent 150–200 nA, horizontally striped circles represent 100–150 nA, and vertically striped circles represent 50–100 nA (adapted from Wang et al. 2011).

(Baker et al. 2006; Vickers 2006a,b). Other studies have combined extensive QTL and candidate gene analyses of pheromone ORs, their functionalization (ascribing their response specificity to a range of candidate pheromone-component ligands), and neuroanatomical studies definitively locating certain ORs populating OSNs within stereotypical trichoid sensilla (Große-Wilde et al. 2007; Gould et al. 2010; Wang et al. 2011; Vásquez et al. 2013). In the latter studies, several *H. virescens* ORs (HvOR6, HvOR14, HvOR15, and HvOR16) were mapped to a single chromosome, Hv/Hs-C27 (Gould et al. 2010). By introgressing Hs-C27 into an *H. virescens* backcrossed background, it was discovered that minor DNA sequence differences between orthologs of *H. subflexa* and *H. virescens* for this chromosome (and presumably Hv/HsOR6) determine attraction and ORN receptivity to either Z9-14Ald or Z9-16Ald (Gould et al. 2010). These studies corroborated the collaborative neuroethological studies of hybrids by Baker et al. (2006) and Vickers (2006a,b), all of which provided evidence that male response can be strongly influenced by shifts in the expression levels of one or a few OR genes and affect the tuning curves of OSNs to create novel behavioral responses to new pheromone components and blends. Furthermore, through such hybrid and backcross studies, the framework for potential speciation was established via novel trait emergence by introgression of pheromone ORs and OSNs in the background of closely related species (Gould et al. 2010).

## BASIC ARCHITECTURE OF THE HELIOTHINE MALE MOTH PHEROMONE OLFACTORY SYSTEM

The OSNs of heliothine moths respond quite narrowly and specifically with action potentials to one pheromone component of their conspecific pheromone blend. Their response specificity is determined by the OR that is expressed on them, such as is shown for the OSNs of *Heliothis virescens* (figure 21.3). The axons of pheromone-sensitive OSNs such as these for *H. virescens*, as in other moth species, project to pheromone-component-specific knots of neuropil called glomeruli in the AL of the brain (figure 21.4). Second-order neurons, called projection interneurons (PNs), exit each glomerulus after synapsing within them and send pheromone-component stimulation along their axons to higher centers of the brain (figures 21.5 and 21.6). In the heliothine moths, the specificities of response of PNs to particular pheromone components have been well worked out, as have their pathways to, and arborization locations in, higher neuropils such as the mushroom body and lateral protocerebrum (see more concerning architectures of higher-order neuronal pathways in the next sections). Some of these PN pathways synapse with integrative circuits in these higher centers and create the odor sensation "pheromone," whereas other PN integrations result in an odor that is behaviorally antagonistic (figures 21.5 and 21.6).

## COORDINATED BEHAVIORAL AND SINGLE-CELL STUDIES OF HYBRID *HELIOTHIS VIRESCENS* AND *H. SUBFLEXA*

Some major findings from the collaborative wind-tunnel behavioral and single-cell studies of parental- and hybrid-type males of *Heliothis virescens* and *H. subflexa* (Baker et al. 2006; Vickers 2006a) revealed how the olfactory pathways of hybrids can shift their tuning profiles to allow broadened behavioral responsiveness to new combinations of components. The key changes in behavioral attraction responses and the concomitant changes in tuning profiles of the OSNs in hybrid males of *H. subflexa* and *H. virescens* are as follows and complement the extensive QTLs and OR-gene expression neuroanatomical studies cited above.

*1. Z11-16OH Is Required for Attraction of Hybrid* Heliothis virescens × H. subflexa *Males*

The response of *Heliothis virescens* × *H. subflexa* hybrid males to different blends of the two species' pheromone components was found to be more similar to the parental *H. subflexa* response type than to the *H. virescens* type (Vickers 2006a). Unlike the *H. virescens* parental type, but similar to *H. subflexa* males, hybrid males all required the presence of Z11-16OH in whatever blend mixture was tested for upwind flight to any of the blends (Vickers 2006a). This is consistent with peripheral neurophysiological recordings that found that nearly all of the OSNs responding to Z11-16OH in the "C-type" sensilla of hybrid males retained fidelity to only Z11-16OH, similar to *H. subflexa* males but unlike C-type OSNs in *H. virescens* males (Baker et al. 2006). This would mean that the Z11-16OH-dedicated OSN line of *H. subflexa* to the anteromedial (AM) glomerulus that is related to positive upwind flight to pheromone was retained in hybrid males, identical to that in *H. subflexa* parental-type males (figure 21.5B). Had OSNs in hybrid males been constructed more like those of *H. virescens* (figure 21.5A), the line to this glomerulus would have switched to now be tuned to Z11-16Ac with a minor responsiveness to Z9-14Ald, and the Z11-16OH-tuned OSNs would now be arborizing in the ventromedial (VM) glomerulus (figure 21.5A). Recordings from PNs (Vickers 2006b) confirmed that the AM glomerulus in hybrids retained its responsiveness to Z11-16OH (figure 21.5C), just as in parental-type *H. subflexa* PNs arborizing in the AM glomerulus (Vickers 2006b) (figure 21.5B). This result with the PNs shows that the AM is the target glomerulus of the Z11-16OH-tuned OSNs found in hybrids (Baker et al. 2006) (figure 21.5C), just as it is in parental-type *H. subflexa* males (Lee et al. 2006b).

*2. Z9-14Ald Can Substitute for Z9-16Ald in Attracting Hybrid* Heliothis virescens × H. subflexa *Males*

A second major finding was that in hybrid males, Z9-14Ald could substitute behaviorally for Z9-16Ald in any of the blends (Vickers 2006a); this flexibility does not exist in *Heliothis subflexa* males (Vickers 2002, 2006a). Similarly, Z9-16Ald could not substitute for Z9-14Ald in *H. virescens* males (Vickers et al. 1991; Vickers 2006a). Therefore, this ability to substitute Z9-16Ald for Z9-14Ald and still get good attraction in hybrid males was surprising, and correlated with single-cell recordings from hybrid B-type sensilla in hybrids (Baker et al. 2006). Hybrid B-type OSNs exhibited *equal* sensitivity to *both* Z9-14Ald and Z9-16Ald (figure 21.5C), which was quite unusual compared to the complete inactivity of B-type OSNs to Z9-16Ald in *H. virescens* (Baker et al. 2004, 2006) (figure 21.5A) and only slight co-responsiveness of B-type OSNs of *H. subflexa* to Z9-14Ald in combination with high responsiveness to Z9-16Ald (Baker et al. 2004, 2006) (figure 21.5B). Recordings from PNs exiting the dorsomedial (DM) glomerulus of hybrid males corroborated the OSN tuning shift; the PNs of hybrid males exiting the DM glomerulus were now equally responsive to Z9-14Ald and Z9-16Ald (Vickers 2006b) (figure 21.5C), whereas in parental *H. subflexa* they were only responsive to Z9-16Ald (Vickers and Christensen 2003) (figure 21.5B) and in *H. virescens* only responsive to Z9-14Ald (Vickers et al. 1998) (figure 21.5A).

Baker et al. (2006) had speculated that the tuning shift in B-type sensillar OSNs may have been caused by the

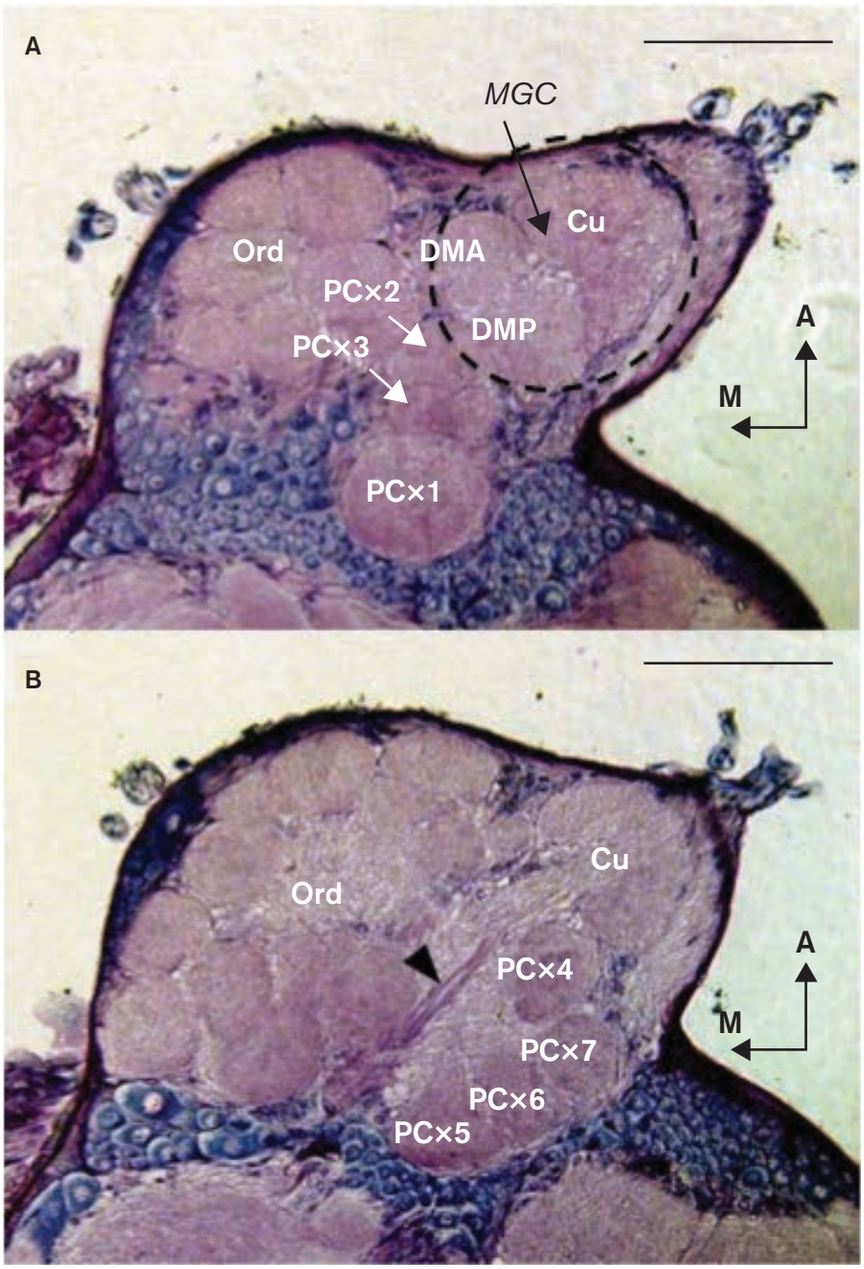



FIGURE 21.4 Horizontal (top-down) view of sections of the right antennal lobe of the brain of a *Helicoverpa zea* male. The pinkish-stained areas are the knots of neuropil called glomeruli, and the small blue-stained ovoids are the cell bodies of olfactory sensory neurons, local interneurons, and projection neurons.

A Section taken from approximately 70 µm from the most dorsal surface showing the macroglomerular complex (MGC) (dashed circled area) with its three glomeruli: cumulus (Cu) and dorsomedial posterior (DMP) and dorsomedial anterior (DMA) glomeruli of the pheromone-component-related MGC. This section also shows the location of PCx1, PCx2, and PCx3 glomeruli immediately posteriomedial to the three MGC glomeruli. PCx1 is the arborization destination of the second OSN co-compartmentalized with the Z11-16Ald–responding OSN in A-type trichoid sensilla. The second OSN that targets PCx1 is unresponsive to all odorants that have been tested on it, and as such no ligand can be assigned to it. Ord designates ordinary glomeruli that receive general odorant-related OSN inputs in the antennal lobe.

B A more ventral section taken at a depth of approximately 130 µm from the dorsal surface of the same animal, still showing the Cu of the MGC, but now other PCx glomeruli—PCx4, PCx5, PCx6, and PCx7—are apparent. A confluent bundle of fibers from interneurons of the MGC and PCx is indicated by the arrowhead.

ABBREVIATIONS: A, anterior; M, medial; MGC, macroglomerular complex; Cu, cumulus; DMA, dorsomedial anterior; DMP, dorsomedial posterior; Ord, ordinary glomeruli; PCx1, posterior complex glomerulus 1; PCx2, posterior complex glomerulus 2; PCx3, posterior complex glomerulus 3; PCx4, posterior complex glomerulus 4; PCx5, posterior complex glomerulus 5; PCx6, posterior complex glomerulus 6; PCx7, posterior complex glomerulus 7.

Scale bars = 100 µm.

SOURCE: Adapted from Lee et al. (2006a).

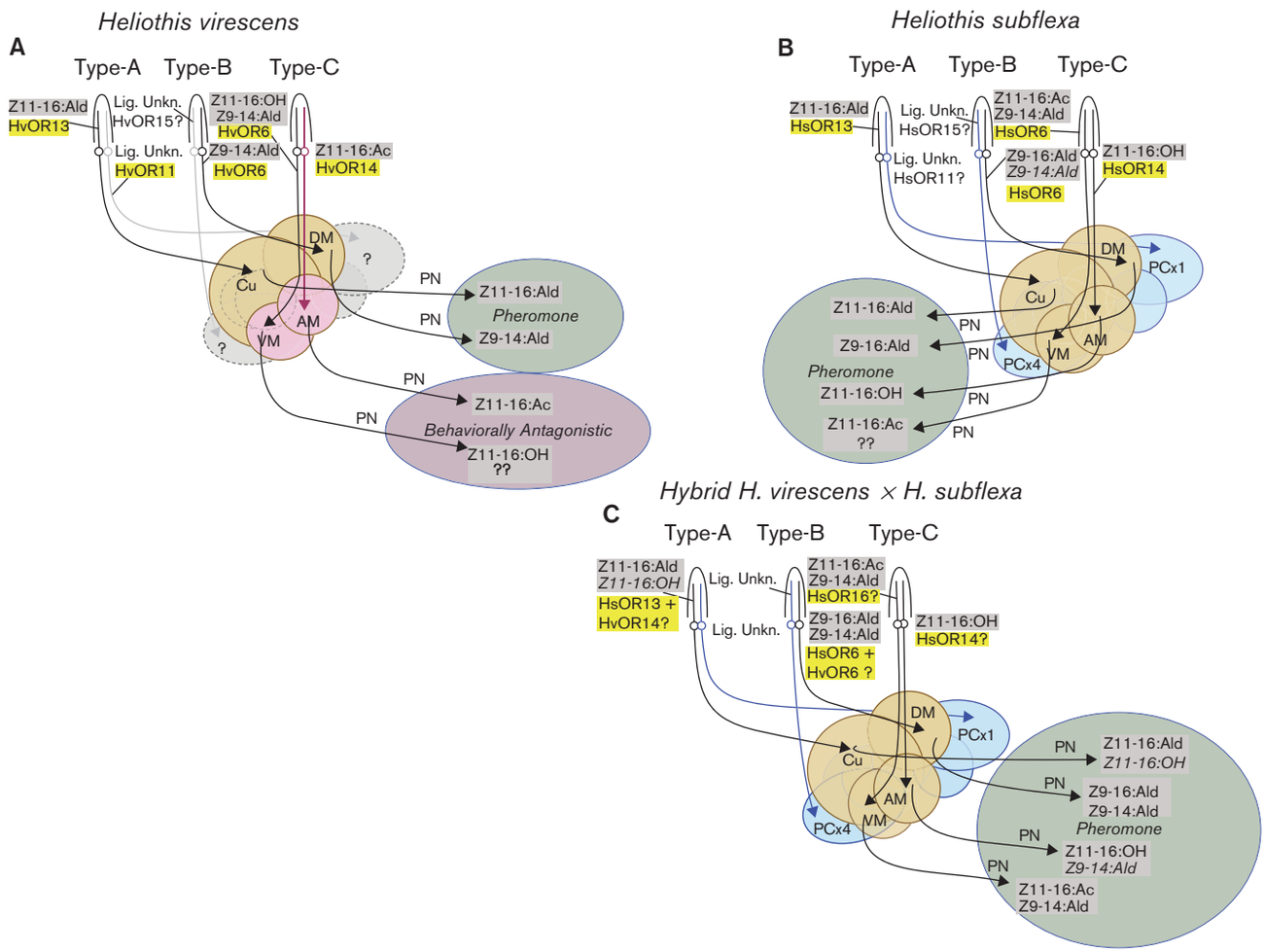



FIGURE 21.5 Neuroanatomical depictions of the known olfactory pathways of male *Heliothis virescens*, *H. subflexa*, and *H. virescens* × *H. subflexa* hybrids. Sensilla are represented by black-outlined conical figures, and OSNs are represented by the black, gray, or blue lines with cell bodies (tiny circles) within the sensilla having axons that project (arrows) to arborize in glomeruli in the MGC (brown or pink ovoid figures) or to the posterior complex (PCx; gray or blue ovoid figures). The targeting of particular glomeruli by pheromone-component-specific OSNs has been shown definitively by cobalt backfilling in *H. virescens* (A) (Berg et al. 1998) and *H. subflexa* (B) (Lee et al. 2006b). For the hybrids (C), the targeting of glomeruli by OSNs has not been demonstrated by cobalt backfilling, but it is assumed to be as depicted due to the demonstrated response profiles and arborization locations of PNs leaving the MGC glomeruli (Vickers 2006b). The known tuning profiles of OSNs are shown in gray rectangles. The ORs of *H. virescens* having tuning profiles matching particular OSNs and that have also been characterized via in situ hybridization studies as being expressed on particular OSNs (Große-Wilde et al. 2007; Krieger et al. 2009) are shown in yellow rectangles (A). Orthologous ORs of *H. subflexa* (Vásquez et al. 2011) (yellow rectangles in B) are placed on respective OSNs according to the known tuning profiles of OSNs (Baker et al. 2004) shown to reside in particular sensilla (Lee et al. 2006b). Placement of ORs of hybrids (yellow rectangles in C) on particular OSNs has been deduced from the OSN tuning profiles of hybrids found by Baker et al. (2006). OSNs having no known ligand are designated as such with the abbreviation, "Lig. Unkn." and have no rectangle. PCx1 and PCx4 arborization destinations of the axons of particular "no-ligand" OSNs of *H. subflexa* from A- and B-type sensilla are shown as blue axons due to their proven anatomies from cobalt staining. Because of the proven inputs after cobalt staining, PCx1 and PCx4 are likewise designated with blue ovoid figures. Conversely, PCx OSN arborization destinations of "no-ligand" OSNs of *H. virescens* are shown as gray ovoids, and their OSN inputs as gray axons following the careful reassessment of the stained OSNs shown in Berg et al. (1998) by Lee et al. (2006b). In particular, Lee et al. (2006b) strongly suggested that the co-localized unknown-ligand OSNs from *H. virescens* A- and B-type sensilla that had previously been designated by Berg et al. as arborizing in "ordinary" glomeruli actually are targeting PCx1 and PCx4 of this species. PNs are shown leaving their known arborization locations within component-specific glomeruli and projecting to protocerebral centers such as the mushroom body or the inferior lateral protocerebrum (see Chapter 10) to be integrated as "pheromone" (green ellipses) or as a "behaviorally antagonistic" odor feature (lavender ellipse). PN tuning profiles and PN arborization locations within particular MGC glomeruli have been determined through neuroanatomical studies of PNs in *H. virescens* (Berg et al. 1998; Vickers et al. 1998; Vickers and Christensen 2003), *H. subflexa* (Vickers and Christensen 2003), and hybrid *H. virescens* × *H. subflexa* (Vickers 2006b). These studies show a predominantly linear relationship of pheromone-component-specific OSN-to-glomerulus-to-PN pathways in these two parental species and their hybrids (Berg et al. 1998; Vickers and Christensen 2003; Vickers 2006b). Graphic scheme adapted from Lee et al. (2006b) which was also adapted and employed by Berg et al. (2014).

ABBREVIATIONS: Cu, cumulus; AM, anteromedial; VM, ventromedial; DM, dorsomedial; PCx1, posterior complex glomerulus 1; PCx4, posterior complex glomerulus 4; PN, projection neuron.

SOURCE: Adapted from Lee et al. (2006b).

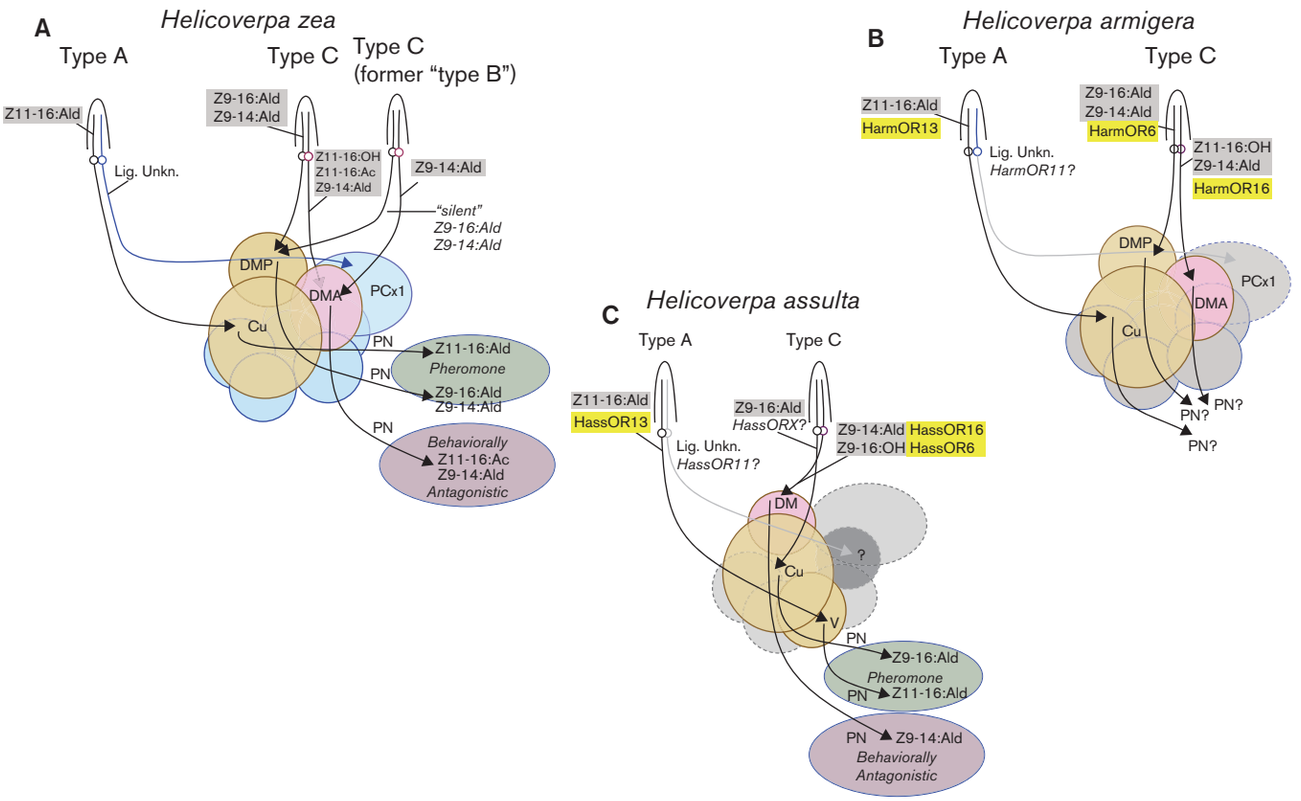



FIGURE 21.6 Neuroanatomical depictions of the known olfactory pathways of male *Helicoverpa zea*, *H. assulta*, and *H. armigera*. Sensilla are represented by black-outlined conical figures, and OSNs are represented by the black, gray, or blue lines with cell bodies (tiny circles) within the sensilla having axons that project (arrows) to arborize in glomeruli in the MGC (brown or pink ovoid figures) or to the posterior complex (PCx; gray or blue ovoid figures). The optimal pheromone-component-related odorant molecules for the OSNs (gray rectangles) and OSN co-localization relationships within A- or C-type sensilla have all been proven using single sensillum recordings for *H. zea* (Cossé et al. 1998; Lee et al. 2006a), *H. assulta* (Berg and Mustaparta 1995; Berg et al. 2005; Wu et al. 2013), and *H. armigera* (Wu et al. 2013; except for the non-Z9-16Ald-responding OSN in the C-type sensillum whose profile is merely conjectured here). OSNs having no known ligand are designated as such with the abbreviation "Lig. Unkn." or with the word "silent," and have no rectangle. For *H. armigera* and *H. assulta*, the names of ORs that have been shown to have the particular indicated tuning profile (Liu et al. 2013; Jiang et al. 2014) and to be expressed on particular OSNs based on OSNs' particular tuning profiles (Berg and Mustaparta 1995; Berg et al. 2005; Wu et al. 2013) are shown in yellow rectangles. No ORs are labeled as residing on any OSNs for *H. zea* because none have been characterized. The targeting of particular glomeruli by component-specific OSNs has been shown by cobalt backfilling in *H. zea* (Lee et al. 2006a) and *H. assulta* (Berg et al. 1998). For *H. armigera*, the targeting of glomeruli by OSNs has not been demonstrated by cobalt backfilling, but it is assumed to be as depicted due to the tuning profiles of OSNs (Wu et al. 2013) and *H. armigera*'s pheromonal similarity to *H. zea*. The PCx1 arborization destination of the "no-ligand" OSN of *H. zea* from A-type sensilla was proven via cobalt backfilling (blue axon from the OSN from the A-type sensillum) and is designated as a blue ovoid figure, as are the other *H. zea* PCx glomeruli. The OSN arborization destination of the no-ligand OSN (gray axon) from the A-type sensilla of *H. assulta* into a PCx is shown in gray and was done (Lee et al. 2006b) by reassessing older figures and data from Berg et al. (2005). The gray axon from the co-localized OSN in the A-type sensillum of *H. armigera* arborizing in PCx1 of this species is just suggested here due to *H. armigera*'s pheromonal similarity to *H. zea*. PNs are shown leaving their known arborization locations within component-specific glomeruli and projecting to protocerebral centers such as the mushroom body or the inferior lateral protocerebrum (see figure 21.3C) to be integrated as "pheromone" (green ellipses) or as a "behaviorally antagonistic" odor feature (lavender ellipse). PN tuning profiles and PN arborization locations within particular MGC glomeruli have been determined through neuroanatomical studies of PNs in *H. zea* (Vickers et al. 1998) and *H. assulta* (Berg et al. 2005). No PN analyses have yet been performed in *H. armigera*, as indicated by the question marks for this species. These studies of the PNs of *H. zea* and *H. assulta* (Vickers et al. 1998; Berg et al. 2005) again demonstrate the predominantly linear relationship of pheromone-component-specific OSN-to-glomerulus-to-PN pathways in heliothine moths (graphic scheme adapted from Lee et al. 2006b, also employed by Berg et al. 2014).

ABBREVIATIONS: Cu, cumulus; DMA, dorsomedial anterior; DMP, dorsomedial posterior; DM, dorsomedial; V, ventral; PCx1, posterior complex glomerulus 1; PN, projection neuron.

SOURCE: Adapted from Lee et al. (2006a).

co-expression of two different ORs on the same sensillum, with one OR being tuned to Z9-14Ald and the other OR tuned to Z9-16Ald. Although such co-expression of multiple pheromone OR types on a single OSN has been demonstrated in moths (Koutroumpa et al. 2014), another possibility is that a completely different and new OR might now be being expressed in the membranes of OSN dendrites of the hybrids. These different alleles would code for ORs having significantly different affinities for different ranges of pheromone-component ligands.

A striking example of this broadened acceptance of ligands comes from the work of Leary et al. (2012). They showed in the *Ostrinia nubilalis* and *O. furnicalis* sex pheromone receptor systems that a change in a single amino acid in the third transmembrane domain (of seven) of an OR that usually is optimally responsive to (*E*)-11-tetradecenyl acetate (E11-14Ac) plus several other pheromone-related ligands now severely restricts its tuning profile to respond only to (*Z*)-12-tetradecenyl acetate (Z12-14Ac) and (*E*)-12-tetradecenyl acetate (E12-

14Ac) (Leary et al. 2012). The fact that an OR, "HarmOR6," has now been found in *H. armigera* that is equally stimulated by either Z9-14Ald or Z9-16Ald (Liu et al. 2013) supports the idea that there may be a type of OR in heliothine moths amenable to a tuning shift similar to that occurring in *O. nubilalis* and *O. furnicalis*. This OR would be exhibiting a shifted, broadened acceptance of ligands due to a minor amino acid substitution, and an OR similar to HarmOR6 might be being expressed in hybrid *H. virescens* × *H. subflexa* B-type OSNs and explain their tuning to both Z9-14Ald and Z9-16Ald.

*3. Addition of Z11-16Ac Does Not Adversely Affect Attraction of Hybrid* Heliothis virescens × H. subflexa *Males*

A third major finding in *Heliothis virescens* × *H. subflexa* hybrid males was that the addition of Z11-16Ac to the optimal hybrid blend did not affect attraction of the hybrid males significantly (Vickers 2006a), a finding that is quite different than the antagonistic effect on upwind flight that even 0.1% or 1.0% Z11-16Ac has on *H. virescens* males when added to its two-component blend (Vickers and Baker 1997). It was clear from OSN recordings that in hybrids, the OSN that previously would have responded only to Z11-16Ac in parental type *H. virescens* now responded to Z9-14Ald as well (figure 21.5C). This arrangement is more like the parental-type *H. subflexa* OSN in C-type sensilla (figure 21.5B), except in hybrids the sensitivity to Z9-14Ald relative to Z11-16Ac in this OSN had been elevated (figure 21.5C). This pattern is yet a third way that the hybrids exhibit a more *H. subflexa*-like behavior and olfactory pathway phenotype than a parental *H. virescens* phenotype (Baker et al. 2006).

The behavior and OSN tuning profiles for hybrid C-type sensilla are borne out in the responsiveness of PNs of hybrid males that were recorded from and responded to Z11-16Ac, Z11-16OH, and Z9-14Ald (Vickers 2006b) (figure 21.4C). The PNs responsive to Z11-16OH did not respond to Z9-14Ald, a lack of Z9-14Ald-responsiveness that is similar to that of the PNs usually found in *H. subflexa* (Vickers and Christensen 2003). These Z11-16OH-responding PNs of hybrid males arborized in the AM glomerulus of the macroglomerular complex (MGC), again just like those of parental *H. subflexa* (Vickers and Christensen 2003) (figure 21.5C). This type of PN in *H. virescens* would have arborized in the VM glomerulus and would have responded to high doses of Z9-14Ald as well as Z11-16OH (Christensen et al. 1995; Vickers et al. 1998), mirroring the response profiles of *H. virescens* OSNs in C-type sensilla that were tuned to Z11-16OH (Baker et al. 2004, 2006) (figure 21.5A).

Furthermore, the Z11-16Ac-responding PNs in the hybrid males arborized in the VM glomerulus, exactly like Z11-16Ac-responding PNs of *H. subflexa* males (Vickers and Christensen 2003) (figures 21.5B,C) and unlike those of *H. virescens* males, which arborize in the AM glomerulus (Christensen et al. 1995) (figure 21.5A). These hybrid PNs exhibited an elevated responsiveness to Z9-14Ald compared to the parental-type PNs of *H. subflexa*, which are tuned to Z11-16Ac (Vickers and Christensen 2003), and again mirrors the OSN response profiles found in these same hybrid males (Baker et al. 2006) (figure 21.5C).

## DIFFERENTIAL EXPRESSION OF ORs ON OSNs DETERMINES OSN RESPONSE PROFILES AND BEHAVIORAL RESPONSE SPECIFICITY

Studies with hybrids between *Heliothis virescens* and *H. subflexa* showed behavioral response shifts in hybrids to different blends of components that corresponded to shifts in OSN tuning curves as well as to shifts in PN tuning profiles and associated MGC arborization locations. All of these changes in the response spectra of hybrids illustrate how important OSN tuning profiles are in determining the subsequent neuronal activities, ending with behavior. Furthermore, the determinants of OSN tuning profiles are the ligand-specific response profiles of the ORs that are expressed on OSN dendritic membranes. For each OSN type, the selectors of which OR out of all the many possible ORs will populate its dendritic membrane are the presumed transcription factors that choose one *OR* gene to be expressed on an OSN over all the others (Ray et al. 2007; Fujii et al. 2011).

Years of work have gone into de-orphanizing putative pheromone-component-tuned ORs that are expressed on the dendrites of heliothine OSNs. These OR response profiles have been characterized using heterologous expression systems, the first and most widespread of which is the *Xenopus* oocyte system (see Baker and Hansson, this volume), in which the ORs are expressed in the oocyte membrane and pheromone-related compounds are solubilized to flow over the oocyte in the aqueous bath to contact the oocyte membrane. With the *Xenopus* system, the magnitude of the change in membrane conductance as the potential ligands contact the ORs bound to the membrane is measured electrophysiologically (Wang et al. 2011). A second heterologous expression system used in studying heliothine moth OR tuning profiles is the *Drosophila* "empty neuron" system in which the OR is expressed in the *Drosophila* OSN and its activity is monitored electrophysiologically (Dobritsa et al. 2003; Hallem et al. 2004; Hallem and Carlson 2004, 2006). A third heterologous expression system that has been used successfully with helothine moths (Große-Wilde et al. 2007) is a Flp-In-System coupled with a human embryonic kidney 293 (HEK293) cell line stably carrying a mouse $G\alpha_{15}$ gene; this system is named Flp-In T-Rex293/$G\alpha_{15}$. With this HEK293 cell line expression system, the magnitude of the change in membrane conductance is measured via calcium imaging; this imaging involves monitoring increases in fluorescence due to a calcium-sensitive dye responding to calcium release by the cells when they are contacted by the prospective ligands (Große-Wilde et al. 2007; Krieger et al. 2009).

Several of these heterologous expression studies showed clear tuning profiles of putative *H. virescens* pheromone-component-responsive ORs that corresponded with previously performed single-cell neurophysiological assays and to neuroanatomical tracings of OSN axons to specific MGC glomeruli (Große-Wilde et al. 2007; Krieger et al. 2009; Wang et al. 2011; Liu et al. 2013; Vásquez et al. 2013; Jiang et al. 2014). Importantly, in situ hybridization studies, electrophysiological recordings, and cobalt backfilling of OSNs from single sensilla showed definitively that certain pairs of ORs could be characterized as being expressed on co-localized OSNs within the same sensilla. Thus, specific ORs could be mapped to their sensillar locations on stereotypical pairs of OSNs, and the axonal projections to AL glomeruli could be surmised (Baker et al. 2004, 2006; Lee et al. 2006a,b; Große-Wilde et al. 2007; Baker 2009; Krieger et al. 2009; Vásquez et al. 2011; Wang et al. 2011; Jiang et al. 2014) (figure 21.3).

These studies culminated in a definitive map of OR expression on certain OSNs for the *H. virescens* sex pheromone olfaction system (figure 21.3). The OR that is expressed on the OSN in *H. virescens* "A-type" sensilla that respond optimally to Z11-16Ald is HvOR13 (previously known as HR13; Große-Wilde et al. 2007; Krieger et al. 2009). The response spectrum of the

HvOR13 expressed in the *Drosophila* empty neuron system was shown to be very specific for Z11-16Ald (Vásquez et al. 2013), and as such mirrors the OSN responsive to Z11-16Ald in A-type sensilla (Berg et al. 1995; Baker et al. 2004). The OSN in A-type sensilla that is co-localized with the Z11-16Ald-responsive OSN was shown in in situ hybridization studies (Krieger et al. 2009) to have HvOR11 (previously known as HR11) expressed on its dendritic membranes, but as yet there is no known ligand for this OR that excites the corresponding OSN (Große-Wilde et al. 2007; Wang et al. 2011) (figures 21.3 and 21.5A).

The C-type sensilla of *H. virescens* have two known co-localized OSNs whose electrophysiological response profiles mirror those of two ORs, HvOR14 and HvOR16 (Große-Wilde et al. 2007; Krieger et al. 2009). HvOR14 (previously known as HR14) was shown to be optimally responsive to Z11-16Ac, whereas HvOR16 (previously known as HR16) was responsive to both Z11-16OH and Z9-14Ald, just like the response profiles of the two OSNs in C-type sensilla recorded in vivo (Baker et al. 2004, 2006; Große-Wilde et al. 2007; Baker 2009; Krieger et al. 2009) (figures 21.3 and 21.5A). In situ hybridization studies had shown these two ORs to be expressed in co-localized OSNs (Große-Wilde et al. 2007). The OSNs expressing these Z11-16Ac-tuned ORs had been shown to project to the AM glomerulus of the MGC (Berg et al. 1998) with PNs exiting the AM, confirming this pathway for reporting the presence of Z11-16Ac to protocerebral centers (Vickers et al. 1998; Vickers and Christensen 2003) (figure 21.5A). OSNs expressing the Z11-16OH-plus-Z9-14Ald-tuned ORs have been shown to project to the VM glomerulus (Berg et al. 1998), projections that presumably would be substantiated with further neuroanatomical studies of PNs, because VM is the only MGC glomerulus that has had no PNs neuroanatomically characterized and Z11-16OH- and Z9-14Ald-responding PNs are the only remaining unrepresented types of PNs for *H. virescens* (Vickers et al. 1998; Vickers and Christensen 2003; Vickers 2006b).

After years of uncertainty, it was finally shown definitively that in B-type sensilla, the OSN responsive to Z9-14Ald expresses HvOR6 (Wang et al. 2011) (previously known as HR6). It is still unknown what the co-localized OSN expresses, but it is suggested that it might be HvOR15 (Wang et al. 2011) (previously known as HR15). As with HvOR11, there is currently no known ligand for HvOR15. It will take double-staining in situ hybridization studies to determine whether in fact HvOR15 and HvOR6 are expressed on co-localized OSNs in B-type sensilla of *H. virescens*. We now know at least that the HvOR6 must be expressed on the Z9-14Ald-responsive OSN in B-type sensilla, but we do not know about the companion OSN (figures 21.4A and 21.6). Studies of PNs substantiate the conclusion that the DM glomerulus receives input from Z9-14Ald-responding OSNs in *H. virescens*, because this is the arborization location of PNs responding to Z9-14Ald, but to no other ligands in *H. virescens* (Vickers et al. 1998; Vickers and Christensen 2003).

The OSN in B-type sensilla that responds to Z9-14Ald in *H. virescens* and Z9-16Ald in *H. subflexa* (figures 21.5A and 21.5B) seems to be the OSN that determines behavioral response specificity in the two species, yet allows a broadened response in hybrids to both Z9-16Ald and Z9-14Ald (figure 21.5C). Orthologous ORs in *H. subflexa* have been isolated using QTL techniques (Gould et al. 2010), with the expression of HsOR6, along with HsOR14, HsOR16, HsOR13, HsOR11, and HsOR15, being implicated as orthologs to the *H. virescens* ORs of corresponding sequence similarity. However, the functionalization of *H. subflexa* ORs measuring their actual responses to ligands has not been performed.

In reviewing the data on the intermediate type of responsiveness of the B-type OSN of hybrids that responded equally well to both Z9-14Ald and Z9-16Ald, Baker et al. (2006) conjectured that perhaps this new, dual responsiveness to both components was due to a co-expression of two different types of ORs on the dendrites of a single type of OSN, with one OR being tuned to Z9-14Ald and the other OR to Z9-16Ald. Vásquez et al. (2011) examined the relative expression levels of HvOR6 and HsOR6 in *H. subflexa* and *H. virescens* and concluded that HvOR6 and HsOR6 are not differentially expressed in male antennae; therefore, interspecific sequence differences between these two genes must explain the species-specificity of their tuning on their B-type OSNs and the resultant differential responsiveness to their pheromone blends. Therefore, the possibility exists that a co-expression of HvOR6 and HsOR6 on hybrid B-type OSNs as conjectured by Baker et al. (2006) might explain the co-responsiveness of these OSNs to both Z9-14Ald and Z9-16Ald and the mutual interchangeability of Z9-14Ald and Z9-16Ald with regard to successful upwind flight behavior to the hybrid sex pheromone blend.

## Heliothine Moth Pheromone Olfactory Pathways Common across Species

The underlying circuitry of the pheromone olfactory systems of moths is quite similar across all species (see Baker and Hansson, this volume), and this similarity holds true especially for *Heliothis* and *Helicoverpa* spp. that have been thoroughly examined neuroanatomically. Because the pheromone olfactory pathways ascending from the AL to higher neuropils are so similar across heliothines, the major changes that can be expected to be at work in changing the specificity of behavioral response by male heliothines will be at the periphery. OSNs can be selected to express different ORs and change the types of pheromone compounds that can be registered by the existing circuitry platform of the olfactory system. Because the target glomeruli of OSNs of insects are not known to change when new ORs are expressed on them (Dobritsa et al. 2003; Hallem et al. 2004; Goldman et al. 2005), the malleability of pheromone-component reporting by OSNs to glomeruli causing changes in behavioral response profiles will rest predominantly with any shifts in expression of ORs on OSNs. Males can be expected to "track" shifting female-emitted blends (Phelan 1992) by being selected to have new and different ORs expressed on their antennal OSNs. The action potentials of these OSNs in response to now-different pheromone-component ligands will be received and integrated as representing the same pheromone blend as before due to the unvarying, underlying synaptic circuitry. Such shifts in OSN tuning will be what optimizes conspecific mate finding and minimizes heterospecific attraction and mating mistakes.

The general theme across all of the heliothine species that have been intensely looked at is one of balanced olfactory antagonism (Baker 2008) that is orchestrated first by OSNs that are optimally responsive to each of the species' own conspecific pheromone components. These OSN inputs are carried by OSN axons to their sex pheromone-component-specific glomerular targets in the MGC of the AL (see Baker and Hansson, this volume) (figure 21.4A). The balance of excitations across the MGC glomeruli will determine the pheromone blend sensation that is registered by protocerebral neurons after they receive inputs from PNs carrying information out of the AL to the protocerebrum (see Baker and Hansson, this volume).

In heliothines, there are either three or four glomeruli that comprise the MGC. In the genus *Helicoverpa*, there are three MGC glomeruli that receive pheromone-component-related inputs from pheromone-component-tuned OSNs: the "cumulus" or largest glomerulus in all three species, a second "dorsomedial anterior" (DMA) glomerulus in *H. zea* and *H. armigera*, which is called "ventral" in *H. assulta*, and a third glomerulus called "dorsomedial posterior" (DMP) in *H. zea* and *H. armigera*, and just "dorsomedial" in *H. assulta*) (Vickers et al. 1998; Berg et al. 2002, 2005, 2014) (figure 21.4B). In the genus *Heliothis*, there are four MGC glomeruli, including the cumulus, the DM, AM, and VM (Vickers et al. 1998; Berg et al. 2002; Vickers and Christensen 2003) (figure 21.5). In both *Heliothis* and *Helicoverpa* spp., there is an unusual "posterior complex" of glomeruli, immediately behind the MGC (figure 21.3), some of whose glomeruli receive inputs from thus far unexcitable OSNs whose ligands have yet to be discovered. The OSNs arborizing in posterior complex glomeruli are co-compartmentalized in A-type trichoid sensilla with OSNs responsive to Z11-16Ald and in B-type sensilla with OSNs responsive to Z9-14Ald or Z9-16Ald (Lee et al. 2006a,b; Baker 2009) (figures 21.4–21.6).

The correct ratio of conspecific sex pheromone components results in an optimal ratio of excitatory action potential inputs to the target glomeruli, and these excitations, usually still component specific even after the MGC AL level, are then conducted by PNs to integrative neuropils in the protocerebrum, including the calyces of the mushroom body (MBC) and the inferior lateral protocerebrum (ILPC) (see Baker and Hansson, this volume). Incorrect sex pheromone-component ratio inputs to the glomeruli of the MGC (i.e., too little or too much of any of the components), or else the presence of heterospecific female pheromone components in the plume strands reported to other MGC glomeruli, can result in a suboptimal balance of MGC glomerular activity that now results in poor behavioral response and little or no attraction.

## A-, B-, and C-Type Sensilla House Stereotypically Paired, Differentially Tuned, Olfactory Sensory Neurons

The differentially tuned pheromone-component-responsive OSNs of heliothine pheromone olfactory systems reside in trichoid sensilla on the male antennae (Almaas and Mustaparta 1990, 1991; Almaas et al. 1991; Vickers et al. 1991; Wu 1993; Cossé et al. 1998). Sensilla are named according to the responsiveness of the OSNs that are housed in them. For instance, OSNs tuned to Z11-16Ald have been deemed to reside in A-type sensilla (Cossé et al. 1998; Baker et al. 2004), along with a second OSN for which no ligand has yet been found (figures 21.5 and 21.6). Across all the species examined thus far, except for *Helicoverpa assulta*, the A-type sensilla are the most abundant and are correlated with Z11-16Ald being the most abundant sex pheromone component in the blend (Baker et al. 2004). A-type sensilla for these species include >70% of the trichoid sensilla and thus 70% of the pheromone-component-tuned OSNs in these species are Z11-16Ald-responding OSNs (Berg et al. 1995; Cossé et al. 1998; Baker et al. 2004; Lee et al. 2006a,b; Wu et al. 2013). *H. assulta* differs from these species that use Z11-16Ald as their major component, yet it follows the rule of greater abundance of OSNs tuned to the major component because >80% of the OSNs of *H. assulta* are tuned to its major pheromone component *Z*9-16Ald (Berg et al. 2005; Wu et al. 2013) and reside in C-type sensilla (Cossé et al. 1998; Lee et al. 2006a,b) (figure 21.6).

In the C-type sensilla of four of the five closely examined species (e.g., all but *Heliothis subflexa*), one of the two OSNs is always tuned to behaviorally antagonistic compounds emitted by other species. For instance, a single C-type OSN is tuned to both Z9-14Ald and Z9-16OH in *H. assulta* (Berg et al. 1995, 2005, 2014) and to Z11-16Ac, Z11-16OH, and Z9-14Ald in *H. zea* (Cossé et al. 1998). The two OSNs in C-type sensilla of *H. virescens* are tuned to Z11-16Ac and Z11-16OH, respectively (Baker et al. 2004, 2006; Baker 2009; Krieger et al. 2009). Although no direct electrophysiological recordings have been made from the second OSN in C-type sensilla of *H. armigera* (the first OSN of the pair has been shown electrophysiologically to be tuned to Z9-16Ald; Wu et al. 2013), the second OSN has been labeled as being tuned to behavioral antagonists Z11-16OH and Z9-14Ald, based on OR de-orphanization results (Liu et al. 2013; Jiang et al. 2014) (figure 21.6).

In three of these four species, the second OSN in C-type sensilla is tuned to one of the minor conspecific pheromone components, e.g., to Z9-16Ald in *H. zea* and *H. armigera* (Cossé et al. 1998; Wu et al. 2013), or to the major conspecific component Z9-16Ald in *H. assulta* (Berg et al. 1995, 2005, 2014).

The exception for C-type sensilla having at least one OSN that is tuned to a heterospecific antagonist is *H. subflexa*. In this species, no heterospecific behavioral antagonists have been found to date. One of the *H. subflexa* C-type OSNs is tuned to its third sex pheromone component Z11-16OH (Baker et al. 2004, 2006), with the second co-localized OSN tuned to Z11-16OAc (figure 21.5). This compound has been shown in field tests to function as a fourth sex pheromone component across the entirety of its geographical range (Groot et al. 2007).

For *H. virescens* and *H. subflexa*, B-type sensilla house an OSN tuned either to strictly Z9-14Ald (*H. virescens*) or to Z9-16Ald with slight responsiveness to Z9-14Ald (*H. subflexa*) (Baker et al. 2004, 2006). This type of OSN in B-type sensilla is critical for reporting the presence of these two species' minor sex pheromone components, either Z9-14Ald (*H. virescens*) or Z9-16Ald (*H. subflexa*). In both species, there is a second OSN co-localized in B-type sensilla that has not yet been found to respond to any ligands (Berg et al. 1998; Lee et al. 2006b) (figure 21.5).

Lee et al. (2006a) concluded that for a third species, *H. zea*, their initial designation of the presence of a B-type sensillum needed to be reconsidered. The two OSNs in the purportedly B-type sensilla that were recorded from and stained were observed to be anatomically C-type sensilla in their glomerular targets (Lee et al. 2006a). They found that OSNs in these sensilla projected their axons to the DMP and DMA glomeruli (see next section) just like the OSNs of C-type sensilla, with one OSN responding only to Z9-14Ald (figure 21.6). For some reason in these few sensilla, the large-spiking OSN that usually would have responded to Z9-16Ald, Z9-14Ald, or both seemed to have become silent. In addition, the small spiking OSN in this sensillum had become unresponsive to Z11-16OH and Z11-16Ac, remaining responsive only to Z9-14Ald (Lee et al. 2006a). The conclusion that the "B-type" sensilla of *H. zea* are actually C-type, with some of the C-type OSNs having become totally or partially silenced in response to some ligands, makes sense when comparing *H. zea* sensilla to those of other *Helicoverpa* species. For instance, to date no B-type sensilla have been recorded from and characterized for either *H. armigera* or *H. assulta*; there are only A- and C-type sensilla in these two species (Berg et al. 1995, 2005, 2014; Wu et al. 2013; Jiang et al. 2014) (figure 21.6).

### A-, B-, and C-Type Olfactory Receptor Neurons Project to Stereotypical Antennal Lobe Glomeruli

For all of the heliothine spp. whose olfactory systems have been thoroughly examined, each species' major pheromone-component inputs from antennal OSNs tuned to the major component arborize in the largest glomerulus, the cumulus (Hansson et al. 1991), of the MGC (Berg et al. 1998, 2005, 2014; Lee et al. 2006a,b) (Cu in figures 21.4–21.6). The cumulus sits immediately at the base of the antenna, at the confluence of all the axons from the antenna entering the AL through the antennal nerve. For most species, the cumulus-arborizing OSNs are tuned to Z11-16Ald, but for *Helicoverpa assulta* they are tuned to Z9-16Ald (Berg et al. 2005) (figure 21.6). Minor component-tuned OSNs arborize in smaller companion glomeruli that usually are either dorsomedial (DM or DMP in figures 21.5 and 21.6), anteriomedial (AM or DMA in figures 21.5 and 21.6), or ventral (VM or V in figures 21.5 and 21.6) to the cumulus (Berg et al. 1998, 2005; Lee et al. 2006a,b).

It seems that the contributions of the activities within these small glomeruli vary between species with regard to the overall antagonistic balance of the signal that is integrated in protocerebral neuropils. Rather than considering that different glomeruli represent neuronal activities having a positive or negative odor "valence" (Knaden et al. 2012), we prefer to consider the activity, for instance of Z11-16Ac stimulation within the AM glomerulus of *Heliothis virescens*, as having extra gain (strength) compared to the same activity in the AM glomerulus of *H. subflexa*. Thus, although the activity in this glomerulus in *H. virescens* appears to have a negative valence due to its negative effect on behavior, and the activity in the corresponding glomerulus in *H. subflexa* seems to have a positive valence due to its positive behavioral effect, the antagonistic influence of Z11-16Ac in *H. virescens* might be viewed as being extra strong and creating an extra-heavy weight on the overall blend-balance between all the glomeruli and thus results in behavioral antagonism. Similarly, in *H. subflexa* the Z11-16OH-related activity within the AM glomerulus can be considered as exerting a moderate antagonism that serves as a perfect counterweight to balance the antagonistic strengths of the synaptic activities within the Cu and the DM glomeruli, thus contributing to balanced olfactory antagonism and male attraction to the blend. In both *H. virescens* and *H. subflexa*, inputs to the DM glomerulus by either Z9-14Ald from OSNs in the B-type sensilla of *H. virescens* or Z9-16Ald from the OSNs of the B-type sensilla of *H. subflexa* could be contributing an appropriate amount of antagonistic counterweight to the antagonistic inputs of other glomeruli in these species, resulting in balanced olfactory antagonism and male attraction (Baker 2008).

The PNs whose pheromone-related response profiles and neuroanatomies have been characterized for *H. virescens*, *H. subflexa* (figures 21.5A and 21.5B), *H. zea*, and *H. assulta* (figures 21.6A and 21.6C) have been found to project along linear, component-specific pathways, emerging from the same MGC glomeruli that are visited by OSNs tuned to the same major and minor pheromone components of these species that the PNs are tuned to (Vickers et al. 1998; Vickers and Christensen 2003; Zhao and Berg 2010). This theme of one-component-to-one-glomerulus-and-PN linear design is typical of heliothine pheromone olfactory systems (Vickers et al. 1998; Vickers and Christensen 2003; Zhao and Berg 2010), but it is not typical of moth sex pheromone olfactory systems in general (see Baker and Hansson, this volume).

ORs of other heliothine moth species have now begun to be functionalized, and data concerning their tuning profiles mirror OR homology with regard to their sequence similarity versus their ligand responsiveness. The orthologs HarmOR6 and HassOR6 have recently been expressed in *Xenopus* heterologous expression systems and provided a first glimpse of their tuning profiles in response to possible ligands (Liu et al. 2013; Jiang et al. 2014). HarmOR13 was found to be tuned as strongly to Z11-16Ald as its ortholog HvOR13 is, and it shares a 91% sequence identity with HvOR13 (Liu et al. 2013; Jiang et al. 2014). However, the *H. armigera* ortholog to HvOR6 (HarmOR6) in *H. virescens* has a distinctly different tuning profile, despite having an 88% sequence identity to HvOR6 (Liu et al. 2013). HarmOR6 responded equally well to Z9-16Ald and Z9-14Ald, whereas HvOR6 was specifically tuned to Z9-14Ald. This HarmOR6 profile fits well with the behavioral and female pheromone emission data showing that Z9-14Ald is a third pheromone component in this species (Zhang et al. 2012). This is consistent with the congruence between OR response profiles and their respective OSNs' tuning profiles in *H. virescens* and *H. subflexa*. There was increased male *H. armigera* behavioral responsiveness (trap catch) when both Z9-14Ald and Z9-16Ald were added into blends containing Z11-16Ald (Zhang et al. 2012).

Jiang et al. (2014) found that the *H. assulta* ortholog HassOR6 was optimally responsive to Z9-16OH, with lower, and equivalent, levels of reactivity to both Z9-16Ald and Z9-14Ald. For *H. assulta*, it is not clear why there should be equal responsiveness to Z9-16Ald and Z9-14Ald on this type of pheromone-component-tuned OSN (Wu et al. 2013), because Z9-14Ald is highly behaviorally antagonistic (Boo et al. 1995), as is Z9-16OH (Cork et al. 1992). However, Z9-16Ald is the most abundant of the two pheromone components and should predominate in the stimulation of HassOR6 on this OSN, Furthermore, because the behavioral antagonists Z9-14Ald and Z9-16OH are detected by HassOR16 on the other co-localized C-type OSN (Berg et al. 2005), it should not matter if either of these compounds also coincidentally stimulate HassOR6 on the large-spiking C-type OSN (Jiang et al. 2014). It is clear that work on the complete functionalization coupled with studies of in situ hybridization of these HassORs and HarmORs has only just begun. More research is needed to characterize fully their response profiles and to map them to their resident OSNs to determine patterns of co-localization in sensilla. Further work on these *H. armigera* and *H. assulta* ORs (along with OR11 and OR15 in all heliothines) also needs to be performed to reconcile some of these seemingly conflicting and confusing early results. It would be particularly helpful to have different research groups use the same panel of prospective pheromone-component odorants in their experiments.

## Courtship Pheromones

Given their relevance for agricultural applications, such as monitoring programs, much research has focused on female sex pheromones of the Heliothinae. However, male-produced courtship pheromones are also quite prevalent within this group and influence mating encounters between males and females. The majority of studies have focused on documenting the behavioral relevance and impact of such compounds on females (and in some instances males) during a courtship bout (Agee 1969; Teal and Tumlinson 1989; Cibrian-Tovar and Mitchell 1991; Hillier and Vickers 2004).

The pre-copulatory behavior of heliothines has been documented in several species, along with the impact of various factors on such behavior, including geographic region (Colvin et al. 1994), host plant distribution and moth age (Kvedaras et al. 2000), disruption of courtship with pheromone components or ultrasound (Callahan 1958; Agee 1969; Huang et al. 1997), and general ethology (Hendricks and Tumlinson 1974; Mitchell et al. 1974; Mitchell 1976; Teal et al. 1981b). Similar to many other Lepidoptera, male Heliothinae release volatile courtship pheromones from eversible structures (hairpencils) associated with the distal eighth abdominal segment (Birch et al. 1990). Hairpencil structures, closely associated with male claspers, disperse pheromones when everted during courtship. In different species, the effects of these pheromones have been proposed to either increase female receptivity to courting males, attract females to males, induce or arrest female calling, arrest female movement to facilitate copulation, or inhibit approach of competing males (Birch 1974; Baker 1981; Dong et al. 2005; Hillier and Vickers 2004, 2007; Jurenka and Rafaeli 2011). Overall, these compounds seem to modulate mating and courtship, offering a secondary pre-copulatory (prezygotic) barrier to mating mistakes beyond species-specific female pheromone production and male attraction.

A similar general pattern of courtship behavior has been documented in multiple heliothine species (*Heliothis virescens*, *H. subflexa*, *Helicoverpa zea*, and, to a lesser degree, *H. armigera* and *H. assulta*) (Agee 1969; Teal et al. 1981b, 1986; Cibrian-Tovar and Mitchell 1991; Hillier and Vickers 2004, 2011b; Ming et al. 2007). Behaviors may be separated in to pre-courtship behaviors (calling by females and activation or orientation by males) and courtship behaviors (hairpencil display, abdominal extension, clasping attempts) (Teal et al. 1989; Hillier and Vickers 2004, 2011b). Generally, a male will approach a stationary calling female from downwind. On arrival, the male will typically tap his antennae on her abdomen, near her ovipositor. The male then moves adjacent to the female and exposes his hairpencils, followed shortly thereafter by curling his abdomen toward the female and attempting clasping and copulation. The female typically either moves or flies away from the male, or she curls her own abdomen to accept the copulation attempt. There is considerable variation among and within species regarding the length of time spent conducting a given behavior within the sequence, and there are likely various modalities of feedback (visual, chemical, tactile, acoustic) that are likely used during courtship.

Teal et al. (1981b) documented the composition of such male-produced compounds from *H. virescens* as primarily being a combination of 16–18 chain-length acetates, alcohols, and carboxylic acids. Interestingly, these compounds share similarity to pheromone components of female Heliothinae, suggesting some common biosynthetic pathways, and perhaps functional homology in reception between sexes. However, to date, there has been no evidence of desaturase activity (commonly found in biosynthetic pathways for female-produced sex pheromones), from either the examination of male hairpencil gland extracts or hairpencil airborne emissions. Furthermore, gene transcripts and immunoassays have indicated the presence of PBAN in male *H. armigera* (Hirsch 1991; Rafaeli 2009; Ma et al. 1998). RNAi receptor studies in *H. armigera* also demonstrated that PBAN influences the production of male pheromone components, stimulating similar fatty acid biosynthetic pathways (Bober and Rafaeli 2010; for review, see Jurenka and Rafaeli 2011).

The composition, ratio, and concentration of male pheromone components can differ dramatically among species. For example, *H. subflexa* and *H. virescens* have opposite ratios of hexadecan-1-ol (16OH) to hexadecyl acetate (16Ac), and the concentration found in *H. subflexa* males is often substantially (20–100 times) lower than that for *H. virescens* males (Teal and Oostendorp 1995). Teal and Oostendorp (1995) also found that in hybrids and backcross progeny between *H. virescens* and *H. subflexa*, the ratio of 16OH/16Ac production was determined by dominant autosomal inheritance of *H. subflexa* alleles. Irrespective of cross direction, hairpencil pheromone titers of hybrid males were quantitatively and qualitatively similar to those of *H. subflexa* adults. In backcrosses between F1 hybrids and either *H. virescens* or *H. subflexa*, the composition and ratio of hairpencil components 16OH and 16Ac varied with cross direction, producing phenotypes similar to each species or intermediate phenotypes between species (Teal and Oostendorp 1993, 1995). Results suggest that the production of hairpencil pheromone by hybrids and backcrosses is under dominant, sex-linked control of alleles on the *H. subflexa* Z chromosome (Teal and Oostendorp 1993, 1995; Teal and Tumlinson 1997). Despite this, the morphology of hairpencils seems to be dictated by sex-linked inheritance from the male (Z) *H. virescens* sex chromosome (Teal and Oostendorp 1993).

Jacobson et al. (1984) reported quantities of 1 mg/male of Z9-14Ald in hairpencil extracts from *H. virescens*. Furthermore, this compound was proposed by Jacobson et al. (1984) to repel males during a courtship bout. However, subsequent studies on *H. virescens* and *H. subflexa* have not isolated Z9-14Ald from male glands, and further have not shown the presence of large quantities of any unsaturated hydrocarbons, suggesting that desaturases may not be present in the male pheromone glands of these species (Teal and Tumlinson 1989; Teal and Oostendorp 1993, 1995; Hillier and Vickers 2004). Finally, Huang et al. (1997) documented a similar complement of compounds (saturated 14–18 chain-length acetates, alcohols, and corresponding carboxylic acids) in the hairpencil gland extracts and headspace of *H. armigera*. Huang et al. (1997) also documented that the titers of hairpencil compounds peak 2–5 days after emergence and that titers are typically highest during scotophase.

The detection and behavioral effects of courtship pheromones have been investigated in multiple species, but perhaps most extensively in *H. virescens*. Male and female *H. virescens* share two short sensillar types that house olfactory receptor neurons that have been shown to selectively respond to both 16Ac and octadecyl acetate (18Ac), or to both 16OH and octadecan-1-ol (18OH) from among a selection of behaviorally relevant compounds (Hillier et al. 2006; Hillier and Vickers 2007). In *H. virescens*, OSNs from these sensilla selectively stain glomeruli near the entrance of the AL; the OSNs responding to acetate project to glomerulus 24 (adjacent and medial to the MGC for males), and to the possibly homologous glomerulus 59 in females (adjacent and medial to the large female glomeruli [LFG]) (Hillier and Vickers 2007; Hillier et al. 2007). OSNs responding to 16OH and 18OH project to glomerulus 41, adjacent and ventral to the LFG (Hillier and Vickers 2007). The proximity of these glomeruli to the MGC, along with similarity in position in males and females, suggests that there may be a "map" in the organization of the AL based upon molecular structure. Another option is that there is further behavioral segregation of pheromone and non-pheromone odor processing with processing of male hairpencil components and female sex pheromone

components in a similar region, thus representing a co-localization of conspecific odorant processing, irrespective of the producing sex.

In female *H. virescens*, hairpencil compounds induce quiescence during a courtship bout, thereby increasing male success in mating by preventing females from moving away (and increasing female receptivity) (Teal et al. 1981b; Hillier and Vickers 2004). Furthermore, the efficacy of hairpencil extracts to induce quiescence in females is species specific, potentially linked to differential ratios of acetates and alcohols in the hairpencil composition of related species. Consequently, females can distinguish conspecific and heterospecific males by hairpencil composition, an important feature, as there are costs associated with mating mistakes (e.g., irreversible locking of genitalia or inviable male progeny) (Hardwick 1965; Goodpasture et al. 1980; Stadelbacher et al. 1983). It is possible that the initial choosiness by females for signals indicative of conspecific males may be the initial step in subsequent runaway female choice sexual selection. Females will then become even more discriminating and proceed to evaluate the quality and reproductive fitness of conspecific males according to the quality and quantity of their hairpencil volatiles, which will lead to increasingly amplified and specific courtship pheromone signals (Birch et al. 1990; Hillier and Vickers 2004, 2011b).

Moreover, a series of experiments comparing the mating behavior and success of interspecific mating trials between *H. subflexa* and *H. virescens* confirmed that hairpencil composition differentially influences courtship success between these species (Hillier and Vickers 2011b). Hairpencil-ablated males were more successful mating with the opposing species, provided females were stimulated with an artificial odor source with male conspecific pheromone. This effect was much more pronounced in trials involving male *H. virescens*. *H. subflexa* males were significantly more successful clasping and mating with both *H. virescens* and *H. subflexa* females in the absence of hairpencils, suggesting that there are fundamental differences in each species' requirements for these compounds to increase female receptivity or quiescence.

Field-cage studies with *H. virescens* suggest that female calling (and associated pheromone release) are inhibited by exposure to 50-male-equivalents of hairpencil extracts or exposure to 2-day-old virgin males (Hendricks and Shaver 1975). In *H. armigera*, an opposing effect has been found, as onset of female calling behavior may also be slightly influenced by saturated acids found within male hairpencil glands (percentage of calling females and duration of calling do not appear to be influenced) (Dong et al. 2005).

Wind-tunnel studies with male *H. virescens* also suggest 16Ac and 18Ac inhibit upwind male flight toward a synthetic female sex pheromone blend (Hillier and Vickers 2007). In *H. armigera*, saturated alcohols have also been tested as potential inhibitors of male approach, but they did not significantly influence male behavior (whereas addition of 5% Z11-16OH to an attractive blend inhibited approach) (Huang et al. 1997). Induction of quiescence in females and inhibition of approach by conspecific males may facilitate copulation and reduce competition from other suitors, ultimately increasing mating success. However, it remains unclear what the costs are for males to continue to orient upwind and attempt mating, despite the presence of conspecific males releasing courtship pheromone. Further research is required to reveal the potential behavioral roles of these compounds in courtship behavior.

## Conclusions

The role of pheromones in male and female moth reproductive behaviors has doubtlessly been most comprehensively documented in the heliothine moths. Well-studied species of the genera *Heliothis* and *Helicoverpa* share biosynthetic pathways that produce similar compounds, but they exhibit considerable variation in pheromone composition between allopatric and sympatric populations around the world. In particular, a discrete balance is maintained between female pheromone production of key conspecific attractant blends (often including heterospecific antagonists) and male attraction to blends containing key compounds within a critical range of ratios. Field studies, laboratory behavioral tests, chemical analyses, and hybridization and crossing studies have provided a wealth of information on the probable evolution of pheromone communication in this group. Recent advances in neurophysiology, genetic analyses, and molecular biology offer great promise to continue to expand on and unlock key mechanisms that drive selection and diversification of these pheromone chemical signals and receptors.

## References Cited


Agee, H. R. 1969. Mating behavior of bollworm moths. *Annals of the Entomological Society of America* 62:1120–1122.

Almaas, T. J., and H. Mustaparta. 1990. Pheromone reception in tobacco budworm moth, *Heliothis virescens*. *Journal of Chemical Ecology* 16:1331–1347.

Almaas, T. J., and H. Mustaparta. 1991. *Heliothis virescens*: response characteristics of receptor neurons in sensilla trichodea type 1 and type 2. *Journal of Chemical Ecology* 17:953–972.

Almaas, T. J., T. A. Christensen, and H. Mustaparta. 1991. Chemical communication in heliothine moths. I. Antennal receptor neurons encode several features of intra-and interspecific odorants in the male corn earworm moth *Helicoverpa zea*. *Journal of Comparative Physiology A* 69:249–258.

Baker, T. C. 2008. Balanced olfactory antagonism as a concept for understanding evolutionary shifts in moth sex pheromone blends. *Journal of Chemical Ecology* 34:971–981.

Baker, T. C. 2009. Nearest neural neighbors: moth sex pheromone receptors HR11 and HR13. *Chemical Senses* 34:465–468.

Baker, T. C. and R. T. Cardé. 1979. Analysis of pheromone-mediated behaviors in male *Grapholitha molesta*, the oriental fruit moth (Lepidoptera: Tortricidae). *Environmental Entomology* 8:956–968.

Baker, T. C., Nishida, R., and W. L. Roelofs. 1981. Close-range attraction of female oriental fruit moths to herbal scent of male hairpencils. *Science* 214:1359–1361.

Baker, T. C., A. A. Cossé, and J. L. Todd. 1998a. Behavioral antagonism in the moth *Helicoverpa zea* in response to pheromone blends of three sympatric heliothine moth species is explained by one type of antennal neuron. *Annals of the New York Academy of Sciences* 855:511–513.

Baker, T. C., H. Y. Fadamiro, and A. A. Cossé. 1998b. Moth uses fine tuning for odour resolution. *Nature* 393:530.

Baker, T. C., A. A. Cossé, S. G. Lee, J. L. Todd, C. Quero, and N. J. Vickers. 2004. A comparison of responses from olfactory receptor neurons of *Heliothis subflexa* and *Heliothis virescens* to components of their sex pheromone. *Journal of Comparative Physiology A* 190:155–165.

Baker, T. C., C. Quero, S. A. Ochieng', and N. J. Vickers. 2006. Inheritance of olfactory preferences II. Olfactory receptor neuron responses from *Heliothis subflexa* × *Heliothis virescens* hybrid male moths. *Brain, Behavior and Evolution* 68:75–89.

Behere, G. T., W. T. Tay, D. A. Russell, D. G. Heckel, B. R. Appleton, K. R. Kranthi, and P. Batterham. 2007. Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea*. *BMC Evolutionary Biology* 7:117.

Berg, B.G., and H. Mustaparta. 1995. The significance of major pheromone components and interspecific signals as expressed by receptor neurons in the oriental tobacco budworm moth, *Helicoverpa assulta*. *Journal of Comparative Physiology A* 177:683–694.

Berg, B.G., J.H. Tumlinson, and H. Mustaparta. 1995. Chemical communication in heliothine moths. IV. Receptor neuron responses to pheromone compounds and formate analogues in the male tobacco budworm moth *Heliothis virescens*. *Journal of Comparative Physiology A* 177:527–534.

Berg, B.G., T.J. Almaas, J.G. Bjaalie, and H. Mustaparta. 1998. The macroglomerular complex of the antennal lobe in the tobacco budworm moth *Heliothis virescens*: specified subdivision in four compartments according to information about biologically significant compounds. *Journal of Comparative Physiology A* 183:669–682.

Berg, B.G., C.G. Galizia, R. Brandt, and H. Mustaparta. 2002. Digital atlases of the antennal lobe in two species of tobacco budworm moths, the oriental *Helicoverpa assulta* (male) and the American *Heliothis virescens* (male and female). *Journal of Comparative Neurology* 446:123–134.

Berg, B.G., T.J. Almaas, J.G. Bjaalie, and H. Mustaparta. 2005. Projections of male-specific receptor neurons in the antennal lobe of the oriental tobacco budworm moth, *Helicoverpa assulta:* a unique glomerular organization among related species. *Journal of Comparative Neurology* 486:209–220.

Berg, B.G., X.-C. Zhao, and G. Wang. 2014. Processing of pheromone information in related species of heliothine moths. *Insects* 5:742–761. doi:10.3390/insects5040742.

Birch, M.C. 1974. Aphrodisiac pheromones in insects. Pp. 115–134. In M.C. Birch, ed.. *Pheromones*. Amsterdam: North Holland.

Birch, M.C., G.M. Poppy, and T.C. Baker. 1990. Scents and eversible scent structures of male moths. *Annual Review of Entomology* 35:25–58.

Bober, R., and Rafaeli, A. 2010. Gene-silencing reveals the functional significance of pheromone biosynthesis activating neuropeptide receptor (PBAN-R) in a male moth. *Proceedings of the National Academy of Sciences of the United States of America* 107:16858–16862.

Boo, K.S., K.C. Park, D.R. Hall, A. Cork, B.G. Berg, and H. Mustaparta. 1995. (Z)-9-Tetradecenal: a potent inhibitor of pheromone-mediated communication in the oriental tobacco budworm moth, *Helicoverpa assulta*. *Journal of Comparative Physiology A* 177:695–699.

Butlin, R. 1987. Species, speciation, and reinforcement. *The American Naturalist* 130:461–464.

Byers, J. R., and D. L. Struble. 1987. Monitoring population levels of eight species of noctuids with sex-attractant traps in southern Alberta, 1978–1983: specificity of attractants and effect of target species abundance. *The Canadian Entomologist* 119:541–556.

Callahan, P.S. 1958. Behavior of the imago of the corn earworm, *Heliothis zea* (Boddie), with special reference to emergence and reproduction. *Annals of the Entomological Society of America* 51:271–283.

Cho, S., A. Mitchell, C. Mitter, J. Regier, M. Matthews, and R.O.N. Robertson. 2008. Molecular phylogenetics of heliothine moths (Lepidoptera: Noctuidae: Heliothinae), with comments on the evolution of host range and pest status. *Systematic Entomology* 33:581–594.

Choi, M. Y., Fuerst, E. J., Rafaeli, A., and R. Jurenka. 2003. Identification of a G protein-coupled receptor for pheromone biosynthesis activating neuropeptide from pheromone glands of the moth *Helicoverpa zea*. *Proceedings of the National Academy of Sciences of the United States of America* 100:9721–9726.

Choi, M.Y., A. Groot, and R.A. Jurenka. 2005. Pheromone biosynthetic pathways in the moths *Heliothis subflexa* and *Heliothis virescens*. *Archives of Insect Biochemistry and Physiology* 59:53–58.

Christensen, T.A., S.C. Geofrion, and J.G. Hildebrand. 1990. Physiology of interspecific chemical communication in *Heliothis* moths. *Physiological Entomology* 15:275–283.

Christensen, T.A., H. Itagaki, P.E. Teal, R.D. Jasensky, J.H. Tumlinson, and J.G. Hildebrand. 1991. Innervation and neural regulation of the sex pheromone gland in female *Heliothis* moths. *Proceedings of the National Academy of Sciences of the United States of America* 88:4971–4975.

Christensen, T.A., H.K. Lehman, P.E.A. Teal, H. Itagaki, J.H. Tumlinson, and J.G. Hildebrand. 1992. Diel changes in the presence and physiological actions of octopamine in the female sex-pheromone glands of heliothine moths. *Insect Biochemistry and Molecular Biology* 22:841–849.

Christensen, T.A., H. Mustaparta, and J.G. Hildebrand. 1995. Chemical communication in heliothine moths. VI: Parallel pathways for information processing in the macroglomerular complex of the male tobacco budworm moth *Heliothis virescens*. *Journal of Comparative Physiology A* 177:545–557.

Cibrian-Tovar, J., and E.R. Mitchell. 1991. Courtship behavior of *Heliothis subflexa* (Gn.) (Lepidoptera: Noctuidae) and associated backcross insects obtained from hybridization with *H. virescens* (F.). *Environmental Entomology* 20:419–426.

Colvin, J., R.J. Cooter, and S. Patel. 1994. Laboratory mating behavior and compatibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) originating from different geographical regions. *Journal of Economic Entomology* 87:1502–1506.

Cork, A., and E.A. Lobos. 2003. Female sex pheromone components of *Helicoverpa gelotopoeon*: first heliothine pheromone without (Z)-11-hexadecenal. *Entomologia Experimentalis et Applicata* 107:201–206.

Cork, A., K.S. Boo, E. Dunkelblum, D.R. Hall, K. Jee-Rajunga, M. Kehat, E. Kong Jie, K.C. Park, P. Tepgidagarn, and L. Xun. 1992. Female sex pheromone of oriental tobacco budworm, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae): identification and field testing. *Journal of Chemical Ecology* 18:403–418.

Cossé, A.A., J.L. Todd, and T.C. Baker. 1998. Neurons discovered in male *Helicoverpa zea* antennae that correlate with pheromone-mediated attraction and interspecific antagonism. *Journal of Comparative Physiology A* 182:585–594.

Davis, M.T., V.N. Vakharia, J. Henry, T.G. Kempe, and A.K. Raina. 1992. Molecular cloning of the pheromone biosynthesis-activating neuropeptide in *Helicoverpa zea*. *Proceedings of the National Academy of Sciences of the United States of America* 89:142–146.

Descoins, C., Silvain, J. F., Lalanne-Cassou, B., and H. Cheron. 1988. Monitoring of crop pests by sexual trapping of males in Guadeloupe and Guyana. *Agriculture, Ecosystems & Environment* 21:53–56.

Dobritsa, A.A., W. Van der Goes van Naters, C.G. Warr, R.A. Steinbrecht, and J.R. Carlson. 2003. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* 37:827–841.

Domingue, M.J., C.J. Musto, C.E. Linn, Jr., W.L. Roelofs, and T.C. Baker. 2007. Evidence of olfactory antagonistic inhibition as a facilitator of evolutionary shifts in pheromone blend usage in *Ostrinia* spp. (Lepidoptera: Crambidae). *Journal of Insect Physiology* 53:488–496.

Dong, W.X., B.Y. Han, and J.W. Du. 2005. Inhibiting the sexual behavior of female cotton bollworm *Helicoverpa armigera*. *Journal of Insect Behavior* 18:453–463.

Dunkelblum, E., and M. Kehat. 1989. Female sex pheromone components of *Heliothis peltigera* (Lepidoptera: Noctuidae). Chemical identification from gland extracts and male response. *Journal of Chemical Ecology* 15:2233–2245.

Dunkelblum, E., Gothilf, S., and M. Kehat. 1980. Identification of the sex pheromone of the cotton bollworm, *Heliothis armigera*, in Israel. *Phytoparasitica* 8:209–211.

Eltahlawy, H., J.S. Buckner, and S.P. Foster. 2007. Evidence for two-step regulation of pheromone biosynthesis by the pheromone biosynthesis-activating neuropeptide in the moth *Heliothis virescens*. *Archives of Insect Biochemistry and Physiology* 64:120–130.

Fadamiro, H.Y., and T.C. Baker. 1997. *Helicoverpa zea* males (Lepidoptera: Noctuidae) respond to the intermittent fine structure of their sex pheromone plume and an antagonist in a flight tunnel. *Physiological Entomology* 22:316–324.

Fadamiro, H.Y., A.A. Cossé, and T.C. Baker. 1999. Fine-scale resolution of closely spaced pheromone and antagonist filaments by flying male *Helicoverpa zea*. *Journal of Comparative Physiology A* 185:131–141.

Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* 34:17–52.

Foster, S.P., and K.G. Anderson. 2012. Synthetic rates of key stored fatty acids in the biosynthesis of sex pheromone in the moth *Heliothis virescens*. *Insect Biochemistry and Molecular Biology* 42:865–872.

Fujii, T., T. Fujii, S. Namiki, H. Abe, T. Sakurai, A. Ohnuma, R. Kanzaki, S. Katsuma, Y. Ishikawa, and T. Shimada. 2011. Sex-linked transcription factor involved in a shift of sex-pheromone preference in the silkmoth *Bombyx mori*. *Proceedings of the*

*National Academy of Sciences of the United States of America* 108:18038–18043.
Goldman, A.L., W. Van der Goes van Naters, D. Lessing, C.G. Warr, and J.R. Carlson. 2005. Coexpression of two functional odor receptors in one neuron. *Neuron* 45:661–666.
Goodpasture, C., R.D. Richard, D. Martin, and M. Laster. 1980. Sperm cell abnormalities in progeny from interspecific crosses between *Heliothis virescens* and *H. subflexa*. *Annals of the Entomological Society of America* 73:529–532.
Gothilf, S., Kehat, M., Dunkelblum, E., and M. Jacobson. 1979. Efficacy of (Z)-11-hexadecenal and (Z)-11-tetradecenal as sex attractants for *Heliothis armigera* on two different dispensers. *Journal of Economic Entomology* 72:718–720.
Gould, F., M. Estock, N.K. Hillier, B. Powell, A.T. Groot, C.M. Ward, J.L. Emerson, C. Schal, and N.J. Vickers. 2010. Sexual isolation of male moths explained by a single pheromone response QTL containing four receptor genes. *Proceedings of the National Academy of Sciences of the United States of America* 107:8660–8665.
Große-Wilde, E., T. Gohl, E. Bouché, H. Breer, and J. Krieger. 2007. Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. *European Journal of Neuroscience* 25:2364–2373.
Groot, A.T., C. Ward, J. Wang, A. Pokrzywa, J. O'Brien, J. Bennett, R.G. Santangelo, C. Schal, and F. Gould. 2004. Introgressing pheromone QTL between species: towards an evolutionary understanding of differentiation in sexual communication. *Journal of Chemical Ecology* 30:2495–2514.
Groot, A.T., Y. Fan, C. Brownie, R.A. Jurenka, F. Gould, and C. Schal. 2005. Effect of PBAN on pheromone production by mated *Heliothis virescens* and *Heliothis subflexa* females. *Journal of Chemical Ecology* 31:15–28.
Groot, A.T., J.L. Horovitz, J. Hamilton, R.G. Santangelo, C. Schal, and F. Gould. 2006. Experimental evidence for interspecific directional selection on moth pheromone communication. *Proceedings of the National Academy of Sciences of the United States of America* 103:5858–5863.
Groot, A.T., R.G. Santangelo, E. Ricci, C. Brownie, F. Gould, and C. Schal. 2007. Differential attraction of *Heliothis subflexa* males to synthetic pheromone lures in eastern US and western Mexico. *Journal of Chemical Ecology* 33:353–368.
Groot, A.T., M.L. Estock, J.L. Horovitz, J. Hamilton, R.G. Santangelo, C. Schal, and F. Gould. 2009a. QTL analysis of sex pheromone blend differences between two closely related moths: insights into divergence in biosynthetic pathways. *Insect Biochemistry and Molecular Biology* 39:568–577.
Groot, A.T., O. Inglis, S. Bowdridge, R.G. Santangelo, C. Blanco, J.D. López, Jr., A.T. Vargas, F. Gould, and C. Schal. 2009b. Geographic and temporal variation in moth chemical communication. *Evolution* 63:1987–2003.
Groot, A.T., C.A. Blanco, A. Claßen, O. Inglis, R.G. Santangelo, J. Lopez, D.G. Heckel, and C. Schal. 2010a. Variation in sexual communication of the tobacco budworm, *Heliothis virescens*. *Southwestern Entomologist* 35:367–372.
Groot, A.T., A. Classen, H. Staudacher, C. Schal, and D.G. Heckel. 2010b. Phenotypic plasticity in sexual communication signal of a noctuid moth. *Journal of Evolutionary Biology* 23:2731–2738.
Groot, A.T., H. Staudacher, A. Barthel, O. Inglis, G. Schöfl, R.G. Santangelo, S. Gebauer-Jung et al. 2013. One quantitative trait locus for intra- and interspecific variation in a sex pheromone. *Molecular Ecology* 22:1065–1080.
Hagström, A.K., M.A. Liénard, A.T. Groot, E. Hedenström, and C. Löfstedt. 2012. Semi-selective fatty acyl reductases from four heliothine moths influence the specific pheromone composition. *PLOS ONE* 7:e37230.
Hallem, E.A., and J.R. Carlson. 2004. The odor coding system of Drosophila. *Trends in Genetics* 20:453–459.
Hallem, E.A., and J.R. Carlson. 2006. Coding of odors by a receptor repertoire. *Cell* 125:143–160.
Hallem, E.A., M.G. Ho, and J.R. Carlson. 2004. The molecular basis of odor coding in the *Drosophila* antenna. *Cell* 117:965–979.
Hansson, B.S., T.A. Christensen, and J.G. Hildebrand. 1991. Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *Journal of Comparative Neurology* 312:264–278.
Hardwick, D.F. 1958. Taxonomy, life history, and habits of the elliptoid-eyed species of *Schinia* (Lepidoptera: Noctuidae), with notes on the Heliothidinae. *Memoirs of the Entomological Society of Canada* 90:5–116.
Hardwick, D.F. 1965. The corn earworm complex. *Memoirs of the Entomological Society of Canada* 97(Suppl 40):5–247.
Hardwick, D.F. 1970. The biological status of *Heliothis stombleri*. *Canadian Entomologist* 102:339–341.
Hartstack, A.W., J.A. Witz, and D.R. Buck. 1979. Moth traps for the tobacco budworm. *Journal of Economic Entomology* 72:519–522.
Heath, R.R., E.R. Mitchell, and J.C. Tovar. 1990. Effect of release rate and ratio of (Z)-11-hexadecen-1-ol from synthetic pheromone blends on trap capture of *Heliothis subflexa* (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 16:1259–1268.
Hendricks, D.E., and T.N. Shaver. 1975. Tobacco budworm: male pheromone suppressed emission of sex pheromone by the female. *Environmental Entomology* 4:555–558.
Hendricks, D.E., and J.H. Tumlinson. 1974. A field cage bioassay system for testing candidate sex pheromones of the tobacco budworm. *Annals of the Entomological Society of America* 67:547–552.
Hendricks, D.E., B.A. Leonardt, and T.N. Shaver. 1989. Development of optimized blends of two sex pheromone components impregnated in PVC dispensers for tobacco budworm bait. *Southwestern Entomologist* 14:17–25.
Hillier, N.K., and N.J. Vickers. 2004. The role of heliothine hairpencil compounds in female *Heliothis virescens* (Lepidoptera: Noctuidae) behavior and mate acceptance. *Chemical Senses* 29:499–511.
Hillier, N.K., and N.J. Vickers. 2007. Physiology and antennal lobe projections of olfactory receptor neurons from sexually isomorphic sensilla on male *Heliothis virescens*. *Journal of Comparative Physiology A* 193:649–663.
Hillier, N.K., and N.J. Vickers. 2011a. Mixture interactions in moth olfactory physiology: examining the effects of odorant mixture, concentration, distal stimulation, and antennal nerve transection on sensillar responses. *Chemical Senses* 36:93–108.
Hillier, N.K., and N.J. Vickers. 2011b. Hairpencil volatiles influence interspecific courtship and mating between two related moth species. *Journal of Chemical Ecology* 37:1127–1136.
Hillier, N.K., C. Kleineidam, C., and N.J. Vickers. 2006. Physiology and glomerular projections of olfactory receptor neurons on the antenna of female *Heliothis virescens* (Lepidoptera: Noctuidae) responsive to behaviorally relevant odors. *Journal of Comparative Physiology A* 192:199–219.
Hillier, N.K., D. Kelly, and N.J. Vickers. 2007. A specific male olfactory sensillum detects behaviorally antagonistic hairpencil odorants. *Journal of Insect Science* 7:4.
Huang, Y., S. Xu, X. Tang, Z. Zhao, and J. Du. 1997. Male orientation inhibitor of cotton bollworm: inhibitory effects of alcohols in wind-tunnel and in the field. *Insect Science* 4:173–181.
Jacobson, M., V.E. Adler, and A.H. Baumhover. 1984. A male tobacco budworm pheromone inhibitory to courtship. *Journal of Environmental Science and Health A* 19:469–476.
Jiang, X.-J., H. Guo, C. Di, S. Yu, L. Zhu, L.-Q. Huang, and C.-Z. Wang. 2014. Sequence similarity and functional comparisons of pheromone receptor orthologs in two closely related *Helicoverpa* species. *Insect Biochemistry and Molecular Biology* 48:63–74.
Jurenka, R.A. 2003. Biochemistry of female moth sex pheromones. Pp. 53–80. In G.J. Blomquist and R. Vogt, eds. *Insect Pheromone Biochemistry and Molecular Biology*. Amsterdam: Elsevier.
Jurenka, R., and A. Rafaeli. 2011. Regulatory role of PBAN in sex pheromone biosynthesis of heliothine moths. *Frontiers in Endocrinology* 2:46.
Jurenka, R.A., E. Jacquin, and W.L. Roelofs. 1991. Control of the pheromone biosynthetic pathway in *Helicoverpa zea* by the pheromone biosynthesis activating neuropeptide. *Archives of Insect Biochemistry and Physiology* 17:81–91.
Kaae, R.S., H.H. Shorey, S.U. McFarland, and L.K. Gaston. 1973. Sex pheromones of Lepidoptera. XXXVII. Role of sex pheromones and other factors in reproductive isolation among ten species of Noctuidae. *Annals of the Entomological Society of America* 66:444–448.
Kakizaki, M., and H. Sugie. 2003. Sex pheromone of the flax budworm, *Heliothis maritima adaucta* Butler (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* 38:73–78.
Kehat, M., and E. Dunkelblum. 1990. Behavioral responses of male *Heliothis armigera* (Lepidoptera: Noctuidae) moths in a flight

tunnel to combinations of components identified from female sex pheromone glands. *Journal of Insect Behavior* 3:75–83.
Kehat, M., S. Gothilf, E. Dunkelblum, and S. Greenberg. 1980. Field evaluation of female sex pheromone components of the cotton bollworm, *Heliothis armigera*. *Entomologia Experimentalis et Applicata* 27:188–193.
Kim, Y.J., R.J. Nachman, K. Aimanova, S. Gill, and M.E. Adams. 2008. The pheromone biosynthesis activating neuropeptide (PBAN) receptor of *Heliothis virescens*: identification, functional expression, and structure–activity relationships of ligand analogs. *Peptides* 29:268–275.
Kingan, T.G., M.B. Blackburn, and A.K. Raina. 1992. The distribution of pheromone-biosynthesis-activating neuropeptide (PBAN) immunoreactivity in the central nervous system of the corn earworm moth, *Helicoverpa zea*. *Cell and Tissue Research* 270:229–240.
Kingan, T.G., P.A. Thomas-Laemont, and A.K. Raina. 1993. Male accessory gland factors elicit change from 'virgin' to 'mated' behaviour in the female corn earworm moth *Helicoverpa zea*. *Journal of Experimental Biology* 183:61–76.
Klun, J.A., B.A. Bierl-Leonhardt, J.R. Plimmer, A.N. Sparks, M. Primiani, O.L. Chapman, and G.H. Lee. 1980a. Sex pheromone chemistry of the female tobacco budworm moth, *Heliothis virescens*. *Journal of Chemical Ecology* 6:177–183.
Klun, J. A., Plimmer, J. R., Bierl-Leonhardt, B. A., Sparks, A. N., Primiani, M., Chapman, O. L., Lee, G.H., and G. Lepone. 1980b. Sex pheromone chemistry of female corn earworm moth, *Heliothis zea*. *Journal of Chemical Ecology* 6:165–175.
Klun, J. A., Leonhardt, B. A., Lopez, J. D., and L. E. Lachance. 1982. Female *Heliothis subflexa* (Lepidoptera: Noctuidae) sex pheromone: chemistry and congeneric comparisons. *Environmental Entomology* 11:1084–1090.
Knaden, M., A. Strutz, J. Ahsan, S. Sachse, and B.S. Hansson. 2012. Spatial representation of odorant valence in an insect brain. *Cell Reports* 1:392–399.
Koutroumpa, F.A., Z. Kárpáti, C. Monsempes, S.R. Hill, B.S. Hansson, E. Jacquin-Joly, J. Krieger, and T. Dekker. (2014) Shifts in sensory neuron identity parallel differences in pheromone preference in the European corn borer. *Frontiers in Ecology and Evolution* 2:65. doi:10.3389/fevo.2014.00065.
Krieger, J., E. Grosse-Wilde, T. Gohl, Y.M.E. Dewer, K. Raming, and H. Breer. 2004. Genes encoding candidate pheromone receptors in a moth (*Heliothis virescens*). *Proceedings of the National Academy of Sciences of the United States of America* 101:11845–11850.
Krieger, J., I. Gondesen, M. Forstner, T. Gohl, Y. Dewer, and H. Breer. 2009. HR11 and HR13 receptor-expressing neurons are housed together in pheromone-responsive sensilla trichodea of male *Heliothis virescens*. *Chemical Senses* 34:469–477.
Kvedaras, O.L., P.C. Gregg, and A.P. Del Socorro. 2000. Techniques used to determine the mating behaviour of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in relation to host plants. *Australian Journal of Entomology* 39:188–194.
Kvedaras, O.L., A.P. Del Socorro, and P.C. Gregg. 2007. Effects of phenylacetaldehyde and (Z)-3-hexenyl acetate on male response to synthetic sex pheromone in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology* 46:224–230.
Lambert, D.M., B. Michaux, and C.S. White. 1987. Are species self-defining? *Systematic Zoology* 36:196–205.
Landolt, P.J., C.L. Smithhisler, R.S. Zack, and L. Camelo. 2006. Attraction of *Heliothis belladonna* (Henry and Edwards) to the sex pheromone of the corn earworm moth, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). *Journal of the Kansas Entomological Society* 79:303–308.
Laster, M.L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa*. *Environmental Entomology* 1:682–687.
Laster, M. L., Martin, D. F., and D. W. Parvin, Jr. 1976. Potential for suppressing tobacco budworm (Lepidoptera: Noctuidae) by genetic sterilization. *Technical Bulletin of the Mississippi State University Agriculture and Forestry Experimental Station*.
Leary, G.P., J.E. Allen, P.L. Bunger, J.B. Luginbill, C.E. Linn, Jr., I.E. Macallister, and K.W. Wanner. 2012. Single mutation to a sex pheromone receptor provides adaptive specificity between closely related moth species. *Proceedings of the National Academy of Sciences of the United States of America* 109:14081–14086.
Lee, S.G., M.A. Carlsson, B.S. Hansson, J.T. Todd, and T.C. Baker. 2006a. Antennal lobe projection destinations of *Helicoverpa zea* male olfactory receptor neurons responsive to heliothine sex pheromone components. *Journal of Comparative Physiology A* 192:351–363.
Lee, S.G., N.J. Vickers, and T.C. Baker. 2006b. Glomerular targets of *Heliothis subflexa* male olfactory receptor neurons housed within long trichoid sensilla. *Chemical Senses* 31:821–834.
Lelito, J.P., A.J. Myrick, and T.C. Baker. 2008. Interspecific pheromone-plume interference among sympatric heliothine moths: a wind tunnel test using live, calling females. *Journal of Chemical Ecology* 34:725–733.
Liu, Y., C. Liu, K. Lin, and G. Wang. 2013. Functional specificity of sex pheromone receptors in the cotton bollworm *Helicoverpa armigera*. *PLOS ONE* 8:e62094.
Löfstedt, C., J. Löfqvist, B.S. Lanne, J.N.C. van der Pers, and B.S. Hansson. 1986. Pheromone dialects in European turnip moths *Agrotis segetum*. *Oikos* 46:250–257.
Ma, P.W.K., W.L. Roelofs, and R.A. Jurenka. 1996. Characterization of PBAN and PBAN-encoding gene neuropeptides in the central nervous system of the corn earworm moth, *Helicoverpa zea*. *Journal of Insect Physiology* 42:257–266.
Ma, P.W., D.C. Knipple, and W.L. Roelofs. 1998. Expression of a gene that encodes pheromone biosynthesis activating neuropeptide in the central nervous system of corn earworm, *Helicoverpa zea*. *Insect Biochemistry and Molecular Biology* 28:373–385.
Matthews, M. 1999. *Heliothine Moths of Australia: A Guide to Pest Bollworms and Related Noctuid Groups*. Melbourne: CSIRO.
Mbata, G.N., and S.B. Ramaswamy. 1990. Rhythmicity of sex pheromone content in female *Heliothis virescens*: impact of mating. *Physiological Entomology* 15:423–432.
McElfresh, J.S., and J.G. Millar. 1999. Geographic variation in sex pheromone blend of *Hemileuca electra* from southern California. *Journal of Chemical Ecology* 25:2505–2525.
McElfresh, J.S., and J.G. Millar. 2001. Geographic variation in the pheromone system of the saturniid moth *Hemileuca eglanterina*. *Ecology* 82:3505–3518.
Ming, Q.L., Y.H. Yan, and C.Z. Wang. 2007. Mechanisms of premating isolation between *Helicoverpa armigera* (Hübner) and *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* 53:170–178.
Mitchell, E. R. 1982. Attraction of *Schinia mitis* males to southern armyworm females. *The Florida Entomologist* 65:291.
Mitchell, E. R., Tumlinson, J. H., and A. H. Baumhover. 1978. *Heliothis virescens*: attraction of males to blends of (Z)-9-tetradecen-1-ol formate and (Z)-9-tetradecenal. *Journal of Chemical Ecology* 4:709–716.
Mitter, C., R.W. Poole, and M. Matthews. 1993. Biosystematics of the Heliothinae (Lepidoptera: Noctuidae). *Annual Review of Entomology* 38:207–225.
Nesbitt, B.F., P.S. Beevor, D.R. Hall, and R. Lester. 1979. Female sex pheromone components of the cotton bollworm, *Heliothis armigera*. *Journal of Insect Physiology* 25:535–541.
Park, K.C., A. Cork, K.S. Boo, and D.R. Hall. 1994. Biological activity of female sex pheromone of the oriental tobacco budworm, *Helicoverpa assulta* (Guenee) (Lepidoptera: Noctuidae): electroantennography, wind tunnel observation and field trapping. *Korean Journal of Applied Entomology* 33:26–32.
Park, K.C., A. Cork, and K.S. Boo. 1996. Intrapopulational changes in sex pheromone composition during scotophase in oriental tobacco budworm, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 22:1201–1210.
Paterson, H.E.H. 1985. The recognition concept of species. Pp. 21–29. In E.S. Vrba, ed. *Species and Speciation. Transvaal Museum Monograph No. 4*. Pretoria, South Africa: Transvaal Museum.
Phelan, P.L. 1992. Evolution of sex pheromones and the role of asymmetric tracking. Pp. 265–314. In B.D. Roitberg and M.B. Isman, eds. *Insect Chemical Ecology*. New York: Chapman & Hall.
Piccardi, P., A. Capizzi, G. Cassani, P. Spinelli, E. Arsura, and P. Massardo. 1977. A sex pheromone component of the Old World bollworm *Heliothis armigera*. *Journal of Insect Physiology* 23:1443–1445.
Pope, M.M., L.K. Gaston, and T.C. Baker. 1982. Composition, quantification, and periodicity of sex pheromone gland volatiles from individual *Heliothis virescens* females. *Journal of Chemical Ecology* 8:1043–1055.

Pope, M. M., L. K. Gaston, and T. C. Baker. 1984. Composition, quantification, and periodicity of sex pheromone volatiles from individual *Heliothis zea* females. *Journal of Insect Physiology* 30:943–945.

Proshold, F. I., and L. E. LaChance. 1974. Analysis of sterility in hybrids from interspecific crosses between *Heliothis virescens* and *H. subflexa*. *Annals of the Entomological Society of America* 67:445–449.

Quero, C., and T. C. Baker. 1999. Antagonistic effect of (*Z*)-11-hexadecen-1-ol on the pheromone-mediated flight of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). *Journal of Insect Behavior* 12:701–709.

Quero, C., H. Y. Fadamiro, and T. C. Baker. 2001. Responses of male *Helicoverpa zea* to single pulses of sex pheromone and behavioural antagonist. *Physiological Entomology* 26:106–115.

Rafaeli, A. 2009. Pheromone biosynthesis activating neuropeptide (PBAN): regulatory role and mode of action. *General and Comparative Endocrinology* 162:69–78.

Rafaeli, A., and C. Gileadi. 1995. Modulation of the PBAN-stimulated of pheromonotropic activity in *Helicoverpa armigera*. *Insect Biochemistry and Molecular Biology* 25:827–834.

Rafaeli, A., and R. A. Jurenka. 2003. PBAN regulation of pheromone biosynthesis in female moths. Pp. 107–136. In G. J. Blomquist and R. G. Vogt, eds. *Insect Pheromone Biochemistry and Molecular Biology*. Oxford: Elsevier.

Rafaeli, A., J. Hirsch, V. Soroker, B. Kamensky, and A. K. Raina. 1991. Spatial and temporal distribution of pheromone biosynthesis-activating neuropeptide in *Helicoverpa* (*Heliothis*) *armigera* using RIA and in vitro bioassay. *Archives of Insect Biochemistry and Physiology* 18:119–129.

Raina, A. K. 1993. Neuroendocrine control of sex pheromone biosynthesis in Lepidoptera. *Annual Review of Entomology* 38:329–349.

Raina, A. K., and Kempe, T. G. 1990. A pentapeptide of the C-terminal sequence of PBAN with pheromonotropic activity. *Insect Biochemistry* 20:849–851.

Raina, A. K., and T. G. Kempe. 1992. Structure activity studies of PBAN of *Helicoverpa zea* (Lepidoptera: Noctuidae). *Insect Biochemistry and Molecular Biology* 22:221–225.

Raina, A. K., and J. A. Klun. 1984. Brain factor control of sex pheromone production in the female corn earworm moth. *Science* 225:531–533.

Raina, A. K., J. A. Klun, J. D. Lopez, and B. A. Leonhardt. 1986. Female sex pheromone of *Heliothis phloxiphaga* (Lepidoptera: Noctuidae): chemical identification, male behavioral response in the flight tunnel, and field tests. *Environmental Entomology* 15: 931–935.

Raina, A. K., H. Jaffe, J. A. Klun, R. L. Ridgway, and D. K. Hayes. 1987. Characteristics of a neurohormone that controls sex pheromone production in *Heliothis zea*. *Journal of Insect Physiology* 33:809–814.

Ramaswamy, S. B. 1990. Periodicity of oviposition, feeding, and calling by mated female *Heliothis virescens* in a field cage. *Journal of Insect Behavior* 3:417–427.

Ramaswamy, S. B., and R. T. Roush. 1986. Sex pheromone titers in females of *Heliothis virescens* from three geographical locations (Lepidoptera: Noctuidae). *Entomologia Generalis* 12:19–23.

Ramaswamy, S. B., S. A. Randle, and W. K. Ma. 1985. Field evaluation of the sex pheromone components of *Heliothis virescens* (Lepidoptera: Noctuidae) in cone traps. *Environmental Entomology* 14:293–296.

Ramaswamy, S. B., R. A. Jurenka, C. E. Linn, Jr., and W. L. Roelofs. 1995. Evidence for the presence of a pheromonotropic factor in hemolymph and regulation of sex pheromone production in *Helicoverpa zea*. *Journal of Insect Physiology* 41:501–508.

Ray, A., W. van der Goes van Naters, T. Shiraiwa, and J. R. Carlson. 2007. Mechanisms of odor receptor gene choice in *Drosophila*. *Neuron* 53:353–369.

Roelofs, W. L., A. S. Hill, R. T. Cardé, and T. C. Baker. 1974. Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. *Life Sciences* 14:1555–1562.

Rothschild, G. H. L. 1978. Attractants for *Heliothis armigera* and *H. punctiger*. *Journal of the Australian Entomological Society* 17:389–390.

Rothschild, G. H. L., B. F. Nesbitt, P. S. Beevor, A. Cork, D. R. Hall, and R. A. Vickers. 1982. Studies of the female sex pheromone of the native budworm, *Heliothis punctiger*. *Entomologia Experimentalis et Applicata* 31:395–401.

Shaver, T. N., J. D. Lopez, Jr., and A. W. Hartstack, Jr. 1982. Effects of pheromone components and their degradation products on the response of *Heliothis* spp. to traps. *Journal of Chemical Ecology* 8:755–762.

Shaver, T. N., D. E. Hendricks, and J. D. Lopez. Jr. 1989. Influence of (Z)-11-hexadecen-1-ol on field performance of *Heliothis virescens* pheromone in a PVC dispenser as evidenced by trap capture. *Journal of Chemical Ecology* 15:1637–1644.

Sheck, A. L., Groot, A. T., Ward, C. M., Gemeno, C., Wang, J., Brownie, C., Schal, C., and F. Gould. 2006. Genetics of sex pheromone blend differences between *Heliothis virescens* and *Heliothis subflexa*: a chromosome mapping approach. *Journal of Evolutionary Biology* 19:600–617.

Shorey, H. H., and L. K. Gaston. 1965. Sex pheromones of noctuid moths. V. Circadian rhythm of pheromone-responsiveness in males of *Autographa californica*, *Heliothis virescens*, *Spodoptera exigua*, and *Trichoplusia ni* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 58:597–600.

Shorey, H. H., L. K. Gaston, and J. S. Roberts. 1965. Sex pheromones of noctuid moths. VI. Absence of behavioral specificity for the female sex pheromones of *Trichoplusia ni* versus *Autographa californica*, and *Heliothis zea* versus *H. virescens* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* 58:600–603.

Stadelbacher, E. A., M. W. Barry, A. K. Raina, and J. R. Plimmer. 1983. Fatal interspecific mating of two *Heliothis* species induced by synthetic sex pheromone. *Experientia* 39:1174–1176.

Steck, W., Underhill, E. W., and M. D. Chisholm. 1982. Structure-activity relationships in sex attractants for North American noctuid moths. *Journal of Chemical Ecology* 8:731–754.

Sugie, H., Tatsuki, S., Nakagaki, S., Rao, C. B. J., and Yamamato, A. 1991. Identification of the sex pheromone of the oriental tobacco budworm, *Heliothis assulta* (Guenee) (Lepidoptera: Noctuidae). *Applied Entomology and Zoology* 26:151–153.

Szõcs, G., A. Raina, M. Tóth, and B. A. Leonhardt. 1993. Sex pheromone components of *Heliothis maritima*: chemical identification, flight tunnel and field tests. *Entomologia Experimentalis et Applicata* 66:247–253.

Tay, W. T., M. F. Soria, T. Walsh, D. Thomazoni, P. Silvie, G. T. Behere, C. Anderson, and S. Downes. 2013. A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PlOS ONE* 8:e80134.

Teal, P. E. A., and A. Oostendorp. 1993. Interspecific hybridization between *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae) affects the presence and structure of hairpencil glands of males. *Annual Review of Entomology* 86:322–326.

Teal, P. E. A., and A. Oostendorp. 1995. Production of pheromone by hairpencil glands of males obtained from interspecific hybridization between *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 21:59–67.

Teal, P. E. A., and J. H. Tumlinson. 1986. Terminal steps in pheromone biosynthesis by *Heliothis virescens* and *H. zea*. *Journal of Chemical Ecology* 12:353–366.

Teal, P. E. A., and J. H. Tumlinson. 1989. Isolation, identification, and biosynthesis of compounds produced by male hairpencil glands of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 15:413–427.

Teal, P. E. A., and J. H. Tumlinson. 1997. Effects of interspecific hybridization between *Heliothis virescens* and *Heliothis subflexa* on the sex pheromone communication system. Pp. 535–547. In R. T. Cardé and A. K. Minks, eds. *Insect Pheromone Research: New Directions*. New York: Chapman & Hall.

Teal, P. E. A., R. R. Heath, J. H. Tumlinson, and J. R. McLaughlin. 1981a. Identification of a sex pheromone of *Heliothis subflexa* (Gn.) (Lepidoptera: Noctuidae) and field trapping studies using different blends of components. *Journal of Chemical Ecology* 7:1011–1022.

Teal, P. E. A., J. R. McLaughlin, and J. H. Tumlinson. 1981b. Analysis of the reproductive behavior of *Heliothis virescens* (F.) under laboratory conditions. *Annals of the Entomological Society of America* 74:324–330.

Teal, P. E. A., J. H. Tumlinson, J. R. McLaughlin, R. Heath, and R. A. Rush. 1984. (Z)-11-Hexadecen-1-ol: a behavioral modifying chemical present in the pheromone gland of female *Heliothis zea* (Lepidoptera: Noctuidae). *Canadian Entomologist* 116:777–779.

Teal, P. E. A., J. H. Tumlinson, and R. R. Heath. 1986. Chemical and behavioral analyses of volatile sex pheromone components

released by calling *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 12:107–126.
Teal, P. E. A., J. H. Tumlinson, and H. Oberlander. 1989. Neural regulation of sex pheromone biosynthesis in *Heliothis* moths. *Proceedings of the National Academy of Sciences of the United States of America* 86:2488–2492.
Tillman, J. A., S. J. Seybold, R. A. Jurenka, and G. J. Blomquist. 1999. Insect pheromones—an overview of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology* 29:481–514.
Tsfadia, O., A. Azrielli, L. Falach, A. Zada, W. L. Roelofs, and A. Rafaeli. 2008. Pheromone biosynthetic pathways: PBAN-regulated rate-limiting steps and differential expression of desaturase genes in moth species. *Insect Biochemistry and Molecular Biology* 38:552–567.
Underhill, E. W., Chisholm, M. D., and W. Steck. 1977. Olefinic aldehydes as constituents of sex attractants for noctuid moths. *Environmental Entomology* 6:333–337.
Vásquez, G. M., P. Fischer, C. M. Grozinger, and F. Gould. 2011. Differential expression of odorant receptor genes involved in the sexual isolation of two *Heliothis* moths. *Insect Molecular Biology* 20:115–124.
Vásquez, G. M., Z. Syed, P. A. Estes, W. S. Leal, and F. Gould. 2013. Specificity of the receptor for the major sex pheromone component in *Heliothis virescens*. *Journal of Insect Science* 13:160.
Vetter, R. S., and T. C. Baker. 1983. Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female glands. *Journal of Chemical Ecology* 9:747–749.
Vetter, R. S., and T. C. Baker. 1984. Behavioral responses of male *Heliothis zea* moths in sustained flight-tunnel to combinations of four compounds identified from female sex pheromone gland. *Journal of Chemical Ecology* 10:193–202.
Vickers, N. J. 2002. Defining a synthetic pheromone blend attractive to male *Heliothis subflexa* under wind tunnel conditions. *Journal of Chemical Ecology* 28:1255–1267.
Vickers, N. J. 2006a. Inheritance of olfactory preferences I. Pheromone-mediated behavioral responses of *Heliothis subflexa* × *Heliothis virescens* hybrid male moths. *Brain, Behavior and Evolution* 68:63–74.
Vickers, N. J. 2006b. Inheritance of olfactory preferences. III. Processing of pheromonal signals in the antennal lobe of *Heliothis subflexa* × *Heliothis virescens* hybrid male moths. *Brain Behavior & Evolution* 68:90–108.
Vickers, N. J., and T. C. Baker. 1994. Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proceedings of the National Academy of Sciences of the United States of America* 91:5756–5760.
Vickers, N. J., and T. C. Baker. 1997. Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. *Journal of Comparative Physiology A* 180:523–536.
Vickers, N. J., and T. A. Christensen. 1998. A combinatorial model of odor discrimination using a small array of contiguous, chemically defined glomeruli. *Annals of the New York Academy of Sciences* 855:514–516.
Vickers, N. J., and T. A. Christensen. 2003. Functional divergence of spatially conserved olfactory glomeruli in two related moth species. *Chemical Senses* 28:325–338.
Vickers, N. J., T. A. Christensen, H. Mustaparta, and T. C. Baker. 1991. Chemical communication in heliothine moths. III. Flight behavior of male *Helicoverpa zea* and *Heliothis virescens* in response to varying ratios of intra-and interspecific sex pheromone components. *Journal of Comparative Physiology A* 169:275–280.
Vickers, N. J., T. A. Christensen, and J. G. Hildebrand. 1998. Combinatorial odor discrimination in the brain: attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. *Journal of Comparative Neurology* 400:35–56.
Vogel, H., A. J. Heidel, D. G. Heckel, and A. T. Groot. 2010. Transcriptome analysis of the sex pheromone gland of the noctuid moth *Heliothis virescens*. *BMC Genomics* 11:29.
Wang, C. 2007. Interpretation of the biological species concept from interspecific hybridization of two *Helicoverpa* species. *Chinese Science Bulletin* 52:284–286.
Wang, C., and J. Dong. 2001. Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae). *Chinese Science Bulletin* 46:489–491.
Wang, G., G. M. Vásquez, C. Schal, L. J. Zwiebel, and F. Gould. 2011. Functional characterization of pheromone receptors in the tobacco budworm *Heliothis virescens*. *Insect Molecular Biology* 20:125–133.
Wang, H.-L., Zhao, C.-H., and C.-Z. Wang. 2005. Comparative study of sex pheromone composition and biosynthesis in *Helicoverpa armigera*, *H. assulta* and their hybrid. *Insect Biochemistry and Molecular Biology* 35:575–583.
Wang, H.-L., Q. L. Ming, C. H. Zhao, and C.-Z. Wang. 2008. Genetic basis of sex pheromone blend difference between *Helicoverpa armigera* (Hübner) and *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae). *Journal of insect physiology* 54: 813–817.
Wu, C.-I.. 1993. Responses from sensilla on the antennae of male *Heliothis armigera* to its sex pheromone components and analogs. *Acta Entomologica Sinica* 36:385–389.
Wu, H., C. Hou, L.-Q. Huang, F.-S. Yan, and C.-Z. Wang. 2013. Peripheral coding of sex pheromone blends with reverse ratios in two *Helicoverpa* species. *PLOS ONE* 8:e70078.
Zhao, X. C., and B. G. Berg. 2010. Arrangement of output information from the 3 macroglomerular units in the heliothine moth *Helicoverpa assulta*: morphological and physiological features of male-specific projection neurons. *Chemical Senses* 35: 511–521.
Zhao, X.-C., J.-F. Dong, Q.-B. Tang, Y.-H. Yan, I. Gelbic, J. J. A. Van Loon, and C.-Z. Wang. 2005. Hybridization between *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera: Noctuidae): development and morphological characterization of $F_1$ hybrids. *Bulletin of Entomological Research* 95:409–416.
Zhao, X.-C., Y.-H. Yan, and C.-Z. Wang. 2006. Behavioral and electrophysiological responses of *Helicoverpa assulta*, *H. armigera* (Lepidoptera: Noctuidae), their $F_1$ hybrids and backcross progenies to sex pheromone component blends. *Journal of Comparative Physiology A* 192:1037–1047.
Zhang, D. D., K. Y. Zhu, and C.-Z. Wang. 2010. Sequencing and characterization of six cDNAs putatively encoding three pairs of pheromone receptors in two sibling species, *Helicoverpa armigera* and *Helicoverpa assulta*. *Journal of Insect Physiology* 56: 586–593.
Zhang, J.-P., C. Salcedo, Y.-L. Fang, R.-L. Zhang, and Z.-N. Zhang. 2012. An overlooked component: (Z)-9-tetradecenal as a sex pheromone in *Helicoverpa armigera*. *Journal of Insect Physiology* 58:1209–1216.