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# 1

## **Biosynthesis and detection of pheromones and plant volatiles – introduction and overview**

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### **1.1 Introduction and overview**

The first half of this book deals with the production of pheromones, primarily in female insects, and the second half deals with reception of pheromones and other odorants, the former primarily in male insects and the latter in males, females and juveniles. Most of the work on pheromone production and reception is recent, all occurring in the past three decades. The emphasis in this book is on work done since 1987, when *Pheromone Biochemistry* (Prestwich and Blomquist, 1987) was published. From the work presented in this edition, it can readily be seen that the field has undergone tremendous advances in the last one and a half decades.

Our understanding of pheromone production has evolved from identifying biochemical pathways towards unraveling the molecular biology of key regulatory enzymes, and in one system, a genomics approach has been initiated. Our understanding of the regulation of pheromone production has similarly advanced from simply knowing that a particular hormone increased pheromone production in a few species to developing an understanding of which enzymes are regulated at the molecular level.

Our understanding of pheromone reception had undergone dramatic change just prior to 1987 with the proposal that Pheromone Binding Proteins (PBPs) and pheromone degrading enzymes transported and inactivated pheromonal signals

within the sensilla (see Figure 1.2B). The general framework of this process is now known to be widespread for most insects and for the reception of pheromones, plant volatiles and other odorants. Biochemical transduction pathways have been identified, including the all important olfactory receptor proteins. A marriage of molecular genetics and genomics with biomechanics, behavior, anatomy and physiology is giving new understanding of how pheromones are detected, and raising many new questions in the process.

## 1.2 Pheromone production: biosynthesis of pheromones

The first insect sex pheromone identified was bombykol, (*E*, *Z*)-10,12-hexadecadien-1-ol (Butenandt *et al.*, 1959) from the silkworm moth, *Bombyx mori* (L.). The elucidation of the structure spanned 20 years and required 500 000 female abdomens. A few years later, (*Z*)-7-dodecenyl acetate was identified as the sex pheromone of the cabbage looper, *Trichoplusia ni* (Berger, 1966). By 1970, following the pioneering work by Silverstein on bark beetles, in which three terpenes were identified as a synergistic pheromone blend for *Ips paraconfusus* (Silverstein *et al.*, 1966), it became recognized that most insect pheromones consisted of multicomponent blends. This has since been shown to be true for most insects, and single-component pheromones are rare. Over the past four decades, extensive research on insect pheromones has resulted in the chemical and/or behavioral elucidation of pheromone components from several thousand species of insects, with much of the work concentrating on sex pheromones from economically important pests.

One of the early issues addressed in pheromone production was the origin of pheromone components. Ultimately, all precursors for pheromone biosynthesis can be traced through dietary intake. A question asked in several systems was whether pheromone components were derived from dietary components that were altered only minimally or whether they were synthesized *de novo*. This simple question proved surprisingly difficult to answer, and different answers were obtained for different groups of insects. By the mid – 1980s, isotope studies had demonstrated that in the Lepidoptera, most of the sex pheromone components were synthesized *de novo* (Bjostad *et al.*, 1987), with some exceptions (see Eisner and Meinwald Chapter 12.). Early studies in the boll weevil, *Anthonomus grandis*, gave evidence that the monoterpenoid pheromone components could arise both from modification of dietary precursors (Thompson and Mitlin, 1979) and from *de novo* biosynthesis (Mitlin and Hedin, 1974). The relative contribution of each has still not been fully resolved (Tillman *et al.*, 1999). Early studies in bark beetles yielded convincing evidence that aggregation pheromone components such as 2-methyl-6-methylene-7-octen-4-ol (ipsenol), 2-methyl-6-methylene-2,7-octadien-4-ol (ipsdienol) and *cis*- and *trans*-verbenol

were synthesized by the slight modification, usually hydroxylation, of host tree-derived monoterpene precursors (Hughes, 1974; Byers *et al.*, 1979; Byers, 1981). Indeed, Hendry *et al.* (1980) convincingly demonstrated that deuterium labeled myrcene was directly converted to ipsenol and ipsdienol. This led to the widely accepted dogma that bark beetles obtain their pheromones differently than most other insects, where *de novo* biosynthesis is the norm. Not until the last decade did it become apparent that the situation in bark beetles was more complicated, and radiotracer studies demonstrated that some bark beetle pheromone components are synthesized *de novo* (Seybold *et al.*, 1995). This led to renewed interest in both the biochemistry (Seybold and Vanderwel, Chapter 6) and molecular biology (Tittiger, Chapter 7) of bark beetle pheromone production.

By 1987, when *Pheromone Biochemistry* (Prestwich and Blomquist, 1987) was published, the biosynthetic pathways of pheromones for a number of species had been determined, and work was progressing toward the characterization of some of the unique enzymes involved. It became apparent that the products of normal metabolism, particularly those of the fatty acid and isoprenoid pathways, were modified by a few pheromone gland-specific enzymes to produce the myriad of pheromone molecules. The elegant work of the Roelofs laboratory (Bjostad *et al.*, 1987) demonstrated that many of the lepidopteran pheromones could be formed by the appropriate interplay of highly selective chain shortening and a unique delta-11 desaturase followed by modification of the carboxyl carbon. This work has been extended, and a clear understanding of the biosynthetic pathways for many of the lepidopteran pheromones is now known (Jurenka, Chapter 3). Also, the delta-11 and other pheromone-specific desaturases in Lepidoptera have been characterized at the molecular level (Knipple and Roelofs Chapter 4). Chain shortening of fatty acids is also involved in producing the queen pheromone in honeybees (Plettner *et al.*, 1996, 1998) and this is reviewed in Chapter 11 (Blomquist and Howard). In some insects, fatty acid elongation followed by decarboxylation produce the hydrocarbon pheromones, and these include examples of lepidopterans (Jurenka, Chapter 3), dipterans (Blomquist, Chapter 8; Jallon and Wicker-Thomas, Chapter 9), the German cockroach (Schal *et al.*, Chapter 10) and the social insects (Blomquist and Howard, Chapter 11). More recent work in bark beetles has shown that *Ips* and *Dendroctonus* spp. produce their monoterpene-derived pheromones ipsenol, ipsdienol and frontalin by modifications of isoprenoid pathway products (Seybold and Vanderwel, Chapter 6). Until that work, it was considered very rare for animals to produce monoterpene derivatives (C10 isoprenoids).

Sex pheromone gland cells can be individual cells or clusters of cells forming glandular tissue, and these structures can be located almost everywhere externally on insects, including antennae, the head, thorax, legs and abdomen (Ma and Ramaswamy Chapter 2). Recent work at the molecular level has shown that midgut tissue (Hall *et al.*, 2002a, 2002b; Nardi *et al.* 2002) in *I. pini* and

*Dendroctonus jeffreyi* upregulate key mevalonate pathway enzymes during the induction of pheromone biosynthesis.

The powerful tools of molecular biology have recently been applied to studies on pheromone production. Knipple and Roelofs discuss their work on these unique desaturases involved in lepidopteran pheromone production in Chapter 4, and Tittiger reviews the work on the characterization of the mevalonate pathway enzymes in bark beetle pheromone production in Chapter 7. The sequencing of the *Drosophila* genome has resulted in advances in our understanding of the genetics in *Drosophila* and, combined with biochemical and molecular approaches, has led to new insights in a host of areas, including pheromone production (Jallon and Wicker-Thomas, Chapter 9).

### 1.3 Endocrine regulation of pheromone production

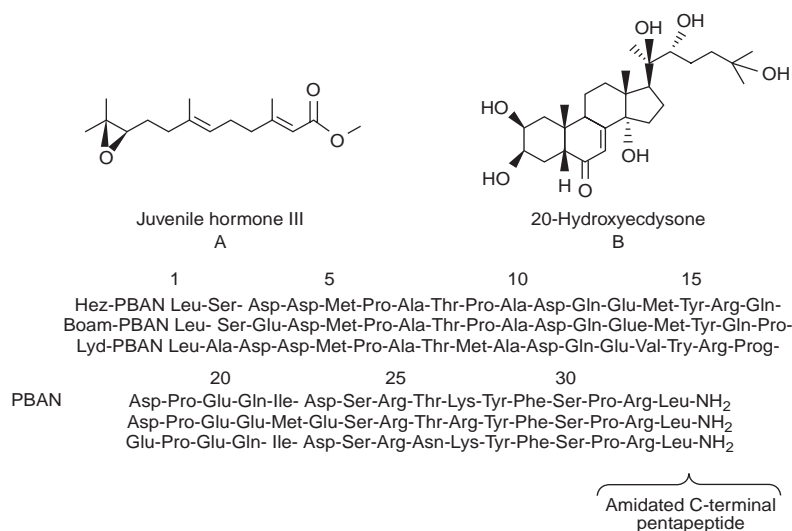
The production and/or release of sex pheromones is influenced by a variety of environmental factors (Shorey, 1974). In general, insects do not release pheromones until they are reproductively competent, although exceptions occur. Pheromone production is usually age related and coincides with the maturation of ovaries or testes, and in some cases with feeding. The observation that females of certain species have repeated reproductive cycles and that mating occurs only during defined periods of each cycle led to the proposal that pheromone production might be under hormonal control (Barth, 1965). Early work on cockroaches established that females require the presence of functional corpora allata in order to produce sex pheromones. Allatectomized females produce no pheromone and this prevents successful mating (Barth, 1961, 1962). Within a few years, the role of juvenile hormone (JH) in regulating pheromone production was established, and JH was shown to regulate pheromone production in a number of cockroach and beetle species (Hughes and Renwick, 1977a, b; Vanderwel and Oehlschlager, 1987; Vanderwel, 1994; Blomquist and Dillwith, 1983; Schal, Chapter 10; Seybold and Vanderwel, Chapter 6).

A unifying theme of this work was that the same hormone that regulated ovarian maturation (JH) also regulated pheromone production, coordinating sexual maturity with mating. Thus, in retrospect, it was not surprising that ovarian-produced 20-hydroxyecdysone, which plays an important role in reproduction in female Diptera, was shown to be the key hormone inducing sex pheromone production in the female housefly, *Musca domestica* (Adams *et al.*, 1984; Dillwith *et al.*, 1983; Blomquist *et al.*, 1987). This work has been extended to show that 20-hydroxyecdysone regulates the fatty acyl-CoA elongase enzymes to induce muscature production (Blomquist, Chapter 8).

It was recognized by the mid-1980s that Lepidoptera regulated pheromone production through a different mechanism than flies, cockroaches and beetles

(Raina and Klun, 1984), but it wasn't until 1989 that the structure of the pheromone biosynthesis activating neuropeptide (PBAN) was elucidated (Raina *et al.*, 1989). The rapid advances in this area in the last decade are chronicled by Rafaeli and Jurenka (Chapter 5).

The three hormones that regulate pheromone production in insects are shown in Figure 1.1 PBAN alters enzyme activity through second messengers at one or more steps during or subsequent to fatty acid synthesis during pheromone production (Rafaeli and Jurenka, Chapter 5). In contrast, 20-hydroxyecdysone and JH induce or repress the synthesis of specific enzymes at the transcription level (Tittiger, Chapter 7; Blomquist, Chapter 9).



**Figure 1.1** The three major types of hormones that regulate pheromone production in insects. A Juvenile Hormone III (C16 JH), B 20-Hydroxyecdysone and C PBANs from the corn earworm, *Helicoverpa zea* (Raina *et al.*, 1989), the silkworm moth *Bombyx mori* (Kitamura *et al.*, 1989) and the gypsy moth, *Lymantria dispar* (Masler *et al.*, 1994). The minimum sequence (pentapeptide) required for activity is indicated.

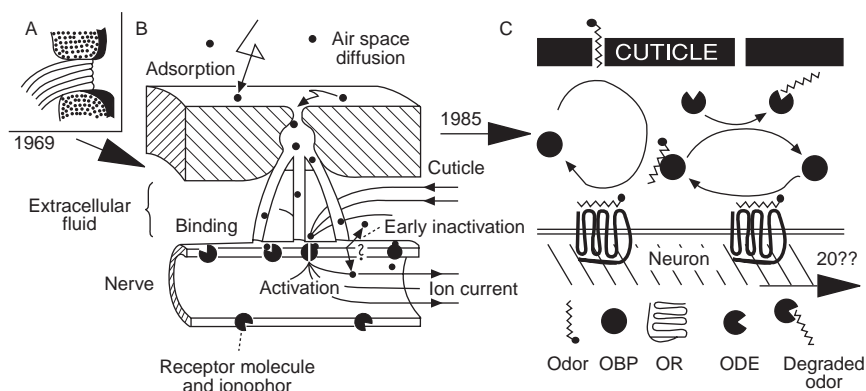
In no model pheromone biosynthetic system is the molecular mechanism of hormonal regulation completely understood. The mechanism of action of JH and the nature of its receptor remain one of the mysteries of insect science, and the clear-cut action of JH by itself in inducing specific genes in pheromone production in bark beetles offers an excellent model for study. A better understanding of the PBAN receptor and the second messenger system it triggers as well as the steps regulated in pheromone biosynthesis is also needed. The next several years should see some of the key questions answered in model insects.

#### 1.4 Detection of pheromones and plant volatiles

One reads that olfaction is the oldest sense, although this is generally stated from a human/vertebrate perspective and with the implication that olfaction was invented in fish. Chemoreception is certainly one of the oldest senses, present in bacteria and presumably one of those life essentials that was required of the earliest of chemotrophic single celled organisms. Life would be quite irrelevant in the absence of sensory input: no food, no escaping those who want to eat you, and no sex. There would be no learning since there would be no input through which one could learn anything, and no memory since there would be no means of experiencing. Chemoreception presumably became established in prokaryotes near the onset of life on earth, and became increasingly diversified and specialized through the evolution of eukaryotes. Plant, fungus and animal lineages diverged when organisms were still single celled; multicellularity developed independently within each of these lineages. Animals developed unique means to communicate between cells (cell–cell interactions and endocrine systems) and to coordinate their body movements and behaviors (nervous systems and endocrine systems). The detection of chemicals external to the animal body presumably became transformed into what we now think of as taste and smell. Chemoreception is not necessarily neuronal, but what we consider as smell and taste are clearly neuronal processes. The mechanisms underlying smell and taste are those common to the nervous system: neurons respond to external chemical stimuli (neurotransmitters and neuropeptides) via receptor proteins in their membranes which activate ion channels either directly (many receptors are themselves ion channels) or via second messenger transducing systems (e.g. G-protein coupled receptors). These processes are common to all those organisms that we consider animals, except for sponges, which are somewhat transitional between single and multicellularity and lack nervous systems. But even jellyfish have nervous systems that perform the same cellular functions as our own.

So, at the onset of this new millennium, we quite readily accept that olfaction and taste work via sensory neurons and that odor and taste molecules stimulate these neurons by binding to receptor proteins. But this is a remarkably recent view, certainly not preceding the experiments which elucidated the mechanisms underlying acetylcholine stimulation of nerve-muscle synapses (Eccles *et al.*, 1941; Fatt and Katz, 1951, 1952; del Castillo and Katz, 1954). It is perhaps, then, not surprising that Vincent Dethier (e.g. Dethier, 1962) and Dietrich Schneider (e.g. Schneider, 1969) should have been in the positions to establish insect taste and olfaction as neurobiological systems. There is no doubt that Schneider and his colleagues are responsible for establishing the basics of how pheromones and other odors are detected by insects (e.g. Schneider, 1957; Boeckh, 1962; Schneider *et al.*, 1964; Boeckh *et al.*, 1965; Kaissling and Priesner, 1970; Steinbrecht and Kasang, 1972; Kaissling 1974a).

In 1980, there were no identified gene products that functioned in the detection of odors, pheromone or otherwise. This is not to say there had been no efforts to identify olfactory proteins. The degradation of pheromone molecules had been characterized in *B. mori* (e.g. Kasang, 1971, 1972, 1974; Kasang and Kaissling, 1972) and *T. ni* (Mayer, 1975; Ferkovich *et al.*, 1973a, b, 1980). Structure–activity studies suggested pheromones were detected by receptor proteins (e.g. Kikuchi, 1975; Kafka and Neuwirth, 1975), and chemicals that disrupt protein structure had been used to uncouple odor response pathways (e.g. Villet, 1974; Frazier and Heitz, 1975). Other studies suggested roles of second messengers in olfactory transduction (e.g. Villet, 1978; see Wiczorek and Schweickl, 1985). But these studies yielded no actual proteins or genes. In 1974, Karl-Ernst Kaissling (Kaissling, 1974b) proposed a model for pheromone detection in silk moths in which pheromone molecules were transported to neuronal receptor proteins via pore-tubules (after Steinbrecht and Müller, 1971; also see Steinbrecht, 1997) and were subsequently inactivated by some rapid but non-enzymatic process (after Kasang, 1973) (see Figure 1.2a). But in 1980, matters were about to change.



**Figure 1.2** Historical models of insect odor detection. “A” is taken from a figure in Slifer *et al.* (1959) and depicts neuronal cilia making contact with air through a pore penetrating the cuticle hair wall. In 1969, K. D. Ernst published images showing that these “cilia” were not neuronal but rather were tubular extensions from the cuticular pores (pore-tubules) (Ernst, 1969; Steinbrecht, 1997). “B” is taken from a figure of Kaissling (1974a or b) and depicts pore-tubules serving as conduits to transport pheromone molecules from the air to the neuronal membrane. “C” is after Vogt *et al.* (1985) and depicts OBPs serving to transport odor molecules from pores to ORs, and ODEs as odor inactivators (see Chapter 14 for more information). “20??” is to imply the coming emergence of new understanding of the mechanisms underlying odor detection.

In 1981, the pheromone binding protein (PBP) and sensilla esterase (SE) of *Antheraea polyphemus* were identified (Vogt and Riddiford, 1981); and in 1985 a new model for pheromone detection was proposed. In this model, PBPs transported pheromone to receptor proteins (replacing pore-tubules in this role) and SE rapidly inactivated pheromone by enzymatic degradation (Vogt *et al.*, 1985) (Figure 1.2b). In 1987, when the previous edition of this book was published, not a great deal more was known. PBPs had been characterized in the gypsy moth, *Lymantria dispar* (Vogt, 1987; see Vogt *et al.*, 1989), and Glenn Prestwich was designing pheromone analogs to chemically dissect the biochemical pathways of pheromone detection (Prestwich, 1987a, b). In retrospect, this new model for pheromone reception (Vogt *et al.*, 1985) was somewhat of a watershed for biochemical studies of insect olfaction, and the previous edition of this book a description of the calm before a coming storm that has yet to show any sign of letting up.

Much has occurred since 1987. PBPs have become established as only a subclass of a much larger family of insect OBPs that are represented at least throughout the neopterous insects, from cockroach to honeybee. The SE of *A. polyphemus* is known to be only one type of odor degrading enzyme (ODE) (and has recently been cloned: Ishida and Leal, 2002); a variety of ODEs are now known from diverse species. Odor receptors (ORs) have been characterized from *D. melanogaster*, *Anopheles gambiae* and *Heliothis virescens*, and the transducing processes that follow OR activation have been characterized. The differential expression of many olfactory genes has been described, providing explanations for the diverse functional phenotypes of olfactory sensilla. And *Drosophila* has become the “new kid on the block,” contributing its genome and genetic manipulations as important new tools for elucidating olfactory mechanisms and interpreting the olfactory genomes of other insects.

The chapters of Part 2 reflect well the expansion of molecular genetic studies in insect odor detection that has occurred since 1987. The part is introduced (Chapter 13) with a thought-provoking review of the current state and future opportunities of the field. This part then presents discussions about several pre-receptor proteins, including odor degrading enzymes and odorant binding proteins as well as proteins we refer to as if in some secret code as CSPs and SNMPs (Chapters 14–18), followed by discussions of odor receptors and olfactory transduction mechanisms (Chapters 19, 20). The part closes with chapters providing critical context in which to view olfactory biochemistry, including discussions of antennal form and function (Chapter 21), the biosynthesis and ecology of plant volatiles that serve as pollination cues (Chapter 22), and the physiology and genetics underlying olfactory behaviors (Chapters 23, 24).

The contributing authors represent considerable expertise for the subject. Larry Zwiebel (Chapter 13) has opened the malaria mosquito *A. gambiae* to studies of olfactory molecular genetics, with profound implications for human

health (e.g. Fox *et al.*, 2002; Hill *et al.*, 2002). Richard Vogt (Chapter 14) identified the first OBPs and ODEs and continues to provide the odd tidbit now and then (e.g. Vogt and Riddiford, 1981; Rogers *et al.*, 1999, 2001; Vogt *et al.*, 2002). Walter Leal (Chapter 15) and Erika Plettner (Chapter 16) continually make ground-breaking advances in our understanding of the mechanisms underlying OBP function (e.g. Wojtasek and Leal, 1999; Sandler *et al.*, 2000; Plettner *et al.*, 2000; Kowcun *et al.*, 2001). Patricia Nagnan-Le Meillour and Emmanuelle Jacquin-Joly (Chapter 17) have focused on the olfactory biochemistry of *Mamestra brassicae*, providing important insights into a lepidopteran family (Noctuidae) of profound agricultural importance (Maïbèche-Coisné *et al.*, 1997; Bohbot *et al.*, 1998; Campanacci *et al.*, 2001). Jean-François Picimbon (Chapter 18) has expanded our understanding and appreciation of the CSP/SAP family of proteins, some of which may have OBP-like functions, and is as well exploring the evolution of PBPs in noctuid moths (Picimbon and Leal, 1999; Picimbon *et al.*, 2000; Picimbon and Gadenne, 2002).

Leslie Vosshall (Chapter 19) was among the first to identify and characterize OR genes in insects, using *Drosophila*, and has provided important insights into the mechanisms underlying the conveyance of olfactory information from ORs to the brain (Vosshall *et al.*, 1999, 2000; Vosshall, 2000, 2001). Vosshall has additionally made significant contributions to the field of circadian behavior (Vosshall *et al.*, 1994; Vosshall, and Young (1995), a subject of historical importance to insect olfactory biology (e.g. Rau and Rau, 1929). Jürgen Krieger and Heinz Breer (Chapter 20) were among the first to clone insect OBPs, and have made major contributions to our understanding of pre-receptor, receptor and transducing mechanisms in both insects and vertebrates (Raming *et al.*, 1989; Freitag *et al.*, 1995; Krieger and Breer, 1999; Bette *et al.*, 2002). Recently, they were the first to characterize OR genes in Lepidoptera (Krieger *et al.*, 2002).

Catherine Loudon (Chapter 21) is renewing studies of the how the design of an insect's antennae relates to its ability to capture odor molecules (Loudon and Koehl, 2000). This subject has been kicked around several times in our history (e.g. Adam and Delbrück, 1968; Vogel, 1983) but until Loudon's work not fully addressed. Robert Raguso (Chapter 22) has been a student of floral scent chemistry and insect pollination behavior. His studies are informing us that plant volatiles contain olfactory signals as specific as pheromones in eliciting behavioral responses from insects (Raguso and Pichersky, 1995; Raguso *et al.*, 1996; Raguso and Roy, 1998; Levin *et al.*, 2001). Marien de Bruyne (Chapter 23) has made important contributions to olfactory coding in *Drosophila* at the periphery, characterizing odor responsiveness and sensitivities of specific classes of olfactory sensilla, with a strong orientation towards genetics and genomics (de Bruyne *et al.*, 1999, 2001; Clyne *et al.*, 1999; Warr *et al.*, 2001). Mikael Carlsson and Bill Hansson (Chapter 24) have an extensive history with olfactory coding in moths and orthopteroids, and are in a unique position to understand olfactory coding in the

brain in a manner that relates across the neopterous insects (Anton and Hansson, 1995; Carlsson *et al.*, 2002). Hansson in particular was the first to characterize the topographic organization of the interface between primary and secondary olfactory neurons in any animal (Hansson *et al.*, 1991, 1992).

This book is designed as a sourcebook for the next decade of research, and we hope it fills this expectation. Chapters have been assembled from experts who are at the frontiers of pheromone physiology, biochemistry, morphology, neurobiology and molecular biology. Ultimately, just as behavioral chemicals themselves have been extended to pest management, research on pheromone biosynthesis, hormonal regulation and reception may be directed toward application and ultimately used in insect control.

## References

- Adam G. and Delbrück M. (1968) Reduction of dimensionality in biological diffusion processes. In *Structural Chemistry and Molecular Biology* eds. A. Rich and N. Davidson, pp. 198–215. W. H. Freeman, San Francisco.
- Adams T. S., Dillwith J. W. and Blomquist G. J. (1984) The role of 20-hydroxyecdysone in housefly sex pheromone biosynthesis. *J. Insect Physiol.* **30**, 287–294.
- Anton S. and Hansson B.S. (1995) Sex pheromone and plant-associated odour processing in antennal lobe interneurons of male *Spodoptera littoralis* (Lepidoptera: Noctuidae). *J. Comp. Physiol. A.* **176**, 773–789.
- Barth R. H., Jr (1961) Hormonal control of sex attractant production in the Cuban cockroach. *Science* **133**, 1598–1599.
- Barth R. H., Jr (1962) The endocrine control of mating behavior in the cockroach *Byrsotria fumigata* (Guerin). *Gen. Comp. Endocrinol.* **2**, 53–69.
- Barth R. H., Jr (1965) Insect mating behavior: endocrine control of a chemical communication system. *Science* **149**, 882–883.
- Berger R. S. (1966) Isolation, identification and synthesis of sex attractant of the cabbage looper, *Trichoplusia ni*. *Ann. Entomol. Soc. Amer.* **59**, 767–771.
- Bette S. Breer H. and Krieger J. (2002) Probing a pheromone binding protein of the silkworm *Antheraea polyphemus* by endogenous tryptophan fluorescence. *Insect Biochem. Mol. Biol.* **32**, 241–246.
- Bjostad L. B., Wolf W. A. and Roelofs W. L. (1987) Pheromone biosynthesis in lepidopterans: desaturation and chain shortening. In *Pheromon Biochemistry*, eds G. J. Blomquist and G. D. Prestwich, pp. 77–120. Academic Press, Orlando, FL.
- Blomquist G. J. and Dillwith J. W. (1983) Pheromones: biochemistry and physiology. In *Endocrinology of Insects*, eds R. G. H. Downer and H. Laufer, pp. 527–542. Alan R. Liss, Inc., New York, NY.
- Blomquist G. J., Dillwith J. W. and Adams T. S. (1987) Biosynthesis and endocrine regulation of sex pheromone production in Diptera. In *Pheromone Biochemistry*, eds G. J. Blomquist and G. D. Prestwich, pp. 217–250. Academic Press, Orlando, FL.
- Boeckh J. (1962) Elektrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen des Totengräbers (*Necrophorus*, Coleoptera). *Z. Vergl. Physiol.* **46**, 212–248.
- Boeckh J., Kaissling K. E. and Schneider D. (1965) Insect olfactory receptors. *Cold Spring Harbor Symposium on Quantitative Biology* **30**, 263–280.

- Bohbot J., Sobrio F., Lucas P. and Nagnan-Le Meillour P. (1998) Functional characterization of a new class of odorant-binding proteins in the moth *Mamestra brassicae*. *Biochem Biophys. Res. Commun.* **253**, 489–494.
- Butenandt A., Beckmann R., Stamm D. and Hecker E. (1959) Über den Sexual-Lockstoff des Seidenspinners *Bombyx mori*. Reindarstellung und Konstitution. *Z. Naturforsch.* **14**, 283–284.
- Byers J. A. (1981) Pheromone biosynthesis in the bark beetle, *Ips paraconfusus*, during feeding or exposure to vapours of host plant precursors. *Insect Biochem.* **11**, 563–569.
- Byers J. A. (1983) Bark beetle conversion of a plant compound to a sex-specific inhibitor of pheromone attraction. *Science* **220**, 624–626.
- Byers J. A., Wood D. L., Browne L. E., Fish R. H., Piatek B. and Hendry L. B. (1979) Relationship between a host plant compound, myrcene, and pheromone production in the bark beetle, *Ips paraconfusus*. *J. Insect Physiol.* **25**, 477–482.
- Campanacci V., Mosbah A., Bornet O., Wechselberger R., Jacquin-Joly E., Cambillau C., Darbon H. and Tegoni M. (2001) Chemosensory protein from the moth *Mamestra brassicae*. Expression and secondary structure from <sup>1</sup>H and <sup>15</sup>N NMR. *Eur. J. Biochem.* **268**, 4731–4739.
- Carlsson M. A., Galizia C. G. and Hansson B. S. (2002) Spatial representation of odours in the antennal lobe of the moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Chem. Senses.* **27**(3), 231–244.
- Clyne P. J., Certel S., de Bruyne M., Zaslavsky L., Johnson W., Carlson J. R. (1999) The odor-specificities of a subset of olfactory receptor neurons are governed by *acj6*, a POU domain transcription factor. *Neuron* **22**, 339–347.
- de Bruyne M., Clyne P. J., and Carlson J. R. (1999) Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* **19**, 4520–4532.
- de Bruyne M., Foster K. and Carlson J. R. (2001) Odor coding in the *Drosophila* antenna. *Neuron* **30**, 537–552.
- del Castillo J. and Katz B. (1954) Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573.
- Dethier, V. 1962. *To Know a Fly*. Holden-Day, San Francisco.
- Dillwith J. W., Adams T. S. and Blomquist G. J. (1983) Correlation of housefly sex pheromone production with ovarian development. *J. Insect Physiol.* **29**, 377–386.
- Eccles J. C., Katz B. and Kuffler S. W. (1941) Nature of the “endplate potential” in curarized muscle. *J. Neurophysiol.* **4**, 362–387.
- Ernst, K. D. (1969) Die Feinstruktur von Reichsensilen auf der Antenne des Aaskäfers *Necrophorus* (Coleoptera). *Z. Zellforsch. Mikrosk. Anat.* **94**, 72–102.
- Fatt P. and Katz B. (1951) An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.* **115**, 320–370.
- Fatt P. and Katz B. (1952) Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.
- Ferkovich S. M., Mayer M. S. and Rutter R. R. (1973a) Conversion of the sex pheromone of the cabbage looper. *Nature* **242**, 53–55.
- Ferkovich S. M., Mayer M. S. and Rutter R. R. (1973b) Sex pheromone of the cabbage looper: reactions with antennal proteins *in vitro*. *J. Insect Physiol.* **19**, 2231–2243.
- Ferkovich S. M., Van Essen F. and Taylor T. R. (1980) Hydrolysis of sex pheromone by antennal esterases of the cabbage looper, *Trichoplusia ni*. *Chem. Senses Flavour* **5**, 33–45.
- Fox A. N., Pitts R. J. and Zwiebel L. J. (2002) A cluster of candidate odorant receptors from the malaria vector mosquito, *Anopheles gambiae*. *Chem. Senses* **27**, 453–459.
- Frazier J. L. and Heitz J. R. (1975) Electrophysiological studies of the interactions of sulfhydryl reagents with insect olfactory receptors. *J. Miss. Acad. Sci* **19**, 188.

- Freitag J., Krieger J., Strotmann J. and Breer H. (1995) Two classes of olfactory receptors in *Xenopus laevis*. *Neuron* **15**, 1383–1392.
- Hall G. M., Tittiger C., Andrews G., Mastick G., Kuenzli M., Luo X., Seybold S. J. and Blomquist G. J. (2002a) Male pine engraver Beetles, *Ips pini*, synthesize the Naturwissenschaften **89**, 79–83.
- Hall G. M., Tittiger C., Blomquist G. J., Andrews G., Mastick G., Barkawi L. A., Bengoa C. S. and Seybold S. J. (2002b) Male Jeffrey Pine Beetles, *Dendroctonus jeffreyi*, synthesize the pheromone component frontalin in anterior midgut tissue. *Insect Biochem. Mol. Biol.* **32**, 1525–1532.
- Hansson B. S., Christensen T. A. and Hildebrand J. G. (1991). Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *J. Comp. Neurol.* **312**, 264–278.
- Hansson B. S., Ljungberg H., Hallberg E. and Löfstedt, C. (1992). Functional specialization of olfactory glomeruli in a moth. *Science* **256**, 1313–1315.
- Hendry L. B., Piatek B., Browne L. E., Wood D. L., Byers J. A., Fish R. H. and Hicks R. A. (1980) *In vivo* conversion of a labelled host plant chemical to pheromones of the bark beetle, *Ips paraconfusus*. *Nature* **284**, 485.
- Hill C. A., Fox A. N., Pitts R. J., Kent L. B., Tan P. L., Chrystal M. A., Cravchik A., Collins FH., Robertson H. M. and Zwiebel L. J. (2002) G protein-coupled receptors in *Anopheles gambiae*. *Science* **298**, 176–178.
- Hughes P. R. (1974) Myrcene: a precursor of pheromones in *Ips* beetles. *J. Insect Physiol.* **20**, 1271–1275.
- Hughes P. R. and Renwick J. A. A. (1977a) Hormonal and host factors stimulating pheromone synthesis in female western pine beetles, *Dendroctonus brevicomis*. *Physiol. Entomol.* **2**, 289–292.
- Hughes P. R. and Renwick J. A. A. (1977b) Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus*. *Physiol. Entomol.* **2**, 117–123.
- Ishida Y. and Leal S. (2002) Cloning of putative odorant-degrading enzyme and integumental esterase cDNAs from the wild silkworm, *Antheraea polyphemus*. *Insect Biochem. Mol. Biol.* **32**, 1775–1780.
- Kafka W. A. and Neuwirth J. (1975) A model of pheromone molecule-acceptor interaction. *Z. Naturforsch.* **30**, 278–282.
- Kaissling K. E. (1974a) Sensory transduction in insect olfactory receptors. In *Biochemistry of Sensory Functions*, ed. L. Jaenicke, pp. 275–278. Springer-Verlag, New York.
- Kaissling K. E. (1974b) Sensory transduction in insect olfactory receptors. In *Biochemistry of Sensory Functions*. eds L. Jaenicke. Springer, Berlin, pp. 243–273.
- Kaissling K.-E. and Priesner E. (1970) Die Riechschwelle des Seidenspinners. *Naturwissenschaften* **57**, 23–28.
- Kasang G. (1971) Bombykol reception and metabolism on the antennae of the silkworm *Bombyx mori*. In *Gustation and Olfaction* eds Ohloff G. and Thomas A. F. pp. 245–250. Academic Press, New York.
- Kasang G. (1973) Physikomichemische Vorange beim Riechen des Seidenspinners. *Naturwissenschaften* **60**, 95–101.
- Kasang G. (1974) Uptake of the sex pheromone 3H-bombykol and related compounds by male and female *Bombyx* antennae. *J. Insect Physiol.* **20**, 2407–2422.
- Kasang G. and Kaissling K. E. (1972) Specificity of primary and secondary olfactory processes in *Bombyx* antennae. In *Int. Symp. Olfaction and Taste IV*, ed. D. Schneider 200–206. Verlagsgesellschaft, Stuttgart.
- Kikuchi T. (1975) Correlation of moth sex pheromone activities with molecular characteristics involved in conformers of bombykol and its derivatives. *Proc. Natl. Acad. Sci. USA* **72**, 3337–3341.

- Kitamura A., Nagasawa H., Kataoka H., Inoue T., Matsumoto S., Ando T. and Suzuki A. (1989) Amino acid sequence of pheromone biosynthesis activating neuropeptide (PBAN) of the silkworm, *Bombyx mori*. *Biochem. Biophys. Res. Commun.* **163**, 520–526.
- Kowcun A., Honson N. and Plettner E. (2001) Olfaction in the gypsy moth, *Lymantria dispar*: effect of pH, ionic strength, and reductants on pheromone transport by pheromone-binding proteins. *J. Biol. Chem.* **276**, 44770–44776.
- Krieger J. and Breer H. (1999) Olfactory reception in invertebrates. *Science* **286**, 720–723.
- Krieger J., Raming K., Dewer Y. M., Bette S., Conzelmann S. and Breer H. (2002) A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. *Eur. J. Neurosci.* **16**(4), 619–628.
- Levin R. A., Raguso R. A. and McDade L. A. (2001) Fragrance chemistry and pollinator affinities in Nyctaginaceae. *Phytochemistry* **58**, 429–440.
- Loudon, C. and Koehl, M. A. R. (2000) Sniffing by a silkworm moth: wing fanning enhances air penetration through and pheromone interception by antennae. *Journal of Experimental Biology* **203**, 2977–2990.
- Maïbèche-Coisné M., Sobrio F., Delaunay T., Lettere M., Dubroca J., Jacquin-Joly E. and Nagnan-LeMeillour P. (1997) Pheromone Binding Proteins of the moth *Mamestra brassicae*: specificity of ligand binding. *Insect Biochem. Mol. Biol.* **27**, 213–221.
- Masler E. P., Raina A. K., Wagner R. M. and Kochansky J. P. (1994) Isolation and identification of a pheromonotropic neuropeptide from the brain–subesophageal ganglion complex of *Lymantria dispar*: a new member of the PBAN family. *Insect Biochem. Mol. Biol.* **24**, 829–836.
- Mayer M. S. (1975) Hydrolysis of sex pheromone by the antennae of *Trichoplusia ni*. *Experientia* **31**, 452–454.
- Mitlin N. and Hedin P. A. (1974) Biosynthesis of grandlure, the pheromone of the boll weevil, *Anthonomus grandis*, from acetate, mevalonate, and glucose. *J. Insect Physiol.* **20**, 1825–1831.
- Nardi J. B., Gilg Young A., Ujhelyi E., Tittiger C., Lehane M. J. and Blomquist G. J. (2002) Specialization of midgut cells for synthesis of male isoprenoid pheromone in two scolytid beetles, *Dendroctonus jeffreyi* and *Ips pini*. *Tissue and Cell.* **226**, 221–231.
- Picimbon J. F. and Gadenne C. (2002) Evolution and noctuid pheromone binding proteins: identification of PBP in the black cutworm moth, *Agrotis ipsilon*. *Insect Biochem. Molec. Biol.* **32**, 839–846.
- Picimbon J. F. and Leal W. S. (1999) Olfactory soluble proteins of cockroaches. *Insect Biochem. Mol. Biol.*, **29**, 973–978.
- Picimbon J. F., Dietrich K., Breer H. and Krieger J. (2000). Chemosensory proteins of *Locusta migratoria* (Orthoptera: Acrididae). *Insect Biochem. Mol. Biol.* **30**, 233–241.
- Plettner E., Slessor K. N., Winston M. L. and Oliver J. E. (1996) Caste-selective pheromone biosynthesis in honeybees. *Science* **271**, 1851–1853.
- Plettner E., Slessor K. N. and Winston M. L. (1998) Biosynthesis of mandibular acids in hone bees (*Apis mellifera*): de novo synthesis, route of fatty acid hydroxylation and caste selective  $\beta$ -oxidation. *Insect Biochem. Molec. Biol.* **28**, 31–42.
- Plettner E., Lazar J., Prestwich E. G. and Prestwich G. D. (2000) Discrimination of pheromone enantiomers by two pheromone binding proteins from the gypsy moth *Lymantria dispar*. *Biochemistry* **39**, 8953–8962.
- Prestwich G. D. (1987a) Chemical studies of pheromone reception and catabolism. In *Pheromone Biochemistry* eds, G. D. Prestwich and G. J. Blomquist, pp. 473–527. Academic Press, New York.

- Prestwich G. D. (1987b) Chemistry of pheromone and hormone metabolism in insects. *Science* **237**, 999–1006.
- Prestwich G. D. and Blomquist G. J. (1987) *Pheromone Biochemistry*, 565 pp. Academic Press, Orlando, Florida.
- Raguso R. and Pichersky E. (1995). Floral volatiles of *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral aroma and moth pollination. *Plant Systematics and Evolution* **194**, 55–67.
- Raguso R., Light D. M. and Pichersky E. (1996). Electroantennogram responses of *Hyles lineata* (Sphingidae: Lepidoptera) to floral volatile compounds from *Clarkia breweri* (Onagraceae) and other moth-pollinated flowers. *Journal of Chemical Ecology* **22**, 1735–1766.
- Raguso R. A. and Roy B. A. (1998) “Floral” scent production by *Puccinia* rust fungi that mimic flowers. *Molecular Ecology* **7**, 1127–1136.
- Raina A. K. and Klun J. A. (1984) Brain factor control of sex pheromone production in the female corn earworm moth. *Science* **225**, 531–533.
- Raina A. K., Jaffe H., Kempe T. G., Keim P., Blacher R. W., Fales H. M., Riley C. T., Klun J. A., Ridgway R. L. and Hayes D. K. (1989) Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. *Science* **244**, 796–798.
- Raming K., Krieger J. and Breer H. (1989) Molecular cloning of an insect pheromone-binding protein. *FEBS Lett.* **256**, 215–218.
- Rau P. and Rau N. (1929) The sex attraction and rhythmic periodicity in giant saturniid moths. *Trans. Acad. Sci. St. Louis* **26**, 83–221.
- Rogers M. E., Jani M. K., Vogt R. G. (1999) An olfactory-specific glutathione-S-transferase in the sphinx moth *Manduca sexta*. *J. Exp. Biol.* **202**, 1625–1637.
- Rogers M. E., Krieger J. and Vogt R. G. (2001) Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. *J. Neurobiol.* **49**, 47–61.
- Sandler B. H., Nikonova L., Leal W. S. and Clardy J. (2000) Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein-bombykol complex. *Chem. Biol.* **7**, 143–151.
- Schneider D. (1957) Elektrophysiologische Untersuchungen von Chemo- und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori*. *Z. Vergl. Physiol.* **40**, 8–41.
- Schneider D. (1969) Insect olfaction: deciphering system for chemical messages. *Science* **163**, 1031–1037.
- Schneider D., Lacher V. and Kaissling K.-E. (1964) Die Reaktionsweise und das Reaktionsspektrum von Riechzellen bei *Antheraea pernyi* (Lepidoptera, Saturniidae). *Z. Vergl. Physiol.* **48**, 632–662.
- Seybold S. J., Quilici D. R., Tillman J. A., Vanderwel D., Wood D. L. and Blomquist G. J. (1995) *De novo* biosynthesis of the aggregation pheromone components ipsenol and ipsdienol by the pine bark beetles *Ips paraconfusus* Lanier and *Ips pini* (Say) (Coleoptera: Scolytidae). *Proc. Natl. Acad. Sci. USA* **92**, 8393–8397.
- Shorey H. H. (1974) Environmental and physiological control of insect sex pheromone behavior. In *Pheromones*, ed. M.C. Birch, pp 62–80. New York, American Elsevier.
- Silverstein R. M., Rodin J. O. and Wood D. L. (1966) Sex attractants in frass produced by male *Ips confusus* in ponderosa pine. *Science* **154**, 509–510.
- Slifer E. H., Prestage J. J., Beams H. W. (1959) The chemoreceptors and other sense organs on the antennal flagellum of the grasshopper (Orthoptera: Acrididae). *J. Morph.* **105**, 145–191.
- Steinbrecht R. A. (1997) Pore structures in insect olfactory sensilla: a review of data and concepts. *Int. J. Insect Morphol. & Embryol.* **26**, 229–245.

- Steinbrecht R. A. and Kasang G. (1972) Capture and conveyance of odour molecules in an insect olfactory receptor. In *Olfaction and Taste IV*, ed. D. Scheeder, pp. 193–199. Wiss. Verl. Ges., Stuttgart.
- Steinbrecht R. A. and Müller B. (1971) On the stimulus conducting structures in insect olfactory receptors. *Z. Zellforsch.* **117**, 570–575.
- Thompson A. C. and Mitlin N. (1979) Biosynthesis of the sex pheromone of the male boll weevil from monoterpene precursors. *Insect Biochem.* **9**, 293–294.
- Tillman J. A., Seybold S. J., Jurenka J. A. and Blomquist G. J. (1999) Insect pheromones – an overview of biosynthesis and endocrine regulation. *Insect Biochem. Mol. Biol.* **29**, 481–514.
- Vanderwel D. (1994) Factors affecting pheromone production in beetles. *Arch. Insect Biochem. Physiol.* **25**, 347–362.
- Vanderwel D. and Oehlschlager A. C. (1987) Biosynthesis of pheromones and endocrine regulation of pheromone production in Coleoptera. In *Pheromone Biochemistry*, eds G. J. Blomquist and G. D. Prestwich, pp. 175–215. Academic Press, Orlando, FL.
- Villet R. H. (1974) Involvement of amino and sulfhydryl groups in olfactory transduction in silkworms. *Nature* **248**, 707–709.
- Villet R. H. (1978) Mechanism of insect sex-pheromone sensory transduction: role of adenylyl cyclase. *Comp. Biochem. Physiol.* **61C**, 389–394.
- Vogel S. (1983). How much air passes through a silkworm's antenna? *Journal of Insect Physiology* **29**, 597–602.
- Vogt R. G. (1987) The molecular basis of pheromone reception: its influence on behavior. In *Pheromone Biochemistry*, eds G. D. Prestwich and G. J. Blomquist, pp. 385–431. Academic Press, New York.
- Vogt R. G., Köehne A. C., Dubnau J. T. and Prestwich G. D. (1989) Expression of pheromone binding proteins during antennal development in the gypsy moth *Lymantria dispar*. *J. Neurosci.* **9**, 3332–3346.
- Vogt R. G. and Riddiford L. M. (1981) Pheromone binding and inactivation by moth antennae. *Nature* **293**, 161–163.
- Vogt R. G., Riddiford L. M. and Prestwich G. D. (1985) Kinetic properties of a sex pheromone-degrading enzyme: the sensillar esterase of *Antheraea polyphemus*. *Proc. Natl. Acad. Sci. USA* **82**, 8827–8831.
- Vogt R. G., Rogers M. E., Franco M. D. and Sun M. (2002) A comparative study of odorant binding protein genes: differential expression of the PBP1-GOBP2 gene cluster in *Manduca sexta* (Lepidoptera) and the organization of OBP genes in *Drosophila melanogaster* (Diptera). *J. Exp. Biol.* **205**, 719–744.
- Vosshall L. B. (2000) Olfaction in *Drosophila*. *Curr. Opin. Neurobiol.* **10**, 498–503.
- Vosshall L. B. (2001) How the brain sees smells. *Dev. Cell* **1**, 588–590.
- Vosshall L. B., Amrein H., Morozov P. S., Rzhetsky A. and Axel R. (1999) A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**, 725–736.
- Vosshall L. B., Price J. L., Sehgal A., Saez L. and Young M. W. (1994) Block in nuclear localization of period protein by a second clock mutation, timeless. *Science* **263**, 1606–1609.
- Vosshall L. B., Wong A. M. and Axel R. (2000) An olfactory sensory map in the fly brain. *Cell* **102**, 147–159.
- Vosshall L. B. and Young M. W. (1995) Circadian rhythms in *Drosophila* can be driven by period expression in a restricted group of central brain cells. *Neuron* **15**, 345–360.
- Warr C. G., Clyne P. J., de Bruyne M., Kim J., Carlson J. R. (2001) Olfaction in *Drosophila*: coding, genetics and e-genetics. *Chem. Senses* **26**, 201–206.
- Wieczorek H. and Schweikl H. (1985) Concentrations of cyclic nucleotides and activities

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of cyclases and phosphodiesterases in an insect, chemosensory organ. *Insect Biochem.* **6**, 723–728.

Wojtasek H. and Leal W. S. (1999) Conformational change in the pheromone-binding protein from *Bombyx mori* induced by pH and by interaction with membranes. *J. Biol. Chem.* **274**, 30950–30956.