Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Insect Science

Review

Regulation of insect cuticular hydrocarbon biosynthesis

Zinan Wang[1,2,3], Ignatius P Andika[1,4] and Henry Chung[1,2]

Cuticular hydrocarbons (CHCs) play pleiotropic roles in insect survival and reproduction. They prevent desiccation and function as pheromones influencing different behaviors. While the genes in the CHC biosynthesis pathway have been extensively studied, the regulatory mechanisms that lead to different CHC compositions received far less attention. In this review, we present an overview of how different hormones and transcriptional factors regulate CHC synthesis genes, leading to different CHC compositions. Future research focusing on the regulatory mechanisms underlying CHC biosynthesis can lead to a better understanding of how insects could produce dynamic chemical profiles in response to different stimuli.

Addresses
[1] Department of Entomology, Michigan State University, East Lansing, MI, United States
[2] Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, United States
[3] Department of Entomology, University of Kentucky, Lexington, KY, United States
[4] Department of Biology, Faculty of Biotechnology, University of Atma Jaya Yogyakarta, Indonesia

Corresponding authors: Wang, Zinan (wangzina@uky.edu), Chung, Henry (hwchung@msu.edu)

Current Opinion in Insect Science 2025, **67**:101287

This review comes from a themed issue on **Development and regulation**

Edited by **Shigeyuki Koshikawa** and **Yoichiro Tamori**

https://doi.org/10.1016/j.cois.2024.101287

Introduction

Cuticular hydrocarbons (CHCs) form a lipid layer on the body surface of most insect species. These molecules play pleiotropic roles in insect survival and reproduction [1–3]. CHCs are important in preventing desiccation and facilitating adaptation to various terrestrial environments [4–7] while also functioning as contact pheromones that modulate various behaviors in diverse insects, such as mating success [8–10], species recognition [11–13], and social interactions [14]. CHCs have also been implicated in insecticide resistance by reducing insecticide penetration [15–17]. Due to these critical roles of CHCs across insect species, the genes underlying CHC synthesis have garnered significant attention in recent years [2,18].

CHC synthesis in insects occurs in specialized cells called oenocytes located underneath the cuticle and co-opts the fatty acyl-CoA synthesis pathway involving multiple enzymes from gene families, such as fatty acyl-CoA synthases, elongases, desaturases, and reductases, to produce very-long-chained fatty acyl-CoA (≥22 carbons) [19] and other derived molecules [2,20]. The final step of CHC synthesis in the oenocytes involves the conversion of some of these molecules into hydrocarbons by an insect-specific cytochrome P450 (Cyp4g1) expressed in the oenocytes [21,22]. Evolutionary changes in the genes that encode these enzymes, such as birth-and-death evolution [23–25], protein-coding evolution [5], and *cis*-regulatory evolution [10,26], can lead to very different CHC profiles within and between species.

While CHC biosynthesis pathways have been intensively studied and many reviews have been published recently [1,2,18,27], the factors that regulate CHC biosynthesis received far less attention. Understanding how CHC biosynthesis is regulated is important for determining how sexually dimorphic CHCs are produced, how different CHC profiles are generated between different castes in social insects, and how CHC profiles can vary within an individual at different times of the day. These different CHC compositions can play essential roles in modulating behaviors such as mating [8,9,28], aggression [13,29], and kin or caste recognition [30,31]. In this review, we discuss the hormonal and transcriptional factors (TFs) regulating CHC biosynthesis and highlight recent studies on this topic.

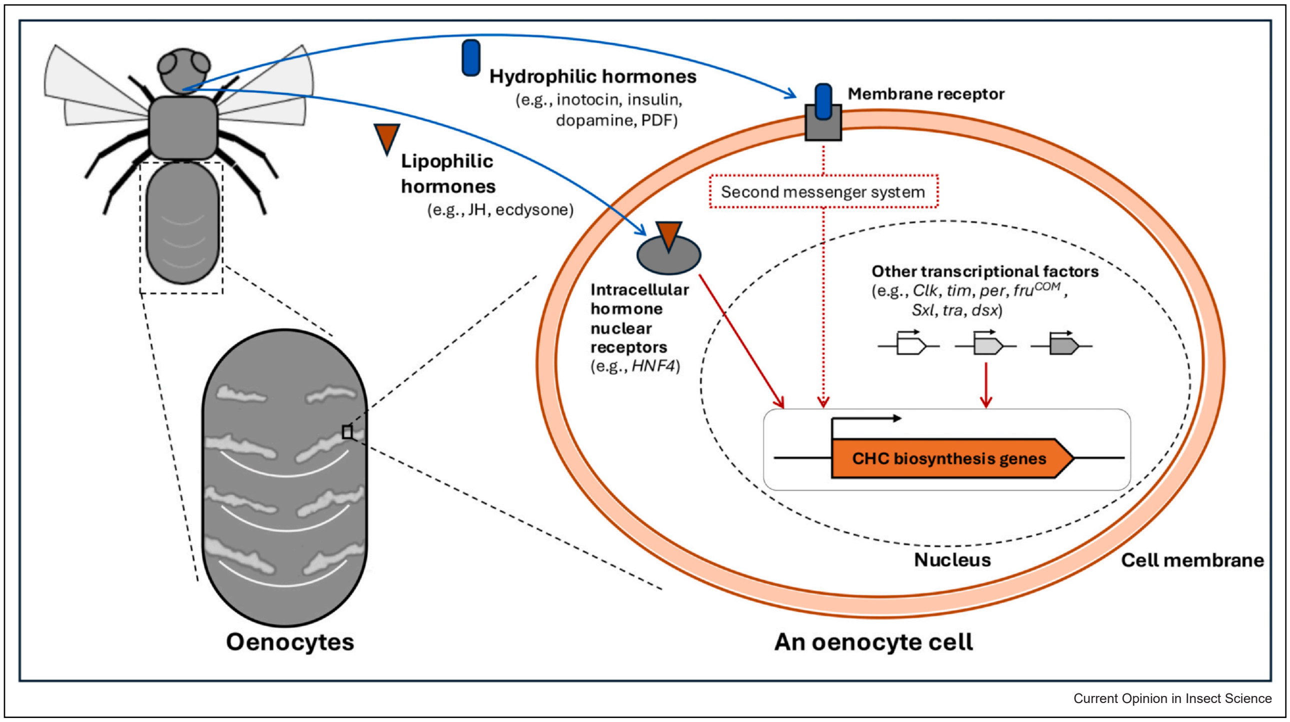
Hormonal control of cuticular hydrocarbon biosynthesis

In insects, hormones play essential roles in development and other important physiological processes. These hormones include lipophilic hormones, such as ecdysone and juvenile hormone (JH), hydrophilic neuropeptides, such as insulin and pigment-dispersing factor (PDF), and hydrophilic biogenic amines, such as dopamine and serotonin. Hormones act in a complex manner to enter cells and regulate gene expression. Lipophilic hormones directly pass through cell membranes and regulate gene expressions through binding nuclear receptors, while hydrophilic hormones require the binding of corresponding membrane receptors and activate a second messenger system to regulate gene expression [32].

Several key hormones were reported to affect CHC biosynthesis, including insulin [33], JH [34], ecdysone [35], and dopamine [36] (Figure 1). Insulin, including insulin-like peptides and insulin-like growth factors, controls the rate of glucose transport across cell membranes and affects insect reproduction, growth, and metabolism. Global disruption of insulin/insulin-like growth factor signaling pathway (IIS) leads to reduced CHC production [33]. Interestingly, not only does IIS in the oenocytes affect CHC production [37] but also IIS in the fat body and late follicle cells in the ovary indirectly affect the production of several CHCs that are related to female sexual attractiveness in *Drosophila melanogaster* [38,39]. Although the increased transcription of an insulin-like peptide (*dIlp6*) in the fat body has been identified as a key component associated with these indirect effects [38,40], the molecular mechanisms underlying this cross-tissue effects of CHC biosynthesis are not clear, along with how specific CHC synthesis genes were regulated. As the effects of IIS on CHC production were mainly on the CHCs related to female sexual attractiveness, the related biosynthesis genes such as the desaturase *desat1* and the elongase *eloF* are likely involved with the regulation of IIS in the oenocytes [37,38]. Insulin is a conserved hormone in insects that affects reproductive and courtship behaviors in insects [41]. Although the connection of CHC biosynthesis between IIS and related behaviors is not examined in insects other than *D. melanogaster*, the connection through CHCs is likely conserved in the various insect species.

Similar to insulin, experiments that disrupted JH and ecdysone synthesis (e.g. by ablating the JH-secreting corpora allata) or mutated hormonal receptors showed delayed or reduced production of CHCs [34,35]. Using similar experimental approaches, dopamine was also found to influence the synthesis of a female-specific CHC (7,11-heptacosadiene) in *D. melanogaster* [36].

Figure 1



An overview of hormonal and transcriptional regulation on CHC biosynthesis. Insect CHCs are produced in a group of cells underneath the abdominal cuticle named oenocytes. Hormones that are produced in secretory cells affect CHC biosynthesis in the oenocytes. Although some hormones such as inotocin and insulin regulate CHC biosynthesis genes via binding with corresponding membrane receptors, whether and how the other hormones, including JH, ecdysone, dopamine, and PDF, regulate CHC biosynthesis are not clear. Several TFs have been found regulating gene expressions of different CHC biosynthesis genes. The TFs in the sex determination pathway, including *Sex lethal* (*Sxl*) and *transformer* (*tra*), and the sex-specifically spliced TF, *doublesex* (*dsx*), are the main regulators leading to sex-specific CHC compositions. In addition, the recently found oenocyte-specific *fruitless* (*fru*) transcript Fru^{COM} and *HNF4* are expressed in the oenocytes of both sexes, and the collaboration of these two TFs regulates several CHC biosynthesis genes and contributes to CHC production.

Although none of these studies specifically tested the direct hormonal regulations on oenocytes, a recent study using RiboTag sequencing on the *D. melanogaster* oenocytes reported the expression of corresponding hormonal receptors in these cells [42], suggesting the roles of these essential hormones in regulating CHC biosynthesis. Similar effects of the key hormones on CHC compositions are also observed in other distantly related species; for example, JH regulates CHCs in wasps [43,44] and ants [45] and ecdysone in houseflies [46] and flesh flies [47]. The regulatory mechanisms of JH and ecdysone on CHC biosynthesis are likely conserved widely in insects.

In addition to these conserved hormones, a recent interesting finding in social insects showed another example of hormonal regulation of CHC production. This was uncovered from the worker of the carpenter ant *Camponotus fellah*, of which the CHC production increases when its worker ages and switches tasks from nursing to foraging [48]. The increased CHC production is regulated by higher gene expression of *inotocin* in the head, which produces an oxytocin/vasopressin-like neuropeptide hormone and the upregulation of its receptor in the oenocytes (Figure 1). The upregulation of both *inotocin* and *inotocin receptor* led to the upregulation of the key P450 enzyme gene for CHC synthesis, *Cyp4g1*, in the oenocytes. Oxytocin/vasopressin-like peptides are conserved neuropeptides in arthropods, and they modulate various behaviors in social insects [49]. In subsocial insects such as the burying beetles (*Nicrohorus* spp.), the oxytocin/vasopressin peptide is associated with their parenting behaviors [50]. Interestingly, CHCs are a key group of chemical signals that modulate these behaviors, including the parenting behaviors in the burying beetle [51]. There may be a possibility in burying beetles that the oxytocin/vasopressin peptide-regulated parenting behaviors are mediated through changes in CHC production. A mechanistic investigation of the connection between oxytocin/vasopressin peptides, CHC biosynthesis, and various behaviors in subsocial and social insects may uncover mechanisms underlying the evolution of diverse behaviors in insects and lead to a better understanding of how certain behaviors in social insects were maintained.

Regulation of cuticular hydrocarbon biosynthesis by the circadian clock

The production of CHCs varies throughout the day [52,53] and follows the daily circadian rhythm [54]. There is evidence that the circadian clock synchronizes the increased production of some CHCs with sex pheromone activities at the time of the day when the highest frequency of mating occurs [54]. In *D. melanogaster*, core circadian clock genes such as *timeless* (*tim*), *period* (*per*), and *Clock* (*Clk*) in oenocytes display the typical cyclical expression pattern, suggesting oenocytes are also under circadian clock control. In addition, the expression level of the key *Drosophila* CHC pheromone synthesis gene, *desat1*, in oenocytes and the amount of CHC pheromones showed the same cyclical pattern [54]. The modulation of CHC production in the oenocytes is coupled to the central nervous system by the neuropeptide PDF [55] (Figure 1). PDF is rhythmically released by the ventral lateral neurons, and it acts through its receptor (PDFR) and cAMP to stabilize the PER protein and synchronize molecular activities in the clock neurons [56]. The receptor gene *pdfr* was also found to be expressed in the oenocytes [42], indicating that PDF may be released to the hemolymph and remotely regulate the circadian clock of genes in the oenocytes.

Although there is still a gap in knowledge of how PDF remotely regulates the clock mechanism in different tissues, two recent studies showed the connection between CHC synthesis and the circadian clock genes in several mosquito species from different genera, including *Anopheles*, *Aedes*, and *Culex* [57,58]. Mosquitoes use CHCs as pheromones, modulating their swarming and mating behaviors [59]. Expression of the *desat1* gene of the mosquito species from these two studies is correlated with the expression of core circadian clock genes *tim*, *per*, or *Clk*, similar to *D. melanogaster*. Global disruption of these genes leads to reduced expression of *desat1*, decreased mating pheromone, and inhibited mating behaviors. However, these studies only investigated one desaturase gene. In *D. melanogaster*, almost all CHCs change cyclically according to the circadian rhythm; for example, the abundance of methyl-branched CHCs is higher in the day and the abundance of linear CHCs is higher at night [53]. This suggests that, in addition to *desat1*, other CHC biosynthesis genes may also be regulated by the circadian clock.

So far, the investigation on the circadian regulation of CHC biosynthesis is limited to fruitfly and mosquito species in Diptera. However, as the circadian clock system is conserved across insect species and also regulates the production of other non-CHC pheromones [60,61], the circadian clock system may also modulate the oscillation of CHC production in other insect species. Moreover, the mechanistic link between the core circadian clock genes and CHC biosynthesis genes has been elusive. It remains unknown what the other TFs downstream of these circadian clock pathways are, how they initiate or modulate transcriptions of CHC biosynthesis genes, and whether this mechanistic link between the clock genes and CHC biosynthesis genes is also conserved across insect species. Considering the diverse roles of different CHC components in mediating insect behaviors, determining how the circadian clock regulates CHC biosynthesis genes will provide a promising perspective for understanding behavioral changes in many insect species.

Transcription regulation of cuticular hydrocarbon biosynthesis

TFs are regulators of gene expression. One known TF regulation of insect CHC synthesis genes is through the HNF4 nuclear receptor (Figure 1) [62,63]. In *D. melanogaster*, the oenocyte-specific knockdown of *Drosophila* HNF4 (dHNF4) leads to reduced expression of multiple fatty acyl-CoA elongase genes and less CHC production [62]. The regulation of HNF4 on CHC production in oenocytes potentially starts from the direct binding of fatty acid precursors [64]. This leads to a plausible hypothesis that HNF4 regulation can be co-opted into the other regulatory pathways in oenocytes and contribute to the different CHC compositions within species.

A recent study provided the first evidence that dHNF4 is the connection between one isoform of the TF *fruitless* (Fru) and CHC biosynthesis (Figure 1) [65]. In *D. melanogaster*, *fru* encodes a set of TFs generated through alternative splicing. The most widely studied transcripts of *fru* are the sex-specifically spliced fru^{M} and fru^{F} that are expressed in the neurons of the central nervous system of each sex and are the master regulators of many behaviors in *D. melanogaster*, including courtship and aggression [66]. In addition to the sex-specific alternative splicing, *fru* produces several non-sex-specific TF isoforms in different tissues. In this study, the authors showed that in the oenocytes of *D. melanogaster*, *fru* produces one of the non-sex-specific isoforms $\mathrm{Fru}^{\mathrm{COM}}$ in both sexes and regulates the expression of *dHNF4* and multiple CHC biosynthesis genes, including a fatty acyl-CoA synthase, a desaturase, multiple elongases, and *Cyp4g1*. Further experiments showed that both oenocyte-specific knockdown of *fru* and *dHNF4* reduced most CHCs to very low amounts in males but only reduced half of most female CHCs, while overexpression of *dHNF4* in the *fru*-knockdown background can rescue the reduced CHCs and produce even more CHCs in females. This demonstrated that dHNF4 is an essential part of $\mathrm{Fru}^{\mathrm{COM}}$-mediated CHC biosynthesis [65].

In addition to *fru*, the other TFs that are known to regulate CHC biosynthesis include TFs from the sex determination pathway such as *sex lethal* (*sxl*) [67] and *transformer* (*tra*) [68] and a sex-specifically spliced TF *doublesex* (*dsx*) (Figure 1) [65], but their regulation on CHC compositions are different from the regulation of fru^{COM}. It could be due to the sexually dimorphic expression pattern of *Sxl*, *tra*, and *dsx*, which mainly regulate genes that contribute to the synthesis of sexually dimorphic CHCs. Such sexually dimorphic regulation is also reported in other distantly related insect species, including cockroach [69] and a *Nasonia* wasp [70], in which knockdown of *dsx* led to reductions of CHC sex pheromones in both species and knockdown of *tra* in female cockroaches converted the CHC phenotype to a male-like one.

Is HNF4 also co-opted in the regulatory pathway of *dsx* or the other sex determination components on CHC biosynthesis? If so, then the knockdown of *dHNF4* in the *D. melanogaster* oenocytes would lead to similar phenotypes as the knockdown of *dsx*. However, Sun et al. (2023) showed that the oenocyte-specific knockdown of *dsx* led to the reduction of CHCs in females and the production of female-specific CHCs in males, which is different from the *dHNF4* knockdown. Previous studies also showed *dsx*-binding sites in the enhancer regions of *desatF*, a desaturase gene that is only expressed in female oenocytes [71]. These two pieces of evidence suggest that DSX may directly bind and regulate the expressions of a few CHC synthesis genes for female-specific CHC synthesis without working through *dHNF4*, independent of fru^{COM}. This also explains why the knockdown of *fru* in the oenocytes only reduces half of the CHCs in females, and other pathways, including female-specific gene regulations, could regulate the other half. Nevertheless, the current understanding suggests that the collaboration of *dsx* and fru^{COM} regulates different sets of CHC biosynthesis genes and contributes to the differences in CHC production between sexes in *D. melanogaster*. The *fru* gene homologs have been found in various distantly related insect species with alternatively spliced transcripts [72]. Future studies on the roles of Fru isoforms, the master-controlling TFs in the sex determination pathway, and other TFs in regulating CHC compositions will be needed to understand how dynamic CHC compositions are produced in various insect species.

Conclusion

In this review, we present an overview of hormonal and transcriptional regulation of CHC biosynthesis in insects. Although many essential hormones that are related to development and reproduction also affect CHC production, how they regulate gene expression in oenocytes is still not clear. The neuropeptides, including PDF and oxytocin/vasopressin-like peptides, have been demonstrated to play important roles in regulating CHC compositions and modulating different behaviors. Elucidating the roles of various neuropeptides in regulating CHC biosynthesis and corresponding behaviors will be promising for understanding how insects respond to changes in the environment. We also summarized current knowledge of the main TFs that regulate CHC biosynthesis, including *HNF4*, *fru*, and *dsx*. As a dual trait that contributes to both ecological adaptation and chemical communication, the compositions of CHCs in insects are highly dynamic, influenced by various abiotic factors, such as temperature [8,73] and diet [74], as well as biotic factors, including aging [75], social interaction [29], and diapause [76]. A mechanistic understanding of how these factors regulate genes involved in CHC biosynthesis may uncover new hormones and TFs that contribute to the dynamic production of CHC in insects. Determining the regulatory mechanisms will provide a clear picture of how these chemicals can change in responding to ecological and environmental changes.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

HC is supported by a National Science Foundation grant (2054773) and Michigan State University AgBioresearch (MICL02789).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Chung H, Carroll SB: **Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating**. *Bioessays* 2015, **37**:822-830.
2. Blomquist GJ, Ginzel MD: **Chemical ecology, biochemistry, and molecular biology of insect hydrocarbons**. *Annu Rev Entomol* 2021, **66**:45-60.
3. Pei X-J, Schal C, Fan Y-L: **Genetic underpinnings of cuticular hydrocarbon biosynthesis in the German cockroach, *Blattella germanica* (L.): progress and perspectives**. *J Chem Ecol* 2024,1-14.
4. Wang Z, Receveur JP, Pu J, Cong H, Richards C, Liang M, Chung H: **Desiccation resistance differences in Drosophila species can be largely explained by variations in cuticular hydrocarbons**. *eLife* 2022, **11**:e80859.
5. Wang Z, Pu J, Richards C, Giannetti E, Cong H, Lin Z, Chung H: **Evolution of a fatty acyl-CoA elongase underlies desert adaptation in *Drosophila***. *Sci Adv* 2023, **9**:eadg0328.
6. Lei G, Zhou H, Chen Y, Vasseur L, Gurr GM, You M, You S: **A very long-chain fatty acid enzyme gene, PxHacd2 affects the temperature adaptability of a cosmopolitan insect by altering epidermal permeability**. *Sci Total Environ* 2023, **891**:164372.
7. Yang Y, Li X, Liu D, Pei X, Khoso AG: **Rapid changes in composition and contents of cuticular hydrocarbons in *Sitobion avenae* (Hemiptera: Aphididae) clones adapting to desiccation stress**. *J Econ Entomol* 2022, **115**:508-518.
8. Kárpáti Z, Deutsch F, Kiss B, Schmitt T: **Seasonal changes in photoperiod and temperature lead to changes in cuticular hydrocarbon profiles and affect mating success in *Drosophila suzukii***. *Sci Rep* 2023, **13**:5649.
9. Mitchell C, Wylde Z, Del Castillo E, Rapkin J, House CM, Hunt J: **Beauty or function? The opposing effects of natural and sexual selection on cuticular hydrocarbons in male black field crickets**. *J Evolut Biol* 2023, **36**:1266-1281.
10. Rusuwa BB, Chung H, Allen SL, Frentiu FD, Chenoweth SF: **Natural variation at a single gene generates sexual antagonism across fitness components in Drosophila**. *Curr Biol* 2022, **32**:3161-3169 e7..
11. Caliari Oliveira R, van Zweden J, Wenseleers T: **Similarities in recognition cues lead to the infiltration of non-nestmates in an ant species**. *J Chem Ecol* 2022, **48**:16-26.
12. Li Y, Parven N, Akimoto S-i: **Interspecific recognition based on cuticular hydrocarbons mediates reproduction control in aphids**. *Sci Rep* 2024, **14**:4079.
13. Hertaeg C, Vorburger C, De Moraes CM, Mescher MC: **Effects of genotype and host environment on the cuticular hydrocarbon profiles of *Lysiphlebus* parasitoids and aggression by aphid-tending ants**. *Proc R Soc B* 2023, **290**:20231642.
14. Wittke M, Baumgart L, Menzel F: **Acclimation in ants: interference of communication and waterproofing through cuticular hydrocarbons in a multifunctional trait**. *Funct Ecol* 2022, **36**:1973-1985.
15. Balabanidou V, Grigoraki L, Vontas J: **Insect cuticle: a critical determinant of insecticide resistance**. *Curr Opin Insect Sci* 2018, **27**:68-74.
16. Pu J, Chung H: **New and emerging mechanisms of insecticide resistance**. *Curr Opin Insect Sci* 2024, **63**:101184.
17. Pu J, Wang Z, Chung H: **Climate change and the genetics of insecticide resistance**. *Pest Manag Sci* 2020, **76**:846-852.
18. Holze H, Schrader L, Buellesbach J: **Advances in deciphering the genetic basis of insect cuticular hydrocarbon biosynthesis and variation**. *Heredity* 2021, **126**:219-234.
19. Kyselová L, Vítová M, Řezanka T: **Very long chain fatty acids**. *Prog Lipid Res* 2022, **87**:101180.
20. Yew JY, Chung H: **Insect pheromones: an overview of function, form, and discovery**. *Prog Lipid Res* 2015, **59**:88-105.
21. Feyereisen R: **Origin and evolution of the CYP4G subfamily in insects, cytochrome P450 enzymes involved in cuticular hydrocarbon synthesis**. *Mol Phylogenetics Evol* 2020, **143**:106695.
22. Qiu Y, Tittiger C, Wicker-Thomas C, Le Goff G, Young S, Wajnberg E, Fricaux T, Taquet N, Blomquist GJ, Feyereisen R: **An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis**. *Proc Natl Acad Sci* 2012, **109**:14858-14863.
23. Buellesbach J, Holze H, Schrader L, Liebig J, Schmitt T, Gadau J, Niehuis O: **Genetic and genomic architecture of species-specific cuticular hydrocarbon variation in parasitoid wasps**. *Proc R Soc B* 2022, **289**:20220336.
24. Di Z, Tian J, Ali MY, Zhang B, Yang S, Fu Y, Li F, Luo C: **Diversity, domain duplication, birth-and-death and adaptive evolution of fatty acyl-CoA reductase genes in Hemiptera**. *Physiol Entomol* 2023, **48**:97-106.
25. Finet C, Slavik K, Pu J, Carroll SB, Chung H: **Birth-and-death evolution of the fatty acyl-CoA reductase (FAR) gene family and diversification of cuticular hydrocarbon synthesis in *Drosophila***. *Genome Biol Evol* 2019, **11**:1541-1551.
26. Chung H, Loehlin DW, Dufour HD, Vaccarro K, Millar JG, Carroll SB: **A single gene affects both ecological divergence and mate choice in *Drosophila***. *Science* 2014, **343**:1148-1151.
27. Sun W, Lange MI, Gadau J, Buellesbach J: **Decoding the genetic and chemical basis of sexual attractiveness in parasitic wasps**. *Elife* 2023, **12**:e86182.
28. Jin B, Barbash DA, Castillo DM: **Divergent selection on behavioural and chemical traits between reproductively isolated populations of *Drosophila melanogaster***. *J Evolut Biol* 2022, **35**:693-707.
29. Lima LD, Michelutti KB, Cardoso CAL, Lima-Junior SE, Graciolli G, Antonialli-Junior WF: **Low intraspecific aggression level, cuticular hydrocarbons, and polydomy in the bullet ant**. *J Chem Ecol* 2024, **50**:351-363.
30. Buellesbach J, Rinke J, Reuter L, Thomas JM, Hartmann V, Pohl M, Gadau J, Ernst UR: **Conserved worker policing in African carpenter ants with drastically different egg chemotypes**. *Evolut Ecol* 2023, **37**:815-834.

31. de Fouchier A, Leroy C, Khila A, d'Ettorre P: **, Discrimination of non-nestmate early brood in ants: behavioural and chemical analyses**. *Anim Behav* 2023, **197**:81-95.

32. Klowden MJ, Palli SR: **Signaling systems**. In *Physiological Systems in Insects*. Edited by Klowden MJ, Palli SR. Academic Press; 2023.

33. Kuo T-H, Fedina TY, Hansen I, Dreisewerd K, Dierick HA, Yew JY, Pletcher SD: **Insulin signaling mediates sexual attractiveness in Drosophila**. *PLoS Genet* 2012, **8**:e1002684.

34. Bilen J, Atallah J, Azanchi R, Levine JD, Riddiford LM: **Regulation of onset of female mating and sex pheromone production by juvenile hormone in *Drosophila melanogaster***. *Proc Natl Acad Sci* 2013, **110**:18321-18326.

35. Baron A, Denis B, Wicker-Thomas C: **Control of pheromone production by ovaries in *Drosophila***. *J Insect Physiol* 2018, **109**:138-143.

36. Marican C, Duportets L, Birman S, Jallon JM: **Female-specific regulation of cuticular hydrocarbon biosynthesis by dopamine in Drosophila melanogaster**. *Insect Biochem Mol Biol* 2004, **34**:823-830.

37. Lin WS, Yeh SR, Fan SZ, Chen LY, Yen JH, Fu TF, Wu MS, Wang PY: **Insulin signaling in female Drosophila links diet and sexual attractiveness**. *FASEB J* 2018, **32**:3870-3877.

38. Fedina TY, Arbuthnott D, Rundle HD, Promislow DE, Pletcher SD: **Tissue-specific insulin signaling mediates female sexual attractiveness**. *PLoS Genet* 2017, **13**:e1006935.

39. Zhang L, Guo X, Zhang W: **Nutrients and pheromones promote insulin release to inhibit courtship drive**. *Sci Adv* 2022, **8**:eabl6121.

40. Chatterjee D, Katewa SD, Qi Y, Jackson SA, Kapahi P, Jasper H: **Control of metabolic adaptation to fasting by dILP6-induced insulin signaling in Drosophila oenocytes**. *Proc Natl Acad Sci* 2014, **111**:17959-17964.

41. Weger AA, Rittschof CC: **The diverse roles of insulin signaling in insect behavior**. *Front Insect Sci* 2024, **4**:1360320.

42. Huang K, Chen W, Zhu F, Li PW-L, Kapahi P, Bai H: **RiboTag translatomic profiling of Drosophila oenocytes under aging and induced oxidative stress**. *BMC Genom* 2019, **20**:1-19.

43. Kelstrup HC, Hartfelder K, Esterhuizen N, Wossler TC: **Juvenile hormone titers, ovarian status and epicuticular hydrocarbons in gynes and workers of the paper wasp *Belonogaster longitarsus***. *J Insect Physiol* 2017, **98**:83-92.

44. Prato A, da Silva RC, Assis DS, Mateus S, Hartfelder K, do Nascimento FS: **Juvenile hormone affects age polyethism, ovarian status and cuticular hydrocarbon profile in workers of the wasp Polybia occidentalis**. *J Exp Biol* 2021, **224**:jeb240200.

45. Lengyel F, Westerlund S, Kaib M: **Juvenile hormone III influences task-specific cuticular hydrocarbon profile changes in the ant *Myrmicaria eumenoides***. *J Chem Ecol* 2007, **33**:167-181.

46. Blomquist GJ: **Biosynthesis and ecdysteroid regulation of housefly sex pheromone production**. Insect Pheromone Biochemistry and Molecular Biology. Elsevier; 2003:231-252.

47. Armold M, Regnier F: **Stimulation of hydrocarbon biosynthesis by ecdysterone in the flesh fly *Sarcophaga bullata***. *J Insect Physiol* 1975, **21**:1581-1586.

48. Koto A, Motoyama N, Tahara H, McGregor S, Moriyama M, Okabe T, Miura M, Keller L: **Oxytocin/vasopressin-like peptide inotocin regulates cuticular hydrocarbon synthesis and water balancing in ants**. *Proc Natl Acad Sci* 2019, **116**:5597-5606.
••

This paper showed that in the worker of an ant species, the increased levels of gene expression of an oxytocin/vasopressin-like neuropeptide in the head and its receptor in the oenocytes upregulate the expression of the key P450 enzyme gene, *Cyp4g1*, for CHC synthesis in the oenocytes.

49. Hou L, Wang N, Sun T, Wang X: **Neuropeptide regulations on behavioral plasticity in social insects**. *Curr Opin Insect Sci* 2023, **60**:101119.

50. Potticary AL, Cunningham CB, McKinney EC, Moore PJ, Belay AT, Moore AJ: **Insect homolog of oxytocin/vasopressin associated with parenting of males but not females in a subsocial beetle**. *Evolution* 2023, **77**:2029-2038.
•

This paper showed that inotocin is associated with the parenting behavior of a subsocial insect.

51. Steiger S, Peschke K, Francke W, Müller JK: **The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides***. *Proc R Soc B Biol Sci* 2007, **274**:2211-2220.

52. Gershman SN, Toumishey E, Rundle HD: **Time flies: time of day and social environment affect cuticular hydrocarbon sexual displays in *Drosophila serrata***. *Proc R Soc B Biol Sci* 2014, **281**:20140821.

53. Kent C, Azanchi R, Smith B, Chu A, Levine J: **A model-based analysis of chemical and temporal patterns of cuticular hydrocarbons in male *Drosophila melanogaster***. *PLoS One* 2007, **2**:e962.

54. Krupp JJ, Kent C, Billeter J-C, Azanchi R, So AK-C, Schonfeld JA, Smith BP, Lucas C, Levine JD: **Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster***. *Curr Biol* 2008, **18**:1373-1383.

55. Krupp JJ, Billeter J-C, Wong A, Choi C, Nitabach MN, Levine JD: **Pigment-dispersing factor modulates pheromone production in clock cells that influence mating in Drosophila**. *Neuron* 2013, **79**:54-68.

56. Li Y, Guo F, Shen J, Rosbash M: **PDF and cAMP enhance PER stability in Drosophila clock neurons**. *Proc Natl Acad Sci* 2014, **111**:E1284-E1290.

57. Liu S, Zhou J, Kong L, Cai Y, Liu H, Xie Z, Xiao X, James AA, Chen X-G: **Clock genes regulate mating activity rhythms in the vector mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus***. *PLoS Negl Trop Dis* (12) 2022, **16**:e0010965.
•

This paper added evidence from two different mosquito species to support that clock genes regulate CHC compositions.

58. Wang G, Vega-Rodríguez J, Diabate A, Liu J, Cui C, Nignan C, Dong L, Li F, Ouedrago CO, Bandaogo AM: **Clock genes and environmental cues coordinate *Anopheles pheromone* synthesis, swarming, and mating**. *Science* 2021, **371**:411-415.
••

This paper was the first to show that clock genes play essential roles in regulating the synthesis of CHC pheromones, a key part in mosquitoes' swarming and mating behaviors.

59. Triana MF, Melo N: **The dynamics of *Aedes aegypti* mating behavior**. *Curr Opin Insect Sci* 2024, **65**:101237.

60. Bloch G, Herzog ED, Levine JD, Schwartz WJ: **Socially synchronized circadian oscillators**. *Proc R Soc B Biol Sci* 2013, **280**:20130035.

61. Ghosh S, Suray C, Bozzolan F, Palazzo A, Monsempès C, Lecouvreur F, Chatterjee A: **Pheromone-mediated command from the female to male clock induces and synchronizes circadian rhythms of the moth *Spodoptera littoralis***. *Curr Biol* 2024, **34**:1414-1425.

62. Storelli G, Nam H-J, Simcox J, Villanueva CJ, Thummel CS: **Drosophila HNF4 directs a switch in lipid metabolism that supports the transition to adulthood**. *Dev Cell* 2019, **48**:200-214.

63. Vonolfen MC, Zu Altenschildesche FLM, Nam H-J, Brodesser S, Gyenis A, Buellesbach J, Lam G, Thummel CS, Storelli G: **Drosophila HNF4 acts in distinct tissues to direct a switch between lipid storage and export in the gut**. *Cell Rep* 2024, **43**:114693.

64. Wisely GB, Miller AB, Davis RG, Thornquest AD, Johnson R, Spitzer T, Sefler A, Shearer B, Moore JT, Miller AB: **Hepatocyte nuclear factor 4 is a transcription factor that constitutively binds fatty acids**. *Structure* 2002, **10**:1225-1234.

65. Sun J, Liu W-K, Ellsworth C, Sun Q, Pan Y, Huang Y-C, Deng W-M: **Integrating lipid metabolism, pheromone production and perception by Fruitless and Hepatocyte nuclear factor 4**. *Sci Adv* 2023, **9**:eadf6254.
••

This paper showed that the collaboration of *Fru*^{COM} and *HNF4* regulates CHC production in the oenocytes, and this regulation is different from the *dsx*-regulated CHC biosynthesis.

66. Yamamoto D, Koganezawa M: **Genes and circuits of courtship behaviour in Drosophila males**. *Nat Rev Neurosci* 2013, **14**:681-692.

67. Tompkins L, McRobert SP: **Behavioral and pheromonal phenotypes associated with expression of loss-of-function mutations in the sex-lethal gene of *Drosophila melanogaster***. *J Neurogenet* 1995, **9**:219-226.

68. Ferveur J-F, Savarit F, O'Kane CJ, Sureau G, Greenspan RJ, Jallon J-M: **Genetic feminization of pheromones and its behavioral consequences in Drosophila males**. *Science* 1997, **276**:1555-1558.

69. Pei X-J, Fan Y-L, Bai Y, Bai T-T, Schal C, Zhang Z-F, Chen N, Li S, Liu T-X: **Modulation of fatty acid elongation in cockroaches sustains sexually dimorphic hydrocarbons and female attractiveness**. *PLoS Biol* 2021, **19**:e3001330.

70. • Wang Y, Sun W, Fleischmann S, Millar JG, Ruther J, Verhulst EC: **Silencing Doublesex expression triggers three-level pheromonal feminization in *Nasonia vitripennis* males**. *Proc R Soc B* 2022, **289**:20212002.

This paper provided evidence that *dsx* directs the synthesis of male-specific CHC pheromones in a hymenopteran species.

71. Shirangi TR, Dufour HD, Williams TM, Carroll SB: **Rapid evolution of sex pheromone-producing enzyme expression in Drosophila**. *PLoS Biol* 2009, **7**:e1000168.

72. Salvemini M, Polito C: **Fruitless alternative splicing and sex behaviour in insects: an ancient and unforgettable love story?** *J Genet* 2010, **89**:287-299.

73. Baleba SB, Jiang N-J, Hansson BS: **Temperature-mediated dynamics: Unravelling the impact of temperature on cuticular hydrocarbon profiles, mating behaviour, and life history traits in three *Drosophila* species**. *Heliyon* 2024, **10**:e36671.

74. Lima LD, Ceballos-González AV, Prato A, Cavalleri A, Trigo JR, Nascimento FSd: **Chemical camouflage induced by diet in a pest treehopper on host plants**. *Plants* 2024, **13**:216.

75. Caselli A, Favaro R, Petacchi R, Valicenti M, Angeli S: **The cuticular hydrocarbons of *Dasineura oleae* show differences between sex, adult age and mating status**. *J Chem Ecol* 2023, **49**:369-383.

76. Khabir M, Izadi H, Mahdian K: **Impact of diapause on the cuticular hydrocarbons and physiological status of *Hippodamia variegata***. *J Insect Sci* 2024, **24**:11.