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REVIEW

Genetic Underpinnings of Cuticular Hydrocarbon Biosynthesis in the German Cockroach, *Blattella germanica* (L.): Progress and Perspectives

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Abstract

Insect cuticular hydrocarbons (CHCs) serve as important waterproofing barriers and as signals and cues in chemical communication. Over the past 30 years, numerous studies on CHCs have been conducted in the German cockroach, *Blattella germanica*, leading to substantial progress in the field. However, there has not been a systematic review of CHC studies in this species in recent years. This review aims to provide a concise overview of the chemical composition, storage, transport, and physical properties of different CHCs in *B. germanica*. Additionally, we focus on the biosynthetic pathway and the genetic regulation of HC biosynthesis in this species. A considerable amount of biochemical evidence regarding the biosynthetic pathway of insect CHCs has been gathered from studies conducted in *B. germanica*. In recent years, there has also been an improved understanding of the molecular mechanisms that underlie CHC production in this insect. In this article, we summarize the biosynthesis of different classes of CHCs in *B. germanica*. Then, we review CHCs reaction to various environmental conditions and stressors and internal physiological states. Additionally, we review a body of work showing that in *B. germanica*, CHC profiles exhibit significant sexual dimorphism, specific CHCs act as essential precursors for female contact sex pheromone components, and we summarize the molecular regulatory mechanisms that underlie sexual dimorphism of CHC profiles. Finally, we highlight future directions and challenges in research on the biosynthesis and regulatory mechanisms of CHCs in *B. germanica*, and also identify potential applications of CHC studies in the pest control.

Keywords Cockroach · Cuticular hydrocarbons · Biosynthesis · Sex pheromone · Sexual dimorphism

Introduction

The German cockroach, *Blattella germanica*, is a persistent household pest that degrades indoor environmental quality and poses a significant threat to human health worldwide. *B. germanica* has a diverse diet, and nymphs tend to hide in dark, damp, cluttered, and often unsanitary environments. Furthermore, *B. germanica* can spread various pathogenic microorganisms and allergens, posing a serious risk to human health (Wei et al. 2001; Gore and Schal 2007; Hamu et al. 2014; Kleine-Tebbe et al. 2019; Pomés and Schal 2020; Schal and DeVries 2021). Additionally, *B. germanica* is highly adaptable, difficult to control, and often resistant to available chemical pesticides. The epicuticle of *B. germanica* contains a lipid layer composed of aliphatic hydrocarbons (HCs), alcohols, aldehydes, ketones, fatty acids, esters, and other lipids (Jurenka et al. 1989; Eliyahu et al. 2009; Paszkiewicz et al. 2016; Pei et al. 2022). This lipid layer helps the cockroach avoid excessive dehydration, which

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is especially important for this species as it can easily face water shortages in the indoor environment. In *B. germanica*, long-chain saturated HCs serve as the main class of its cuticular lipids. These HCs are synthesized in the oenocytes and are then transported to the cuticle, ovaries and ultimately to the offspring, playing an important role in retaining water in newly hatched nymphs (Fan et al. 2008). Therefore, studying the cuticular hydrocarbons (CHCs) of *B. germanica* holds potential for providing valuable insights and targets for controlling this species. Furthermore, the CHCs of *B. germanica* are key precursors for the female's contact sex pheromone, which is crucial for mating in *B. germanica* (Nishida et al. 1979; Nishida and Fukami 1983; Chase et al. 1992). In recent years, the publication of genome resources (Harrison et al. 2018) and the use of RNAi technology and gene knockout technology have opened up possibilities for pest control through genetic modification (Lin et al. 2017; Shirai et al. 2022). Genes involved in the synthesis of HCs in the epidermis of *B. germanica* could be a promising target for such control methods. Interfering with the synthesis of these HCs can suppress mating and reproduction of *B. germanica*, offering an alternative method for controlling this pest. Therefore, understanding the synthesis and regulation of HCs in *B. germanica* is of fundamental importance and a promising target for pest control.

The primary function of CHCs in insects is to prevent evaporation of water from the epidermis (Gibbs 1998; Qiu et al. 2012). In many insect species, CHCs in the outermost layer of the cuticle (epicuticle) have been coopted for intra- and inter-specific chemical communications (Howard and Blomquist 2005; Blomquist and Ginzel 2021). The earliest studies on insect CHCs can be traced back to the 1930s. However, due to technical limitations, the CHC components have not been identified at that time. Wigglesworth (1933) initially proposed the existence of a layer of aliphatic or wax esters in the outermost layer of the insect cuticle. Chibnall et al. (1934) were the first to identify the presence of aliphatic HCs in the waxy outer layer of insects and plants, but it was not until the 1960s and 1970s that a large number of insect CHCs were identified due to the development and application of gas chromatography–mass spectrometry (GC–MS) and the capillary column (Nelson and Sukkestad 1970). Studies on the CHCs of *B. germanica* started relatively late, and its CHC components were first analyzed in the 1980s (Augustynowicz et al. 1987; Jurenka et al. 1989). However, in the past 30 years, research on *B. germanica* CHCs has made significant progress. This includes the identification of CHC components and their physicochemical properties, exploration of the synthesis and storage location of HCs, identification of the biosynthetic pathway of CHCs and related catalytic enzymatic genes, revealing the biological significance of CHCs in *B. germanica*. Aspects of the CHCs in *B. germanica* have been reviewed in general reviews of insect CHCs (Gemeno and Schal 2004; Blomquist et al. 2011; Blomquist and Ginzel 2021). In this paper, we aim to review the recent progress in the biosynthesis and regulation of HCs in *B. germanica*, and discuss future research directions in this field.

Characteristics, Biosynthetic Location, Transport and Storage of Hydrocarbons in *B. germanica*

As early as 1918, entomologists were aware of the existence of cuticular lipids in *B. germanica*. However, at that time, the complex surface lipid composition had not been explored (Dusham 1918). The CHC profile of *B. germanica* was first reported in 1987 (Augustynowicz et al. 1987) and further characterized in 1989 (Jurenka et al. 1989). Later, to differentiate the external and internal lipids, Young and Schal improved and standardized methods for extracting CHCs of *B. germanica*, and they also verified the reliability of these extraction and analysis methods (Young and Schal 1997). The CHC profile of *B. germanica* obtained under different extraction conditions were found to be roughly the same, and the sample profile measured in our laboratory is shown in Fig. 1A; Table 1. The CHCs of *B. germanica* are primarily composed of C27–C32 saturated alkanes, with C29 CHCs being the most abundant, followed by C27 CHCs, and monomethyl and dimethyl derivatives were the main components. One of the main functions of these HCs is to prevent cuticular evaporation and water loss, and the physical properties of these HCs, such as melting temperature, largely determines their water retention performance. It is generally believed that longer carbon chain lengths result in higher melting temperatures and better water retention performance, and the introduction of unsaturated bonds or methyl branches lowers the melting temperature and introduces gaps in the CHC layer (Gibbs 1998, 2002). Higher melting temperature indicates better waterproofing ability, but it is important to note that a higher melting temperature of HCs in insect cuticle may compromise other functions of the CHCs, such as in chemical communications. Longer-chain CHCs with higher melting temperatures have lower or even no volatility, which makes them poor candidates for chemical communication. Thus, insects have evolved a delicate balance between various functions of CHCs (Chung and Carroll 2015). The CHCs of *B. germanica* have relatively low melting temperatures. The melting temperature of CHCs from10-day-old female adults is around 37°C, which may be due to the high content of methyl branched components. However, it is worth noting that the melting temperature of CHCs on the epicuticle of *B. germanica* varies

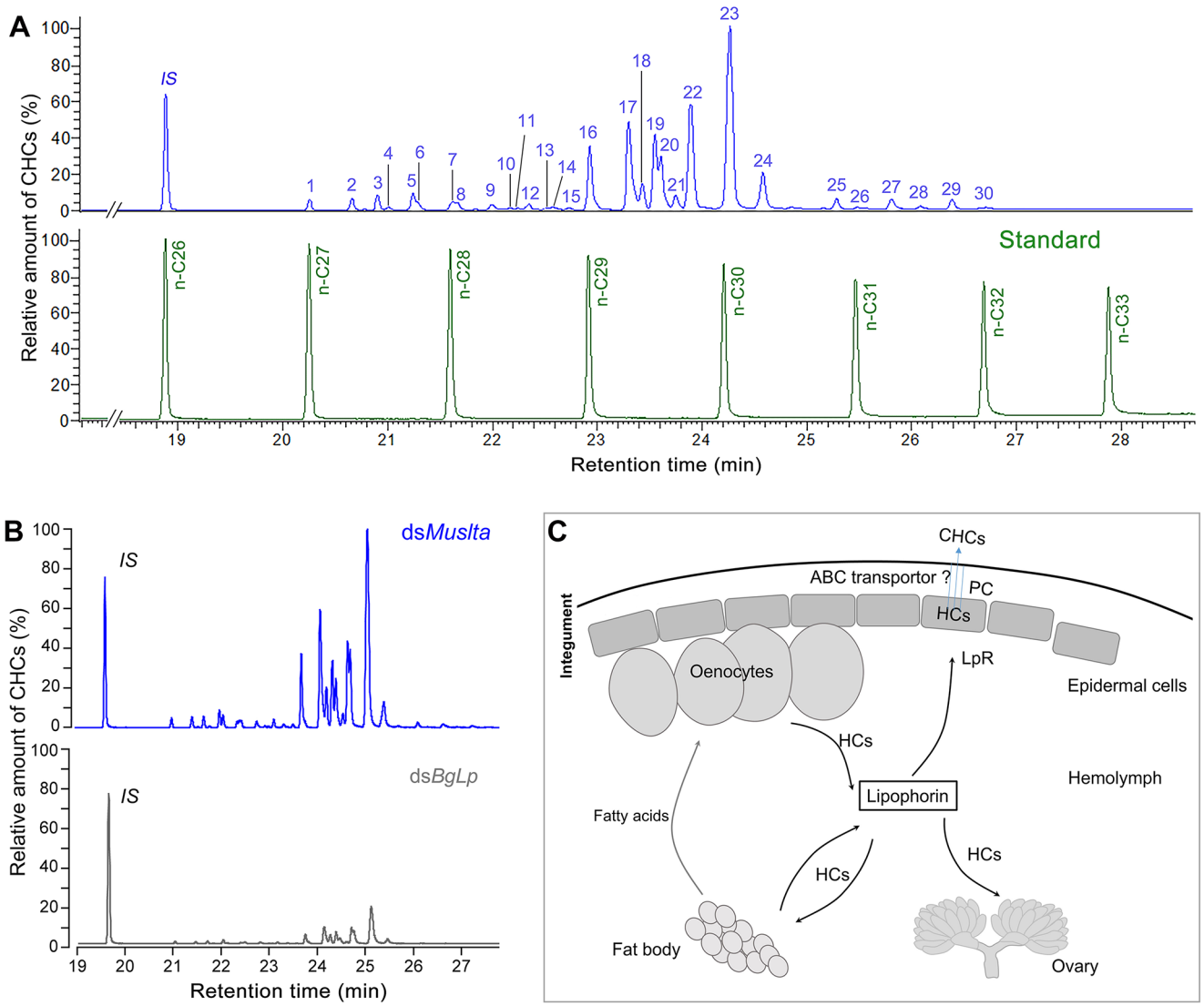

Fig. 1 Cuticular hydrocarbon profiles and its transport and storage in *Blattella germanica*. **(A)** Cuticular hydrocarbon profiles of two-day-old adult female *Blattella germanica*. *IS* represents the peak of n-hexadecane (constant 15 µg) which functions as an internal standard. Different GC peaks are numbered and annotated in Table 1. The lower spectrum depicts the retention times of the normal alkane standards across various carbon chain lengths. Detailed descriptions can be found in Pei et al. (2019). (**B**) Effect of *BgLp*-RNAi on the amount of CHCs in the German cockroach *B. germanica* (Our unpublished data). (**C**) Schematic diagram of the transport and storage of HCs in the German cockroach *B. germanica*. The precursor fatty acids of hydrocarbons can be directly produced by oenocytes or transported to oenocytes from other tissues. HCs: hydrocarbons; CHCs: cuticular hydrocarbons; LpR: Lipophorin receptor; PC: Pore canal

among different developmental stages. Older nymphs and adults have higher melting temperatures than newly molted cockroaches. Furthermore, the melting temperature differs significantly among different tissues, with the CHC melting temperature on the ootheca being the highest at around 49°C, which may be related to the high concentration of *n*-alkanes and monomethyl branched alkanes on the ootheca and lower amounts of dimethyl alkanes of the same chain lengths (Young et al. 2000). The heterogeneity of CHC distribution on the epicuticle of *B. germanica* holds important biological significance since the cost of dehydration can vary greatly among different parts of the body. The ootheca is particularly susceptible to desiccation and *B. germanica* gravid females provision the embryos with water throughout their 3-week embryogenesis (Mullins et al. 2002). However, further investigation is needed to understand how *B. germanica* achieves this heterogeneity of CHC distribution.

Insect CHCs can be directly obtained from food and transferred to the epidermis, but this is not the main route for HC acquisition in insects. Only a small portion of CHCs is transferred to the outer epicuticle of insects that are fed isotopically labeled alkanes, indicating the CHCs are mainly derived from *de novo* biosynthesis (Nelson et al. 1971; Blomquist and Jackson 1973). Initially, insect CHCs were

Table 1 Cuticular hydrocarbon components in the German cockroach *Blattella germanica* (Pei et al. 2019)

Peak No.	Compound	Peak No.	Compound	Peak No.	Compound
1	*n*-C27	11	5-MeC28	21	7,11-DimeC29
2	11-; 13-MeC27	12	4-MeC28	22	3-MeC29 and 5,9-; 5,11-DimeC29
3	5-MeC27	13	Unknown	23	3,7-; 3,9-;3,11-DimeC29
4	11,15-DimeC27	14	3-MeC28	24	7,11-DimeC30
5	3-MeC27	15	Unknown	25	4,8-; 4,10-DimeC30
6	5, 9-; 5,11-DimeC27	16	n-C29	26	Unknown
7	n-C28	17	9-; 11-; 13-; 15-MeC29	27	11-; 13-; 15-MeC31
8	3, 9-; 3,11-DimeC27	18	7-MeC29	28	11,15-; 13,17-DimeC31
9	12-;14-MeC28	19	5-MeC29	29	5,9-; 5,11-DimeC31
10	6-MeC28	20	11,15-; 13,17-DimeC29	30	10,12-DimeC32

Peak numbers are consistent with Fig. 1A, and the writing of different compounds is in shorthand (Me: Methyl; Dime: Dimethyl; n: Normal; Unknown means the identity of the compound was not determined)

believed to be synthesized in the epidermal cells. Locke (1965) proposed that insect CHCs might be synthesized in the oenocytes that are located under the epidermal cellular layer or the surrounding peripheral fat body, and then transported to the outermost layer of the cuticle through pore canals. Later, Diehl demonstrated, using isotopically labeled substrates, that the oenocytes of the desert locust (*Schistocerca gregaria*) catalyzed the synthesis of HCs and found that the newly generated HCs were further released into the hemolymph (Diehl 1973, 1975). Different insect species have varying distributions and localization of oenocytes. The oenocytes of *B. germanica* are located under the epidermis cell layer in the abdomen (Fan et al. 2003; Makki et al. 2014; Chen et al. 2020).

Collaborative studies, particularly by the Schal and Blomquist labs (North Carolina State University and the University of Nevada, respectively), have elucidated the biosynthetic pathways, transport, and storage of HCs using *B. germanica* as a model insect. Gu et al. (1995) dissected different tissues of *B. germanica* for in vitro incubation of isotopically labeled substrates and found that only the abdominal tergites (dorsal cuticle) and sternites (ventral cuticle) synthesize HCs. Additionally, by tracing the appearance sequence of markers after the injection of isotopically labeled substrates, they discovered that labeled HCs first appeared in the hemolymph, and then in the outer surface of the cuticle. Furthermore, blocking the hemolymph circulation significantly reduced the accumulation of isotopically labeled CHCs in the wings, suggesting that HCs synthesized in the oenocytes were first released into the hemolymph and then transported to various tissues. Additionally, the analysis of HC content in various internal organs confirmed the significance of the ovaries, fat body, and hemolymph as crucial internal distribution and storage sites for HCs. *B. germanica* females also provision their oocytes with large amounts of HCs that are later used by neonates to coat their cuticle with the maternal HCs (Fan et al. 2008). Fan et al. (2003) successfully separated the epidermal cells and oenocytes through enzymatic dissociation and Percoll density gradient centrifugation, and provided conclusive evidence that the oenocytes are the site of HC synthesis in *B. germanica* by incubating the two cell types with isotopically labeled propionate, which efficiently gets incorporated into the methyl branches of HCs. The sources of HC precursors may be diverse, even within a species, based on the observations that acetate is incorporated into the aliphatic HC chain, and acetate (see below) can come from a large variety of dietary sources. Propionate as well as the amino acids valine, isoleucine, and methionine serve as the methyl branch donors in *B. germanica* and other species; however, all three amino acids can be metabolized to propionate (see pathways in Jurenka et al. 2017). Thus, it is not surprising that lipids stored in the fat body of *D. melanogaster* can be transported to the oenocytes for the synthesis of HCs (Wicker-Thomas et al. 2015), and our recent study showed that carbohydrates in the diet of *B. germanica* can be converted into lipid precursors that are likely used in HC synthesis (Pei et al. 2023).

HCs synthesized in oenocytes need to be transported to other tissues and organs for storage and use. Due to the strong hydrophobicity of long-chain HCs, which cannot be directly dissolved in the insect hemolymph, the transport of HCs in insects requires the participation of the lipid transport system. During vitellogenesis, large amounts of HCs accumulate in the developing basal oocytes, and a hemolymph high-density lipoprotein, lipophorin (Lp), was found to facilitate the uptake and storage of large amounts of HCs in the egg (Fan et al. 2002). In subsequent studies, Lp was isolated and purified from the hemolymph of *B. germanica*, and was found to contain a large amount of HCs. Since the transport of Lp-loaded HCs to their target sites requires the involvement of Lp receptors which mediate the reception of the HCs (Zhao et al. 2023), these findings raised the possibility that the differences in the HC profiles in different tissues of *B. germanica* may be caused by the selective receptor-mediated unloading of certain HCs from Lp at various target sites (Gu et al. 1995; Sevala et al. 1997; Schal

et al. 1998). In our recent work, we identified a Lp gene from *B. germanica*, *BgLp*, and showed that suppression of *BgLp* through RNAi (The RNAi efficiency surpasses 75%, as is the case for all subsequent RNAi experiments) resulted in significantly smaller amounts of CHCs recovered from the cuticular surface of *B. germanica* (Fig. 1B). This technology should facilitate studies to determine how the selective deposition of HCs in different tissues is accomplished. Additionally, two *BgLpR* genes were identified in *B. germanica* (Ciudad et al. 2007), but the function of *BgLpR* in CHC deposition has not been studied. Based on studies in *B. germanica* and other insects, it is speculated that the HCs are transported to various internal organs or epidermis through Lp after synthesis in the oenocytes (Fig. 1C). However, it is noteworthy that Lp is only responsible for transporting HCs to the epidermal cells, and ABCG transporters are required to further transport HCs from epidermal cells to the outer epidermis through the pore canals in *Locusta migratoria* and *Drosophila melanogaster* (Yu et al. 2017; Zuber et al. 2018; Wang et al. 2020). Whether a similar mechanism exists in *B. germanica* needs further exploration.

Biochemistry and Molecular Biology of HC Biosynthesis in *B. germanica*

ACC Insect HCs originate from the conserved fatty acid biosynthesis pathway. The initial step in HC synthesis involves the conversion of acetyl-CoA to malonyl-CoA, which is catalyzed by acetyl-CoA carboxylase (ACC). ACC is considered to be the limiting factor in fatty acid biosynthesis (Barber et al. 2005). Insects have been extensively studied in relation to the *ACC* gene (Alabaster et al. 2011; Zhang et al. 2015; Wicker-Thomas et al. 2015; Moraes et al. 2022). However, experimental evidence suggesting the direct involvement of *ACC* gene in HC biosynthesis has been available only in *D. melanogaster* (Parvy et al. 2012). In our recent study, we identified a single acetyl-CoA carboxylase gene (*BgACC*) in *B. germanica*. Through RNAi, we successfully inhibited *BgACC* and observed significantly lower amounts of CHCs in *B. germanica*. Our findings suggest that *BgACC* not only patriciates in the HC synthesis process in *B. germanica*, but also converts sugars into lipids, which may contribute to HC synthesis (Pei et al. 2023).

FAS Malonyl-CoA, synthesized by ACC, is further cyclically combined with acetyl-CoA precursor under the catalysis of fatty acid synthase (FAS) to generate long-chain fatty acids with carbon chain lengths of C14, C16, or C18 (Blomquist and Bagnères 2010). The introduction of methyl branches occurs during the early stage of fatty acid chain extension in the German cockroach, as in other insects (Blomquist et al. 1994, 1995; Juárez et al. 1996). The synthesis of 3-methyl or internal methyl branched fatty acids is triggered by acetyl-CoA, and methyl malonyl-CoA, derived from propionate and the amino acids valine, isoleucine, and methionine (which can be converted to propionate) serve as the 2-carbon unit donors. The internal methyl-branched HCs are synthesized, and different classes of methyl-branched HCs are eventually synthesized through differences in the location and number of additions of methyl malonyl-CoAs (Chase et al. 1990). Subsequent studies have shown that microsomal fatty acid synthase and cytoplasmic fatty acid synthase exist in the epidermal tissue of *B. germanica*. Microsomal fatty acid synthase efficiently uses methyl malonyl-CoA to synthesize methyl-branched fatty acids which serve as a substrate of methyl-branched HCs. However, cytoplasmic fatty acid synthase has a low efficiency in the synthesis of methyl-branched fatty acids (Juárez et al. 1992). In the molecular biology exploration of HC synthesis in *B. germanica*, a total of seven fatty acid synthase genes (*BgFas1-7*) were identified. Through RNAi screening, we found that inhibition of *BgFas1* by RNAi reduced the content of both internal and cuticular HCs by about 70%. Among them, methyl-branched CHCs are strongly down-regulated, *n*-C28 and *n*-C29 CHCs are weakly down-regulated, and *n*-C27 CHC is significantly increased (Pei et al. 2019). Therefore, we hypothesize that *BgFas1* is a microsomal fatty acid synthase gene. However, the fatty acid synthase gene involved in the synthesis of straight-chain HCs has not been identified because its precursor fatty acid sources may be diverse, and it may be catalyzed by multiple fatty acid synthase genes.

Elongases Cuticular HCs identified in *B. germanica* are all alkanes, indicating that the biosynthetic pathway does not involve any desaturases. Long-chain acyl-CoA is synthesized under the catalysis of fatty acid synthase and further catalyzed by the fatty acid elongation system to synthesize very-long-chain fatty acids with a carbon chain length exceeding C18. The long-chain fatty acid elongation system consists of four different enzymes, including β-ketoacyl-CoA synthase (KCS), which is often referred to as elongase (Elo) in insects; β-ketoacyl-CoA reductase (KAR); β-hydroxyacyl-CoA dehydrase (HADC); and trans-2-enoyl CoA reductase (TER). The process of carbon chain extension is similar to that of long-chain fatty acid synthesis, and the first condensation reaction is usually the rate-limiting step (Leonard et al. 2004). Previous studies have shown that microsomal proteins from houseflies can convert ^{14}C-labeled stearoyl-CoA into C20 and other carbon chain length products (Vaz et al. 1988), and similar results have been obtained in the *Triatoma infestans* (Juárez and Brenner 1989). Later, Juárez (2004) found that the epidermal microsomal protein of *B. germanica* can extend C16 palmitoyl-CoA into extremely long-chain fatty acyl-CoA of

C18–C32, confirming the involvement of microsomal Elo in insect HC synthesis. In recent years, 24 different fatty acid elongase genes (*BgElo1–24*) have been identified from *B. germanica* based on genomic data (Harrison et al. 2018) and full-length transcriptomic data. Using gene expression analysis and RNAi screening, two key fatty acid elongase genes, *BgElo12* and *BgElo24*, involved in HC synthesis were identified. RNAi of *BgElo12* strongly down-regulates C29 CHCs but up-regulates C27 CHCs. RNAi of *BgElo24* results in strong downregulation of most CHC components. In addition, yeast expression analysis revealed that *BgElo12* can synthesize triacontanoic acid with the addition of octacosanoic acid as the substrate, while BgElo24 enzyme can catalyze the synthesis of octacosanoic acid and triacontanoic acid, indicating that BgElo24 can provide a catalytic substrate for BgElo12 and participate in the synthesis of HCs in *B. germanica* (Pei et al. 2021). Studies on the carbon chain extension system of *B. germanica* have mainly focused on fatty acid elongases, while the other three steps of the enzyme extension system have not been extensively explored.

FAR and CYP4G The last two steps of insect HC synthesis involve the reduction and decarbonylation reaction which are catalyzed by fatty acyl-CoA reductase (FAR) and cytochrome P450 subfamily 4G (CYP4Gs), respectively. It was previously believed that FAR first catalyzed the conversion of very-long-chain fatty acyl-coA into fatty aldehydes, and the fatty aldehydes were converted to HCs under the catalysis of CYP4G (Reed et al. 1994). At present, there is no direct evidence on the catalytic products of FAR, but the FARs that have been identified in insects can only synthesize fatty alcohols (Carot-Sans et al. 2015; Hu et al. 2018; Lienard et al. 2010; Teerawanichpan et al. 2010). Recent studies have reported that CYP4Gs derived from mountain pine beetle (*Dendroctonus ponderosae*) can convert both long and short chain fatty alcohols or aliphatic aldehydes into HCs, and its catalytic activity on fatty alcohols is stronger, suggesting that CYP4Gs may first convert fatty alcohols into aliphatic aldehydes. Fatty aldehydes are then converted to HCs by decarbonylation, i.e. the catalytic product of FAR may be fatty alcohols rather than fatty aldehydes (MacLean et al. 2018). There are few studies on the function of FAR in HC synthesis in insects. Two FAR genes have been identified in the planthopper *Nilaparvata lugens* to be involved in the synthesis of HCs, among which *NlFAR7* has a greater effect on HC synthesis. *NlFAR* RNAi prevents *N. lugens* from walking or jumping on the water surface, making *N. lugens* more likely to die upon contact with water (Li et al. 2019, 2020). A recent study on the mealybug *Phenacoccus solenopsis* also identified a *PsFAR* gene involved in CHC and cuticular wax synthesis (Tong et al. 2022).

In *B. germanica*, we recently found that multiple *FAR* genes participate in the synthesis of HCs at the same time. Simultaneous RNAi inhibition of five *FAR* genes with high expression in the integument strongly suppressed the amount of CHCs on the cuticle, while RNAi of each *BgFAR* gene found that inhibition of *BgFAR5* interfered with the synthesis of C27 components of CHCs. Meanwhile, simultaneous inhibition of *BgFAR1 + 2 + 3 + 4* decreased C29 CHCs but increased C27 CHCs (Fig. 2), indicating that *BgFAR5* is involved in C27 HC synthesis in *B. germanica*, while the synthesis of other HCs may be catalyzed by multiple *BgFAR* genes. We speculated that the FAR product in the HC synthesis pathway of *B. germanica* might be a fatty alcohol, but this needs to be verified, possibly with yeast expression analysis. The last step of HC synthesis is catalyzed by the cytochrome P450 4G family. There is only one CYP4G gene, *CYP4G19*, in *B. germanica*, and inhibition of *CYP4G19* by RNAi significantly decreased the amount of CHCs on the cuticular surface (Chen et al. 2020). We speculate that CYP4G19 can convert fatty alcohols with different carbon chain lengths into aldehydes, and then synthesize HCs with different chain lengths through decarbonylation. However, more studies need to be performed to verify this hypothesis.

At present, using genome data, transcriptomic methods and RNAi technology, we have explored the major enzymatic genes involved in HC biosynthesis of *B. germanica* as summarized in Fig. 3. A major challenge is to understand how these genes achieve tissue- and sex-specific plasticity of the CHCs in *B. germanica*, and how the cockroach adjusts the expression of key genes in response to changes in the external environment to generate CHC profiles that are consistent with functional requirements.

Research Progress in the Regulation of HC Biosynthesis in *B. germanica*

Plasticity Insects display a high level of plasticity in their CHC profiles (Thomas and Simmons 2011; Stinziano et al. 2015; Li et al. 2021). The CHCs of *B. germanica* are composed of approximately 30 compounds and can be categorized based on carbon chain lengths and the number and positions of methyl branches. The composition of CHCs in *B. germanica* is not fixed and shows significant variations in different tissues, developmental stages, and external environments. The presence of methyl-branched HCs in *B. germanica* is primarily determined by FAS. Although the upregulation of the *BgFas1* gene has been observed in dry environments, the proportional changes in methyl-branched CHC components in *B. germanica* CHCs under long-term dry conditions have not been analyzed (Pei et al. 2019).

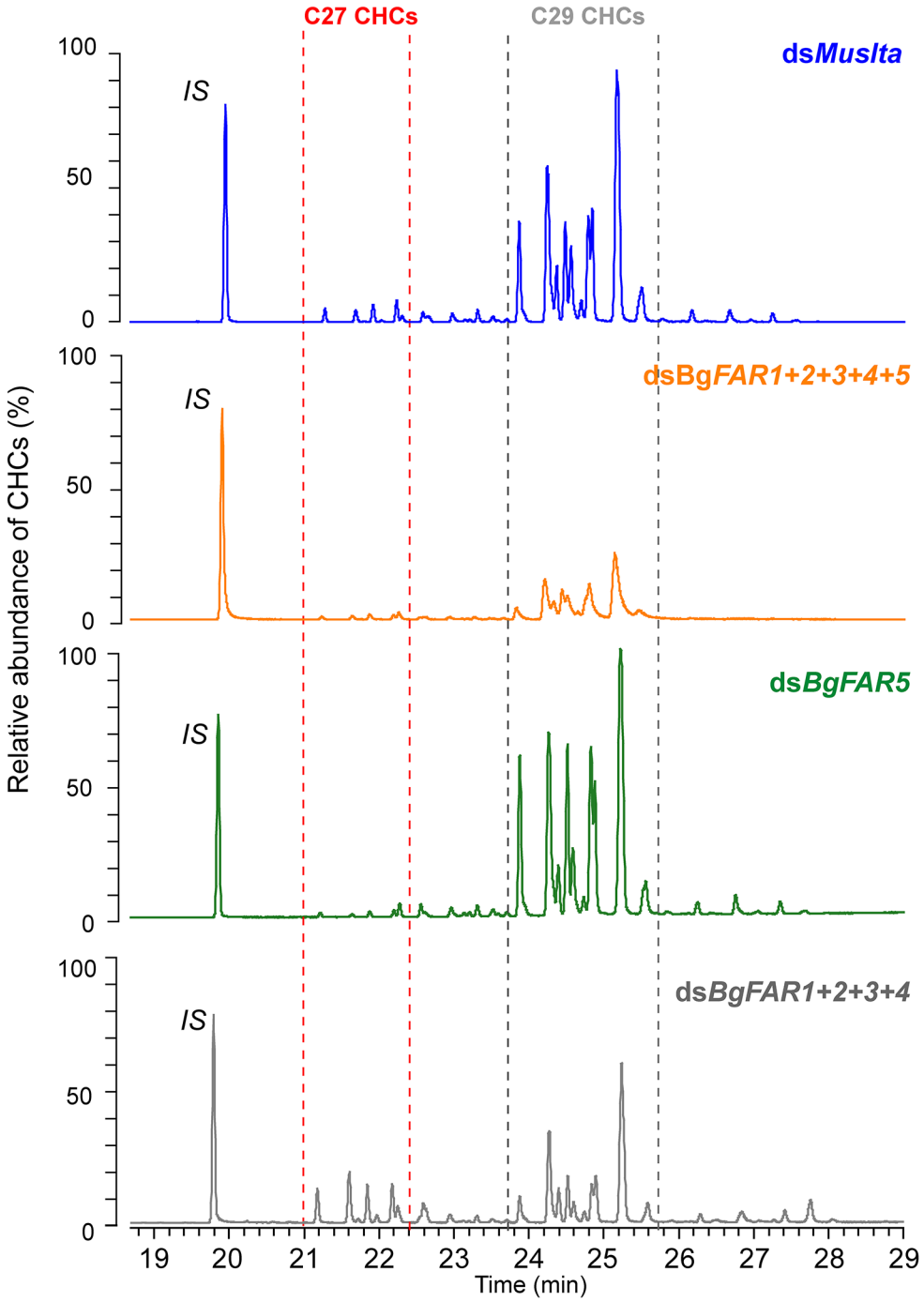

Fig. 2 Effects of *BgFAR*-RNAi on the content of CHCs in the German cockroach *Blattella germanica*. RNAi knockdown of *BgFAR1 + 2 + 3 + 4 + 5* dramatically reduced C29 and C27 CHCs, RNAi knockdown of *BgFAR5* only reduced C27 CHCs, RNAi knockdown of *BgFAR1 + 2 + 3 + 4* reduced C29 CHCs, but significantly increased C27 CHCs (Our unpublished data)

Another aspect of plasticity of CHCs in *B. germanica* is the alteration of the average carbon chain length, which has previously been attributed to elongases. However, our research suggests that the FAR gene also plays a role in selectively changing the carbon chain length of HCs. Female *B. germanica* have longer average carbon chain lengths and also survive longer in dry environments. However, we have not extensively examined how the average carbon chain length of CHCs in *B. germanica* adapts to fluctuations in the internal and external environments, specifically through modifications in gene expression levels.

Abiotic influence Many insects undergo changes in the composition of their CHC profile during external environmental fluctuations (Etges et al. 2016; Menzel et al. 2017; Otte et al. 2018). One of the main functions of CHCs in insects is to counteract excessive evaporation and water loss in dry environments, and inhibiting the synthesis of CHCs in *B. germanica* can lead to death due to excessive dehydration in such conditions (Pei et al. 2019). In addition, we found that

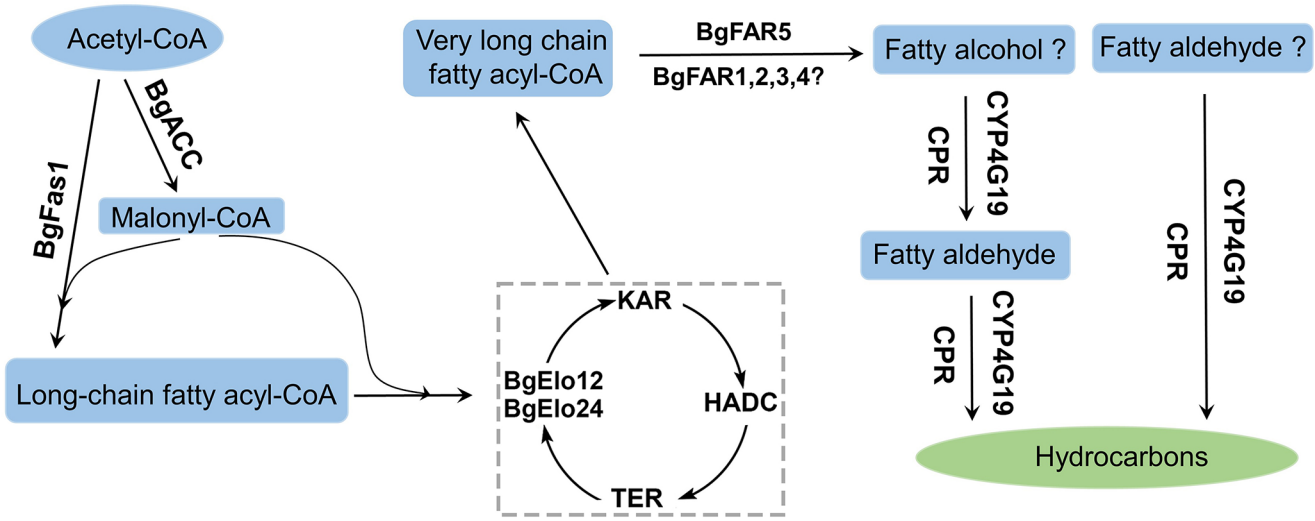

Fig. 3 Summary of the current knowledge for the biosynthetic pathway of CHCs in the German cockroach *Blattella germanica*. Detailed descriptions can be found in Pei et al. (2021). Figure is adapted and modified from Howard and Blomquist (2005), Blomquist and Bagnères (2010), Chung and Carroll (2015), and Blomquist and Ginzel (2021)

disrupting the melanin synthesis gene impairs the cuticular melanization process in *B. germanica*, which significantly increased cuticular permeability, making the cockroach more susceptible to dehydration and ultimately causing its death. We also observed a substantial increase in the amount of CHCs in this scenario. Furthermore, the expression level of the *CYP4G19* gene was significantly up-regulated (Bai et al. 2022), a likely adaptive compensation mechanism for the CHC levels in response to severe dehydration.

Another function of CHCs is to act as a barrier to xenobiotics, and the composition of the CHCs in the German cockroach undergoes significant changes in response to sublethal doses of chlorpyrifos (Paszkiewicz et al. 2016). In resistant strains of German cockroaches, the amounts of CHC are significantly higher compared to susceptible strains. The expression level of the *CYP4G19* gene is also significantly higher in the resistant strains. Inhibiting the *CYP4G19* gene leads to lower CHC amounts increased cuticular permeability and sensitivity to chemical pesticides (Chen et al. 2020). These findings suggest that CHCs serve as adaptive barriers to exogenous substances such as chemical pesticides, which can change in response to external abiotic conditions.

Nutrition and hormones The biosynthesis of HCs in *B. germanica* is influenced by feeding and developmental stages (Schal et al. 1994; Young and Schal 1997). Building on these reports, we conducted further research to examine the impact of nutritional and hormonal signals on the synthesis of HCs in *B. germanica*. Inhibition of the insulin receptor (*BgInR*) using RNAi resulted in a slight but significant decrease in the overall amount of CHCs in *B. germanica*, and *BgInR*-RNAi shows similar effects on CHCs with different carbon chain lengths (Fig. 4). The insulin signal has also been reported to regulate the biosynthesis of HCs in *D. melanogaster* (Kuo et al. 2012), indicating that nutrient signals can indeed regulate the biosynthesis of HCs in insects, and this regulation may be relatively consistent across different species. Additionally, we discovered that the down-regulation of the primary response gene *Krh1*, which is involved in juvenile hormone signaling, did not have a significant effect on CHCs in *B. germanica* (Fig. 4). This suggests that juvenile hormone in *B. germanica* does not play a major role in regulating the biosynthesis of HCs. However, in *D. melanogaster*, juvenile hormone receptors (Met and Gce) indeed regulate the biosynthesis of specific HC components (Bilen et al. 2013), indicating that there may be variations in the regulatory mechanisms among different insect species. Furthermore, ecdysone and bioamines have been reported to participate in the regulation of HC biosynthesis in various insects (Adams et al. 1984; Marican et al. 2004; Baron et al. 2018). Therefore, it is necessary to conduct further research to determine whether these factors also play a role in regulating HC synthesis in *B. germanica*. Overall, understanding the regulation of HC synthesis in insects is a complex and diverse topic, with potentially different modes of regulation across different insect lineages and species. Further investigation in *B. germanica* will provide valuable insights into this process.

Sexual dimorphism Cuticular hydrocarbons of *B. germanica* adults exhibit obvious sexually dimorphic characteristics. The CHC patterns of males and females are nearly identical within two days after adult emergence. However, at day 3, differences in CHC profiles between males and females begin to emerge and gradually expand, resulting in distinct differences on the sixth day. With sexual maturity,

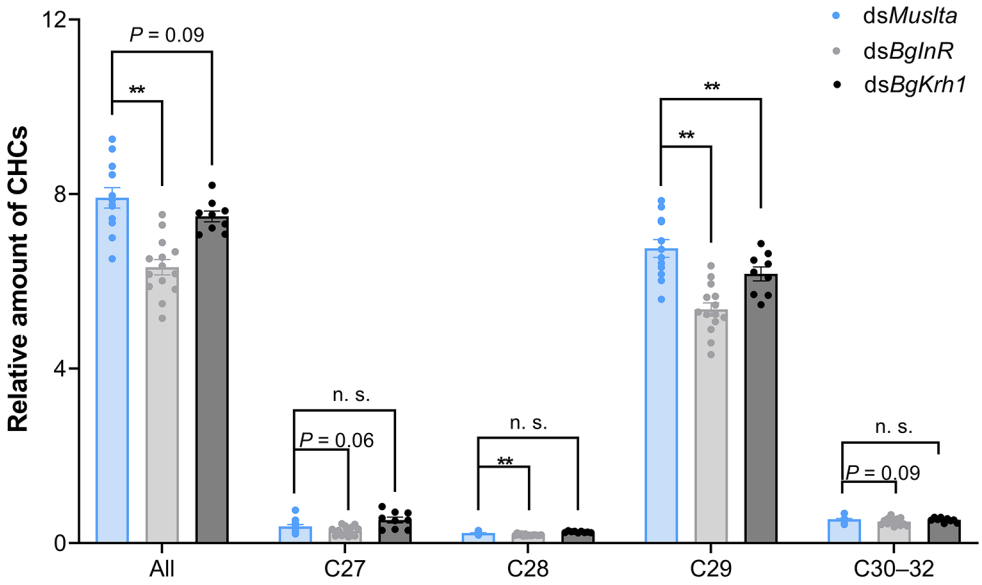

Fig. 4 Effect of *BgInR*- and *BgKrh1*-RNAi on the content of CHCs in the German cockroach *Blattella germanica*. Data are shown as mean ± SD, and each replicate is shown as a dot. $^{**}P < 0.01$, two-tailed Student *t*-test; $n = 9–14$

there is a significant reduction in male C29 CHCs and 3,7-; 3,9-; and 3,11-DiMeC29 (the main female contact sex pheromone precursor), while there is a significant increase in C27 CHCs and 9-; 11-; and 13-; 15-MeC29. Conversely, the CHC profile of adult females remains relatively stable, consistently maintaining a high proportion of C29 CHCs and 3,7-; 3,9-; and 3,11-DiMeC29 (Wexler et al. 2019; Pei et al. 2021). The sexual dimorphism of CHCs in *B. germanica* holds significant biological importance, as females-enriched 3,7-; 3,9-; and 3,11-DiMeC29 serve as the immediate precursor for the biosynthesis of the major components of contact sex pheromones (Jurenka et al. 1989; Schal et al. 1990; Chase et al. 1992; Eliyahu et al. 2008a, b). The high concentration of contact sex pheromone precursors in females contributes to maintaining a heightened level of mating competitiveness. Our recent studies have identified the highly expressed fatty acid elongase gene *BgElo12* in females as a key determinant of the formation of sexually dimorphic CHCs (Pei et al. 2021). Inhibition of *BgElo12* hampers the establishment of the female-like CHC profile and significantly decreases the content of contact sex pheromone. This also leads to longer latency of male courtship wing raising responses and lower frequency of male wing raising during courtship (Fig. 5A).

Furthermore, the expression of *BgElo12* is transcriptionally regulated by the sex-differentiation genes. In females, *Doublesex-M*ale (*Doublesex-M*) undergoes splicing of Transformer to form the *Doublesex-Female* (*Doublesex-F*) isoform, enabling the normal expression of *BgElo12*. Conversely, in males, the lack of functional splicer *Transformer* results in the *Doublesex-M* isoform, which represses the expression of *BgElo12*. As a result, sexually mature male cockroaches possess only a minimal amount of C29 CHCs, while sexually mature females maintain a high level of sex pheromone precursors (Fig. 5B) (Wexler et al. 2019; Pei et al. 2021). Similarly, our early studies demonstrated that the catalytic oxidation of precursor HC to contact pheromone in *B. germanica* is positively regulated by juvenile hormone (Chase et al. 1992). Our recent studies have shown that the female-specific *CYP4PC1*, which catalyzes the synthesis of contact sex pheromones, is not only regulated by juvenile hormone, but also transcriptionally inhibited by *Doublesex-M* in male cockroaches (Chen et al. 2022). In *D. melanogaster*, sex differentiation genes also regulate the synthesis of CHCs and sex pheromone (Ferveur et al. 1997; Chertemps et al. 2007). Many other insects exhibit sexual dimorphism in their CHCs (Thomas and Simmons 2008; Jennings et al. 2014; Butterworth et al. 2020), and we speculate that sex differentiation signals may play a key role in regulating sex-specific CHCs and sex pheromone biosynthesis in some of them.

Conclusions and Future Directions

In the past 35 years, significant progress has been made in the study of HCs in *B. germanica*, specifically in the fields of chemical ecology, biochemistry, and molecular biology of HC synthesis. Important catalytic enzymes and corresponding genes related to HC biosynthesis have been extensively studied in the past decade. However, there are still unresolved issues that warrant further exploration. Currently, the primary method used to identify the CHC components in *B. germanica* is GC-MS, and only CHCs with carbon chain length of C27–C32 were identified. However, using matrix-assisted laser desorption/ionization mass spectrometry, Cvačka et al. (2006) discovered that insects possess HCs with chain length up to more than 70 carbons. Additionally, Sutton et al. (2013) utilized high-temperature GC-MS to identify HC components in insects with carbon chain lengths ranging

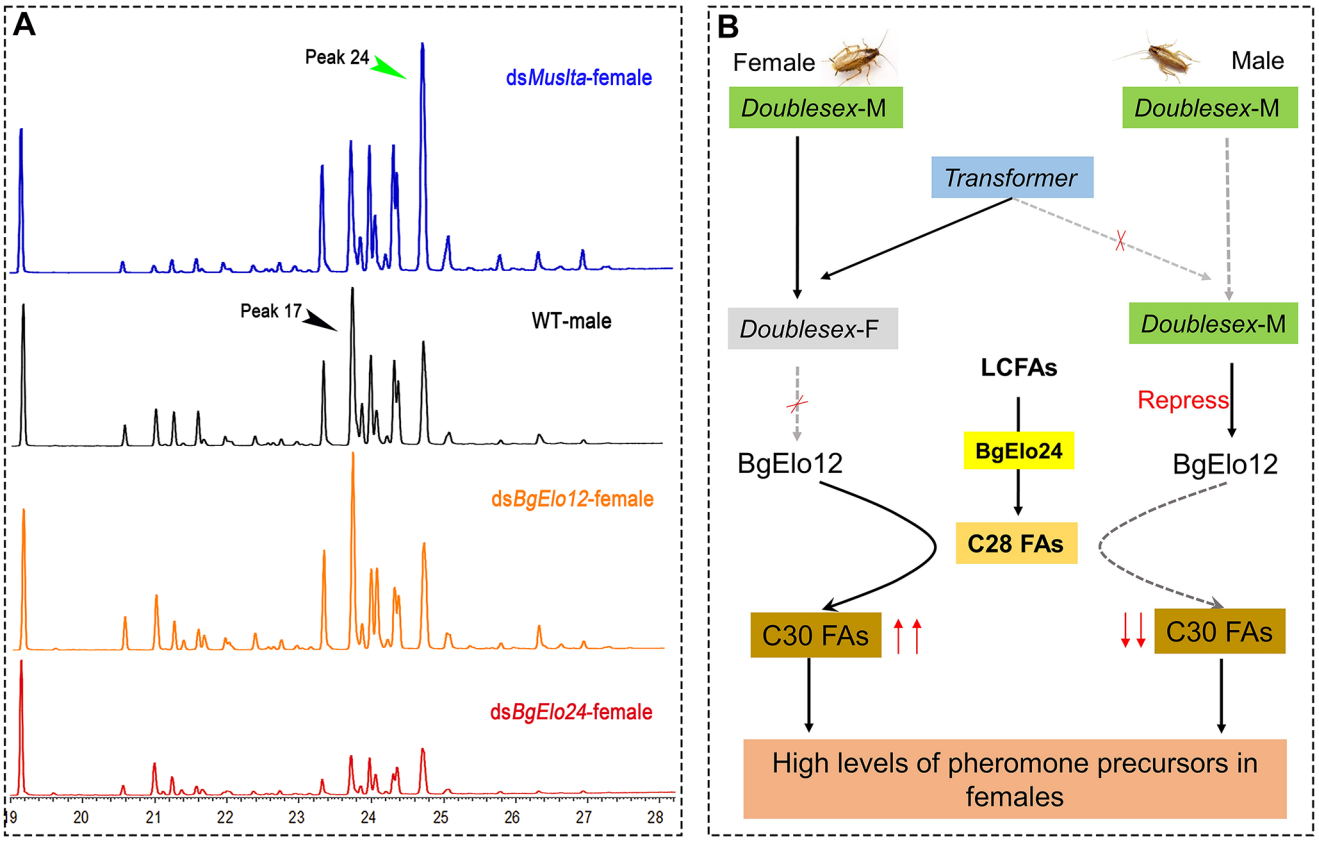

Fig. 5 The mechanism underlying the formation of sexually dimorphic cuticular hydrocarbon (CHC) profiles. (**A**)Effect of *BgElo12*- and *BgElo24*-RNAi on the sexually dimorphic CHC profile in the German cockroach *B. germanica*. Detailed descriptions can be found in Pei et al. (2021). (**B**) Model for the generation of sexually dimorphic CHC profiles in *B. germanica*. Both BgElo12 and BgElo24 participated in HC biosynthesis, but BgElo24 is a basic fatty acid elongase which catalyzes a wide range of substrates and provides C28 FAs for BgElo12 to generate specific C30 FAs. The male-specific *Doublesex* (*Doublesex-M*) represses the transcription of *BgElo12* in males. However, Transformer only functions in females, and can splice *Doublesex-M* to the non-functional female type *Doublesex* (*Doublesex-F*); thus, *BgElo12* is highly expressed in females and generates more C30 FAs. A high level of C30 FAs produces more contact sex pheromone precursors in females. Detailed descriptions can be found in Pei et al. (2021)

from C25 to C62. Golian et al. (2022) used a novel approach of silver-assisted laser desorption/ionization mass spectrometry (Ag-LDI-MS) which can also detect higher chain length compounds (approx. C39–C50) and discovered CHCs with chain lengths even beyond C40 for the German cockroach *B. germanica*. Therefore, it remains to be elucidated if and how these very long chain HC components affect desiccation tolerance and chemical communication in *B. germanica*.

The genes involved in the HC biosynthesis pathway of *B. germanica* – fatty acid synthases, fatty acid elongases, and fatty acyl-coA reductases – are all members of large gene families. Our research suggests that there may be multiple homologous genes responsible for catalyzing HC synthesis in *B. germanica*. However, we have only utilized RNAi technology to suppress the expression of these genes, without considering gene variants and isoforms, or employing genome editing techniques to screen for other potential catalytic enzyme genes that might be involved in HC synthesis. Taking the *BgFAR* gene as an example, we found five genes involved in HC synthesis, but it is puzzling why *B. germanica* evolved multiple genes with repetitive functions for HC synthesis, how these five *BgFAR* genes coordinate to participate in HC synthesis, and how they respond to internal and external environmental conditions. Additionally, when we expressed *BgElo* in yeast, C28 and C30 very-long-chain fatty acids were generated. Consequently, this approach provides an opportunity to further verify *BgFAR* products through the expression of various *BgFAR* genes and their co-expression with *BgElo*. The precise role of *FAR* gene products in HC synthesis in insects remains unclear, making it of great importance to uncover the catalytic products of *BgFAR* in HC biosynthesis.

Understanding the transcriptional regulation of HC synthetic genes is another important research direction where the *B. germanica* system might be valuable. The German cockroach is highly adaptable to environmental stressors, including the onslaught of insecticides to suppress infestations and the low humidity of indoor spaces. Desiccation stress, pesticide challenge, and internal physiological state fluctuations can all

induce the up- or down-regulation of HC biosynthetic genes in *B. germanica*, thereby altering the CHC profile and enhancing stress tolerance and resistance. However, currently, no relevant studies have investigated the mechanism through which *B. germanica* detects environmental stress, signal transduction, and transcriptional regulation of genes involved in HC biosynthesis. In other insects, it has been reported that HC synthesis is regulated by factors such as Hepatocyte Nuclear Factor 4 and Oxytocin/vasopressin-like peptide, which respond to physiological and environmental changes, and modulate the expression of HC biosynthetic genes (Koto et al. 2019; Storelli et al. 2019; Sun et al. 2023). Therefore, further exploration of the factors governing HC synthesis in *B. germanica* is necessary.

Cuticular hydrocarbons play crucial physiological and chemical communication roles in *B. germanica*, contributing to its adaptation to the domestic environment, high proliferation rate, and rapid worldwide spread as a public health pest. Achieving control of *B. germanica* through flourishing biotechnology is one of the main objectives of our ongoing research. At present, dsRNA pesticides have emerged as a potentially effective method in pest control (Baki et al. 2020; Long et al. 2023; Ligonniere et al. 2024). Many genes from the HC biosynthesis pathways and contact pheromone synthesis are potential targets for dsRNA pesticides. However, further experimental assessment is needed in this research field. In addition, direct parental CRISPR has been successfully implemented in *B. germanica* (Shirai et al. 2022), and our laboratory experiments have also demonstrated the simplicity and efficiency of this method in obtaining homozygous mutants. By using genome editing technology to obtain mutants of crucial genes in the HC (or contact sex pheromone) biosynthetic pathway in *B. germanica*, we can effectively assess their stress tolerances and mating abilities. By integrating these findings with population control principles, substantial advancements can be achieved in the integrated management and eradication of *B. germanica* populations.

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Declarations

Competing Interests The authors declare no competing interests.

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