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Extraction and analysis of cuticular hydrocarbons in the weaver ant *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae)

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Abstract
Ants (Hymenoptera: Formicidae) are one of the most diverse and ubiquitous groups of social insects the largest family under the order Hymenoptera. In insects, cuticular hydrocarbons (CHC) function as chemical cues for the recognition of mates, species, and nest-mates in social insects. The surface of insects is covered by a complex mixture of cuticular hydrocarbons (CHCs) to prevent desiccation. In this study, the cuticular hydrocarbons profiles (CHCs) of weaver ant *Oecophylla smaragdina* workers was extracted and analyzed in Gas Chromatography and Mass Spectrometry (GC-MS). The results reveal that *O. smaragdina* CHCs extract composed straight-chain and branched-chain alkanes and alkenes. A total of 18 CHCs were identified in the workers of *O. smaragdina*. The major cuticular hydrocarbons such as Hexadecane ($C_{16}H_{34}$), Octacosane ($C_{28}H_{58}$), Octadecane,9-ethyl-9-heptyl ($C_{27}H_{56}$), Heptacosane ($C_{27}H_{56}$), Eicosane,9-octyl ($C_{28}H_{58}$) and Pentatriacontane ($C_{35}H_{72}$). These cuticular hydrocarbons might be acted as a chemical messenger between the ant colony, nest, nest-mate recognition of the weaver ant *O. smaragdina.*

Keywords: Formicida, *Oecophylla smaragdina,* Cuticular hydrocarbons, GC-MS and Hexadecane

1. Introduction
Ant is one of the most diverse and universal groups of the social insect. Ant belongs to a single large family Formicidae, largest of order Hymenoptera. The ant, *Oecophylla smaragdina* Fab. (Subfamily: Formicinae) is also known as the weaver ant, green ant, Kerengga red ant, emerald leaf dweller or Indian tree ant. It is distributed in the tropical Old World from India to Taiwan and across Southeast Asia to Australia [1]. This ant nest is arboreal, seen on trees or shrubs and made of living leaves. The workers construct the nest by gluing the edges of adjacent leaves using the sticky silk secreted by the larvae [1].

Oecophylla ants also feed on nectaries and insects. The ant *O. smaragdina* is a vigilant and territorial predator of many insect pests in a number of horticultural crops [1]. Ambethgar [2] studied, that the prevalence of the weaver ant *Oecophylla smaragdina* is effectively controlled the pest *Helopeltis* populations in cashew plantings of Tamil Nadu and Kerala in South India. The tree inhabiting red weaver ants *Oecophylla smaragdina* (Fabricius) (Formicidae: Hymenoptera) are known to control over fifty species of insect pests on many tropical tree crops across the world [2].

The cuticular hydrocarbons (CHC) profiles of social insects encode the information necessary for maintaining colony identity and social unity [3]. In ants, hydrocarbons comprise a vital constituent in nestmate recognition. They are spread throughout the epi-cuticular surface so that recognition of nestmate or extraterrestrial ants is instantly attained upon antennal contact [4, 5].

Hydrocarbons are distributed all over the epicuticular surface of all insects. In most insects, they serve as an antidesiccant layer, but can also serve as communication signals [5-7]. The epicuticle of insects is protected against dehydration and pathogen attack by a hydrophobic layer of lipids [8, 9]. This layer is typically composed of a complex mixture of straight-chain and methyl-branched alkanes and alkenes (commonly referred to as cuticular hydrocarbons, CHCs) [3, 10]. Cuticular hydrocarbons (CHCs) are synthesized in tissues from the abdomen of the ants (epidermis, fat body, oenocytes) [5].

The cuticular hydrocarbons profiles of insects usually are composed primarily of straight-chain and branched-chain alkanes and alkenes of 19–35 carbons [11, 3] and small quantity of long-chain hydrocarbons, triacylglycerides and oxygenated compounds were also present [12-14].
The non-polar cuticular hydrocarbons have been expansively investigated by a number of authors, with respect to their role as semiochemicals [5, 6, 9]. Soroker and Hefetz [5] studied the *Cataglyphis niger* cuticular hydrocarbons are biosynthesized in the fat body and secreted to the haemolymph where they are transported both to the postpharyngeal gland and the epicuticle The non-hydrocarbon cuticular compounds (triacylglycerides) are important components of pheromone blends [9], interacting synergistically with cuticular hydrocarbons [15]. Brunner [16] *et al*. found that the profiles of the queen and worker ants of six *Temnothorax* species were dominated by linear and branched alkanes.
All members of a colony bear a universal odor, the colonial odor, but some members may show individual chemical signals on their cuticle that are not mixed in the common pool. Denis [17] *et al*., reported that cuticular hydrocarbons are involved in fertility signaling in ants. When numerous queens occur in the same colony, the cuticular hydrocarbons offer information on the degree of fecundity and workers are competent to differentiate among queens with disparate fecundities [18].
Several authors have confirmed the usefulness of cuticular hydrocarbons for identifying insect species, including parasitic wasps [19], Culicids [20], Anophelines [21, 22], Triatomines [23, 24], Phlebotomines [25, 26] and Sarcophagidae [27]. The recognized cuticular hydrocarbons were usually a fusion of n-alkanes, monomethyl alkanes and dimethyl alkanes with linear chain lengths varying from 23 to 33 carbons [19, 21, 27].
Weaver ant nest are formed basically of living leaves and stems bound together with larval silk. *Oecophylla smaragdina* species was rich in the coconut field in cultivated areas [28]. In this study, it was found that weaver ant nests hanging on the trees (Pungam and Neem) being aggressive predators and territory defense, they sometimes drop down their nest and tree branches on the ground for foraging and defense. The present study, extraction, and analysis of cuticular hydrocarbons in the ant weaver *Oecophylla smaragdina* (Fabricius, 1775) has been discussed.

2. Materials and Methods

2.1. Ant collection

Colonies of red ant *Oecophylla smaragdina* (Fabricius, 1775) belong to the subfamily Formicinae. Colonies were collected from the *Pongamia pinnata* tree, near the department of Zoology, Pachaiyappa's College for Men Campus (PACM), Kanchipuram (KPM) during January 2015. The ant *Oecophylla smaragdina* nest was covered by thin plastic covers (60 micron thickness) for minimize the disturbances and collection of the worker ants. Kanchipuram is situated on the northern East Coast of Tamil Nadu and is adjacent to Bay of Bengal and Chennai (Latitude: 12.8341735ºN and Longitude: 79.7036402ºE) India.

2.2. Cuticular Hydrocarbons extraction (CHCs).

In the zoology laboratory, the worker ant *O. smaragdina* (1:1 ratio: 30no's/30mL) whole body were freeze killed for 20 min and submerged, in 30 mL of non-polar solvent n- hexane (HPLC grade; Sigma-Aldrich) for 48 hrs, in 25^{0}C for extraction of CHCs. The weaver ant *O. smaragdina* extract (CHCs) was filtered in Whatman No.1. filter paper, the filtered extract (CHCs) was directly injected to Gas Chromatography and Mass Spectrometry (GC -MS).

2.3. Gas Chromatography and Mass Spectrometry analysis (GC-MS).

The weaver ant, *Oecophylla smaragdina* workers cuticular hydrocarbons were analyzed by Gas Chromatography and Mass Spectrometry (GC-MS). Gas Chromatography and Mass Spectrometry [JEOL GCMATE II -GC-MS] system equipped with a quantitative analysis by SIM mode detector, An HP5 Column 30.0m x 250µm and the 0.25µm film thickness were used. The oven was programmed from an initial temperature 60 ºC (hold for 2 min) to the final temperature 300 ºC at the rate of 20 (36.0 minutes). The final temperature hold up time was 06 minutes. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode (Oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min, Inj A auto=250°C, Split=10:1, Carrier Gas=Helium, Solvent Delay=2.00 min, Transfer Temp=240°C, Source Temp=240°C and Scan: 10 to 600Da). NIST version 2 library search was used for CHCs analysis.

Fig 1: The weaver ant *O. smaragdina* colony in Pachaiyappa's College for Men, campus, Kanchipuram Tamilnadu (January -2015)

3. Results and Discussion

3.1. Cuticular hydrocarbon profiles

In this study, the ant *O. smaragdina* CHCs extract composed mainly straight-chain and branched-chain alkanes and alkenes. A total of 18 CHCs were identified in the workers of *O. smaragdina* (Fig.2). The cuticular hydrocarbon (CHCs) of *Oecophylla smaragdina* are a complex mixture of alkanes, alkenes, and methyl-branched alkanes ranging from C16 to C35.

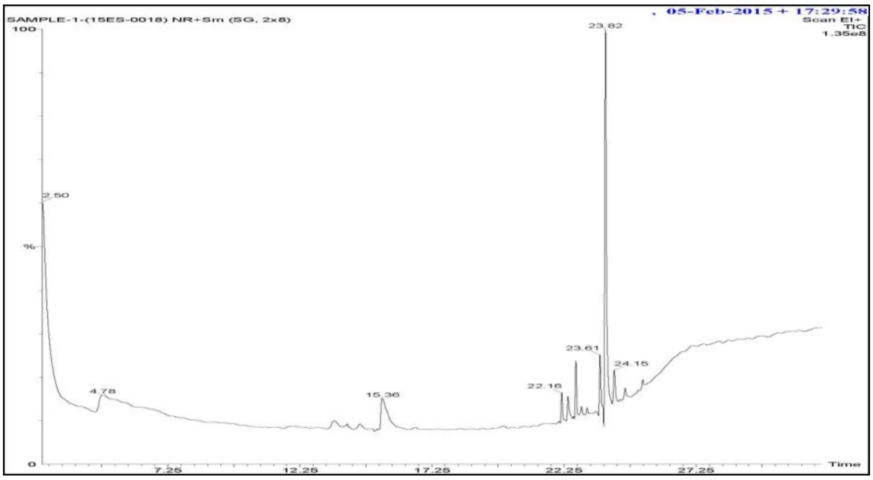


Fig 2: GC-MS chromatograms of cuticular extracts of ant *O. smaragdina* worker.

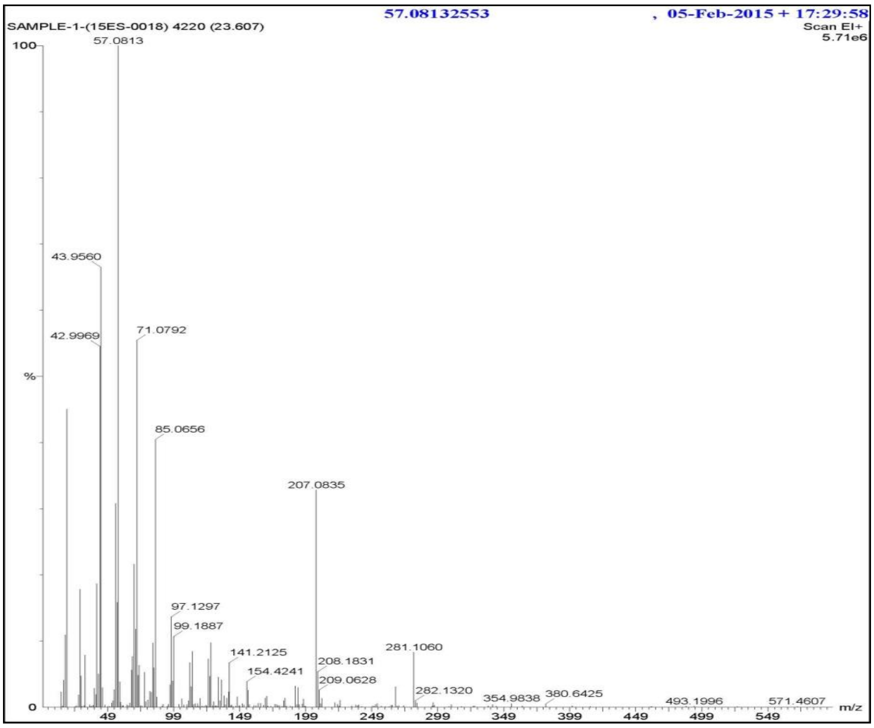


Fig 3: GC-MS chromatograms of cuticular extracts of ant *O. smaragdina* worker.

Among them, the four hydrocarbons, Pentatriacontane, Eicosane, 9-octyl, Hexacosane, 9-octyl, and Octacosane were present in high proportions (area range 12.65 -59 %) in the *O. smaragdina* CHCs extract. The identified CHCs were mostly a mixture of n-alkanes, monomethyl alkanes, and dimethyl alkanes with linear chain lengths varying from C16 to C35 carbons (Fig. 3). It may be acted as a chemical messenger between the ant colony and nest recognition.

The weaver ant, *O. smaragdina* workers epicuticle, consists of hydrocarbons such as Hexadecane ($C_{16}H_{34}$), Octacosane ($C_{28}H_{58}$), Octadecane,9-ethyl-9-heptyl ($C_{27}H_{56}$), Heptacosane ($C_{27}H_{56}$), Eicosane,9-octyl($C_{28}H_{58}$), Pentatriacontane ($C_{35}H_{72}$), Hexacosane, 9-octyl ($C_{34}H_{70}$), Nonadecane ($C_{19}H_{40}$), Heneicosane ($C_{21}H_{44}$), Heptadecane,2,6,10,15-tetramethyl- ($C_{21}H_{44}$), Triacontyl- trifluoroacetate ($C_{32}H_{61}O_2F_3$), Tetracosane ($C_{24}H_{50}$), Eicosane ($C_{20}H_{42}$), Heptadecane,8,8-dipentyl ($C_{27}H_{56}$), 2-Methyldocosane ($C_{23}H_{48}$), Docosane, 2,4-dimethyl- ($C_{24}H_{50}$), 15-Methyltritriacontane ($C_{34}H_{70}$) and 9-Methyltritriacontane ($C_{34}H_{70}$) (Fig. 4, 5 and 6).

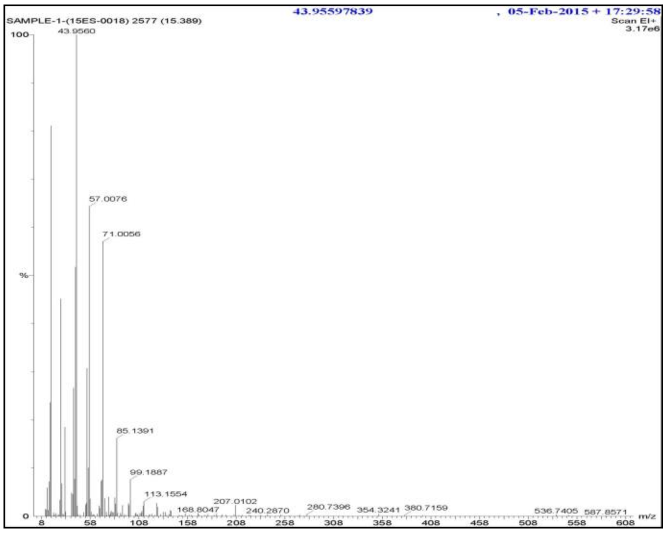
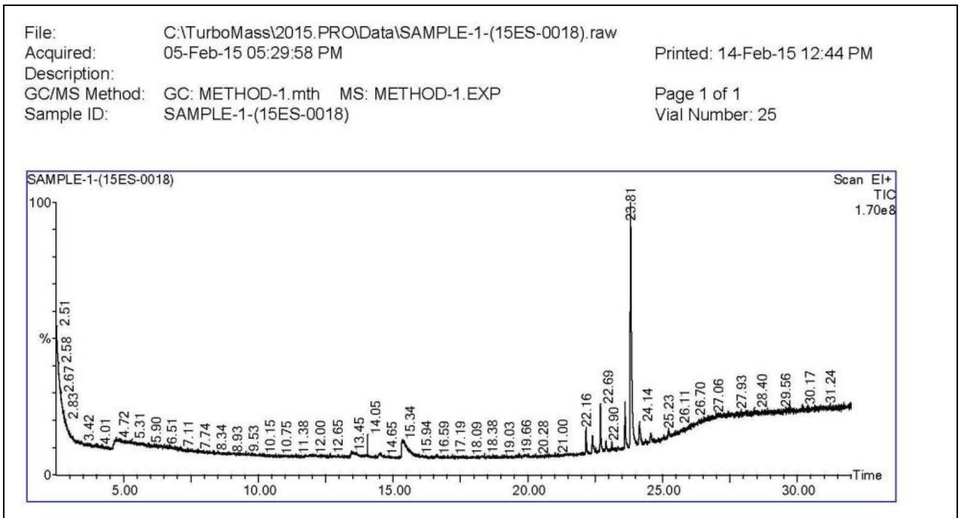


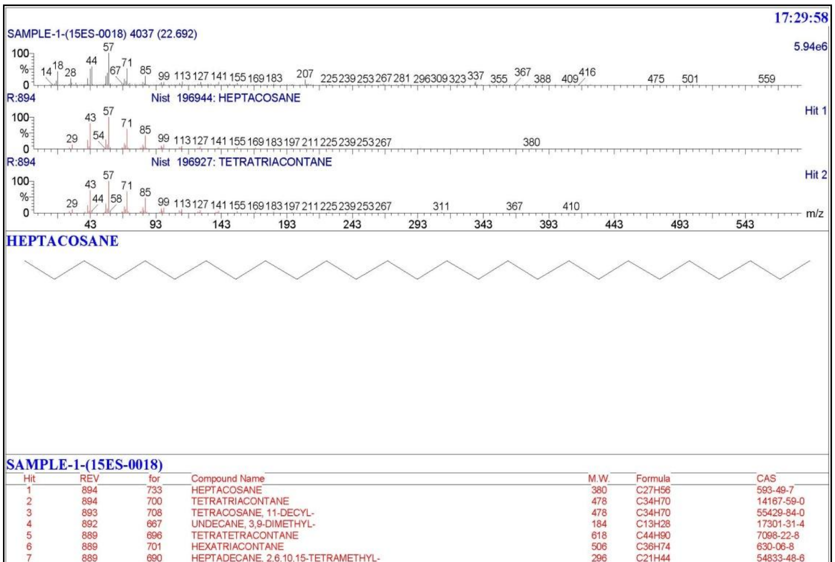
Fig 4: GC-MS chromatograms (peaks) of cuticular extracts of ant *O. smaragdina* worker.



#	RT	Scan	Height	Area	Area %	Norm %
1	15.389	2577	9,615,288	1,646,221.4	12.657	21.16
2	22.161	3931	16,567,361	523,547.9	4.025	6.73
3	22.402	3979	9,632,456	363,966.9	2.798	4.68
4	22.692	4037	28,773,440	959,944.1	7.381	12.34
5	23.607	4220	28,576,412	968,406.1	7.446	12.45
6	23.812	4261	150,691,456	7,779,701.5	59.815	100.00
7	24.142	4327	13,041,568	764,545.4	5.878	9.83

Fig 5: GC-MS chromatograms (area %) of cuticular extracts of ant *O. smaragdina* worker.

Cuticular hydrocarbons (CHCs) have been intensely investigated in social insects as they are known to be involved nestmate recognition [29]. The evolutionary success of organisms relies on their adaptability to various environmental conditions. The integument represents the outer, functional and structured interface between an organism and its habitat [7, 30]. It functions as a physical barrier to pathogens or predators, provides protection against wounding and injuries [31, 32], regulates the exchanges of water, primarily, but also plays a role in respiration, determination of inner temperature, body movements [33, 34] [35], intra and interspecific communications or sensing [36. 37].



SAMPLE-1-(15ES-0018)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	894	733	HEPTACOSANE	380	C27H56	593-49-7
2	894	700	TETRATRIACONTANE	478	C34H70	14167-59-0
3	893	708	TETRACOSANE, 11-DECYL-	478	C34H70	55429-84-0
4	892	667	UNDECANE, 3,9-DIMETHYL-	184	C13H28	17301-31-4
5	889	696	TETRATETRACONTANE	618	C44H90	7098-22-8
6	889	701	HEXATRIACONTANE	506	C36H74	630-06-8
7	889	690	HEPTADECANE, 2,6,10,15-TETRAMETHYL-	296	C21H44	54833-48-6
8	888	695	OCTACOSANE	394	C28H58	630-02-4
9	887	701	DOTRIACONTANE	450	C32H66	544-85-4
10	885	697	TRITRIACONTANE	464	C33H68	630-05-7
11	885	692	HEPTADECANE, 3-METHYL-	254	C18H38	6418-44-6
12	884	695	TRITETRACONTANE	604	C43H88	7098-21-7
13	882	689	TETRACOSANE	338	C24H50	646-31-1
14	882	687	TRIACONTANE, 11,20-DIDECYL-	702	C50H102	55256-09-2
15	882	709	PENTACOSANE, 13-UNDECYL-	506	C36H74	55517-89-0
16	881	679	HENEICOSANE	296	C21H44	629-94-7
17	880	673	HEXATRIACONTANE	506	C36H74	630-06-8

Fig 6: GC-MS chromatograms of cuticular hydrocarbons Heptacosane present in the ant *O. smaragdina* worker.

In ants, CHCs are extremely diverse; collecting data on 78 ant species, Martin and Drijfhout [11], found a total of about 1000 different CHCs occurring in peculiar, species-specific mixtures, irrespectively of species phylogeny. Most abundant are n-alkanes followed by monomethyl alkanes, dimethyl alkanes, alkenes, dienes and, more rarely, trimethyl alkanes. Methylalkenes, methyl alkadienes, trienes and tetramethyl alkanes are seldom produced by very few ant species [11]. In ants, CHCs are synthesized in the oenocytes, which are cells associated with fat bodies or to the epidermis, and afterward conveyed to target tissues by lipid carriers (lipoprotein) through the hemolymph [38].

Six major cuticular hydrocarbons were present in the social insects such as n-Alkanes, Olefins (alkenes and alkadienes), methyl alkanes (monomethylalkanes, dimethylalkanes and trimethyl alkanes), Methylolefins (methyl alkenes and methyl alkadienes) [11]. The CHCs of *Myrmecia gulosa* are a complex mixture of alkanes, alkenes and methyl-branched alkanes ranging from C-23 to C-39 [39]. High amount abundance of n-alkanes on their cuticle of *M. eumenoides* and *P. barbatus* [39]. Soroker and Hefetz [5], reported17 hydrocarbons in the desert ant *Cataglyphis niger.* The ant *C. niger* CHCs were 13,11-Methylheptacosane,7-Methylheptacosane, 5-Methylheptocosane, 3-Methylheptocosane, n-Nonacosane, 5-Methylnonacosane and 3- Methylnonacosane [5]. Smith [15] *et al.*, reported that the CHC*s* of *O. haematodus* were Tricosane, 11-Methyltetracosane, Pentacosane; 5-Methylpentacosane, 3-Methylpentacosane, Hexacosane, 2-Methylhexacosane, Heptacosane, Octacosane, Nonacosane and Hentriacontane [15].

In the present study, the weaver ant *O. smaragdina* CHCs were mostly, n-alkanes such as Hexadecane, Octacosane, Heptacosane, Eicosane,9-octyl, Pentatriacontane, Hexacosane, 9-octyl, Nonadecane, Heneicosan, and Heptadecane (Fig. 4, 5 and 6). The cuticular hydrocarbons profile of *O. smaragdina* is composed primarily of straight-chain and branched-chain alkanes and alkenes It may be acted as a chemical messenger between the ant colony and nest recognition of the weaver ant *O. smaragdina*. Hence, the present study has produced valuable information on ant *O. smaragdina* workers Cuticular hydrocarbons (CHCs) profiles.

4. Conclusion

The cuticular hydrocarbons profiles of the weaver ants *O. smaragdina* were extracted and analyzed. A total of 18 CHCs were identified in the workers of *O. smaragdina*. The ant *O. smaragdina* CHCs extract was composed primarily of straight-chain and branched-chain alkanes and alkenes. In this study, the major cuticular hydrocarbons, Pentatriacontane, Eicosane,9-octyl, Hexacosane, 9-octyl, and Octacosane were present in high proportions. These cuticular hydrocarbons might be acted as a chemical messenger between the ant colony and nest recognition of the weaver ant *O. smaragdina*. Further comparative studies within subfamily level will help to explicate how these vital eusocial signals arose and evolved, and investigations in cuticular chemical differences may provide insights into the evolutionary history of chemical communication in social insects in general.

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6. Conflict of interest:

The authors declare that they have no conflict of interest.

7. References

1. Verghese Abraham PD, Kamala Jayanthi K, Sreedevi K, Sudha Devi, Viyolla Pinto. A quick and non-destructive population estimate for the weaver ant *Oecophylla smaragdina* Fab. (Hymenoptera: Formicidae). Current Science. 2013; 104(5):641-646.
2. Ambethgar V. Recognition of red weaver ants in integrated control of tea mosquito bug in cashew plantations in India. ISHS Acta Horticulturae. 2015, 1080. doi.10.17660/ActaHortic.2015.1080.52.
4. Blomquist GJ, Bagneres AG. Introduction: history and overview of insect hydrocarbons. In Insect Hydrocarbons (ed. G. J. Blomquist and A. G. Bagneres). 2010, 3-18. Cambridge: Cambridge University Press.
5. Vander Meer RK, Morel L. Nestmate Recognition in Ants. In Pheromone Communication in Social Insects; vander Meer, R.K., Breed, M.D., Winston, M.L., Espelie, K.E., Eds.; Westview Press: Oxford, UK. 1998, 79-103.
6. Soroker Victoria, Abraham Hefetz. Hydrocarbon site of synthesis and circulation in the desert an *Cataglyphis niger.* Journal of Insect Physiology. 2000; 46:1097-1102.
7. Howard RW. Cuticular hydrocarbons and chemical communication. In Insect Lipids: Chemistry, Biochemistry, and Biology (ed. D. W. Stanley-Samuelson and D.R. Nelson). Lincoln, NB: University of Nebraska Press. 1993, 179-226.
8. Lockey KH. Lipids of the insect cuticle-Origin, composition and function. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 1988; 89:595-645.
9. Gibbs AG, Rajpurohit S. Cuticular lipids and water balance. In Insect Hydrocarbons (ed. G. J. Blomquist and A. G. Bagneres). Cambridge: Cambridge University Press. 2010, 100-120.
10. Kuhbandner S, Sperling S, Mori K, Ruther J. Deciphering the signature of cuticular lipids with contact sex pheromone function in a parasitic wasp. J. Exp. Biol. 2012; 215:2471-2478.
11. Howard RW, Blomquist GJ. Ecological, behavioral, and biochemical aspects of insect hydrocarbons.In Annual Review of Entomology; Annual Reviews: Palo Alto, CA, USA. 2005; 50:371-393.
12. Martin S, Drijfhout FA. review of ant cuticular hydrocarbons. J. Chem. Ecol. 2009; 32:1151-1161.
13. Cvacka J, Jiros P, Sobotnik J, Hanus R, Svatos A. Analysis of insect cuticular hydrocarbons using matrix-assisted laser desorption/ionization mass spectrometry. J. Chem. Ecol. 2006; 32:409-434.
14. Buckner JS. Oxygenated derivatives of hydrocarbons. In Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology (ed. G. J. Blomquist and A. G. Bagnères). Cambridge: Cambridge University Press. 2010, 187-203.
15. Yew JY, Dreisewerd K, de Oliveira CC, Etges WJ. Male specific transfer and fine scale spatial differences of newly identified cuticular hydrocarbons and triacylglycerides in a *Drosophila* species pair. PLoS One. 2011; 6:168-98.
16. Smith A, Jocelyn G, Millar, Andrew V. Suarez. Comparative analysis of fertility signals and sex-specific cuticular chemical profiles of *Odontomachus* trap-jaw ants. Journal of Experimental Biology. 2016; 219:419-430. doi:10.1242/jeb.128850.
17. Brunner E, Kroiss J, Trindl A, Heinze J. Queen pheromones in Temnothorax ants: control or honest signal? BMC Evol. Biol. 2011; 11:55.
18. Denis Damien, Rumsaïs Blatrix, Dominique Fresneau.

How an Ant Manages to Display Individual and Colonial Signals by Using the Same Channel. J Chem Ecol. 2006; 32:1647-1661. DOI 10.1007/s10886-006-9099-7.

19. Hannonen M, Sledge MF, Turillazzi S, Sundström L. Queen reproduction, chemical signalling andworker behaviour in polygynous colonies of the ant Formica fusca. Anim. Behav. 2002; 64:477-485.
20. Bernier UR, Carlson DA, Geden CJ. Gas chromatography/mass spectrometry analysis of the cuticular hydrocarbons from parasitic wasps of the genus *Muscidifurax*. Journal of the American Society for Mass Spectrometry. 1997; 9:320-332.
21. Horne GL, Priestmann AA. The chemical characterization of the epicuticular hydrocarbons of *Aedes aegypti* (Diptera: Culicidae). Bulletin of Entomological Research. 2002; 92:287-294.
22. Anyanwu GI, Molyneux DH, Phillips A. Variation in cuticular hydrocarbons among strains of the *Anopheles gambiae Sensu stricto* by analysis of cuticular hydrocarbons using gas liquid chromatography of larvae. Memórias do Instituto Oswaldo Cruz. 2000; 95:295-300.
23. Anyanwu GI, Molyneux DH, Priestman A. Cuticular-hydrocarbon discrimination between *Anopheles gambiae* s.s. and An. Arabiensis larval karyotypes. Annals of Tropical Medicine and Parasitology. 2001; 95:843-852.
24. Calderón-Fernández G, Juárez MP, Monroy MC, Menes M, Bustamante DM. Mijailovsky S.Intraspecific variability in Triatoma dimidiata (Hemiptera: Reduviidae) populations from Guatemala based on chemical and morphometric analyses. Journal of Medical Entomology. 2005a; 42:29-35.
25. Calderón-Fernández G, Juárez MP, Ramsey J, Salazar-Schettino PM, Monroy MC, Ordoñez R *et al.* Cuticular hydrocarbon variability among Triatoma dimidiate (Hemiptera: Reduviidae) populations from Mexico and Guatemala. Journal of Medical Entomology. 2005b; 42:780-788.
26. Mahamat H, Hassanali A. Cuticular hydrocarbon composition analysis for taxonomic differentiation of phlebotomine sandfly species (Diptera: Psychodidae) in Kenya. Journal of Medical Entomology. 1998; 35:778-781. 26. Bejarano EE, Rojas W, Uribe S, Vélez ID. Sistemática de espécies de Lutzomyia del grupo verrucarum Theodor, 1965 (Diptera: Psychodidae). Biomedica. 2003; 23:87-102.
27. Bragaa Marina Vianna, Zeneida Teixeira Pinto, Margareth Maria de Carvalho Queiroza, Nana Matsumotod, Gary James Blomquist. Cuticular hydrocarbons as a tool for the identification of insect species: Puparial cases from Sarcophagidae. Acta Trop. 2013, 128(3). doi:10.1016/j.actatropica.2013.07.014.
28. Kumaresan V. Host plant range of arboreal nesting red ants in Kanyakumari District of Tamilnadu (India). Journal of the Bombay Natural History Society. 1998; 95:70-75.
29. Van Zweden JS, d'Ettorre P, Blomquist GJ, Bagneres AG. Nestmate recognition in social insects and the role of hydrocarbons. In Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology; Cambridge University Press: Cambridge, UK. 2010, 222-243.
30. Hadley NF. Cuticle: Ecological significance. In Biology of the Integument; Springer: Berlin, Germany. 1984, 685-693.
31. Eigenbrode SD, Jetter R. Attachment to plant surface waxes by an insect predator. Integr. Comp. Biol. 2002; 42:1091-1099.
32. Serrano M, Coluccia F, Torres M, L'Haridon F, Metraux JP. The cuticle and plant defense to pathogens. Front. Plant Sci. 2014; 5:8.
33. Kerstiens G. Signalling across the divide: A wider perspective of cuticular structure-function relationships. Trends Plant Sci. 1996; 1:125-129.
34. Gibbs AG. Water-proofing properties of cuticular lipids. Am. Zool. 1998; 38:471-482.
35. Riederer M, Schreiber L. Protecting against water loss: Analysis of the barrier properties of plant cuticles. J. Exp. Bot. 2001; 52:2023-2032.
36. Lockey KH. Insect hydrocarbon classes—Implications for chemotaxonomy. Insect Biochem. 1991; 21:91-97.
37. Barbero Francesca. Cuticular Lipids as a Cross-Talk among Ants, Plants and Butterflies. Int. J. Mol. Sci. 2016, 17. 1966; doi:10.3390/ijms17121966.
38. Schal C, Sevala VL, Young HP, Bachmann JA. Sites of synthesis and transport pathways of insect hydrocarbons: Cuticle and ovary as target tissues. Am. Zool. 1998; 38:382-393.
39. Wagner D, Brown MJ, Broun P, Cuevas W, Moses LE, Chao DL *et al.* Task-related differences in the cuticular hydrocarbon composition of harvester ants, *Pogonomyrmex barbatus*. J. Chem. Ecol. 1998; 24:2021-2037.