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Characterization of Cuticular Hydrocarbon Profiles in Southeastern Argentine Ant (Linepithema humile) Colonies

Katherine R. Barrs
Georgia Southern University

Characterization of Cuticular Hydrocarbon Profiles in Southeastern Argentine Ant (*Linepithema humile*) Colonies

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By
Katherine Barrs
Under the mentorship of Dr. Joshua Gibson

Abstract:

Argentine ants, *Linepithema humile*, are a globally invasive species that displaces native species and facilitates agricultural pests. Colonies occupy distinct territories with multiple nests and defend them against other colonies. Ants have a waxy coating of cuticular hydrocarbons (CHCs) and can detect (i.e. smell) differences in these CHCs to identify their own vs foreign colonies. In the US, Argentine ants have been primarily studied in California where they only form four massive colonies showing mutual aggression, some spanning more than 500 miles. In the Southeast, colony recognition remains largely unexplored; however, we have identified several smaller colonies based on behavioral differences across lesser distances. It is currently unknown why the California ants interact as a single colony even across long distances while the Southeastern ants are found in many smaller colonies. The CHC profiles of the four colonies in California are known to contain over 70 chemicals, but it is unclear which components are most important for recognition. We collected Argentine ants from within and across colonies in Southeast Georgia as well as the largest California colony to analyze their CHC profiles using gas chromatography-mass spectroscopy (GC/MS). We present an analysis of the qualitative differences within and between colonies in southeast Georgia. As one of the first studies of CHCs in southeast Georgia, this work increases our understanding of the variability and the role of CHC profiles in facilitating colony recognition in Argentine ants.

Thesis Mentor:_____
Dr. *Joshua Gibson*
Honors Director:_____
Dr. Steven Engel

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Department of Biology
Honors College
Georgia Southern University

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Introduction:

Argentine ants, *Linepithema humile,* are a well-studied species of ant known to form extremely vast colonies termed "supercolonies." Supercolonies are a unique concept characterized by unity, recognition, and the capacity for unrestricted growth across extensive geographic ranges (Moffett, 2012). Argentine ants are distributed natively in Argentina in the Paraná River drainage in distinct small colonies, tens of square meters in size (Suarez et al., 1999; Tsutsui & Case, 2001; Wild, 2004). However, the capacity of Argentine ants to spread in introduced populations is prevalent close and far from their native range. Introduction events mediated by human activities were noted in the region surrounding the Paraná River (Wild, 2004). At a larger scale, Argentine ants have also been introduced to six continents and some oceanic islands (Van Wilgenburg et al., 2010). Introduced populations of Argentine ants across the world form high densities, dominate native species, and grow in size by hundreds of square kilometers maximizing their growth capacity to form supercolonies (Lowe et al., 2000). Supercolonies are a single example of the fascinating variety of social organizations found in insects.

Overview of Colony Structure:

The structure of ant colonies is highly variable, and Pederson et al. in 2006 provided a succinct review of terms central to the study of unicolonial social insects. A nest is a physical structure a colony inhabits, and the individuals inhabiting the same nest are defined as nestmates. The definition of a colony, a society, or a group can be more difficult to describe as context and the social insect species studied can impact the definition. Pederson et al. 2006, defines a colony as grouped individuals who interact cooperatively. In Table 1, an overview of colony structure is described through defining physical and social structure characteristics.

Table 1: An overview of physical and social structure characteristics critical to describing the colony structure of ant species.

Term	Definition	Citation
Monodomy	Single colonies living in a single nest.	(Pedersen et al., 2006)
Polydomy	Colonies exchange individuals among several connected nests.	(Pedersen et al., 2006)
Monogyne	A society with a single-queen characterized by long-range dispersal and high relatedness among nestmates.	(Sundström et al., 2005)
Polygyne	A society with multiple-queens associated with short-range dispersal which in extreme examples can result in polydomous colonies with variable relatedness among nestmates. These colonies generally inhabit an area geographically close to the original nest and freely transfer workers and resources among the nest sites	(Sundström et al., 2005)
Unicolonial	Species that can form populations comprised of one or many supercolonies	(Pedersen et al., 2006)
Multicolonial	Species that can form colonies based on relatedness among nestmates where populations can show intercolonial aggression.	(Pedersen et al., 2006)

The dichotomy among monogyne and polygyne societies, colonies exhibiting monodomy or polydomy, and species identified as unicolonial or multicolonial is not always clear. Expression of these characteristics can vary greatly among ant species, populations, and even within colonies such that invariably defining the characteristics of different ant species strictly within these dichotomies is unlikely.

Broadly, experts agree ant species form a type of anonymous society (Moffett, 2012). Individually, ants do not communicate with each other to determine colony identity. Instead, colonies rely on shared cues. Currently, these cues are understood as chemical blends which generate a colony-specific scent recognized by all individuals (d'Ettorre & Lenoir, 2010). The ability to share colony identity widely through chemical cues enables ant colonies to approach populations numbering in the millions, billions, or trillions, in the case of Argentine ants. In terms of colony recognition, ant societies have two separate but related goals: identifying each

other with shared chemical cues while distinguishing outsider ants with distinct chemical cues (Moffett, 2012). Due to the enormous population size, an individual ant is unlikely to encounter all other individuals of the same supercolony. More critical is the need to maintain recognition to separate the colony mates of the supercolony from outsiders. A unique feature of ant species that establish supercolonies is geographically distinct but recognizing nests. These recognizing nests keep a common identity and persist as a unit within the larger supercolony. Argentine ant colonies from discernibly different environments maintain nestmate recognition among individual ants across large ranges. Thus, the cues for recognition among Argentine ants are consistent despite the movement of individuals among multiple nest sites and the potential variable relatedness among workers resulting from the presence of multiple queens.

Argentine Ants and Their Global Supercolony:

Argentine ants are a damaging invasive species found to exhibit a truly global supercolony (Van Wilgenburg et al., 2010). Introduced populations have spread around the world and have been evaluated through aggression assays to determine colony boundaries. These introduced populations show a characteristic lack of territorial behavior and intraspecific aggression within the range of the supercolony (Van Wilgenburg et al., 2010). Workers show no aggression to individuals of the same supercolony but are highly aggressive to individuals from different colonies (Van Wilgenburg et al., 2010). A recent global-scale analysis of the largest introduced populations of Argentine ants found ants in these populations behave as a single supercolony distributed across North America, Europe, Australia, New Zealand, Japan, and Hawaii (Van Wilgenburg et al., 2010). Despite nests within the single supercolony spreading across the globe, colony identity is maintained even across oceans.

In the United States, Argentine ants are found in a wide introduced range. Despite their extended geographic range, supercolonies still interact aggressively with other supercolonies

(Figure 1). This aggression is based on differences in cuticular hydrocarbons (CHCs) between colonies (Buellesbach et al., 2018). Each supercolony is genetically distinct resulting in unique combinations of CHC components that form their specific CHC profile used in colony recognition. Previous research has suggested native populations of Argentine ants are unicolonial and organized into supercolonies (Pedersen et al., 2006). Currently, the central distinction among native and introduced Argentine ant populations is the size of supercolonies (Pedersen et al., 2006).

Role of Insect Cuticular Hydrocarbons (CHCs):

For insects, their cuticle and a waxy coating of hydrocarbons are an important impedance to desiccation and in social insects also function as a chemical cue promoting colony recognition (Leonhardt et al., 2016). Different classes of cuticular hydrocarbons (CHCs) are commonly found including linear *n*-alkanes, linear *n*-alkenes, and methylated alkanes (Gibbs & Pomonis, 1995). Generally, the chain length of CHCs varies greatly between 20 to 40 carbon atoms, and an increase in carbon chain length has been correlated to increasing desiccation protection (Gibbs & Pomonis, 1995). Similarly, other properties of CHCs are important such as the melting temperature of a hydrocarbon, which is indicative of the switch from solid to liquid which increases the permeability of the cuticle and loss of water (Buellesbach et al., 2018). Different classes of CHCs and their associated properties have been researched revealing melting temperature to be highest for linear alkanes and lower for CHCs with methyl groups or double bonds (Buellesbach et al., 2018).

Argentine ants retain colony identity while experiencing vastly different environmental conditions. How these ants balance colony recognition and desiccation resistance using the broad range of components in the CHC profile is unknown. It is expected that CHC profiles would differ with changes in environmental factors such as temperature and precipitation (Buellesbach

et al., 2018). Previous studies have identified Argentine ants as particularly susceptible to high temperatures and desiccation (Holway et al., 2002). Yet the recognition capacity of the profiles must be maintained for the continued ecological success of the colony. Certain components of the CHC profile may contribute to desiccation resistance while other components are crucial to colony recognition. Currently, there is no conclusive answer to this question. The parsing out of these elements and their roles would be an important finding helping to elucidate how these chemicals facilitate colony recognition.

Statement of Purpose:

My study proposes to increase understanding of the role of CHC profiles in colony recognition in Argentine ants. Specifically, I aim to identify and characterize the CHC profiles of novel southeastern Argentine ant colonies. Previous research and preliminary data from the lab indicate the presence of multiple distinct and aggressive colonies in the southeast (Buczkowski et al., 2004). Comparing my findings to previously obtained CHC profiles could illustrate the presence or absence of continuity in chemicals comprising CHC profiles. The physical properties of more complex compounds make them less suitable for resisting desiccation, yet the complex compounds persist in the CHC profiles indicating a role outside of desiccation resistance. More complex compounds allow more variation in structure which has the potential to encode more information (Chung & Carroll, 2015). Therefore, I developed the following hypothesis: more complex compounds such as those with double bonds or methylated branches will have more signaling capacity and less importance for desiccation resistance. I predict distinct, mutually aggressive southeastern colonies will vary more in complex compounds than in less complex compounds among distinct colonies. Those findings would support the hypothesis that more complex compounds have a greater capacity to signal colony identity. Argentine ants provide an excellent study system for this work as their intercolony aggression can be scored as a binary

rather than a continuous variable. They also are an excellent choice to study CHC recognition because of the volume of previous work in California and the lack of work in the southeast (Buczkowski et al., 2004; Buellesbach et al., 2018; Suarez et al., 1999; Tsutsui & Case, 2001).

Methods:

Figure 2: Map of ant collection sites in Southeast Georgia. Orange color designates the colony in the Swainsboro, GA area. Blue color designates the colony in the Statesboro, GA area.

Ant Collection:

Ants were collected from two different colonies ~32 miles apart, one in the Statesboro, GA area and the other in the Swainsboro, GA area (Figure 2). Collections from the Statesboro colony were made at two sites: Georgia Southern University, Statesboro Campus (GSU, lat: 32.421711, long: -81.790255, collected in February and March 2021, Statesboro, GA), and Statesboro (ST, lat: 32.436071, long: -81.785132, collected in February 2021, Statesboro, GA). Collections from the East Georgia State College colony were made at a single site (EGSC, lat: 32.599878, long: -82.308560, collected in March 2021, Swainsboro, GA). A separate sample of ants from the Albany Bulb collection site was shipped from California (AB, lat: 37.89013, long:−122.3163, collected in June 2020, Albany, CA).

At each site, workers and queens were collected and kept in laboratory conditions to establish colonies for future sampling. Depending on field habitat, ants were collected from small logs, earth, and sand and placed into 5-gallon buckets with water-moistened paper towels to prevent drying out. (Lowe's Companies Inc., Mooresville, North Carolina, USA). To prevent ants from escaping during transport, buckets were coated with Insect-a-Slip (BioQuip). In the laboratory, the bucket contents were poured into plastic bins (Sterilite 28qt storage boxes) coated with Insect-a-Slip. The plastic bins with nest materials were covered with water-moistened paper towels and placed on top of the bricks, which were placed in another bin partially filled with the water and detergent. Ants acclimated to laboratory conditions overnight. New plastic bins were set up as permanent nest boxes with 2 to 3 water-filled glass tubes sealed with cotton balls (VWR International, Radnor, PA), 2 to 3 sugar water-filled glass tubes sealed with cotton, and 2 to 3 plastic petri dishes spray-painted black (60mm diameter, 15mm height) with a small hole (3 to 4mm) and filled halfway with plaster-of-Paris. Cardstock bridges and tape were used to connect bins with nesting materials to the new bins. To stimulate the movement of workers to the new bins, tap water was slowly added to the box with nesting materials for up to 6 hours. Workers and queens transported their brood into the new bins. Ants were maintained on a diet of *ad lib* water, sugar water, and fed frozen crickets three times per week.

Chemical Extractions:

CHC extractions were performed using the methods of Buellesbach et al. in 2018. For extracts of pooled samples, 500 ants were collected and frozen for 30 min at -20℃ (Figure 3). One mL of hexane (HPLC Grade, Fisher Chemical, Hampton, New Hampshire, USA) and 500 ants were added to a 2 mL vial (Agilent Technologies, Santa Clara, California, USA) which was capped and swirled for 10 minutes in an orbital shaker (CO-Z) at 180 r/min. To separate the CHCs from the ants, a 5 ¾'' Pasteur pipette was used to draw off the hexane extract,

approximately 750 µL of supernatant and placed in a separate 2 mL screw top vial (Agilent Technologies, Santa Clara, California, USA). The ants were left in the 2 mL vial and later discarded. A flow of nitrogen inside a chemical fume hood was used to evaporate the hexane extract until the volume was less than 200 µL, around 15 minutes. The 100-200 µL of hexane extract + CHCs was placed in a GC/MS insert (Agilent Technologies, Santa Clara, California, USA) in the 2 mL vial. A flow of nitrogen was used on the 100-200 µL of extract to evaporate all the hexane, leaving the CHCs. The dried extract in the insert was resuspended with 150 µL of hexane + a dodecane internal standard (7.5 ng/µL, MilliporeSigma, St. Louis, Missouri, USA). (Ant per µL concentration calculation, 500 ants /150 µL = 3.33 ants/uL * 5µL = 16.667 ants/µL).

Qualitative Analysis:

After acclimation to room temperature, five µL of the resuspended CHC extract was manually injected (10 µL Syringe, ACE Glass Incorporated, Vineland, NJ) into a gas chromatograph-mass spectrometer (GC: 2010, MS: QP2010S, Shimadzu Corporation, Kyoto, Kyoto, Japan). The injection was performed in splitless mode with a temperature of 200℃. Compounds were separated using an XTI-5 column (30 m x 0.25 mm x 0.25 µm, Restek GC Columns, Restek Corporation, Bellefonte, PA) with a temperature program beginning at 80℃ held for 5 minutes and increased by 80℃ per minute until 200℃, followed by an increase of 5℃ per minute until 300℃ which was held for 20 minutes. The total program time was 46.5 minutes. Helium with a column flow of 1.8 mL/min was used as the carrier gas. A solvent cut time of 3.8 minutes was used.

GC/MS data file processing and figure generation were performed using the GC/MS analysis software “OpenChrom®” version 1.4.0 (Lablicate GmbH, Hamburg, Germany) and “GCMSsolution, Postrun Analysis” version 4.50 (Shimadzu Corporation, Kyoto, Kyoto, Japan).

Briefly, GC/MS data were processed in OpenChrom using SNIP Baseline Detection, First Derivative Peak Detection, and Peak Integration (Trapezoid Method). Manually identified peaks were compared with system identified peaks to maintain consistency. To highlight the highest quality peaks, a signal to noise minimum of 60 was used. Identification of CHCs was determined based on retention time, diagnostic ions, and mass spectra. An internal Alkane Library for Argentine ant CHCs was also used (Jan Buellesbach, Personal Communication). The *n*-dodecane internal standard (7.5 ng/ul) was used to determine absolute CHC quantities of the chemical components of the CHC profile. Calculations for percent area and dodecane standard determined the total amount of each CHC in ng/ant concentration.

Sensitivity Analysis:

In collaboration with the authors of Buellesbach et al. 2018, I used a standardized mixture of CHC profile components, an alkane standard solution (C_{21}-C_{40}) (MilliporeSigma, Burlington, MA), to evaluate my ability to identify CHC components using the equipment at my university. My collaborators use an ultra inert GC/MS column which is more sensitive and can be run at higher temperatures than my available equipment. My collaborator's GC/MS column can reach 325℃ while the available GC/MS column at my university is limited to 300℃. Larger CHC compounds, up to C_{40}, are resolved at higher temperatures. To determine the impact of the maximum running temperature, my collaborators tested the alkane standard on their equipment at both 300°C and 325°C. A longer hold time was programmed to offset peak stretching after C_{31} at lower temperatures and to attempt to resolve C_{40}.

Results:

Sensitivity Analysis Results:

Testing the alkane standard at 300°C and 325°C showed reaching only 300°C fails to resolve C_{40} and stretches the time between signal peaks of the larger compounds (Figure 4). By

comparing the results of running the alkane standard on both machines, I found the available equipment could not resolve larger CHC compounds at the column's maximum running temperature, C_{39} and C_{40} (Figure 5). In addition, the column is less sensitive to these larger compounds demonstrated by relatively smaller peaks beyond C_{35} (Figure 5).

Further, I obtained a sample of Argentine ants from California (Albany Bulb, AB), from the authors of Buellesbach et al. 2018, to evaluate my ability to reproduce the identification of the CHC components using the equipment at my university. The identification detailed in Table 1 of Buellesbach et al. 2018 highlights 64 CHCs across five classes (*n*-alkanes, *n*-alkenes, monomethyl alkanes, dimethyl alkanes, and trimethyl alkanes) that were found in samples from AB. With my methodology, 36 CHCs were found in the AB sample across three classes (*n*-alkanes, *n*-alkenes & diene, and methyl alkanes) (Table 3).

CHC Identification Results:

To describe the variation across Argentine ant collection sites, the CHCs common to both samples from each collection site were identified (Table 4, Table 5, and Table 6). The CHCs common to each collection site pair (EGSC vs GSU, EGSC vs ST, and GSU vs ST) were compared to find the CHCs unique to each collection site within the pair. For EGSC and GSU, Pentadecene (C_{15}ene), C_{28} (Octacosane), and C_{37} (Heptatriacontane) were unique to ESGC while C_{31} (Hentriacontane), unknown (KB-DI:386), and C_{34} (Tetratriacontane) were found in samples from the GSU collection site (Figure 6). For EGSC and ST, C_{28} (Octacosane) and C_{37} (Heptatriacontane) were unique to ESGC while 5-MeC_{29} and C_{34} (Tetratriacontane) were found in samples from the ST collection site (Figure 7). Comparing GSU and ST, C_{31} (Hentriacontane) and unknown (KB-DI:386) were found for GSU and 5-MeC_{29} was found in samples from the ST collection site (Figure 8). The compound 5-MeC_{29} was only found in samples from the ST collection site. The compound unknown (KB-DI:386) was only found in samples from GSU. The

compound Pentadecene (C_{15}ene) was found only in samples from EGSC. To analyze the variation across Argentine ant colonies in southeast Georgia, the CHCs common to the two colonies were identified (Table 7). Those CHCs were Pentadecene (C_{15}ene) and C_{28} (Octacosane) for EGSC and C_{34} (Tetratriacontane) for the colony with two collection sites, GSU and ST (Figure 9).

In Table 2, the OpenChrom analysis resulted in 43 unique CHCs across 3 different classes, *n*-alkanes from C_{15} to C_{37}, *n*-alkenes and a diene, and methyl branched alkanes. The identification detailed in Table 1 of Buellesbach et al. 2018 highlights 72 CHCs across five classes (*n*-alkanes, *n*-alkenes, monomethyl alkanes, dimethyl alkanes, and trimethyl alkanes) that were found in their samples.

Discussion:

While this study used methods in line with Buellesbach et al. 2018, there are differences in methodology which impacts the sensitivity of the GC/MS analysis. Buellesbach et al. 2018 used a Pasteur pipette plugged with glass wool filled with ~1 inch of silica gel desiccant to separate the non-polar CHC fraction from polar surface lipids in the extractions of ~100 ants. In the preliminary extractions I performed using ~500 ants with the silica gel column, a low concentration of CHCs was observed. Therefore to gain more distinct results from the GC/MS, the silica gel column was excluded from my method. Further analysis of the extracted CHCs revealed two large and wide peaks (RT: 7.058-7.2 and RT: 7.85-8.242) corresponding to a polar compound, Iridomyrmecin (Figure 6, Figure 7, Figure 8, and Figure 9). Previous research on iridomyrmecin showed it is a primary chemical constituent of the Argentine ant trail pheromone (Choe et al., 2012).

To compare the single AB sample run through a silica desiccant column with my extractions which did not use a silica desiccant column, I completed a quantitative analysis to

calculate the abundances of CHCs (ng/ant) relative to the C_{12} Standard, Dodecane. The analysis showed a 4x greater CHC concentration in the sample extracted without the silica gel column (Table 1). The concentration discrepancy discovered between the samples run with and without the silica gel column could explain the lower overall number of CHCs identified in the samples from southeast Georgia.

OpenChrom is an open source software independent of the operating system, but documentation and literature exploring the operations offered by OpenChrom are scarce (Wenig & Odermatt, 2010). In this analysis, the settings for the detection of peaks, identification, and quantification in OpenChrom relied on a signal to noise ratio minimum of 60. While the analysis parameter helped reduce the number of extraneous GC/MS peaks, important components of the variation across sites and colonies could be found in less prevalent peaks. More information on the distribution and seasonality of CHC variation would be needed to draw further conclusions.

Overall, I found CHCs that differ across colonies, C_{34}, C_{15}ene, and C_{28}. C_{28} and C_{34} are especially interesting as they are even-numbered alkanes. Most CHCs found in my analysis and the Buellesbach et al. 2018 study were odd-numbered. I predicted distinct, mutually aggressive southeastern colonies will differ more in complex compounds than in less complex compounds. My finding that the compounds which differed between colonies were mostly less complex compounds does not support my hypothesis. However, other factors are impacting my results including the low quantity of samples and the general levels of variability observed in the CHC profiles among different nest sites. One of the key results of Buellesbach et al. 2018 was the associations of certain classes of CHCs with monthly average temperature, monthly average precipitation, and signaling. My samples were collected at different times and environmental

conditions. The local conditions and temporal variability could also play a role in the level of variation observed in my analysis.

When testing my ability to identify CHC components using the equipment at my university, I concluded the current equipment cannot resolve larger CHC compounds at the column's maximum running temperature, C_{39} and C_{40}. The column was also less sensitive to larger CHC compounds. These physical limitations inherent to laboratory equipment also produce limitations for identifying and classifying CHC compounds. Though preliminary, this work is one of the first comparative analyses of the CHCs in the southeast Georgia region. Continued work in southeast Georgia could fill a gap in the study of Argentine ants by providing a greater number of distinct colonies for chemical analysis, behavioral comparison, and genetic study.

Impact Statement and Significance:

The threat posed to native species by invasive species has become increasingly apparent. Argentine ants are one of many invasive species in the United States and are listed as one of the world's 100 most damaging invasive species by the International Union for Conservation of Nature. Increases in the understanding of the mechanisms that allow Argentine ants to display contrasting supercolony sizes in their native and introduced ranges would provide critical information to possibly limit the spread of these ants. More broadly, the identification of the individual roles of the components of CHC profiles would increase understanding of the chemical's responsiveness to environmental conditions and their facilitation of colony recognition (Sprenger & Menzel, 2020). The approach proposed in this study using chemistry and biology is well established. However, previous studies have focused on finding statistical differences in CHC profiles of known colonies in California but have not investigated populations of Argentine ants in southeast Georgia (Buellesbach et al., 2018). Broadly, this form

of colony recognition is not unique to Argentine ants. My findings contribute to current knowledge on Argentine ants and may inspire research inquiries in related social insect species.

Future Directions:

The methods tested and refined in this project will be applied to a greater number of collection sites. Behavioral assays will also be used to determine the number of colonies for sampling through behavioral assays with southeastern and California colonies. To better describe CHC variation and generate statistical information, the number of samples will be increased per site and per colony. Further improvement could also include refining the methodology, filtering out polar compounds, developing a CHC library to aid identification, and using an ultra-inert column to reduce background noise in the samples.

The colony structure, characterization of CHC profiles, and genetic information on Argentine ants in the southeast remain largely unexplored. Thorough studies on genetic relatedness, colony structure, CHC profiles, and behavioral interaction have already been completed for many introduced populations of Argentine ants across the globe and native populations in Argentina. Working with Argentine ants in the southeast represents a nascent study system. Identifying and characterizing southeastern colonies will enable further work on the role of chemicals within CHC profiles, the investigation of interactions among colonies, and will be fundamental to future projects essential to the Gibson Lab research program. The fundamental concepts underlying this project related broadly to interactions among genotype and phenotype. This project offers a unique opportunity to study the evolutionarily based factors which influence the relationship among individual ants, individual ants and their colonies, and different ant species.

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1215.

Appendix I: Figures & Tables

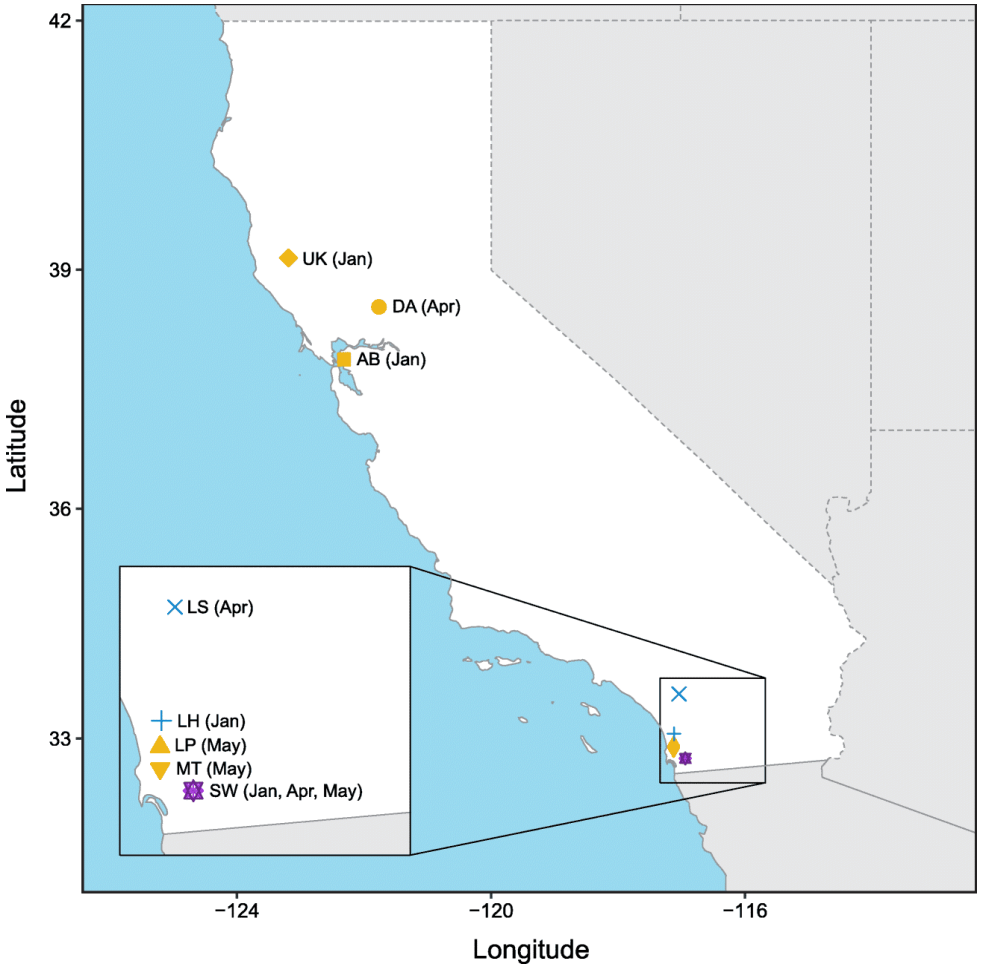

Figure 1: Colonies and nestsites found in California. Yellow shapes indicate nestsites in the main supercolony with an extended range (500 + miles). Blue and purple shapes indicate secondary supercolonies that are genetically distinct from the main supercolony. Secondary supercolonies are found in smaller ranges in southern California.
Image Credit: Buellesbach et al. 2018

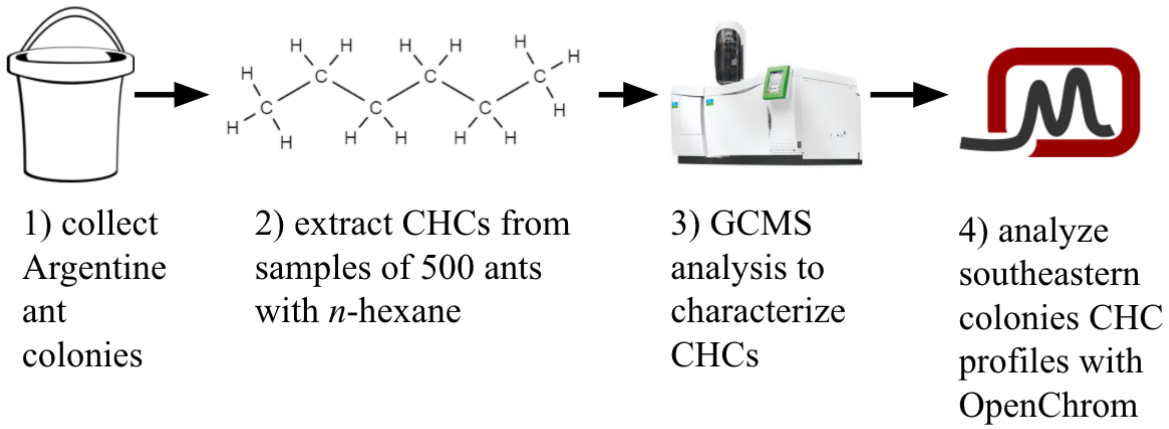

Figure 3: A Schematic Diagram of Methods. Colonies will be collected from the southeast. *n*-hexane extractions of CHCs will be performed. The resulting CHCs will be characterized through GC/MS analysis which will allow the identification of the chemical components of the CHC profile.

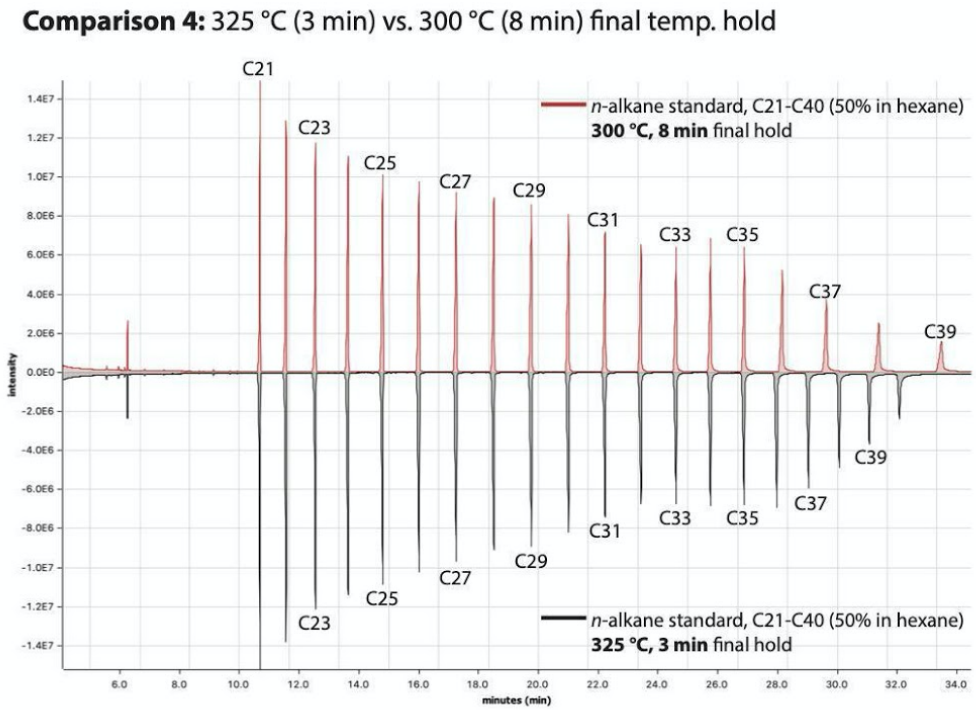

Figure 4: Comparison of *n*-alkane standard solution (C_{21}-C_{40}) at 300℃ and 325℃ final hold temperatures. Both temperatures were run on an ultra-inert column. The top panel shows an *n*-alkane standard solution run at 300℃ for eight minutes. The bottom panel shows an *n*-alkane standard solution run at 325℃ for three minutes. Five additional minutes were added to the 300℃ final hold time to match the 325℃ total run time.

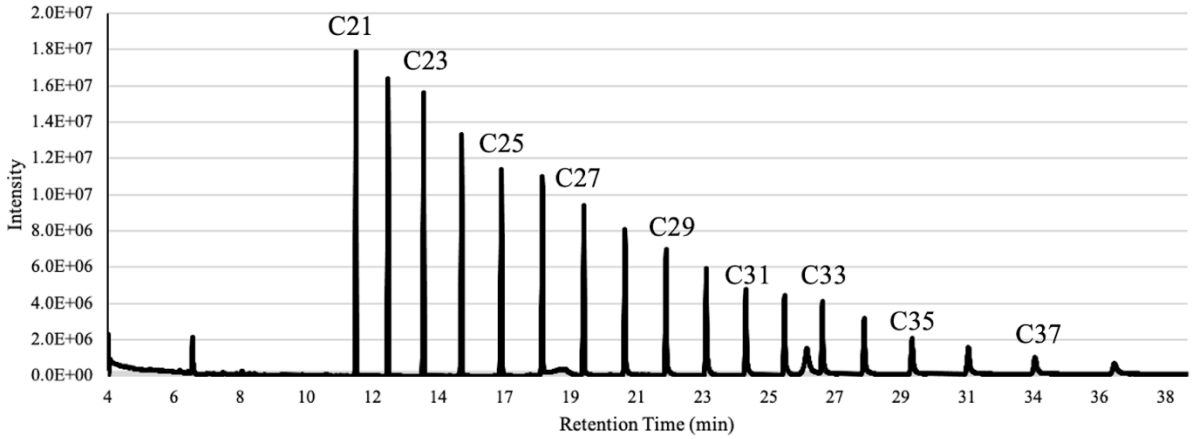

Figure 5: *n*-alkane standard solution (C_{21}-C_{40}) run on Georgia Southern GC/MS at a 300°C final hold temperature for 12 minutes to match the total run time of 38.5 minutes.

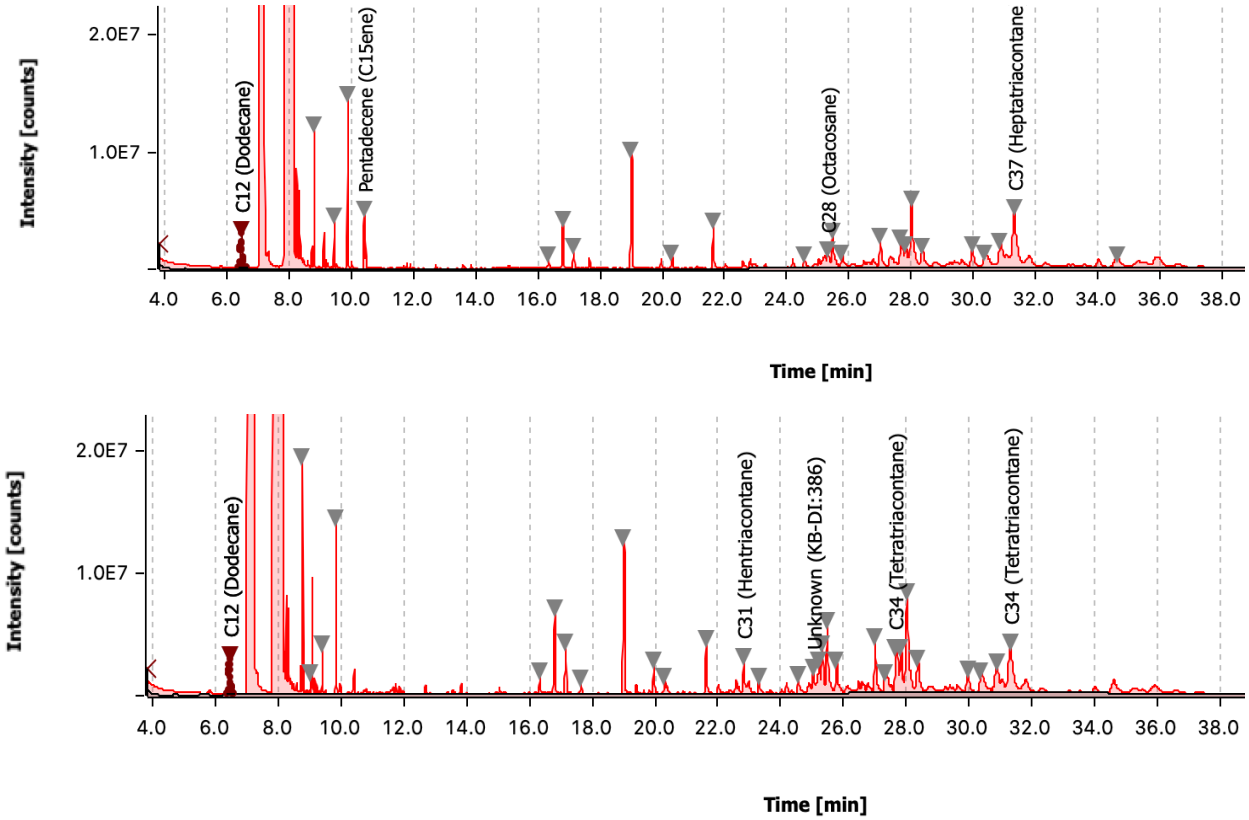

Figure 6: GC/MS chromatograms from collection sites EGSC (top) and GSU (bottom). Peaks are labeled with CHC identification. The peak resulting from an *n*-dodecane internal standard (7.5ng/ul) is found at RT: 6.5. Peaks at (RT: 7.058-7.2 and RT: 7.85-8.242) correspond to a polar compound, Iridomyrmecin. Labeled peaks after RT: 10 are the CHCs unique to each site within the collection site pair.

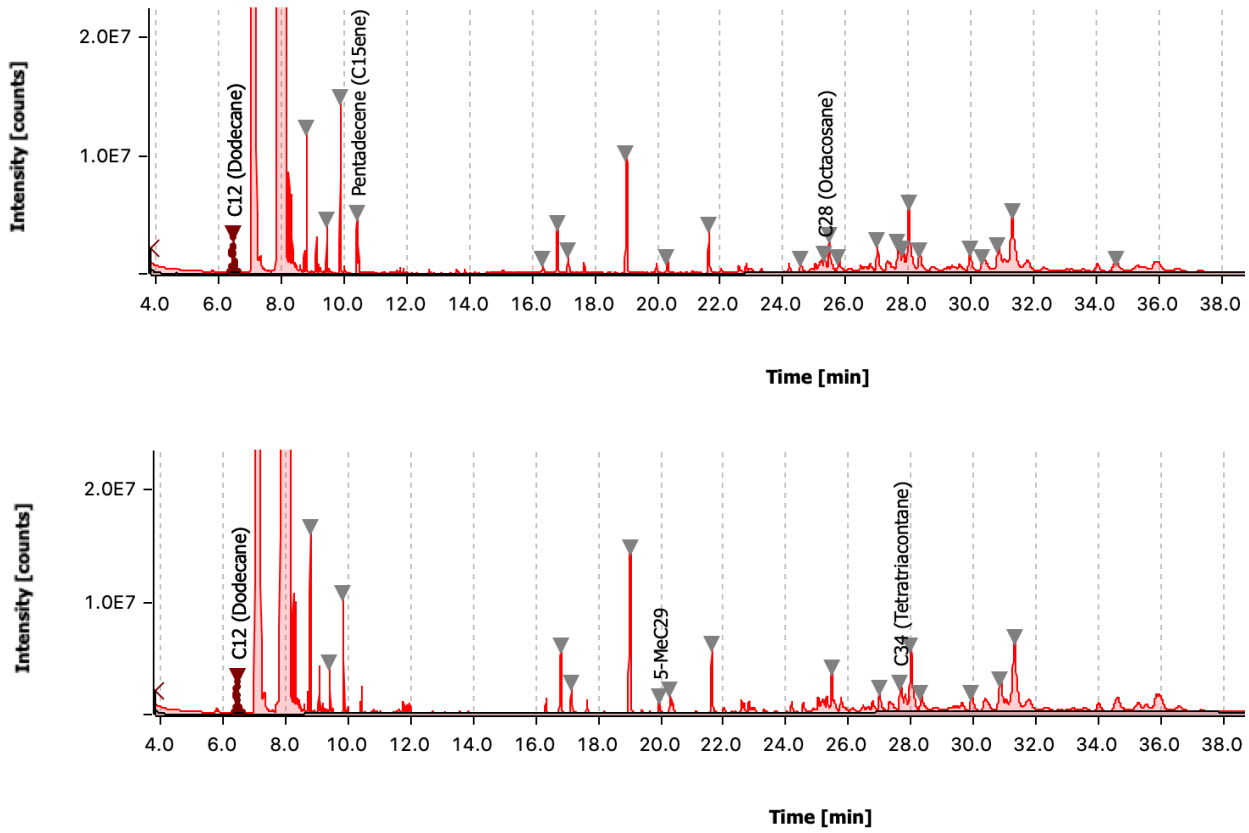

Figure 7: GC/MS chromatograms from collection sites EGSC (top) and ST (bottom). Peaks are labeled with CHC identification. The peak resulting from an *n*-dodecane internal standard (7.5ng/ul) is found at RT: 6.5. Peaks at (RT: 7.058-7.2 and RT: 7.85-8.242) correspond to a polar compound, Iridomyrmecin. Labeled peaks after RT: 10 are the CHCs unique to each site within the collection site pair.

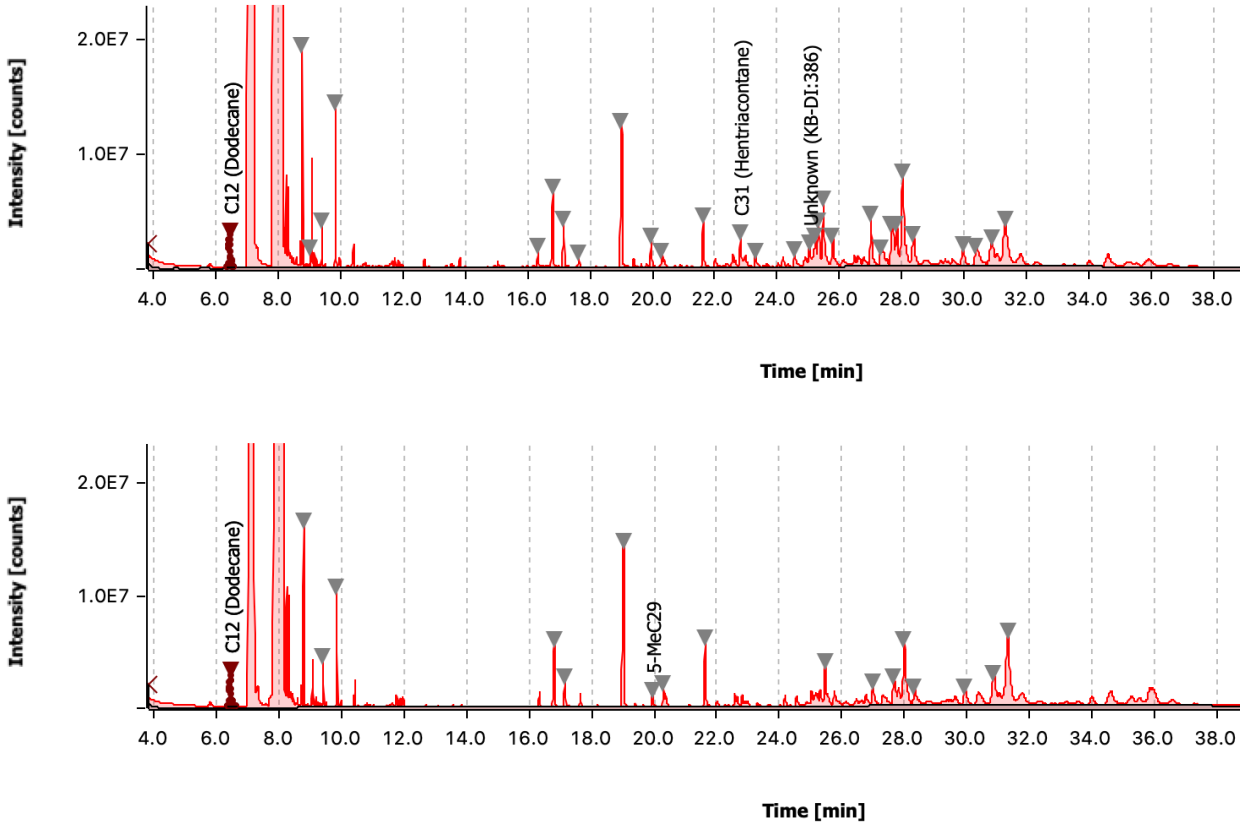

Figure 8: GC/MS chromatograms from collection sites GSU (top) and ST (bottom). Peaks are labeled with CHC identification. The peak resulting from an *n*-dodecane internal standard (7.5ng/ul) is found at RT: 6.5. Peaks at (RT: 7.058-7.2 and RT: 7.85-8.242) correspond to a polar compound, Iridomyrmecin. Labeled peaks after RT: 10 are the CHCs unique to each site within the collection site pair.

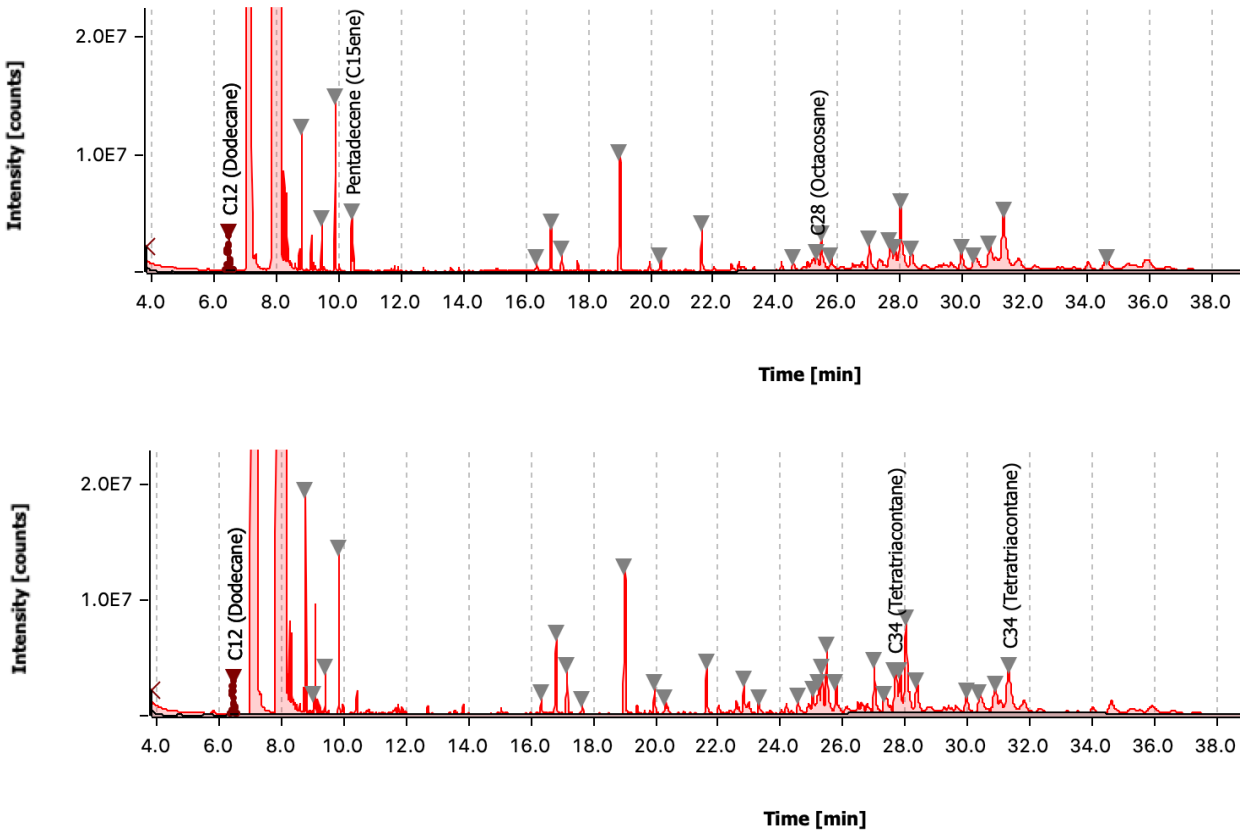

Figure 9: GC/MS chromatograms from colonies, EGSC (top) and GSU & ST (bottom). Peaks are labeled with CHC identification. The peak resulting from an *n*-dodecane internal standard (7.5ng/ul) is found at RT: 6.5. Peaks at (RT: 7.058-7.2 and RT: 7.85-8.242) correspond to a polar compound, Iridomyrmecin. Labeled peaks after RT: 10 are the CHCs unique to each colony in southeast Georgia.

Table 2: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from all samples from each collection site.

		Georgia Southern		East Georgia State College		Statesboro		California
RT [min]	Identification	GSUA	GSUB	EGSCA	EGSCB	STA	STB	AB
6.467-6.483	C12 (Dodecane)	0.450	0.450	0.450	0.450	0.450	0.450	0.450
7.058-7.2	Iridomyrmecin	21.503	12.722	12.566	11.009	5.982	20.659	X
7.708	Dodecadiene	X	X	0.084	X	X	X	X
7.85-8.242	Iridomyrmecin	40.235	34.053	39.982	33.986	21.031	33.579	X
7.883	C15 (Pentadecane)	X	X	X	X	X	X	0.019
8.1	Me-alkane Pentadecane, DiMe-Alkane Tetradecane, TriMe-Alkane Tridecane	X	X	X	X	X	X	0.005
8.333	C16 (Hexadecane)	0.460	X	X	X	X	X	X
8.35	Me-Alkane Dodecane (C12), DiMe-Alkane Undecane (C11), TriMe-Alkane Decane (C10)	X	X	0.601	X	X	X	X
8.583	Me-alkane Hexadecane, DiMe-Alkane Pentadecane, TriMe-Alkane Tetradecane	X	X	X	X	X	X	0.007
8.683-8.7	Heptadecene (C17ene)	X	X	0.076	X	0.107	X	X
8.775-8.817	C17 (Heptadecane)	0.574	0.704	0.576	0.493	0.919	0.657	0.287
9.042	Unknown (KB-DI:239)	X	0.029	X	X	X	X	X
9.067-9.092	Pentadecene (C15ene)	X	X	0.309	X	0.482	X	X
9.183	Me-alkane Heptadecane, DiMe-Alkane Hexadecane, TriMe-Alkane Pentadecane	X	X	X	X	X	X	0.030
9.342	C18 (Octadecane)	X	X	X	X	X	X	0.006
9.4-9.45	Heptadecene (C17ene)	0.214	0.208	0.300	0.192	0.217	0.215	
9.842-10.008	Nonadecene (C19ene)	0.649	0.778	0.650	0.774	1.749	0.570	0.046
10.008	C19 (Nonadecane)	X	X	X	X	X	X	0.012
10.375-10.417	Pentadecene (C15ene)	0.133	X	0.059	0.242	0.660	X	X
10.567	Me-Alkane Octadecane, DiMe-Alkane Heptadecane, TriMe-Alkane Hexadecane	X	X	X	X	X	X	0.007
10.75	C20 (Eicosane)	X	X	0.098	X	X	X	X

		Georgia Southern		East Georgia State College		Statesboro		California
RT [min]	Identification	GSUA	GSUB	EGSCA	EGSCB	STA	STB	AB
11.658-11.708	C21 (Heneicosane)	X	X	0.274	X	X	X	0.016
12.7-12.742	C22 (Docosane)	X	X	0.478	X	X	X	0.024
13.842-13.892	C23 (Tricosane)	X	X	0.492	X	X	X	0.032
15.058-15.117	C24 (Tetracosane)	X	X	0.402	X	X	X	0.028
16.325-16.408	C25 (Pentacosane)	X	0.150	0.577	X	0.166	X	0.055
16.35	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X	X	X	0.106	X	X	X
16.792-16.825	Unknown (KB-DI:332)	0.705	0.793	0.968	0.441	0.447	0.677	X
17.133-17.158	Unknown (KB-DI:332)	0.291	0.413	0.465	0.169	0.187	0.276	X
17.633-17.725	C26 (Hexacosane)	X	X	0.432	X	0.141	X	0.035
17.65	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X	0.117	X	X	X	X	X
18.992-19.1	C27 (Heptacosane)	0.961	1.491	1.958	1.077	2.193	1.881	0.278
19.95-19.967	5-MeC29	X	0.283	0.276	X	0.173	0.139	X
20.3-20.317	Unknown (KB-DI:395)	X	X	0.365	X	0.295		X
20.308-20.392	C28 (Octacosane)	X	X	X	X	X	0.202	0.037
20.308	Me-alkane Tricosane (C23), DiMe-Alkane Docosane (C22), TriMe-Alkane Heneicosane (C21)	X	0.114	X	X	X	X	X
20.317	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X	X	X	0.149	X	X	X
21.617-21.725	C29 (Nonacosane)	0.426	0.533	0.773	0.455	0.868	0.760	0.135
22.575	Me-alkane Octacosane (C28), DiMe-Alkane Heptacosane (C27), TriMe-Alkane Hexacosane (C26)	X	X	X	X	0.139	X	X
22.817-22.825	C31 (Hentriacontane)	0.289	0.353	X	X	X	X	X
22.825	5-MeC29	X	X	0.143	X	X	X	X
22.933	Me-alkane Tetracosane (C24), DiMe-Alkane Tricosane (C23), TriMe-Alkane Docosane (C22)	X	X	0.340	X	X	X	X
23.308-24.2	Unknown3 (JB)	X	0.199	0.234	X	X	X	X

		Georgia Southern		East Georgia State College		Statesboro		California
RT [min]	Identification	GSUA	GSUB	EGSCA	EGSCB	STA	STB	AB
24.275	C27 (Heptacosane)	X	X	X	X	X	X	0.032
24.558-24.575	Unknown3 (JB)	0.254	0.257	0.258	0.186	X	X	X
25.033-25.042	Unknown (KB-DI:386)	0.148	0.201	X	X	X	X	X
25.033	Unknown3 (JB)	X	X	X	X	0.212	X	X
25.225	5-MeC29	0.282	X	X	X	X	X	X
25.242-25.317	Unknown3 (JB)	X	0.289	X	X	0.168	X	X
25.258	5,11-/5,13-/5,15-/ 5,17-DiMeC31	X	X	X	X	X	X	0.031
25.325-25.5	C33 (Tritriacontane)	0.372	0.405	0.759	X	0.777	0.659	X
25.333	C28 (Octacosane)	X	X	0.166	0.138	X	X	X
25.475-25.5	C33 (Tritriacontane)	0.837	0.935	X	X	X	X	X
25.483	Me-alkane Triacontane (C30), DiMe-Alkane Nonacosane (C29), TriMe-Alkane Octacosane (C28)	X	X	X	0.515	X	X	X
25.558	11-/13-/15-/17-MeC33	X	X	X	X	X	X	0.042
25.767-25.792	Unknown3 (JB)	0.427	X	0.308	0.248	0.348	X	X
25.792	5-MeC29	X	0.468	X	X	X	X	X
27-27.092	C33 (Tritriacontane)	0.630	0.636	0.485	0.397	X	X	0.050
27	Me-alkane Tritriacontane (C33), DiMe-Alkane Dotriacontane (C32), TriMe-Alkane Hentriacontane (C31)	X	X	X	X	0.394	X	X
27-27.342	Unknown3 (JB)	0.470	0.464	X	X	X	0.346	X
27.408	C30 (Triacontane)	X	X	X	X	X	X	0.035
27.667-27.783	C34 (Tetratriacontane)	0.855	0.808	0.575	X	0.651	0.560	0.111
27.683	C31 (Hentriacontane)	X	X	X	0.495	X	X	X
27.833	Unknown3 (JB)	0.427	X	0.181	0.151	X	X	X
27.85-28.117	C35 (Pentatriacontane)	1.749	0.411	1.432	1.153	1.501	1.307	0.171
28.042	C35 (Pentatriacontane)		1.722	X	X	X	X	X
28.342-28.383	Unknown3 (JB)	0.618	0.618	0.426	0.372	0.444	0.364	X
28.467	Unknown (KB-DI:341)	X	X	X	X	X	X	0.026

		Georgia Southern		East Georgia State College		Statesboro		California
RT [min]	Identification	GSUA	GSUB	EGSCA	EGSCB	STA	STB	AB
29.942-29.975	Unknown3 (JB)	0.488	0.430	0.469	0.411	0.449	0.417	X
30.1	C35 (Pentatriacontane)	X	X	X	X	X	X	0.096
30.4-30.408	Unknown3 (JB)	0.591	0.572	0.473	0.431	X	X	X
30.508	Me-Alkane Pentacosane (C25), DiMe-Alkane Tetracosane (C24), TriMe-Alkane Tricosane (C23)	X	X	X	X	X	X	0.085
30.858-30.9	C33 (Tritriacontane)	0.740	0.638	0.661	0.584	X	0.754	X
30.867-31.042	C36 (Hexatriacontane)	X	X	X	X	0.892		0.202
31.292-31.325	C34 (Tetratriacontane)	1.338	1.228	X	X	X	X	X
31.325-31.533	C37 (Heptatriacontane)	X	X	1.533	1.401	2.314	2.000	0.467
31.983	Me-Alkane Pentacosane (C25), DiMe-Alkane Tetracosane (C24), TriMe-Alkane Tricosane (C23)	X	X	X	X	X	X	0.071
34.25-34.6	Unknown3 (JB)	0.469	X	X	X	0.668	X	0.092
34.633	Me-alkane Heptacosane (C27), DiMe-Alkane Hexacosane (C26), TriMe-Alkane Pentacosane (C25)	X	X	X	0.369	X	X	X
34.808	C27 (Heptacosane)	X	X	X	X	X	X	0.123
35.542	C35 (Pentatriacontane)	X	X	X	X	X	X	0.108
35.858	Unknown3 (JB)	X	X	X	X	0.337	X	X
36.192	C36 (Hexatriacontane)	X	X	X	X	X	X	0.161

Table 3: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from AB *L. humile* sample. The value "X" designates that this component was identified but was not included in the calculations of abundance.

RT [min]	Compound Name	California (AB)
6.467-6.483	C12 (Dodecane)	0.450
7.058-7.2	Iridomyrmecin	X
7.708	Dodecadiene	X
7.85-8.242	Iridomyrmecin	X
7.883	C15 (Pentadecane)	0.019
8.1	Me-alkane Pentadecane, DiMe-Alkane Tetradecane, TriMe-Alkane Tridecane	0.005
8.333	C16 (Hexadecane)	X
8.35	Me-Alkane Dodecane (C12), DiMe-Alkane Undecane (C11), TriMe-Alkane Decane (C10)	X
8.583	Me-alkane Hexadecane, DiMe-Alkane Pentadecane, TriMe-Alkane Tetradecane	0.007
8.683-8.7	Heptadecene (C17ene)	X
8.775-8.817	C17 (Heptadecane)	0.287
9.042	Unknown (KB-DI:239)	X
9.067-9.092	Pentadecene (C15ene)	X
9.183	Me-alkane Heptadecane, DiMe-Alkane Hexadecane, TriMe-Alkane Pentadecane	0.030
9.342	C18 (Octadecane)	0.006
9.4-9.45	Heptadecene (C17ene)	X
9.842-10.008	Nonadecene (C19ene)	0.046
10.008	C19 (Nonadecane)	0.012
10.375-10.417	Pentadecene (C15ene)	X
10.567	Me-Alkane Octadecane, DiMe-Alkane Heptadecane, TriMe-Alkane Hexadecane	0.007
10.75	C20 (Eicosane)	X
11.658-11.708	C21 (Heneicosane)	0.016
12.7-12.742	C22 (Docosane)	0.024
13.842-13.892	C23 (Tricosane)	0.032
15.058-15.117	C24 (Tetracosane)	0.028
16.325-16.408	C25 (Pentacosane)	0.055
16.35	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X
16.792-16.825	Unknown (KB-DI:332)	X

17.133-17.158	Unknown (KB-DI:332)	X
17.633-17.725	C26 (Hexacosane)	0.035
17.65	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X
18.992-19.1	C27 (Heptacosane)	0.278
19.95-19.967	5-MeC29	X
20.3-20.317	Unknown (KB-DI:395)	X
20.308-20.392	C28 (Octacosane)	0.037
20.308	Me-alkane Tricosane (C23), DiMe-Alkane Docosane (C22), TriMe-Alkane Heneicosane (C21)	X
20.317	Me-alkane Eicosane (C20), DiMe-Alkane Nonadecane (C19), TriMe-Alkane Octadecane (C18)	X
21.617-21.725	C29 (Nonacosane)	0.135
22.575	Me-alkane Octacosane (C28), DiMe-Alkane Heptacosane (C27), TriMe-Alkane Hexacosane (C26)	X
22.817-22.825	C31 (Hentriacontane)	X
22.825	5-MeC29	X
22.933	Me-alkane Tetracosane (C24), DiMe-Alkane Tricosane (C23), TriMe-Alkane Docosane (C22)	X
23.308-24.2	Unknown3 (JB)	X
24.275	C27 (Heptacosane)	0.032
24.558-24.575	Unknown3 (JB)	X
25.033-25.042	Unknown (KB-DI:386)	X
25.033	Unknown3 (JB)	X
25.225	5-MeC29	X
25.242-25.317	Unknown3 (JB)	X
25.258	5,11-/5,13-/5,15-/ 5,17-DiMeC31	0.031
25.325-25.5	C33 (Tritriacontane)	X
25.333	C28 (Octacosane)	X
25.475-25.5	C33 (Tritriacontane)	X
25.483	Me-alkane Triacontane (C30), DiMe-Alkane Nonacosane (C29), TriMe-Alkane Octacosane (C28)	X
25.558	11-/13-/15-/17-MeC33	0.042
25.767-25.792	Unknown3 (JB)	X
25.792	5-MeC29	X

27-27.092	C33 (Tritriacontane)	0.050
27	Me-alkane Tritriacontane (C33), DiMe-Alkane Dotriacontane (C32), TriMe-Alkane Hentriacontane (C31)	X
27-27.342	Unknown3 (JB)	X
27.408	C30 (Triacontane)	0.035
27.667-27.783	C34 (Tetratriacontane)	0.111
27.683	C31 (Hentriacontane)	X
27.833	Unknown3 (JB)	X
27.85-28.117	C35 (Pentatriacontane)	0.171
28.042	C35 (Pentatriacontane)	X
28.342-28.383	Unknown3 (JB)	X
28.467	Unknown (KB-DI:341)	0.026
29.942-29.975	Unknown3 (JB)	X
30.1	C35 (Pentatriacontane)	0.096
30.4-30.408	Unknown3 (JB)	X
30.508	Me-Alkane Pentacosane (C25), DiMe-Alkane Tetracosane (C24), TriMe-Alkane Tricosane (C23)	0.085
30.858-30.9	C33 (Tritriacontane)	X
30.867-31.042	C36 (Hexatriacontane)	0.202
31.292-31.325	C34 (Tetratriacontane)	X
31.325-31.533	C37 (Heptatriacontane)	0.467
31.983	Me-Alkane Pentacosane (C25), DiMe-Alkane Tetracosane (C24), TriMe-Alkane Tricosane (C23)	0.071
34.25-34.6	Unknown3 (JB)	0.092
34.633	Me-alkane Heptacosane (C27), DiMe-Alkane Hexacosane (C26), TriMe-Alkane Pentacosane (C25)	X
34.808	C27 (Heptacosane)	0.123
35.542	C35 (Pentatriacontane)	0.108
35.858	Unknown3 (JB)	X
36.192	C36 (Hexatriacontane)	0.161

Table 4: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from the *L. humile* samples from the EGSC and GSU collection sites. Colored cells indicate a compound unique to both samples from a single collection site. The compounds represented in this table were included only if common to both samples from a single collection.

		East Georgia State College		Georgia Southern	
RT [min]	Identification	EGSCA	EGSCB	GSUA	GSUB
6.467-6.483	C12 (Dodecane)	0.450	0.450	0.450	0.450
7.058-7.2	Iridomyrmecin	12.566	11.009	21.503	12.722
7.85-8.242	Iridomyrmecin	39.982	33.986	40.235	34.053
8.775-8.817	C17 (Heptadecane)	0.576	0.493	0.574	0.704
9.4-9.45	Heptadecene (C17ene)	0.300	0.192	0.214	0.208
9.842-10.008	Nonadecene (C19ene)	0.650	0.774	0.649	0.778
10.375-10.417	Pentadecene (C15ene)	0.059	0.242	X	X
16.792-16.825	Unknown (KB-DI:332)	0.968	0.441	0.705	0.793
17.133-17.158	Unknown (KB-DI:332)	0.465	0.169	0.291	0.413
18.992-19.1	C27 (Heptacosane)	1.958	1.077	0.961	1.491
21.617-21.725	C29 (Nonacosane)	0.773	0.455	0.426	0.533
22.817-22.825	C31 (Hentriacontane)	X	X	0.289	0.353
24.558-24.575	Unknown3 (JB)	0.258	0.186	0.254	0.257
25.033-25.042	Unknown (KB-DI:386)	X	X	0.148	0.201
25.325-25.5	C33 (Tritriacontane)	X	X	0.372	0.405
25.333	C28 (Octacosane)	0.166	0.138	X	X
25.475-25.5	C33 (Tritriacontane)	X	X	0.837	0.935
25.767-25.792	Unknown3 (JB)	0.308	0.248	X	X
27-27.092	C33 (Tritriacontane)	0.485	0.397	0.630	0.636
27-27.342	Unknown3 (JB)	X	X	0.470	0.464
27.667-27.783	C34 (Tetratriacontane)	X	X	0.855	0.808
27.833	Unknown3 (JB)	0.181	0.151	X	X
27.85-28.117	C35 (Pentatriacontane)	1.432	1.153	1.749	0.411
28.342-28.383	Unknown3 (JB)	0.426	0.372	0.618	0.618
29.942-29.975	Unknown3 (JB)	0.469	0.411	0.488	0.430
30.4-30.408	Unknown3 (JB)	0.473	0.431	0.591	0.572
30.858-30.9	C33 (Tritriacontane)	0.661	0.584	0.740	0.638
31.292-31.325	C34 (Tetratriacontane)	X	X	1.338	1.228
31.325-31.533	C37 (Heptatriacontane)	1.533	1.401	X	X

Table 5: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from the *L. humile* samples from the EGSC and ST collection sites. Colored cells indicate a compound unique to both samples from a single collection site. The compounds represented in this table were included only if common to both samples from a single collection.

		East Georgia State College		Statesboro	
RT [min]	Compound Name	EGSCA	EGSCB	STA	STB
6.467-6.483	C12 (Dodecane)	0.450	0.450	0.450	0.450
7.058-7.2	Iridomyrmecin	12.566	11.009	5.982	20.659
7.85-8.242	Iridomyrmecin	39.982	33.986	21.031	33.579
8.775-8.817	C17 (Heptadecane)	0.576	0.493	0.919	0.657
9.4-9.45	Heptadecene (C17ene)	0.300	0.192	0.217	0.215
9.842-10.008	Nonadecene (C19ene)	0.650	0.774	1.749	0.570
10.375-10.417	Pentadecene (C15ene)	0.059	0.242	X	X
16.792-16.825	Unknown (KB-DI:332)	0.968	0.441	0.447	0.677
17.133-17.158	Unknown (KB-DI:332)	0.465	0.169	0.187	0.276
18.992-19.1	C27 (Heptacosane)	1.958	1.077	2.193	1.881
19.95-19.967	5-MeC29	X	X	0.173	0.139
21.617-21.725	C29 (Nonacosane)	0.773	0.455	0.868	0.760
24.558-24.575	Unknown3 (JB)	0.258	0.186	X	X
25.325-25.5	C33 (Tritriacontane)	X	X	0.777	0.659
25.333	C28 (Octacosane)	0.166	0.138	X	X
25.767-25.792	Unknown3 (JB)	0.308	0.248	X	X
27-27.092	C33 (Tritriacontane)	0.485	0.397	X	X
27.667-27.783	C34 (Tetratriacontane)	X	X	0.651	0.560
27.833	Unknown3 (JB)	0.181	0.151	X	X
27.85-28.117	C35 (Pentatriacontane)	1.432	1.153	1.501	1.307
28.342-28.383	Unknown3 (JB)	0.426	0.372	0.444	0.364
29.942-29.975	Unknown3 (JB)	0.469	0.411	0.449	0.417
30.4-30.408	Unknown3 (JB)	0.473	0.431	X	X
30.858-30.9	C33 (Tritriacontane)	0.661	0.584	X	X
31.325-31.533	C37 (Heptatriacontane)	1.533	1.401	2.314	2.000

Table 6: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from the *L. humile* samples from the GSU and ST collection sites. Colored cells indicate a compound unique to both samples from a single collection site. The compounds represented in this table were included only if common to both samples from a single collection.

		Georgia Southern		Statesboro	
RT [min]	Compound Name	GSUA	GSUB	STA	STB
6.467-6.483	C12 (Dodecane)	0.450	0.450	0.450	0.450
7.058-7.2	Iridomyrmecin	21.503	12.722	5.982	20.659
7.85-8.242	Iridomyrmecin	40.235	34.053	21.031	33.579
8.775-8.817	C17 (Heptadecane)	0.574	0.704	0.919	0.657
9.4-9.45	Heptadecene (C17ene)	0.214	0.208	0.217	0.215
9.842-10.008	Nonadecene (C19ene)	0.649	0.778	1.749	0.570
16.792-16.825	Unknown (KB-DI:332)	0.705	0.793	0.447	0.677
17.133-17.158	Unknown (KB-DI:332)	0.291	0.413	0.187	0.276
18.992-19.1	C27 (Heptacosane)	0.961	1.491	2.193	1.881
19.95-19.967	5-MeC29	X	X	0.173	0.139
21.617-21.725	C29 (Nonacosane)	0.426	0.533	0.868	0.760
22.817-22.825	C31 (Hentriacontane)	0.289	0.353	X	X
24.558-24.575	Unknown3 (JB)	0.254	0.257	X	X
25.033-25.042	Unknown (KB-DI:386)	0.148	0.201	X	X
25.325-25.5	C33 (Tritriacontane)	0.372	0.405	0.777	0.659
25.475-25.5	C33 (Tritriacontane)	0.837	0.935	X	X
27-27.092	C33 (Tritriacontane)	0.630	0.636	X	X
27-27.342	Unknown3 (JB)	0.470	0.464	X	X
27.667-27.783	C34 (Tetratriacontane)	0.855	0.808	0.651	0.560
27.85-28.117	C35 (Pentatriacontane)	1.749	0.411	1.501	1.307
28.342-28.383	Unknown3 (JB)	0.618	0.618	0.444	0.364
29.942-29.975	Unknown3 (JB)	0.488	0.430	0.449	0.417
30.4-30.408	Unknown3 (JB)	0.591	0.572	X	X
30.858-30.9	C33 (Tritriacontane)	0.740	0.638	X	X
31.292-31.325	C34 (Tetratriacontane)	1.338	1.228	X	X
31.325-31.533	C37 (Heptatriacontane)	X	X	2.314	2.000

Table 7: CHC compounds with their respective retention time in minutes (RT), identification, and abundances (in ng) from the *L. humile* samples from the EGSC and (GSU & ST) colonies. Colored cells indicate a compound unique to a colony, appearing in both samples from a single collection site. The compounds represented in this table were included only if common to all samples from a colony.

		East Georgia State College		Georgia Southern		Statesboro	
RT [min]	Identification	EGSCA	EGSCB	GSUA	GSUB	STA	STB
6.467-6.483	C12 (Dodecane)	0.450	0.450	0.450	0.450	0.450	0.450
7.058-7.2	Iridomyrmecin	12.566	11.009	21.503	12.722	5.982	20.659
7.85-8.242	Iridomyrmecin	39.982	33.986	40.235	34.053	21.031	33.579
8.775-8.817	C17 (Heptadecane)	0.576	0.493	0.574	0.704	0.919	0.657
9.4-9.45	Heptadecene (C17ene)	0.300	0.192	0.214	0.208	0.217	0.215
9.842-10.008	Nonadecene (C19ene)	0.650	0.774	0.649	0.778	1.749	0.570
10.375-10.417	Pentadecene (C15ene)	0.059	0.242	X	X	X	X
16.792-16.825	Unknown (KB-DI:332)	0.968	0.441	0.705	0.793	0.447	0.677
17.133-17.158	Unknown (KB-DI:332)	0.465	0.169	0.291	0.413	0.187	0.276
18.992-19.1	C27 (Heptacosane)	1.958	1.077	0.961	1.491	2.193	1.881
19.95-19.967	5-MeC29	X	X	X	X	0.173	0.139
21.617-21.725	C29 (Nonacosane)	0.773	0.455	0.426	0.533	0.868	0.760
22.817-22.825	C31 (Hentriacontane)	X	X	0.289	0.353	X	X
24.558-24.575	Unknown3 (JB)	0.258	0.186	0.254	0.257	X	X
25.033-25.042	Unknown (KB-DI:386)	X	X	0.148	0.201	X	X
25.325-25.5	C33 (Tritriacontane)	X	X	0.372	0.405	0.777	0.659
25.333	C28 (Octacosane)	0.166	0.138	X	X	X	X
25.475-25.5	C33 (Tritriacontane)	X	X	0.837	0.935	X	X
25.767-25.792	Unknown3 (JB)	0.308	0.248	X	X	X	X
27-27.092	C33 (Tritriacontane)	0.485	0.397	0.630	0.636	X	X
27-27.342	Unknown3 (JB)	X	X	0.470	0.464	X	X
27.667-27.783	C34 (Tetratriacontane)	X	X	0.855	0.808	0.651	0.56
27.833	Unknown3 (JB)	0.181	0.151	X	X	X	X
27.85-28.117	C35 (Pentatriacontane)	1.432	1.153	1.749	0.411	1.501	1.307
28.342-28.383	Unknown3 (JB)	0.426	0.372	0.618	0.618	0.444	0.364
29.942-29.975	Unknown3 (JB)	0.469	0.411	0.488	0.430	0.449	0.417
30.4-30.408	Unknown3 (JB)	0.473	0.431	0.591	0.572	X	X
30.858-30.9	C33 (Tritriacontane)	0.661	0.584	0.740	0.638	X	X

31.292-31.325	C34 (Tetratriacontane)	X	X	1.338	1.228	X	X
31.325-31.533	C37 (Heptatriacontane)	1.533	1.401	X	X	2.314	2.000