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Consistency of Cuticular Hydrocarbon Mixtures of Five *Reticulitermes* (Blattodea: Rhinotermitidae) Taxa From Northern California: Similarity Among Colonies and Seasonal Variation

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Abstract

Cuticular hydrocarbon (CHC) mixtures from workers of five distinct CHC phenotypes of *Reticulitermes* Holmgren 1913 from two locations in northern California were examined from monthly collections taken over a 3-yr period. The objectives of this study were (1) to identify and quantify variations of the CHCs of multiple colonies of each of these phenotypes (= species or subspecies) to demonstrate consistency, (2) to assess the potential of CHC mixtures to separate or identify colonies within each phenotype, and (3) to detect any temporal changes in each of the hydrocarbons in the CHC mixtures. Nonmetric multidimensional scaling of all CHC mixtures of all samples collected at both locations separated the samples into five clearly visible, different groups of CHC phenotypes (taxa or species) of *Reticulitermes*. The degree of variability of the CHC mixtures among colonies of each phenotype was such that nonmetric multidimensional scaling did not separate or identify colonies. Strong seasonal fluctuations were evident in some of the CHCs of all five phenotypes and were significantly consistent with a sine curve. Maximum proportions of seasonal CHCs within a phenotype occurred in all seasons of the year but occurred mostly in the winter and summer. In general, the CHCs displaying maximum values in the winter were short-chained (C23–C27) methyl-branched alkanes, whereas the CHCs displaying maximum values in the summer were long-chained (C35–C43) methyl-branched alkanes, which likely influences water retention. These consistent chemical fingerprints are probably responsible for inter-phenotype recognition patterns and are thus useful for chemical taxonomy.

Key words: chemotaxonomy, colony identification, nestmate recognition, subterranean termite, water loss

The taxonomy of subterranean termites in the genus *Reticulitermes* Holmgren 1913 is in need of rigorous and comprehensive revision throughout its distribution to resolve ambiguity about species identity and regional distributions (Haverty et al. 1996a, 1999c; Haverty and Nelson 1997, 2007; Takematsu 1999; Takematsu and Yamaoka 1999; Clément et al. 2001; Nelson et al. 2001, 2008; Austin et al. 2002; Velonà et al. 2010; Dedeine et al. 2016; Ke et al. 2017; Wu et al. 2019; Johnson and Forschler 2022). Traditional morphological identification of *Reticulitermes* to species level is challenging. Workers are often the only individuals collected, however existing keys to North American species are based on the morphology of soldier and/or alate castes (Banks and Snyder 1920, Snyder 1954, Weesner 1965, Nutting 1990, Scheffrahn and Su 1994, Lim and Forschler 2012).

While conducting studies of the foraging ecology and control methods of *Reticulitermes* in northern California (Haverty et al. 1999b, 2000), termite workers and soldiers were collected from monitoring stations and their cuticular hydrocarbons (CHCs)

characterized with the goal of determining whether multiple taxa were present at the study sites. Attempts at morphological identification of soldier specimens using the key to North American species by Nutting (1990) were unsuccessful, and alates were rarely available, thus chemical characters were used to distinguish taxa (Haverty and Nelson 1997). Five distinct CHC phenotypes were characterized from two study sites using diagnostic CHCs empirically determined by Haverty and Nelson (1997). Three of these phenotypes, designated as CA-A, CA-B, and CA-C, were sympatric at a wildland study site near Placerville, CA, and two phenotypes, designated as CA-A' and CA-D, were sympatric at a residential site in Novato, CA. Evidence that these five CHC phenotypes or taxa represent at least four separate species and one subspecies of *Reticulitermes* in California has been reported (Haverty et al. 1991a,b; Haverty and Nelson 1997; Nelson et al. 2001, 2008; Page et al. 2002). These separate taxa have been corroborated by studies of mtDNA COII sequences (Copren et al. 2005), soldier defense secretion (SDS) mixtures (Nelson et al. 2001, 2008), agonistic behavior (Haverty et al. 1999a, Getty et al. 2000a, Delphia et al. 2003), and phenology of alate flights (Haverty et al. 2003). While there is ample evidence of multiple species, the definitive assignment of combined suites of characters (CHC, mtDNA, SDS, behavior) to a described species has been hindered by the inadequacy of the existing keys and original descriptions (Banks and Snyder 1920).

Earlier taxonomic literature described only two species of *Reticulitermes* occurring in California, *R. hesperus* Banks and *R. tibialis* Banks (Weesner 1965, 1970; Nutting 1990), and their exact geographic distributions were the subject of much debate (Banks and Snyder 1920; Pickens 1934a,b; Weesner 1970). Recently *R. flavipes* (Kollar), the eastern subterranean termite, was found to have been established in California, (Austin et al. 2005, Su et al. 2006, Tripodi et al. 2006). Another putative species, *R. okanaganensis* (T.G. Myles unpublished, Szalanski et al. 2006), has been proposed to occur in western North America, including California. This putative species has since been declared *nomen nudum* by Krishna et al. (2013), as there has been no formal description of this species published to date. In addition, studies by Su et al. (2006) and Tripodi et al. (2006) each reported two possible undescribed species of *Reticulitermes* collected in California based on the results of phylogenetic analysis.

The value of CHCs for distinguishing species of termites, ants, and other insects is well established (Kaib et al. 1991, Page et al. 1997, Takematsu 1999, Takematsu and Yamaoka 1999, Clément et al. 2001, Howard and Blomquist 2005, Bagnères and Wicker-Thomas 2010, Perdereau et al. 2010, Seppä et al. 2011, Chung and Carroll 2015, Guillem et al. 2016, Sprenger and Menzel 2020). Correspondence of chemical and genetic information in identifying taxa within *Reticulitermes* has been demonstrated (Jenkins et al. 2000, Clément et al. 2001, Kutnik et al. 2004, Uva et al. 2004, Copren et al. 2005, Austin et al. 2007, Lim and Forschler 2012). Environmentally induced plasticity of CHC profiles has been shown to occur (Woodrow et al. 2000, Otte et al. 2018, Martin et al. 2019), but is most often quantitative in nature, rather than qualitative (Bagnères and Wicker-Thomas 2010, Perdereau et al. 2010, Sprenger and Menzel 2020).

The repeatability of CHC mixtures among colonies within a phenotype has been demonstrated for *Reticulitermes* from California and Arizona (Page et al. 2002, Haverty and Nelson 2007, Nelson et al. 2008). However, the consistency or repeatability in CHC mixtures within colonies has not been examined for *Reticulitermes* as it has for *Coptotermes formosanus* Shiraki in Hawaii (Haverty et al. 1996b) or for *C. gestroi* Wasmann in Florida (Gordon et al. 2020). Furthermore, stability of the proportions of the CHCs within a colony and slight, but consistent, quantitative differences among colonies could also provide an additional tool for associating different foraging groups (i.e., from different monitoring stations or different sources of wood in/on the soil) from the same colony.

In this study, we quantitatively compared CHC mixtures of individual colonies of five CHC phenotypes of *Reticulitermes* over a 2- or 3-yr period from two locations in northern California. We used all samples from all monitoring stations and unequivocally separated these samples into phenotypes to demonstrate the utility of CHC mixtures for definitively identifying separate phenotypes (and putative species) of *Reticulitermes*. We subjected these data to nonmetric multidimensional scaling analysis (Cox and Cox 2001) to determine that all samples could be assigned to a phenotypic group and whether there was enough consistency to separate colonies within a phenotype based on the CHC mixtures. Additionally, we combined all data from all colonies for each CHC within a phenotype to determine whether any CHCs exhibited seasonal variation in percentage of the total CHC.

Materials and Methods

Collection of Termites

Samples of *Reticulitermes* were collected from termite monitoring stations (Lewis et al. 1998) at permanent field sites established in northern California at the Institute of Forest Genetics (IFG), Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture, near Placerville, El Dorado County, and at St. Francis of Assisi Church in Novato, Marin County. Monitoring stations installed at these field sites were checked monthly for termite activity from August 1994 through November 1996 at the IFG site and from January 1995 through December 1996 at the Novato site (Haverty et al. 1999b). CHC phenotypes were determined (as per Haverty and Nelson 1997) for all active monitoring stations at least once during the study period at both sites. When present in sufficient numbers (100 to 200 workers), termite samples from every monitoring station were returned to the laboratory and kept frozen at −15°C until the CHC mixtures were determined. At the end of the study period (1994–1996) we had samples from all active stations in the freezer except for those used for confirmation of CHC phenotype of the termites in each monitoring station before the study reported here.

To evaluate variation in CHC profiles within and among colonies of the same phenotype and to assess the consistency of CHC mixtures over time, we selected monitoring stations that were occupied over most of the duration of our studies (Haverty et al. 1999b, 2000). We chose four monitoring stations from disparate locations (>25 m apart) at the IFG site for phenotype CA-A (Wc7, Xb8, Yh27, and Zp49) and phenotype CA-B (Wb36, Yd16, Yq23, and Yv34) so that we were confident that the termites within the stations listed above were from different colonies. Termites were found in each of these stations on ≥90.0% of visits, except for Wb36, which was occupied only 65.6% of the time, but was located in a position to ensure that it was a separate colony. Phenotype CA-C was less common, and we selected only three monitoring stations (Wi74, Yt2, and Zn11) that were occupied by this phenotype on at least 81.0% of visits during the 3-yr period of study (Haverty et al. 1999b, 2000). At the Novato site we chose four monitoring stations (St18, St25, St57, and St63) that were consistently occupied (90.0% of visits) by phenotype CA-A'. We chose the three monitoring stations (St21, St253, and St314) that were continuously occupied (100% of the visits) by phenotype CA-D over the 2-yr study period.

Our initial goals at these two sites were somewhat different. At the IFG site our initial focus was (1) to determine the number of phenotypes present and their relative abundance and dispersion,

and (2) to quantify their foraging activity. During the 3-yr observation period we were not concerned with the number of colonies present. At the Novato site we were also focused on the number of phenotypes present and the quantification of their foraging activity, but we also needed to know the number of each phenotype and their dispersion around two residential structures.

Therefore, for this present study the distances among monitoring stations occupied by the same CHC phenotypes at IFG was the metric we used to assume monitoring stations were occupied by different colonies of the same phenotype. Furthermore, we assumed that the termites occupying the same monitoring station over the period of the study met the definition of a colony: 'a group of individuals, other than a single mated pair, that constructs nests or rears offspring in a cooperative manner (as opposed to an aggregation).' (Hölldobler and Wilson 2009). We knew that each of the monitoring stations we report here always produced termites of the same phenotype, but cannot be absolutely sure that only one colony occupied the selected monitoring stations over the 3-yr period.

At the Novato site we determined that the termites in each of the monitoring stations reported in this study were from different colonies as determined by mark-release-recapture and agonistic behavior studies (Getty et al. 2000a,b; Haverty et al. 1999a, 2000). This knowledge was critical to studies of colony elimination using baits at this site (Getty 2000b).

Voucher specimens (usually soldiers and workers) for many of the collection dates for each of the colonies in this study were placed in 70% ethanol and have been retained by the authors (LJN and MIH).

Characterization of CHC Mixtures

Beginning in July 2002 and ending in October 2002, CHCs were characterized by extracting samples of 100–200 worker termites. Samples were removed from the freezer, brought to room temperature, then dried at 70°C for 30 min. Dried samples were then transferred to clean 20-ml vials and immersed in 10 ml of *n*-hexane for 10 min. The resulting extracts of cuticular lipids were pipetted through 4 cm of activated silica gel in a Pasteur pipette mini-column to isolate the hydrocarbon components. The CHC extract was then evaporated to dryness under nitrogen and redissolved in 60 µl of hexane.

Gas chromatography (GC) analyses of the CHCs were performed on a Hewlett-Packard 6890 gas chromatograph equipped with a capillary column (HP-1 #19091z-433, 30 m × 0.25 mm ID × 0.25 µm film thickness) and a flame ionization detector (FID). The system was interfaced with Hewlett Packard ChemStation software for data acquisition and integration. Helium was the carrier gas, column flow was 1.5 ml/min, and the GC was operated in the split mode with a ratio of 10:1. Injection volume was 1 µl. The GC was temperature programmed from 200 to 320°C at 3°C/min with an initial hold at 200°C for 4 min and a final isothermal hold at 320°C for 16 min. For the FID, hydrogen flow was 40 ml/min, and total air flow was 450 ml/min.

To confirm the identity of all peaks, ca. 10% of the samples were also run on a gas chromatograph-mass spectrometer (GC-MS). Analyses were performed on an Agilent 6890 GC coupled with an Agilent 5973 Mass Selective Detector. The injection parameters, column, and temperature program were the same as for the FID analyses. Helium was the carrier gas, column flow was 1.0 ml/min, and the inlet was operated in the split mode with a ratio of 10:1. Electron impact (EI) mass spectra were obtained at 70 eV, and scanning was done from 55 to 640 mass units every 1.0 s. The system was interfaced with Agilent ChemStation software for data acquisition and analysis of mass spectral data. CHC peak assignments were verified using the mass spectral interpretation methods reported in Page et al. (1997) and Nelson et al. (2008).

Shorthand descriptors were used to label each CHC as defined in Nelson et al. (2008). Double bond positions were not determined for the unsaturated compounds, thus equivalent chain lengths (ECL) were calculated, and lowercase letters assigned were necessary to distinguish isomers.

Quantitative Analysis Techniques

Only CHC characterization and quantification data obtained from the GC-FID analyses were used in this study. The GC-MS characterizations were performed solely to confirm the identification of the CHCs in each peak. All samples of one CHC phenotype (i.e., CA-A) were analyzed before any samples from a different phenotype were analyzed. The order of the samples quantified within a phenotype was randomized to prevent potential bias due to time of year or potential changes in the sensitivity of the gas chromatograph over time. Quantification of individual CHC peaks was done by calculating the area of each peak then summing the areas of all peaks. The quantity of each CHC or peak was expressed as a percentage of the total CHC fraction. The response variable for all statistical evaluations was the percentage of each individual CHC or peak. Some peaks represented two or more co-eluting compounds (i.e., 11-meC27 and C27:2) or isomers (13-; 11-meC37); the sum of the compounds in these peaks was used to generate the response variable. To calculate the percentages of CHCs belonging to different hydrocarbon classes, the areas of co-eluting peaks were simply divided by the number of components. Positional isomers that co-elute such as 13-; 11-meC37 or 13,17-; 11,15-dimeC37 were counted as one component.

For each CHC (or peak) in each phenotype, the mean, standard deviation, minimum value, maximum value, and the number of samples with a percentage under 0.1% were determined. Percentage values for each CHC within a phenotype were examined to identify extreme outliers. Values were determined to be unusable outliers if their value for a given sample date exceeded four standard deviations from all other values for that particular CHC for that phenotype for the duration of the study. Some of the CHCs identified in this study were rare: their mean value was less than 0.1 percent of the total CHC and/or were not detected in over 25% of the samples. These CHCs were not included in any of the analyses.

Separation of Samples into Phenotypes by their CHC Mixtures.

Potential differences of CHC mixtures among all samples were examined by nonmetric multidimensional scaling analysis to provide 2-dimensional displays of chosen variables. Nonmetric multidimensional scaling analysis is a dimension-reduction technique related to principal component analysis and canonical correlation that derives a linear combination of quantitative variables (in this case, the percentages of CHC components) that summarizes between-class variation. That analysis was conducted in R (R Core Team 2021) using the package MASS (Venables and Ripley 2002) and figures produced using packages ggplot2 (Wickham 2016) and factoextra (Kassambara and Mundt 2020). The resulting groupings would be considered separation of samples into quantitatively determined phenotypes based on all CHCs, rather than by single or multiple diagnostic CHCs.

Table 1. Comparison of mean relative percentages of diagnostic cuticular hydrocarbons for the five cuticular hydrocarbon phenotypes of *Reticulitermes* from the current study analyzed by GC-FID to previously published results of GC-MS analyses

	CA-A		CA-A'		CA-B		CA-C		CA-D	
	2022	1997/2008[a]	2022	1997/2008[a]	2022	1997/2008[a]	2022	1997/2008[a]	2022	1997/2008[a]
Hydrocarbon	*N* = 81	*N* = 8	*N* = 36	*N* = 8	*N* = 70	*N* = 5	*N* = 43	*N* = 4	*N* = 51	*N* = 6
C25 3a (ECL[b] = 25.98)			2.85	4.51						
C25 3b (ECL = 26.07)			3.74	5.86						
C27:2; C27:1[c] (ECL = 26.75)							11.94	11.23		
5-meC25					12.44	7.78			13.17	7.65
5-meC27					5.54	4.67			0.46	0.65
11,15-dimeC27; 3-meC27[d]	0.11	0.25	0.00	0.24			8.96	7.05		
5,17-dimeC27[e]					22.01	18.45			1.12	1.51
5,17-dimeC35					2.38	3.61			1.09	1.68
5,17-dimeC37					2.02	3.37			1.98	2.78

[a]Haverty and Nelson 1997 and Nelson et al. 2008.
[b]Equivalent chain length.
[c]These two compounds co-elute along with 2-meC26, but are present only in CA-C
[d] These compounds eluted separately in 1997/2008 (GC-MS) and were added together for this comparison.
[e]This compound co-elutes with C25:3 in CA-D.

Identification of Colonies Within a Phenotype by Their CHC Mixtures

Potential differences of CHC mixtures among termite samples from each monitoring station for each phenotype were also examined by nonmetric multidimensional scaling analysis to provide 2-dimensional displays of chosen variables for each phenotype. This analysis was conducted using the same methods as described above. The goal was to determine whether we could assess the similarity of CHC mixtures to separate or identify samples from the same colonies within each phenotype.

Potential Seasonal Changes in Hydrocarbon Levels

Quantities (percent of the total) of each CHC or peak were plotted against the date of collection for each of the five phenotypes (Supp Figs. 1–5 [online only]). Because we saw what appeared to be increases and decreases in a cyclical manner for some of the CHCs, we decided to evaluate seasonal trends for each CHC for all five phenotypes. To further investigate that trend, a sine wave model was considered:

$$Percent = a + b\sin\left(2\pi\left(d+\theta\right)/365\right) + \epsilon$$

where *Percent* is a particular CHC percentage for samples of termites on Julian day of the year (d) from all colonies of a particular phenotype. Constants to be estimated are a (mean percent), b (amplitude of sine wave), and θ (phase adjustment for the sine wave in units of days). The error term ϵ is assumed to have constant variance σ^2. That analysis was conducted in R (R Core Team 2021) using the nls function.

Only a potential annual cycle was considered and while having the data follow a perfect sine wave is likely unrealistic, the estimated amplitude was considered a reasonable summary statistic to characterize the strength of the cyclical nature. The overall quality of the fit was summarized with an R^2 statistic. Another characterization of the strength of any potential cycle consists of the 'signal-to-noise' ratio and time of year of the cycle peak. For the 'signal-to-noise' ratio (*S*/*N*) we used the ratio of the estimate of b (amplitude of the sine wave) to the standard error of the regression: $\hat{b}/\hat{\sigma}$. For the cycle peak we used the Julian day estimate of the peak: $\frac{365\left(\pi-2\hat{\theta}\right)}{4\pi}$. R^2 and signal-to-noise ratios (*S*/*N*) are highly correlated, and our focus was on CHCs for each phenotype with a *S*/*N* ≥1.0.

For each CHC for each phenotype with an *S*/*N* greater than or equal to 1.0 within a particular season, primarily winter (December 21–March 19) and summer (June 21–September 21) (See Supp Tables 6–10 [online only]), we summed the percentages for each observation date and plotted these data against the dates throughout the study. The resulting plots are presented to display obvious seasonal trends.

Results

CHCs Found in Five Phenotypes

The CHCs identified from the 18 colonies sampled (4 each from phenotypes CA-A, CA-A', and CA-B, and 3 each from phenotypes CA-C and CA-D) over a 2- or 3-yr period are presented as relative percentages in Supp Tables 1–5 (online only). The compositions of the five CHC phenotypes in the present study were comparable to those previously reported by Haverty and Nelson (1997) and Nelson et al. (2008). Classes of CHCs reported here again included normal alkanes, olefins (alkenes, alkadienes, and alkatrienes), and mono-, di- and trimethyl alkanes. The resolution of certain compounds in GC-FID analyses was not identical to previous analyses by GC-MS, so there were some differences in co-eluting constituents. The diagnostic CHCs used to separate phenotypes (Haverty and Nelson 1997) were present in equivalent amounts (Table 1).

Phenotype CA-A

In phenotype CA-A the internally branched monomethyl alkanes were the most plentiful class with a total of 29.23% of the CHCs; 13-; 11-meC25 was the most abundant of the internally branched monomethyl alkanes; it co-eluted with C25:2 (Supp Table 1 [online only]). The olefins, *n*-alkanes, terminally branched monomethyl alkanes, and dimethyl alkanes were found in relatively similar percentages (20.57, 17.74, 16.12, and 16.05%, respectively). The CHCs with the largest quantity in each class had 25 carbons in the parent chain and accounted for 64.08% of the total CHC.

Phenotype CA-A'

In phenotype CA-A' the olefins were the most abundant class with a total of 23.88% of the CHC mixture (Supp Table 2 [online only]). The internally branched monomethyl alkanes were nearly as abundant,

comprising 22.20% of the total CHC. The dimethyl alkanes, terminally branched alkanes, and *n*-alkanes occurred in slightly lesser amounts (18.43, 18.23, and 16.85% of the total CHCs, respectively). As with phenotype CA-A, CHCs with the highest amount in each class had 25 carbons in the parent chain with a total of 61.43% of the total CHC.

Phenotype CA-B

The predominant class of CHCs in phenotype CA-B was the dimethyl alkanes (38.78% of the total); 5,17-dimeC27 contributed the largest amount with 21.79% of the total (Supp Table 3 [online only]). The terminally branched monomethyl alkanes (methyl branches at positions 2–6) and internally branched alkanes made up 27.39 and 20.98% of the total CHC, respectively. The *n*-alkanes comprised only 11.11% of the total CHCs; no olefins were found. The trimethyl alkanes represented only 1.51% of the total CHC. 5,9,17-trimeC27; 5,9,17-trimeC35 and 5,9,17-trimeC37 contributed 0.62, 0.48, and 0.41% of the total CHC, respectively (Supp Table 3 [online only]). CHCs with 25 carbons in the parent chain comprised 31.74% of the total CHC; CHCs with 27 carbons in the parent chain represented 40.94% of the total CHC.

Phenotype CA-C

Terminally branched monomethyl alkanes, internally branched monomethyl alkanes, and dimethyl alkanes were the most abundant CHC classes in phenotype CA-C, with 24.03, 22.34, and 20.41% of the total, respectively (Supp Table 4 [online only]). Olefins and *n*-alkanes comprised 19.89 and 13.04%, respectively. The peaks with co-eluting CHCs of different classes predominated (13-; 11-; 9-; 7-meC25 + 11,13-dimeC25 totaled 12.53%; 2-meC26 + C27:2 + C27:1 totaled 11.88%; and 13-; 11-meC27 + C27:2 + 7-meC27 + 11,13-dimeC27 totaled 16.51%). CHCs with 25 carbons in the parent chain comprised 36.26% of the total CHC; CHCs with 27 carbons in the parent chain represented 36.70% of the total CHC.

Phenotype CA-D

Methyl-branched alkanes dominated the mixture of CHCs in phenotype CA-D (Supp Table 5 [online only]). Internally branched monomethyl alkanes were the most abundant at 35.12% of the total; 13-; 11-; 9-meC25 contributed the most with 26.26% of the total. Terminally branched monomethyl alkanes and dimethyl alkanes comprised similar totals at 24.51 and 23.70% of the total, respectively. Each of the latter two classes had one dominant compound: 5-meC25 at 13.30% and 5,17-dimeC25 at 13.79% of the total. The *n*-alkanes from C23 to C27 amounted to 13.97% of the total. Olefins were rare (1.56% of the total), consisting of three trienes. Only one trimethyl alkane (5,9,17-trimeC25) was found and co-eluted with C25:3. CHCs with 25 carbons in the parent chain comprised 69.57% % of the total CHC.

Separation of Samples Into Phenotypes by Their CHC Mixtures

Nonmetric multidimensional scaling analysis performed with the isoMDS function of the R package MASS (Venables and Ripley 2002) resulted in complete separation of the phenotypes using just two dimensions with a small stress value of 0.083, which is lower than the commonly accepted threshold of 0.2 for a good reproduction of the distances between sample points (Dexter et al. 2018). The complete separation (Fig. 1) is consistent with previous designations of phenotypes of *Reticulitermes* from northern California based on diagnostic CHCs (Haverty and Nelson 1997, Page et al. 2002) and phylogenetic analyses of mtDNA sequences (Copren et al. 2005).

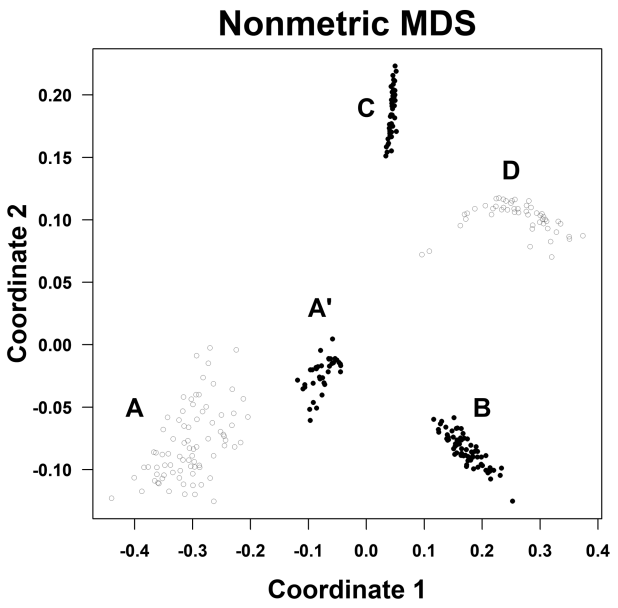

Fig. 1. Separation of phenotypes CA-A, CA-A′, CA-B, CA-C, and CA-D using nonmetric multidimensional scaling of all CHCs exceeding 0.09% of the total CHC.

Comparison of Colonies Within Each of the Five Phenotypes

Nonmetric multidimensional scaling analysis was also performed to look for any separation of colonies with each of the five phenotypes. While there might be some different mean component scores, there was a great deal of overlap of the individual colonies within a phenotype (Fig. 2), which suggests that this particular analysis cannot distinguish colonies within phenotypes.

Seasonal Variation in CHCs Within All Five Phenotypes

With our experience finding seasonal differences in the quantities of some CHCs in *C. formosanus* (Haverty et al. 1996b), we decided to investigate any possible seasonal trends in the five CHC phenotypes of *Reticulitermes* in this study. When we plotted CHC quantities against the Julian dates of the 2- or 3-yr periods, we found a seasonal trend in some, but not all, of the CHCs in all five phenotypes. The seasonal cycle strongly fit a sine curve and achieved a maximum value usually in the summer or winter months; occasionally maximum values occurred in the spring or fall (Supp Figs. 1–5 [online only]). As a measure of the goodness of fit, we report here the R^2 value and signal-to-noise ratio (*S/N*) for each CHC peak (Supp Tables 6–10 [online only]). The R^2 value will be more familiar to entomologists and the *S/N* is often used in the physical sciences. The R^2 and *S/N* values are highly correlated. With each highly significant CHC peak, we include the R^2 and the *S/N* as a measure of the fit to the sine curve, the date and Julian Day (JD) of the maximum value of the curve, and the season of the year when the maximum value occurred (Tables 2–6; Supp Tables 6–10 [online only]). The seasonal limits we used were: winter (December 21–March 19), spring (March 20–June 20), summer (June 21–September 21), and fall (September 22–December 20). For each phenotype, the CHCs

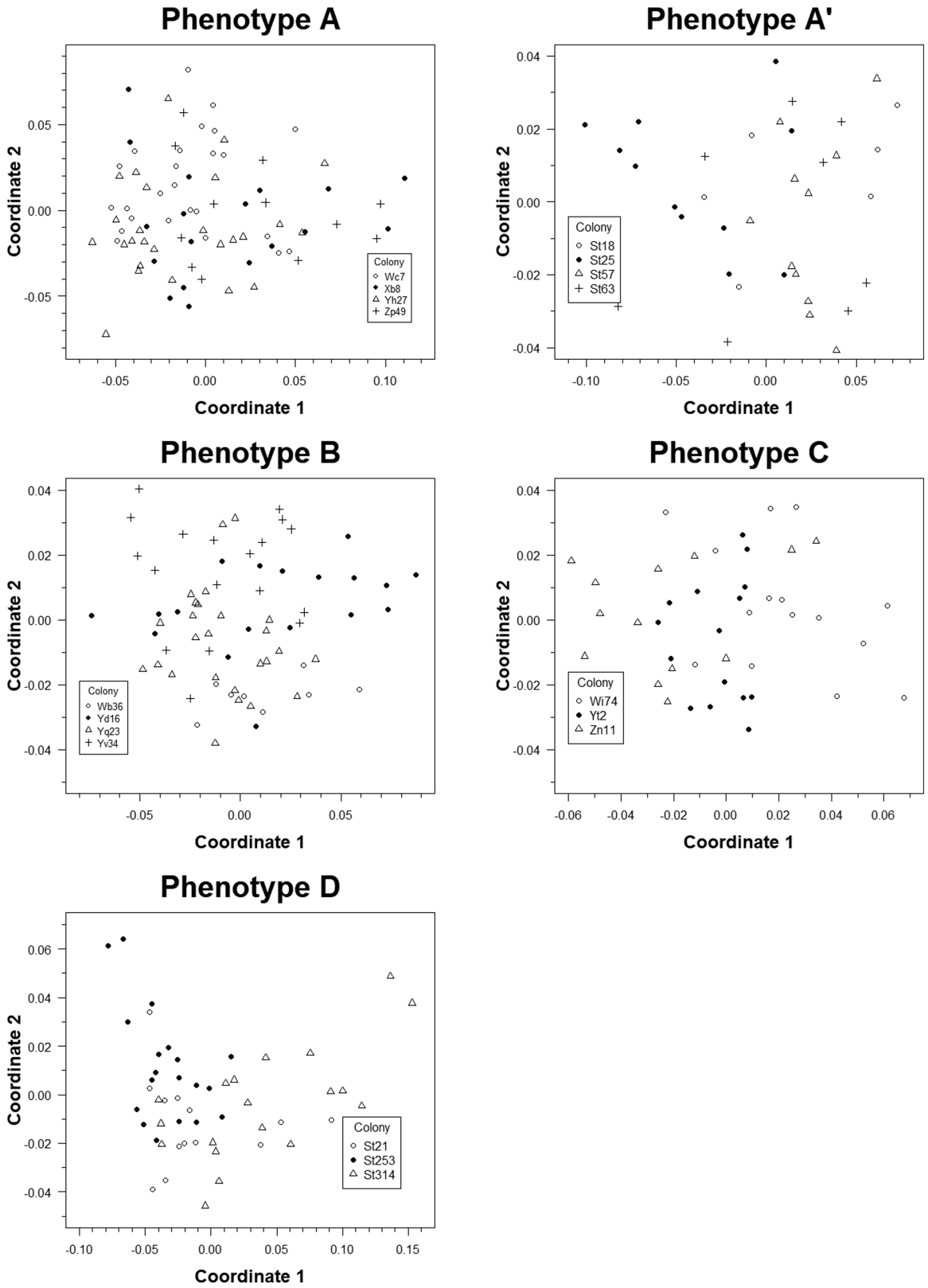

Fig. 2. Results of nonmetric multidimensional scaling analysis on each phenotype testing for separation among colonies.

having *S/N* ≥1.0 are presented by season and decreasing order of *S/N* in Tables 2–6.

Phenotype CA-A

Nine CHC peaks demonstrated a strong seasonal cycle (Table 2). Eight of the nine were either internally branched monomethyl or internally branched dimethyl alkanes; one was a terminally branched monomethyl alkane. The R^2 values of the fit to the sine curve ranged from 0.36 to 0.72 with sample sizes of 77 to 81 (p values <0.001). The four CHCs with an apex (that reached a maximum value) in the winter months (Julian Days 36–60; February 5–March 1) were: 3-meC23, 11,15-dimeC25, 11,15-dimeC27, and 11,15-dimeC26.

Table 2. R^2, signal-to-noise ratio (*S*/*N*), date and Julian day of maximum percentage for cuticular hydrocarbons of *Reticulitermes* phenotype CA-A from Placerville, CA. Data are grouped by season in descending order of signal-to-noise ratio. Only peaks with signal-to-noise ratio ≥ 1.0 are shown

Hydrocarbon[a]	R^2	*S*/*N*[b]	Max Date	Julian Day	Season
3-meC23	0.72	2.31	5-Feb.	36	Winter
11,15-dimeC25	0.57	1.64	25-Feb.	56	Winter
11,15-dimeC27	0.41	1.21	22-Feb.	53	Winter
11,15-dimeC26	0.41	1.20	1-Mar.	60	Winter
11,15-dimeC35	0.36	1.04	26-Mar.	85	Spring
13-; 11-meC39	0.62	1.87	13-Aug.	225	Summer
13-; 11-meC37	0.61	1.81	3-Aug.	215	Summer
11,15-dimeC39	0.61	1.81	22-Aug.	234	Summer
12-; 11-meC36	0.37	1.11	17-July	198	Summer

[a]Sample sizes range from 77 to 81 for each CHC. See Supp Table 1 (online only).
[b]Ratio of amplitude to σ.

Table 3. R^2, signal-to-noise ratio (*S*/*N*), and date and Julian day of maximum percentage for cuticular hydrocarbons of *Reticulitermes* phenotype CA-A' from Novato, CA. Data are grouped by season in descending order of signal-to-noise ratio. Only peaks with signal-to-noise ratio ≥ 1.0 are shown

Hydrocarbon[a]	R^2	*S*/*N*[b]	Max Date	Julian Day	Season
11,15-dimeC35	0.60	1.83	16-Feb.	47	Winter
11,15-dimeC25	0.58	1.74	10-Feb.	41	Winter
3-meC23	0.55	1.67	25-Jan.	25	Winter
12,16-; 11,15-dimeC36	0.51	1.52	31-Jan.	31	Winter
C25:2	0.34	1.06	8-Feb.	39	Winter
11,15-dimeC39	0.87	3.73	22-Aug.	234	Summer
15-; 13-; 11-meC39	0.84	3.41	1-Aug.	213	Summer
15-; 13-; 11-meC41	0.74	2.52	30-July	211	Summer
11,15-dimeC41	0.72	2.34	18-Aug.	230	Summer
13-; 11-meC37	0.65	2.01	22-July	203	Summer
12,16-; 11,15-dimeC38	0.58	1.56	18-Sept.	261	Summer
12-; 11-meC38	0.44	1.31	15-July	196	Summer
11-; 9-meC23	0.42	1.17	16-Dec.	350	Fall
12-; 11-; 10-; 9-meC24	0.38	1.00	20-Nov.	324	Fall

[a]Sample sizes range from 35 to 36 for each CHC. See Supp Table 2 (online only).
[b]Ratio of amplitude to σ.

Table 4. R^2, signal-to-noise ratio (*S*/*N*), and date and Julian day of maximum percentage of cuticular hydrocarbons of *Reticulitermes* phenotype CA-B from Placerville, CA. Data are grouped by season in descending order of signal-to-noise ratio. Only peaks with signal-to-noise ratio ≥ 1.0 are shown

Hydrocarbon[a]	R^2	*S*/*N*[b]	Max Date	Julian Day	Season
5,17-dimeC33	0.41	1.17	22-Feb.	53	Winter
5,9,17-trimeC35	0.36	1.02	3-Mar.	62	Winter
13-meC37	0.51	1.41	22-Aug.	234	Summer
14-meC34	0.52	1.40	9-Sept.	252	Summer
5,17-dimeC43	0.44	1.25	21-Aug.	233	Summer
15-; 13-; 11-meC35	0.44	1.21	31-Aug.	243	Summer
13-meC39	0.42	1.20	18-Aug.	230	Summer
14-; 13-; 12-meC36	0.40	1.16	9-Aug.	221	Summer
5,17-dimeC41	0.34	1.00	27-Aug.	239	Summer
2-meC25	0.35	1.00	2-Sept.	245	Summer

[a]Sample sizes range from 69 to 70 for each CHC. See Supp Table 3 (online only).
[b]Ratio of amplitude to σ.

One peak with a maximum value in the spring (Julian Day 85; March 26) was 11,15-dimeC35. The four peaks with an apex in the summer (Julian Days from 198 to 234; July 17–August 22) were: 13-; 11-meC39, 13-; 11-meC37, 11,15-dimeC39, and 12-; 11-meC36 (Table 2).

Phenotype CA-A'

Fourteen CHC peaks demonstrated a strong seasonal cycle (Table 3). Twelve of the fourteen were either internally branched monomethyl or internally branched dimethyl alkanes; one was a terminally branched monomethyl alkane, and one was an alkadiene. The R^2

Table 5. R^2, signal-to-noise ratio (*S/N*), and date and Julian day of maximum percentage for cuticular hydrocarbons of *Reticulitermes* phenotype CA-C from Placerville, CA. Data are grouped by season in descending order of signal-to-noise ratio. Only peaks with signal-to-noise ratio ≥ 1.0 are shown

Hydrocarbon[a]	R^2	S/N[b]	Max Date	Julian Day	Season
2-meC25	0.61	1.83	19-Feb.	50	Winter
13,17-; 11,15-dimeC35	0.58	1.74	14-Feb.	45	Winter
11,15-dimeC27; 3-meC27	0.50	1.51	6-Feb.	37	Winter
11-meC23	0.32	1.02	31-Dec.	365	Winter
2-meC26; C27:2; C27:1	0.42	1.08	2-May	122	Spring
C27	0.56	1.75	16-July	197	Summer
13-; 11-meC39	0.45	1.39	24-July	205	Summer
C29:1	0.43	1.36	28-July	209	Summer
15-; 13-; 11-meC35	0.47	1.36	26-Aug.	238	Summer
13,17-dimeC41	0.48	1.32	7-Sept.	250	Summer
13,17-dimeC43	0.42	1.24	21-Aug.	233	Summer
14-; 12-meC36	0.35	1.13	4-Aug.	216	Summer
13-meC43	0.36	1.13	10-Aug.	222	Summer
C24	0.34	1.08	13-Aug.	225	Summer
C23	0.32	1.05	4-Aug.	216	Summer
13-; 11-; 9-meC25; 7-meC25; 11,13-dimeC25	0.53	1.48	13-Dec.	347	Fall

[a]Sample sizes range from 41 to 43 for each CHC. See Supp Table 4 (online only).
[b]Ratio of amplitude to σ.

Table 6. R^2, signal-to-noise ratio (*S/N*), and date and Julian day of maximum percentage for cuticular hydrocarbons of *Reticulitermes* phenotype CA-D from Novato, CA. Data are grouped by season in descending order of signal-to-noise ratio. Only peaks with signal-to-noise ratio ≥ 1.0 are shown

Hydrocarbon[a]	R^2	S/N[b]	Max Date	Julian Day	Season
5-meC23	0.56	1.50	31-Jan.	31	Winter
5,17-dimeC35	0.54	1.46	1-Mar.	60	Winter
11-; 9-; 7-meC23	0.52	1.38	1-Feb.	32	Winter
5-meC25	0.46	1.22	30-Jan.	30	Winter
3-meC25	0.62	1.70	9-Aug.	221	Summer
17-; 15-; 13-; 11-meC39	0.60	1.63	18-Aug.	230	Summer
5,17-dimeC43	0.53	1.41	18-Aug.	230	Summer
15-; 13-; 11-meC37	0.47	1.25	30-July	211	Summer
2-meC23	0.39	1.07	28-July	209	Summer

[a]Sample sizes range from 49 to 50 for each CHC. See Supp Table 5 (online only).
[b]Ratio of amplitude to σ.

values of the fit to the sine curve ranged from 0.34 to 0.87 with sample sizes of 35–36 (p values <0.001). The five peaks with a maximum value in the winter (Julian Days 25–47; January 25–February 16) were: 11,15-dimeC35, 11,15-dimeC25, 3-meC23, 12,16-; 11,15,-dimeC36, and C25:2. The seven peaks with an apex in the summer (Julian Days from 196 to 234; July 15–August 22) were: 11,15-dimeC39, 15-; 13-; 11-meC39, 15-; 13-; 11-meC41, 11,15-dimeC41, 13-; 11-meC37, 12,16-; 11,15-dimeC38, and 12-; 11-meC38. Two peaks that had a maximum value in the fall (Julian Days 324–350; November 20–December 16) were: 11-; 9-meC23 and 12-; 11-; 10-; 9-C24 (Table 3).

Phenotype CA-B

Ten CHC peaks demonstrated a moderate seasonal cycle (Table 4). Five of the eight were internally branched monomethyl alkanes, one was a terminally branched monomethyl alkane, two were 5,17-dimethyl alkanes and one was a 5,9,17-trimethyl alkane. The R^2 values of the fit to the sine curve ranged from 0.34 to 0.52 with sample sizes of 69–70 (p values <0.001). The two CHCs with an apex in the winter (Julian Days 53–62, February 22–March 3) were: 5,17-dimeC33 and 5,9,17-trimeC35. The eight peaks with a maximum value in the summer (Julian Days 221–252, August 9–September 9) were: 13-meC37, 14-meC34, 5,17-dimeC43, 15-; 13-; 11-meC35, 13-meC39, 14-; 13-; 12-meC36, 5,17-dimeC41, and 2-meC25 (Table 4).

Phenotype CA-C

Sixteen CHC peaks demonstrated a strong seasonal cycle (Table 5). Eight of the sixteen were either internally branched monomethyl or internally branched dimethyl alkanes; one was a terminally branched monomethyl alkane, one peak was a mixture of a terminally branched alkane + one alkene + one alkadiene, one peak was a mixture of internally branched dimethyl alkane + a terminally branched monomethyl alkane, three were normal alkanes, one peak was a mixture of isomeric, internally branched monomethyl alkanes + an internally branched 11,13- dimethyl alkane, and one was an alkene. The R^2 values of the fit to the sine curve ranged from 0.32 to 0.61 with sample sizes of 41–43 (p values <0.001). The four peaks with a maximum value in the winter (Julian Days 365–50, December 31–February 19) were: 2-meC25, 13,17-; 11,15-dimeC35, 11,15-dimeC27 + 3-meC27, and 11-meC23. One peak with a mixture of 2-meC26; C27:2; C27:1 increased in the spring and reached its

apex on May 2 (Julian Day = 122). The ten peaks with an apex in the summer (Julian Days 197–250, July 16–September 7) were: C27, 13-; 11-meC39, C29:1, 15-; 13-; 11-meC35, 13,17-dimeC41, 13,17-dimeC43, 14-; 12-meC36, 13-meC43, C24, and C23. One peak consisting of a mixture of 13-; 11-; 9-meC25; 7-meC25; 11,13-dimeC25 increased in the fall (Julian Day 347, December 13) (Table 5).

Phenotype CA-D

Nine CHC peaks demonstrated a strong seasonal cycle (Table 6). Three of the nine were internally branched monomethyl alkanes, two were 5,17-dimethyl alkanes and four were terminally branched monomethyl alkanes. The R^2 values of the fit to the sine curve ranged from 0.39 to 0.62 with a sample size of 51 (p values <0.001). The four peaks with a maximum value in the winter (Julian Days 30–60, January 31–March 1) were: 5-meC23, 5,17-dimeC35, 11-; 9-; 7-meC23, and 5-meC25. The five peaks with an apogee in the summer (Julian Days 209–230, July 28–August 18) were: 3-meC25, 17-; 15-; 13-; 11-meC39, 5,17-dimeC43, 15-; 13-; 11-meC37, and 2-meC23 (Table 6).

To demonstrate the strength of the seasonal cycles of some of the CHCs for each phenotype, we summed the percentages of CHCs with a significant R^2 or S/N for each observation date (See Tables 2–6). When these data were plotted against the dates of the study periods, highly significant sine-wave curves resulted (Fig. 3). Each phenotype had a cohort of CHCs with maximum values in either the winter or summer (Tables 2–6; Fig. 3).

Discussion

Taxonomic Characters

CHCs are involved in several functions in insects. Primarily they are known to function in waterproofing to prevent desiccation of insects but have many additional roles as semiochemicals and barriers to abrasion and to pathogens. CHCs are not the only chemicals on or in the cuticles of insects; other polar lipids may also be involved in waterproofing or be used as semiochemicals (Blomquist and Bagnères 2010). However, the nonpolar CHCs have been the most commonly characterized cuticular lipids, and they have proven useful for separating many insect taxa. Bagnères and Wicker-Thomas (2010) have prepared an extensive review of chemical taxonomy of insects based on CHCs. Many of our previous studies (LJN and MIH) are featured in this review on page 135. In their studies of ants, Sprenger and Menzel (2020) affirm that CHC profiles (the variety of CHCs and their relative quantities) are species-specific and mostly genetically heritable, which makes them suitable characters for species delineation. Seppä et al. (2011) suggest that while CHC profiles are useful for delimiting and identifying species, using multiple types of information, such as CHCs, morphology, and genetics, is the ideal approach. In a recent paper surveying the distribution of five species of *Reticulitermes* in the southeastern USA (Johnson and Forschler 2022) the authors emphasized the need to use an integrative taxonomic approach, combining morphology, behavioral attributes, and chemical signatures to provide taxonomic support to a given molecular marker.

The focus of the work presented here is confirming the taxonomic utility of CHCs of *Reticulitermes* phenotypes from northern California by demonstrating the consistency of CHC mixtures from multiple samples taken from the same colonies over time. Many studies of the taxonomy of *Reticulitermes* (including our own) utilize single samples from many different colonies taken at different times; the variation that is presented in those studies is intercolonial, and perhaps seasonal, variation. Our goal here is to provide context to the amount of potential variation that likely will occur within multiple samples taken from a given colony. All our samples were handled in the same way; the groups of termites were frozen and then dried before extraction and separation of the CHCs (see Materials and Methods). Our approach was to sample whole bodies that would include hydrocarbons throughout the cuticle. Other approaches to CHC sampling have been used that isolate specific cuticle layers or body segments, such as SPME (solid-phase microextraction) (Bland et al. 2001, Ginzel 2010) and direct assay of sections of cuticle (Brill and Bertsch 1986, Bagnères and Morgan, 1990).

In our study, all classes of CHCs were found, but not necessarily in each phenotype/species: We identified alkanes, monomethyl alkanes, dimethyl alkanes, trimethyl alkanes, alkenes, alkadienes, and alkatrienes. The chain length of the hydrocarbons that we consistently found ranged from C22 to C43, with the number and position of the methyl branches and the number and position of double bonds providing inter-phenotype variation. Within each phenotype we found many homologous series of CHCs, which may arise from common biosynthetic pathways (Martin and Drijfhout 2009). These were mainly monomethyl alkanes with the methyl branches in the same position, such as 2-; 3-; 5-, or 11-meCXX, but with a different parent chain length, and dimethyl alkanes with the pair of methyl branches, such as 5,17- or 11,15-dimeCXX in the same positions, but with a different chain length.

Comparison of Cuticular Hydrocarbons Among Colonies Within a Phenotype

We specifically chose monitoring stations that were far enough apart to be sure they contained different colonies of the same phenotype at the IFG site or were determined to be different by using mark/release/recapture techniques or agonistic behavior studies in the laboratory at the Novato site (Getty et al. 2000a,b; Haverty et al. 2000). Even with our confidence that we were sampling different colonies, all colonies sampled within a phenotype were from the same population of colonies within a relatively small geographic area. This would likely result in less genetic diversity within phenotypes. Dronnet et al. (2006) found correspondence between CHC profiles and genetic variation in colonies of *R. santonensis* in France. It should be noted that *R. santonensis* has been synonymized with *R. flavipes* (Austin et al. 2005, Su et al. 2006). In a global-scale study of Argentine ant populations, Brandt et al (2009) reported that more genetically diverse populations are more diverse chemically, and that among-colony variation is genetically determined. Sprenger and Menzel (2020) reiterate that CHC profiles of social insects are usually colony-specific, and function as cues to discriminate nestmates from nonnestmates. They also generalize that within a species, variation in CHC profiles is largely quantitative, i.e., the same CHCs are present but the relative proportions may vary. In ants, particularly predaceous ants, dietary differences can affect colony-specific cuticular hydrocarbon profiles and can disrupt nestmate recognition and result in intercolonial aggression (Liang and Silverman 2000, Sprenger and Menzel 2020).

Reticulitermes workers can also recognize nonkin and nonnestmates, as demonstrated by observations of agonistic behavior among colonies of the same or different phenotypes (Haverty et al. 1999a, Getty et al. 2000a, Delphia et al. 2003). When different individuals or groups from the same colony (even if collected at different times) are paired, no aggressive behaviors were observed and mortality after 24 hr was no different than zero. When individuals

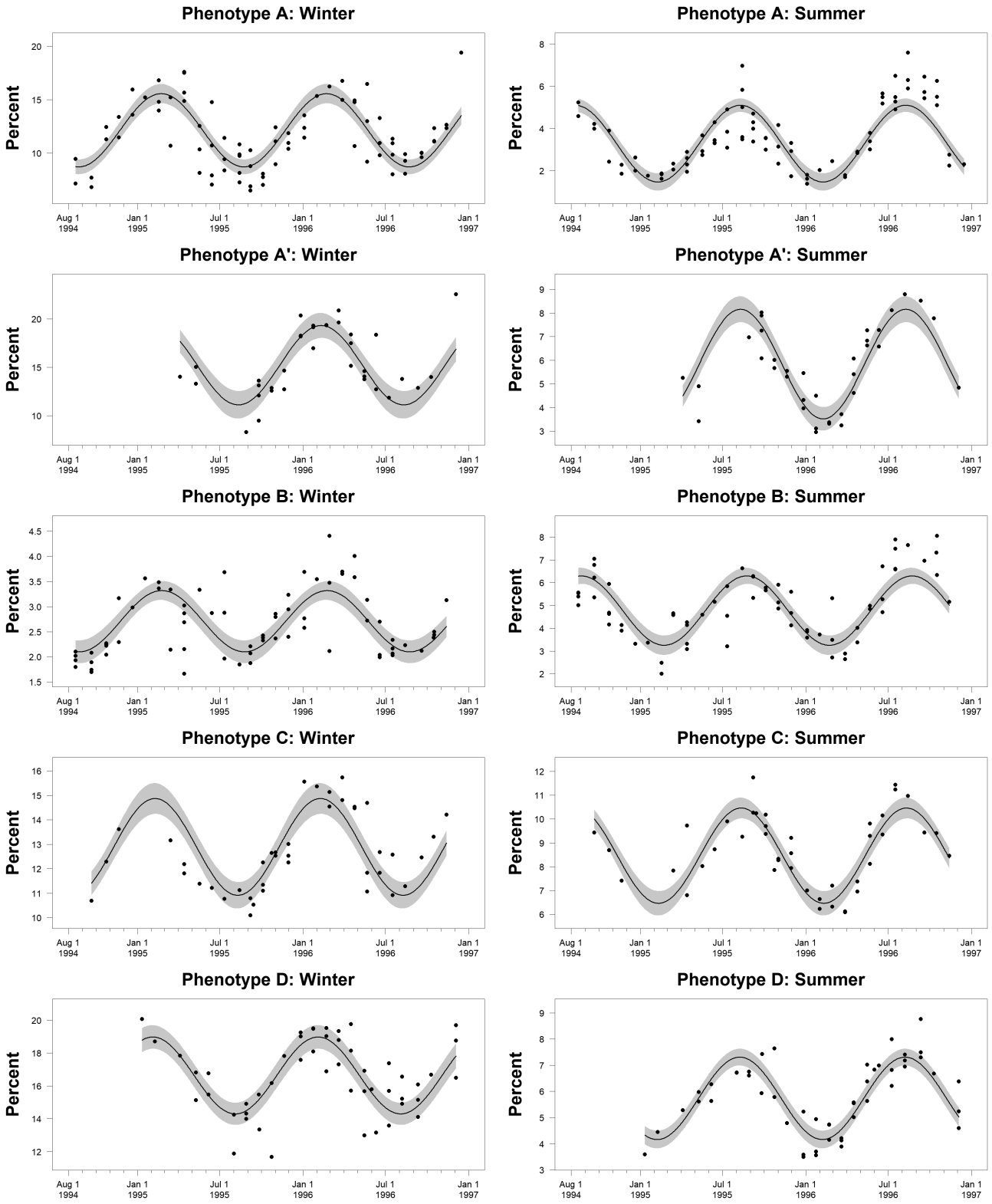

Fig. 3. Sine wave curves depicting the seasonal cycle of cuticular hydrocarbons from five phenotypes of *Reticulitermes* in northern California. For each of the five phenotypes (A, A′, B, C, and D) the CHCs with *S*/*N* ratios above 1.0 are combined for those with a maximum value in the winter or in the summer to display the cyclical and seasonal changes in a selection of the CHC components. The shaded area surrounding the sine curve represents the 95% confidence level.

or groups from different colonies of the same phenotype were paired, workers initially investigated the other(s) by antennation or grooming. Immediate aggressive behavior was not common, but mortality after 24 hr was usually high (>50 percent). Whatever signals they initially received did not elicit an agonistic response. But after investigating one another for several hours, a cue or cues was/were received that resulted in aggressive grooming, grappling, and/or biting. When individuals or groups from colonies of different

phenotypes were paired, aggression was immediate, and all pairings resulted in high mortality after 24 hr.

Clearly, worker termites use chemical cues to determine nestmates or conspecifics. The difference in the amount of time to recognize members of different colonies with the same CHC phenotype and the amount of time to recognize different CHC phenotypes (i.e., different species) suggests different agonistic cues. CHCs may provide the cue or cues, however, it is possible that polar lipids present in the cuticle may be involved (Blomquist and Bagnères 2010). We did not investigate the polar fraction of cuticular extracts in this study. Our nonmetric multidimensional scaling analyses of different colonies within each of the five cuticular hydrocarbon phenotypes indicate that we were unable to use CHCs to separate or identify colonies of *Reticulitermes*.

Seasonal Cycles

The seasonal cycles of some of the CHCs are likely important in restricting water loss via transpiration, even though the cuticle is not the only avenue for water loss: others include respiration through spiracles, trophallaxis, and production of liquid feces. Cuticular transpiration has been shown to account for the majority of overall water loss (Quinlan and Gibbs 2006, Gibbs and Rajpurohit 2010). *n*-Alkanes and terminally branched monomethyl alkanes can pack together tightly due to their linear configuration, resulting in increased viscosity which is key in preventing water loss (Gibbs, 1998, Gibbs and Rajpurohit 2010, Brooks et al. 2015, Sprenger and Menzel 2020). Increasing chain length in homologous series of hydrocarbons, with correspondingly increased melting temperatures, is also correlated with protection against water loss. Methyl-branched alkanes and unsaturated hydrocarbons cannot pack as tightly, reducing the melting temperature, and thus provide less protection against water loss (Gibbs and Pomonis 1995, Gibbs 2002, Sprenger and Menzel 2020).

Seasonal temperature changes affect variation in CHC production and differences in water loss rates (Hadley 1977, Toolson 1982, Gibbs and Rajpurohit 2010). Warmer temperatures, leading to higher risk of desiccation, should result in a CHC mixture with a higher melting point due to a higher proportion of linear and/or long-chain alkanes. Environmental changes (e.g., temperature gradients) can induce adaptive changes in CHC profiles. CHC variation in response to higher seasonal temperatures generally involves an increase in the proportion of *n*-alkanes and terminally branched monomethyl alkanes, whereas cooler temperatures are associated with an increase in internally branched methyl alkanes, dimethyl alkanes, and unsaturated components. Desiccation stress leads to a shift towards longer chain CHCs, a higher proportion of saturated hydrocarbons, and a larger proportion of straight vs. branched chain hydrocarbons. The response is similar for high and low humidity (Otte et al. 2018, Sprenger and Menzel 2020).

In this study, we reported only quantified relative proportions of various CHCs, not quantitative differences in the amount of total hydrocarbon. There could be seasonal changes in the total amounts of lipid produced, as well as changes in the physical arrangement of CHCs on the cuticle surface, but these aspects were not investigated here. Our results generally fit the pattern one might expect: the relative proportions of long-chain *n*-alkanes or terminally branched long-chain alkanes increased in summer months, whereas short-chain hydrocarbons, internally branched mono- or dimethyl alkanes and unsaturated hydrocarbons increased in winter months. In all five phenotypes there is a bimodal distribution: early eluting CHCs with carbon chain lengths of 23–27, and later eluting CHCs with carbon chain lengths of 33–43 carbons. We designate CHCs with 23–27 carbons as short-chain, and those with 33–43 carbons as long-chain for this discussion.

In phenotype CA-A, 4 of 4 hydrocarbons with a maximum in the winter were short-chain alkanes and 4 of 4 hydrocarbons with a maximum in the summer were long-chain alkanes (Supp Table 6 and Supp Fig. 1 [online only]). In phenotype CA-A', 3 of 5 winter hydrocarbons were short-chain, 1 of which was an alkadiene, and 2 of 5 were long-chain; 7 of 7 summer hydrocarbons were long-chain. Both CHCs with a maximum value in the fall were short chain (Supp Table 7 and Supp Fig. 2 [online only]). In phenotype CA-B, 2 of 2 winter CHCs were long-chain, however, they were di- and trimethyl alkanes, and 7 of 8 summer CHCs were long-chain (Supp Table 8 and Supp Fig. 3 [online only]). In phenotype CA-C, 3 of 4 winter hydrocarbons were short-chain mono- or dimethyl alkanes; 6 of 10 summer hydrocarbons were long-chain mono- or dimethyl alkanes; 3 of 10 were normal alkanes, 1 of 10 was a C29 alkene. The spring and fall peaks were a mixture of CHCs, but all were short-chained (Supp Table 9 and Supp Fig. 4 [online only]). In phenotype CA-D, 3 of 4 winter hydrocarbons were short-chain monomethyl alkanes, and one was a long-chain dimethyl alkane; 2 of 5 summer hydrocarbons were terminally branched monomethyl, short-chain alkanes, 3 of 5 were long-chain monomethyl or dimethyl alkanes. (Supp Table 10 and Supp Fig. 5 [online only]).

Seasonal temperature changes have been shown to impact both lipid variation and changes in water loss rates. For example, summer-collected scorpions and tenebrionid beetles have lower water loss rates than fall/winter collected arthropods, as well as a distinct seasonal complement of cuticular hydrocarbons (Hadley 1977, Toolson and Hadley 1979, Toolson 1982, Gibbs and Rajpurohit 2010). Although we have shown that *Reticulitermes* displays a seasonal change in relative proportions for a selection of cuticular hydrocarbons, the transpiration situation is much different for subterranean termites like *Reticulitermes*, as they live in an environment with very high humidity (near saturation) inside their subterranean chambers and gallery system, which should reduce cuticular transpiration. Changes in the colony-wide CHC profile may be affected by changes in caste composition, such as seasonal production of alates (Haverty et al. 1996b, 2003; Gordon et al. 2020). During the course of this study, we did not observe alates of any CHC phenotype in the monitoring stations.

In addition to quantitative changes in their CHC profile, changes in the behavior of subterranean termites can be modified to minimize water loss. For example, desert subterranean termites alter their foraging times at the soil surface depending on soil and wood temperature and moisture. In the Sonoran Desert grassland ca. 40 km south of Tucson, Arizona, at an elevation of 950 m, the subterranean termites *Heterotermes aureus* (Snyder) and *Gnathamitermes perplexus* (Banks) forage throughout the year. *H. aureus* foraged day and night with minimal activity from December through February. Foraging intensity increased moderately in the spring and fall and was high, but erratic, during the summer months. The number of foragers feeding on paper rolls at the soil surface generally increased with increasing temperature. Rainfall had little effect on foraging when daily mean soil temperatures were below 20°C (at the food/soil interface). However, even the slightest amount of rain during the hot summer months increased foraging intensity. Above 33°C, even with precipitation, foraging numbers plummeted (Haverty et al. 1974). *G. perplexus* foraging was limited by upper level (0–15 cm) soil temperatures within a range of 9–49°C. Periodic rises in soil moisture resulting from rainfall increased foraging intensity (La Fage et al. 1976).

In laboratory tests survival of *H. aureus* and *G. perplexus* was high after warming from 27 to 53°C lasting for ca. 30 min (84.4% for *H. aureus* and 70.6% for *G. perplexus*). Survival for *H. aureus* was abruptly reduced (19.7%) after warming to 54°C, however, survival of *G. perplexus* was significantly higher (50.0%). Both species are capable of working and surviving for short periods at ambient temperatures somewhat above 50°C; they avoid water stress by returning to the relatively cooler and moister environment below the soil surface (Collins et al. 1973).

Characterization of CHC profiles of *Reticulitermes* has facilitated the detection of previously unidentified taxa or species in California (Haverty and Nelson 1997, Haverty et al. 2003, Copren et al. 2005, Nelson et al. 2008). The results of the current study show that CHC profiles were very consistent for multiple samples from individual colonies of each phenotype and were so similar between colonies that we were unable to separate colonies within a phenotype based on the quantification of their CHC mixtures. All five phenotypes of *Reticulitermes* from northern California demonstrated strong seasonal variation in some of their CHCs. The CHCs with maximum values in the winter were generally short-chain mono- or dimethyl alkanes. CHCs with maximum values in the summer tended to be long-chain mono- or dimethyl alkanes, or normal alkanes. CHCs with increased proportions in the spring and fall tended to follow the winter pattern. Much remains to be learned, but we are confident that variation in CHC profiles does not diminish their value in delineating *Reticulitermes* species.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

References Cited

Austin, J. W., A. L. Szalanski, P. Uva, A. G. Bagnères, and A. Kence. 2002. A comparative genetic analysis of the subterranean termite genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am.* 95: 753–760.

Austin, J. W., A. L. Szalanski, R. H. Schreffahn, M. T. Messenger, S. Dronnet, and A. G. Bagnères. 2005. Genetic evidence for the synonymy of two *Reticulitermes* species: *Reticulitermes flavipes* and *Reticulitermes santonensis*. *Ann. Entomol. Soc. Am.* 98: 395–401.

Austin, J. W., A. G. Bagnères, A. L. Szalanski, R. H. Scheffrahn, B. P. Heintschel, M. T. Messenger, J. L. Clément, and R. E. Gold. 2007. *Reticulitermes malletei* (Isoptera: Rhinotermitidae): a valid nearctic subterranean termite from eastern North America. *Zootaxa*. 1554: 1–26.

Bagnères, A. G., and E. D. Morgan. 1990. A simple method for analysis of insect cuticular hydrocarbons. *J. Chem. Ecol*. 16: 3263–3276.

Bagnères, A. G., and C. Wicker-Thomas. 2010. Chemical taxonomy with hydrocarbons (chapter 7), pp. 121–162. *In* G. J. Blomquist and A. G. Bagnères (eds.), *Insect hydrocarbons: biology, biochemistry, and chemical ecology*. Cambridge University Press, New York.

Banks, N., and T. E. Snyder. 1920. A revision of the Nearctic termites. *Bull. U.S. Natl. Mus.* 108: 1–228.

Bland, J. M., W. L. A. Osbrink, M. L. Cornelius, A. R. Lax, and C. B. Vigo. 2001. Solid-phase microextraction for the detection of termite cuticular hydrocarbons. *J. Chromatogr*. 932: 119–127.

Blomquist, G. J., and A. G. Bagnères. 2010. Introduction: history and overview of insect hydrocarbons, pp. 3–18. *In* G.J. Blomquist and A. G. Bagnères (eds.), *Insect hydrocarbons. biology, biochemistry, and chemical ecology*. Cambridge University Press, New York.

Brandt, M., E. van Wilgenburg, and N. D. Tsutsui. 2009. Global-scale analyses of chemical ecology and population genetics in the invasive Argentine ant. *Mol. Ecol*. 18: 997–1005.

Brill, J. H., and W. Bertsch. 1986. Headspace analysis of small biological samples. Cuticular hydrocarbons from different body regions of ants, as an example. *J. High Resolut. Chromatogr*. 9: 462–464.

Brooks, L., M. Brunelli, P. Pattison, P. Jones, and A. Fitch. 2015. Crystal structures of eight mono-methyl alkanes (C26-C32) via single-crystal and powder diffraction and DFT-D optimization. *IUCrJ*. 2: 490–497.

Chung, H., and S. B. Carroll. 2015. Wax, sex and the origin of species: Dual roles of insect cuticular hydrocarbons in adaptation and mating. *Bioessays*. 37: 822–830.

Clément, J. L., A. G. Bagnères, P. Uva, L. Wilfert, A. Quintana, J. Reinhard, and S. Dronnet. 2001. Biosystematics of *Reticulitermes* termites in Europe: morphological, chemical and molecular data. *Insectes Soc*. 48: 202–215.

Collins, M. S., M. I. Haverty, J. P. La Fage, and W. L. Nutting. 1973. High-temperature tolerance in two species of subterranean termites from the Sonoran Desert in Arizona. *Environ. Entomol*. 2: 1122–1123.

Copren, K. A., L. J. Nelson, E. L. Vargo, and M. I. Haverty. 2005. Phylogenetic analyses of mtDNA sequences corroborate taxonomic designations based on cuticular hydrocarbons in subterranean termites. *Mol. Phylogenet. Evol*. 35: 689–700.

Cox, T. F. and M. A. A. Cox. 2001. *Multidimensional scaling*, 2nd ed. Chapman and Hall, Boca Raton, FL.

Dedeine, F., S. Dupont, S. Guyot, K. Matsuura, C. Wang, B. Habibpour, A. G. Bagnères, B. Mantovani, and A. Luchetti. 2016. Historical biogeography of *Reticulitermes* termites (Isoptera: Rhinotermitidae) inferred from analyses of mitochondrial and nuclear loci. *Mol. Phylogenet. Evol*. 94: 778–790.

Delphia, C. M., K. A. Copren, and M. I. Haverty. 2003. Agonistic behavior between individual worker termites from three cuticular hydrocarbon phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. *Ann. Entomol. Soc. Am*. 96: 585–593.

Dexter, E., G. Rollwagen-Bollens, and S. M. Bollens. 2018. The trouble with stress: a flexible method for the evaluation of nonmetric multidimensional scaling. *Limnol. Oceanogr. Methods*. 16: 434–443.

Dronnet, S., C. Lohou, J. P. Christides, and A. G. Bagnères. 2006. Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* Feytaud. *J. Chem. Ecol*. 32: 1027–1042.

Getty, G. M., M. I. Haverty, and V. R. Lewis. 2000a. Agonistic behavior between recently collected and laboratory cultured *Reticulitermes* spp. (Isoptera: Rhinotermitidae) from northern California. *Pan-Pac. Entomol*. 76: 243–250.

Getty, G. M., M. I. Haverty, K. A. Copren, and V. R. Lewis. 2000b. Response of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in northern California to baiting with hexaflumuron with Sentricon Termite Colony Elimination System. *J. Econ. Entomol*. 93: 1498–1507.

Gibbs, A. G. 1998. The role of lipid physical properties in lipid barriers. *Am. Zool*. 38: 268–279.

Gibbs, A. G. 2002. Lipid melting and cuticular permeability: new insights into an old problem. *J. Insect Physiol*. 48: 391–400.

Gibbs, A. G., and J. G. Pomonis. 1995. Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. *Comp. Biochem. Physiol*. 112B: 243–249.

Gibbs, A. G., and S. Rajpurohit. 2010. Cuticular lipids and water balance, pp. 100–120. *In* G.J. Blomquist and A-G. Bagnères (eds.), *Insect hydrocarbons. biology, biochemistry, and chemical ecology*. Cambridge University Press, New York.

Ginzel, M. D. 2010. Hydrocarbons as contact pheromones of longhorned beetles (Coleoptera: Cerambycidae), pp. 375–389. *In* G. J. Blomquist and A. G. Bagnères (eds.), *Insect hydrocarbons. biology, biochemistry, and chemical ecology*. Cambridge University Press, New York.

Gordon, J. M., J. Šobotník, and T. Chouvenc. 2020. Colony-age-dependent variation in cuticular hydrocarbon profiles in subterranean termite colonies. *Ecol. Evol*. 10: 10095–10104.

Guillem, R. M., F. P. Drijfhout, and S. J. Martin. 2016. Species-specific cuticular hydrocarbon stability within European *Myrmica* ants. *J. Chem. Ecol*. 42: 1052–1062.

Hadley, N. F. 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armatus*: seasonal and acclimatory effects on chemical composition. *Insect Biochem*. 7: 277–283.

Haverty, M. I., and L. J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California indicate undescribed species. *Comp. Biochem. Physiol*. 118B: 869–880.

Haverty, M. I., and L. J. Nelson. 2007. *Reticulitermes* (Isoptera: Rhinotermitidae) in Arizona: multiple cuticular hydrocarbon phenotypes indicate additional taxa. *Ann. Entomol. Soc. Am*. 100: 206–221.

Haverty, M. I., J. P. La Fage, and W. L. Nutting. 1974. Seasonal activity and environmental control of foraging of the subterranean termite, *Heterotermes aureus* (Snyder), in a desert grassland. *Life Sci*. 15: 1091–1101.

Haverty, M. I., M. Page, B. L. Thorne, and P. Escoubas. 1991a. Cuticular hydrocarbons: Species and population-level discrimination in termites. pp. 15–23. *In* M. I. Haverty and W. W. Wilcox (technical coordinators), Proc. Symposium on Current Research on Wood-destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1989, Bend, Oregon, USDA Forest Service, General Technical Report PSW-128.

Haverty, M. I., L. J. Nelson, and M. Page. 1991b. Preliminary investigations of the cuticular hydrocarbons of North American *Reticulitermes* and tropical *Coptotermes* (Isoptera: Rhinotermitidae) for chemosystematic studies. *Sociobiology*. 19: 51–76.

Haverty, M. I., B. T. Forschler, and L. J. Nelson. 1996a. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the southeastern United States based on cuticular hydrocarbons. *Sociobiology*. 28: 287–318.

Haverty, M. I., J. K. Grace, L. J. Nelson, and R. T. Yamamoto. 1996b. Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *J. Chem. Ecol*. 22: 1813–1834.

Haverty, M. I., K. A. Copren, G. M. Getty, and V. R. Lewis. 1999a. Agonistic behavior and cuticular hydrocarbon phenotypes of colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. *Ann. Entomol. Soc. Am*. 92: 269–277.

Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 1999b. Seasonal foraging and feeding behavior of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. *Environ. Entomol*. 28: 1077–1084.

Haverty, M. I., L. J. Nelson, and B. T. Forschler. 1999c. New cuticular hydrocarbon phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from the United States. *Sociobiology*. 34: 1–21.

Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 2000. Size and dispersion of colonies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. *Environ. Entomol*. 29: 241–249.

Haverty, M. I., G. M. Getty, L. J. Nelson, and V. R. Lewis. 2003. Flight phenology of sympatric populations of *Reticulitermes* (Isoptera: Rhinotermitidae) in northern California: disparate flight intervals indicate reproductive isolation among cuticular hydrocarbon phenotypes. *Ann. Entomol. Soc. Am*. 96: 828–833.

Hölldobler, B., and E.O. Wilson. 2009. *The superorganism. The beauty, elegance, and strangeness of insect societies*. W.W. Horton & Company, New York.

Howard, R. W., and G. J. Blomquist. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol*. 50: 371–393.

Jenkins, T. M., M. I. Haverty, C. J. Basten, L. J. Nelson, and M. Page. 2000. Correlation of mitochondrial haplotypes with cuticular hydrocarbon phenotypes of sympatric *Reticulitermes* species from the southeastern United States. *J. Chem. Ecol*. 26: 1525–1542.

Johnson, A., and B. T. Forschler. 2022. Biodiversity and distribution of *Reticulitermes* in the southeastern USA. *Insects*. 13: 565.

Kaib, M., R. Brandl, and R. K. N. Bagine. 1991. Cuticular hydrocarbon profiles: a valuable tool in termite taxonomy. *Naturwissenschaften*. 78: 176–179.

Kassambara, A. and F. Mundt. 2020. factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. https://CRAN.R-project.org/package=factoextra

Ke, Y., W. Wu, S. Zhang, and Z. Li. 2017. Morphological and genetic evidence for the synonymy of *Reticulitermes* species: *Reticulitermes dichrous* and *Reticulitermes guangzhouensis* (Isoptera: Rhinotermitidae). *Fla. Entomol*. 100: 101–108.

Krishna, K., D. A. Grimaldi, V. Krishna, and M. S. Engel. 2013. Treatise on the Isoptera of the world: vol. 3: Neoisoptera excluding Termitidae. *Bull. Am. Mus. Nat. Hist*. 377: 6231–6973.

Kutnik, M., P. Uva, L. Brinkworth, and A. G. Bagnères. 2004. Phylogeography of two European *Reticulitermes* (Isoptera) species: the Iberian refugium. *Mol. Ecol*. 13: 3099–3113.

La Fage, J. P., M. I. Haverty, and W. L. Nutting. 1976. Environmental factors correlated with the foraging behavior of a desert subterranean termite, *Gnathamitermes perplexus* (Banks) (Isoptera: Termitidae). *Sociobiology*. 2: 155–169.

Lewis, V. R., M. I. Haverty, G. M. Getty, K. A. Copren, and C. Fouche. 1998. Monitoring station for studying populations of *Reticulitermes* (Isoptera: Rhinotermitidae) in California. *Pan-Pac. Entomol*. 74: 121–133.

Liang, D., and J. Silverman. 2000. You are what you eat: Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften*. 87: 412–416.

Lim, S. Y., and B. T. Forschler. 2012. *Reticulitermes nelsonae*, a new species of subterranean termite (Rhinotermitidae) from the Southeastern United States. *Insects*. 3: 62–90.

Martin, S. J., and F. P. Drijfhout. 2009. How reliable is the analysis of complex cuticular hydrocarbon profiles by multivariate statistical methods?. *J. Chem. Ecol*. 35: 375–382.

Martin, S. J., F. P. Drijfhout, and A. G. Hart. 2019. Phenotypic plasticity of nestmate recognition cues in *Formica exsecta* ants. *J. Chem. Ecol*. 45: 735–740.

Nelson, L. J., L. G. Cool, B. T. Forschler, and M. I. Haverty. 2001. Correspondence of soldier defense secretion mixtures with cuticular hydrocarbon phenotypes for chemotaxonomy of the termite genus *Reticulitermes* in North America. *J. Chem. Ecol*. 27: 1449–1479.

Nelson, L. J., L. G. Cool, C. W. Solek, and M. I. Haverty. 2008. Cuticular hydrocarbons and soldier defense secretions of *Reticulitermes* in southern California: a critical analysis of the taxonomy of the genus in North America. *J. Chem. Ecol*. 34: 1452–1475.

Nutting, W. L. 1990. Insecta: Isoptera. pp. 997–1032. *In* D.L. Dindal (ed.), *Soil biology guide*. John Wiley & Sons, Inc., New York.

Otte, T., M. Hilker, and S. Geiselhardt. 2018. Phenotypic plasticity of cuticular hydrocarbon profiles in insects. *J. Chem. Ecol*. 44: 235–247.

Page, M., L. J. Nelson, G. J. Blomquist, and S. J. Seybold. 1997. Cuticular hydrocarbons as chemotaxonomic characters of pine engraver beetles (*Ips* spp.) in the *grandicollis* subgeneric group. *J. Chem. Ecol*. 23: 1053–1099.

Page, M., L. J. Nelson, B. T. Forschler, and M. I. Haverty. 2002. Cuticular hydrocarbons suggest three lineages in *Reticulitermes* (Isoptera: Rhinotermitidae) from North America. *Comp. Biochem. Physiol*. 131B: 305–324.

Perdereau, E., F. Dedeine, J. P. Christides, and A. G. Bagnères. 2010. Variations in worker cuticular hydrocarbons and soldier isoprenoid defensive secretions within and among introduced and native populations of the subterranean termite, *Reticulitermes flavipes*. *J. Chem. Ecol*. 36: 1189–1198.

Pickens, A. L. 1934a. The biology and economic significance of the western subterranean termite, *Reticulitermes hesperus*, pp. 157–183. *In* C. A. Kofoid, S. F. Light, A. C. Horner, M. Randall, W.B. Herms, and E. E. Bowe (eds.), *Termites and termite control*, 2nd ed. University of California Press, Berkeley, CA.

Pickens, A. L. 1934b. The barren-lands subterranean termite, *Reticulitermes tibialis*, pp. 184–186. *In* C. A. Kofoid, S. F. Light, A. C. Horner, M. Randall, W. B. Herms, and E. E. Bowe (eds.), *Termites and termite control*, 2nd ed. University of California Press, Berkeley, CA.

Quinlan, M. C., and A. G. Gibbs. 2006. Discontinuous gas exchange in terrestrial insects. *Resp. Physiol. Neurobiol*. 154: 18–29.

R Core Team. **2021**. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/

Scheffrahn, R. H., and N. Y. Su. 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. *Fla. Entomol*. 77: 460–474.

Seppä, P., H. Helanterä, K. Trontti, P. Punttila, A. Chernenko, S. J. Martin, and L. Sundström. 2011. The many ways to delimit species: hairs, genes and surface chemistry. *Myrmecol. News*. 15: 31–41.

Snyder, T. E. 1954. *Order Isoptera. The termites of the United States and Canada*. Technical Bulletin, National Pest Control Association, New York. 64 pp.

Sprenger, P. P., and F. Menzel. 2020. Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: how and why they differ among individuals, colonies, and species. *Myrmecol. News*. 30: 1–26.

Su, N. Y., W. Ye, R. Ripa, R. H. Scheffrahn, and R. M. Giblin-Davis. 2006. Identification of Chilean *Reticulitermes* (Isoptera: Rhinotermitidae) inferred from three mitochondrial gene DNA sequences and soldier morphology. *Ann. Entomol. Soc. Am*. 99: 352–363.

Szalanski, A. L., J. W. Austin, J. Mckern, and M. T. Messenger. 2006. Genetic evidence of a new subterranean termite species (Isoptera: Rhinotermitidae) from western United States and Canada. *Fla. Entomol*. 89: 299–304.

Takematsu, Y. 1999. The genus *Reticulitermes* (Isoptera: Rhinotermitidae) in Japan, with description of a new species. *Entomol. Sci*. 2: 231–243.

Takematsu, Y., and R. Yamaoka. 1999. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) in Japan and neighboring countries as chemotaxonomic characters. *Appl. Entomol. Zool*. 34: 179–188.

Toolson, E. C. 1982. Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *J. Exp. Zool*. 222: 249–253.

Toolson, E. C., and N. F. Hadley. 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *J. Comp. Physiol. B*. 129: 319–325.

Tripodi, A. D., J. W. Austin, A. L. Szalanski, J. Mckern, M. K. Carroll, R. K. Saran, and M. T. Messenger. 2006. Phylogeography of *Reticulitermes* termites (Isoptera:Rhinotermitidae) in California inferred from mitochondrial DNA sequences. *Ann. Entomol. Soc. Am*. 99: 697–706.

Uva, P., J. L. Clément, and A. G. Bagnères. 2004. Colonial and geographic variations in agonistic behaviour, cuticular hydrocarbons and mtDNA of Italian populations of *Reticulitermes lucifugus* (Isoptera, Rhinotermitidae). *Insectes Soc*. 51: 163–170.

Velonà, A., S. Ghesini, A. Luchetti, M. Marini, and B. Mantovani. 2010. Starting from Crete, a phylogenetic re-analysis of the genus *Reticulitermes* in the Mediterranean area. *Mol. Phylogenet. Evol*. 56: 1051–1058.

Venables, W. N. and B. D. Ripley. 2002. *Modern applied statistics with S*. 4th ed. Springer, New York.

Weesner, F. M. 1965. *The termites of the United States. A handbook*. The National Pest Control Association, Elizabeth, New Jersey.

Weesner, F. M. 1970. Termites of the Nearctic Region. pp. 477–525. *In* F.M. Weesner and K. Krishna (eds.), *Biology of termites*, vol. I. Academic Press, New York.

Wickham, H. 2016. *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York.

Woodrow, R. J., J. K. Grace, L. J. Nelson, and M. I. Haverty. 2000. Modification of cuticular hydrocarbons of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in response to temperature and relative humidity. *Environ. Entomol*. 29: 1100–1107.

Wu, C. C., C. L. Tsai, W. R. Liang, Y. Takematsu, and H. F. Li. 2019. Identification of subterranean termite genus, *Reticulitermes* (Blattodea: Rhinotermitidae) in Taiwan. *J. Econ. Entomol*. 112: 2872–2881.