



HAL
open science

Nest structures display specific hydrocarbon profiles: insights into the chemical ecology of the invasive yellow-legged hornet *Vespa velutina nigrithorax*

Mélissa Haouzi, Jeremy Gevar, Alix Khalil, Éric Darrouzet

► **To cite this version:**

Mélissa Haouzi, Jeremy Gevar, Alix Khalil, Éric Darrouzet. Nest structures display specific hydrocarbon profiles: insights into the chemical ecology of the invasive yellow-legged hornet *Vespa velutina nigrithorax*. *Chemoecology*, In press, 31 (4), pp.227-238. <10.1007/s00049-021-00343-7>. <hal-03188794>

HAL Id: hal-03188794

<https://hal.science/hal-03188794v1>

Submitted on 8 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1 **Nest structures display specific hydrocarbon profiles: insights into the chemical ecology**
2 **of the invasive yellow-legged hornet *Vespa velutina nigrithorax***

3 Mélissa Haouzi¹, Jérémy Gévar², Alix Khalil¹, E. Darrouzet^{1*}

4 ¹*IRBI, UMR CNRS 7261, University François Rabelais of Tours, Parc de Grandmont, 37200*
5 *Tours, France*

6 ²*iEES Paris, INRA, 78026 Versailles, France*

7 *Corresponding author: eric.darrouzet@univ-tours.fr

8

9 ORCID:

10 MH: 0000-0002-8851-9223

11 ED: [0000-0002-7224-6937](https://orcid.org/0000-0002-7224-6937)

12

13 **Abstract**

14 In insects, chemical communication is the most common form of communication, and cuticular
15 hydrocarbons (CHCs) are employed in recognition processes. In social insects, CHCs also help
16 define colony identity and thus contribute to social cohesion among nestmates. Individuals can
17 deposit their chemical signatures on nest surfaces. This information serves as a reference for
18 newly emerged individuals and allows them to obtain the odor specific to their colony. This
19 study examined nest chemical profiles in an inbred invasive species: the yellow-legged hornet,
20 *Vespa velutina nigrithorax*. We demonstrated that nest structures (i.e., envelopes, combs, and
21 pillars) had specific hydrocarbon profiles, which were colony specific. There were similarities
22 between the chemical profiles of the nests and the CHC signatures of hornets. The loss of
23 genetic diversity previously documented in the yellow-legged hornet population in France does
24 not appear to have constrained nest chemical diversity.

25

26 **Keywords:** cuticular hydrocarbons, chemical marking, Hymenoptera, invasive species

27

28 **Introduction**

29 The members of social insect colonies operate as a unit and are thus considered to form
30 a higher-level organism (Wheeler 1911). The stability of social groups relies on group members
31 being able to differentiate between those who belong to the group and those who do not
32 (Hamilton 1987). This recognition system is based on chemical cues (Hölldobler 1995).

33 In insects, chemical communication can be carried out by pheromones, and substrates
34 may be marked with volatile or non-volatile compounds. In social insects, nestmates can

35 communicate using cuticular hydrocarbons (CHCs). CHCs are non-volatile compounds and
36 form a complex layer on the insect cuticle, providing essential protection against desiccation
37 (Hadley 1984; Gibbs 1998). They also play a key role in kin recognition (Espelie and Hermann
38 1990), nestmate recognition (Hefetz 2007; D’Ettorre and Lenoir 2010), caste recognition
39 (Howard et al. 1982; Smith and Taylor Jr 1990; Bagnères et al. 1998; Kaib et al. 2000), and the
40 division of labor (Bonavita-Cougourdan et al. 1993; Scholl and Naug 2011; Rahman et al.
41 2016). The CHC signatures of insects are often complex and dynamic, with compound levels
42 that vary qualitatively and quantitatively (Bonavita-Cougourdan et al. 1993; Lenoir et al. 1999,
43 2001). Indeed, insects have personalized chemical signatures that are shaped by endogenous
44 factors (Fan et al. 2004; Lengyel et al. 2007; Darrouzet et al. 2014), exogenous factors
45 (Bagnères et al. 1990), and parasitism (Bagnères et al. 1996; Darrouzet et al. 2010; Lebreton et
46 al. 2010). An individual’s chemical signature serves as a type of highly specific “visa,”
47 conveying the individual’s identity to conspecifics (Fresneau and Errard 1994).

48 Some invasive social insects are tolerant of small differences in CHC signatures, a trait
49 that enhances their invasive success (Suarez et al. 1999; Tsutsui et al. 2001; Pedersen et al.
50 2006; Brandt et al. 2009). In such species, colonies differ less in their chemical profiles and
51 thus compete less for resources. One example of this phenomenon is the Argentine ant,
52 *Linepithema humile*, which has successfully colonized several continents (Holway and Case
53 1999; Suarez et al. 2000). In this context, attention has turned to a species that recently invaded
54 Europe: the yellow-legged hornet, *Vespa velutina nigrithorax*. This hornet is native to China
55 and, more specifically, comes from two provinces near Shanghai (Arca 2012; Arca et al. 2015).
56 It was accidentally introduced into southwestern France around 2004 (Haxaire et al. 2006) and
57 then spread rapidly throughout Europe (Robinet et al. 2018). It is now found in Spain, Portugal,
58 Italy, Germany, and, more recently, in Belgium, the Netherlands, Switzerland, and the United
59 Kingdom (Bruneau 2011; López et al. 2011; Grosso-silva and Maia 2012; Demichelis et al.
60 2014; Monceau and Thiéry 2017; Robinet et al. 2017, 2018). To feed its larvae, it consumes
61 large quantities of insects (Villemant et al. 2011) and, more particularly, of honey bees
62 (Darrouzet and Gévar 2014; Darrouzet 2019). Bee colonies suffer as a result, and their foraging
63 activity decreases, thus impacting beekeeping (Requier et al. 2018, 2019; Leza et al. 2019). Not
64 only is the yellow-legged hornet a threat to biodiversity, but it is also responsible for drastic
65 economic losses as well as public health concerns (Beggs et al. 2011; Darrouzet 2019; Laborde-
66 Castérot et al. 2020).

67 Despite the lack of genetic diversity in the European yellow-legged hornet population
68 (Arca et al. 2015; Darrouzet et al. 2015), hornet chemical signatures, composed of CHCs, were

69 found to vary tremendously based on caste, sex, and colony (Gévar et al. 2017). This result was
70 surprising because such heterogeneity has not been observed in other invasive social insects,
71 such as ants [*Linepithema humile* (Tsutsui et al. 2000; Brandt et al. 2009); *Wasmannia*
72 *auropunctata* (Errard et al. 2005)] or termites [*Reticulitermes flavipes* (Perdereau et al. 2010)].
73 Previous studies have suggested that this lower level of chemical variability may arise from the
74 reduced genetic diversity that can result from genetic bottlenecks (Tsutsui et al. 2001) or
75 selection against less-common recognition alleles (Giraud et al. 2002). Gévar et al. (2017)
76 suggested that the heterogeneity of CHC profiles in *Vespa velutina nigrithorax* may be affected
77 by environmental and/or epigenetic factors. For example, in termites, changes in temperature
78 or diet can erase or modify colony odor in *Coptotermes formosanus* (Shelton and Grace 1997;
79 Florane et al. 2004). In *Reticulitermes flavipes*, seasonal variation can modify chemical
80 signatures (Bagnères et al. 1990); in *Cryptotermes brevis*, profiles shift depending on specific
81 combinations of temperature and relative humidity (Woodrow et al. 2000). The composition of
82 the intestinal bacteria community can also affect nestmate recognition in *Reticulitermes*
83 *speratus* (Matsuura 2001, 2003).

84 In eusocial insects, nest odors are present and attractive to colony members (Kukuk et
85 al. 1977; Gamboa et al. 1986; Ferguson et al. 1987). Indeed, colony odor helps maintain colony
86 unity in ants and termites (Wilson 1971). Insects may use visual cues for nest recognition, but
87 olfactory cues have also been observed in *Apis mellifera* (Butler et al. 1969, 1970; Free 1987)
88 and *Lasioglossum zephyrum* (Kukuk et al. 1977). Cuticular hydrocarbons may be deposited on
89 nest structures, like the walls and soil surfaces of ant nests (Bos et al. 2011), the waxy combs
90 of beehives (Couvillon et al. 2007), and the paper nests of social wasps (Espelie and Hermann
91 1990; Elia et al. 2017). For example, the ant *Lasius niger* passively deposits CHCs on the inner
92 walls of its nest via its tarsi (Devigne and Detrain 2006; Lenoir et al. 2009). *Camponotus*
93 *aethiops* indirectly transfers CHCs to its nest, creating a uniform colony odor (Bos et al. 2011).
94 In the social wasp *Polistes metricus*, the CHCs of adult wasps may be similar to those of the
95 nest (Espelie et al. 1990). The chemical compounds laid down within the nest allow newly
96 emerged individuals to recognize and obtain their colony's signature (Singer and Espelie 1992).
97 In the social wasp *Polistes biglumis*, there is temporal variation in nest chemical profiles, which
98 can also shift when colonies are invaded by the social parasite *Polistes atrimandibularis* (Elia
99 et al. 2017).

100 This study aimed to investigate (1) whether nests built by *Vespa velutina nigrithorax*
101 displayed hydrocarbon-based chemical profiles, (2) whether the three main nest structures
102 (envelopes, combs, and pillars) had similar or different chemical profiles, and (3) whether nests

103 differed in their chemical profiles. We hypothesized that, since hornet workers display colony-
104 specific CHC signatures (Gévar et al. 2017), the latter should be reflected in the chemical
105 profiles of nests, since hydrocarbons should be deposited onto nest materials. This process
106 could influence colony signatures across generations (Singer and Espelie 1992), overcoming
107 the effects of inbreeding within the invasive population (Darrouzet et al. 2015).

108

109 **Materials and Methods**

110 Vespidae nests are built with plant fibers mixed with water and saliva (Edwards 1980). Yellow-
111 legged hornet nests are located in different habitats but frequently occur in tree tops; they can
112 generally be distinguished from the nests of other social Vespidae in France by the presence of
113 a lateral entrance (Rome et al. 2009; Darrouzet 2013, 2019). Nests are composed of a stack of
114 several combs that are connected by pillars and covered by a protective envelope (Darrouzet
115 2019). The combs are composed of alveolar cells that open downward; these cells harbor larvae
116 and nymphs at different developmental stages. The top of the nest does not contain cells;
117 instead, it consists of a sponge-like structure that likely protects the lower part of the nest (where
118 the hornets are found) against harsh environmental conditions (e.g., rain). Nest construction
119 follows a cyclical pattern and varies depending on the colony's degree of development. After
120 overwintering, the foundress will begin nest building in early spring (March–April): she will
121 first affix the pedicel and then build an initial comb of a dozen alveolar cells that are surrounded
122 by a protective envelope. Around May, workers will emerge. They will enlarge and strengthen
123 the nest along the vertical axis by adding new combs and along the horizontal axis by adding
124 new cells to already established combs (Rome et al. 2015; Darrouzet 2019).

125

126 *Chemical analysis of different nest structures*

127 *Nest collection*

128 A total of 10 *Vespa velutina nigrithorax* nests (Table S1) were collected in France's Centre-
129 Val-de-Loire region between 2015 and 2017. Each nest was processed immediately and stored
130 at -20°C. Chemical extractions were carried out in 2018.

131

132 *Hydrocarbon extractions*

133 Hydrocarbons were extracted from three distinct nest structures: the envelopes, the combs, and
134 the pillars. For each structure, three replicates were performed per nest (n = 10 nests). To extract
135 the hydrocarbons, 150 mg of structural material was placed in a 20-ml glass vial and crushed

136 down to small particles. Then, 5 ml of heptane was added, and the whole mixture was vortexed
137 for 1 minute at 300 rpm. It was left to rest for 24 hours, allowing the particles to settle.
138 Subsequently, 1.5 ml of the supernatant was transferred into 2-ml glass vials and fully
139 evaporated under nitrogen flux. Then, 150 μ l of heptane was added to each vial, and the mixture
140 was vortexed for 30 seconds at 300 rpm. Afterward, 100- μ l aliquots of these extracts were
141 transferred into vials. Before the chemical analyses were performed, 3 μ l of C20 (n-eicosane
142 10^{-3} g/ml) was added as an internal standard.

143

144 *Detailed comb analyses*

145 *Nest collection and sampling*

146 To carry out a more detailed chemical analysis of the nests, three end-of-season nests (i.e., large
147 nests; Table S2) were collected in the Centre-Val-de-Loire, Brittany, and Pays-de-la-Loire
148 regions from February to March 2018. The combs' hydrocarbon profiles were analyzed by
149 taking samples from different parts of the nest: from the top comb to the bottom comb and from
150 the center, middle, and periphery of the individual combs. In each case, three replicates were
151 performed (n = 3 nests).

152

153 *Hydrocarbon extraction*

154 To extract the hydrocarbons, 150 mg of structural material was collected. Then, 5 ml of heptane
155 was added. The whole mixture was vortexed for 1 minute at 300 rpm and left to rest for 24
156 hours. Subsequently, 100 μ l of the supernatant was transferred to 2-ml glass vials containing
157 an insert. Before the chemical analyses were performed, 3 μ l of the internal standard (C20 n-
158 eicosane at 10^{-3} g/ml) was added to samples.

159

160 *Chemical analyses*

161 Samples were analyzed using gas chromatography (GC), employing an Agilent Technologies
162 7000C GC/MS Triple Quad with a 7890 BGC System equipped with a capillary column (HP-5
163 Agilent Technology, Santa Clara, USA; 30 m \times 0.32 mm \times 0.25 μ m); helium was the carrier
164 gas (1.7 ml/min). The following temperature program was used: an initial temperature ramp
165 from 50 $^{\circ}$ C to 200 $^{\circ}$ C at 8 $^{\circ}$ C/min, a second temperature ramp from 200 $^{\circ}$ C to 315 $^{\circ}$ C at 5
166 $^{\circ}$ C/min, and a 5-min final hold at 315 $^{\circ}$ C. The surface areas of the 27 major peaks were
167 determined using ChemStation software (Agilent Technologies). The hydrocarbon profiles of
168 the nests were compared to the species' chemical signature. The identities of the chemical
169 compounds present on the nest structures were determined via comparison with the *Vespa*

170 *velutina nigrithorax* CHCs analyzed using the same GC system (Gévar et al. 2017). Compound
171 identities were confirmed using GC-MS analyses performed with an Agilent Technologies
172 7000C GC/MS Triple Quad with a 7890B GC System equipped with the same column as above;
173 we also used the same temperature program as above.

174

175 *Calculations and statistical analyses*

176 Principal component analysis (PCA) was carried out using R software (v. 3.4.3) and the
177 packages FactoMineR (Lê et al. 2008) and RcmdrPluginFactoMineR (Fox 2005), which are
178 included in the software. Differences in the hydrocarbon profiles of the three nest structures
179 (envelopes, combs, and pillars) among and within nests were quantified using the Nei distance
180 (Nowbahari et al. 1990; Dronnet et al. 2006). A dissimilarity matrix, which included all possible
181 pairwise comparisons (within structures, among structures within nests, and among nests for a
182 given structure), was constructed using the mean relative areas of the hydrocarbon peaks. The
183 matrix was then analyzed using a Kruskal-Wallis test implemented in RStudio (v. 1.0.143;
184 ©2009–2016 RStudio, Inc). For each structure, hydrocarbon quantity was estimated using the
185 following formula: $Q_i = (QC_{20} \times x_i) / (x_{C_{20i}})$ (Gévar et al. 2017). The quantity of each
186 hydrocarbon was then divided by the mass of the nest sample to obtain the mass-specific
187 quantity of each compound. Then, Kruskal-Wallis nonparametric tests were performed using
188 RStudio software.

189

190 **Results**

191 *Hydrocarbon analyses*

192 The nest structures bore the same hydrocarbon compounds as the cuticle of *Vespa velutina*
193 *nigrithorax* (Gévar et al. 2017) (Table 1). There were 8 alkanes, 10 methyl alkanes, 3 dimethyl
194 alkanes, 1 di/trimethyl alkane, and 5 alkenes among the 27 major peaks analyzed.

195

196 *Differences in nest structure hydrocarbons*

197 The three nest structures (envelopes, combs, and pillars) had distinct hydrocarbon profiles (Fig.
198 1) in which certain compounds predominated. In particular, there were two alkanes and three
199 methyl alkanes specifically found in the envelopes and combs; one alkane and one methyl
200 alkane specifically found in the combs and pillars; and one alkane, five methyl alkanes, two
201 dimethyl alkanes, and two alkenes specifically found in the envelopes and pillars (Table 2).

202

203 *Chemical distances for nest structures*

204 Chemical distances were compared within and among nest structures (Fig. S1). Chemical
205 distances within structures (i.e., among samples from a given structure, regardless of nest
206 identity) were significantly smaller than chemical distances among structures (i.e., among nest
207 structures within the same nest, regardless of nest identity) (Kruskal-Wallis; p-value < 0.001).
208 Similarly, for each structure, chemical distances among nests were significantly greater than
209 chemical distances within structures (Kruskal-Wallis; p-value < 0.001).

210

211 *Quantitative analyses of the nest structures*

212 The envelopes had significantly lower quantities of total hydrocarbons than did the other two
213 structures (Kruskal-Wallis; p-value < 0.001). The combs had significantly higher quantities of
214 total hydrocarbons than did the pillars (Kruskal-Wallis; p-value < 0.05) (Fig. 2).

215 The structures all differed from each other in specific hydrocarbon quantities (Kruskal-Wallis;
216 p-value < 0.001 for most comparisons, with p-value < 0.05 for the comparisons of alkanes and
217 branched alkanes between the pillars and combs). For each compound, the envelopes displayed
218 the lowest quantities, and the combs displayed the highest quantities (Fig. 3).

219

220 *Qualitative analyses of the combs*

221 Within combs, we found no significant differences among center samples or between center
222 and middle samples (regardless of comb or nest) (Kruskal-Wallis; p-value > 0.05; Fig. S2). The
223 chemical distances between center and periphery samples were significantly higher than the
224 chemical distances among center samples (Kruskal-Wallis; p-value < 0.001; Fig. S2). Finally,
225 the chemical distances among middle samples and among periphery samples were significantly
226 different as were the chemical distances between middle and periphery samples (Kruskal-
227 Wallis; p-value < 0.001; Fig. S2).

228 The results for the chemical distances among combs (Fig. S3) revealed that only comb 1 (the
229 oldest comb, found at the top of the nest) was significantly different from all the others
230 (Kruskal-Wallis; p-value < 0.05), with the exception of comb 7 (Kruskal-Wallis; p-value >
231 0.05). The other combs were not significantly different from each other (Kruskal-Wallis; p-
232 value > 0.05).

233

234 *Quantitative analyses of the combs*

235 Within combs, hydrocarbon quantities decreased significantly from the center to the periphery
236 (Kruskal-Wallis; p-value < 0.001 for the center/periphery and middle/periphery comparisons
237 and p-value < 0.05 for center/middle comparison) (Fig. 4).

238 Across combs, hydrocarbon quantities differed significantly between combs 1, 2, 3, and 4 and
239 combs 6 and 7 (Kruskal-Wallis; p-value < 0.05); comb 5 was only significantly different from
240 comb 7 (Kruskal-Wallis; p-value < 0.05) (Fig. 5).

241

242 **Discussion**

243 Many studies have shown that nest recognition in social insects is based on chemical cues. Such
244 is the case in bumble bees (Saleh et al. 2007), in honey bees and wasps (Butler et al. 1969), and
245 in ants (Depickère et al. 2004). In this study, hydrocarbons previously found on the hornet's
246 cuticle (Gévar et al. 2017) were discovered to occur on different parts of the hornet's nest. This
247 finding suggests *Vespa velutina nigrithorax* deposits CHCs on nest structures. However, we do
248 not know if deposition is active or passive. Indeed, no data on *Vespa velutina* behavior within
249 the nest are currently available. Consequently, we do not know if the hornet interacts in a
250 particular way with the nest substrate. Some species are known to directly interact with nest
251 materials and/or with nestmates to obtain/deposit chemical markings (Akino 2008). For
252 example, some European *Polistes* species apply chemical markings via rubbing (Turillazzi and
253 Ugolini 1979). Conversely, in *Lasius niger*, ants passively mark the nest with their tarsi as they
254 walk across the substrate (Depickère et al. 2004; Devigne and Detrain 2006; Lenoir et al. 2009).
255 Further behavioral studies are thus needed to clarify marking mechanisms in *V. velutina*.

256 Chemical signatures are colony and caste specific in *V. velutina* (Gévar et al. 2017). In
257 our study, we observed pronounced qualitative and quantitative chemical differences among
258 nest structures. This finding may indicate that individuals differing in caste or reproductive
259 status (fertile vs. non-fertile females) occur in separate parts of the nest. For example, chemical
260 signatures can vary among castes: *V. velutina* queens bear four specific alkenes and have higher
261 levels of CHCs than do other castes (Gévar et al. 2017). However, the queen-specific
262 compounds were not found on the nest structures. The chemical signatures of nest structures
263 are likely shaped by the chemical deposits of colony members, whose CHC signatures vary in
264 their absolute and relative composition. This variation is functional and is likely influenced by
265 a variety of recognition-oriented behaviors and discrimination among individuals (Hölldobler
266 and Carlin 1987). For example, monomethyl alkanes often serve as signals (Nelson 1993).
267 Hydrocarbons could therefore function in recognition and communication within nests. Indeed,

268 the nest's interior is opaque, creating dark conditions. Individuals thus need a mechanism other
269 than vision to locate each other within the nest. Labeling nest structures so that they have
270 different chemical profiles could allow insects to orient themselves and communicate with each
271 other. The fact that combs and envelopes had the highest and lowest quantities of hydrocarbons,
272 respectively, might support this hypothesis. However, these differences could also arise from
273 the higher contact frequencies among individuals and between individuals and comb surfaces
274 inside the nest. Furthermore, there could be smaller quantities of hydrocarbons on the envelopes
275 because they are exposed to exterior conditions (e.g., rainfall could wash them away). More
276 experiments are necessary to explore these issues. Moreover, nest structures were also
277 qualitatively different from each other. This finding is interesting because branched alkanes and
278 alkenes help define the chemical identity of colonies and are involved in communication among
279 individuals (Howard and Blomquist 2005). Linear alkanes may play more of a role in physically
280 protecting individuals against desiccation (Hadley 1984; Singer 1998). Consequently, their high
281 levels on the nest's surface could provide protection against wet and humid conditions. Indeed,
282 because the hornet's nest is composed of "paper mache", it is likely that chemical protection
283 would be required. When n-alkanes and monomethyl alkanes are combined, they produce an
284 impermeable layer whose solid-liquid phase transition extends over a wide temperature range
285 (Gibbs 1995), allowing the nest to benefit from seasonal waterproofing. Finally, the chemical
286 heterogeneity of nest structures could be explained by temporal shifts. In *Bombus terrestris*,
287 cuticular lipids convey colony identity and change over time (Rottler-Hoermann et al. 2016).
288 Further research is needed to clarify the chemical dynamics of nests.

289 We also found that there was spatial heterogeneity in the combs' chemical profiles.
290 Hydrocarbon quantity decreased from the center to the periphery of the combs and from the top
291 combs to the bottom combs. It appeared that the oldest parts of the nest (i.e., the center of the
292 comb and the top comb) had greater quantities of hydrocarbons. This observation could be
293 explained by structure age, these structures had spent more time in contact with adult hornets.
294 There were qualitative differences along the horizontal axis of the individual combs, signaling
295 differences associated with time of construction. In contrast, there were no differences along
296 the vertical axis of the combs. These results suggest that marking patterns within nests may be
297 somewhat complex. Further research is needed to clarify hydrocarbon deposition mechanisms
298 and dynamics within nests and across time.

299 Finally, the chemical diversity among nests and among structures highlights the
300 existence of a colony signature. Indeed, chemical diversity among nests was high. As described
301 by Gévar et al. (2017), individual *V. velutina* hornets vary in their chemical signatures, and we

302 observed the same variability in the species' nests. Consequently, the chemical heterogeneity
303 observed among individuals could explain the heterogeneity seen in the nests themselves. This
304 result was somewhat surprising, however, because hornet populations in France are highly
305 related (Darrouzet et al. 2015) as the result of a genetic bottleneck (Arca et al. 2015). Previous
306 studies of invasive species all found that chemical variability was reduced in introduced
307 populations. Indeed, this is the case in the ants *Linepithema humile* (Brandt et al. 2009) and
308 *Wasmannia auropunctata* (Errard et al. 2005) as well as in the termite *Reticulitermes flavipes*
309 (Perdereau et al. 2010). This phenomenon could be related to the limited number of
310 introductions that have taken place. In the case of *V. velutina* in France, the population is inbred;
311 the heterogeneity in hydrocarbon profiles within nests could result from environmental and/or
312 epigenetic factors (Gévar et al. 2017). Additional research is clearly necessary to assess the
313 roles played by different factors in shaping nest chemical profiles.

314

315 **Acknowledgments**

316 We gratefully acknowledge C. Poirier, R. Cuartéro, and L. Marteil for their help in collecting
317 colonies in the field, L. Berville for her comments on the manuscript, and J. Pearce for her
318 English-language editing services. This study was supported by funding from the Centre-Val-
319 de-Loire regional government (FRELON2 project).

320

321 **Bibliography**

- 322 Akino T (2008) Chemical strategies to deal with ants: a review of mimicry, camouflage,
323 propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods.
324 Myrmecological News 11:173–181
- 325 Arca M (2012) Caractérisation génétique et étude comportementale d'une espèce
326 envahissante en France : *Vespa velutina* Lepeletier (Hymenoptera, Vespidae). Université
327 Pierre et Marie Curie
- 328 Arca M, Mougél F, Guillemaud T, et al (2015) Reconstructing the invasion and the
329 demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe. Biol
330 Invasions 17:2357–2371. <https://doi.org/10.1007/s10530-015-0880-9>
- 331 Bagnères AG, Clément JL, Blum MS, et al (1990) Cuticular hydrocarbons and defensive
332 compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis* (Feytaud):
333 Polymorphism and chemotaxonomy. J Chem Ecol 16:3213–3244.
334 <https://doi.org/10.1007/BF00982094>

335 Bagnères AG, Lorenzi MC, Dusticier G, et al (1996) Chemical Usurpation of a Nest by Paper
336 Wasp Parasites. *Am Assoc Adv Sci* 272:889–892

337 Bagnères AG, Rivière G, Clément JL (1998) Artificial neural network modeling of caste odor
338 discrimination based on cuticular hydrocarbons in termites. *Chemoecology* 8:201–209.
339 <https://doi.org/10.1007/s000490050026>

340 Beggs JR, Brockerhoff EG, Corley JC, et al (2011) Ecological effects and management of
341 invasive alien Vespidae. *BioControl* 56:505–526. [https://doi.org/10.1007/s10526-011-](https://doi.org/10.1007/s10526-011-9389-z)
342 [9389-z](https://doi.org/10.1007/s10526-011-9389-z)

343 Bonavita-Cougourdan A, Clement JL, Lange C (1993) Functional subcaste discrimination
344 (foragers and brood-tenders) in the ant *Camponotus vagus* Scop.: Polymorphism of
345 cuticular hydrocarbon patterns. *J Chem Ecol* 19:1461–1477.
346 <https://doi.org/10.1007/BF00984890>

347 Bos N, Grinsted L, Holman L (2011) Wax On, Wax Off: Nest Soil Facilitates Indirect
348 Transfer of Recognition Cues between Ant Nestmates. *PLoS One* 6:2–7.
349 <https://doi.org/10.1371/journal.pone.0019435>

350 Brandt M, Van Wilgenburg E, Tsutsui ND (2009) Global-scale analyses of chemical ecology
351 and population genetics in the invasive Argentine ant. *Mol Ecol* 18:997–1005.
352 <https://doi.org/10.1111/j.1365-294X.2008.04056.x>

353 Bruneau E (2011) Le frelon asiatique, déjà là. *Actu Api* 55:1–6

354 Butler CG, Fletcher DJC, Watler D (1969) Nest-entrance marking with pheromones by the
355 honeybee- *Apis mellifera* L. , and by a wasp, *Vespula vulgaris* L. *Anim Behav* 17:142–
356 147. [https://doi.org/http://dx.doi.org/10.1016/0003-3472\(69\)90122-5](https://doi.org/http://dx.doi.org/10.1016/0003-3472(69)90122-5)

357 Butler CG, Fletcher DJC, Watler D (1970) Hive entrance finding by honeybee (*Apis*
358 *mellifera*) foragers. *Anim Behav* 18:78–91. [https://doi.org/10.1016/0003-](https://doi.org/10.1016/0003-3472(70)90074-6)
359 [3472\(70\)90074-6](https://doi.org/10.1016/0003-3472(70)90074-6)

360 Couvillon MJ, Caple JP, Endsor SL, et al (2007) Nest-mate recognition template of guard
361 honeybees (*Apis mellifera*) is modified by wax comb transfer. *Biol Lett* 3:228–230.
362 <https://doi.org/10.1098/rsbl.2006.0612>

363 D’Ettorre P, Lenoir A (2010) Nestmate Recognition. *Ant Ecol* 11:194–209.
364 <https://doi.org/10.1093/acprof:oso/9780199544639.003.0011>

365 Darrouzet E (2019) Le frelon asiatique, un redoutable prédateur. Syndicat National
366 d’Apiculture

367 Darrouzet E (2013) Les Insectes bâtisseurs, nids de termites, de guêpes et de frelons.
368 *Connaissances et Savoirs*

369 Darrouzet E, Gévar J (2014) Le frelon asiatique à la conquête de l'Europe. *La Santé l'Abeille*
370 259:49–59

371 Darrouzet E, Gévar J, Guignard Q, Aron S (2015) Production of Early Diploid Males by
372 European Colonies of the Invasive Hornet *Vespa velutina nigrithorax*. *PLoS One* 10:1–9.
373 <https://doi.org/10.1371/journal.pone.0136680>

374 Darrouzet E, Labédan M, Landré X, et al (2014) Endocrine control of cuticular hydrocarbon
375 profiles during worker-to-soldier differentiation in the termite *Reticulitermes flavipes*. *J*
376 *Insect Physiol* 61:25–33. <https://doi.org/10.1016/j.jinsphys.2013.12.006>

377 Darrouzet E, Lebreton S, Gouix N, et al (2010) Parasitoids Modify Their Oviposition
378 Behavior According to the Sexual Origin of Conspecific Cuticular Hydrocarbon Traces.
379 *J Chem Ecol* 36:1092–1100. <https://doi.org/10.1007/s10886-010-9845-8>

380 Demichelis S, Manino A, Minuto G, et al (2014) Social wasp trapping in north west italy:
381 comparison of different bait-traps and first detection of *Vespa velutina*. *Bull Insectology*
382 67:307–317

383 Depickère S, Fresneau D, Detrain C, Deneubourg J-L (2004) Marking as a decision factor in
384 the choice of a new resting site in *Lasius niger*. *Insectes Soc* 51:243–246.
385 <https://doi.org/10.1007/s00040-004-0739-z>

386 Devigne C, Detrain C (2006) How does food distance influence foraging in the ant *Lasius*
387 *niger*: The importance of home-range marking. *Insectes Soc* 53:46–55.
388 <https://doi.org/10.1007/s00040-005-0834-9>

389 Dronnet S, Lohou C, Christides JP, Bagnères AG (2006) Cuticular Hydrocarbon Composition
390 Reflects Genetic Relationship Among Colonies of the Introduced Termite *Reticulitermes*
391 *santonensis* Feytaud. *J Chem Ecol* 32:1027–1042. [https://doi.org/10.1007/s10886-006-](https://doi.org/10.1007/s10886-006-9043-x)
392 9043-x

393 Edwards R (1980) Social wasps. Their biology and control. Rentokild Ltd, East Grinstead

394 Elia M, Blancato G, Picchi L, et al (2017) Nest signature changes throughout colony cycle
395 and after social parasite invasion in social wasps. *PLoS One* 12:1–19.
396 <https://doi.org/10.1371/journal.pone.0190018>

397 Errard C, Delabie J, Jourdan H, Hefetz A (2005) Intercontinental chemical variation in the
398 invasive ant *Wasmannia auropunctata* (Roger) (Hymenoptera Formicidae): A key to the
399 invasive success of a tramp species. *Naturwissenschaften* 92:319–323.
400 <https://doi.org/10.1007/s00114-005-0628-y>

401 Espelie KE, Hermann HR (1990) Surface lipids of the social wasp *Polistes annularis* (L.) and
402 its nest and nest pedicel. *J Chem Ecol* 16:1841–1852.

403 <https://doi.org/10.1007/BF01020498>

404 Espelie KE, Wenzel JW, Chang G (1990) Surface lipids of social wasp *Polistes metricus* say
405 and its nest and nest pedicel and their relation to nestmate recognition. *J Chem Ecol*
406 16:2229–2241. <https://doi.org/10.1007/BF01026933>

407 Fan Y, Schal C, Vargo EL, Bagnères AG (2004) Characterization of termite lipophorin and its
408 involvement in hydrocarbon transport. *J Insect Physiol* 50:609–620.
409 <https://doi.org/10.1016/j.jinsphys.2004.04.007>

410 Ferguson ID, Gamboa GJ, Jones JK (1987) Discrimination Between Natal and Non-Natal
411 Nests by the Social Wasps *Dolichovespula maculata* and *Polistes fuscatus*
412 (Hymenoptera: Vespidae). *J Kansas Entomol Soc* 60:65–69

413 Florane CB, Bland JM, Husseneder C, Raina AK (2004) Diet-mediated inter-colonial
414 aggression in the formosan subterranean termite *Coptotermes formosanus*. *J Chem Ecol*
415 30:2559–2574. <https://doi.org/10.1007/s10886-004-7950-2>

416 Fox J (2005) The R Commander: A Basic-Statistics Graphical User Interface to R. *J Stat*
417 *Softw* 14:1–42. <https://doi.org/10.1109/ISCAS.2015.7169035>

418 Free JB (1987) Pheromones of social bees. Chapman and Hall, London

419 Fresneau D, Errard C (1994) L'identité coloniale et sa “représentation” chez les fourmis.
420 *Intellectica* 2:91–115

421 Gamboa GJ, Reeve HK, Pfennig DW (1986) The evolution and ontogeny of nestmate
422 recognition in social wasps. *Annu Rev Entomol* 31:431–454.
423 <https://doi.org/10.1146/annurev.ento.31.1.431>

424 Gévar J, Bagnères AG, Christidès JP, Darrouzet E (2017) Chemical Heterogeneity in Inbred
425 European Population of the Invasive Hornet *Vespa velutina nigrithorax*. *J Chem Ecol*
426 43:763–777. <https://doi.org/10.1007/s10886-017-0874-4>

427 Gibbs A (1998) Water-Proofing Properties of Cuticular Lipids. *Am Zool* 38:471–482.
428 <https://doi.org/10.1093/icb/38.3.471>

429 Gibbs A (1995) Physical properties of insect cuticular hydrocarbons: Model mixtures and
430 lipid interactions. *Comp Biochem Physiol -- Part B Biochem* 112B:667–672.
431 [https://doi.org/10.1016/0305-0491\(95\)00119-0](https://doi.org/10.1016/0305-0491(95)00119-0)

432 Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: The Argentine ants of
433 southern Europe. *Proc Natl Acad Sci U S A* 99:6075–6079.
434 <https://doi.org/10.1073/pnas.092694199>

435 Grosso-silva JM, Maia M (2012) NOTA / NOTE *Vespa velutina* Lepeletier, 1836
436 (Hymenoptera, Vespidae), new species for Portugal. *Arq Entomoloxicos* 6:53–54.

437 <https://doi.org/10.7764/onomazein.28.24>

438 Hadley NF (1984) Cuticle: Ecological Significance. In: Bereiter-Hahn J, Matoltsy AG,
439 Richards KS (eds) *Biology of the Integument*. Springer-Verlag Berlin Heidelberg,
440 Tempe, Arizona, pp 685–692

441 Hamilton W (1987) Discrimination nepotism: expectable, common, overlooked. *Kin Recognit*
442 *Anim* 417–437

443 Haxaire J, Tamisier J-P, Bouget J-P (2006) *Vespa velutina* Lepeletier, 1836, une redoutable
444 nouveauté pour la faune de France (Hym., Vespidae). *Bull la Société Entomol Fr*
445 111:194–194

446 Hefetz A (2007) The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera:
447 Formicidae) -- interplay of colony odor uniformity and odor idiosyncrasy. A review.
448 *Myrmecol News* 10:59–68

449 Hölldobler B (1995) The chemistry of social regulation: Multicomponent signals in ant
450 societies. *Proc Natl Acad Sci U S A* 92:19–22. <https://doi.org/10.1073/pnas.92.1.19>

451 Hölldobler B, Carlin NF (1987) Anonymity and specificity in the chemical communication
452 signals of social insects. *J Comp Physiol A* 161:567–581.
453 <https://doi.org/10.1007/BF00603662>

454 Holway DA, Case TJ (1999) Mechanisms of dispersed central-place foraging in polydomous
455 colonies of the Argentine ant. *Anim Behav* 59:433–441.
456 <https://doi.org/10.1006/anbe.1999.1329>

457 Howard RW, Blomquist GJ (2005) Ecological, Behavioral, and Biochemical Aspects of
458 Insect Hydrocarbons. *Annu Rev Entomol* 50:371–393.
459 <https://doi.org/10.1146/annurev.ento.50.071803.130359>

460 Howard RW, McDaniel CA, Nelson DR, et al (1982) Cuticular hydrocarbons of
461 *Reticulitermes virginicus* (Banks) and their role as potential species-and caste-
462 recognition cues. *J Chem Ecol* 8:1227–1239

463 Kaib M, Eisermann B, Schoeters E, et al (2000) Task-related variation of postpharyngeal and
464 cuticular hydrocarbon compositions in the ant *Myrmicaria eumenoides*. *J Comp Physiol -*
465 *A Sensory, Neural, Behav Physiol* 186:939–948. <https://doi.org/10.1007/s003590000146>

466 Kukuk PF, Breed MD, Solti A, Bell WJ (1977) The Contributions of Kinship and
467 Conditioning to Nest Recognition and Colony Member Recognition in a Primitively
468 Eusocial Bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Behav Ecol*
469 *Sociobiol* 2:319–327

470 Laborde-Castérot H, Darrouzet E, Le Roux G, et al (2020) Ocular Lesions Other Than Stings

471 following Yellow-Legged Hornet (*Vespa velutina nigrithorax*) Projections, as Reported
472 to French Poison Control Centers. *JAMA Ophthalmol* 1–4.
473 <https://doi.org/10.1001/jamaophthalmol.2020.4877>

474 Lê S, Josse J, Husson F (2008) FactoMineR: An R Package for Multivariate Analysis. *J Stat*
475 *Softw* 25:1–18. <https://doi.org/10.18637/jss.v025.i01>

476 Lebreton S, Christidès JP, Bagnères AG, et al (2010) Modifications of the chemical profile of
477 hosts after parasitism allow parasitoid females to assess the time elapsed since the first
478 attack. *J Chem Ecol* 36:513–521. <https://doi.org/10.1007/s10886-010-9781-7>

479 Lengyel F, Westerlund SA, Kaib M (2007) Juvenile Hormone III Influences Task-Specific
480 Cuticular Hydrocarbon Profile Changes in the Ant *Myrmecaria eumenoidea*. *J Chem Ecol*
481 33:167–181. <https://doi.org/10.1007/s10886-006-9185-x>

482 Lenoir A, Cuisset D, Hefetz A (2001) Effects of social isolation on hydrocarbon pattern and
483 nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae).
484 *Insectes Soc* 48:101–109. <https://doi.org/10.1007/PL00001751>

485 Lenoir A, Depickère S, Devers S, et al (2009) Hydrocarbons in the Ant *Lasius niger*: From
486 the Cuticle to the Nest and Home Range Marking. *J Chem Ecol* 35:913–921.
487 <https://doi.org/10.1007/s10886-009-9669-6>

488 Lenoir A, Fresneau D, Errard C, Hefetz A (1999) Individuality and colonial identity in ants:
489 the emergence of the social representation concept. *Inf Process Soc Insects* 219–237.
490 https://doi.org/10.1007/978-3-0348-8739-7_12

491 Leza M, Herrera C, Marques A, et al (2019) The impact of the invasive species *Vespa*
492 *velutina* on honeybees: A new approach based on oxidative stress. *Sci Total Environ*
493 689:709–715. <https://doi.org/10.1016/j.scitotenv.2019.06.511>

494 López S, González M, Goldarazena A (2011) *Vespa velutina* Lepeletier, 1836 (Hymenoptera:
495 Vespidae): First records in Iberian Peninsula. *EPPO Bull* 41:439–441.
496 <https://doi.org/10.1111/j.1365-2338.2011.02513.x>

497 Matsuura K (2001) Nestmate recognition mediated by intestinal bacteria in a termite,
498 *Reticulitermes speratus*. *Oikos* 92:20–26. [https://doi.org/10.1034/j.1600-](https://doi.org/10.1034/j.1600-0706.2001.920103.x)
499 [0706.2001.920103.x](https://doi.org/10.1034/j.1600-0706.2001.920103.x)

500 Matsuura K (2003) Symbionts Affecting Termite Behavior. In: Bourtzis K, Miller TA (eds)
501 *Insect Symbiosis*. CRC Press, Boca Raton, Florida, pp 131–143

502 Monceau K, Thiéry D (2017) *Vespa velutina* nest distribution at a local scale: An 8-year
503 survey of the invasive honeybee predator. *Insect Sci* 24:663–674.
504 <https://doi.org/10.1111/1744-7917.12331>

505 Nelson DR (1993) Methyl-branched lipids in insects. In: Stanley-Samuelson DW, Nelson DR
506 (eds) *Insect Lipids: Chemistry, Biochemistry & Biology*. University of Nebraska Press,
507 Lincoln and London, pp 270–315

508 Nowbahari E, Lenoir A, Clément JL, et al (1990) Individual, Geographical and Experimental
509 Variation of Cuticular Hydrocarbons of the Ant *Cataglyphis cursor* (Hymenoptera:
510 Formicidae): Their Use in Nest and Subspecies Recognition. *Biochem Syst Ecol* 18:63–
511 73. [https://doi.org/10.1016/0305-1978\(90\)90036-F](https://doi.org/10.1016/0305-1978(90)90036-F)

512 Pedersen JS, Krieger MJB, Vogel V, et al (2006) Native supercolonies of unrelated
513 individuals in the invasive Argentine ant. *Evolution* (N Y) 60:782–791.
514 <https://doi.org/10.1111/j.0014-3820.2006.tb01156.x>

515 Perdereau E, Dedeine F, Christidès JP, Bagnères AG (2010) Variations in Worker Cuticular
516 Hydrocarbons and Soldier Isoprenoid Defensive Secretions Within and Among
517 Introduced and Native Populations of the Subterranean Termite, *Reticulitermes flavipes*.
518 *J Chem Ecol* 36:1189–1198. <https://doi.org/10.1007/s10886-010-9860-9>

519 Rahman S, Hajong SR, Gévar J, et al (2016) Cuticular Hydrocarbon Compounds in Worker
520 Castes and Their Role in Nestmate Recognition in *Apis cerana indica*. *J Chem Ecol*
521 42:444–451. <https://doi.org/10.1007/s10886-016-0700-4>

522 Requier F, Antúnez K, Morales CL, et al (2018) Trends in beekeeping and honey bee colony
523 losses in Latin America. *J Apic Res* 57:657–662.
524 <https://doi.org/10.1080/00218839.2018.1494919>

525 Requier F, Rome Q, Chiron G, et al (2019) Predation of the invasive Asian hornet affects
526 foraging activity and survival probability of honey bees in Western Europe. *J Pest Sci*
527 (2004) 92:567–578. <https://doi.org/10.1007/s10340-018-1063-0>

528 Robinet C, Darrouzet E, Suppo C (2018) Spread modelling: a suitable tool to explore the role
529 of human-mediated dispersal in the range expansion of the yellow-legged hornet in
530 Europe. *Int J Pest Manag* 65:258–267. <https://doi.org/10.1080/09670874.2018.1484529>

531 Robinet C, Suppo C, Darrouzet E (2017) Rapid spread of the invasive yellow-legged hornet in
532 France: the role of human-mediated dispersal and the effects of control measures. *J Appl*
533 *Ecol* 54:205–215. <https://doi.org/10.1111/1365-2664.12724>

534 Rome Q, Muller F, Gargominy O, Villemant C (2009) Bilan 2008 de l'invasion de *Vespa*
535 *velutina* Lepeletier en France (Hymenoptera, Vespidae). *Bull la Société Entomol Fr*
536 114:297–302

537 Rome Q, Muller FJ, Touret-Alby A, et al (2015) Caste differentiation and seasonal changes in
538 *Vespa velutina* (Hym.: Vespidae) colonies in its introduced range. *J Appl Entomol*

539 139:771–782. <https://doi.org/10.1111/jen.12210>

540 Rottler-Hoermann AM, Schulz S, Ayasse M (2016) Nest wax triggers worker reproduction in
541 the bumblebee *Bombus terrestris*. *R Soc Open Sci* 3:150599.
542 <https://doi.org/10.1098/rsos.150599>

543 Saleh N, Scott AG, Bryning GP, Chittka L (2007) Distinguishing signals and cues:
544 bumblebees use general footprints to generate adaptive behaviour at flowers and nest.
545 *Arthropod Plant Interact* 1:119–127. <https://doi.org/10.1007/s11829-007-9011-6>

546 Scholl J, Naug D (2011) Olfactory discrimination of age-specific hydrocarbons generates
547 behavioral segregation in a honeybee colony. *Behav Ecol Sociobiol* 65:1967–1973.
548 <https://doi.org/10.1007/s00265-011-1206-2>

549 Shelton TG, Grace JK (1997) Impact of Low Temperature Conditioning on Intercolonial
550 Agonism in *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Sociobiology* 30:197–
551 211

552 Singer TL (1998) Roles of Hydrocarbons in the Recognition Systems of Insects. *Am Zool*
553 38:394–405. <https://doi.org/https://dx.doi.org/10.1093/icb/38.2.394>

554 Singer TL, Espelie KE (1992) Social wasps use nest paper hydrocarbons for nestmate
555 recognition. *Anim Behav* 44:63–68. [https://doi.org/10.1016/S0003-3472\(05\)80755-9](https://doi.org/10.1016/S0003-3472(05)80755-9)

556 Smith R-K, Taylor Jr OR (1990) Unsaturated Extracted Hydrocarbon Caste Differences
557 between European Queen and Worker Honey Bees , *Apis mellifera* L . (Hymenoptera :
558 Apidae). *J Kansas Entomol Soc* 63:369–374

559 Suarez A V., Holway DA, Case TJ (2000) Patterns of spread in biological invasions
560 dominated by long-distance jump dispersal: Insights from Argentine ants. *Proc Natl*
561 *Acad Sci* 98:1095–1100. <https://doi.org/10.1073/pnas.98.3.1095>

562 Suarez A V., Tsutsui ND, Holway DA, Case TJ (1999) Behavioral and genetic differentiation
563 between native and introduced populations of the Argentine ant. *Biol Invasions* 1:43–53.
564 <https://doi.org/10.1023/A>

565 Tsutsui ND, Suarez AV, Holway DA, Case TJ (2001) Relationships among native and
566 introduced populations of the Argentine ant (*Linepithema humile*) and the source of
567 introduced populations. *Mol Ecol* 10:2151–2161

568 Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the
569 success of an invasive species. *Proc Natl Acad Sci U S A* 97:5948–5953.
570 <https://doi.org/10.1073/pnas.100110397>

571 Turillazzi S, Ugolini A (1979) Rubbing behaviour in some European Polistes (Hymenoptera
572 Vespidae). *Ital J Zool* 13:129–142

573 Villemant C, Muller F, Haubois S (2011) Bilan des travaux (MNHN et IRBI) sur l'invasion
574 en France de *Vespa velutina*, le frelon asiatique prédateur d'abeilles. Journée Sci Apic 3–
575 12

576 Wheeler WM (1911) The ant-colony as an organism. *J Morphol* 22:307–325

577 Wilson EO (1971) *The insect societies*. Harvard University Press, Cambridge,
578 Massachusetts, USA

579 Woodrow RJ, Grace JK, Nelson LJ, Haverty MI (2000) Modification of Cuticular
580 Hydrocarbons of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in Response to
581 Temperature and Relative Humidity. *Environ Entomol* 29:1100–1107.
582 <https://doi.org/10.1603/0046-225X-29.6.1100>

583

584 **Legends**

585 **Table 1.** Identities and relative proportions of the hydrocarbons found on the three nest
586 structures: envelopes, combs, and pillars.

587 **Table 2.** Differences in hydrocarbon quantities among nest structures (Kruskal-Wallis chi-
588 squared test). Levels of 5 compounds differed between the envelopes and combs; levels of 10
589 compounds differed between the envelopes and pillars; and levels of 2 compounds differed
590 between the combs and pillars.

591 **Figure 1.** Results of the principal component analysis of the proportion of hydrocarbons found
592 on the three nest structures; the first two axes are shown. Three comparisons were performed:
593 envelopes vs. combs, envelopes vs. pillars, and combs vs. pillars.

594 **Figure 2.** Total hydrocarbon quantities found on the envelopes, combs, and pillars (mg of
595 hydrocarbons/gram of nest material).

596 **Figure 3.** Quantities of alkanes, branched alkanes, and alkenes found on the envelopes, combs,
597 and pillars (mg of hydrocarbons/gram of nest material).

598 **Figure 4.** Total hydrocarbon quantities (mg of hydrocarbons/gram of nest material) at the
599 comb's center, middle, and periphery.

600 **Figure 5.** Total hydrocarbon quantities (mg of hydrocarbons/gram of nest material) from the
601 top comb to the bottom comb within nests.

602

603 **Supplementary materials**

604 **Table S1.** Collection locations and dates for the nests used in the chemical analyses of nest
605 structures.

606 **Table S2.** Collection locations and dates for the nests used in the detailed chemical analyses of
607 the combs.

608 **Figure S1.** Chemical distances between the hydrocarbon profiles of the three nest structures:
609 envelopes, combs, and pillars. The among-nest chemical distances represent the chemical
610 diversity of the same structure across different nests; the among-structure chemical distances
611 represent the chemical diversity among nest structures within the same nest; and the within-
612 structure chemical distances represent the chemical diversity within a specific structure for a
613 given nest.

614 **Figure S2.** Chemical distances between hydrocarbon profiles for the comb center, middle, and
615 periphery. The within-zone chemical distances represent the distances among samples taken
616 from within the same comb zone; the between-zone chemical distances represent the distances
617 among samples taken from different comb zones.

618 **Figure S3.** Chemical distances between hydrocarbon profiles within combs from nest top to
619 bottom.

620

621 **Table S1**

Nest number	Latitude	Longitude	Collection date
1	47.401366	0.66336	07/13/2017
2	47.388264	0.827383	08/14/2017
3	47.413326	0.984407	08/17/2017
4	47.498614	0.57948	08/24/2017
5	47.543367	0.744155	10/06/2017
6	47.388264	0.827383	12/01/2017
7	47.388309	0.658258	07/29/2016
8	47.351861	0.66131	08/05/2016
9	47.394144	0.68484	08/29/2016
10	47.388264	0.827383	07/07/2015

622

623

624 **Table S2**

Nest Number	Latitude	Longitude	Height (cm)	Width (cm)	Comb number	Collection date
1	48.066152	-2.967055	73	41	10	20.02.2018
2	47.3404240	-1.526116	53	47	7	14.02.2018
3	47.3898439	0.7224790	44	40	7	20.03.2018

625

626

627 **Table 1**

RT (min)	Compound Identity	ECL	Envelopes		Combs		Pillars	
			%	+SD	%	+SD	%	+SD
23.8	n-C20	20	X	X	X	X	X	X
28.8	n-C23	23	3.57	0.56	1.92	0.11	2.62	0.13
31.5	C25 :1	24.72	0.68	0.11	0.76	0.10	0.66	0.04
31.9	n-C25	25	16.49	0.53	15.72	0.84	17.23	0.32
32.4	13-+11-MeC25	25.37	1.86	0.19	1.51	0.09	2.14	0.13
32.7	5-MeC25	25.47	0.36	0.11	0.33	0.03	0.35	0.04
33.1	3-MeC25	25.70	3.88	0.22	3.10	0.16	3.28	0.19
33.5	n-C26	26	1.72	0.05	1.59	0.13	1.60	0.09
34.1	12-+10-MeC26	26.31	0.55	0.14	0.45	0.04	0.50	0.03
34.7	C27 :1	26.75	4.30	0.35	5.25	0.46	4.94	0.22
34.8	C27 :1	26.83	1.14	0.14	1.07	0.10	0.92	0.06

35.1	n-C27	27	23.71	1.28	32.01	2.58	29.63	0.96
35.5	13-+11- +9MeC27	27.32	4.66	0.56	4.91	0.87	5.82	0.71
35.6	7,MeC27	27.37	0.90	0.23	0.80	0.06	0.87	0.08
35.9	11,15-diMeC27	27.64	4.04	0.52	2.77	0.27	2.65	0.16
36.1	3-MeC27	27.81	8.41	0.31	6.38	0.74	7.48	0.22
36.2	5,15-diMeC27	27.89	1.13	0.49	1.68	0.64	0.95	0.11
36.4	n-C28	28	3.00	150	0.79	0.07	0.87	0.08
36.7	diMe or triMeC27	28.13	1.05	0.23	1.02	0.16	0.97	0.12
36.9	12-MeC28	28.35	2.33	0.47	0.62	0.18	0.48	0.04
37.6	C29 :1	28.78	2.32	0.15	3.24	0.36	3.59	0.14
37.7	C29 :1	28.84	1.13	0.17	2.08	0.41	1.29	0.08
37.9	n-C29	29	5.39	0.39	5.80	0.66	5.43	0.25
38.4	13-+11-+9- MeC29	29.37	1.81	0.18	2.21	0.23	2.61	0.07
38.8	11,15-+11,17- diMeC29	29.68	1.80	0.26	1.37	0.32	1.06	0.15
39.7	4-MeC30	30.44	0.64	0.22	0.20	0.05	0.32	0.14
40.7	n-C31	31	3.32	1.05	2.39	0.37	1.69	0.13

628

629 **Table 2**

Compound	Structure comparison		
	Envelopes/Combs	Envelopes/Pillars	Combs/Pillars
n-C23	NS	NS	p < 0.01
C25 :1	NS	NS	NS
n-C25	NS	NS	NS
13-+11-MeC25	NS	p = 0.01	p < 0.01
5-MeC25	NS	NS	NS
3-MeC25	p = 0.01	p < 0.01	NS
n-C26	NS	NS	NS
12-+10-MeC26	NS	NS	NS
C27 :1	NS	p = 0.01	NS
C27 :1	NS	NS	NS
n-C27	p < 0.05	p < 0.01	NS
13-+11-+9MeC27	NS	NS	NS
7,MeC27	NS	NS	NS

11,15-diMeC27	NS	p < 0.05	NS
3-MeC27	p < 0.05	p = 0.01	NS
5,15-diMeC27	NS	NS	NS
n-C28	p < 0.01	NS	NS
diMe or triMeC27	NS	NS	NS
12-MeC28	p < 0.05	p < 0.01	NS
C29 :1	NS	p < 0.01	NS
C29 :1	NS	NS	NS
n-C29	NS	NS	NS
13-+11-+9-MeC29	NS	p < 0.01	NS
11,15-+11,17-diMeC29	NS	p = 0.05	NS
4-MeC30	NS	NS	NS
n-C31	NS	NS	NS

630

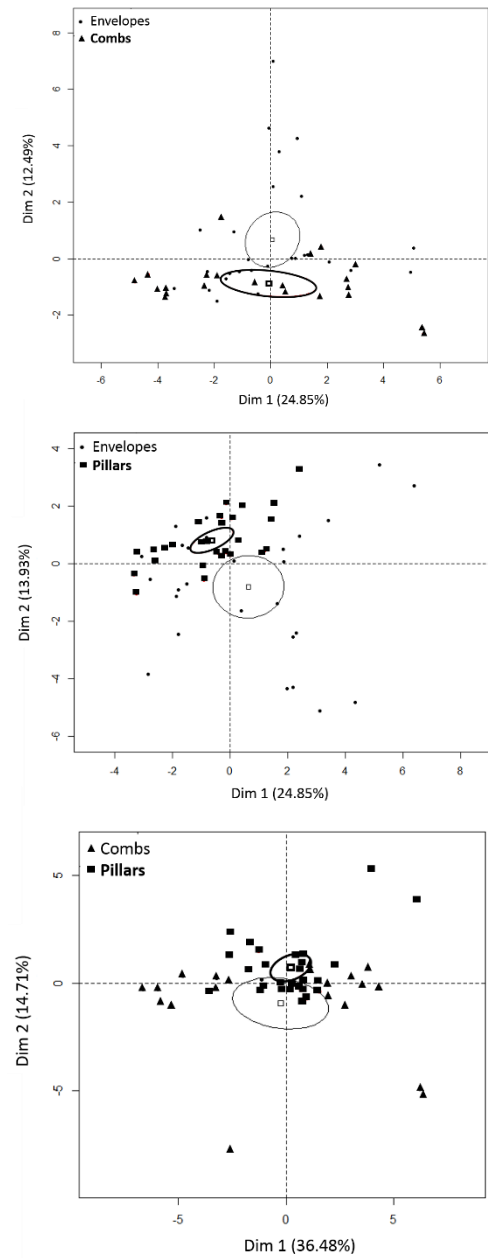


Figure 1

Figure 2

Hydrocarbon quantities

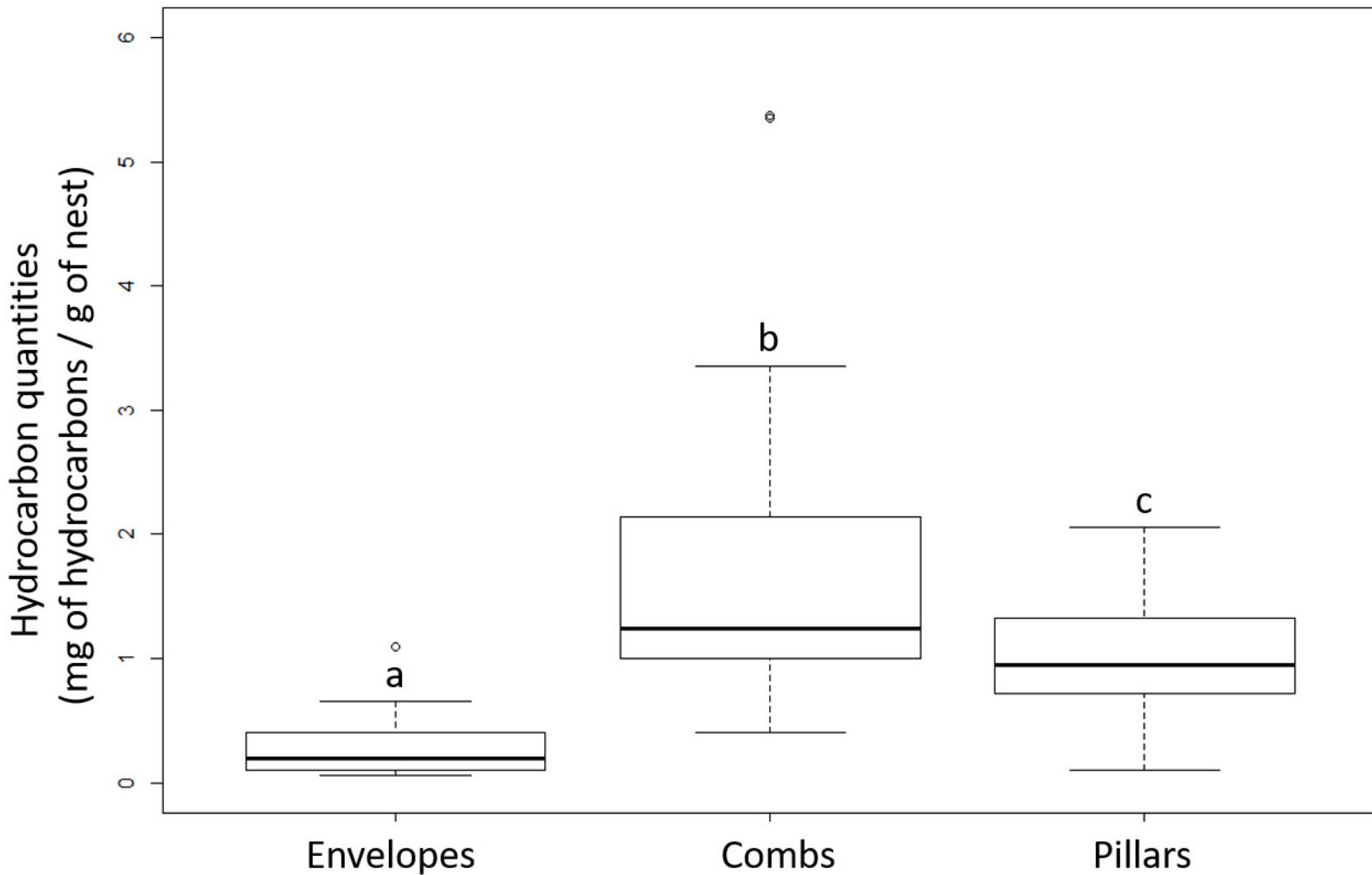


Figure 3

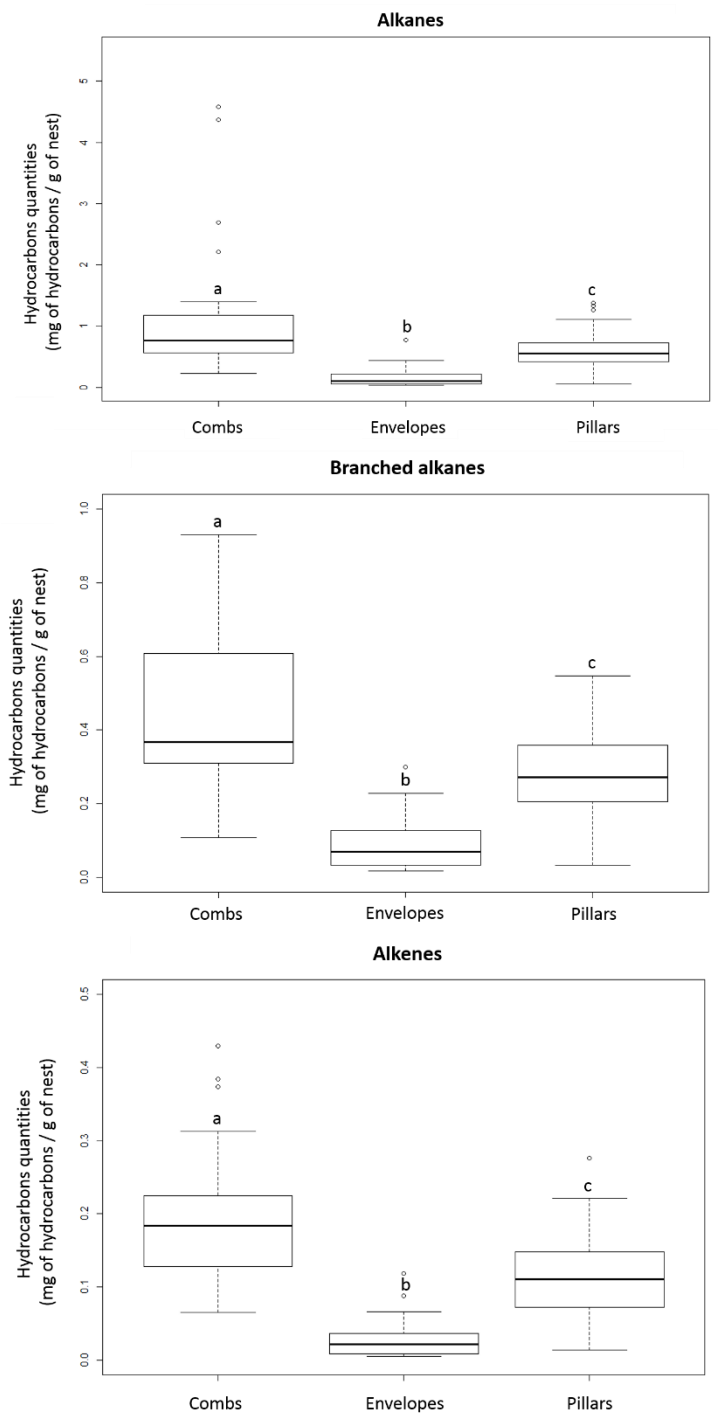


Figure 4

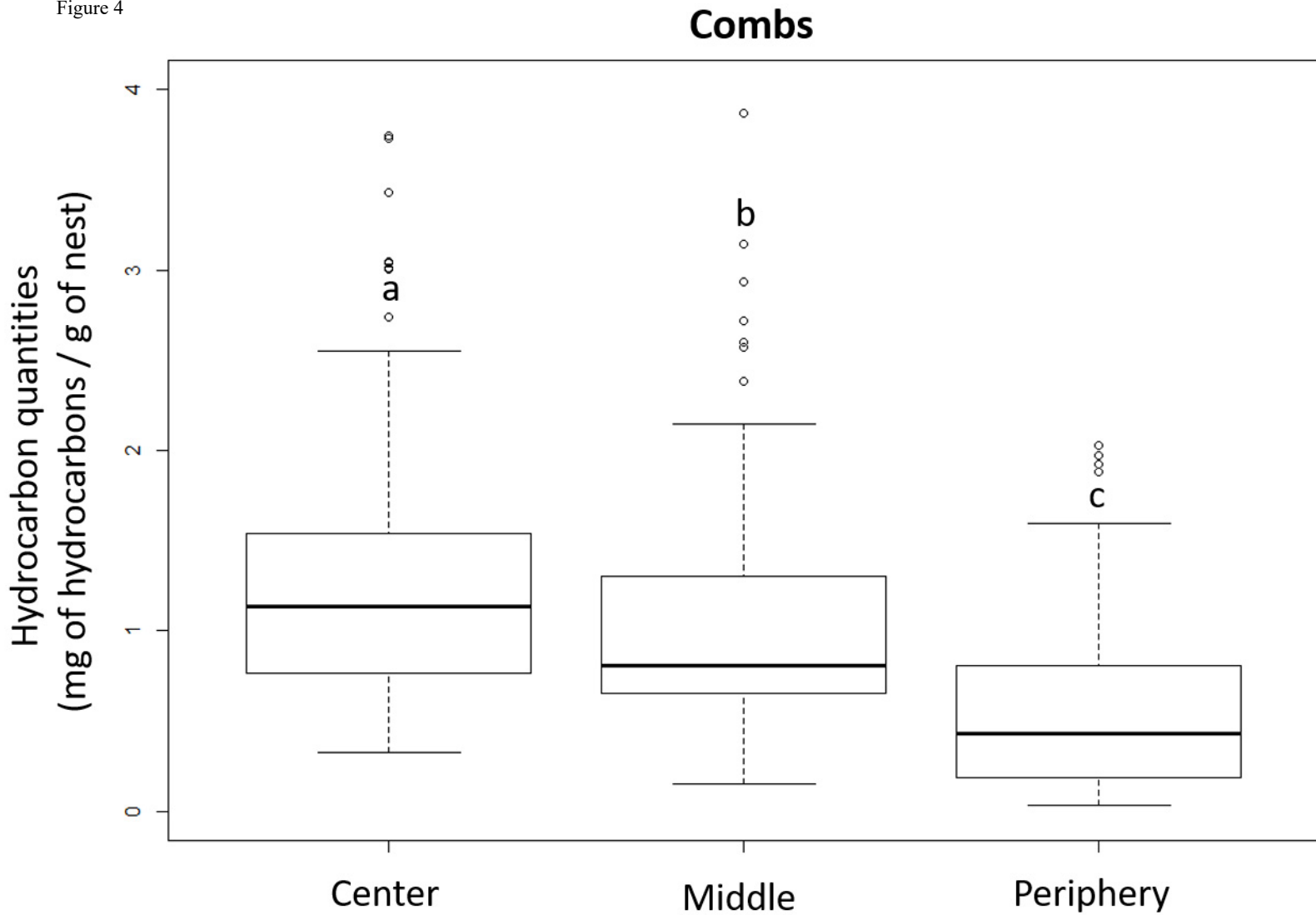


Figure 5

Combs

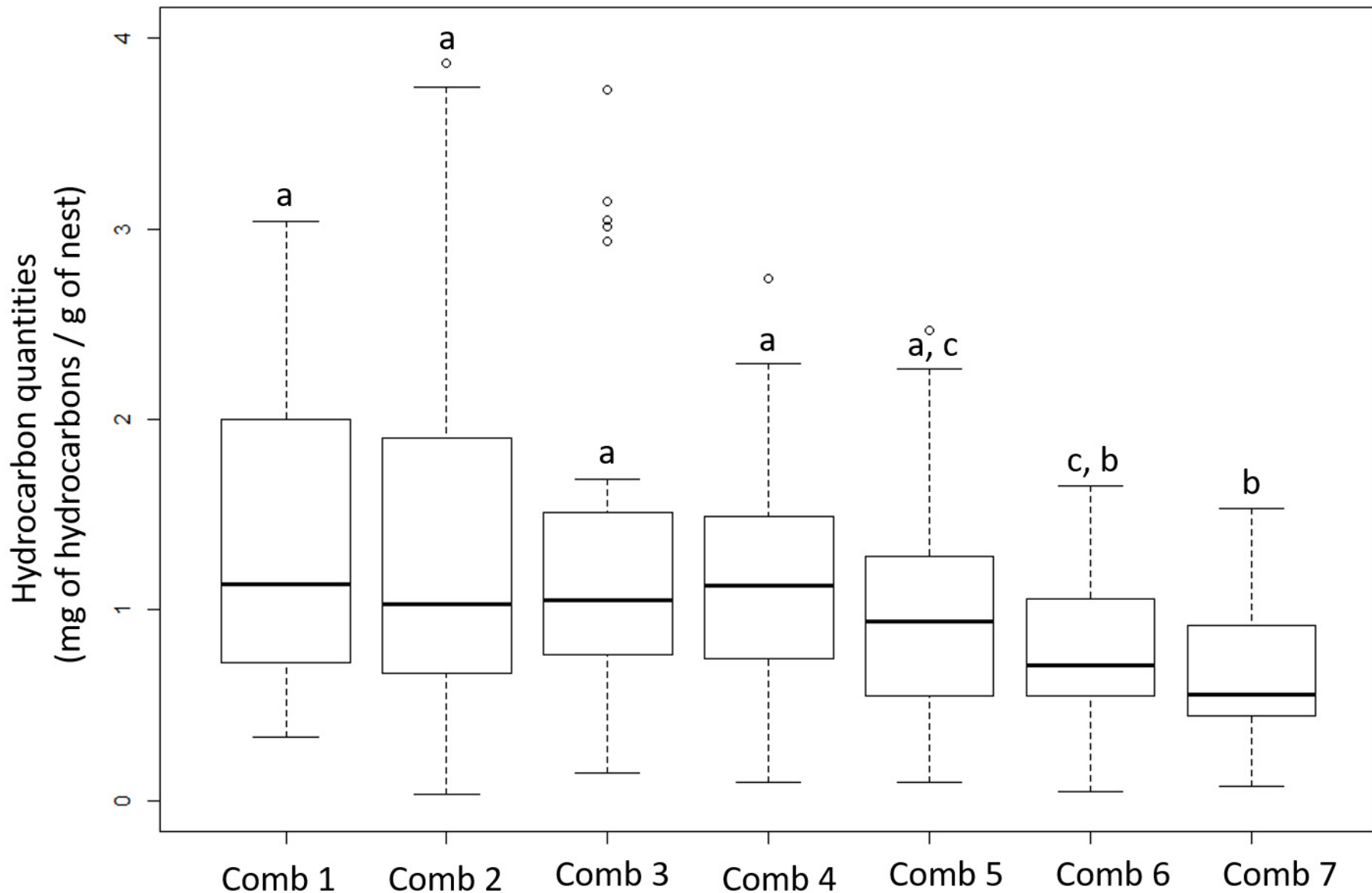


Figure S3

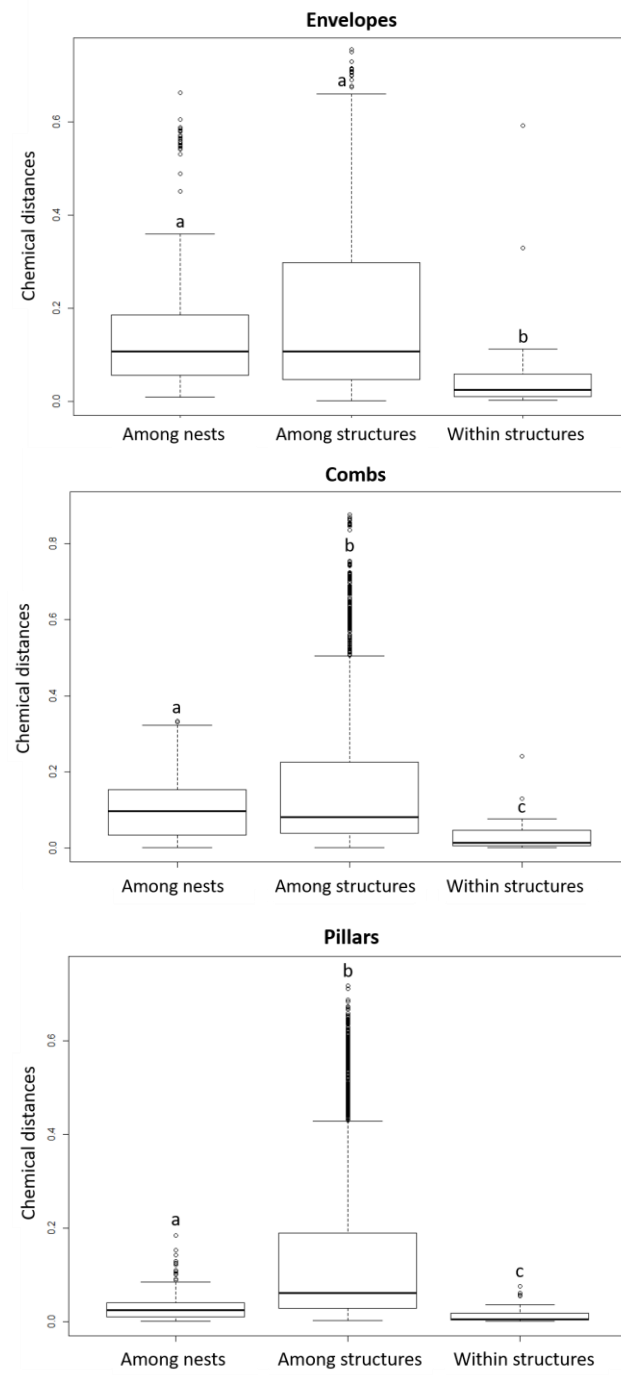


Figure S2

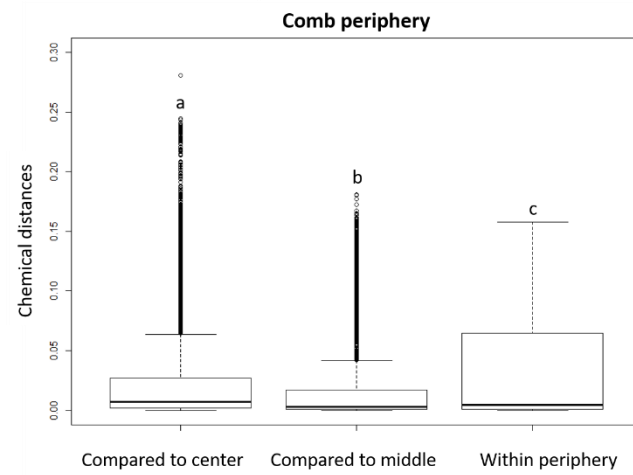
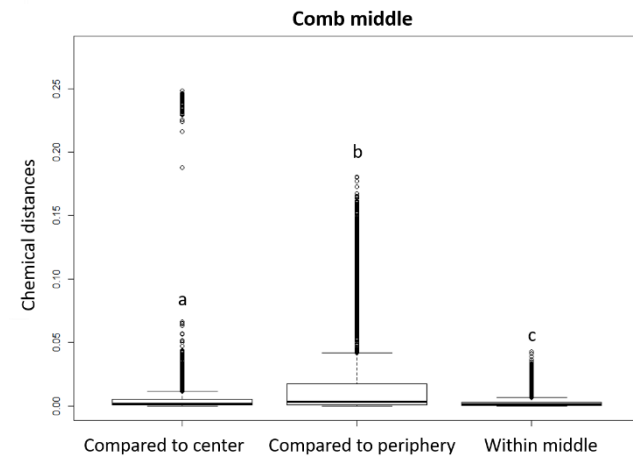
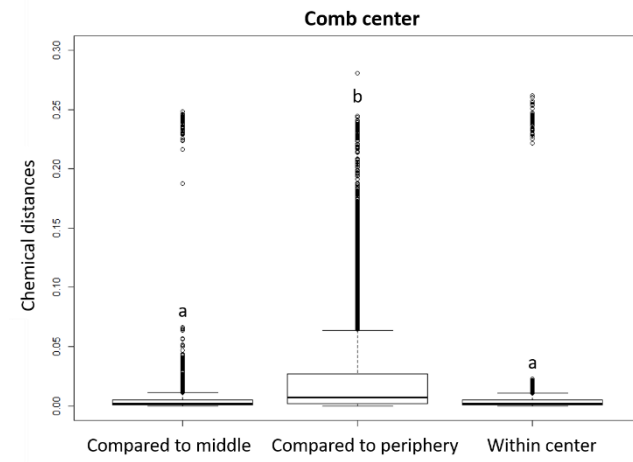


Figure S1

Within combs

