1   # Within and among population differences in cuticular hydrocarbons in the

2   # seabird tick *Ixodes uriae*.

3   Marlène Dupraz[a]*, Chloe Leroy[b], Þorkell Lindberg Þórarinsson[c], Patrizia d'Ettorre[b], Karen D. McCoy[a].

4

5   [a] MIVEGEC, IRD, CNRS, Univ. Montpellier, Centre IRD, 911 Avenue Agropolis, BP 64501, 34394

6   Montpellier, France

7   [b] Laboratoire d'Ethologie Expérimentale et Comparée UR 4443 (LEEC), Université Sorbonne Paris

8   Nord, F-93430 Villetaneuse, France

9   [c] Northeast Iceland Nature Research Centre, 640 Húsavík, Iceland

10

11   *Corresponding author: marleneupraz@gmail.com; Tel. +33 4 67 41 61 78;

12

13

Abstract

The hydrophobic layer of the arthropod cuticle acts to maintain water balance, but can also serve to transmit chemical signals via cuticular hydrocarbons (CHC), essential mediators of ~~insect~~ behavior. CHC signatures typically vary qualitatively among species, but also quantitatively among populations within a species, and have been used as taxonomic tools to differentiate species or populations in a variety of taxa. Most work in this area to date has focused on insects, with little known for other arthropod classes such as ticks. The worldwide distribution and extensive host-range of the seabird tick *Ixodes uriae* make it a good model to study the factors influencing CHC composition. Genetically differentiated host-races of *I. uriae* have evolved across the distribution of this species but the factors promoting sympatric population divergence are still unknown. To test for a potential role of host-associated CHC in population isolation, we collected *I. uriae* specimens from two of its seabird hosts, the Atlantic puffin (*Fratercula arctica)* and the common guillemot (*Uria aalge*) in different colonies in Iceland. Using gas-chromatography and mass-spectrometry, we detected a complex cuticular mixture of 22 hydrocarbons, including *n*-alkanes, methyl-alkanes and alkenes ranging from 17 to 33 carbons in length. We found that each population had a distinct CHC profile, with long-chain hydrocarbons tending to be more abundant in puffin tick populations. As profiles also varied between host-associated groups, future work will now be required to tests whether the different CHC signals may reinforce assortative mating patterns, and thus *I. uriae* population divergence.

Keywords: Host race formation; GC-MS; colonial seabirds; Ixodidae; environmental variation

Introduction

The arthropod cuticle acts as both an exoskeleton and a barrier from the external environment (Andersen, 1979). The outermost layer, the epicuticle, is covered by a lipid layer (Lockey, 1988) made-up of esters, carboxylic acids, alcohols, carbonyls and long-chain hydrocarbons (Andersen, 1979) that protects the organism from desiccation (Filshie, 1982). However, cuticular hydrocarbons (CHC) are also involved in chemical communication serving as sex pheromones, kairomones and/or signature mixtures allowing recognition of social identity (van Zweden and d'Ettorre, 2010; Wyatt, 2010). Using analytic techniques such as gas chromatography-mass spectrometry (GC-MS) and MALDI-TOF mass spectrometry, researchers have described hydrocarbons of up 70 carbons in chain length in insects, principally *n*-alkanes, methyl-branched alkanes and alkenes (Blomquist and Bagnères, 2010). The array of CHC on the cuticle constitute a species-specific chemical signature, varying qualitatively between species and quantitatively within species (Lockey, 1988). CHC patterns are genetically controlled, but the relative abundance of particular components can be linked to environmental conditions (Estrada-Peña et al., 1993; Gibbs et al., 1991). CHC patterns have been used as taxonomic tools to characterize hundreds of arthropod species (Howard and Blomquist, 2005), and to discriminate closely-related populations (Bagnères et al., 1991; Bartelt et al., 1986; Jallon and David, 1987; Kruger et al., 1991; Simmons et al., 2014).

In ticks, hematophagous arthropods widely distributed across the globe and parasitizing a diverse array of vertebrate species (McCoy and Boulanger, 2015), little work has been performed to describe CHC profiles and their variation among species and populations. The only studies to date have focused on relatively few species and used CHC profiles in an attempt to differentiate closely-related taxa (Estrada-Peña et al., 1992, 1994, 1996; Hunt, 1986; Estrada-Peña and Dusbabek, 1993). However, CHC profiles in ticks may play essential roles in several aspects of tick life histories. First,

57   the tick epicuticle is perforated with numerous channels providing a large surface for exchange with

58   the external environment. As most tick species spend the major part of their life cycle in the off-

59   host environment, maintaining water balance across this surface under different environmental

60   conditions will directly dictate survival (Randolph and Storey, 1999); the presence of CHC likely plays

61   an important role in this. Second, as obligate parasites, access to the vertebrate host for the

62   bloodmeal is a key aspect of the tick life cycle and, as such, ticks have adapted key traits to locate

63   and successfully exploit their host (McCoy and Boulanger, 2015). For example, Shimshoni et al.

64   (2013) found *Rhipicephalus* tick species had different cuticular fatty acid compositions in relation to

65   host use. However, whether these differences are linked to adaptive survival or by-product

66   variation due to the host resource is unknown as yet. Finally, ticks aggregate both on hosts and in

67   the off-host environment (Randolph, 1998). This behavior is thought to facilitate blood feeding on

68   the host and increase survival in the off-host environment. It may also enable ticks to find

69   appropriate mates for reproduction. Some ticks, such as the tropical bont tick *Amblyomma*

70   *variegatum*, produce a multicomponent pheromone to provoke ~~this behavior~~ (Schöni et al., 1984)

71   but the potential role of these pheromones in assortative mating has never been examined.

72   Seabird ticks are generally nidicolous, exploiting different local host species that use diverse micro-

73   habitats within the colony (Dietrich et al., 2011). Due to this diversity, these ticks may experience

74   diverse selective pressures coming from both the hosts and from the temperature and humidity

75   conditions of the nest micro-habitat. In particular, *Ixodes uriae*, a tick associated with seabird

76   colonies in the polar regions of both hemispheres, is known to form host-specific races that show

77   genetic (McCoy et al., 2001, 2003, 2005) and morphological (Dietrich et al., 2013) differences in

78   relation to host use. Differential performance on alternative hosts has also been experimentally

79   demonstrated (Dietrich et al., 2014). Nevertheless, the factors driving divergence within this species

80   have yet to be specifically identified. A potential role for isolating mechanisms that lead to

81   assortative mating based on host use have been suggested (McCoy et al., 2013).

82   Here, we examine the degree of cuticular hydrocarbon diversity in *I. uriae* and the factors

83   influencing these chemical signatures. Based on current knowledge, we predicted that cuticular

84   hydrocarbon patterns could vary in ticks 1) exploiting different host species and 2) sampled in

85   different geographic locations. If host exploitation modifies CHC diversity and abundance, we

86   expected that signatures in ticks from the same seabird host in different locations should be more

87   similar than signatures in ticks from different host species in the same geographic location. To test

88   these predictions, we collected *I. uriae* specimens in the nest material of two seabird host species,

89   the common guillemot (CG) *Uria aalge,* and the Atlantic puffin (PF) *Fratercula arctica,* at three

90   locations in Iceland. We then extracted cuticular hydrocarbons and analyzed them using gas

91   chromatography-mass spectrometry (GC-MS).

92

Material and methods

Population samples

95   Flat adult female ticks were collected off-host in three sites in Iceland in June 2016: guillemot ticks

96   were collected under rocks in the middle of the colony in Langanes (66°22'07.2"N 14°38'33.0"W)

97   and on Grimsey Island (66°32'57.3"N 17°59'31.1"W); puffin ticks were collected in burrows on

98   Lundey Island (66°06'53.2"N 17°22'13.1"W) and on Grimsey Island (66°32'39.1"N 18°01'12.9"W)

99   (Fig.1). Ticks were kept in plastic tubes before hydrocarbon extraction. Four replicates of 10 flat

100   ticks were extracted for each host species at each site.

5
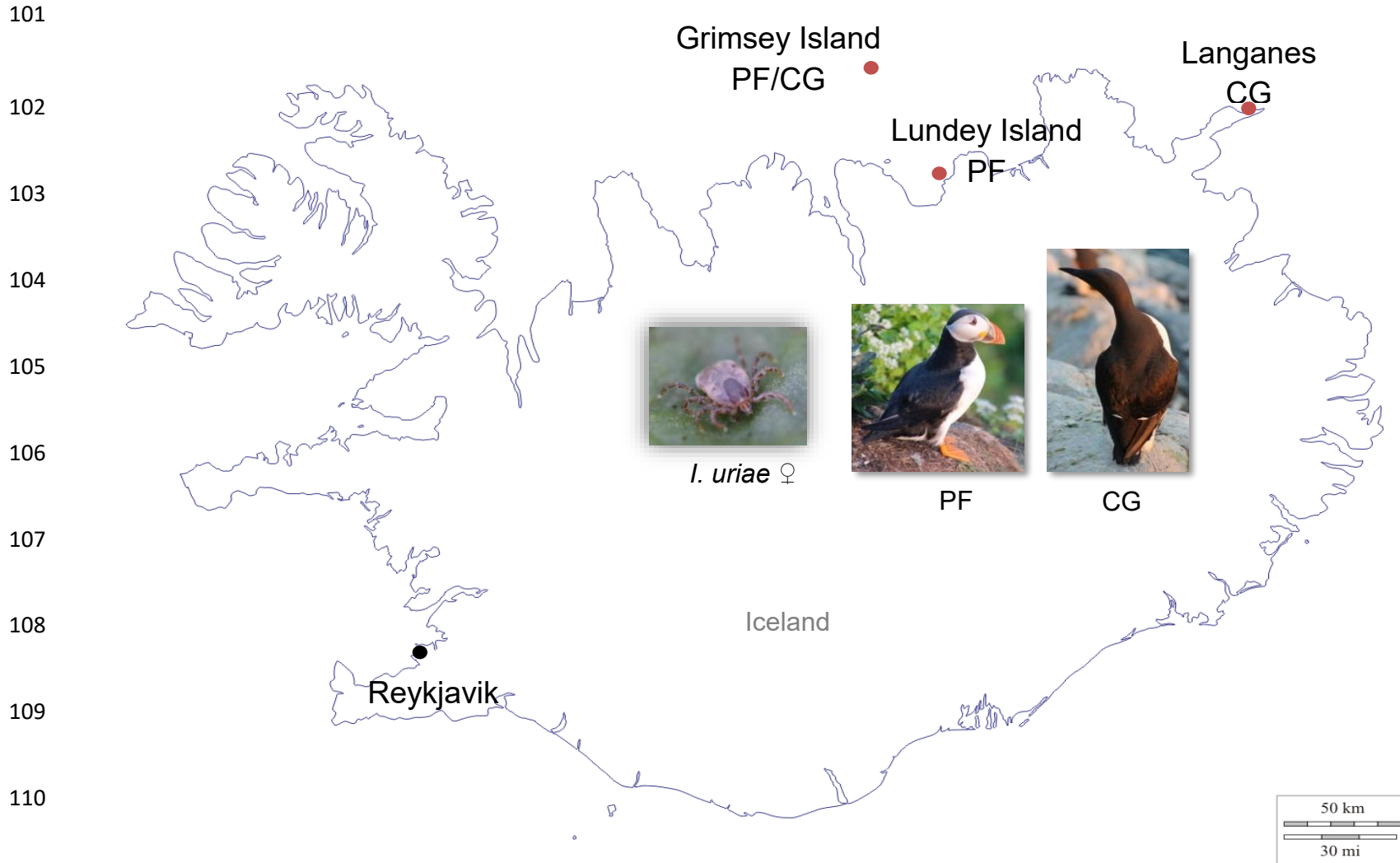
Fig.1: Map showing the sampling hosts and locations in Iceland. Acronyme of sampling location are Grimsey: G; Lundey Island: Li; Langanes: L. PF refers to puffin (Fratercula arctica) and CG to common guillemot (Uria aalge).

Hydrocarbon extraction

114   Cuticular compounds were extracted by immersing 10 living flat female ticks in 200 µl of pentane

115   (HPLC grade, Sigma-Aldrich) in glass vials. The vials were agitated for 1 minute, set down to rest for

116   5 minutes and then re-agitated for 1 minute. Ticks were then removed from the vials and preserved

117   in 70% ethanol. The pentane was then evaporated off over 30 minutes and the vials were closed

118   and kept at 4°C until analyses. A negative control containing only 200 µl of pentane was added to

119   each extraction session to control for potential contamination.

Chemical analyses

121   Samples were re-diluted in 40µl of pentane and 3µl were injected into an Agilent Technologies

122   7890A gas chromatograph (capillary column: Agilent HP-5MS, 30 m × 0.25 mm × 0.25 µm; split–

123   splitless injector; carrying helium gas at 1 mL/min) coupled with an Agilent 5975C mass

124   spectrometer with 70 eV electron impact ionization. The oven temperature was programmed at

125   70°C for 1min, and was increased at 30°C/min to 200°C, then to 320°C at 5°C/min and held for 5

126   min. Compounds were identified on the basis of their mass spectra and retention time and by

127   comparison with standards and published spectra. The areas under the peaks were extracted using

128   the Agilent MSD ChemStation software (E.02.01.1177). The relative amount of each hydrocarbon

129   was calculated using peak area and the mean of the four replicates for each peak was used for the

130   colony average.

Multivariate analyses

132   If the relative abundance of a CHC was 0, the value was replaced by 0.00001 which is several times

133   smaller than the smallest quantity found for a CHC in our dataset. The data were then transformed

134   by centered log ratio (data available in supplementary material, Table S1 and uploaded on Zenodo

135   at https://doi.org/10.5281/zenodo.5889077) and analyzed by Partial Least Squares coupled with a

136   Discriminant analysis (PLS-DA) using the R software (v 3.4.3) and "RVAidememoire" package (Hervé,

137   2014) (https://cran.r-project.org/web/packages/RVAideMemoire/index.html). PLS-DA is the key

138   analysis when the dataset contains less groups than explanatory variables, as in the case of the

139   present quantitative dataset. PLS-DA is a supervised technique, so class memberships of the CHC

140   need to be predefined. Here, we only used the eight first axes produced by the PLS to performed

141   two PLS-DA tests: the first analysis took into account the four population samples as four different

142   classes (LCG, GCG, LiPF, GPF). The second analysis was based on two classes only, representing the

143   two host types (PF, CG). The number of significant PLS components was determined by cross model

144   validation (2CV). We also calculated a numerical value representing the importance of the CHC

145   variable in the projection (abbreviated VIP), i.e. VIP values larger than 1 are most influential (Hervé,

146   2014).

147

148

149

150

151

152

153

154

155

Results

Hydrocarbon profiles

We detected a complex pattern of 22 hydrocarbons and two non-identified components in the cuticle of *I. uriae* ticks. The hydrocarbon mixture was composed of: 14 *n*-alkanes ranging from 17 to 33 carbon atoms in length, 4 monomethyl-alkanes with 17 to 24 carbon atoms and 4 alkenes with 21 to 27 carbon atoms (Table 1).

Table 1: List of detected cuticular molecules in the cuticle of *I. uriae* ticks including name and type.

Cuticular component	Name	Type
C_{17}	*n*-heptadecane	alkane
meC_{17}	methylheptadecane	methyl-branched alkane
C_{18}	*n*-octadecane	alkane
C_{20}	*n*-eicosane	alkane
$C_{21:1}$	heneicosene	alkene
C_{21}	*n*-heneicosane	alkane
$C_{22:1}$	docosene	alkene
C_{22}	*n*-docosane	alkane
$2meC_{22}$	2methyldocosane	methyl-branched alkane
C_{23}	*n*-tricosane	alkane
C_{24}	*n*-tetracosane	alkane
$9meC_{24}$	9methyltetracosane	methyl-branched alkane
$2meC_{24}$	2methyltetracosane	methyl-branched alkane
$C_{25:1}$	pentacosene	alkene
C_{25}	*n*-pentacosane	alkane
C_{26}	*n*-hexacosane	alkane
$C_{27:1}$	heptacosene	alkene
C_{27}	*n*-heptacosane	alkane
C_{28}	*n*-octacosane	alkane
C_{29}	*n*-nonacosane	alkane
C_{31}	*n*-hentriacontane	alkane
C_{33}	*n*-tritriacontane	alkane
?1	Non-identified	-
?2	Non-identified	-

All hydrocarbons were shared by the four tick populations, except $2meC_{24}$ (2methyltetracosane) and C_{33} (*n*-triacontane) which were only found in puffin ticks (Fig.2).

9
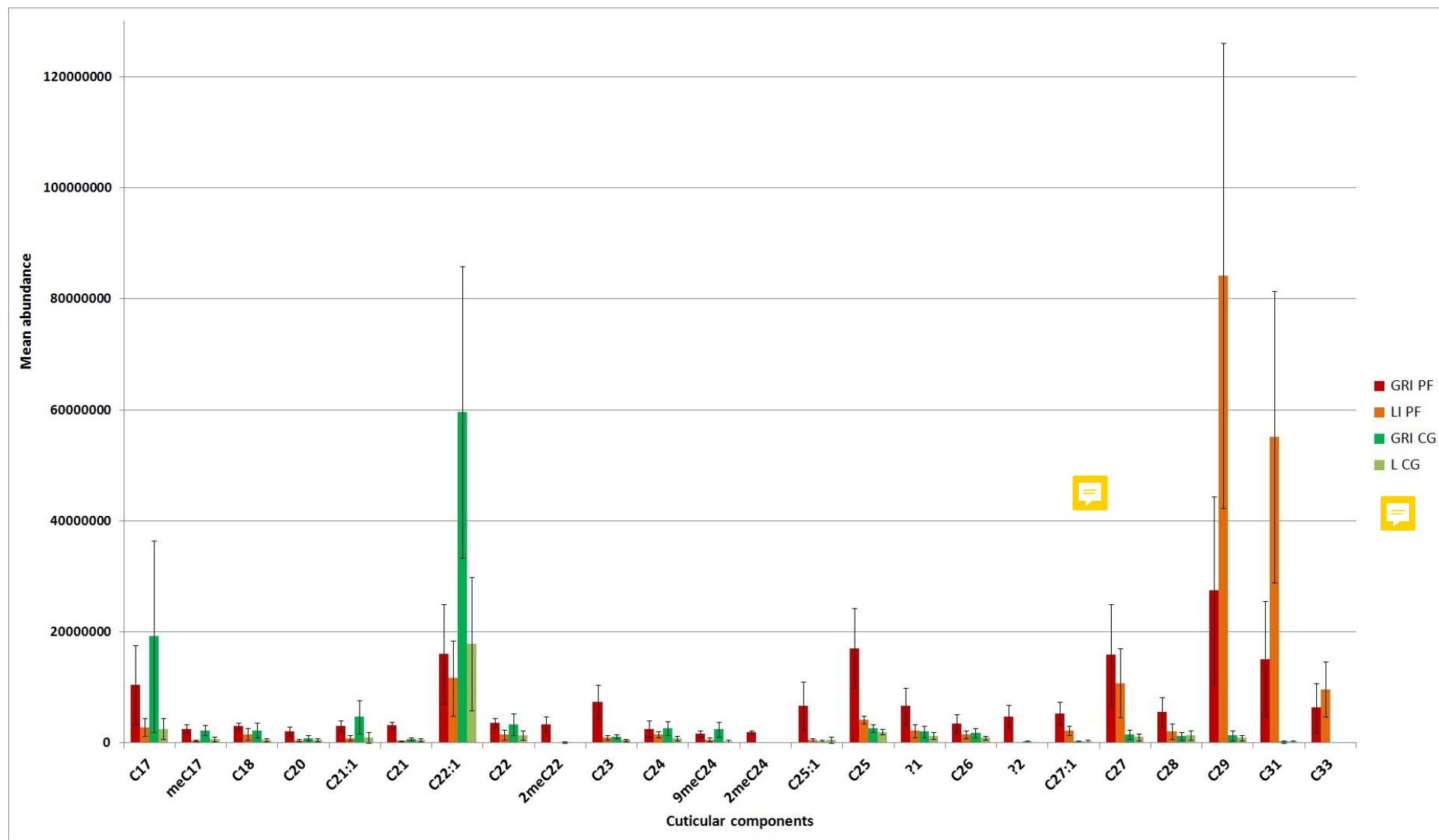
166
167   **Fig.2**: Detected cuticular hydrocarbons from *I. uriae* ticks ranged from 17 to 33 carbon atoms in length. The mean abundance was calculated based on values
168   of each of the four replicate tick pools. Error bars represent standard deviations. See Table 1 for population abbreviations.

169   A high degree of variation in mean abundance of cuticular components among samples was obvious

170   (Fig.2). Hydrocarbons $C_{22:1}$ (docosene), C_{29} (n-nonacosane) and C_{31} (n-hentriacontane) were the

171   most predominant, whereas many hydrocarbons were detected in low quantity, as for example C_{20}

172   (n-eicosane), $2meC_{22}$ (2methyldocosane) and C_{26} (n-hexacosane).

173   The abundance pattern of cuticular hydrocarbons varied between ticks of the two host species

174   (Fig.3), but also among the same host in different colony sites. Nevertheless, results of the pairwise

175   comparisons were non-significant ($p > 0.2$). No CHC was specific to CG samples, although C_{17} (n-

176   heptadecane) and $C_{22:1}$ (docosene) were highly abundant in the GRI colony (Fig.2 and 3). In contrast,

177   long chain cuticular hydrocarbons tended to be present in both PF samples: C_{27} (n-heptacosane), C_{29}

178   (n-nonacosane) and C_{31} (n-hentriacontane). CHC components tended to be in lower overall

179   abundance in samples from Langanes (L CG), whereas samples from Lundey Island (LI PF) had the

180   highest overall abundance (Fig. 2).

181
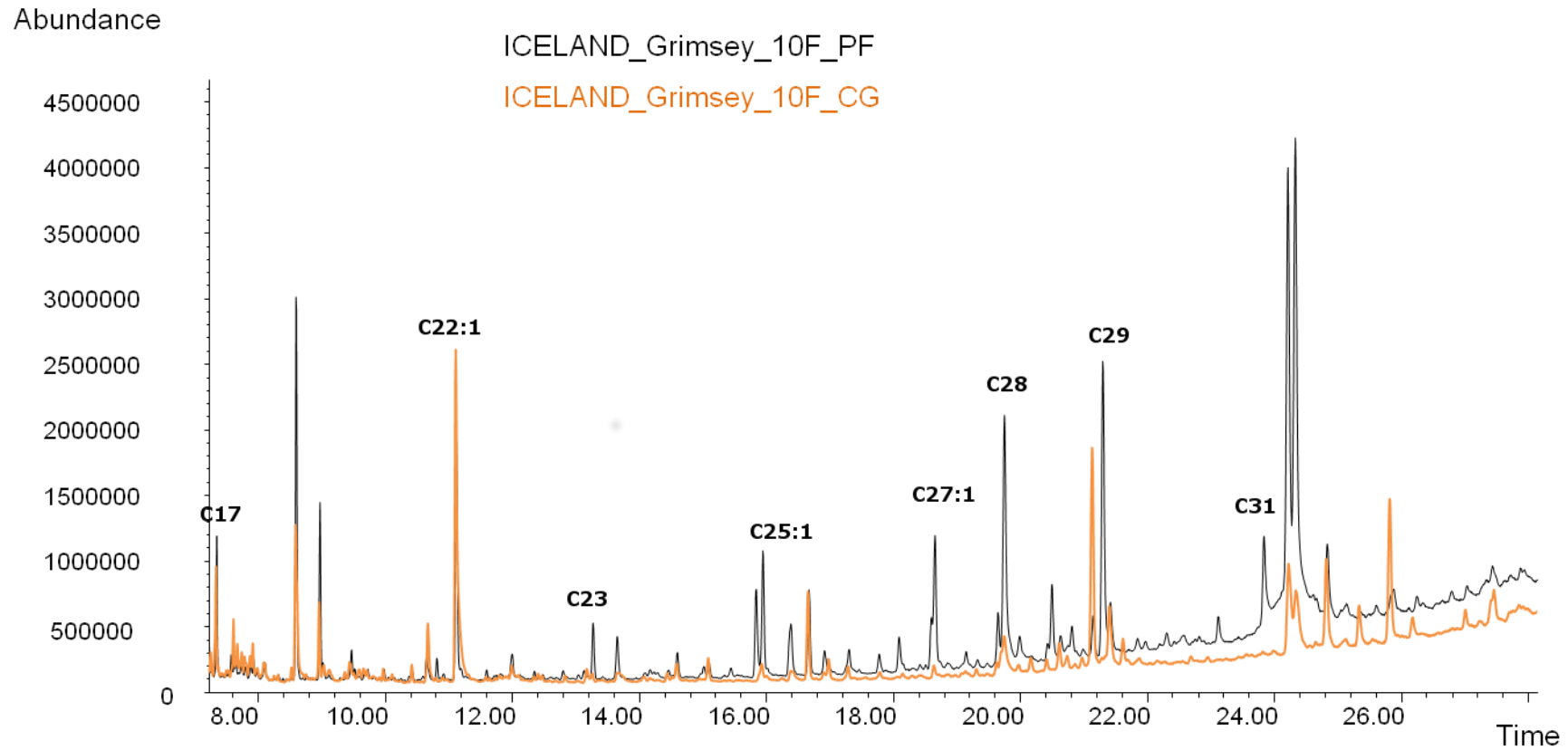
Fig.3: Gas-chromatograms showing the comparison of cuticular hydrocarbon profiles for pools of 10 female ticks from Atlantic puffins (PF: black line) and
Common guillemots (CG: orange line) on Grimsey Island, Iceland.

184 **PLS-DA analyses**

185 *Population samples*

186 Cross model validation for population showed that the quality of the analysis was good: 50.6% of

187 the samples were assigned to the population of origin. PLS-DA analysis showed a discriminating

188 population effect (p=0.008) separating the two host species on first axis (Fig.4). The three first axes

189 explained respectively 11.94, 7.99 and 3.99 % of the total variance among samples.
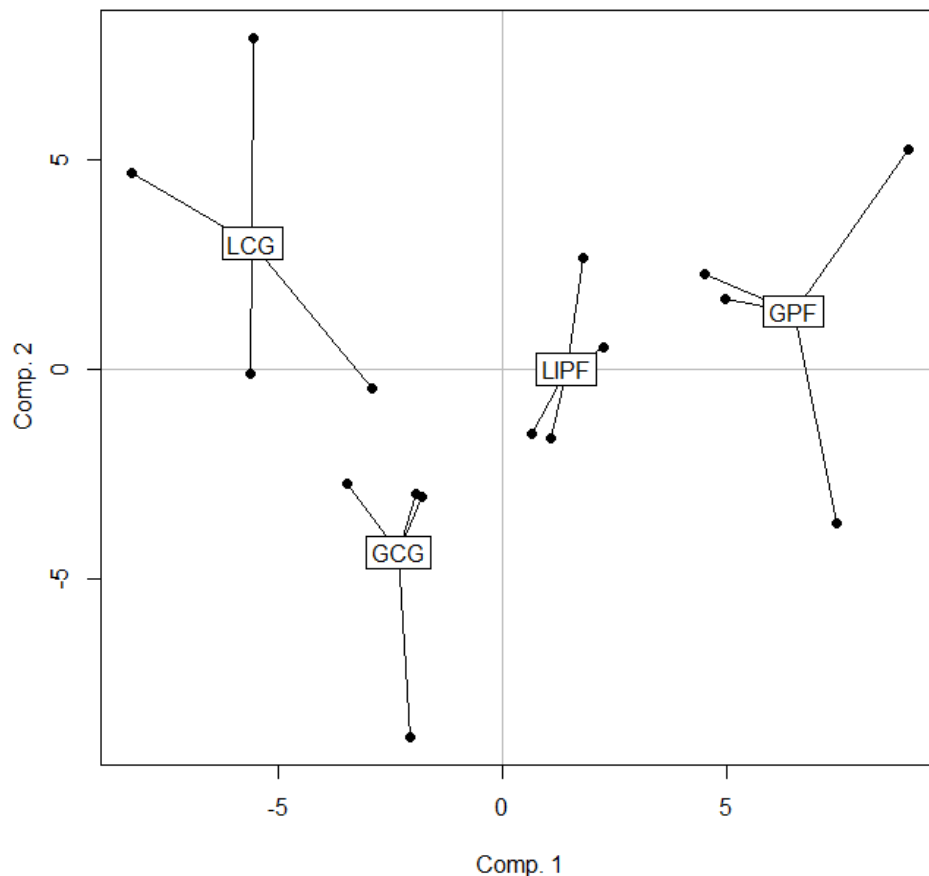
190

191 **Fig.4**: Graphical representation of the four population samples on the two first axes of the PLS-DA analysis.
192 The first and second axes explained respectively 11.94 and 7.99% of the total variance among samples
193 (p=0.008).

194 C_{33} (*n*-tritriacontane), 2meC$_{24}$ (2methyltetracosane) and C_{29} (*n*-nonacosane) appeared as the most

195 influential components separating the four population samples (Fig.5).

13
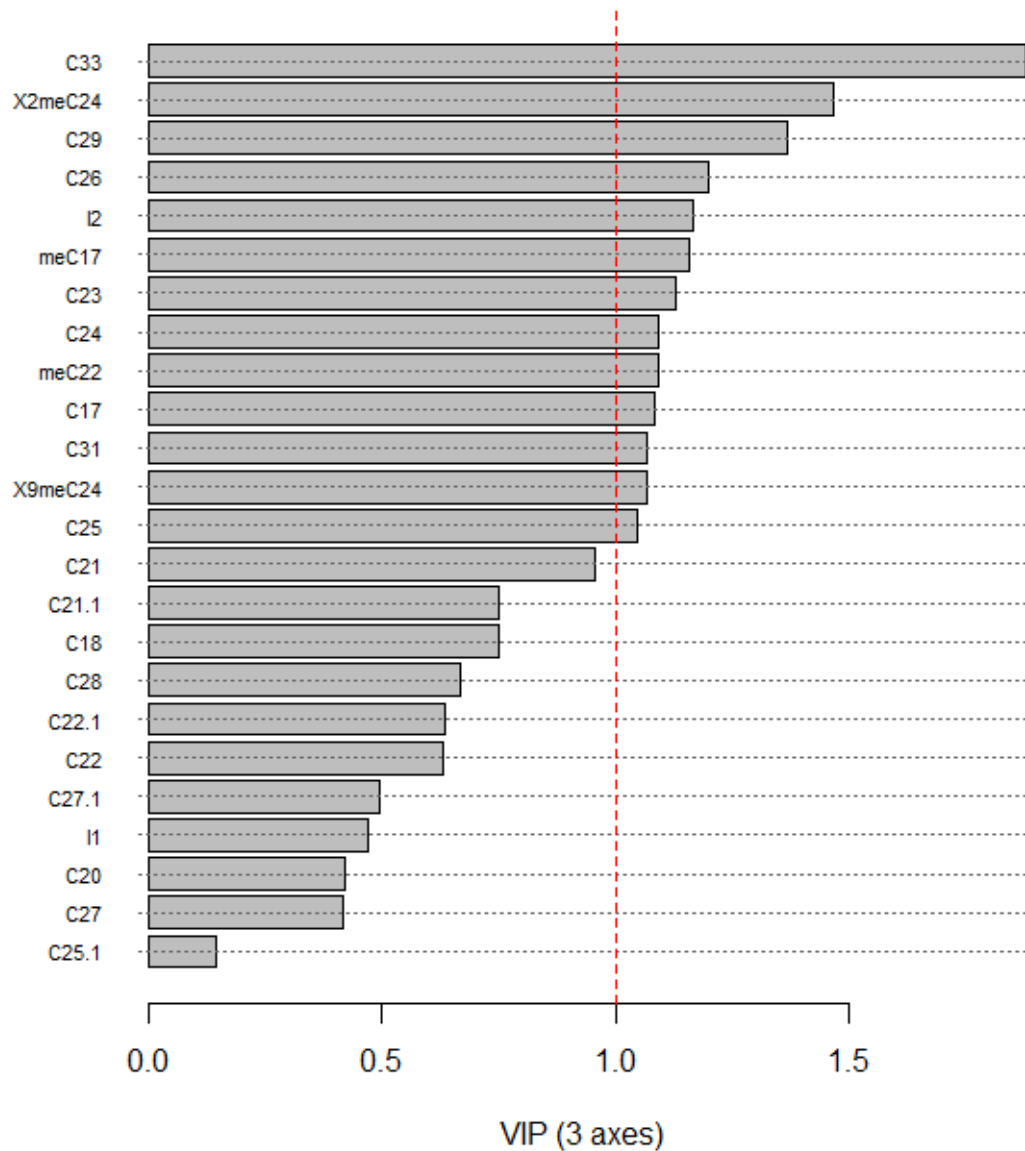
Fig.5: VIP classification showing the influence of CH in the discrimination of the four population samples. VIP values larger than 1 (bar goes beyond the dashed red line) are most influential.

Host types

The cross-validation test for host type revealed that 75% of the tick pools were assigned to the host of origin (PF, CG). The PLS-DA analysis revealed no significant difference in CHC profiles between the host types, although a tendency was found (PLS-DA: p=0.064).

14

Discussion

Cuticle composition and function

Hydrocarbons on the arthropod cuticle help prevent desiccation, but may also be involved in chemical communication, constituting essential mediators of insect behavior (Blomquist and Bagnères, 2010). Using GC-MS techniques to analyze extracts from female *I. uriae* ticks, we detected a complex mixture of cuticular hydrocarbons containing linear and monomethyl-alkanes and alkenes from C_{17} to C_{33}. The qualitative composition is similar to that reported for many other arthropods, containing predominantly linear alkanes (C_{23}, C_{25}, C_{27}, C_{29} and C_{31}) (Howard and Blomquist, 2005; Lockey, 1988). A majority of the detected cuticular hydrocarbons were already reported in other hard ticks: *I. persulcatus* (Tkachev et al., 2000), *Amblyomma variegatum* (Estrada-Peña et al., 1994b) and *Rhipicephalus* spp. (Estrada-Peña et al., 1992a). Nevertheless, alkenes were only detected once in low quantities in *Rhipicephalus* spp. (Estrada-Peña et al., 1992a). Here, we report the presence of alkenes as heneicosene, pentacosene, heptacosene and a high quantity of docosene, particularly in one of the CG samples. Alkenes were demonstrated to act as sex pheromones in the Alfalfa leaf-cutter bee *Megachile rotundata* and the rove beetle *Aleochara curtula* (Paulmier et al., 1999; Peschke and Metzler, 1987). Heneicosene is also described as an aggregation pheromone in drosophila species (Bartelt et al., 1988; Bartelt and Jackson, 1984). Pentacosene is implicated in the mating process of different fly species as stimulant pheromones (Uebel et al., 1978). The presence of these alkenes in *I. uriae* likely corresponds to the biological state of the female ticks when they were collected, as it was during the active reproduction season. This suggests a possible role for these molecules in tick reproduction.

We found also large amount of long-chain hydrocarbons in PF samples (*n*-nonacosane C_{29}, *n*-hentriacontane C_{31} and *n*-tritriacontane C_{33}). Mixed with other compounds, *n*-tritriacontane was

227   demonstrated to induce copulation in males of the stable fly *Stomoxys calcitrans* (Uebel et al.,

228   1975). Other long chain hydrocarbons, including C_{23} to C_{31}, produced in large quantities and acting

229   in combination, were found to serve in colony recognition by bumblebees *Bombus terrestris* (Rottler

230   et al., 2013). Moreover, $2MeC_{24}$, only detected in one PF CHC profile, has been shown to serve as

231   contact pheromone in peach twig borers *Anarsia lineatella* (Schlamp, 2005). As puffin burrows are

232   deep, densely distributed and interconnected (Harris and Wanless, 2011), the large production of

233   these cuticular hydrocarbons in *I. uriae* may enable ticks to find their way in the host nesting

234   environment. The use of this particular blend of CHC may also help ticks to find individuals that

235   smell similar, favoring assortative mating (van Zweden and d'Ettorre, 2010). This type of pheromone

236   may not be necessary for CG ticks because guillemots breed in extremely dense numbers on cliff

237   ledges with no constructed nest; ticks tend to aggregate under and around rocks on the cliff ledge

238   such that finding a mate from the same host type may be easier than in the case of puffin hosts.

239   The presence of complex compounds in the CHC pattern of *I. uriae* highlight that chemical

240   communication may be important in this tick species, enabling host-adapted ticks to find a suitable

241   host and a mating partner (Sonenshine and Roe, 2014).

242   **Site or host-associated patterns?**

243   As expected, chemical analyses revealed that each tick population had a distinct CHC profile, but

244   that specific CHCs were also associated with different host types. In particular, C_{33}, $2MeC_{24}$ and C_{29}

245   were most frequently or exclusively detected in PF populations. The quantitative variability among

246   detected hydrocarbons could be related to different factors. First, aging and development have

247   been demonstrated to impact cuticular hydrocarbon patterns in different taxa (Desena et al., 1999;

248   Ichinose and Lenoir, 2009). For example, aging favors the production of longer hydrocarbon chains

249   and decreased attractiveness in *Drosophila melanogaster* (Kuo et al., 2012). The high quantities of

16

250 long-chain hydrocarbons (C_{27}, C_{29}, C_{31}), particularly observed in the PF samples, could be a

251 consequence of tick age variability between samples. Samples used in the present study were

252 collected in the field and the relative age of the specimens could not be determined. However, as

253 tick activity is synchronous with seabird breeding, we did not expect the overall timing in the adult

254 female activity to differ in a systematic way between ticks exploiting the different host species, nor

255 among distinct colony locations within Iceland.

256 Second, climatic conditions can also shape CHC composition. Differences in cuticular hydrocarbon

257 composition were observed in geographically distant tick populations of *Amblyomma variegatum*

258 (Estrada-Peña et al., 1994) and some extreme climatic parameters were shown to be correlated

259 with methyled-alkanes in *Rhipicephalus sanguineus*, highlighting that variation of these compounds

260 is potentially linked to adaptation to environmental temperatures (Estrada-Peña, 1993). The off-

261 host environment (rock or burrow) of *I. uriae* may display significant variation in terms of

262 temperature and relative humidity due to differential exposure to climatic factors such as sun, rain

263 and/or snow (Buckley and Buckley, 1980). These selective factors can impact tick survival and could

264 lead to the quantitative variation in the cuticular components observed in this study. Howard et al

265 (1978) argued that the effectiveness of CHC in prevention of desiccation is dependent on the

266 quantity of CHC and that saturated CHCs are important components to protect against water loss.

267 This hypothesis does not seem to explain the pattern of long chain CHC we observed in PF samples;

268 the micro-habitat used by PF ticks, i.e. deep burrows, is expected to be more stable in terms of

269 temperature and humidity than more exposed areas used by CG ticks.

270 Third, although the production of CHC is genetically controlled, studies have revealed the

271 importance of the environment in the acquisition of new hydrocarbons (D'ettorre et al., 2006;

272 D'Ettorre et al., 2002). Indeed, Singer and Espelie (1996) showed that the social wasp *Polistes*

17

273   *metricus* Say (1831) is only recognized as a nest mate by siblings if exposed to nest surface

274   hydrocarbons after hatching. Likewise, in the leaf-cutting ant *Acromyrmex octospinosus*, it was

275   demonstrated that individuals foraging on different host plants are aggressive towards each other

276   (Jutsum et al., 1979). The authors proposed that the mediation of inter-colony interactions have

277   evolved by the acquisition of colony-specific odor components. The *I. uriae* specimens used in the

278   present study were collected under rocks in the middle of the guillemot colony or within individual

279   puffin burrows. Living in these different substrates could lead to differences in cuticle composition

280   based on environmental acquisition.

281   Fourth, diet also appears to be an important factor shaping the cuticular hydrocarbon profile. In the

282   Argentine ant *Linepithema humile*, colonies eating different prey items present particular

283   hydrocarbon profiles that include components coming from the prey (Liang and Silverman, 2000). In

284   the same way, Geiselhardt et al. (2012) showed that males of the phytophagous mustard leaf beetle

285   *Phaedon cochleariae* preferred to mate with females reared on the same host plant compared to

286   females from a different host plant, even though they originated from the same laboratory stock

287   population. This phenomenon appears to be due to divergent, host-specific cuticular hydrocarbon

288   profiles. In ticks, it was demonstrated that the cuticular fatty acid profiles of *Rhipicephalus* spp.

289   presented significant differences in fatty acid abundance according to host use (Shimshoni et al.,

290   2013). Here, the blood composition of the two different hosts of *I. uriae* could result in the

291   acquisition of specific hydrocarbon mixtures.

292   In general, making inferences on environmental versus host effects will require the examination of

293   ticks from additional sites and host types. However, regardless of the origin of host-associated

294   differences in CHC profiles of *I. uriae*, what is important to now determine is whether these

295   differences reinforce assortative mating patterns, favouring the divergence of sympatric

18

296 populations and the rapid formation of host races. Future analyses should focus on the

297 characterization and isolation of the main components of the cuticular mixture from ticks of each

298 seabird host type to test for their biological activity and potential role in tick behavior.

299

300

Acknowledgements

305

Funding

310

Conflict of interest disclosure

312 The authors declare they have no conflict of interest relating to the content of this article.

313

314

315

316

Supplementary data

Table S1: Relative abundance of the CHC extracts of each population replicate's after log centered ratio transformation (also available on Zenodo at https://doi.org/10.5281/zenodo.5889077).

320

	C17	meC17	C18	C20	C21.1	C21
CHC_GPF1	1,258262	-1,675478	-1,225611	-1,358771	-0,456587	-1,093444
CHC_GPF2	-0,983004	-0,983004	-0,783199	-1,164937	-1,056892	-0,184697
CHC_GPF3	4,151361	3,580785	4,724183	4,617548	3,807260	3,540713
CHC_GPF4	3,597384	3,540178	2,810370	-5,405227	2,969631	3,222111
CHC_LIPF1	1,518413	-6,017122	1,043470	-1,007387	0,183513	-1,120153
CHC_LIPF2	0,687396	-0,588309	-0,053014	-0,504584	0,166784	-1,747215
CHC_LIPF3	2,953938	1,854215	2,570707	-4,252033	-4,252033	2,319610
CHC_LIPF4	3,334867	2,899295	1,532183	2,736876	-4,685930	2,599998
CHC_GCG1	4,385533	1,306893	1,157818	0,389237	1,944246	0,212800
CHC_GCG2	1,730616	1,703967	1,991403	1,042497	2,791342	0,217889
CHC_GCG3	5,149559	5,171603	4,695299	-4,141914	-4,141914	-4,141914
CHC_GCG4	6,607656	6,507890	-2,764376	-2,764376	-2,764376	6,667177
CHC_LCG1	4,213065	2,588977	1,956074	2,221551	-5,462891	1,390069
CHC_LCG2	1,933896	1,561455	1,352674	1,168282	2,599910	1,493046
CHC_LCG3	5,173815	-3,295400	-3,295400	-3,295400	-3,295400	5,004318
CHC_LCG4	-2,395630	-2,395630	-2,395630	-2,395630	-2,395630	6,592884

	C22.1	C22	meC22	C23	C24	X9meC24
CHC_GPF1	1,50623611	-0,50555381	-1,70555949	0,12096462	-0,42768644	-1,2206834
CHC_GPF2	1,18425441	-0,49071836	-0,98066082	0,94030298	-0,28189781	-1,02914483
CHC_GPF3	4,32918786	4,60171239	4,02667532	-4,77602802	-4,77602802	-4,77602802
CHC_GPF4	2,85991037	2,24131129	3,95590755	3,57761114	-5,4052267	2,55126443
CHC_LIPF1	2,6847376	0,88062159	-6,01712163	0,22189045	0,56501804	-0,13693536
CHC_LIPF2	2,96343585	0,37650416	-5,92683159	0,07138213	0,62140857	-0,18069582
CHC_LIPF3	-4,25203345	-4,25203345	-4,25203345	2,48114139	2,91217821	-4,25203345
CHC_LIPF4	-4,68592953	2,30293014	-4,68592953	2,17284599	2,95775455	-4,68592953
CHC_GCG1	4,72183226	2,16796305	-5,97456355	0,50141786	1,61476737	1,69619514
CHC_GCG2	4,63765818	1,96470376	-6,27678757	0,9386865	1,89458577	1,80575829
CHC_GCG3	-4,14191373	-4,14191373	4,50899999	4,55481651	5,23921344	4,3019409
CHC_GCG4	-2,76437623	-2,76437623	-2,76437623	6,56677188	-2,76437623	-2,76437623

20

CHC_LCG1	5,14840786	2,88187094	-5,46289066	2,15974193	2,64600173	1,30457536
CHC_LCG2	5,19919581	2,44288367	-5,83634374	0,96429749	1,55031557	1,00827119
CHC_LCG3	-3,2954001	-3,2954001	-3,2954001	4,10398495	5,06658076	-3,2954001
CHC_LCG4	-2,39563008	-2,39563008	-2,39563008	6,38876341	-2,39563008	-2,39563008

	X2meC24	C25.1	C25	I1	C26	I2
CHC_GPF1	-1,49255919	0,710483	1,09996476	0,56276083	-0,17627985	0,07387322
CHC_GPF2	-0,85129625	0,41550683	1,72274199	0,15326967	-0,25663413	-0,00255114
CHC_GPF3	3,04100103	-4,77602802	-4,77602802	3,46602618	3,89103385	-4,77602802
CHC_GPF4	2,74237448	-5,4052267	4,27702093	3,00740146	-5,4052267	3,29174679
CHC_LIPF1	-6,01712163	-0,1488945	1,29293382	1,12637653	0,69030705	-6,01712163
CHC_LIPF2	-5,92683159	-0,64688596	1,31627079	0,90987066	0,41085836	-5,92683159
CHC_LIPF3	-4,25203345	-4,25203345	4,71776728	3,3029661	2,56104558	-4,25203345
CHC_LIPF4	-4,68592953	-4,68592953	4,6771672	-4,68592953	2,68595365	-4,68592953
CHC_GCG1	-5,97456355	-0,42396916	1,22349549	1,72540467	1,38243536	-1,39612756
CHC_GCG2	-6,27678757	-0,2065063	1,72213495	1,06728457	1,33585569	-0,21747187
CHC_GCG3	-4,14191373	-4,14191373	5,71433159	-4,14191373	4,75085336	-4,14191373
CHC_GCG4	-2,76437623	-2,76437623	7,51121475	7,01077959	-2,76437623	-2,76437623
CHC_LCG1	-5,46289066	-5,46289066	3,01341895	2,82230157	2,69452068	-5,46289066
CHC_LCG2	-5,83634374	2,0586822	1,72477962	2,25712355	1,39064679	-5,83634374
CHC_LCG3	-3,2954001	-3,2954001	7,60591058	5,62063417	5,2526945	-3,2954001
CHC_LCG4	-2,39563008	-2,39563008	7,96176069	-2,39563008	7,19765085	-2,39563008

	C27.1	C27	C28	C29	C31	C33
CHC_GPF1	-0,00016215	1,30711449	0,33827026	2,04171998	1,59307887	0,72564782
CHC_GPF2	-0,19069859	1,69885837	-0,1473274	1,99452145	1,07445349	0,20275287
CHC_GPF3	-4,77602802	-4,77602802	4,75882151	-4,77602802	-4,77602802	-4,77602802
CHC_GPF4	4,00281861	-5,4052267	-5,4052267	-5,4052267	-5,4052267	-5,4052267
CHC_LIPF1	1,02468304	2,57346606	1,2522136	4,63157554	4,23145935	2,56117937
CHC_LIPF2	-1,29244538	2,91653471	0,74631061	4,80493688	4,29222175	2,5097294
CHC_LIPF3	4,42572948	-4,25203345	-4,25203345	6,29373406	6,22187233	4,15746354
CHC_LIPF4	3,97386234	-4,68592953	-4,68592953	5,79395404	5,57504998	3,61655868
CHC_GCG1	-2,31093524	1,17815423	0,96870906	1,45238521	-5,97456355	-5,97456355
CHC_GCG2	-2,63802842	1,37672655	1,18012447	0,76792459	-6,27678757	-6,27678757
CHC_GCG3	4,78876251	-4,14191373	-4,14191373	-4,14191373	4,9694991	-4,14191373
CHC_GCG4	6,12290594	-2,76437623	-2,76437623	-2,76437623	-2,76437623	-2,76437623
CHC_LCG1	-5,46289066	3,03426816	2,96262862	2,66565265	-5,46289066	-5,46289066
CHC_LCG2	-5,83634374	1,89920237	2,48119674	1,9322039	-5,83634374	-5,83634374
CHC_LCG3	6,05677419	-3,2954001	-3,2954001	-3,2954001	5,54628953	-3,2954001
CHC_LCG4	7,47336812	-2,39563008	-2,39563008	-2,39563008	7,50691468	-2,39563008

321

322

323

References

Andersen, S.O., 1979. Biochemistry of Insect Cuticle. Annu. Rev. Entomol. 24, 29–59. https://doi.org/10.1146/annurev.en.24.010179.000333

Bagneres, A.-G., Killian, A., Clement, J.-L., Lange, C., 1991. Interspecific recognition among termites of the genusReticulitermes: Evidence for a role for the cuticular hydrocarbons. J. Chem. Ecol. 17, 2397–2420. https://doi.org/10.1007/BF00994590

Bartelt, R.J., Armold, M.T., Schaner, A.M., Jackson, L.L., 1986. Comparative analysis of cuticular hydrocarbons in the Drosophila virilis species group. Comp. Biochem. Physiol. Part B Comp. Biochem. 83, 731–742. https://doi.org/10.1016/0305-0491(86)90138-0

Bartelt, R.J., Jackson, L.L., 1984. Hydrocarbon Component of the Drosophila virilis (Diptera: Drosophilidae) Aggregation Pheromone: (Z)-10-Heneicosene. Ann. Entomol. Soc. Am. 77, 364–371. https://doi.org/10.1093/aesa/77.4.364

Bartelt, R.J., Schaner, A.M., Jackson, L.L., 1988. Aggregation pheromones inDrosophila borealis andDrosophila littoralis. J. Chem. Ecol. 14, 1319–1327.

Blomquist, G.J., Bagnères, A.-G., 2010. Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology. Cambridge University Press.

Buckley, F.G., Buckley, P.A., 1980. Habitat Selection and Marine Birds, in: Behavior of Marine Animals. Springer, Boston, MA, pp. 69–112. https://doi.org/10.1007/978-1-4684-2988-6_3

Desena, M.L., Edman, J.D., Clark, J.M., Symington, S.B., Scott, T.W., 1999. Aedes aegypti (Diptera: Culicidae) Age Determination by Cuticular Hydrocarbon Analysis of Female Legs. J. Med. Entomol. 36, 824–830. https://doi.org/10.1093/jmedent/36.6.824

D'Ettorre, P., Mondy, N., Lenoir, A., Errard, C., 2002. Blending in with the crowd: social parasites integrate into their host colonies using a flexible chemical signature. Proc. R. Soc. Lond. B Biol. Sci. 269, 1911–1918. https://doi.org/10.1098/rspb.2002.2110

D'ettorre, P., Wenseleers, T., Dawson, J., Hutchinson, S., Boswell, T., Ratnieks, F.L.W., 2006. Wax combs mediate nestmate recognition by guard honeybees. Anim. Behav. 71, 773–779. https://doi.org/10.1016/j.anbehav.2005.05.014

Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick Ixodes uriae. Biol. J. Linn. Soc. 108, 323–334. https://doi.org/10.1111/j.1095-8312.2012.02021.x

Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. Vector-Borne Zoonotic Dis. 11, 453–470. https://doi.org/10.1089/vbz.2010.0009

Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? J. Anim. Ecol. 83, 576–587. https://doi.org/10.1111/1365-2656.12170

Estrada-Peña, A., 1993. Climate and cuticular hydrocarbon variation inRhipicephalus sanguineus ticks (Acari: Ixodidae). Parasitol. Res. 79, 512–516. https://doi.org/10.1007/BF00931594

Estrada-Peña, A., CASTELLÁ, J., MOREL, P.C., 1994. Cuticular Hydrocarbon Composition, Phenotypic Variability, and Geographic Relationships in Allopatric Populations of Amblyomma variegatum (Acari: Ixodidae) from Africa and the Caribbean. J. Med. Entomol. 31, 534–544.

Estrada-Peña, A., Estrada-Peña, R., Peiró, J.M., Estrada-Pena, A., Estrada-Pena, R., Peiro, J.M., 1992. Differentiation of Rhipicephalus Ticks (Acari: Ixodidae) by Gas Chromatography of Cuticular Hydrocarbons. J. Parasitol. 78, 982. https://doi.org/10.2307/3283217

368 Estrada-Peña, A., Gray, J.S., Kahl, O., 1996. Variability in cuticular hydrocarbons and phenotypic
369   discrimination of Ixodes ricinus populations (Acarina: Ixodidae) from Europe. Exp. Appl. Acarol. 20,
370   457–466. https://doi.org/10.1007/BF00053309
371 Estrada-Peña, A., Guglielmone, A.A., Mangold, A.J., Castellá, J., 1993. Patterns of cuticular hydrocarbon
372   variation and genetic similarity between natural populations of Amblyomma cajennense (Acari:
373   Ixodidae). Acta Trop. 55, 61–78. https://doi.org/10.1016/0001-706X(93)90049-H
374 Filshie, B.K., 1982. Fine Structure of the Cuticle of Insects and Other Arthropods, in: Insect Ultrastructure.
375   Springer, Boston, MA, pp. 281–312. https://doi.org/10.1007/978-1-4615-7266-4_10
376 Geiselhardt, S., Otte, T., Hilker, M., 2012. Looking for a similar partner: host plants shape mating preferences
377   of herbivorous insects by altering their contact pheromones. Ecol. Lett. 15, 971–977.
378   https://doi.org/10.1111/j.1461-0248.2012.01816.x
379 Gibbs, A., Mousseau, T.A., Crowe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of
380   insect cuticle lipids. Proc. Natl. Acad. Sci. 88, 7257–7260. https://doi.org/10.1073/pnas.88.16.7257
381 Harris, M.P., Wanless, S., 2011. The Puffin. Bloomsbury Publishing.
382 Hervé, M., 2014. Aide-mémoire de statistique appliquée à la biologie. Constr Son Étude Anal Résultats À Aide
383   Logiciel R Version 5.
384 Howard, R.W., Blomquist, G.J., 2005. Ecological, Behavioral, and Biochemical Aspects of Insect Hydrocarbons.
385   Annu. Rev. Entomol. 50, 371–393. https://doi.org/10.1146/annurev.ento.50.071803.130359
386 Howard, R.W., McDaniel, C.A., Blomquist, G.J., 1978. Cuticular hydrocarbons of the eastern subterranean
387   termite,Reticulitermes flavipes (Kollar) (Isoptera: Rhinotermitidae). J. Chem. Ecol. 4, 233–245.
388   https://doi.org/10.1007/BF00988058
389 Hunt, L.M., 1986. Differentiation between three species of Amblyomma ticks (Acari: Ixodidae) by analysis of
390   cuticular hydrocarbons. Ann. Trop. Med. Parasitol. 80, 245–249.
391 Ichinose, K., Lenoir, A., 2009. Ontogeny of hydrocarbon profiles in the ant Aphaenogaster senilis and effects
392   of social isolation. C. R. Biol. 332, 697–703. https://doi.org/10.1016/j.crvi.2009.04.002
393 Jallon, J.-M., David, J.R., 1987. Variation in Cuticular Hydrocarbons Among the Eight Species of the Drosophila
394   melanogaster Subgroup. Evolution 41, 294–302. https://doi.org/10.2307/2409139
395 Jutsum, A.R., Saunders, T.S., Cherrett, J.M., 1979. Intraspecific aggression is the leaf-cutting ant Acromyrmex
396   octospinosus. Anim. Behav. 27, 839–844. https://doi.org/10.1016/0003-3472(79)90021-6
397 KRUGER, E.L., PAPPAS, C.D., HOWARD, R.W., 1991. Cuticular Hydrocarbon Geographic Variation Among
398   Seven North American Populations of Aedes albopictus (Diptera: Culicidae). J. Med. Entomol. 28,
399   859–864.
400 Kuo, T.-H., Yew, J.Y., Fedina, T.Y., Dreisewerd, K., Dierick, H.A., Pletcher, S.D., 2012. Aging modulates cuticular
401   hydrocarbons and sexual attractiveness in Drosophila melanogaster. J. Exp. Biol. 215, 814–821.
402   https://doi.org/10.1242/jeb.064980
403 Liang, D., Silverman, J., 2000. "You are what you eat": Diet modifies cuticular hydrocarbons and nestmate
404   recognition in the Argentine ant, Linepithema humile. Naturwissenschaften 87, 412–416.
405   https://doi.org/10.1007/s001140050752
406 Lockey, K.H., 1988. Lipids of the insect cuticle: origin, composition and function. Comp. Biochem. Physiol.
407   Part B Comp. Biochem. 89, 595–645.
408 McCoy, K.D., Boulanger, N. (Eds.), 2015. Tiques et maladies à tiques : biologie, écologie évolutive,
409   épidémiologie, Didactiques. IRD, Marseille.
410 McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic
411   evidence of sympatric host races in the seabird tick Ixodes uriae. J. Evol. Biol. 14, 395–405.
412   https://doi.org/10.1046/j.1420-9101.2001.00290.x
413 McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M.,
414   2005. Recurrent evolution of host-specialized races in a globally distributed parasite. Proc. R. Soc. B
415   Biol. Sci. 272, 2389–2395. https://doi.org/10.1098/rspb.2005.3230
416 McCoy, K.D., Léger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases:
417   a review. Front. Cell. Infect. Microbiol. 3. https://doi.org/10.3389/fcimb.2013.00057

418 McCoy, K.D., Tirard, C., Michalakis, Y., 2003. Spatial genetic structure of the ectoparasite Ixodes uriae within
419   breeding cliffs of its colonial seabird host. Heredity 91, 422–429.
420   https://doi.org/10.1038/sj.hdy.6800339

421 Paulmier, I., Bagnères, A.-G., Afonso, C.M., Dusticier, G., Rivière, G., Clément, J.-L., 1999. Alkenes as a sexual
422   pheromone in the alfalfa leaf-cutter bee Megachile rotundata. J. Chem. Ecol. 25, 471–490.

423 Peschke, K., Metzler, M., 1987. Cuticular hydrocarbons and female sex pheromones of the rove beetle,
424   Aleochara curtula (Goeze) (Coleoptera:Staphylinidae). Insect Biochem. 17, 167–178.
425   https://doi.org/10.1016/0020-1790(87)90157-0

426 Randolph, S.E., 1998. Ticks are not Insects: Consequences of Contrasting Vector Biology for Transmission
427   Potential. Parasitol. Today 14, 186–192. https://doi.org/10.1016/S0169-4758(98)01224-1

428 Randolph, S.E., Storey, K., 1999. Impact of Microclimate on Immature Tick-Rodent Host Interactions (Acari:
429   Ixodidae): Implications for Parasite Transmission. J Med Entomol 36, 741–748.
430   https://doi.org/10.1093/jmedent/36.6.741

431 Rottler, A.-M., Schulz, S., Ayasse, M., 2013. Wax Lipids Signal Nest Identity in Bumblebee Colonies. J. Chem.
432   Ecol. 39, 67–75. https://doi.org/10.1007/s10886-012-0229-0

433 Schlamp, K.K., 2005. Contact pheromone components and diel periodicity of sexual communication in peach
434   twig borers, Anarsia lineatella (Lepidoptera: Gelechiidae) (PhD Thesis). Biological Sciences
435   Department-Simon Fraser University.

436 Schöni, R., Hess, E., Blum, W., Ramstein, K., 1984. The aggregation-attachment pheromone of the tropical
437   bont tick Amblyomma variegatum Fabricius (Acari, Ixodidae): Isolation, identification and action of its
438   components. J. Insect Physiol. 30, 613–618. https://doi.org/10.1016/0022-1910(84)90045-3

439 Shimshoni, J.A., Erster, O., Rot, A., Cuneah, O., Soback, S., Shkap, V., 2013. Cuticular fatty acid profile analysis
440   of three Rhipicephalus tick species (Acari: Ixodidae). Exp. Appl. Acarol. 61, 481–489.
441   https://doi.org/10.1007/s10493-013-9713-7

442 Simmons, L.W., Thomas, M.L., Gray, B., Zuk, M., 2014. Replicated evolutionary divergence in the cuticular
443   hydrocarbon profile of male crickets associated with the loss of song in the Hawaiian archipelago. J.
444   Evol. Biol. n/a-n/a. https://doi.org/10.1111/jeb.12478

445 Singer, T.L., Espelie, K.E., 1996. Nest surface hydrocarbons facilitate nestmate recognition for the social
446   wasp,Polistes metricus Say (Hymenoptera: Vespidae). J. Insect Behav. 9, 857–870.
447   https://doi.org/10.1007/BF02208974

448 Sonenshine, D.E., Roe, R.M. (Eds.), 2014. Biology of ticks, 2nd ed. ed. Oxford University Press, New York.

449 Tkachev, A.V., Dobrotvorsky, A.K., Vjalkov, A.I., Morozov, S.V., 2000. Chemical composition of lipophylic
450   compounds from the body surface of unfed adult Ixodes persulcatus ticks (Acari: Ixodidae). Exp. Appl.
451   Acarol. 24, 145–158. https://doi.org/10.1023/A:1006430323587

452 Uebel, E.C., Schwarz, M., Sonnet, P.E., Miller, R.W., Menzer, R.E., 1978. Evaluation of the mating stimulant
453   pheromones of Fannia canicularis, F. pusio, and F. femoralis as attractants. Fla. Entomol. 139–143.

454 Uebel, E.C., Sonnet, P.E., Bierl, B.A., Miller, R.W., 1975. Sex pheromone of the stable flyl: Isolation and
455   preliminary identification of compounds that induce mating strike behavior. J. Chem. Ecol. 1, 377–
456   385.

457 van Zweden, J.S., d'Ettorre, P., 2010. Nestmate recognition in social insects and the role of hydrocarbons.
458   Insect Hydrocarb. Biol. Biochem. Chem. Ecol. 11, 222–243.

459 Wyatt, T.D., 2010. Pheromones and signature mixtures: defining species-wide signals and variable cues for
460   identity in both invertebrates and vertebrates. J. Comp. Physiol. A 196, 685–700.
461   https://doi.org/10.1007/s00359-010-0564-y

462