# Within and among population differences in cuticular hydrocarbons in the seabird tick *Ixodes uriae*.

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# 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

34 # Introduction

35 The arthropod cuticle acts as both an exoskeleton and a barrier from the external environment
36 (Andersen, 1979). The outermost layer, the epicuticle, is covered by a lipid layer (Lockey, 1988)
37 made-up of esters, carboxylic acids, alcohols, carbonyls and long-chain hydrocarbons (Andersen,
38 1979) that protects the organism from desiccation (Filshie, 1982). However, cuticular hydrocarbons
39 (CHC) are also involved in chemical communication serving as sex pheromones, kairomones and/or
40 signature mixtures allowing recognition of social identity (van Zweden and d'Ettorre, 2010; Wyatt,
41 2010). Using analytic techniques such as gas chromatography-mass spectrometry (GC-MS) and
42 MALDI-TOF mass spectrometry, researchers have described hydrocarbons of up 70 carbons in chain
43 length in insects, principally *n*-alkanes, methyl-branched alkanes and alkenes (Blomquist and
44 Bagnères, 2010). The array of CHC on the cuticle constitute a species-specific chemical signature,
45 varying qualitatively between species and quantitatively within species (Lockey, 1988). CHC patterns
46 are genetically controlled, but the relative abundance of particular components can be linked to
47 environmental conditions (Estrada-Peña et al., 1993; Gibbs et al., 1991). CHC patterns have been
48 used as taxonomic tools to characterize hundreds of arthropod species (Howard and Blomquist,
49 2005), and to discriminate closely-related populations (Bagnères et al., 1991; Bartelt et al., 1986;
50 Jallon and David, 1987; Kruger et al., 1991; Simmons et al., 2014).

51 In ticks, hematophagous arthropods widely distributed across the globe and parasitizing a diverse
52 array of vertebrate species (McCoy and Boulanger, 2015), little work has been performed to
53 describe CHC profiles and their variation among species and populations. The only studies to date
54 have focused on relatively few species and used CHC profiles in an attempt to differentiate closely-
55 related taxa (Estrada-Peña et al., 1992, 1994, 1996; Hunt, 1986; Estrada-Peña and Dusbabek, 1993).
56 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

have yet to be specifically identified. A potential role for isolating mechanisms that lead to assortative mating based on host use have been suggested (McCoy et al., 2013).

Here, we examine the degree of cuticular hydrocarbon diversity in *I. uriae* and the factors influencing these chemical signatures. Based on current knowledge, we predicted that cuticular hydrocarbon patterns could vary in ticks 1) exploiting different host species and 2) sampled in different geographic locations. If host exploitation modifies CHC diversity and abundance, we expected that signatures in ticks from the same seabird host in different locations should be more similar than signatures in ticks from different host species in the same geographic location. To test these predictions, we collected *I. uriae* specimens in the nest material of two seabird host species, the common guillemot (CG) *Uria aalge,* and the Atlantic puffin (PF) *Fratercula arctica,* at three locations in Iceland. We then extracted cuticular hydrocarbons and analyzed them using gas chromatography-mass spectrometry (GC-MS).

# Material and methods

**Population samples**

Flat adult female ticks were collected off-host in three sites in Iceland in June 2016: guillemot ticks were collected under rocks in the middle of the colony in Langanes (66°22'07.2"N 14°38'33.0"W) and on Grimsey Island (66°32'57.3"N 17°59'31.1"W); puffin ticks were collected in burrows on 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) (Fig.1). Ticks were kept in plastic tubes before hydrocarbon extraction. Four replicates of 10 flat ticks were extracted for each host species at each site.

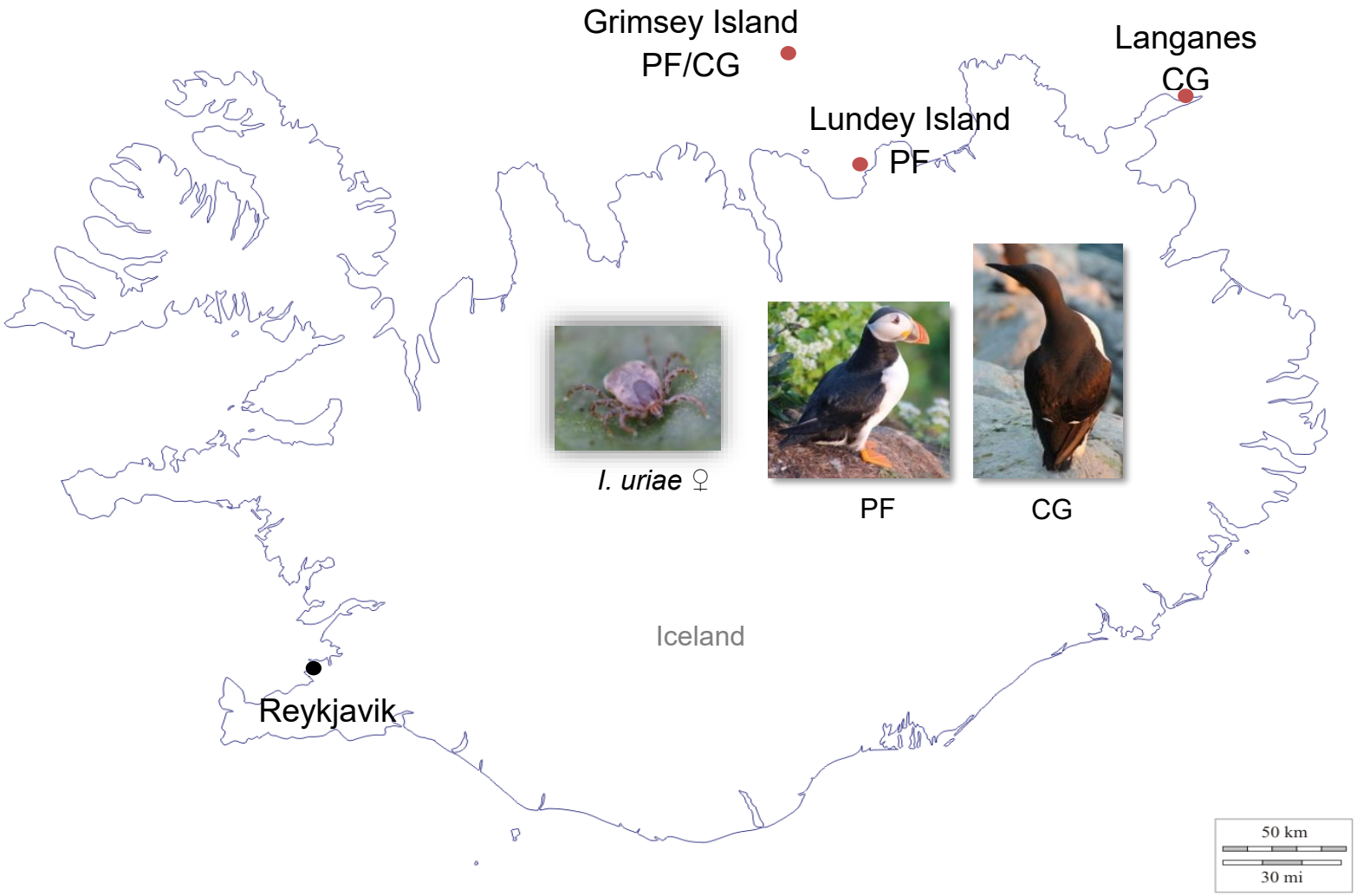



**Fig.1:** Map showing the sampling hosts and locations in Iceland. Acronyme of sampling location are Grimsey: G; Lundey Island: Li; Langanes: L. PF refers to puffin (Fratercula arctica) and CG to common guillemot (Uria aalge).

113 **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.

120 **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.

131 **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

at https://doi.org/10.5281/zenodo.5889077) and analyzed by Partial Least Squares coupled with a Discriminant analysis (PLS-DA) using the R software (v 3.4.3) and "RVAidememoire" package (Hervé, 2014) (https://cran.r-project.org/web/packages/RVAideMemoire/index.html). PLS-DA is the key analysis when the dataset contains less groups than explanatory variables, as in the case of the present quantitative dataset. PLS-DA is a supervised technique, so class memberships of the CHC need to be predefined. Here, we only used the eight first axes produced by the PLS to performed two PLS-DA tests: the first analysis took into account the four population samples as four different classes (LCG, GCG, LiPF, GPF). The second analysis was based on two classes only, representing the two host types (PF, CG). The number of significant PLS components was determined by cross model validation (2CV). We also calculated a numerical value representing the importance of the CHC variable in the projection (abbreviated VIP), i.e. VIP values larger than 1 are most influential (Hervé, 2014).

156 # Results

157 **Hydrocarbon profiles**

158 We detected a complex pattern of 22 hydrocarbons and two non-identified components in the
159 cuticle of *I. uriae* ticks. The hydrocarbon mixture was composed of: 14 *n*-alkanes ranging from 17 to
160 33 carbon atoms in length, 4 monomethyl-alkanes with 17 to 24 carbon atoms and 4 alkenes with
161 21 to 27 carbon atoms (Table 1).

162 **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 | - |

163

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

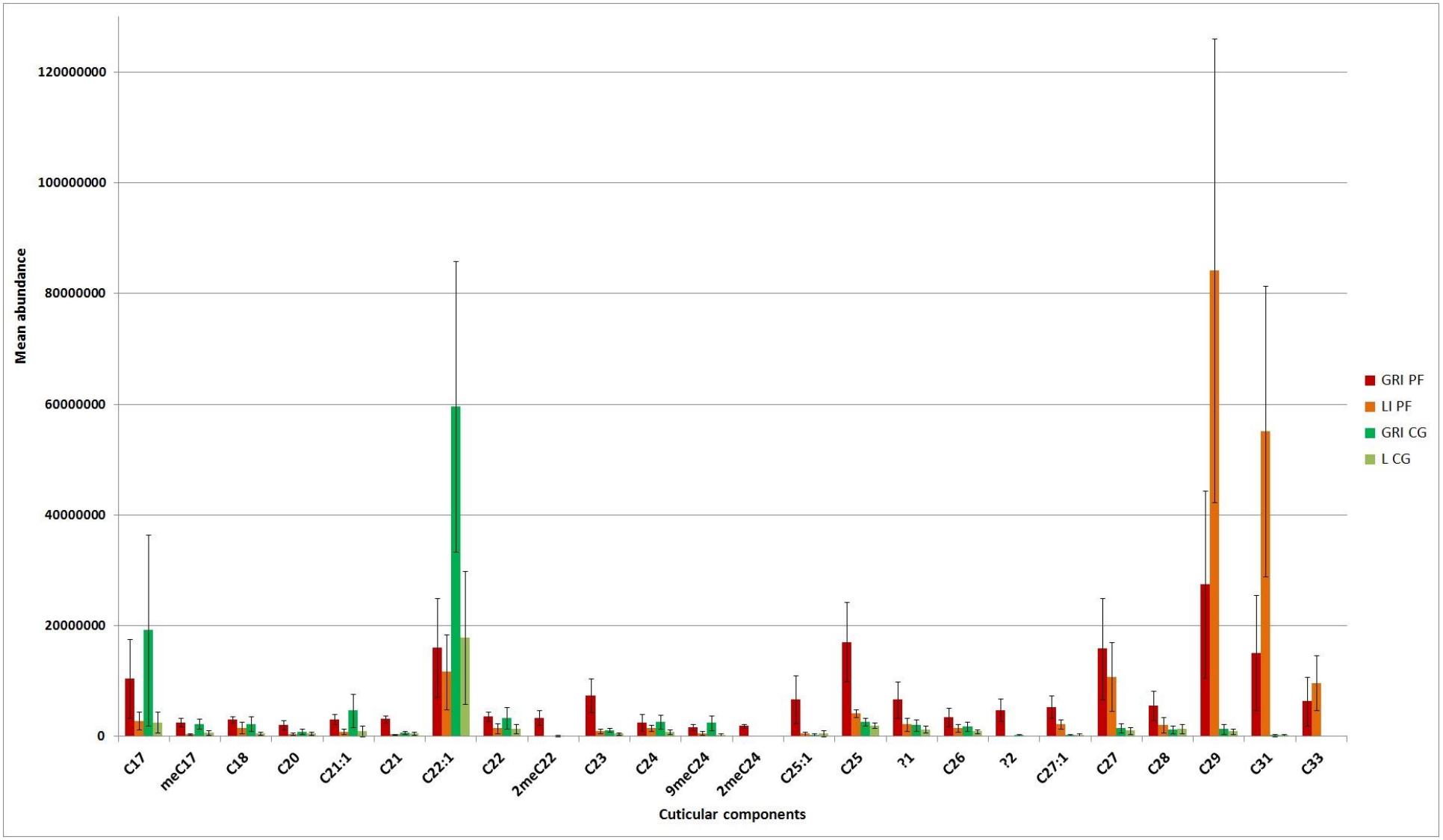



**Fig.2**: Detected cuticular hydrocarbons from *I. uriae* ticks ranged from 17 to 33 carbon atoms in length. The mean abundance was calculated based on values of each of the four replicate tick pools. Error bars represent standard deviations. See Table 1 for population abbreviations.

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).

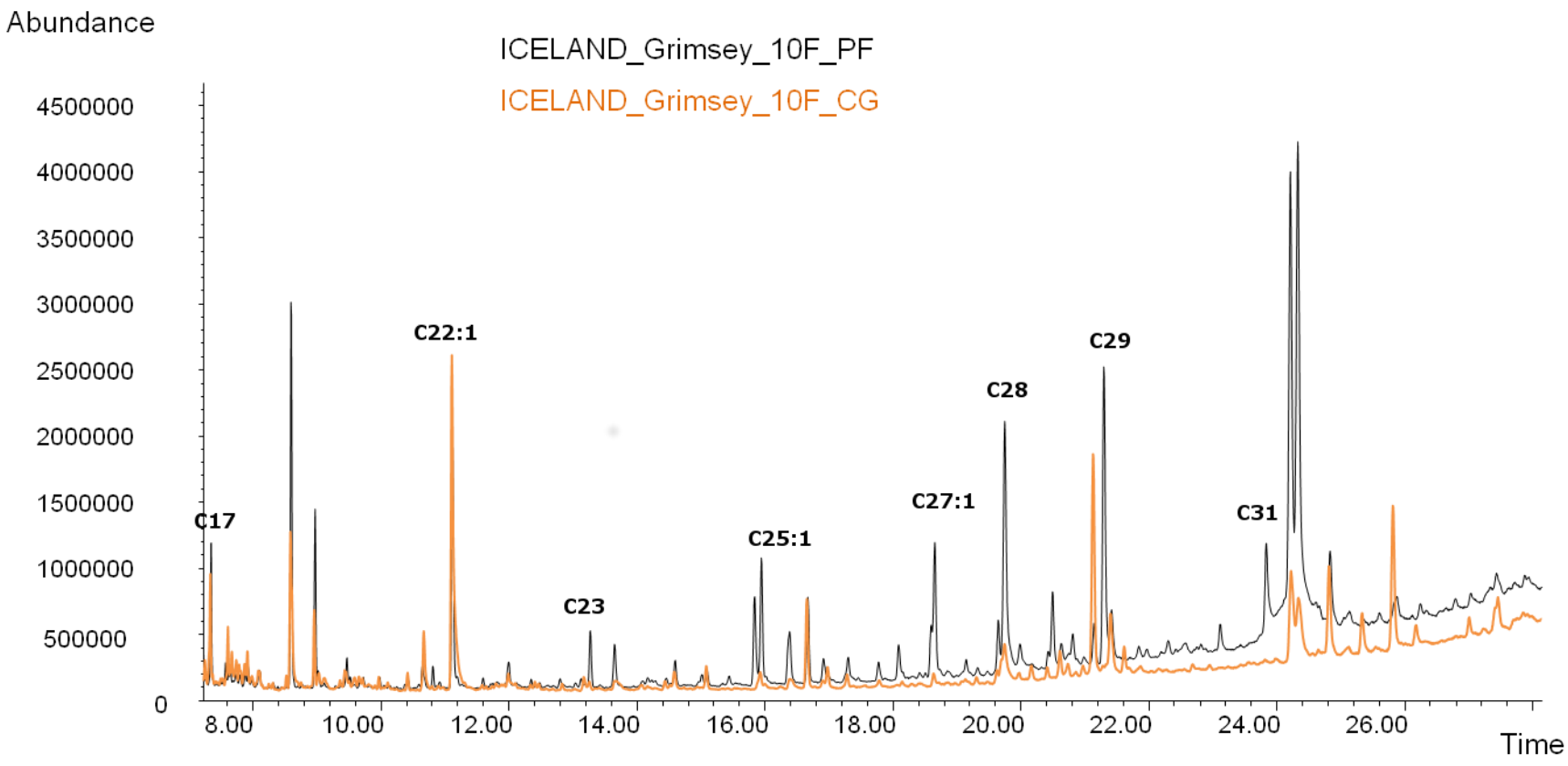
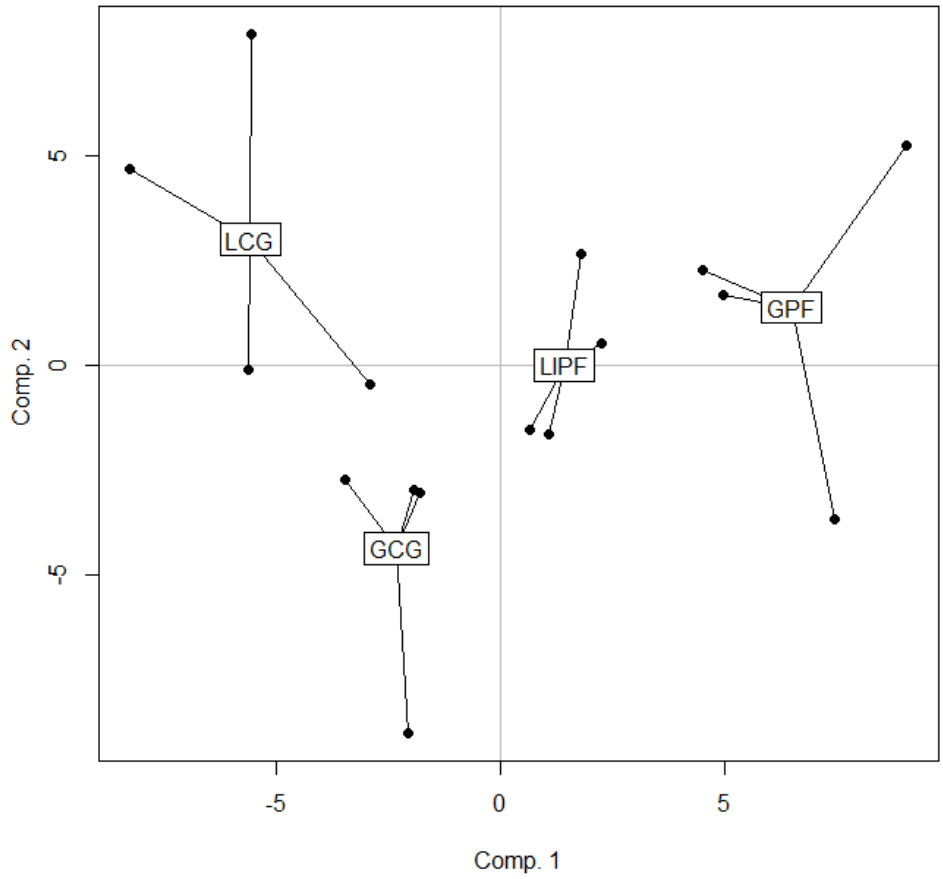



**Fig.3**: Gas-chromatograms showing the comparison of cuticular hydrocarbon profiles for pools of 10 female ticks from Atlantic puffins (PF: black line) and Common guillemots (CG: orange line) on Grimsey Island, Iceland.

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.

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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), 2me$C_{24}$ (2methyltetracosane) and $C_{29}$ (*n*-nonacosane) appeared as the most
195 influential components separating the four population samples (Fig.5).

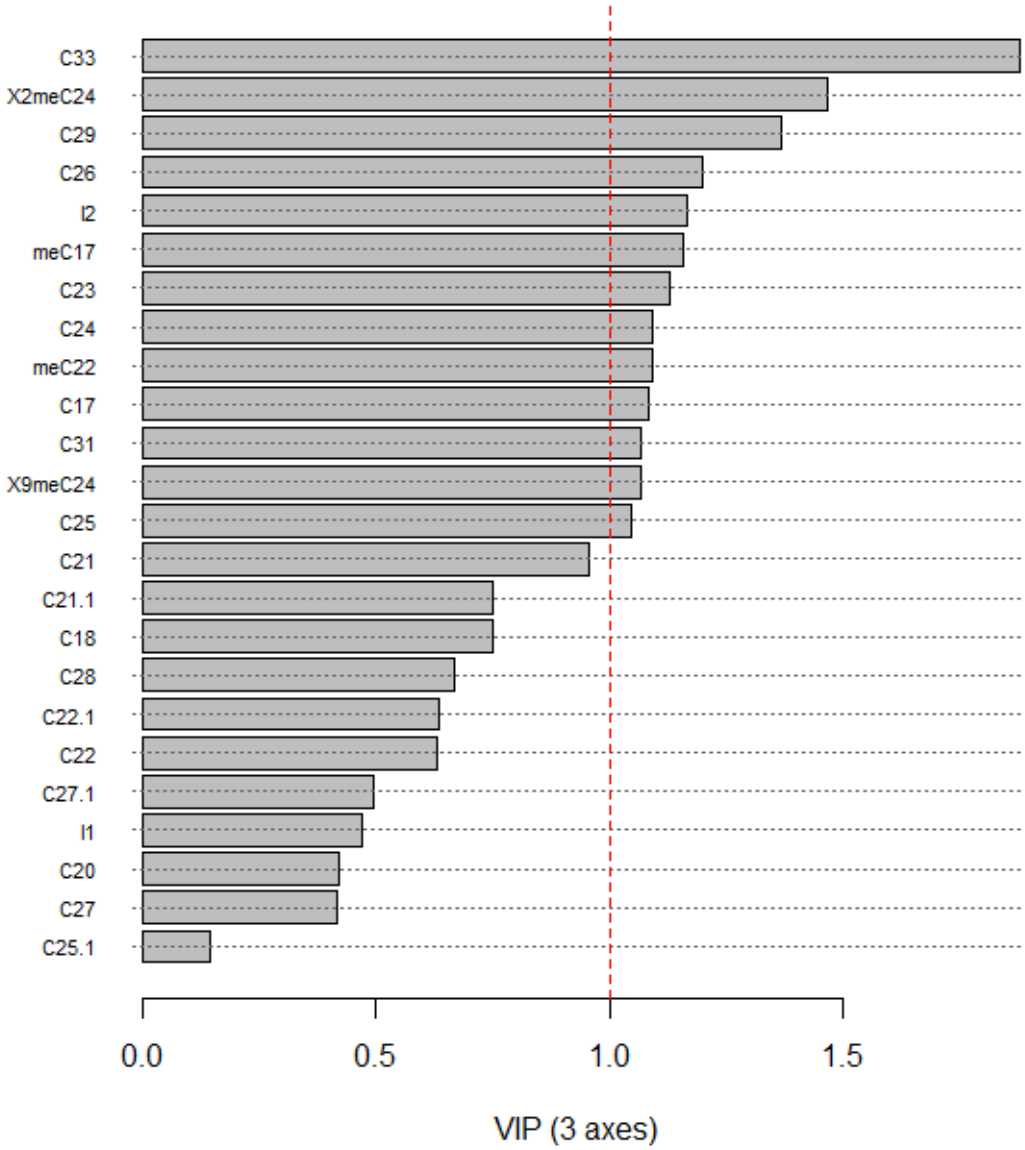


Fig.5: VIP classification showing the influence of CH in the discrimination of the four population samples. VIP values larger than 1 (bar goes beyond the dashed red line) are most influential.

*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).

204 # Discussion

205 **Cuticle composition and function**

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

225 We found also large amount of long-chain hydrocarbons in PF samples (*n*-nonacosane $C_{29}$, *n*-
226 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

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*

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

populations and the rapid formation of host races. Future analyses should focus on the characterization and isolation of the main components of the cuticular mixture from ticks of each seabird host type to test for their biological activity and potential role in tick behavior.

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## Conflict of interest disclosure

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

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317 # Supplementary data

318 **Table S1**: Relative abundance of the CHC extracts of each population replicate's after log centered
319 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 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 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 |

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