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

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,

the tick epicuticle is perforated with numerous channels providing a large surface for exchange with the external environment. As most tick species spend the major part of their life cycle in the off-host environment, maintaining water balance across this surface under different environmental conditions will directly dictate survival (Randolph and Storey, 1999); the presence of CHC likely plays an important role in this. Second, as obligate parasites, access to the vertebrate host for the bloodmeal is a key aspect of the tick life cycle and, as such, ticks have adapted key traits to locate and successfully exploit their host (McCoy and Boulanger, 2015). For example, Shimshoni et al. (2013) found *Rhipicephalus* tick species had different cuticular fatty acid compositions in relation to host use. However, whether these differences are linked to adaptive survival or by-product variation due to the host resource is unknown as yet. Finally, ticks aggregate both on hosts and in the off-host environment (Randolph, 1998). This behavior is thought to facilitate blood feeding on the host and increase survival in the off-host environment. It may also enable ticks to find appropriate mates for reproduction. Some ticks, such as the tropical bont tick *Amblyomma variegatum*, produce a multicomponent pheromone to provoke this behavior (Schöni et al., 1984) but the potential role of these pheromones in assortative mating has never been examined.

Seabird ticks are generally nidicolous, exploiting different local host species that use diverse microhabitats within the colony (Dietrich et al., 2011). Due to this diversity, these ticks may experience diverse selective pressures coming from both the hosts and from the temperature and humidity conditions of the nest micro-habitat. In particular, *Ixodes uriae*, a tick associated with seabird colonies in the polar regions of both hemispheres, is known to form host-specific races that show genetic (McCoy et al., 2001, 2003, 2005) and morphological (Dietrich et al., 2013) differences in relation to host use. Differential performance on alternative hosts has also been experimentally 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; Lundy Island: Li; Langanes: L. PF refers to puffin (*Fratercula arctica*) and CG to common guillemot (*Uria aalge*).

Hydrocarbon extraction

Cuticular compounds were extracted by immersing 10 living flat female ticks in 200 µl of pentane (HPLC grade, Sigma-Aldrich) in glass vials. The vials were agitated for 1 minute, set down to rest for 5 minutes and then re-agitated for 1 minute. Ticks were then removed from the vials and preserved in 70% ethanol. The pentane was then evaporated off over 30 minutes and the vials were closed and kept at 4°C until analyses. A negative control containing only 200 µl of pentane was added to each extraction session to control for potential contamination.

Chemical analyses

Samples were re-diluted in 40µl of pentane and 3µl were injected into an Agilent Technologies 7890A gas chromatograph (capillary column: Agilent HP-5MS, 30 m × 0.25 mm × 0.25 µm; split–splitless injector; carrying helium gas at 1 mL/min) coupled with an Agilent 5975C mass spectrometer with 70 eV electron impact ionization. The oven temperature was programmed at 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 min. Compounds were identified on the basis of their mass spectra and retention time and by comparison with standards and published spectra. The areas under the peaks were extracted using the Agilent MSD ChemStation software (E.02.01.1177). The relative amount of each hydrocarbon was calculated using peak area and the mean of the four replicates for each peak was used for the colony average.

Multivariate analyses

If the relative abundance of a CHC was 0, the value was replaced by 0.00001 which is several times smaller than the smallest quantity found for a CHC in our dataset. The data were then transformed 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).

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	<i>n</i> -heptadecane	alkane
meC ₁₇	methylheptadecane	methyl-branched alkane
C ₁₈	<i>n</i> -octadecane	alkane
C ₂₀	<i>n</i> -eicosane	alkane
C ₂₁ :1	heneicosene	alkene
C ₂₁	<i>n</i> -heneicosane	alkane
C ₂₂ :1	docosene	alkene
C ₂₂	<i>n</i> -docosane	alkane
2meC ₂₂	2methyldocosane	methyl-branched alkane
C ₂₃	<i>n</i> -tricosane	alkane
C ₂₄	<i>n</i> -tetracosane	alkane
9meC ₂₄	9methyltetracosane	methyl-branched alkane
2meC ₂₄	2methyltetracosane	methyl-branched alkane
C ₂₅ :1	pentacosene	alkene
C ₂₅	<i>n</i> -pentacosane	alkane
C ₂₆	<i>n</i> -hexacosane	alkane
C ₂₇ :1	heptacosene	alkene
C ₂₇	<i>n</i> -heptacosane	alkane
C ₂₈	<i>n</i> -octacosane	alkane
C ₂₉	<i>n</i> -nonacosane	alkane
C ₃₁	<i>n</i> -hentriacontane	alkane
C ₃₃	<i>n</i> -tritriacontane	alkane
?1	Non-identified	-
?2	Non-identified	-

All hydrocarbons were shared by the four tick populations, except 2meC₂₄ (2methyltetracosane) and C₃₃ (*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.

A high degree of variation in mean abundance of cuticular components among samples was obvious (Fig.2). Hydrocarbons $C_{22:1}$ (docosene), C_{29} (*n*-nonacosane) and C_{31} (*n*-hentriacontane) were the most predominant, whereas many hydrocarbons were detected in low quantity, as for example C_{20} (*n*-eicosane), 2me C_{22} (2methyldocosane) and C_{26} (*n*-hexacosane).

The abundance pattern of cuticular hydrocarbons varied between ticks of the two host species (Fig.3), but also among the same host in different colony sites. Nevertheless, results of the pairwise comparisons were non-significant ($p>0.2$). No CHC was specific to CG samples, although C_{17} (*n*-heptadecane) and $C_{22:1}$ (docosene) were highly abundant in the GRI colony (Fig.2 and 3). In contrast, long chain cuticular hydrocarbons tended to be present in both PF samples: C_{27} (*n*-heptacosane), C_{29} (*n*-nonacosane) and C_{31} (*n*-hentriacontane). CHC components tended to be in lower overall abundance in samples from Langanes (L CG), whereas samples from Lundey Island (LI PF) had the highest overall abundance (Fig. 2).

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

PLS-DA analyses

Population samples

Cross model validation for population showed that the quality of the analysis was good: 50.6% of the samples were assigned to the population of origin. PLS-DA analysis showed a discriminating population effect ($p=0.008$) separating the two host species on first axis (Fig.4). The three first axes explained respectively 11.94, 7.99 and 3.99 % of the total variance among samples.

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

C_{33} (*n*-tritriacontane), $2meC_{24}$ (2methyltetracosane) and C_{29} (*n*-nonacosane) appeared as the most 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: $p=0.064$).

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 C₁₇ to C₃₃. The qualitative composition is similar to that reported for many other arthropods, containing predominantly linear alkanes (C₂₃, C₂₅, C₂₇, C₂₉ and C₃₁) (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 C₂₉, *n*-hentriacontane C₃₁ and *n*-tritriacontane C₃₃). Mixed with other compounds, *n*-tritriacontane was

demonstrated to induce copulation in males of the stable fly *Stomoxys calcitrans* (Uebel et al., 1975). Other long chain hydrocarbons, including C₂₃ to C₃₁, produced in large quantities and acting in combination, were found to serve in colony recognition by bumblebees *Bombus terrestris* (Rottler et al., 2013). Moreover, 2MeC₂₄, only detected in one PF CHC profile, has been shown to serve as contact pheromone in peach twig borers *Anarsia lineatella* (Schlamp, 2005). As puffin burrows are deep, densely distributed and interconnected (Harris and Wanless, 2011), the large production of these cuticular hydrocarbons in *I. uriae* may enable ticks to find their way in the host nesting environment. The use of this particular blend of CHC may also help ticks to find individuals that smell similar, favoring assortative mating (van Zweden and d’Ettorre, 2010). This type of pheromone may not be necessary for CG ticks because guillemots breed in extremely dense numbers on cliff ledges with no constructed nest; ticks tend to aggregate under and around rocks on the cliff ledge such that finding a mate from the same host type may be easier than in the case of puffin hosts.

The presence of complex compounds in the CHC pattern of *I. uriae* highlight that chemical communication may be important in this tick species, enabling host-adapted ticks to find a suitable host and a mating partner (Sonenshine and Roe, 2014).

Site or host-associated patterns?

As expected, chemical analyses revealed that each tick population had a distinct CHC profile, but that specific CHCs were also associated with different host types. In particular, C₃₃, 2MeC₂₄ and C₂₉ were most frequently or exclusively detected in PF populations. The quantitative variability among detected hydrocarbons could be related to different factors. First, aging and development have been demonstrated to impact cuticular hydrocarbon patterns in different taxa (Desena et al., 1999; Ichinose and Lenoir, 2009). For example, aging favors the production of longer hydrocarbon chains and decreased attractiveness in *Drosophila melanogaster* (Kuo et al., 2012). The high quantities of

long-chain hydrocarbons (C_{27} , C_{29} , C_{31}), particularly observed in the PF samples, could be a consequence of tick age variability between samples. Samples used in the present study were collected in the field and the relative age of the specimens could not be determined. However, as tick activity is synchronous with seabird breeding, we did not expect the overall timing in the adult female activity to differ in a systematic way between ticks exploiting the different host species, nor among distinct colony locations within Iceland.

Second, climatic conditions can also shape CHC composition. Differences in cuticular hydrocarbon composition were observed in geographically distant tick populations of *Amblyomma variegatum* (Estrada-Peña et al., 1994) and some extreme climatic parameters were shown to be correlated with methylated-alkanes in *Rhipicephalus sanguineus*, highlighting that variation of these compounds is potentially linked to adaptation to environmental temperatures (Estrada-Peña, 1993). The off-host environment (rock or burrow) of *I. uriae* may display significant variation in terms of temperature and relative humidity due to differential exposure to climatic factors such as sun, rain and/or snow (Buckley and Buckley, 1980). These selective factors can impact tick survival and could lead to the quantitative variation in the cuticular components observed in this study. Howard et al (1978) argued that the effectiveness of CHC in prevention of desiccation is dependent on the quantity of CHC and that saturated CHCs are important components to protect against water loss. This hypothesis does not seem to explain the pattern of long chain CHC we observed in PF samples; the micro-habitat used by PF ticks, i.e. deep burrows, is expected to be more stable in terms of temperature and humidity than more exposed areas used by CG ticks.

Third, although the production of CHC is genetically controlled, studies have revealed the importance of the environment in the acquisition of new hydrocarbons (D'ettorre et al., 2006; D'ettorre et al., 2002). Indeed, Singer and Espelie (1996) showed that the social wasp *Polistes*

metricus Say (1831) is only recognized as a nest mate by siblings if exposed to nest surface hydrocarbons after hatching. Likewise, in the leaf-cutting ant *Acromyrmex octospinosus*, it was demonstrated that individuals foraging on different host plants are aggressive towards each other (Jutsum et al., 1979). The authors proposed that the mediation of inter-colony interactions have evolved by the acquisition of colony-specific odor components. The *I. uriae* specimens used in the present study were collected under rocks in the middle of the guillemot colony or within individual puffin burrows. Living in these different substrates could lead to differences in cuticle composition based on environmental acquisition.

Fourth, diet also appears to be an important factor shaping the cuticular hydrocarbon profile. In the Argentine ant *Linepithema humile*, colonies eating different prey items present particular hydrocarbon profiles that include components coming from the prey (Liang and Silverman, 2000). In the same way, Geiselhardt et al. (2012) showed that males of the phytophagous mustard leaf beetle *Phaedon cochleariae* preferred to mate with females reared on the same host plant compared to females from a different host plant, even though they originated from the same laboratory stock population. This phenomenon appears to be due to divergent, host-specific cuticular hydrocarbon profiles. In ticks, it was demonstrated that the cuticular fatty acid profiles of *Rhipicephalus* spp. presented significant differences in fatty acid abundance according to host use (Shimshoni et al., 2013). Here, the blood composition of the two different hosts of *I. uriae* could result in the acquisition of specific hydrocarbon mixtures.

In general, making inferences on environmental versus host effects will require the examination of ticks from additional sites and host types. However, regardless of the origin of host-associated differences in CHC profiles of *I. uriae*, what is important to now determine is whether these 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.

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>).

	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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