Deciphering the variation in cuticular hydrocarbon profiles of five European honey bee subspecies

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Abstract

The Western honey bee (*Apis mellifera*) subspecies exhibit local adaptive traits that evolved in response to the different environments that characterize their native distribution ranges. An important trait is the cuticular hydrocarbon (CHC) profile, which helps preventing desiccation and mediating communication. We compared the CHC profiles of six European subspecies (*A. m. mellifera*, *A. m. carnica*, *A. m. ligustica*, *A. m. macedonica*, *A. m. iberiensis*, and *A. m. ruttneri*) and investigated potential factors shaping their composition. We found that forager CHC profiles were more subspecies-specific than those of nurse bees. These differences could be partially explained by their molecular phylogeny, indicating genetic drift as an important factor. We did not find adaptation of the CHC profiles of the subspecies to the climatic conditions in their distribution range. Nurse bees showed a larger variation in subspecies-specific CHC profiles, likely due to a controlled environment with less desiccation stress inside the hive. The strongest profile differences appeared between nurse bees and foragers in all subspecies, suggesting an adaptation to social task and a role in communication. Foragers also showed an increase in the relative amount of alkanes in their profiles compared to nurses, indicating adaptation to climatic conditions.

Keywords: Social insects, honey bees, ecology, chemical communication, climate adaptation.

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Introduction

Subspecies are populations of organisms that show signs of local adaptation and can be discriminated by morphological, behavioral, and molecular characteristics [1]. The Western honey bee (*Apis mellifera*) is an excellent model to investigate adaptation to local climate because it has evolved into 31 subspecies [2–6]. Although *A. mellifera* is currently distributed worldwide due to anthropogenic dispersion [2,7–9], its native range comprises Africa and large parts of Eurasia [2,7,8,10,11]. Throughout this environmentally diverse range, *A. mellifera* expresses a remarkable genetic and phenotypic variation, which was earlier grouped into four evolutionary lineages based on morphological traits [2] and has recently expanded into seven, based on whole genome data [2,6,8,10,12–14]. Europe harbors an important component of the *A. mellifera* diversity, which is represented by 10 subspecies. This diversity clusters into three divergent morphological lineages, including the Northern and Western European lineage (M), the Central Mediterranean and Southeastern European lineage (C), and the African lineage (A) [2,4]. Lineage M extends from Southern Iberia, where the summers are very warm and dry, to southern Scandinavia, where the winters are very long and cold. Lineage

C comprises the Apennine and Balkan Peninsulas, where the climate ranges from continental (in the north and east) to Mediterranean (in the coastal areas). Finally, lineage A occupies the entire African continent but has historically expanded into Europe, where it is present in Malta and Sicily but also in southeastern Iberia, where the mitochondrial, but not the nuclear DNA, is mostly of African ancestry [2,15–17].

In this wide geographical area, *A. mellifera* evolved local adaptive traits to thrive across diverse climatic selective pressures [2,7]. An example of such a trait is the lower thermal tolerance of the European C-lineage *A. m. ligustica* and *A. m. carnica* compared to the Oriental lineage *A. m. jemenitica,* which thrives in the extremely arid habitats of the Arabian Peninsula [18,19]. Differences in climatic adaptations of honey bee subspecies are particularly important under the current climate change scenario, because they can affect the distribution range and/or drive new competitive relationships between honey bee subspecies or wild bee species [20].

Cuticular hydrocarbons (CHCs) represent an important adaptive trait to climate because they form a semi-liquid coating preventing desiccation. In addition, they offer a physical barrier against microorganisms, and mediate intra- and interspecific communication [21–28]. The desiccation barrier of the CHCs depends on the aggregation of the molecules, which, in turn, is determined by the composition of the CHC layer. Tight aggregations are formed by long chain-length hydrocarbons and saturated, non-methyl-branched hydrocarbons (alkanes). The waterproof barrier induced by those hydrocarbons is more efficient than that of shorter chain-length, unsaturated, or methyl-branched hydrocarbons. The latter, however, are known to act as recognition cues for sex, age, nestmate, cast, and task performance [25,29–40]. The trade-off between water-loss prevention and communication necessities might therefore constrain the variation in CHC compositional profiles [23,29,41,42].

The CHC profiles of honey bee workers display stereotypic qualitative and quantitative variations correlating with age and task performance [31,37,43]. These task specific profiles are assumedly an adaptive response of worker bees to contrasting environments (i.e., temperature and humidity).

Workers performing tasks inside the hive (brood care) are naturally exposed to a more controlled environment compared to foraging honey bees, which leave the hive to collect resources and are exposed to a harsher environment. Not surprisingly, foragers have a more waterproof CHC profile than nurse bees [31,39]. Considering that honey bees can discriminate between different CHC profiles, those differences might serve as recognition cues for task performance among worker bees [31,43,44]. Whether the CHC profile compositions exhibited by different *A. mellifera* subspecies is subspecies-specific and adapted to different climatic conditions is an open question.

In the present study, we analyzed the CHC profiles of nurse bees and foragers of six European *A. mellifera* subspecies (*A. m. mellifera* and *A. m. iberiensis* of M-lineage ancestry, *A. m. carnica*, *A. m. ligustica*, and *A. m. macedonica* of C-lineage ancestry, and *A. m. ruttneri* of A-lineage ancestry) raised under the same environmental conditions. We chose a common-garden experiment to exclude the impact of the local environment on the expression of the potential subspecies-specific CHC profiles. We investigated whether (1) honey bee workers of different subspecies display different CHC profiles under the same environmental conditions as a consequence of their innate differences; and (2) whether the subspecies-specific differences of the CHC profiles can be interpreted as an adaptation to the environmental conditions of their geographic origin or due to genetic drift. We hypothesized that foragers should be more different between subspecies than nurse bees because they are more exposed to environmental conditions outside the hive.

Materials and methods

Experimental set-up

We performed a common garden experiment, by keeping queen-right colonies of six different honey bee subspecies (*A. m. carnica*, *A. m. iberiensis*, *A. m. ligustica*, *A. m. macedonica*, *A. m. mellifera*, and *A. m. ruttneri*) in the departmental apiary of the University of Würzburg from April to September 2018 to February 2019. By doing so, we ensure that the colonies experience the same environmental conditions, thus the differences in their CHC composition mainly respond to their genetic differences.

The mated queens came from populations central to the current distribution range of each respective subspecies, namely: Würzburg, Germany: *A. m. carnica*; Bragança, Portugal: *A. m. iberiensis*; Reggio Emilia, Italy: *A. m. ligustica*; Neo Moudania, Greece: *A. m. macedonica*; Malta, Malta: *A. m. ruttneri*. Finally, *A. m. mellifera* queens originated from a protected population in Chimay, Belgium, to avoid analyzing admixed individuals, as this subspecies genetic integrity is threatened in large tracts of its native range by C-derived gene flow [45].

The queens were introduced in queen-less *A. m. carnica* hives for the initial set-up. To avoid drifting of foragers, the hives were placed in pairs of the same subspecies on hive stands, facing the opposite direction, and the hive stands were grouped by subspecies. Furthermore, the hive boxes were sorted in different color combinations, helping the returning foragers to locate their own hive. Thus, intending to reduce the drifting risk within and between the groups of hives [46].

To assure complete turnover in the *A. m. carnica* recipient hives, workers were collected from each subspecies

and colony after a minimum of two months from queen introduction. The workers were immediately killed by immersing them in liquid nitrogen and stored in labeled Eppendorf tubes at -20 °C until hexane extraction. Ten workers were collected per task (nursing and foraging) of each subspecies between 7:00 a.m. and 12:00 p.m. between July and September 2018. Individual workers inside the hive who poked their heads into an open brood cell for at least 10 seconds were identified as nurse bees. Returning pollen foragers were identified by a considerable pollen load in the corbiculae of their hind legs.

Extraction of CHCs and chemical analysis

After defrosting, honey bees were immersed in hexane for 10 minutes in 1.5 ml glass vials to extract their CHCs. Subsequently, the solvent was evaporated using a gentle stream of CO_2, until the total volume was 100 µl to transfer it into a 300 µl glass insert. The insert was placed in a 1.5 ml glass vial. 1 µl of each extract was injected into an Agilent 7890A Series Gas Chromatography System (GC) coupled to an Agilent 5975C Mass Selective Detector (MS) (Agilent Technologies, Waldbronn, Germany). The GC was equipped with a J & W, DB-5 fused silica capillary column (30 m x 0.25 mm ID; df = 0.25 µm; J & W, Folsom, CA, USA). The GC temperature was programmed from 60 to 300 °C with a 5 °C/min heating rate and held for 10 min at 300 °C. Helium was used as carrier gas with a constant flow of 1 ml/min. The injection was carried out at 300 °C in the split-less mode for 1 min with an automatic injector. The electron impact mass spectra (EI-MS) were recorded at 70 eV and with a source temperature of 230 °C.

The chromatograms were analyzed using the data analysis software package 'MSD ChemStation F.01.00.1903' for Windows (Agilent Technologies, Waldbronn, Germany). The area of each peak was determined by integration and the initial threshold of the integration parameters was set on 15. Initial area reject was set on 1, initial peak width was set on 0.02 and shoulder detection was off. The compounds were identified by their retention indices and diagnostic ions of their mass spectra. Double bond positions of monounsaturated hydrocarbons were identified by dimethyl disulfide derivatization [47]. Compounds that represented less than 0.01% of the total ion count of a sample and compounds in less than 50% of the extracts in a group were excluded from the analysis to exclude concentration effects and to compare group-specific profiles. The abundances of the compounds were quantified, for each sample, as the proportion (%) that the area of the corresponding peaks represented from the sum of the area of all the peaks included in the analysis.

Additionally, we calculated the relative abundance of the different hydrocarbon classes (i.e. n-alkanes, alkenes, alkadienes, and methyl-branched alkanes), as well as the mean chain length, in the CHC profile of each bee, and compared them between subspecies, for the nurses and the foragers separately. The relative abundances

of the different hydrocarbon classes were calculated as the sum of the relative abundances of all the individual compounds of a corresponding class that were found in the CHCs extract of a bee. The mean chain length corresponds to the weighted mean of the chain length of all the compounds found in the CHC profile of each bee, using the relative abundance of the compounds as their weights.

Climate data

Climatic conditions were characterized by calculating the average temperature (°C) and precipitation (mm) for the countries of origin of the mated queens of each subspecies, using the WorldClim 2.1 [48] downscaled CRU-TS 4.06 data set [49]. Subsequently, we correlated these parameters with the CHC compositions of the subspecies.

Statistical analysis

The chemical similarity between task and subspecies was visualized via hierarchical cluster analyses, using the mean abundance of each compound for each group, the Bray-Curtis dissimilarity index, and the Wards' grouping method.

A bi-dimensional non-metric multidimensional scaling (NMDS) was used to visualize the CHC composition differences separately for nurse bees and foragers, using the Bray-Curtis dissimilarity index to estimate the inter-individual chemical dissimilarity.

The difference in the CHC composition between subspecies was tested via a one-way PERMANOVA. The permutations were restricted to the same task-performance groups (i.e. Comparing nurses or foragers between subspecies). Additionally, pairwise comparisons with FDR Benjamini & Hochberg adjustments were performed, to test the significance of the differences between subspecies, for the nurses and foragers separately.

The difference in CHC composition variability between nurses and foragers was tested via a one-way permutational test of multivariate homogeneity, with the permutations restricted to the subspecies (i.e. Contrasting the variance in the CHC profiles between nurses and foragers of the same subspecies).

The relative abundance of the different substance classes of hydrocarbons between subspecies was tested, for nurse and forager bees separately, with Kruskal Wallis tests, followed by Dunn's tests with FDR Benjamini & Hochberg adjustment. The relative abundance of each hydrocarbon substance class was calculated by

summing up the relative abundances of all the compounds of different classes in the CHC profile of each honey bee worker.

Likewise, the weighted mean chain length was calculated for every individual CHC profile, and its difference was tested between subspecies, for nurse and forager bees separately, with Kruskal Wallis tests, followed by Dunn's tests with FDR Benjamini & Hochberg adjustment. The weighted mean chain length was calculated using the relative abundance of the compounds as the weights for averaging their chain length.

The correlation of the average temperature (°C) and precipitation (mm) for the countries of origin of each subspecies with the ratio of olefins (unsaturated hydrocarbons) to n-alkanes, and with the mean chain length of the hydrocarbons, in the CHC profile of the honey bee workers was tested via Spearman's correlation tests. The olefins to n-alkanes ratio was calculated by summing the relative abundance of the alkenes and alkadienes in the CHC profiles of every bee and dividing it by the respective relative abundance of n-alkanes.

Statistics were performed using R 4.4.0 [50], with the IDE RStudio v2024.4.1.748 [51], and the packages vegan v2.6.6.1 [52], dunn.test v1.3.6 [53], ggdendro v0.2.0 [54], raster v3.6.26 [55], geodata v0.6.2 [56], and tidyverse v2.0.0 [57].

Results

The average composition of the CHC profiles of the honey bee subspecies differs between nurse bees and foragers, except for *A. m. iberiensis* foragers, which are placed in the same cluster with *A. m. iberiensis and A. m. mellifera* nurses (Figure 1) meaning that the CHC composition of *A. m. iberiensis* foragers is more similar to their and *A. m. mellifera* nurse bees than to foragers of the other subspecies. The characterization of the CHC profiles revealed that, when they are different among *A. mellifera* subspecies, they differ mainly in the relative abundance of their compounds, although, each subspecies displayed one or a few compounds that were not present in the other subspecies (see Supplementary tables S2 and S1). The relative abundance of each substance class differed among subspecies in the CHC profiles of the workers (p-value < 0.05; Kruskal-Wallis test; Table 1), except for the alkenes (p-value > 0.05) in both nurse bees and foragers (Figure 2).

Regarding the foragers, most subspecies differed significantly in their CHC profiles (p-value < 0.05; Figure 3; Tables 2 and 3). Only *A. m. macedonica*, *A. m. carnica* and *A. m. ligustica* did not differ from each other. Although all other subspecies have a specific CHC profiles, the strongest differences appear between *A. m. carnica* and *A. m. iberienses*, with the former having a higher relative amount of alkanes and the latter

having higher relative amounts of methyl-branched hydrocarbons and alkadienes (Figure 2). Interestingly, there was no difference in the weighted mean chain length between the foragers of the studied subspecies (p-value > 0.05; Figure 4; Table 4).

As expected, there were fewer differences in the CHC profiles among subspecies in nurse bees than in foragers (p-value < 0.05; Figure 3; Tables 2 and 3). Regarding the nurse bees *A. m. mellifera* and *A. m. iberiensis* were not different from each other, but they were different from all the other subspecies. There were no differences among *A. m. macedonica*, *A. m. carnica, A. m. ligustica,* and *A. m. mellifera.* Of all subspecies, *A. m. macedonica* has teh lowest relative amounts of alkanes in favor of higher amounts of methyl-branched alkanes and alkadienes. In contrast, *A. m. carnica* shows relatively low amounts of methyl-branched alkanes and alkadienes (Figure 2).

There was more variation in the weighted mean chain length of CHC profiles in nurse bees than foragers with the highest weighted mean chain length in *A. m. macedonica* and the lowest weighted mean chain length in *A. m. mellifera* and *A. m. iberiensis* nurse bees (Figure 4). In addition, the CHC profile of nurse bees occupied a significantly larger chemical space than that of the foragers (p-value < 0.05; Figure 3; Supplementary Table S3).

The average temperature for the country of origin of the subspecies correlated positively with the ratio of unsaturated hydrocarbons to n-alkanes only in the nurse bees, while no correlation was observed for the foragers. In contrast, the average temperature correlated negatively with the weighted mean chain length of the CHC profiles in the foragers, but they did not correlate for the case of the nurse bees (Supplementary Figure S1).

The average precipitation for the country of origin of the subspecies correlated negatively with the ratio of unsaturated hydrocarbons to n-alkanes in the nurse bees and with the weighted mean chain length in the foragers (Supplementary Figure S2). No correlation was observed for the mean precipitation and the unsaturated to n-alkanes ratio in the foragers, nor with the weighted mean chain length of the nurse bees. The correlations with the climate parameters and the CHC profiles followed the prediction of adapting to drought stress only in nurse bees but not in foragers. Overall, the correlation coefficients were low ($R < 0.4$, Spearman's test).

Discussion

In the present study, we investigated (1) whether honey bee subspecies exhibit a differentiated CHC profile, and (2) whether potential CHC differences between subspecies can be explained by adaptation to climatic conditions or by genetic drift. We show that the composition of CHC profiles of honey bee workers differ moderately between subspecies with more pronounced differences between foragers than nurses. Keeping the subspecies under the same environmental conditions suggests that the subspecies' differences in CHC profiles have a genetic basis. The cluster analysis reveals similarities among honey bee subspecies belonging to the same evolutionary lineage. In nurse bees, the members of the lineage M, *A. m. mellifera* and *A. m. iberiensis*, and the members of the lineage C, *A. m. carnica*, *A. m. ligustica*, and *A. m. macedonica*, are more similar among each other than between the different lineages [6,7,58,59]. This indicates an effect of genetic drift on the CHC profiles of the subspecies subsequent to their phylogenetic divergence. The only exception is the A-lineage *A. m. ruttneri*, which clusters with the members of the lineage C, but this subspecies is highly admixed with C-derived *A. m. ligustica* bees [6,58,59]. This contrasts to CHC profiles in ants, which have been shown to evolve independently from phylogeny, allowing them to rapidly adapt their CHC profiles to environmental selection pressures [60]. In foragers, the members of the lineages do not cluster together with the exception of lineage C, suggesting an impact of environmental factors on their CHC profiles.

In contrast to our expectations, we did not find a strong adaptation of the subspecies CHC profiles to their climatic conditions. We had expected that particularly foragers from environments with more drought stress (e.g. *A. m. iberiensis*) would harden their CHC profile by increasing the ratio of unsaturated hydrocarbons to n-alkanes and the weighted mean chain length. However, the correlation of these CHC profile parameters with temperature and precipitation supported the adaptation hypothesis only in the nurse bees but not in foragers and, in addition, was very weak. Thus, we suggest genetic drift having a stronger effect on the composition of subspecies-specific CHC profiles than climatic conditions.

By performing a common garden experiment with all investigated subspecies, we ensured that all colonies were kept under the same environmental conditions. Thus, the differences in the CHC composition among subspecies should be due to genetic differences. Although, there were no consistent signs of adaptation in the CHC profiles of the subspecies to the climatic conditions in their country of origin, we found less variation in the CHC profiles between individual foragers within a subspecies compared to nurse bees of the same subspecies among all investigated honey bee subspecies. This might be due to an overall stronger desiccation stress on foragers, independent of their local environment. Foragers might be restricted from

occupying a large chemical space, as we see in nurse bees, because their CHC profile is already adapted to a harsher environment outside the hive. Alternatively, the smaller chemical space found in foragers is an adaptation to nestmate recognition helping the guard bees to better discriminate between nestmates and non-nestmates. To test this hypothesis, a future study should compare foragers and nurse bees from different honey bee colonies.

Honey bee workers perform several tasks inside and outside the nest during their lifetime. The task performance of each worker is influenced by its age, sucrose responsiveness, genotype, environmental conditions, and previous experience [61–67]. The most pronounced differences in behaviour and physiology occur between nurse bees and foragers. While nurse bees provide brood and queen with food, the foragers leave the hive to collect pollen, nectar, water or propolis [68]. We find that these differences between nurse bees and foragers correlate with differential CHC profiles across subspecies. This could be a result of their different environments. While nurse bees normally do not leave the hive, foragers leave the colony daily for up to several hours. Our findings directly support data from other social insects performing tasks outside and inside the colony [38,39]. Generally, it has been hypothesized that the differences in the CHC composition of foragers compared to nurse bees could be a response to the harsher environment that foragers face outside the hive [31]. This is supported by the fact that foragers display a higher abundance of alkanes and a lower abundance of alkenes than nurse bees. This shift in alkane-to-alkene ratio makes the CHC profile of the foragers more waterproof than that of nurse bees [23,24,41,42,69]. In contrast, foragers display hydrocarbons with shorter mean chain lengths than nurses, which weakens the waterproofing of the foragers CHC profile and thus contradicts a simple response of the CHC profiles to stronger desiccation stress in foragers. Although the shift in abundance of the different substance classes is likely to have a stronger effect on the waterproofing of the CHC layer than a shift in mean chain length, other factors, such as intraspecific chemical signaling using hydrocarbons, might shape the CHC profiles [23,26,29,41,41,42,70,71]. Part of the variation in the CHC profile across nurses and foragers might allow an individual worker to assess the task of an encountered individual in the nest.

Conclusions

Overall, some *Apis mellifera* subspecies show a specific CHC profile when comparing the nurse and forager profiles separately from each other. In addition, our study reveals a very strong shift in the CHC profiles from nurses to foragers in all investigated subspecies. We hypothesize that these CHC differences between subspecies evolved in parallel to morphological traits within these populations due to allopatry, and they

are, at least partly, the result of genetic drift. Although there were no signs of adaptation of subspecific CHC profiles to their native climatic conditions, the adaptation of forager CHC profiles of all subspecies compared to the nurse bee profiles was evident. Thus, we hypothesize that the more restricted chemical space of forager CHC profiles compared to the chemical space of nurse bees is an adaptation to harsher climatic conditions outside of the hive in all subspecies.

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Data availability

The data and code used for this study are not included in this manuscript, but will be made publicly available as supplementary material for the corresponding paper at the time of publication.

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Figures

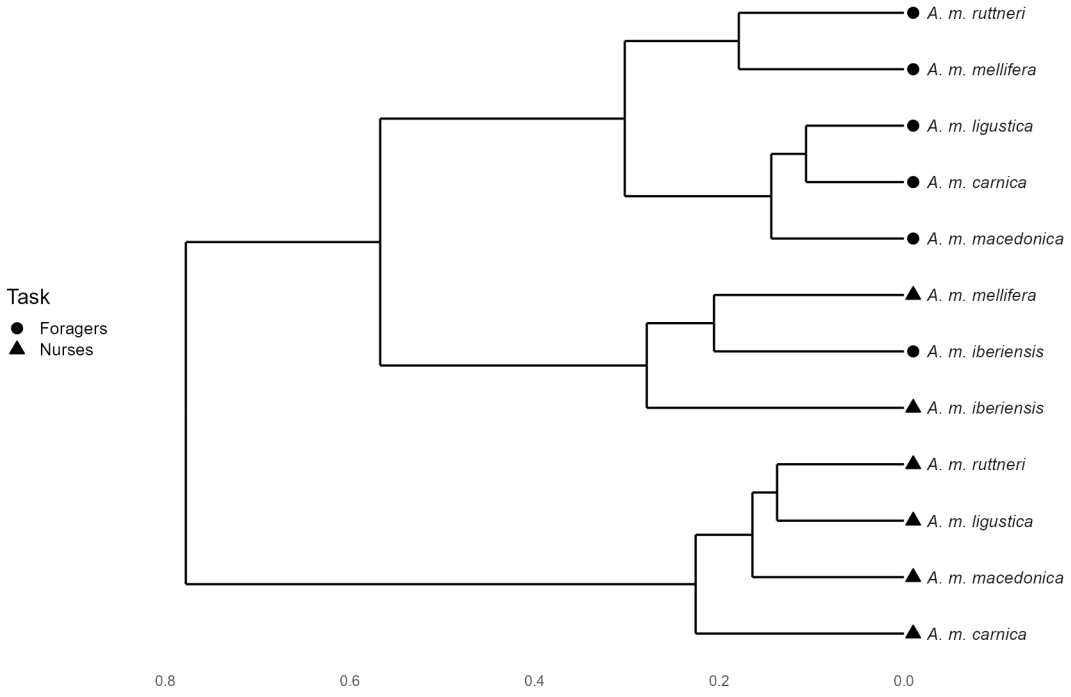

Figure 1: Dendrogram of the CHC profiles hierarchical cluster analysis of *A. mellifera* subspecies worker bees.

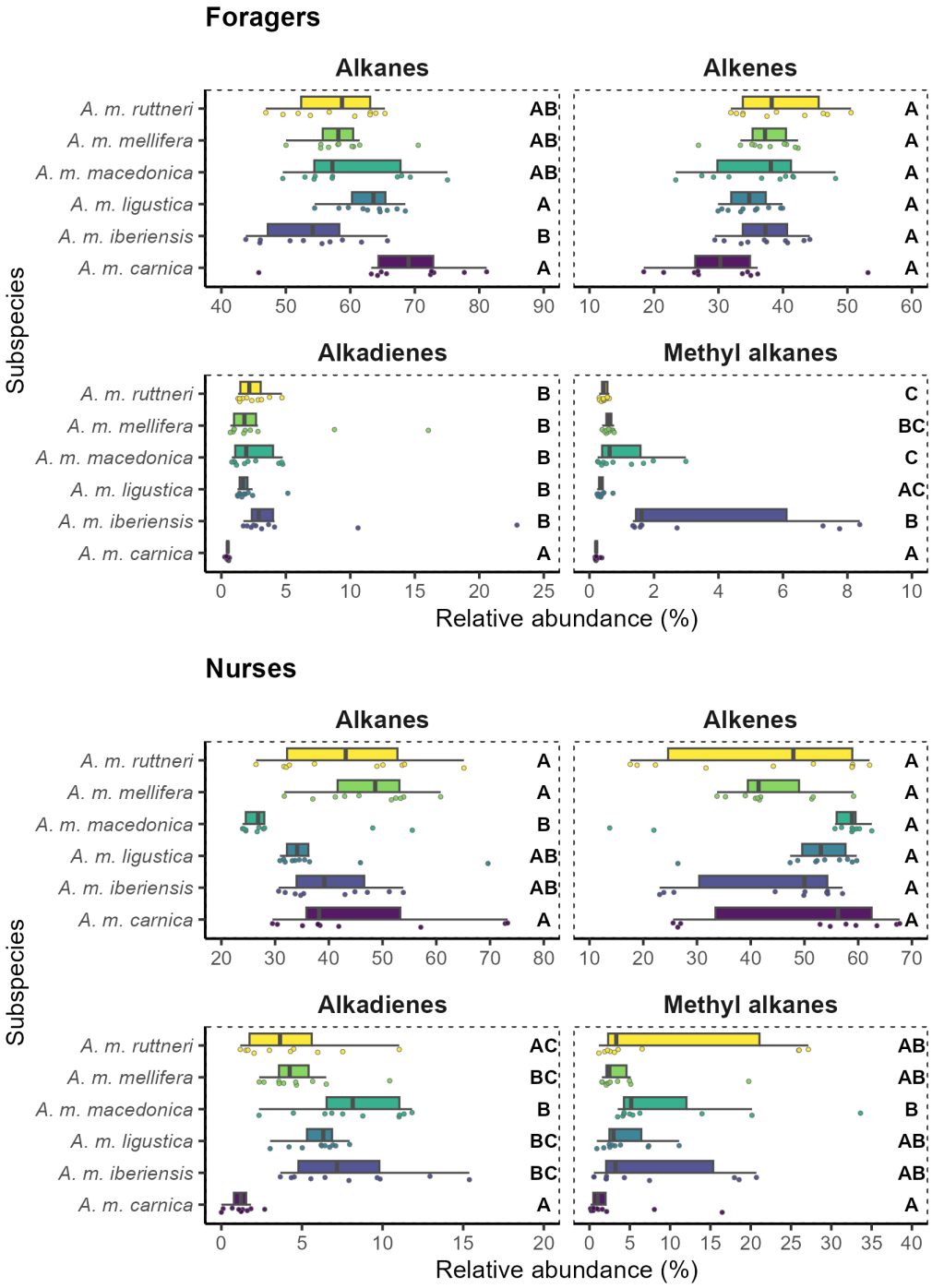

Figure 2: Box plot of the relative abundance of each compound class in the CHC profile of worker bees of different *A. mellifera* subspecies. The figure is divided regarding the task of the worker bees (foragers and nurse bees). Subspecies with significantly different abundance of a hydrocarbon class (p-value < 0.050) are labeled with different letters, within the plot of the corresponding compound class. Significance of pair-wise comparisons was obtained with Dunn's tests and a FDR Benjamini & Hochberg adjustment, after a Kruskal Wallis tests.

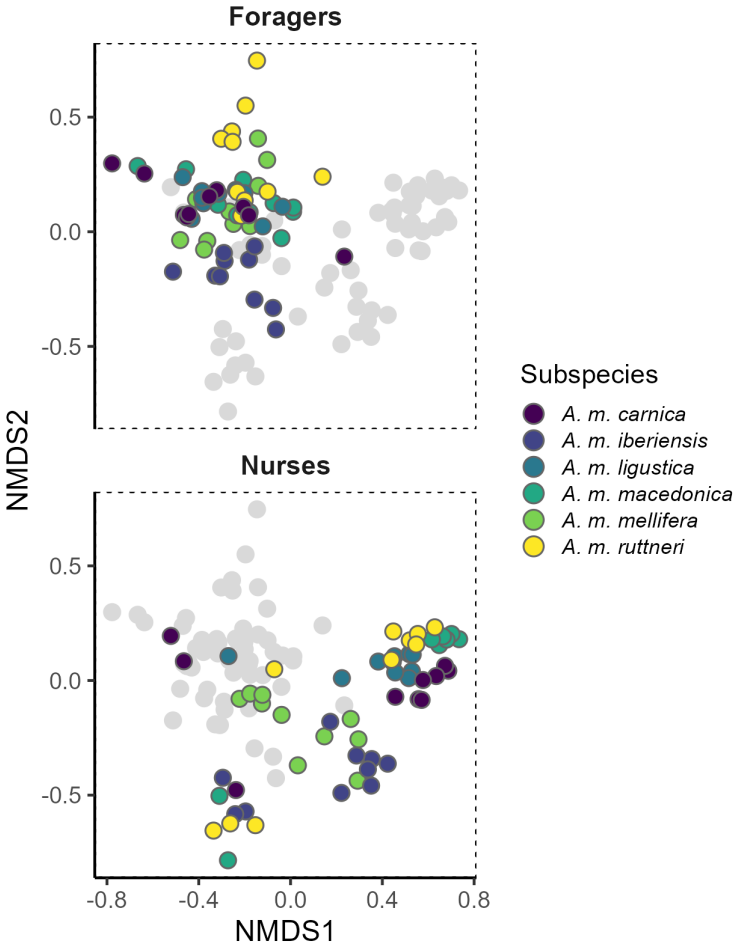

Figure 3: Bi-dimensional Non-metric Multidimensional Scaling analysis on a dissimilarity matrix of the CHC profile of honey bee workers. Stress value: 0.140. The figure is divided in two sections regarding the task of the worker bees (Foragers on the top, and nurse bees at the bottom). Both sections show the same NMDS plot, but only the points representing bees of the corresponding task are colored, while the others are shown in grey.

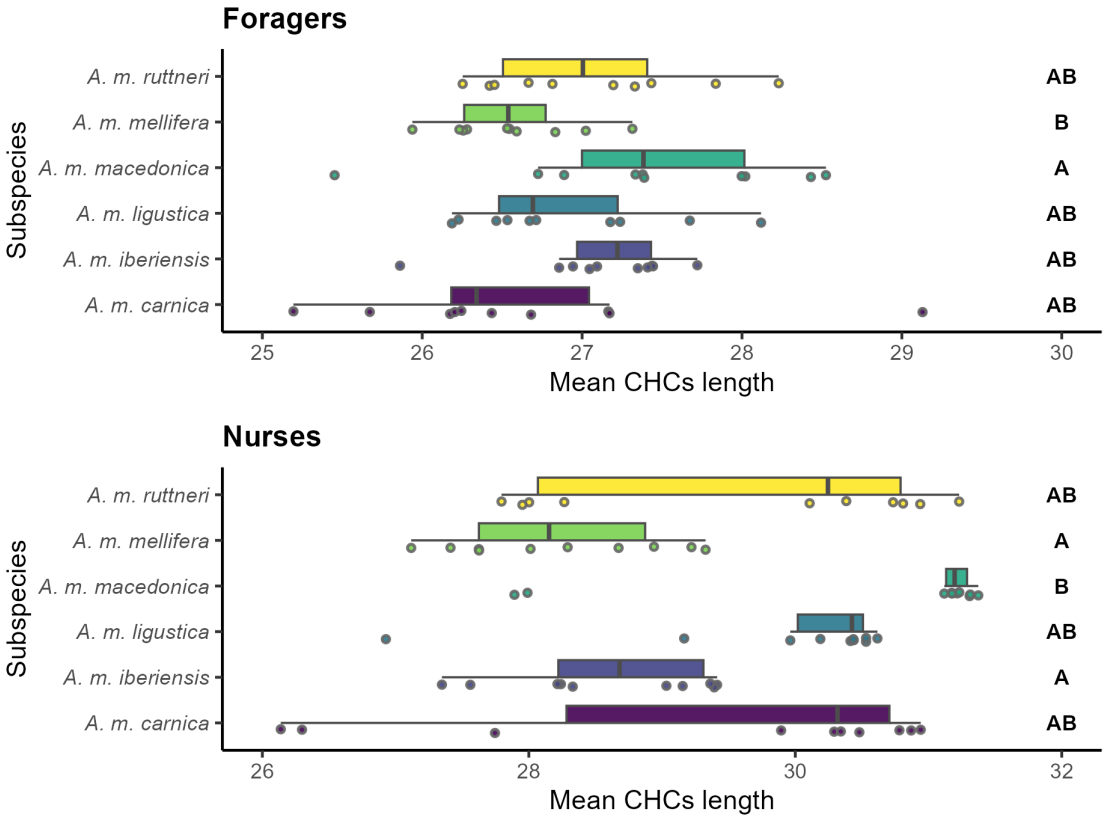

Figure 4: Box plot of the weighted mean chain length of hydrocarbons in the CHC profile of worker bees of different *A. mellifera* subspecies. The figure is divided regarding the task of the worker bees (foragers and nurse bees). Subspecies that significantly differ (p-value < 0.050) are labeled with different letters. Significance of pair-wise comparisons was obtained with Dunn's tests and a FDR Benjamini & Hochberg adjustment, after a Kruskal Wallis tests.

Tables

Table 1: Kruskal-Wallis results for subspecific differences in abundance of hydrocarbon classes in the CHC profile of honey bee workers. Chi2 - χ^2; DF - degrees of freedom.

	Chi^2	DF	p-value
Foragers			
Alkanes	16.260	5	0.006
Alkenes	8.377	5	0.137
Alkadienes	29.250	5	<0.001
Methyl alkanes	40.565	5	<0.001
Nurses			
Alkanes	14.658	5	0.012
Alkenes	7.759	5	0.17
Alkadienes	31.233	5	<0.001
Methyl alkanes	13.154	5	0.022

Table 2: One-way PERMANOVA contrasting the CHC composition between subspecies. The permutations (n=1000) were restricted to the task-performance group. Df - degrees of freedom; SS - sum of squares; R2 - R squared; F - F-value; SES - standard effect size.

	Df	SS	R2	F	SES	p-value
Subspecies	5	2.185	0.203	5.795	25.803	0.001
Residual	114	8.596	0.797			
Total	119	10.781	1.000			

Table 3: Pairwise PERMANOVA results contrasting the CHC composition between honey bee subspecies for the nurses and foragers separately. SS - sum of squares; R2 - R squared; F - F-value, SES - standard effect size. In the case of the SS and R2 the value for each comparison is displayed over that of the corresponding residuals. The adjusted p-values result from a FDR Benjamini & Hochberg adjustment. p-values < 0.05 are marked in bold

	SS	R2	F(1, 18)	SES	p-value	adjusted p-value
Foragers						
A. m. carnica vs A. m. iberiensis	0.384	0.398	11.892	11.463	**0.001**	**0.015**
	0.581	0.602				
A. m. carnica vs A. m. ligustica	0.062	0.124	2.536	1.650	0.076	1.000
	0.443	0.876				
A. m. carnica vs A. m. macedonica	0.107	0.152	3.234	2.273	**0.042**	0.629
	0.597	0.848				
A. m. carnica vs A. m. mellifera	0.278	0.354	9.869	11.516	**0.001**	**0.015**
	0.508	0.646				
A. m. carnica vs A. m. ruttneri	0.357	0.344	9.450	11.338	**0.001**	**0.015**
	0.679	0.656				
A. m. iberiensis vs A. m. ligustica	0.344	0.454	14.952	17.274	**0.001**	**0.015**
	0.414	0.546				
A. m. iberiensis vs A. m. macedonica	0.320	0.361	10.160	12.495	**0.001**	**0.015**
	0.567	0.639				
A. m. iberiensis vs A. m. mellifera	0.292	0.379	10.964	12.841	**0.001**	**0.015**
	0.479	0.621				
A. m. iberiensis vs A. m. ruttneri	0.473	0.421	13.103	15.411	**0.001**	**0.015**
	0.650	0.579				
A. m. ligustica vs A. m. macedonica	0.084	0.164	3.519	3.453	**0.011**	0.165
	0.430	0.836				
A. m. ligustica vs A. m. mellifera	0.210	0.381	11.088	14.649	**0.001**	**0.015**
	0.341	0.619				
A. m. ligustica vs A. m. ruttneri	0.286	0.358	10.053	12.364	**0.001**	**0.015**
	0.513	0.642				
A. m. macedonica vs A. m. mellifera	0.210	0.298	7.659	11.106	**0.001**	**0.015**
	0.494	0.702				

Table 3: *(continued)*

	SS	R2	F(1, 18)	SES	p-value	adjusted p-value
A. m. macedonica vs A. m. ruttneri	0.217	0.246	5.859	7.859	**0.001**	**0.015**
	0.666	0.754				
A. m. mellifera vs A. m. ruttneri	0.166	0.223	5.160	7.754	**0.001**	**0.015**
	0.577	0.777				
Nurses						
A. m. carnica vs A. m. iberiensis	0.417	0.240	5.690	5.017	**0.005**	0.075
	1.320	0.760				
A. m. carnica vs A. m. ligustica	0.128	0.109	2.208	1.123	0.115	1.000
	1.044	0.891				
A. m. carnica vs A. m. macedonica	0.295	0.177	3.876	3.010	**0.027**	0.405
	1.370	0.823				
A. m. carnica vs A. m. mellifera	0.414	0.276	6.865	5.396	**0.006**	0.090
	1.085	0.724				
A. m. carnica vs A. m. ruttneri	0.190	0.111	2.250	1.194	0.095	1.000
	1.522	0.889				
A. m. iberiensis vs A. m. ligustica	0.518	0.390	11.514	13.306	**0.001**	**0.015**
	0.811	0.610				
A. m. iberiensis vs A. m. macedonica	0.747	0.397	11.842	10.586	**0.001**	**0.015**
	1.136	0.603				
A. m. iberiensis vs A. m. mellifera	0.201	0.191	4.245	4.330	**0.006**	0.090
	0.851	0.809				
A. m. iberiensis vs A. m. ruttneri	0.462	0.264	6.461	6.105	**0.002**	**0.030**
	1.288	0.736				
A. m. ligustica vs A. m. macedonica	0.106	0.110	2.222	1.194	0.129	1.000
	0.860	0.890				
A. m. ligustica vs A. m. mellifera	0.472	0.451	14.769	12.138	**0.001**	**0.015**
	0.575	0.549				
A. m. ligustica vs A. m. ruttneri	0.113	0.100	2.001	1.036	0.135	1.000
	1.012	0.900				

Table 3: *(continued)*

	SS	R2	F(1, 18)	SES	p-value	adjusted p-value
A. m. macedonica vs A. m. mellifera	0.721	0.445	14.414	12.417	**0.001**	**0.015**
	0.901	0.555				
A. m. macedonica vs A. m. ruttneri	0.157	0.105	2.106	0.774	0.137	1.000
	1.338	0.895				
A. m. mellifera vs A. m. ruttneri	0.451	0.300	7.715	7.230	**0.001**	**0.015**
	1.053	0.700				

Table 4: Kruskal-Wallis results for subspecific difference in mean chain length of hydrocarbons in the CHC profile of honey bee workers. Chi^2 - χ^2; DF- degrees of freedom.

	Chi2	Df	p-value
Foragers	12.955	5	0.024
Nurses	19.965	5	0.001

Supplementary material

Table S1: CHC profile of forager bees from different *A. mellifera* subspecies showing mean relative abundance (%) and standard deviation (sd) of all compounds. Mean and sd values are in bold for the cases a compound is present in only one subspecies.

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C17:2	1659	0.000	0.000	**0.037**	**0.040**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8-C17:1	1666	0.000	0.000	0.117	0.109	0.000	0.000	0.040	0.051	0.000	0.000	0.000	0.000
C17	1700	0.000	0.000	0.148	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.351	0.689
C19:2	1863	0.000	0.000	**0.021**	**0.018**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C19:1	1875	0.164	0.222	0.365	0.355	0.000	0.000	0.212	0.135	0.163	0.179	0.000	0.000
C19	1900	0.000	0.000	0.382	0.115	0.126	0.193	0.035	0.051	0.922	1.344	0.999	1.149
C20	2000	0.000	0.000	0.038	0.031	0.000	0.000	0.000	0.000	0.140	0.054	0.131	0.061
C21:2	2065	0.000	0.000	**0.020**	**0.019**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C21:1	2073	0.000	0.000	0.199	0.096	0.053	0.032	0.117	0.066	0.091	0.030	0.042	0.031
C21	2100	0.462	0.307	2.169	2.670	0.947	0.460	1.232	1.808	7.911	4.145	7.666	3.052
C22:1	2173	0.000	0.000	0.027	0.016	0.000	0.000	0.000	0.000	0.035	0.028	0.000	0.000
C22	2200	0.231	0.100	0.232	0.078	0.291	0.088	0.235	0.099	0.390	0.138	0.305	0.115
C23:2	2264	0.000	0.000	3.258	6.887	0.000	0.000	0.000	0.000	1.551	4.905	0.000	0.000
9-; 7-C23:1	2271	2.330	0.904	2.557	0.804	3.128	0.870	2.112	1.311	3.124	0.548	6.194	7.084
C23	2300	22.271	6.827	8.712	3.011	19.051	5.173	15.350	7.051	14.868	2.453	12.367	5.246
11-MeC23	2334	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.044**	**0.026**	0.000	0.000
9-C24:1	2371	0.103	0.057	0.108	0.064	0.753	1.879	0.161	0.110	0.000	0.000	0.248	0.207
C24	2400	0.651	0.195	0.542	0.341	0.770	0.200	0.660	0.238	0.485	0.074	0.430	0.113

Table S1: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C25:2_1	2455	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.248**	**0.191**
4-MeC24	2460	0.000	0.000	**0.044**	**0.071**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C25:2_2	2465	0.280	0.120	0.239	0.116	0.779	0.885	0.000	0.000	0.145	0.154	0.000	0.000
9-; 7-C25:1	2471	3.680	1.291	2.343	0.504	4.975	1.352	4.008	2.133	3.944	1.512	3.202	2.028
C25	2500	23.436	9.057	14.301	3.752	19.671	3.011	19.900	3.858	13.483	2.381	15.080	2.412
13-; 11-MeC25	2533	0.000	0.000	0.066	0.038	0.000	0.000	0.046	0.041	0.192	0.094	0.000	0.000
4-MeC25	2561	0.000	0.000	**0.017**	**0.015**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C26:1	2574	0.000	0.000	0.405	1.015	0.000	0.000	0.114	0.081	0.000	0.000	0.000	0.000
C26	2600	0.465	0.074	1.260	1.321	0.596	0.104	0.607	0.200	0.679	0.157	0.531	0.107
C27:2_1	2643	0.000	0.000	**0.040**	**0.026**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4-MeC26	2660	0.000	0.000	**0.041**	**0.034**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C27:1	2671	0.695	0.280	1.243	0.536	1.276	0.282	1.561	0.591	2.476	0.381	0.900	0.682
7-C27:1	2678	0.205	0.082	0.830	0.412	0.402	0.140	0.410	0.184	1.585	0.449	0.910	1.218
C27	2700	13.644	4.010	18.993	3.968	12.207	2.933	11.978	2.224	14.714	3.667	11.788	3.734
13-; 11-MeC27	2727	0.233	0.071	0.305	0.123	0.383	0.139	0.314	0.103	0.355	0.094	0.307	0.092
3-MeC27	2772	0.000	0.000	0.000	0.000	0.000	0.000	**0.324**	**0.446**	0.000	0.000	0.000	0.000
C28	2800	0.348	0.176	0.908	0.218	0.565	0.193	0.358	0.172	0.667	0.554	0.000	0.000
C29:2_1	2847	0.000	0.000	0.000	0.000	0.000	0.000	**0.084**	**0.050**	0.000	0.000	0.000	0.000
4-MeC28	2860	0.000	0.000	0.629	1.093	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.107

Table S1: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
10-; 9-; 8-; 7-C29:1	2875	0.777	0.517	3.357	1.366	0.833	0.758	2.968	3.747	3.096	0.457	0.958	0.448
C29	2900	9.402	4.936	14.411	3.342	8.897	1.982	7.669	2.702	7.637	1.703	4.374	2.234
15- ; 13-MeC29	2928	0.204	0.074	0.299	0.150	0.252	0.146	0.223	0.115	0.239	0.101	0.164	0.075
11, 17-diMeC29	2958	0.000	0.000	0.000	0.000	0.000	0.000	**0.069**	**0.098**	0.000	0.000	0.000	0.000
4-MeC29	2959	0.000	0.000	**0.085**	**0.132**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3-MeC29	2967	0.000	0.000	**0.119**	**0.162**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10-; 9-; 8-; 7-C30:1	2976	0.000	0.000	0.499	0.542	0.000	0.000	0.126	0.110	0.000	0.000	0.000	0.000
C30:1	2983	0.000	0.000	**0.015**	**0.025**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C30	3000	0.130	0.095	0.452	0.119	0.519	0.248	0.293	0.120	0.290	0.080	0.791	1.479
C31:2_1	3051	0.000	0.000	0.173	0.126	0.000	0.000	0.058	0.044	0.147	0.057	0.000	0.000
C31:2_2	3057	0.111	0.103	0.763	0.433	0.000	0.000	0.167	0.133	0.202	0.179	0.000	0.000
4-MeC30	3064	0.000	0.000	1.973	2.238	0.000	0.000	0.298	0.675	0.000	0.000	0.000	0.000
10-; 9-; 8-C31:1	3075	5.316	2.532	6.246	2.320	4.336	0.966	5.918	2.588	4.582	1.799	8.951	4.672
C31:1	3087	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.693**	**0.531**
C31	3100	5.403	3.771	4.147	2.741	5.685	1.676	6.559	2.288	3.865	1.657	3.859	1.550
15-; 13-MeC31	3125	0.000	0.000	**0.222**	**0.238**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10-; 9-C32:1	3173	0.183	0.092	0.278	0.148	0.000	0.000	0.968	0.966	0.380	0.430	1.452	1.408
C32	3200	0.000	0.000	0.179	0.206	0.000	0.000	0.286	0.150	0.000	0.000	0.000	0.000
C33:2_1	3249	0.000	0.000	0.000	0.000	0.000	0.000	1.248	0.761	1.110	2.307	0.905	0.668

Table S1: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C33:2_2	3257	0.000	0.000	1.009	0.374	1.246	0.444	0.000	0.000	0.000	0.000	0.000	0.000
10-C33:1	3278	8.235	3.361	4.149	2.318	10.174	5.150	8.159	4.110	9.939	6.127	10.887	3.840
C33	3300	0.800	0.709	1.026	0.561	2.083	1.023	2.547	2.074	0.000	0.000	3.042	4.541
C35:2_1	3449	0.091	0.050	0.000	0.000	0.000	0.000	0.883	0.718	0.549	0.530	0.589	0.929
C35:2_2	3454	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.707**	**0.629**
12-; 10-C35:1	3470	0.148	0.099	0.000	0.000	0.000	0.000	1.418	0.945	0.000	0.000	0.775	0.545
C35	3500	0.000	0.000	0.000	0.000	0.000	0.000	**0.281**	**0.340**	0.000	0.000	0.000	0.000

Table S2: CHC profile of nurse bees from different *A. mellifera* subspecies showing mean relative abundance (%) and standard deviation (sd) of all compounds. Mean and sd values are in bold for the cases a compound is present in only one subspecies.

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C17:2	1659	0.000	0.000	**0.048**	**0.057**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8-C17:1	1666	0.000	0.000	0.178	0.182	0.000	0.000	0.000	0.000	0.020	0.028	0.000	0.000
C17	1700	0.000	0.000	0.277	0.388	0.000	0.000	0.000	0.000	0.028	0.026	0.000	0.000
C19:2	1863	0.000	0.000	**0.019**	**0.023**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C19:1	1875	0.000	0.000	0.243	0.124	0.230	0.159	0.180	0.199	0.281	0.213	0.053	0.042
C19	1900	0.000	0.000	0.943	0.989	0.137	0.101	0.068	0.090	0.228	0.197	0.131	0.073

Table S2: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C20	2000	0.000	0.000	0.037	0.039	0.000	0.000	0.000	0.000	0.027	0.013	0.000	0.000
9-C21:1	2073	0.000	0.000	0.115	0.101	0.000	0.000	0.000	0.000	0.046	0.027	0.000	0.000
C21	2100	0.000	0.000	2.137	1.301	1.426	1.186	0.626	0.735	1.118	0.528	1.206	0.689
C22:1	2173	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.013**	**0.016**	0.000	0.000
C22	2200	0.000	0.000	0.221	0.098	0.151	0.052	0.086	0.079	0.270	0.086	0.217	0.114
C23:2	2264	0.000	0.000	0.123	0.173	0.000	0.000	0.000	0.000	0.129	0.176	0.000	0.000
9-; 7-C23:1	2271	0.555	0.736	1.010	0.538	0.802	0.736	0.599	0.501	1.890	0.880	1.181	0.276
C23	2300	7.740	9.622	5.336	4.512	4.522	4.518	3.625	4.816	7.146	4.388	5.750	4.269
11-MeC23	2334	0.000	0.000	0.061	0.078	0.000	0.000	0.032	0.057	0.042	0.044	0.000	0.000
9-C24:1	2371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.107**	**0.066**	0.000	0.000
C24	2400	0.000	0.000	0.269	0.068	0.236	0.128	0.069	0.028	0.452	0.120	0.284	0.141
C25:2_1	2455	0.000	0.000	0.171	0.265	0.012	0.015	0.073	0.133	0.035	0.071	0.083	0.124
4-MeC24	2460	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.018**	**0.009**	0.000	0.000
C25:2_2	2465	0.000	0.000	0.043	0.038	0.078	0.157	0.000	0.000	0.105	0.076	0.000	0.000
9-; 7-C25:1	2471	1.269	1.941	0.722	0.345	1.091	1.251	0.549	0.602	1.735	1.209	0.599	0.632
C25	2500	7.888	10.901	4.160	1.682	6.135	4.483	2.783	0.836	9.359	4.760	5.967	4.708
13-; 11-MeC25	2533	0.175	0.315	0.355	0.415	0.142	0.057	0.304	0.529	0.255	0.327	0.358	0.359
5-MeC25	2547	0.000	0.000	0.031	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.033
C26:1_1	2571	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.078**	**0.033**	0.000	0.000

Table S2: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
3-MeC25	2571	0.000	0.000	0.028	0.024	0.010	0.013	0.027	0.032	0.000	0.000	0.042	0.029
9-C26:1	2574	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.092**	**0.056**	0.000	0.000
5, 15-diMeC25	2583	0.000	0.000	0.000	0.000	0.000	0.000	**0.061**	**0.102**	0.000	0.000	0.000	0.000
C26	2600	0.190	0.191	0.581	0.117	0.366	0.080	0.236	0.059	0.837	0.108	0.478	0.184
13-MeC26	2630	0.000	0.000	0.078	0.080	0.039	0.011	0.077	0.094	0.060	0.063	0.077	0.086
C27:2_1	2643	0.000	0.000	0.230	0.378	0.023	0.024	0.059	0.105	0.039	0.050	0.000	0.000
C27:2_2	2649	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.023**	**0.027**	0.000	0.000
4-MeC26	2660	0.000	0.000	**0.012**	**0.019**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9-C27:1	2671	0.201	0.268	0.343	0.071	0.350	0.258	0.238	0.114	1.519	0.933	0.351	0.314
7-C27:1	2678	0.055	0.077	0.314	0.157	0.108	0.077	0.054	0.016	0.933	0.787	0.098	0.068
C27	2700	12.933	4.429	15.776	3.668	10.761	1.993	10.104	3.531	19.477	3.589	13.316	3.680
13-; 11-MeC27	2727	1.866	3.246	2.889	3.679	1.209	0.910	2.027	3.295	1.420	2.345	3.477	4.438
7-MeC27	2738	0.000	0.000	0.118	0.150	0.000	0.000	0.087	0.132	0.000	0.000	0.000	0.000
5-MeC27	2747	0.000	0.000	0.047	0.063	0.000	0.000	0.045	0.065	0.000	0.000	0.062	0.080
11, 15-DiMeC27	2756	0.000	0.000	0.121	0.226	0.000	0.000	0.112	0.160	0.000	0.000	0.000	0.000
x, x-DiMeC27	2758	0.000	0.000	0.000	0.000	0.000	0.000	**0.086**	**0.124**	0.000	0.000	0.000	0.000
y, y-diMeC27	2762	0.000	0.000	0.000	0.000	0.000	0.000	**0.084**	**0.122**	0.000	0.000	0.000	0.000
z, z-diMeC27	2770	0.000	0.000	0.000	0.000	0.000	0.000	**0.118**	**0.155**	0.000	0.000	0.000	0.000
C28:1_1	2772	0.000	0.000	0.151	0.131	0.000	0.000	0.000	0.000	0.115	0.065	0.000	0.000

Table S2: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
3-MeC27	2772	0.000	0.000	0.000	0.000	0.026	0.034	0.000	0.000	0.000	0.000	0.021	0.026
C28:1_3	2781	0.000	0.000	0.288	0.324	0.000	0.000	0.000	0.000	0.156	0.065	0.000	0.000
5, 17-diMeC27	2780	0.000	0.000	0.000	0.000	0.000	0.000	**0.118**	**0.115**	0.000	0.000	0.000	0.000
C28	2800	0.421	0.207	1.136	0.236	0.525	0.123	0.459	0.216	0.699	0.130	0.579	0.353
14-; 13-MeC28	2830	0.000	0.000	0.335	0.451	0.116	0.115	0.300	0.415	0.129	0.255	0.438	0.641
C29:2_1	2847	0.000	0.000	0.025	0.020	0.000	0.000	0.000	0.000	0.038	0.031	0.000	0.000
C29:2_2	2853	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.136**	**0.086**	0.000	0.000
C29:2_3	2860	0.000	0.000	0.120	0.054	0.000	0.000	0.000	0.000	0.114	0.027	0.000	0.000
4-MeC28	2860	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.039	0.000	0.000	0.087	0.068
10-; 9-; 8-; 7-C29:1	2875	0.631	0.185	4.869	2.605	0.855	0.398	0.740	0.131	4.192	1.095	0.680	0.108
C29	2900	13.658	4.739	16.966	5.840	9.602	1.511	7.074	0.990	11.856	3.424	6.837	2.622
15- ; 13-MeC29	2928	2.069	3.428	1.294	3.195	1.622	1.500	3.093	4.182	0.514	0.311	4.580	6.964
13, 17-diMeC29	2951	0.000	0.000	0.000	0.000	0.000	0.000	0.195	0.252	0.086	0.102	0.000	0.000
11, 17-diMeC29	2958	0.000	0.000	0.000	0.000	0.000	0.000	0.414	0.516	0.202	0.276	0.000	0.000
C30:2	2959	0.000	0.000	**0.626**	**0.620**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10-; 9-; 8-; 7-C30:1	2976	0.000	0.000	0.146	0.130	0.118	0.067	0.246	0.083	0.391	0.227	0.000	0.000
5-,17-; 5,15-diMeC29	2980	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.058**	**0.106**	0.000	0.000
C30:1	2983	0.000	0.000	0.362	0.212	0.000	0.000	0.072	0.040	0.000	0.000	0.000	0.000
C30	3000	0.247	0.140	0.494	0.150	0.347	0.050	0.247	0.032	0.305	0.091	0.332	0.063

Table S2: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
15-; 14-MeC30	3030	0.000	0.000	0.205	0.216	0.053	0.078	0.154	0.215	0.058	0.122	0.249	0.360
C31:2_1	3051	0.000	0.000	0.000	0.000	0.429	0.228	0.000	0.000	0.267	0.102	0.000	0.000
C31:2_2	3057	0.079	0.062	2.181	1.791	0.000	0.000	0.000	0.000	0.860	0.517	0.206	0.087
10-; 9-; 8-C31:1	3075	10.199	4.628	10.752	5.823	11.378	3.335	9.924	3.786	12.345	4.861	6.134	3.266
C31	3100	11.501	5.855	6.727	3.540	8.826	1.386	6.147	2.135	5.313	2.579	7.316	4.155
15-; 13-MeC31	3125	1.113	1.687	2.045	2.397	1.498	1.027	2.189	2.337	0.829	1.108	3.151	3.946
11, 17-diMeC31	3149	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.457**	**0.303**	0.000	0.000
13, 17-diMeC31	3150	0.000	0.000	0.000	0.000	0.000	0.000	**0.736**	**0.349**	0.000	0.000	0.000	0.000
C32:2_1	3150	0.000	0.000	**0.542**	**0.467**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C32:2_2	3158	0.000	0.000	0.000	0.000	0.210	0.271	0.000	0.000	0.119	0.206	0.090	0.133
10-; 9-C32:1	3173	0.517	0.330	0.562	0.331	1.005	0.406	1.172	0.416	0.343	0.307	0.749	0.379
C32	3200	0.000	0.000	0.226	0.243	0.270	0.300	0.232	0.117	0.176	0.083	0.285	0.148
C33:2_1	3249	0.872	0.545	0.000	0.000	3.329	1.016	4.565	2.388	2.314	1.772	1.850	2.087
C33:2_2	3257	0.000	0.000	**1.852**	**1.175**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C33:2_3	3265	0.000	0.000	**0.606**	**0.564**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10-C33:1	3278	22.528	11.631	6.226	2.871	23.930	8.950	26.130	13.351	6.975	3.279	23.700	13.826
C33	3300	2.456	1.694	1.033	0.443	2.993	0.801	2.862	0.919	1.122	0.655	2.771	1.614
17-; 15-; 13-MeC33	3327	0.000	0.000	1.307	1.777	1.224	1.185	2.642	2.111	0.602	0.489	2.014	1.630
13, 19-diMeC33	3349	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.343**	**0.158**	0.000	0.000

Table S2: *(continued)*

		A. m. carnica		*A. m. iberiensis*		*A. m. ligustica*		*A. m. macedonica*		*A. m. mellifera*		*A. m. ruttneri*	
Compound	RI	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
C35:2_1	3449	0.223	0.227	1.439	3.113	1.895	0.640	3.464	1.186	0.610	0.294	2.041	1.484
12-; 10-C35:1	3470	0.614	0.443	0.449	0.231	1.921	0.616	3.770	1.477	0.495	0.149	2.129	1.642
C35	3500	0.000	0.000	0.000	0.000	0.000	0.000	**0.406**	**0.218**	0.000	0.000	0.000	0.000
19-; 17-; 15-MeC37	3722	0.000	0.000	0.000	0.000	0.000	0.000	**0.193**	**0.153**	0.000	0.000	0.000	0.000

Table S3: One-way permutational test of multivariate homogeneity of group dispersions contrasting the variance in the CHC profiles between task performance groups. The permutations (n=1000) were restricted to the Subspecies. Df - degrees of freedom; SS - sum of squares; MS - mean of squares; F - F-value; SES - standard effect size.

	Df	SS	MS	F	SES	p-value
Nurses - Foragers	1	0.15	0.150	18.291	14.983	0.001
Residual	118	0.97	0.008			
Total	119	1.12	0.159			

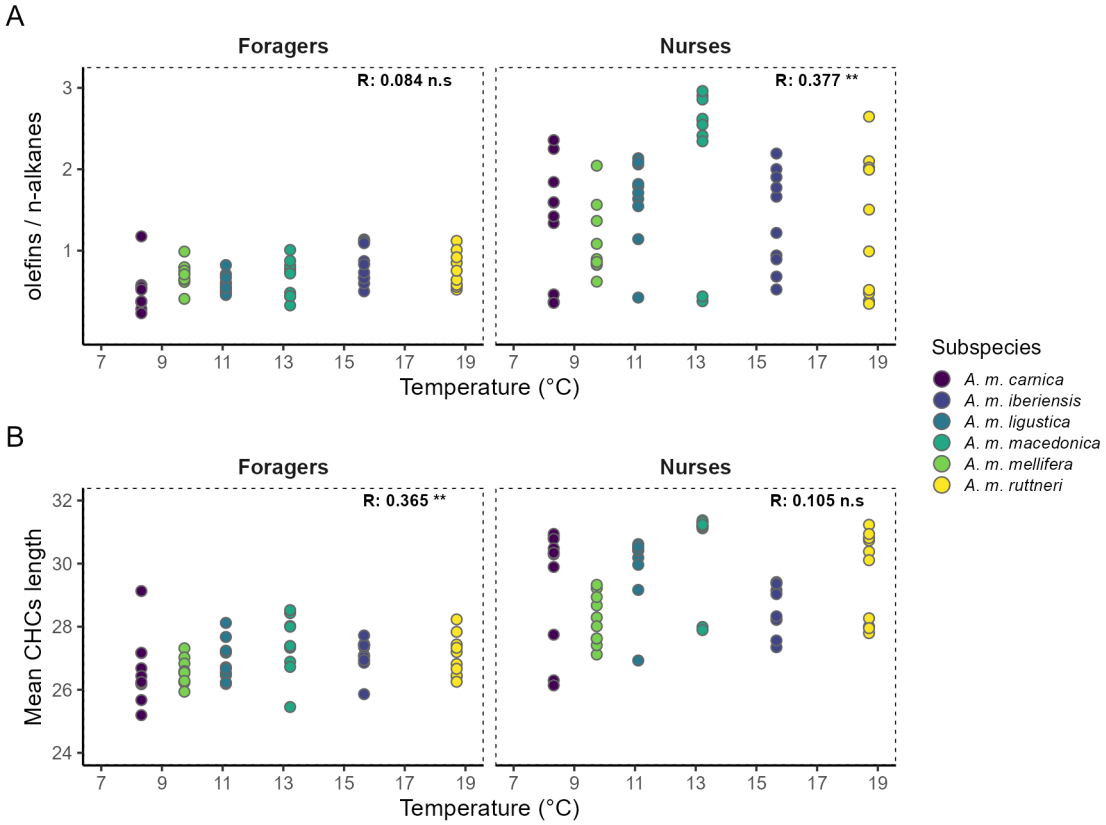

Figure S1: CHC composition vs temperature. A) Spearman's correlation (R) between the olefins to n-alkanes ratio in the CHC profile of worker bees of different *A. mellifera* subspecies and the average temperature (°C) of the country of origin of the honey bee queens. B) Spearman's correlation (R) between the weighted mean chain length of hydrocarbons in the CHC profile of worker bees of different *A. mellifera* subspecies and the average temperature (°C) of the country of origin of the honey bee queens. Significance of the correlation is indicated as n.s (p-value 0.05), * (p-value < 0.05), ** (p-value < 0.01), *** (p-value < 0.001)

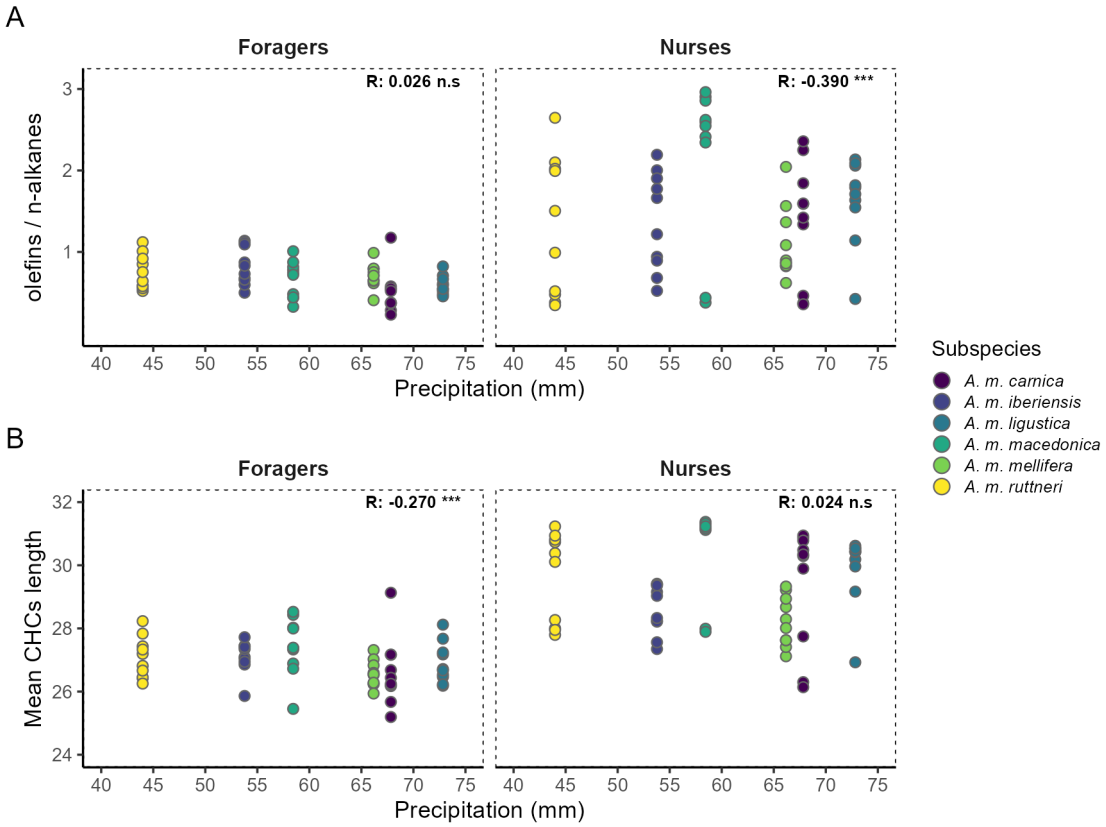

Figure S2: CHC composition vs precipitation A) Spearman's correlation (R) between the olefins to n-alkanes ratio in the CHC profile of worker bees of different *A. mellifera* subspecies and the average precipitation (mm) of the country of origin of the honey bee queens. B) Spearman's correlation (R) between the weighted mean chain length of hydrocarbons in the CHC profile of worker bees of different *A. mellifera* subspecies and the average precipitation (mm) of the country of origin of the honey bee queens. Significance of the correlation is indicated as n.s (p-value 0.05), * (p-value < 0.05), ** (p-value < 0.01), *** (p-value < 0.001)