

Supplemental Material (S1-S9): Cuticular hydrocarbons of alpine bumble bees (Hymenoptera: *Bombus*) are species-specific, but show little evidence of elevation-related climate adaptation

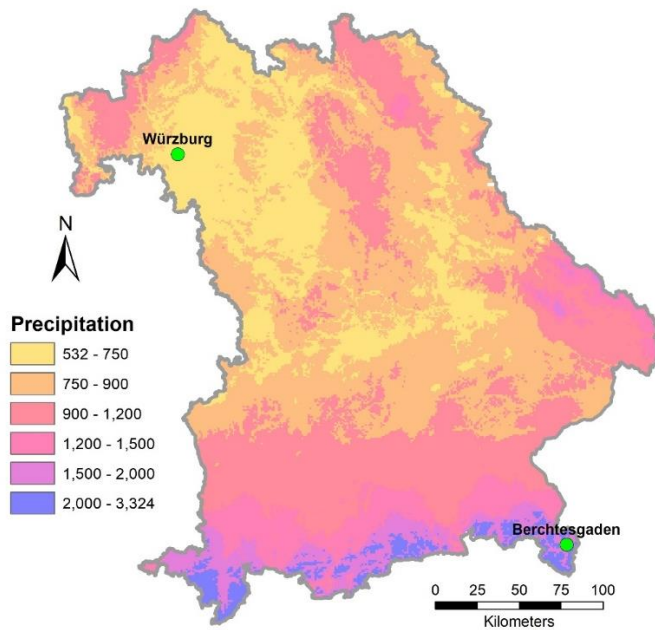
Fabienne Maihoff, Simone Sahler, Simon Schoger, Kristof Brenzinger, Katharina Kallnik, Nikki Sauer, Lukas Bofinger, Thomas Schmitt, Sabine S. Nooten and Alice Classen

S1 Definition of the distribution of species within the study region

Species distribution was extracted from a previous study of intensive wild bee monitoring in 2009 (Maihoff et al., 2022), which was conducted in the same region and on additional sites. The elevational gradient ranged from 641 m to 2032 m above sea level (m.a.s.l.). 33 grassland sites were selected (60 x 60 m) along 5 slopes in 2009. 18 were extensively managed (grazed by livestock or mowed once in late summer) and 15 were not managed (abandoned during the last century or above the treeline). Wild bees and honeybees were recorded from May to September, encompassing approximately the full foraging season. Monitoring was conducted in standardized 50 min- transect walks, repeated six times in lower elevations (<1200 m.a.s.l.) and, due to a shorter season, five times in higher elevations. Monitoring took place from 9:30 to 18:00 when the weather was sunny or when temperature at 650 m.a.s.l. was above 17 °C on cloudy days. We used sweep nets to catch the insects for later morphological determination to species level. Note that even though we did not identify species with molecular tools in this monitoring, we are confident that this data reflects the distribution of *B. lucorum*, given that in another data set of the same year we detected only one male of *B. terrestris* among 117 analyzed samples (Brenzinger et al. 2022). To calculate the mean elevational distribution of each species based on standardized abundance data of workers.

S2 Precipitation in study region

Multi-annual means of precipitation (Supplemental Figure 1) and air temperature for the entire region Bavaria are obtained from DWD Climate Data Center (CDC). Multi-annual means refer to a 30 year mean at a spatial resolution of 1km x 1km.



Supplemental Figure 1 Precipitation characteristics within the translocation experiment. Climate regions differed substantially in annual precipitation. Multi-annual (30 years) mean precipitations (in mm) are presented.

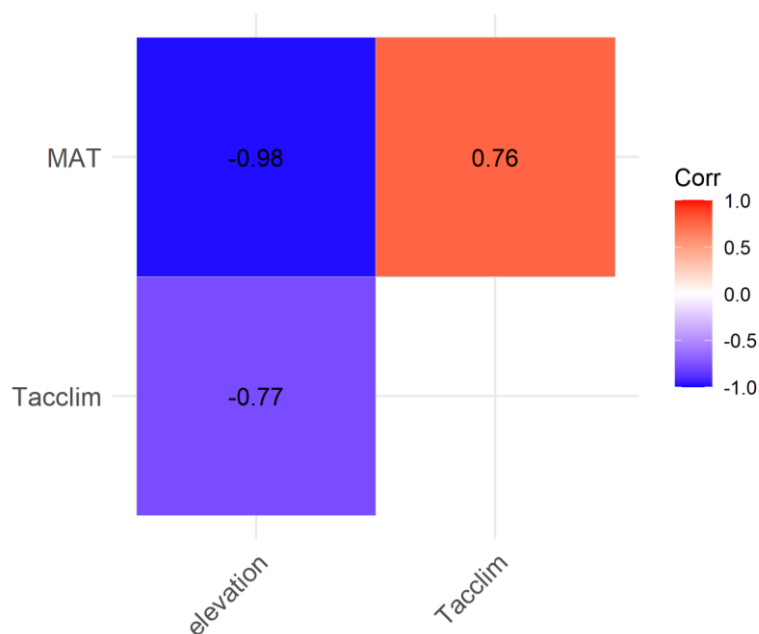
S3 Prediction of temperature data

We first predicted daily mean temperatures between 01.01.2009 and 31.12.2020 for all study sites using hourly temperature data from all available, adjacent climate stations (Supplemental Table 1). As the number of climate stations increased over the decade, we fitted generalized additive models (GAMs) with a smooth term of elevation and day lengths in minutes (as a proxy for season), and date as a factor for each year separately. Deviance explained by the models ranged between 96 and 99%. Elevations for climate stations and study site centers were extracted from a digital elevational model with 1-m resolution, provided by the National Park Berchtesgaden. Slope and exposition, as further potential predictors of temperature, were excluded from the models, as they turned out to reduce the explanatory power; likely because some climate stations are installed on wind-exposed sites like mountain summits, where exposition and slope are less representative. We predicted hourly temperature for each study site for 2019 and 2020, using hourly temperature data from all available climate stations. Due to the large amount of data, we fitted GAMs with a smooth term of elevation and day lengths in minutes (as a proxy for season), and a combined factor for date and hour for each month of 2019 and 2020 separately. The explained deviances of the GAM model predicting hourly temperature data ranged between 83 and 94 percent. From the hourly temperature data, we calculated mean annual temperature and the mean temperature seven days before sampling.

Supplemental Table 1 All climate stations used for temperature calculation per site are presented. Given are the elevation, the name of the climate station, the name of the location and the coordinates (Lat = latitude and Lon = longitude)

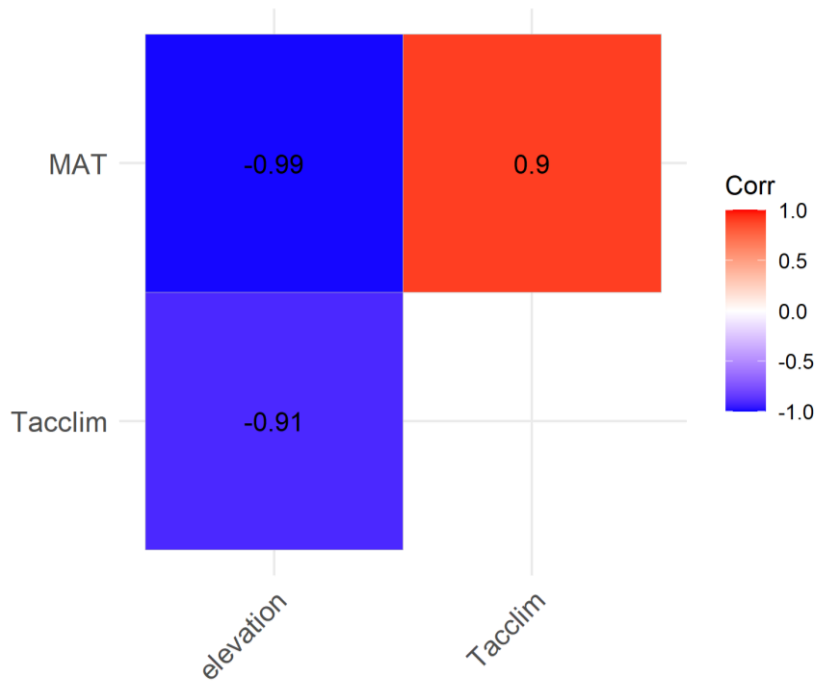
elevation	climatestation_name	name of location	Lat	Lon
913	Alpelwand	Alpelwand	47.577	12.903
1086	Bindalm	Bindalm	47.569	12.8
1650	Blaueishuette	Blaueishuette	47.586	12.87
1240	Brunftbergtiefe	Brunftbergtiefe	47.546	12.878
2520	Funtenseetauern	Kuehroint-Funtensee	47.487	12.971
2493	Hinterberghorn Gipfel	Hinterberghorn	47.554	12.838
839	Hinterseeau	Hinterseeau	47.586	12.825
1991	Hirsch Gipfel	Steinernes Meer	47.498	12.92
660	Hoellgraben	Jenner	47.616	13.006
1200	Jenner	Jenner	47.587	13.019
1670	Reiteralpe Plateau	Reiteralpe	47.651	12.805
2155	Schlunghorn Schneestation	Schlunghorn	47.549	13.045
1764	Trischuebel Schneestation	Trischuebel	47.526	12.911
2635	Watzmanngrat	Watzmann	47.555	12.922
1928	Watzmannhaus	Watzmann	47.571	12.933
1615	Reiteralpe Wartsteinhuette	Reiteralpe	47.65	12.81
1755	Reiteralpe Wartsteinkopf Gipfel	Reiteralpe	47.651	12.804
1420	Kuehroint	Kuehroint	47.571	12.96
272	Würzburg Sieboldshöhe	Würzburg	49.771	9.958

S4 Temperature data correlation in approach 1



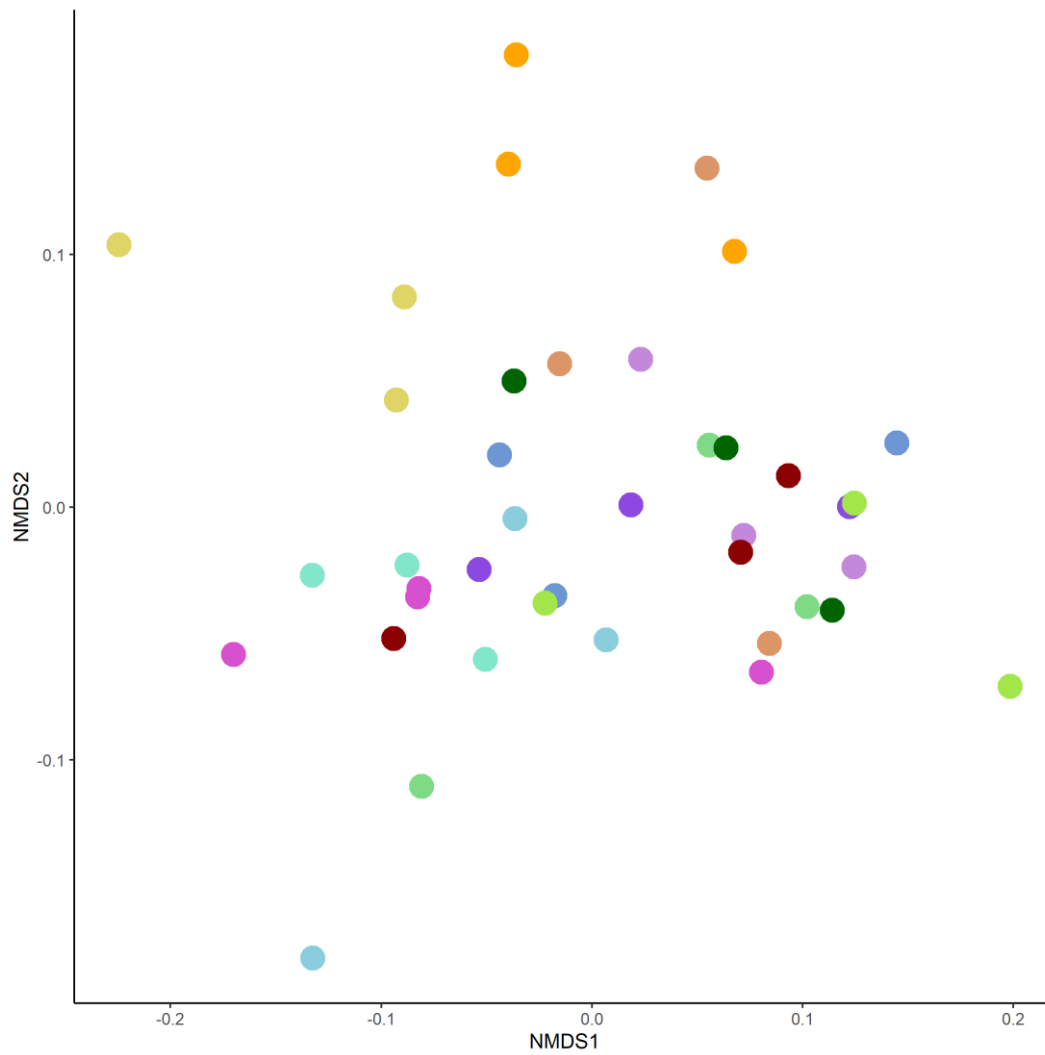
Supplemental Figure 2 Correlation of elevation, MAT and acclimatization temperature (Tacclim) in the dataset of study questions one, where we compared species with distinct distributions long the elevational gradient

S5 Temperature data correlation in approach 2



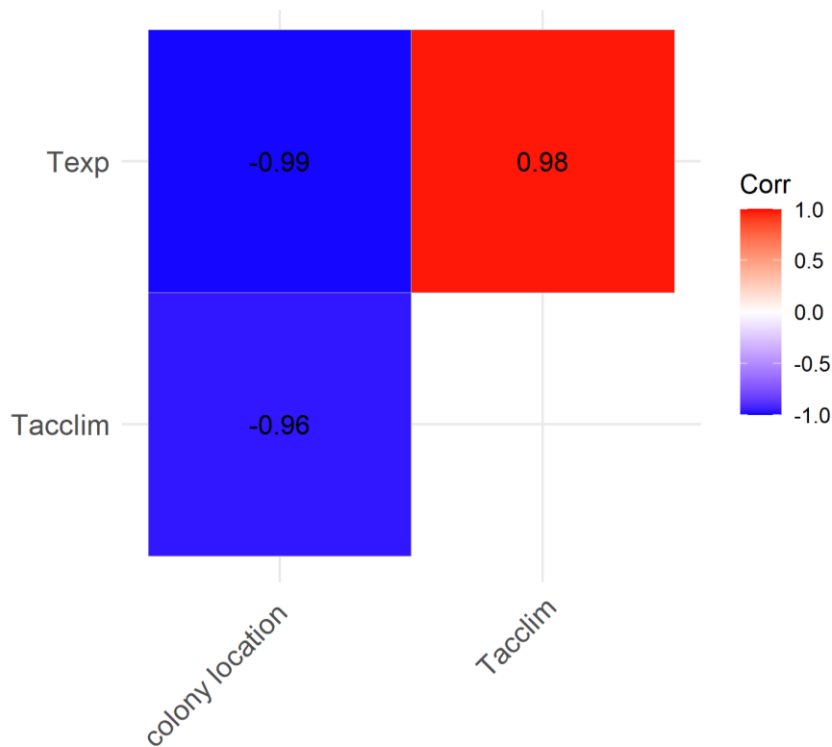
*Supplemental Figure 3 Correlation of elevation, MAT and acclimatization temperature (Tacclim) in the dataset of study questions two, where worker of *B. pascuorum* were sampled along the elevational gradient.*

S6 CHC- profile similarity by colony affinity



Supplemental Figure 4 NMDS Similarity of CHC profiles of individuals displayed in a two-dimensional graph by non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances. Dots represent CHC profiles of individuals. Individuals from the same colony are displayed in the same shape. The closer the dots, the more similar the CHC profile.

S7 Temperature data correlation in approach 3



Supplemental Figure 5 Correlation of colony location (expressed as elevation), exposed temperature during the period of the experiment (Texp) and acclimatization temperature (Tacclim) in the dataset of approach 3, where *B. lucorum*, was settled in different climatic regions and along the mountain gradient.

S8 Pairwise comparison of CHC profile composition between species

Supplemental Table 2. Pairwise PERMANOVA to reveal differences in the CHC profile composition between species pairs

Pairs	Df	SumsOfSqs	F-Model	R ²	p-value
<i>B. lucorum</i> vs <i>B. wurflenii</i>	1	0.931	69.750	0.813	0.001
<i>B. lucorum</i> vs <i>B. mucidus</i>	1	0.147	7.449	0.305	0.001
<i>B. lucorum</i> vs <i>B. monticola</i>	1	0.410	24.993	0.555	0.001
<i>B. lucorum</i> vs <i>B. pascuorum</i>	1	0.662	59.762	0.759	0.001
<i>B. lucorum</i> vs <i>B. soroeensis</i>	1	0.798	46.989	0.734	0.001
<i>B. wurflenii</i> vs <i>B. mucidus</i>	1	1.125	60.276	0.801	0.001
<i>B. wurflenii</i> vs <i>B. monticola</i>	1	0.414	27.303	0.603	0.001
<i>B. wurflenii</i> vs <i>B. pascuorum</i>	1	1.280	140.088	0.892	0.001
<i>B. wurflenii</i> vs <i>B. soroeensis</i>	1	1.562	100.352	0.870	0.001
<i>B. mucidus</i> vs <i>B. monticola</i>	1	0.665	32.049	0.628	0.001
<i>B. mucidus</i> vs <i>B. pascuorum</i>	1	0.637	41.393	0.697	0.001
<i>B. mucidus</i> vs <i>B. soroeensis</i>	1	0.595	26.807	0.626	0.001
<i>B. monticola</i> vs <i>B. pascuorum</i>	1	1.125	87.564	0.807	0.001
<i>B. monticola</i> vs <i>B. soroeensis</i>	1	1.048	57.259	0.751	0.001
<i>B. pascuorum</i> vs <i>B. soroeensis</i>	1	1.138	88.826	0.832	0.001

S9 Average CHC-profile by species

Supplemental Table 3 Mean proportion of components per species in the total profile of the species and standard derivation (SD). Note that if the exact position of the double bond is not given, alkenes with the same chain length but different retention indices are numbered.

component	<i>B. lucorum</i>		<i>B. wurfleinii</i>		<i>B. mucidus</i>		<i>B. monticola</i>		<i>B. pascuorum</i>		<i>B. soroensis</i>	
	mean (%)	SD	mean (%)	SD	mean (%)	SD	mean (%)	SD	mean (%)	SD	mean (%)	SD
9-C21ene	--	--	--	--	--	--	0.09	0.11	--	--	--	--
7-C21ene	--	--	0.44	0.28	--	--	1.98	1.12	--	--	--	--
C21	1.31	1.63	2.66	2.42	0.35	0.68	1.84	2.15	0.52	0.91	0.42	0.36
7-C22ene	--	--	0.14	0.14	--	--	0.51	0.60	--	--	--	--
5-C22ene	--	--	--	--	--	--	0.26	0.25	--	--	--	--
C22	0.15	0.14	0.41	0.14	0.09	0.09	0.38	0.42	0.20	0.10	0.19	0.20
x,y-C23diene	--	--	--	--	--	--	0.02	0.06	--	--	--	--
9-C23ene	1.62	1.31	0.14	0.10	0.39	0.24	5.19	1.16	1.03	0.47	0.85	0.56
7-C23ene	0.33	0.20	8.65	2.36	0.10	0.20	5.53	1.15	0.20	0.20	--	--
5-C23ene	0.02	0.05	0.51	0.17	--	--	0.14	0.16	--	--	--	--
C23	8.53	2.46	17.17	3.11	6.89	1.82	13.00	3.29	11.28	1.58	9.06	3.29
11-; 9-MeC23	0.06	0.08	--	--	--	--	--	--	--	--	0.72	0.51
9-C24ene	0.17	0.15	--	--	0.07	0.15	0.23	0.12	0.82	0.88	--	--
7-C24ene	0.02	0.05	0.62	0.12	--	--	0.20	0.04	--	--	--	--
C24	0.35	0.08	0.71	0.15	0.28	0.09	0.56	0.28	0.66	0.13	0.26	0.15
x,y-C25diene1	--	--	0.03	0.07	0.02	0.07	0.06	0.12	--	--	--	--
x,y-C25diene2	--	--	--	--	--	--	0.04	0.10	--	--	--	--
12-C25ene	--	--	0.03	0.06	--	--	0.06	0.19	--	--	--	--
9-C25ene	7.64	2.22	0.66	0.37	4.59	2.77	6.24	1.42	25.85	3.38	5.93	2.81
7-C25ene	1.90	0.61	19.12	2.17	1.15	1.28	6.40	1.07	1.16	0.37	0.05	0.09
5-C25ene	0.09	0.14	0.79	0.22	0.02	0.05	0.18	0.12	0.10	0.09	--	--
C25	12.89	2.26	16.14	3.59	13.93	4.69	14.40	3.63	15.21	3.09	8.02	3.69
13-; 11-; 9-MeC25	--	--	--	--	--	--	0.17	0.20	--	--	1.07	0.84
7-MeC25	--	--	--	--	--	--	--	--	--	--	0.52	0.24
9-C26ene	0.43	0.55	0.18	0.09	0.30	0.13	0.33	0.32	0.95	1.04	--	--
x-C26ene	0.19	0.15	--	--	0.11	0.18	--	--	--	--	--	--
C26	0.68	0.34	0.33	0.08	0.61	0.16	0.65	0.18	0.53	0.18	0.76	0.16
x,y-C27diene1	--	--	--	--	0.28	0.16	0.09	0.17	--	--	--	--
x,y-C27diene2	--	--	--	--	0.06	0.13	0.05	0.09	--	--	--	--
13-C27ene	0.20	0.44	0.01	0.03	--	--	0.14	0.24	--	--	0.02	0.07
9-C27ene	7.54	2.13	0.47	0.54	13.18	3.85	2.03	0.95	10.15	1.77	1.42	0.61
7-C27ene	5.66	1.22	3.92	0.91	2.60	1.84	4.77	1.39	0.59	0.25	0.10	0.17
5-C27ene	0.02	0.08	0.10	0.14	0.05	0.16	0.11	0.19	0.01	0.04	--	--
C27	13.93	3.02	7.30	1.33	19.25	3.85	14.37	3.44	5.84	1.32	21.88	4.40
13-; 11-; 9-MeC27	--	--	--	--	--	--	--	--	--	--	1.49	1.19
7-MeC27	--	--	--	--	--	--	--	--	--	--	0.18	0.11
9-C28ene	0.54	0.29	0.10	0.28	0.76	0.52	0.47	0.40	0.30	0.25	--	--
x-C28ene	0.25	0.32	0.12	0.11	--	--	--	--	--	--	--	--
C28	0.57	0.27	0.34	0.08	0.59	0.15	0.57	0.24	0.56	0.37	0.76	0.26
x,y-C29diene1	--	--	--	--	0.17	0.13	--	--	--	--	--	--
x,y-C29diene2	--	--	--	--	0.26	0.17	--	--	--	--	--	--
13-; 11-C29ene	0.12	0.24	0.10	0.11	0.28	0.34	0.17	0.17	--	--	0.08	0.10
9-C29ene	10.47	2.56	0.14	0.13	12.53	3.96	1.09	0.72	6.01	1.05	5.18	1.21
7-C29ene	4.45	1.28	2.47	0.85	1.95	1.76	2.34	0.68	0.26	0.25	0.33	0.53
C29	11.56	3.24	8.17	1.43	10.98	2.92	10.22	2.45	4.64	1.13	9.10	3.48
15-; 13-; 11-MeC29	--	--	--	--	--	--	--	--	--	--	1.68	1.13
13-; 11-C30ene	0.19	0.28	--	--	--	--	--	--	--	--	--	--
9-C30ene	0.27	0.58	0.10	0.22	0.24	0.17	0.04	0.10	0.27	0.09	--	--
7-C30ene	0.03	0.11	--	--	0.05	0.08	0.11	0.19	--	--	--	--
5-C30ene	--	--	--	--	0.16	0.27	--	--	--	--	--	--
C30	0.29	0.21	0.33	0.25	0.19	0.14	0.44	0.29	0.40	0.26	--	--
x,y-C31diene1	--	--	--	--	0.10	0.16	--	--	--	--	0.33	0.29
x,y-C31diene2	0.05	0.13	--	--	0.46	0.86	--	--	--	--	0.26	0.16
x,y-C31diene3	--	--	--	--	--	--	--	--	--	--	0.89	0.44
13-C31ene	--	--	0.04	0.08	0.20	0.25	0.47	0.37	--	--	0.46	0.11
9-C31ene	2.18	1.02	1.24	0.94	4.57	3.32	0.69	0.39	5.62	1.02	19.97	2.94
7-C31ene	1.33	0.49	--	--	0.07	0.22	--	--	--	--	--	--
C31	3.97	1.55	4.51	1.21	2.11	1.37	3.38	1.66	3.37	0.60	1.30	0.27
15-; 13-MeC31	--	--	--	--	--	--	--	--	--	--	0.40	0.41
x-C32ene	--	--	0.90	1.27	--	--	--	--	--	--	--	--
C32	--	--	0.90	0.88	--	--	--	--	0.86	0.40	--	--
x,y-C33diene1	--	--	--	--	--	--	--	--	--	--	3.93	1.65
x,y-C33diene2	--	--	--	--	--	--	--	--	--	--	0.63	0.62
9-C33ene	--	--	--	--	--	--	--	--	1.52	0.75	1.76	0.49
C33	--	--	--	--	--	--	--	--	1.05	0.68	--	--