The influence of slavemaking lifestyle, caste and sex on chemical profiles in *Temnothorax* ants: Insights into the evolution of cuticular hydrocarbons

– ELECTRONIC SUPPLEMENTARY MATERIAL –

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Running title: *Cuticular hydrocarbon evolution in ants*

S1: Details on composition of the *Temnothorax* ant community and host species preference

We collected colonies of *T. americanus* with *T. longispinosus* slaves in NY (*N* = 100) and WV (*N* = 56), and with *T. curvispinosus* slaves in OH (*N* = 30). Slavemaker colonies of *T. duloticus* with *T. curvispinosus* slaves (*N* = 100) were collected in southern OH. 42 colonies of the slavemaker *T. pilagens* with either *T. longispinosus* (*N* = 19) or *T. ambiguus* (*N* = 8) slaves or both slave species (*N* = 15) were gathered in MI. We collected on average 47.38 ± SD 32.62 colonies of the non-parasitic species from the same locations as their slavemaker (Table S1). In NY, *T. americanus* occurs with *T. longispinosus* (95%) and *T. ambiguus* (5%; Table S1), but mainly parasitizes the former (Herbers & Foitzik 2002; Foitzik et al. 2009). In West Virginia, *T. longispinosus* and *T. curvispinosus* co-occur, and again *T. americanus* focuses on *T. longispinosus* (Herbers & Foitzik 2002). In southern OH, two slavemaker species, *T. duloticus* and *T. americanus*, are sympatric, and both of them parasitize *T. curvispinosus* (Johnson & Herbers 2006). *T. longispinosus* occurs at low frequencies there and is occasionally exploited by *T. americanus*. *Temnothorax pilagens* from Michigan preferentially uses *T. ambiguus* as its host (Kleeberg et al. 2016), though *T. longispinosus* is sporadically enslaved.

S2: Details on the temperature program for chemical analyses

Ant CHCs were extracted by individually immersing freshly frozen ants in approximately 0.5 ml hexane for 10 min. The extracts were analysed using the GC-MS equipped with a HP5-MS column (30 m x 0.25 mm; coating: 0.25 µm). Sample injection (5µl) was splitless over 2 min at 320°C. Helium was used as carrier gas at a constant flow of 1.2 ml/min. Oven temperature started at 150°C for 3 min, followed by a temperature increase to 300°C in 2 steps (150–250°C with 30°C/min and 250–300°C with 2°C/min). The final temperature of 300°C was held constant for 2 min. After an initial solvent delay of 5 min, a mass range of 40–500 amu was scanned with an ionization voltage of 70 eV. The transfer line was held constant at 320 °C.

Table S1: Collection sites and details for *Temnothorax* colonies. Slavemaker species are listed in bold.

Study site and species	Coordinates	*N* of collected / sampled colonies	*N* of analysed samples from original colonies			*N* of analysed samples that emerged in isolation		
			Workers	Virgin queens	Males	Workers	Virgin queens	Males
T. americanus								
New York	42°31'57.0''N 74°08'45.2''W	100 / 31	17	20	19	2	16	3
West Virginia	38°06'28.8''N 80°07'52.9''W	56 / 12	14	3	9	9	1	0
Ohio	40°14'13.8''N 82°59'06.5''W	30 / 23	3	17	6	1	0	0
T. longispinosus								
New York	42°31'57.0''N 74°08'45.2''W	21 / 13	11	8	17	24	22	14
West Virginia	38°06'28.8''N 80°07'52.9''W	92 / 15	15	10	11	3	11	0
Ohio	40°14'13.8''N 82°59'06.5''W	19 / 9	6	1	4	20	1	2
Michigan	43°26'24.6''N 72°09'20.8''W	88 / 17	13	8	17	17	2	6
T. duloticus								
Ohio	40°14'13.8''N 82°59'06.5''W	30 / 15	11	9	9	16	17	10
T. curvispinosus								
Ohio	40°14'13.8''N 82°59'06.5''W	65 / 13	8	3	3	14	0	10
West Virginia	38°06'28.8''N 80°07'52.9''W	35 / 10	11	4	5	9	0	1
T. pilagens								
Michigan	43°26'24.6''N 72°09'20.8''W	42 / 29	27	6	16	2	0	0
T. ambiguus								
Michigan	43°26'24.6''N 72°09'20.8''W	54 / 14	5	9	13	3	1	5
New York	42°31'57.0''N 74°08'45.2''W	5 / 5	7	2	6	0	0	0

Table S2: Mean relative abundances and standard deviations of all 35 identified substances per species and caste, based on samples that emerged in their original colonies. Putative recognition substances are given in bold. We did not detect 3-MeC31, a putative recognition substance recently identified in *T. longispinosus* (Jongepier & Foitzik 2016). Species names are abbreviated as follows: **TAM = *T. americanus***, **TD = *T. duloticus***, **TP = *T. pilagens***, TL = *T. longispinosus*, TC = *T. curvispinosus*, TA = *T. ambiguus*. Slavemaker species are given in bold.

Substance	Workers						Virgin queens						Males					
	TAM	TL	**TD**	TC	**TP**	TA	**TAM**	TL	**TD**	TC	**TP**	TA	**TAM**	TL	**TD**	TC	**TP**	TA
nC23	0.8±1.3	0.7±0.7	0.4±0.3	1.0±2.0	0.8±0.6	1.3±1.6	2.8±2.0	0.9±1.0	2.1±0.9	0	0.3±0.4	0.3±0.6	1.9±1.3	0.3±0.7	2.9±1.8	0.6±0.5	3.5±2.6	1.2±1.5
nC24	0.1±0.3	0.2±0.3	0.3±0.2	0.4±0.7	0.8±0.6	1.1±1.6	0.7±1.5	0	0.6±0.9	0	0.2±0.2	0.0±0.1	0.6±0.6	0	0.3±0.6	0.7±0.6	1.6±2.4	0.5±0.9
nC25	6.6±6.9	3.5±1.4	7.1±3.1	8.4±9.7	4.1±3.1	3.9±4.2	18.1±9.8	5.9±5.1	6.9±2.6	2.2±3.2	2.3±1.8	2.3±1.7	21.4±10.2	1.2±5.9	8.7±3.1	4.7±4.5	11±6.9	2.9±1.8
9;11;13 MeC25	0.5±0.9	0.3±0.5	1.5±1.0	0.7±0.6	0.7±0.7	0.3±0.6	1.9±1.3	0.6±0.8	1.5±1.0	0.8±0.5	0.3±0.4	0.4±1.2	0.9±0.8	0.2±0.4	1.3±1.3	0.5±0.6	0.6±0.9	0.4±0.7
5;7 MeC25	0.2±0.5	0.5±0.6	1.2±1.0	0.8±0.9	0.9±1.2	0.7±0.9	0.7±0.6	0	1.2±0.5	1.5±1.0	0	0.3±0.6	0.3±0.4	0.3±0.7	0.7±0.9	0.7±0.6	0.9±1.1	0.7±0.8
3 MeC25	0.3±0.5	1.4±2.9	0.9±1.1	1.1±1.0	6.9±16.5	2.1±4.8	1.2±0.8	1.0±1.1	2.5±1.0	1.0±0.6	0.3±0.2	0	1.0±0.9	0.8±0.7	3.7±3.0	0.7±0.6	0.1±0.3	1.7±3.8
nC26	1.3±0.7	0.3±0.3	1.0±0.7	0.6±0.5	0.8±0.5	0.3±0.5	1.3±1.2	0	1.1±0.5	0	0.5±0.1	0.3±0.5	1.5±1.0	0	1.6±1.0	0.9±0.4	2.2±2.5	0.4±0.6
nC27	30.4±13.4	13.6±7.0	22.2±12.0	9.7±7.4	17.9±10.2	9.2±5.4	21.3±8.3	20.2±10.5	18.5±7.3	30.9±13.3	23.9±12.3	16.5±9.4	33.4±10.6	35.3±17.2	25.6±8.1	31.5±7.8	27.5±11.1	17.8±11.0
11;13;15 MeC27	2.7±2.1	3.3±1.6	5.9±5.0	3.6±1.5	0.7±0.4	2.2±1.1	5.0±1.9	5.3±2.6	7.2±2.9	1.0±0.7	0.4±0.4	2.3±2.3	3.8±2.3	1.3±1.3	4.7±1.7	2.1±1.1	2.8±1.4	2.1±1.4
7 MeC27	1.3±1.0	1.1±0.5	1.2±0.7	1.2±0.7	0.8±0.7	0.6±0.8	2.2±0.9	1.5±0.8	2.0±0.9	5.3±2.1	0.3±0.2	0.7±0.9	1.5±0.9	0.1±0.4	1.7±1.0	0.4±0.5	1.1±1.1	0.8±0.7
5 MeC27	**3.2±2.3**	**3.9±1.0**	**4.6±2.7**	**3.7±2.2**	**1.3±0.9**	**4.2±1.8**	**4.8±1.5**	**5.1±2.0**	**6.4±1.5**	**0.2±0.5**	**0.9±0.5**	**2.1±1.6**	**5.3±1.8**	**0.9±2.1**	**8.3±2.4**	**3.2±1.7**	**6.1±2.3**	**4.1±1.8**
11,15 DiMeC27	0.3±0.4	0.3±0.4	0.2±0.3	0.4±0.6	0.4±0.4	1.1±2.6	0.2±0.4	0.4±0.7	0.6±1.2	8.0±3.1	4.0±8.8	0	0.4±0.8	5.6±3.4	0	0.4±0.8	0.4±0.9	1.3±3.3
3 MeC27	**4.5±1.9**	**4.4±1.5**	**5.0±1.1**	**4.4±2.9**	**1.5±0.6**	**4.1±1.9**	**4.4±1.3**	**5.3±2.0**	**8.0±2.3**	**0.8±1.0**	**1.6±0.6**	**2.7±1.2**	**6.2±1.8**	**4.5±10**	**11.3±3.6**	**5.9±4.1**	**6.9±2.4**	**7.0±5.1**
nC28	0.8±0.7	0.3±0.3	0.9±0.7	0.3±0.7	1.9±1.0	0.4±0.6	0.5±0.9	0	0.2±0.4	0	2.0±0.8	0.9±1.1	0.5±0.7	0	0.8±1.7	1.6±1.4	1.2±1.6	0.2±0.4
4 MeC28	**0.9±1.1**	**1.3±0.6**	**1.2±0.8**	**1.8±1.2**	**0.3±0.6**	**0.9±1.3**	**0.7±1.0**	**1.3±1.5**	**1.1±1.2**	**5.1±2.9**	**1.1±0.3**	**0.4±0.7**	**0.9±1.1**	**3.6±1.9**	**0.7±1.1**	**2.0±2.4**	**0.4±0.9**	**1.2±1.5**
nC29	6.2±4.4	2.5±1.0	4.5±2.5	2.0±1.0	16.4±11.2	2.2±1.4	1.7±0.9	2.6±1.5	2.3±1.2	8.2±3.8	14.6±6.7	5.6±4.7	2.2±1.7	7.6±3.1	3.7±1.5	3.7±2.9	5.2±1.3	4.2±1.4
11;13;15;17 MeC29	5.3±4.0	10.0±5.9	10.6±6.3	10.6±5.3	3.7±2.0	15.5±5.9	7.3±3.1	11.0±4.3	12.9±5.3	5.7±3.3	2.9±1.0	13±4.2	4.5±3.4	5.2±3.2	7.3±4.7	5.3±2.3	5.4±3.7	14.4±7.5
7 MeC29	2.5±1.6	5.7±2.3	3.5±1.6	4.5±1.7	2.3±2.1	4.1±2.0	2.8±1.0	6.2±3.2	4.7±1.8	2.7±1.3	1.4±0.7	3.5±1.6	2.6±1.5	3.5±3.4	5.7±1.6	3.3±1.0	4.5±2.8	3.8±2.0
5 MeC29	**2.2±1.1**	**2.5±1.0**	**1.9±0.6**	**1.7±0.6**	**2.2±0.8**	**4.8±3.8**	**2.2±0.9**	**3.2±2.6**	**5.9±5.4**	**0.7±1.1**	**4.3±3.2**	**2.7±1.4**	**2.0±1.2**	**0.9±2.4**	**4.9±1.4**	**3.4±3.9**	**3.3±1.6**	**4.7±3.3**
11,21; 15,19; 13,17; 11,15 DiMeC29	1.0±1.2	2.2±1.6	1.9±1.1	3.6±3.6	0.8±0.9	2.8±1.8	1.2±1.6	1.6±1.4	0.8±1.0	1.5±1.6	2.7±4.3	3.2±2.1	0.3±0.7	1.6±1.3	0	1.4±1.9	1.9±3.2	1.8±2.2
3 MeC29	**2.3±2.4**	**1.9±1.1**	**1.3±0.9**	**1.6±0.9**	**2.9±1.4**	**0.6±0.9**	**0.7±1.1**	**2.1±1.3**	**1.2±1.1**	**0**	**3.6±0.5**	**4.3±2.6**	**0.9±0.9**	**0.4±0.7**	**0.8±0.9**	**1.9±0.6**	**1.7±1.6**	**2.0±2.0**
nC30	0.4±0.6	1.5±1.2	0.8±0.5	0.5±0.5	0.8±0.7	0.4±0.6	0.3±0.7	0.9±1.0	0	1.2±1.1	1.0±0.9	1.4±1.6	0.1±0.3	0.4±0.9	0.1±0.3	2.0±3.2	0.5±1.2	0.3±0.7
14 MeC30	0.7±0.6	1.8±0.8	1.3±0.9	1.9±0.9	1.0±0.6	2.4±1.4	0.9±0.8	1.7±1.3	0	1.7±2.5	1.0±0.7	1.9±1.2	0.7±0.8	1.1±1.0	0.1±0.3	1.0±0.5	0.9±1.0	2.1±1.4
3 MeC30	1.1±1.4	1.6±1.5	0.4±0.6	0.4±0.6	1.1±0.7	1.5±1.2	0.4±0.9	1.7±2.0	1.6±1.2	0.5±0.6	1.0±0.8	2.3±1.5	0	1.6±2.0	1.2±1.3	0.1±0.2	1.9±1.9	1.7±1.3
7 MeC31	**0.7±0.7**	**1.7±1.3**	**1.8±2.7**	**1.3±1.2**	**1.1±1.2**	**0.1±0.4**	**0.8±0.9**	**1.2±1.2**	**7.5±2.4**	**0**	**1.2±1.3**	**0.3±0.6**	**0.6±0.9**	**1.5±2.5**	**3.8±3.7**	**5.3±4.7**	**0.6±1.1**	**0**
11,15 DiMeC31	**3.2±3.5**	**9.9±5.6**	**3.0±2.4**	**8.2±6.7**	**6.5±6.7**	**16.0±11.0**	**3.2±3.3**	**0**	**0**	**1.0±2.5**	**3.5±1.0**	**14.5±9.9**	**1.9±2.2**	**9.4±9.2**	**0**	**6.2±5.3**	**5.6±4.2**	**11±7.3**
4 MeC31; 11,19 DiMeC33	0.8±0.9	2.1±1.3	1.3±0.7	1.2±1.0	1.6±1.3	2.3±2.5	0.9±1.2	1.6±1.7	0	0.2±0.4	1.0±0.5	2.0±2.8	0.3±0.5	1.0±1.5	0	0.5±0.7	0.5±1.0	2.0±3.4
nC32	0.7±0.8	1.4±1.3	0.7±0.5	0.3±0.6	0.8±0.8	0.5±0.7	0.4±0.7	1.0±1.3	0.8±1.2	0.4±0.9	0.8±0.4	1.1±1.4	0.3±0.7	0.6±0.9	0.2±0.5	0	0	0.3±0.7
12 MeC32	0.9±0.9	1.7±1.1	0.9±0.6	1.2±1.1	0.8±1.0	0.6±0.8	0.3±0.6	1.5±1.3	0.4±1.0	0	1.9±2.1	0.4±0.7	0.1±0.3	0.7±1.1	0.1±0.3	0.9±1.3	0	0.3±0.6
11;13;15;17 MeC33	4.1±2.8	5.6±3.1	2.1±1.7	5.7±4.3	4.2±1.7	4.5±2.0	3.3±2.2	5.2±3.8	0	0	2.3±1.4	4.1±3.0	1.9±2.3	3.6±3.5	0	6.7±5.6	0.2±0.6	1.7±2.2
5,15;**5,17 DiMeC33**	**2.6±2.7**	**6.2±3.8**	**1.9±1.7**	**2.4±3.6**	**3.9±3.2**	**3.5±4.0**	**0.6±1.4**	**6.4±6.0**	**1.0±1.4**	**6.5±6.6**	**2.9±1.8**	**5.3±5.4**	**1.5±2.3**	**3.6±3.9**	**0.6±1.2**	**3.1±4.4**	**0.4±0.9**	**1.1±2.3**
11,13; 11,15 DiMeC33	2.4±3.9	1.3±3.2	1.9±1.5	6.5±6.4	3.9±4.4	3.9±4.9	2.5±3.3	0.8±2.5	0	0.9±1.6	3.1±1.8	2.2±3.8	0.2±0.6	0.9±3.4	0.7±1.4	4.5±6.8	0	1.8±3.4
5,15 DiMeC33; 15,19 DiMeC33	2.3±2.0	1.9±1.5	1.8±1.7	1.4±1.4	0.7±1.1	0.3±0.6	3.3±3.0	1.3±1.3	1.2±2.6	0	1.5±0.8	0.7±1.2	0.5±0.9	0.8±1.4	0	0.8±1.1	0	0.7±1.6
13;15;17 MeC35	1.6±1.8	1.1±1.2	1.1±0.9	1.1±1.5	1.5±1.1	0.2±0.4	1.1±1.5	0.9±1.2	0.1±0.4	5.5±7.3	0.9±0.4	0.2±0.5	0.4±0.8	0.6±1.2	0.6±1.2	0.1±0.1	0	0.3±0.8
15,19 DiMeC35; 11,19 DiMeC35	2.1±2.8	2.4±2.5	2.8±1.7	6.2±6.0	4.7±2.0	1.3±2.1	1.6±2.7	0.7±1.9	0.4±1.2	0	5.1±3.3	2.0±2.8	0.5±1.5	1.9±3.7	0.3±0.7	2.1±2.7	0	0.4±1.1

Table S3: Results of the four Permanova analyses, each comparing a focal slavemaker species with all potential (sympatric and allopatric) host species. All analyses are based on Bray-Curtis similarities of entire CHC profiles. *T. americanus* was compared to *T. longispinosus* (from New York, Michigan, Ohio and West Virginia), *T. curvispinosus* (from West Virginia and Ohio) and *T. ambiguus* (from New York and Michigan), whereas *T. pilagens* was compared to *T. longispinosus* (from New York, Michigan, Ohio and West Virginia) and *T. ambiguus* (from New York and Michigan) and *T. duloticus* to *T. longispinosus* (from New York, Michigan, Ohio and West Virginia) and *T. curvispinosus* (from West Virginia and Ohio), based on the slavemakers respective host preferences (Herbers & Foitzik 2002, Johnson & Herbers 2006; Foitzik et al. 2009, Kleeberg et al. 2016).

Focal slavemaker species (factors)	df	Pseudo-F	*P*
T. americanus from New York	8	5.46	0.0001
T. americanus from West Virginia	8	4.35	0.0001
T. duloticus from Ohio	6	4.81	0.0001
T. pilagens from Michigan	6	4.89	0.0001

Table S4: Pairwise comparisons of slavemakers (within a PERMANOVA analysis) toward their potential host species, either allopatric or sympatric, including the average chemical similarity between groups. The analyses are based on Bray-Curtis similarities of entire CHC profiles. The sympatric host species do not differ in similarity from allopatric host species for any of the four slavemaker populations (*t* tests of similarity of slavemaker to sympatric host populations vs. similarity of slavemaker to allopatric host populations). *T. americanus* NY: $t_{2.31} = 0.099$, $P = 0.93$; *T. americanus* WV: $t_{5.53} = -0.74$, $P = 0.49$, *T. duloticus* OH: $t_{1.89} = 0.42$, $p = 0.72$, *T. pilagens* MI: $t_{3.18} = -0.28$, $p = 0.79$.

	t	P	Unique Permutations	Average similarity	denominator df
***T. americanus* from New York vs.**					
T. longispinosus NY	2.36	0.0003	9930	0.499	57
T. longispinosus WV	3.36	0.0001	9952	0.515	56
T. longispinosus OH	1.73	0.0196	9896	0.513	39
T. longispinosus MI	3.39	0.0001	9950	0.475	57
T. curvispinosus OH	2.68	0.0001	9890	0.461	39
T. curvispinosus WV	2.08	0.0021	9945	0.481	47
T. ambiguus NY	2.83	0.0001	9938	0.472	44
T. ambiguus MI	3.78	0.0001	9941	0.458	50
***T. americanus* from West Virginia vs.**					
T. longispinosus NY	1.87	0.0084	9949	0.537	36
T. longispinosus WV	2.67	0.0001	9936	0.571	35
T. longispinosus OH	1.50	0.0597	7361	0.573	18
T. longispinosus MI	2.55	0.0003	9944	0.526	36
T. curvispinosus OH	2.75	0.0003	7383	0.516	18
T. curvispinosus WV	1.64	0.0205	9937	0.523	26
T. ambiguus NY	2.23	0.0005	9927	0.545	23
T. ambiguus MI	3.22	0.0001	9946	0.508	29
***T. duloticus* from Ohio vs.**					
T. longispinosus NY	2.31	0.0008	9929	0.516	41
T. longispinosus WV	3.66	0.0001	9941	0.517	40
T. longispinosus OH	2.01	0.0023	9097	0.522	23
T. longispinosus MI	3.14	0.0001	9939	0.500	41
T. curvispinosus OH	2.76	0.0002	9049	0.499	23
T. curvispinosus WV	2.35	0.0001	9941	0.486	31
***T. pilagens* from Michigan vs.**					
T. longispinosus NY	2.39	0.0002	9941	0.466	52
T. longispinosus WV	2.86	0.0001	9937	0.500	51
T. longispinosus OH	1.45	0.0552	9801	0.499	34
T. longispinosus MI	2.69	0.0001	9943	0.475	52
T. ambiguus NY	2.55	0.0001	9928	0.456	39
T. ambiguus MI	2.74	0.0001	9946	0.479	45

Table S5: Differences in CHC composition depending on lifestyle (slavemaker vs. non-parasitic species), caste (workers, virgin queens and males) and species. The table shows PERMANOVA results and post-hoc comparisons based on Bray-Curtis similarities. Significant factors are given in bold. A repetition of the analyses with only *n*-alkanes, only monomethyl alkanes, only dimethyl alkanes, or only putative recognition substances resulted in similar main effects (all $p \leq 0.002$).

Factors (main test)	df	Pseudo-F	*P*	
Lifestyle	1	10.89	0.0001	
Caste	2	11.17	0.0001	
Species (Lifestyle)	4	5.24	0.0001	
Lifestyle x caste	2	7.13	0.0001	
Species (Lifestyle) x Caste	8	3.52	0.0001	
Pairwise comparisons for the lifestyle x caste interaction				
slavemaker vs. non-parasitic	unique perms	t	*P*	*den. df*
within males	9950	2.41	0.0001	66
within virgin queens	9952	1.84	0.0062	51
within workers	9946	3.98	0.0001	90
caste comparisons within non-parasitic species				
male vs. virgin queen	9945	1.78	0.0064	58
male vs. worker	9928	3.40	0.0001	81
worker vs. virgin queen	9944	3.20	0.0001	68
caste comparisons within slavemakers				
male vs. virgin queen	9940	2.48	0.0005	60
male vs. worker	9948	3.97	0.0001	76
worker vs. virgin queen	9939	1.84	0.0041	74

Table S6: Pairwise comparisons following the species (lifestyle) x caste interaction for pairs of levels of the factor species. Statistics are based on Bray-Curtis similarities of entire CHC profiles with a PERMANOVA analysis.

Comparisons	*t*	*P*	*Unique Permutations*	*den.df*
Within level "non-parasitic" and "worker"				
T. longispinosus vs. *T. curvispinosus*	1.99	0.0005	9928	39
T. longispinosus vs. *T. ambiguus*	1.99	0.0007	9934	36
T. curvispinosus vs. *T. ambiguus*	1.53	0.0199	9765	19
Within level "non-parasitic" and "virgin queen"				
T. longispinosus vs. *T. curvispinosus*	3.03	0.0009	9959	18
T. longispinosus vs. *T. ambiguus*	1.98	0.0009	9923	23
T. curvispinosus vs. *T. ambiguus*	2.13	0.029	36	7
Within level "non-parasitic" and "male"				
T. longispinosus vs. *T. curvispinosus*	1.50	0.0421	9960	28
T. longispinosus vs. *T. ambiguus*	2.46	0.0004	9932	34
T. curvispinosus vs. *T. ambiguus*	1.87	0.0035	999	12
Within level "slavemaker" and "worker"				
T. americanus vs. *T. pilagens*	2.60	0.001	999	30
T. americanus vs. *T. duloticus*	1.12	0.2706	9939	39
T. duloticus vs. *T. pilagens*	1.98	0.0016	9884	23
Within level "slavemaker" and "virgin queen"				
T. americanus vs. *T. pilagens*	3.07	0.001	973	27
T. americanus vs. *T. duloticus*	2.21	0.0023	9945	25
T. duloticus vs. *T. pilagens*	4.26	0.0052	209	8
Within level "slavemaker" and "male"				
T. americanus vs. *T. pilagens*	1.84	0.004	998	23
T. americanus vs. *T. duloticus*	1.65	0.027	9943	27
T. duloticus vs. *T. pilagens*	1.61	0.0145	5697	14

Table S7: Pairwise comparisons following the species (lifestyle) x caste interaction for pairs of levels of factor caste. The values are based on Bray-Curtis similarities of entire CHC profiles with a PERMANOVA analysis.

Comparisons	*t*	*P*	*Unique Permutations*	*den.df*
Within level "non-parasitic" and "*T. longispinosus*"				
male vs. worker	4.72	0.0001	9939	50
male vs. virgin-queen	4.38	0.0001	9942	39
worker vs. virgin queen	3.09	0.0001	9945	42
Within level "non-parasitic" and "*T. curvispinosus*"				
male vs. worker	1.46	0.065	9918	13
male vs. virgin queen	2.18	0.067	15	4
worker vs. virgin queen	2.67	0.0026	9313	11
Within level "non-parasitic" and "*T. ambiguus*"				
male vs. worker	1.59	0.048	9933	16
male vs. virgin queen	0.92	0.522	9944	14
worker vs. virgin queen	1.30	0.095	9944	13
Within level "slavemaker" and "*T. americanus*"				
male vs. worker	3.32	0.0001	9946	39
male vs. virgin queen	2.28	0.0025	9946	38
worker vs. virgin queen	3.26	0.0001	9952	43
Within level "slavemaker" and "*T. duloticus*"				
male vs. worker	2.01	0.0059	2891	12
male vs. virgin queen	1.31	0.1207	462	10
worker vs. virgin queen	1.98	0.0191	2876	12
Within level "slavemaker" and "*T. pilagens*"				
male vs. worker	3.08	0.0001	9946	25
male vs. virgin queen	2.67	0.0016	1001	12
worker vs. virgin queen	1.22	0.1842	4845	19

Table S8: Differences in CHC composition depending on lifestyle (slavemaker vs. non-parasitic species), worker vs. sexuals and species. The table shows PERMANOVA results based on Bray-Curtis similarities.

Factors	df	Pseudo-F	*P*
Lifestyle	1	12.60	0.0001
Workers vs. sexuals	1	14.65	0.0001
Species (Lifestyle)	4	4.10	0.0001
Lifestyle x workers vs. sexuals	1	10.10	0.0001
Species (Lifestyle) x workers vs. sexuals	4	2.37	0.0004

Table S9: Pairwise comparisons following within the species x workers vs. sexuals interaction. Statistics are based on Bray-Curtis similarities of entire CHC profiles with a PERMANOVA analysis.

Caste comparisons	*t*	*P*	*Unique Perms.*	*den.df*
Within *T. longispinosus* (non-parasitic)				
Workers vs. sexuals	3.22	0.0001	9943	68
Within *T. curvispinosus* (non-parasitic)				
Workers vs. sexuals	1.88	0.0066	9939	15
Within *T. ambiguus* (non-parasitic)				
Workers vs. sexuals	1.58	0.0294	9947	23
Within *T. americanus* (slavemaker)				
Workers vs. sexuals	3.37	0.0001	9946	62
Within *T. duloticus* (slavemaker)				
Workers vs. sexuals	2.36	0.0012	9580	18
Within *T. pilagens* (slavemaker)				
Workers vs. sexuals	2.54	0.0001	9925	29
Species comparisons	*t*	*P*	*Unique Perms.*	*den.df*
Within “non-parasitic” and “workers”				
T. longispinosus vs. *T. curvispinosus*	1.99	0.0012	9951	39
T. longispinosus vs. *T. ambiguus*	1.99	0.0004	9927	36
T. curvispinosus vs. *T. ambiguus*	1.53	0.0245	9754	19
Within “non-parasitic” and “sexuals”				
T. longispinosus vs. *T. curvispinosus*	1.19	0.2003	9948	48
T. longispinosus vs. *T. ambiguus*	2.15	0.0003	9936	59
T. curvispinosus vs. *T. ambiguus*	2.07	0.0007	9942	21
Within “slavemaker” and “workers”				
T. americanus vs. *T. pilagens*	2.63	0.0001	9930	30
T. americanus vs. *T. duloticus*	1.12	0.2674	9937	39
T. duloticus vs. *T. pilagens*	1.99	0.0019	9893	23
Within level “slavemaker” and “sexuals”				
T. americanus vs. *T. pilagens*	2.49	0.0001	9949	52
T. americanus vs. *T. duloticus*	2.61	0.0003	9944	24
T. duloticus vs. *T. pilagens*	2.69	0.0001	9930	24

Table S10: Pairwise comparisons following the lifestyle x (workers vs. sexuals) interaction. Statistics are based on Bray-Curtis similarities of entire CHC profiles with a PERMANOVA analysis.

Comparisons	*t*	*P*	*Unique Permutations*	*den.df*
Within level "sexuals"				
Slavemakers vs. non-parasitic species	2.36	0.0002	9938	126
Within level "workers"				
Slavemakers vs. non-parasitic species	3.98	0.0001	9936	90
Within level "non-parasitic"				
Workers vs. Sexuals	3.37	0.0001	9921	108
Within level "slavemakers"				
Workers vs. Sexuals	3.67	0.0001	9923	109

Table S11. Summaries after model selection of linear mixed models analysing the effects of lifestyle and caste on six dependent variables: mean chain length, total CHC quantity and the proportions of *n*-alkanes, monomethyl-alkanes, dimethyl-alkanes and the putative recognition substances. The reference includes slavemakers or non-parasitic hosts (lifestyle) and workers, virgin queens or males (caste). The reference is always compared to the other lifestyle and castes, if there was in interaction of both. Significant *P*-values are given in bold. For the pairwise tests, df = 7 for comparisons between lifestyles and df = 191 for comparisons between castes. Only for the total CHC quantity, df = 156 for between-caste comparisons.

		Mean chain length		Proportion of *n*-alkanes		Proportion of monomethyl-alkanes		Proportion of dimethyl-alkanes		Proportion of recognition substances		Total CHC quantity
Reference	*Comparison*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	
Slavemaker workers	Host workers	5.53	**< 0.001**	-6.02	**< 0.001**	3.25	**0.014**	4.90	**0.002**	4.27	**0.004**	Effect of caste:
	Slavemaker virgin queens	-4.84	**< 0.001**	-1.32	0.188	2.76	**0.006**	-1.50	0.136	1.19	**0.23**	
	Slavemaker males	-8.46	**< 0.001**	4.07	**< 0.001**	-0.05	0.960	-5.33	**< 0.001**	1.96	0.052	chi^2_2 = 42.94 **P < 0.001**
Host workers	Slavemaker workers	-5.53	**< 0.001**	6.02	**< 0.001**	-3.25	**0.014**	-4.40	**0.002**	4.27	**0.004**	
	Host virgin queens	-4.84	**< 0.001**	3.64	**< 0.001**	-0.01	0.988	-5.48	**< 0.001**	-3.19	**0.002**	Workers vs. Virgin queens:
	Host males	-8.46	**< 0.001**	7.05	**< 0.001**	-6.43	**< 0.001**	-2.69	**0.007**	-2.73	**0.007**	
Slavemaker virgin queens	Host virgin queens	5.53	**< 0.001**	-1.95	0.091	1.32	0.228	0.70	0.507	1.38	0.211	*t* = -5.027
	Slavemaker workers	4.8$	**< 0.001**	1.32	0.188	-2.76	**0.006**	1.50	0.136	-1.19	**0.237**	***P* < 0.001**
	Slavemaker males	-2.91	**< 0.001**	5.60	**< 0.001**	-2.94	**0.004**	-3.79	**< 0.001**	0.72	0.472	
Host virgin queen	Slavemaker virgin queens	-5.53	**< 0.001**	1.95	0.091	-1.32	0.228	-0.70	0.507	-1.38	0.211	Workers vs. Males:
	Host workers	4.84	**< 0.001**	-3.64	**< 0.001**	0.01	0.989	-5.48	**0.001**	3.19	**0.002**	*t* = -6.543
	Host males	2.91	**< 0.001**	2.40	**0.017**	-5.59	**< 0.001**	-3.20	**0.001**	0.87	**0.385**	
Slavemaker males	Host males	5.53	**< 0.001**	-4.39	**0.003**	-0.26	0.803	7.41	**< 0.001**	1.53	0.169	***P* < 0.001**
	Slavemaker workers	8.46	**< 0.001**	-4.07	**< 0.001**	0.05	0.960	5.33	**< 0.001**	-1.96	0.052	
	Slavemaker virgin queens	2.91	**< 0.001**	5.60	**< 0.001**	2.94	**0.004**	3.79	**< 0.001**	-0.72	0.472	Virgin queens vs. Males:
Host males	Slavemaker males	-5.53	**< 0.001**	4.39	**0.003**	0.26	0.803	-7.41	**< 0.001**	1.53	0.169	
	Host workers	8.46	**< 0.001**	-7.05	**< 0.001**	6.43	**< 0.001**	2.69	**0.007**	2.73	**0.007**	*t* = -0.923
	Host virgin queens	2.91	< 0.001	-2.40	**0.017**	5.59	**< 0.001**	-3.20	**0.001**	-0.87	**0.385**	*P* = 0.357

Table S12. Statistical results for phylogenetically independent contrasts. The table shows model coefficients and permutational two-tailed *P* values based on permuted linear models through the origin (command *lmorigin*, package *ape*; [58]; df = 1 in each case). Significant values are given in bold.

	Workers		Virgin queens		Males	
	coefficient	*P*	coefficient	*P*	coefficient	*P*
Proportion *n*-alkanes	**5.19**	**0.0020**	-1.52	0.66	6.30	0.10
Proportion dimethyl-alkanes	**-7.08**	**0.0080**	-2.79	0.40	**-6.00**	**0.0010**
Proportion monomethyl-alkanes	-6.28	0.15	0.92	0.62	4.41	0.24
Mean chain length	**-1.14**	**0.015**	-0.82	0.059	**-0.71**	**0.0010**
Proportion recognition substances	**-9.17**	**0.024**	3.29	0.37	-6.76	0.65
Total CHC quantity	2.28	0.20	5.63	0.17	-6.34	0.42

S3: The effect of the social environment

Social isolation experiment

To study the effect of isolation, we removed pupae of each species and caste from their original colony (worker pupae: N = 23.58 ± SD 7.91; virgin queen pupae: N = 10.75 ± SD 9.23; male pupae: N = 16.33 ± SD 9.05) and placed them into empty slide-nests with watered cotton for humidity. We provided them with the same food (crickets and honey) as the ant colonies. Pupae of the same colony and caste were placed together. After five days we froze the young ants. Adult workers help ants to emerge from the pupae, so unsurprisingly on a fraction emerged and survived until sampling (33.05% of slavemaker pupae; 45.16% of host pupae).

Effect of social isolation

The chemical profile of ants that emerged in isolation contained hydrocarbons of shorter chain lengths than those that emerged in their original colony with contact to nestmates ($Likelihood\ Ratio_1$ = 96.71, P < 0.001; Table S13; Fig. S3a). Moreover, isolated ants had proportionally more *n*-alkanes than those emerged in their original colony ($L.Ratio_1$ = 29.81, P < 0.001; Fig. S3b). They carried less monomethyl-alkanes ($L.Ratio_1$ = 43.73, P < 0.001; Fig. S3c) and less dimethyl-alkanes ($L.Ratio_1$ = 27.84, P < 0.001; Fig. S3d) than individuals, that emerged in their original colony, with the exception of slavemaker males where the social environment had no effect on their proportion of dimethyl alkanes (t_{175} = 0.91; P = 0.365; Table S14). Isolated slavemaker workers did not differ in their proportion of recognition substances from those that emerged in their original nest (t_{175} = 0.75; P = 0.453; Fig. S3e). Isolated slavemaker males (t_{175} = -3.27; P = 0.001) and virgin queens (t_{175} = -3.27; P = 0.001) carried relatively less recognition substances than those that emerged in their original colony. Isolated non-parasitic workers (t_{175} = -5.38; P < 0.001), males (t_{175} = -4.34; P < 0.001) and virgin queens (t_{175} = 2.67; P = 0.008) carried relatively less recognition substances than those that emerged with contact to their nestmates. For the slavemakers, isolated virgin queens (t_{175} = 5.06, P < 0.001) and males (t_{175} = 6.07, P < 0.001) carried more CHCs on their cuticle than those that emerged in their mother nest. There was no significant influence of social environment on slavemaker workers (t_{175} = -1.50, P = 0.135). Finally, for the non-parasitic species, workers carried lower amounts of CHCs when emerging in isolation (t_{175} = -2.27, *P* = 0.025), but isolated males (t_{175} = 3.96, *P* < 0.001) and virgin queens (t_{175} = 5.69, *P* < 0.001) carried more CHCs (Fig. S3f).

Caste differences within each lifestyle

Within the non-parasitic hosts that emerged isolated, workers carried shorter hydrocarbons than males (t_{417} = -5.08, P < 0.001) and virgin queens (t_{417} = -4.74, P < 0.001), whereas the latter two did not differ (t_{417} = -0.13, P = 0.899; Fig. S3a; Table S14). All castes of all slavemakers that emerged in isolation did not differ in their mean chain lengths (workers vs. males: t_{417} = -0.61, *P* = 0.539; workers vs. virgin queens: t_{417} = -1.55, *P* = 0.123; males vs. virgin queens: t_{417} = 0.60, *P* = 0.547; Fig. S3a). Slavemaker individuals that emerged isolated carried proportionally more *n*-alkanes than non-parasitic host individuals that emerged isolated (workers: t_{417} = -3.21, *P* = 0.011; virgin queens: t_{417} = -3.14, *P* = 0.012; males: t_{417} = -2.30, *P* = 0.047; Fig. S3b). Within slavemakers emerged in isolation, workers carried relatively less *n*-alkanes than virgin queens (t_{417} = 3.45, *P* = 0.006) and males (t_{417} = 2.85, *P* = 0.005), whereas males and virgin queens did not differ (t_{417} = 0.09, *P* = 0.921; Fig. S3b). Within non-parasitic hosts emerged in isolation, workers carried relatively less *n*-alkanes than virgin queens (t_{417} = 3.84, *P* < 0.001) and males (t_{417} = 5.96, *P* < 0.001). Males and virgin queens did not differ (t_{417} = -1.26, *P* = 0.208; Fig. S3b). Within the slavemakers that emerged in isolation, workers carried relatively more monomethyl-alkanes than virgin queens (t_{417} = 4.63, *P* < 0.001) and males (t_{417} = 3.51, *P* < 0.001), whereas virgin queens and males did not differ (t_{417} = -0.20, *P* = 0.841; Fig. S3c). Within the non-parasitic species that emerged in isolation workers and virgin queens carried relatively more monomethyl-alkanes than males (workers: t_{417} = 2.22, *P* = 0.026; virgin queens: t_{417} = 1.924, *P* = 0.050), but workers were not different from virgin queens (t_{417} = 0.19, *P* = 0.853; Fig. S3c). Slavemaker individuals that emerged isolated did not differ in their proportion of dimethyl-alkanes from non-parasitic ants (workers: t_9 = -1.32, *P* = 0.220; virgin queens: t_9 = -1.02, *P* = 0.335, males: t_9 = -1.12, *P* = 0.292; Fig. S3d). Within the slavemakers that emerged isolated, castes did not differ in their relative amount of dimethyl-alkanes (workers vs. males: t_{417} = -0.71, *P* = 0.479; workers vs. virgin queens: t_{417} = -1.00, *P* = 0.318; males vs. virgin queens: t_{417} = 0.11, *P* = 0.916). Similarly, within the non-parasitic species that emerged isolated, castes did not differ (workers vs. males: t_{417} = -1.46, P = 0.145; workers vs. virgin queens: t_{417} = -1.83, P = 0.067; males vs. virgin queens: t_{417} = -0.50, P = 0.617; Fig. S3d). Within the slavemakers that emerged isolated, workers carried relatively more recognition substances than males (t_{417} = 2.81, P = 0.005) and virgin queens (t_{417} = 3.55, P < 0.001), whereas the latter two did not differ (t_{417} = -0.05, P = 0.956; Fig. S3e).

Table S13: Model selection results from linear mixed models. The six models differed in the dependent variables, which were the mean chain length, the total CHC quantity, the proportion of *n*-alkanes, monomethyl-alkanes, dimethyl-alkanes and the putative recognition substances (Jongepier & Foitzik 2016), respectively. These traits were analysed in relation to lifestyle, caste and social environment of the individuals.

Predictors	Likelihood Ratio	*P*	df
	Mean chain length		
Social environment x Caste x Lifestyle	10.26	0.006	2
Caste x Lifestyle	10.24	0.006	2
Caste x Social environment	24.37	< 0.001	2
Social environment x Lifestyle	13.73	< 0.001	1
Caste	0.48	0.787	2
Lifestyle	3.11	0.078	1
Social environment	96.71	< 0.001	1
	Total CHC quantity		
Social environment x Caste x Lifestyle	8.50	0.014	2
Caste x Lifestyle	3.41	0.182	2
Caste x Social environment	76.74	< 0.001	2
Social environment x Lifestyle	0.96	0.327	1
Caste	6.59	0.037	2
Lifestyle	0.53	0.466	1
Social environment	15.16	< 0.001	1
	Proportion of n-alkanes		
Social environment x Caste x Lifestyle	6.17	0.046	2
Caste x Lifestyle	5.87	0.053	2
Caste x Social environment	9.45	0.009	2
Social environment x Lifestyle	0.09	0.760	1
Caste	83.39	< 0.001	2
Lifestyle	20.41	< 0.001	1
Social environment	29.81	< 0.001	1
	Proportion of monomethyl alkanes		
Social environment x Caste x Lifestyle	23.50	< 0.001	2
Caste x Lifestyle	8.33	0.016	2
Caste x Social environment	12.02	0.003	2
Social environment x Lifestyle	0.29	0.592	1
Caste	39.52	< 0.001	2
Lifestyle	4.70	0.030	1
Social environment	43.73	< 0.001	1
	Proportion of dimethyl alkanes		
Social environment x Caste x Lifestyle	11.57	0.003	2
Caste x Lifestyle	22.88	< 0.001	2
Caste x Social environment	8.11	0.017	2
Social environment x Lifestyle	0.71	0.400	1
Caste	38.74	< 0.001	2
Lifestyle	15.86	< 0.001	1
Social environment	27.84	< 0.001	1
	Proportion of putative recognition substances		
Social environment x Caste x Lifestyle	21.23	< 0.001	2
Caste x Lifestyle	1.80	0.406	2
Caste x Social environment	1.01	0.605	2
Social environment x Lifestyle	0.99	0.318	1
Caste	7.39	0.025	2
Lifestyle	9.48	0.002	1
Social environment	20.28	< 0.001	1

Table S14: Summaries after model selection of the linear mixed models analysing the effects of lifestyle, caste and social environment on six dependent variables: mean chain length, total CHC quantity and proportions of different substance classes. The reference includes slavemakers or non-parasitic species (lifestyle), workers, virgin queens or males (caste) and original colony or isolation (social environment). The reference is always compared to the other lifestyle, castes and social environment. Abbreviations as follows: Slavemakers = SM, non-parasitic species = HOST, workers = w, virgin queens = vq, males = m, original colony = OC, isolated individuals = II. Significant *P*-values are given in bold. For example, the first line shows a pairwise comparison between “slavemaker workers from original colonies” (reference) and “slavemaker workers, individual isolation”. The pairwise difference for these factor level in mean chain length is significant (t = 4.66, p < 0.001). The second line compares “slavemaker workers from original colonies” (reference) and “slavemaker males from original colonies”. Degrees of freedom are 9 for all pairwise comparisons between lifestyles (SM/HOST), 175 for comparisons between isolated and original colony (II/OC) and 417 for comparisons between castes (w/m/vq), respectively.

		Mean chain length		Total CHC quantity [µg]		Proportion of *n*-alkanes		Proportion of monomethyl alkanes		Proportion of dimethyl alkanes		Proportion of recognition substances	
Reference	*Comparison*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Original colony	SM w II	4.66	**< 0.001**	-1.50	0.135	2.63	**0.009**	-1.85	0.066	-3.39	**< 0.001**	-0.75	0.453
Workers	SM m OC	-3.13	**0.002**	-3.64	**< 0.001**	3.44	**< 0.001**	0.04	0.964	6.01	**< 0.001**	1.97	0.049
Slave-	SM vq OC	-1.73	0.084	-3.08	**0.002**	-1.34	0.181	2.79	**0.006**	-1.88	0.060	1.17	0.244
makers	HOST w OC	1.40	0.195	-0.60	0.566	-6.41	**< 0.001**	3.84	**0.004**	5.26	**< 0.001**	4.85	**< 0.001**
Isolation	SM w OC	4.66	**< 0.001**	1.50	0.135	-2.63	**0.009**	1.85	0.066	3.39	**< 0.001**	0.75	0.453
Workers	SM m II	0.61	0.540	4.95	**< 0.001**	2.85	**0.004**	-3.51	**< 0.001**	-0.71	0.480	-2.81	**0.005**
Slave-	SM vq II	-1.55	0.123	3.87	**< 0.001**	3.45	< 0.001	-4.63	**< 0.001**	-1.00	0.318	-3.55	**< 0.001**
makers	HOST w II	-5.39	**< 0.001**	-0.73	0.483	-3.21	**0.011**	-0.09	0.932	1.32	0.220	0.150	0.884
Original	HOST w II	-14.56	**< 0.001**	-2.27	**0.025**	4.61	**< 0.001**	-6.93	**< 0.001**	-6.11	**< 0.001**	-5.38	**< 0.001**
colony	HOST m II	-1.95	0.051	-2.80	**0.005**	6.36	< 0.001	-6.33	**< 0.001**	-2.91	**0.004**	-2.68	**0.008**
Workers	HOST vq II	-1.21	0.228	-1.90	0.058	3.50	< 0.001	-0.08	0.936	-6.388	**< 0.001**	-3.19	**0.002**
Hosts	SM w OC	-1.40	0.195	0.60	0.566	6.41	**< 0.001**	-3.84	**0.004**	-5.26	**< 0.001**	-4.85	**< 0.001**
Isolation	HOST w OC	14.56	**< 0.001**	2.27	**0.025**	-4.61	**< 0.001**	6.93	**< 0.001**	6.11	**< 0.001**	5.38	**< 0.001**
Workers	HOST m OC	5.08	**< 0.001**	3.83	**< 0.001**	5.96	**< 0.001**	-2.22	**0.026**	-1.46	0.145	-1.06	0.289
	HOST vq OC	4.74	**< 0.001**	7.01	**< 0.001**	3.84	**< 0.001**	0.19	0.853	-1.83	0.067	0.43	0.670
Hosts	SM w II	5.39	**< 0.001**	0.73	0.483	3.21	**0.011**	0.09	0.933	-1.32	0.220	-0.15	0.884
Original colony	SM vq II	4.90	**< 0.001**	5.06	**< 0.001**	5.84	**< 0.001**	-6.66	**< 0.001**	-2.92	**0.004**	-3.27	**0.002**
Virgin	SM m OC	-1.24	0.217	-0.44	0.656	4.72	**< 0.001**	-2.84	**0.005**	-4.21	**< 0.001**	0.76	0.449
queens	SM w OC	1.73	0.084	3.07	**0.002**	1.34	0.181	-2.79	**0.006**	1.88	0.060	-1.17	0.244
Slave-makers	HOST vq OC	1.54	0.158	0.40	0.695	-1.94	0.084	1.65	0.133	0.83	0.429	1.67	0.128
Isolation	SM vq OC	4.90	**< 0.001**	-5.06	**< 0.001**	-5.84	**< 0.001**	6.66	**< 0.001**	2.92	**0.004**	3.27	**0.002**
Virgin	SM m II	0.60	0.547	2.04	**0.041**	0.09	0.921	0.20	0.842	0.11	0.916	0.05	0.956
queens	SM w II	1.55	0.123	3.87	**< 0.001**	-3.45	**< 0.001**	4.63	**< 0.001**	1.00	0.317	3.55	**< 0.001**
Slave-makers	HOST vq II	-0.08	0.939	1.00	0.345	3.14	**0.012**	2.61	**0.028**	1.02	0.335	1.90	**0.090**
Original colony	HOST vq II	-6.36	**< 0.001**	5.69	**< 0.001**	4.09	**< 0.001**	-5.57	**< 0.001**	-2.76	**0.006**	-2.67	**0.008**
Virgin	HOST m OC	-0.50	0.615	-0.54	0.590	2.01	**0.045**	-5.44	**< 0.001**	3.94	**< 0.001**	0.91	0.363
queens	HOST w OC	1.21	0.228	1.90	0.059	-3.50	**< 0.001**	0.08	0.936	6.39	**< 0.001**	3.19	**0.002**
Hosts	SM vq OC	1.54	0.158	-0.40	0.695	1.94	0.084	-1.65	0.133	-0.83	0.429	-1.67	0.128
Isolation	HOST vq OC	6.36	**< 0.001**	-5.69	**< 0.001**	-4.09	**< 0.001**	5.57	**< 0.001**	2.76	**0.006**	2.67	**0.008**
Virgin	HOST m II	-0.13	0.899	-3.05	**0.003**	1.26	0.208	-1.92	0.055	0.50	0.617	-1.22	0.225
queens	HOST w II	-4.74	**< 0.001**	-7.01	**< 0.001**	-3.84	**< 0.001**	-0.19	0.853	1.83	0.067	-0.43	0.671
Hosts	SM vq II	0.08	0.939	-1.00	0.345	3.14	**0.012**	-2.61	**0.028**	-1.02	0.335	-1.90	0.090
Original	SM m II	-2.39	**0.018**	6.07	**< 0.001**	2.79	**0.006**	-4.26	**< 0.001**	-0.91	0.365	-3.27	**0.001**
colony	SM vq OC	1.24	0.217	0.45	0.656	-4.72	**< 0.001**	2.84	**0.005**	4.21	**< 0.001**	-0.76	0.449
Males	SM w OC	3.13	**0.002**	3.64	**< 0.001**	-3.45	**< 0.001**	-0.04	0.964	6.00	**< 0.001**	1.97	0.049
Slave-makers	HOST m OC	2.58	**0.030**	0.35	0.732	-4.32	**0.002**	-0.01	0.992	8.25	**< 0.001**	1.87	0.094
Isolation	SM m OC	2.39	**0.018**	-6.07	**< 0.001**	-2.79	**< 0.006**	4.26	**< 0.001**	0.91	0.365	3.27	**0.001**
Males	SM vq II	0.60	0.547	-2.05	**0.041**	-0.10	0.921	-0.20	0.842	-0.11	0.916	-0.05	0.956
Slave-	SM w II	0.61	0.540	-4.95	**< 0.001**	-2.85	**0.005**	3.51	**< 0.001**	0.71	0.480	2.81	**0.005**
makers	HOST vq II	-0.68	0.507	-3.21	**0.011**	-2.30	**0.047**	1.39	0.197	1.12	0.292	1.32	0.220
Original	HOST m II	-7.01	**< 0.001**	3.96	**< 0.001**	4.31	**< 0.001**	-3.98	**< 0.001**	-5.15	**< 0.001**	-4.34	**< 0.001**
colony	HOST vq OC	0.50	0.615	0.54	0.590	-2.01	**0.045**	5.44	**< 0.001**	-3.94	**< 0.001**	-0.91	0.363
Males	HOST w OC	1.95	0.051	2.80	**0.005**	-6.36	**< 0.001**	6.33	**< 0.001**	2.91	**0.004**	2.68	**0.007**
Hosts	SM m OC	-2.58	**0.030**	-0.35	0.732	4.32	**0.002**	0.01	0.993	-8.25	**< 0.001**	-1.87	0.094
Isolation	HOST m OC	7.01	**< 0.001**	-3.96	**< 0.001**	-4.31	**< 0.001**	3.98	**< 0.001**	5.15	**< 0.001**	4.34	**< 0.001**
Males	HOST vq II	0.13	0.899	3.05	**0.003**	-1.26	0.208	1.92	0.055	-0.50	0.617	1.22	0.225
	HOST w II	-5.08	**< 0.001**	-3.83	**< 0.001**	-5.96	**< 0.001**	2.23	**0.027**	1.46	0.145	1.06	0.289
Hosts	SM m II	0.69	0.508	3.21	**0.011**	2.30	**0.047**	-1.39	0.197	-1.12	0.292	-1.32	0.220

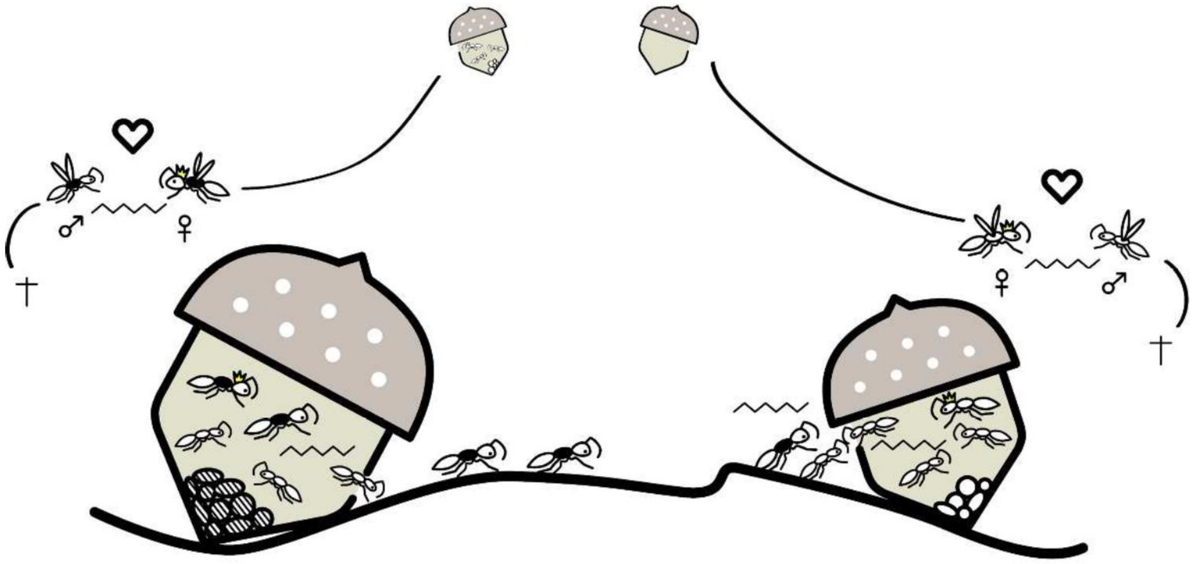

Figure S1: Life histories of queens, males and workers of *Temnothorax* slavemaking ants and their hosts, and the importance of CHCs for the different interaction types. These ants have small colonies (several dozens of workers) and inhabit acorns, nuts or sticks on the forest floor. Ants in black represent slavemakers, those in white, hosts. During nuptial flights, males and queens use CHCs to recognize each other and to avoid inbreeding and hybridization (Beibl *et al.* 2007; Chernenko *et al.* 2012). Mated slavemaker queens go on to invade host colonies to establish a new colony, during which they need to undermine host recognition (Pamminger *et al.* 2012). In contrast, host queens either return to their mother nest or found a new colony independently. Slavemaker and host males die after mating. During slave raids, slavemaker workers force entry to host colonies, so that their chemical profiles are selected to lower host defenses (Brandt et al. 2005). In both lifestyles, workers use specific hydrocarbons for nestmate recognition. In hosts, the presence of slavemakers in a population selects for diversification of host profiles (Jongepier *et al.* 2016). Moreover, queens and in slavemakers, also workers, use hydrocarbons to signal fertility.

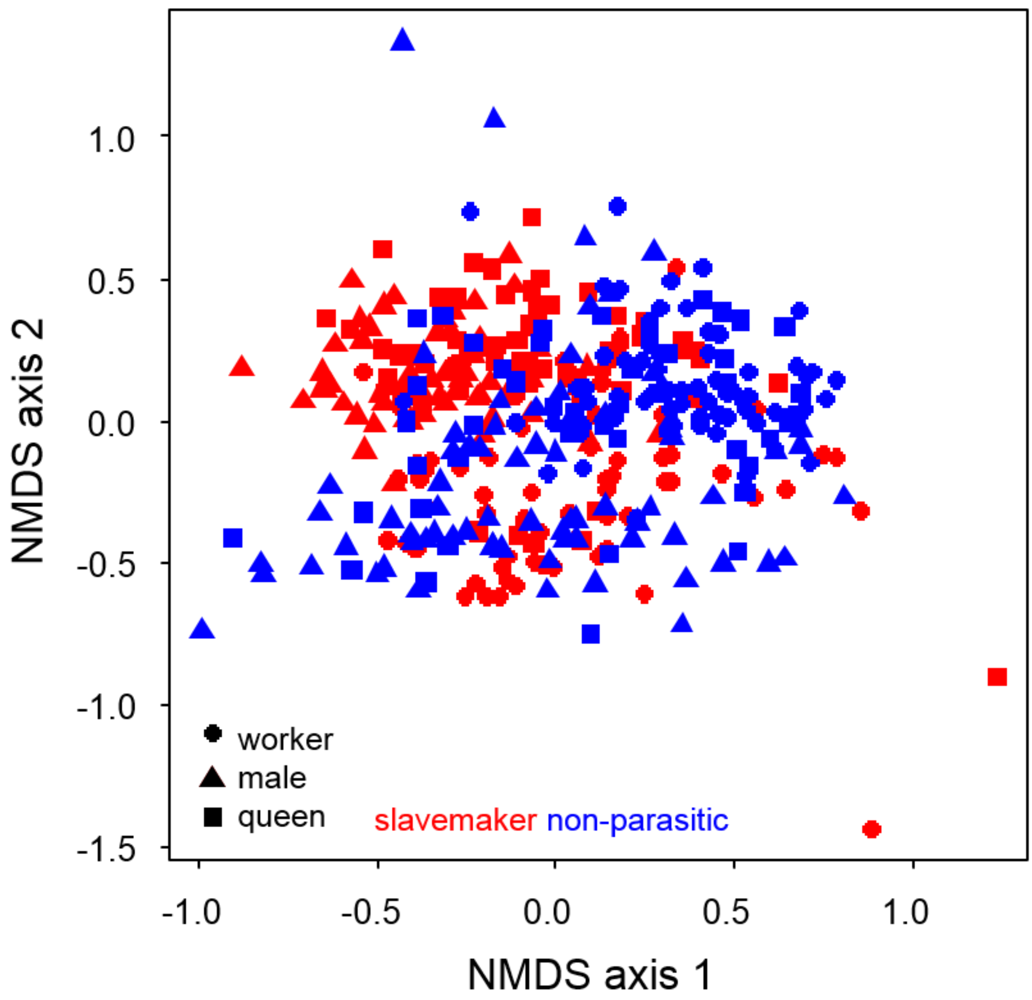

Figure S2: NMDS ordination of cuticular hydrocarbon profiles in slavemakers (red) and non-parasitic species (blue), based on Bray-Curtis similarities between CHC profiles. Different symbols represent different castes (workers = circles, virgin queens = squares and males = triangles). Stress value: 0.19.

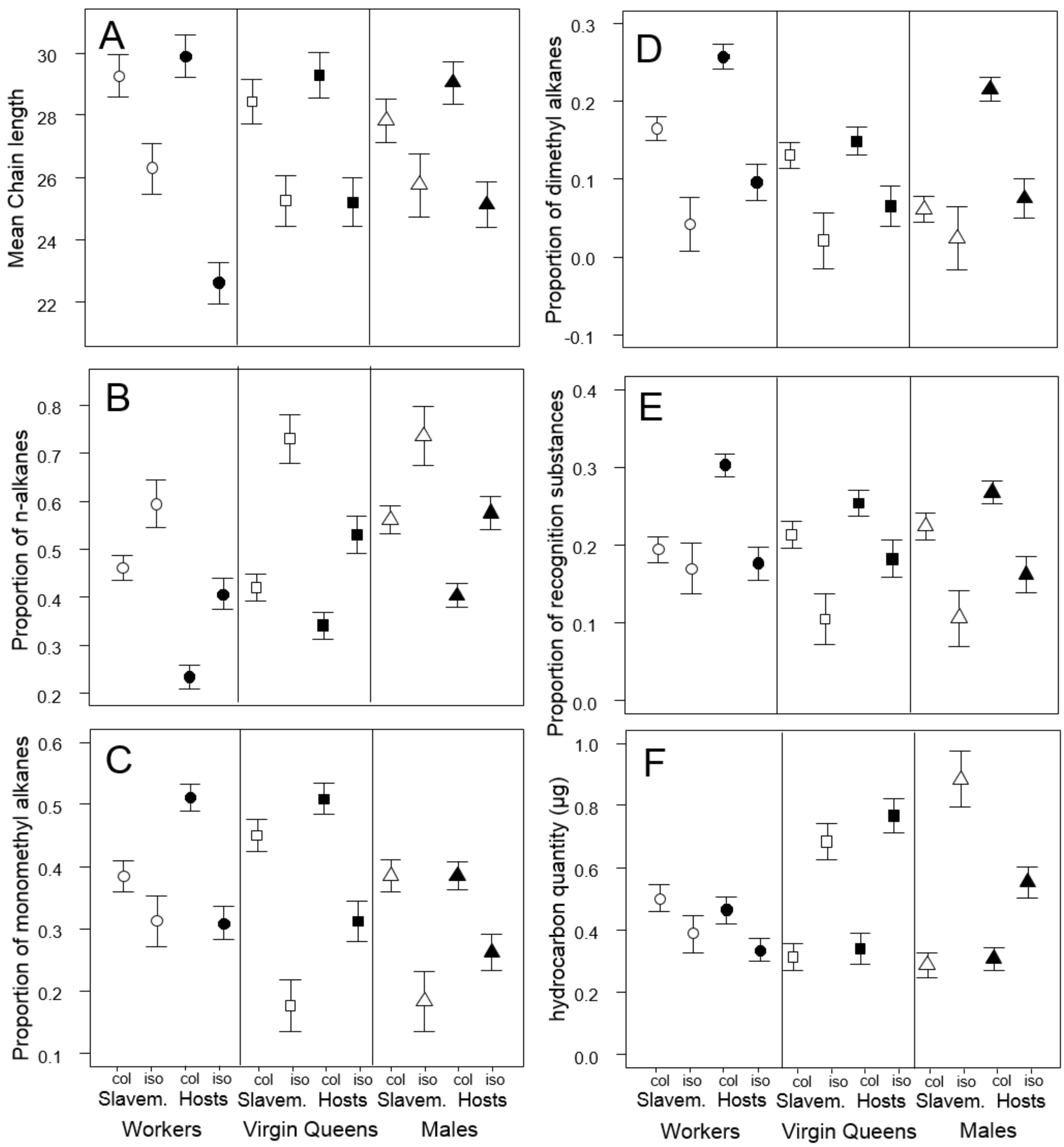

Figure S3: Impact of lifestyle, caste and social environment on different substances classes. The plots show mean chain lengths (a), the relative amounts of *n*-alkanes (b), monomethyl-alkanes (c), dimethyl-alkanes (d), putative recognition substances (e) as well as the total amount of hydrocarbons on the cuticle (f). Slavemakers are shown in white; non-parasitic species are shown in black. Different symbols represent different castes (workers = circles, virgin queens = squares, males = triangles). Data are shown for individuals that emerged in their original colony (*col*) or in isolation (*iso*). The plots show intercepts ± s.e. of the LMM summaries.

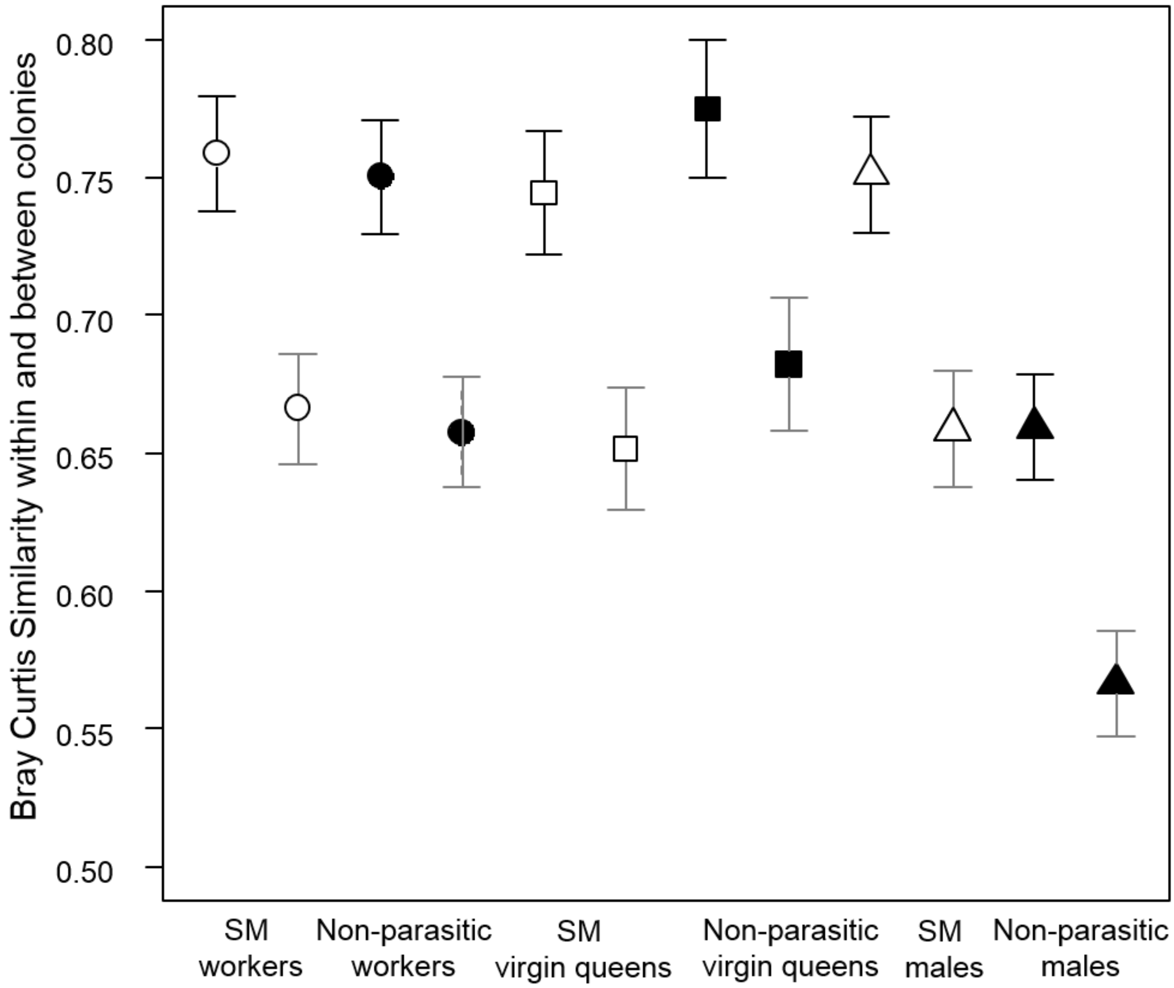

Figure S4: Bray Curtis Similarity indices within and between colonies. Dotted error bars show the chemical similarity between colonies and solid error bars within colonies. Slavemakers (SM) are shown in white; non-parasitic host species are shown in black. Different symbols represent different castes (workers = circles, virgin queens = squares and males = triangles). The plots show intercepts ± s.e. of the LMM summaries.

References: Supplementary Material

Chernenko, A., Holman, L., Helanterä, H. & Sundström, L. 2012 Cuticular Chemistry of Males and Females in the Ant *Formica fusca*. *J. Chem. Ecol.* **38**, 1474–1482.

Beibl, J., D'Ettorre, P. & Heinze, J. 2007 Cuticular profiles and mating preference in a slave-making ant. Insectes Soc. **54**, 174–182.

Brandt, M. & Foitzik, S. 2004 Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology* **85**, 2997–3009.

Brandt, M., Heinze, J., Schmitt, T. & Foitzik, S. 2005 A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. *J. Evol. Biol.* **18**, 576–586.

Foitzik, S., Achenbach, A. & Brandt, M. 2009 Locally adapted social parasite affects density, social structure and life history of its ant hosts. *Ecology* **90**, 1195–1206.

Herbers, J. M. & Foitzik, S. 2002. Ecology of slavemaking ants and their hosts in north-temperate forests. Ecology **83**, 148-163.

Johnson, C., & Herbers, J.M. 2006. Impact of parasite sympatry on the geographic mosaic of coevolution. Ecology **87**, 382–394.

Jongepier, E. & Foitzik, S. 2016 Ant recognition cue diversity is higher in the presence of slavemaker ants. *Behav. Ecol.* **27**, 304–311.

Kleeberg, I. & Foitzik, S. 2016 The placid slavemaker: avoiding detection and conflict as an alternative, peaceful raiding strategy. *Behav. Ecol. Sociobiol.* **70**, 27–39.

Pamminger, T., Modlmeier, A. P., Suette, S., Pennings, P. S. & Foitzik, S. 2012 Raiders from the sky: slavemaker founding queens select for aggressive host colonies. *Biol. Lett.* **8**, 748 – 450.