Myrmecological News

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Digital supplementary material

# Digital supplementary material to

CHOPPIN, M., GRAF, S., FELDMEYER, B., LIBBRECHT, R. MENZEL, F. & FOITZIK, S. 2021: Queen and worker phenotypic traits are associated with colony composition and environment in *Temnothorax rugatulus* (Hymenoptera: Formicidae), an ant with alternative reproductive strategies. – Myrmecological News 31: 61-69.

**Supplementary Table S1a.** Collection sites, elevation, coordinates, and date of collection

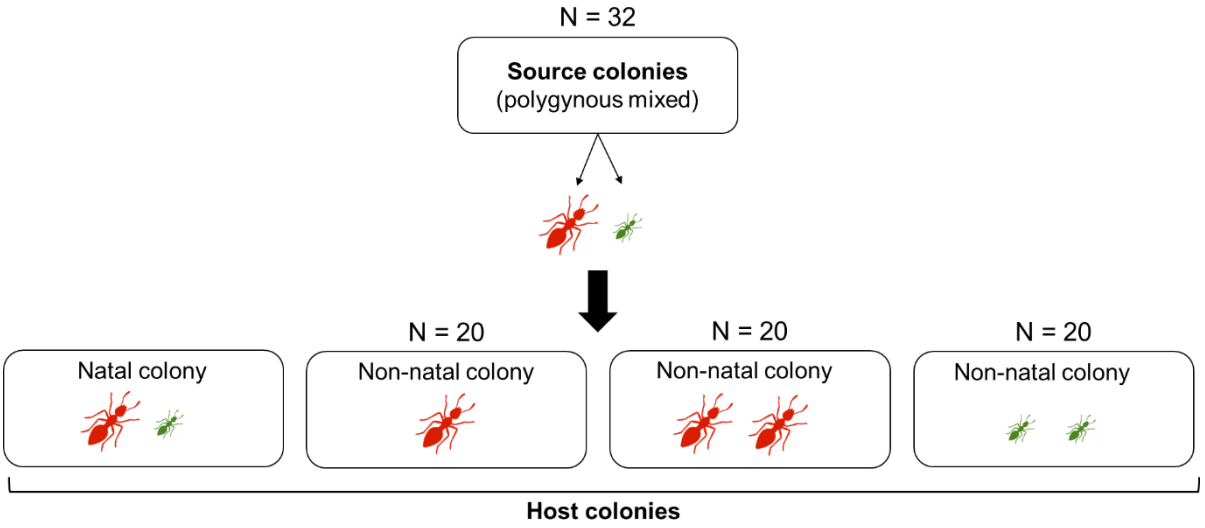
| Site | Elevation (m) | Coordinates | Date of collection (DD/MM/YYYY) |
|---|---|---|---|
| H | 1976 | 31.909071;-109.253337 | 13.08.2018 & 20.08.2018 |
| I | 2607 | 31.919981;-109.277186 | 14.08.2018 |
| J | 1938 | 31.908966;-109.247523 | 14.08.2018 & 20.08.2018 |
| M | 2019 | 31.850508;-109.326524 | 16.08.2018 |
| N | 1951 | 31.855962;-109.329143 | 16.08.2018 |
| P | 1852 | 31.9127347;-109.2401991 | 15.08.2018 & 17.08.2018 |
| Q | 1787 | 31.916534;-109.232811 | 18.08.2018 |
| R | 1796 | 31.898606;-109.226421 | 20.08.2018 |
| S | 1935 | 31.897912; -109.241417 | 21.08.2018 – 26.08.2018 (except 23.08.2018) |

**Supplementary Table S1b.** Collection sites, elevation, coordinates, and date of collection

| Site | Elevation (m) | Coordinates | Date of collection (DD/MM/YYYY) |
|---|---|---|---|
| A | 2543 | 31.918194;-109.268458 | 19.08.2015 & 22.08.2015 |
| B | 2542 | 31.917996;-109.269182 | 19.08.2015 & 22.08.2015 |
| C | 2429 | 31.921641;-109.268378 | 20.08.2015 & 22.08.2015 |
| D | 2584 | 31.904863;-109.278246 | 20.08.2015 & 22.08.2015 |
| E | 2525 | 31.913815;-109.268808 | 22.08.2015 |
| F | 2270 | 31.928994;-109.258134 | 23.08.2015 |
| G | 1984 | 31.910837;-109.252077 | 23.08.2015 |
| H | 1976 | 31.909071;-109.253337 | 24.08.2015 – 28.08.2015 (except 27.08.2015) |
| I | 2607 | 31.919981;-109.277186 | 26.08.2015 |
| J | 1938 | 31.908966;-109.247523 | 26.08.2015 |
| K | 1865 | 31.912552;-109.241957 | 28.-29.08.2015 |
| L | 1891 | 31.910687;-109.244433 | 28.08.2015 |
| M | 2019 | 31.850508;-109.325624 | 29.08.2015 & 01.-02.09.2015 |
| N | 1951 | 31.855962;-109.329143 | 02.-03.09.2015 |
| O | 1967 | 31.855962;-109.329143 | 02.09.2015 |

**Supplementary Table S2.** Number of colonies of each colony composition

| | Macrogynous | Microgynous | Mixed |
|---|---|---|---|
| **Monogynous** | 750 | 36 | |
| **Polygynous** | 358 | 73 | 109 |




**Supplementary Figure S1.** Experimental design of the re-adoption experiments. Source colonies provided one queen of each morph. Each queen was tested for each of four host colony compositions, one after the other in a pseudo-randomized order.

**Supplementary Table S3.** Sample sizes of the re-adoption experiments

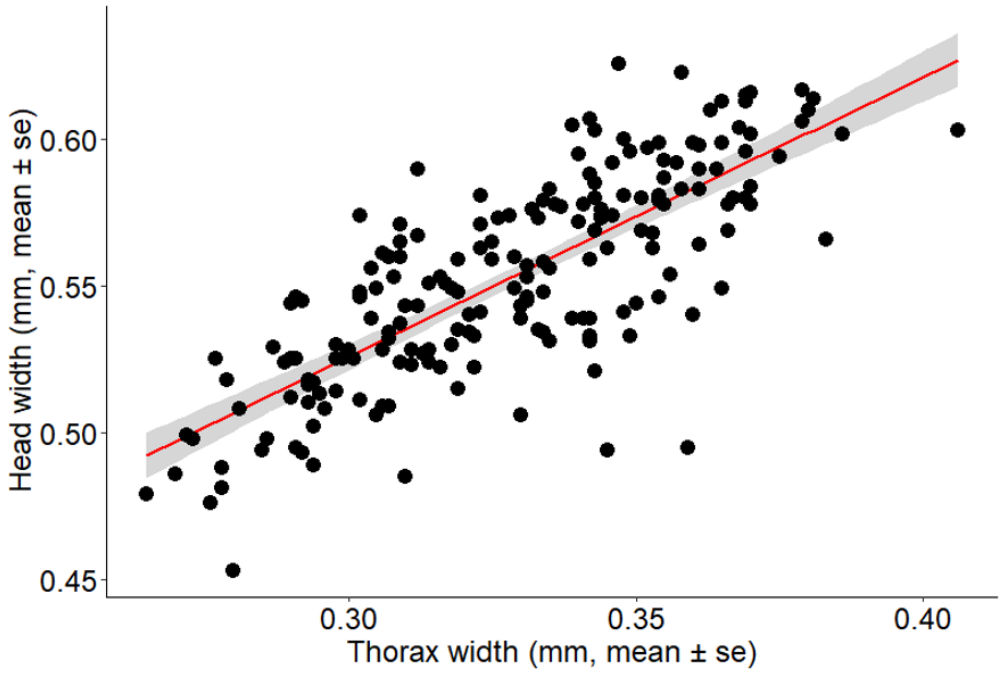
| Tested queen | Host colony | Number of trials |
|---|---|---|
| Macrogyne | Natal | 10 |
| Macrogyne | Monogynous macrogynous | 15 |
| Macrogyne | Polygynous macrogynous | 19 |
| Macrogyne | Polygynous microgynous | 20 |
| Microgyne | Natal | 7 |
| Microgyne | Monogynous macrogynous | 11 |
| Microgyne | Polygynous macrogynous | 12 |
| Microgyne | Polygynous microgynous | 12 |

**Supplementary Table S4.** Experimental design of the chemical analyses

| Data set | Number of queens | Queen morph | Colony composition |
|---|---|---|---|
| A | 10 | Macrogyne | Mixed |
| | 10 | Microgyne | |
| | 10 | Macrogyne | Pure |
| B | 10 | Macrogyne | Pure |
| | 10 | Microgyne | Pure |

**Supplementary Table S5.** Interactive effects of queen morph and colony composition on queen body size

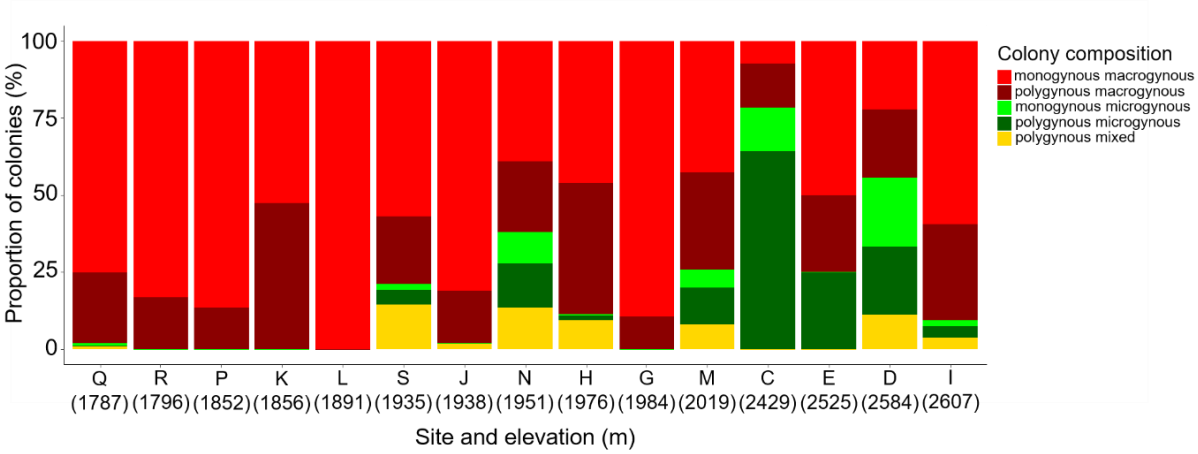
| Queen morph-Colony composition 1 | Queen morph-Colony composition 2 | p-value |
|---|---|---|
| Macrogyne-Polygynous mixed | Macrogyne-Monogynous | < 0.001 |
| Macrogyne-Polygynous pure | Macrogyne-Monogynous | < 0.001 |
| Microgyne-Monogynous | Macrogyne-Monogynous | < 0.001 |
| Microgyne-Polygynous mixed | Macrogyne-Monogynous | < 0.001 |
| Microgyne-Polygynous pure | Macrogyne-Monogynous | < 0.001 |
| Macrogyne-Polygynous pure | Macrogyne-Polygynous mixed | 0.0281 |
| Microgyne-Monogynous | Macrogyne-Polygynous mixed | < 0.001 |
| Microgyne-Polygynous mixed | Macrogyne-Polygynous mixed | < 0.001 |
| Microgyne-Polygynous pure | Macrogyne-Polygynous mixed | < 0.001 |
| Microgyne-Monogynous | Macrogyne-Polygynous pure | < 0.001 |
| Microgyne-Polygynous mixed | Macrogyne-Polygynous pure | < 0.001 |
| Microgyne-Polygynous pure | Macrogyne-Polygynous pure | < 0.001 |
| Microgyne-Polygynous mixed | Microgyne-Monogynous | 0.0497 |
| Microgyne-Polygynous pure | Microgyne-Monogynous | 0.9852 |
| Microgyne-Polygynous pure | Microgyne-Polygynous mixed | 0.0084 |




**Supplementary Figure S2.** Correlation of worker head and thorax widths ($r^2 = 0.602$ and $p < 0.001$)

**Supplementary Table S6.** Interactive effects of social structure and colony morph on worker thorax width

| Social structure-Colony morph 1 | Social structure-Colony morph 2 | p-value |
|---|---|---|
| Monogynous-Microgynous | Monogynous-Macrogynous | < 0.001 |
| Polygynous-Macrogynous | Monogynous-Macrogynous | 0.988 |
| Polygynous-Microgynous | Monogynous-Macrogynous | 0.260 |
| Polygynous-Macrogynous | Monogynous-Microgynous | < 0.001 |
| Polygynous-Microgynous | Monogynous-Microgynous | 0.012 |
| Polygynous-Microgynous | Polygynous-Macrogynous | 0.435 |




**Supplement Figure S3.** Colony composition at different sites (indicated by letters) and corresponding elevations in meters

**Supplementary Table S7.** Effects of queen morph, colony composition and interaction on the location of the queen and aggression and grooming by the workers 1h after her introduction to the host colony, and on queen survival and location after 24h

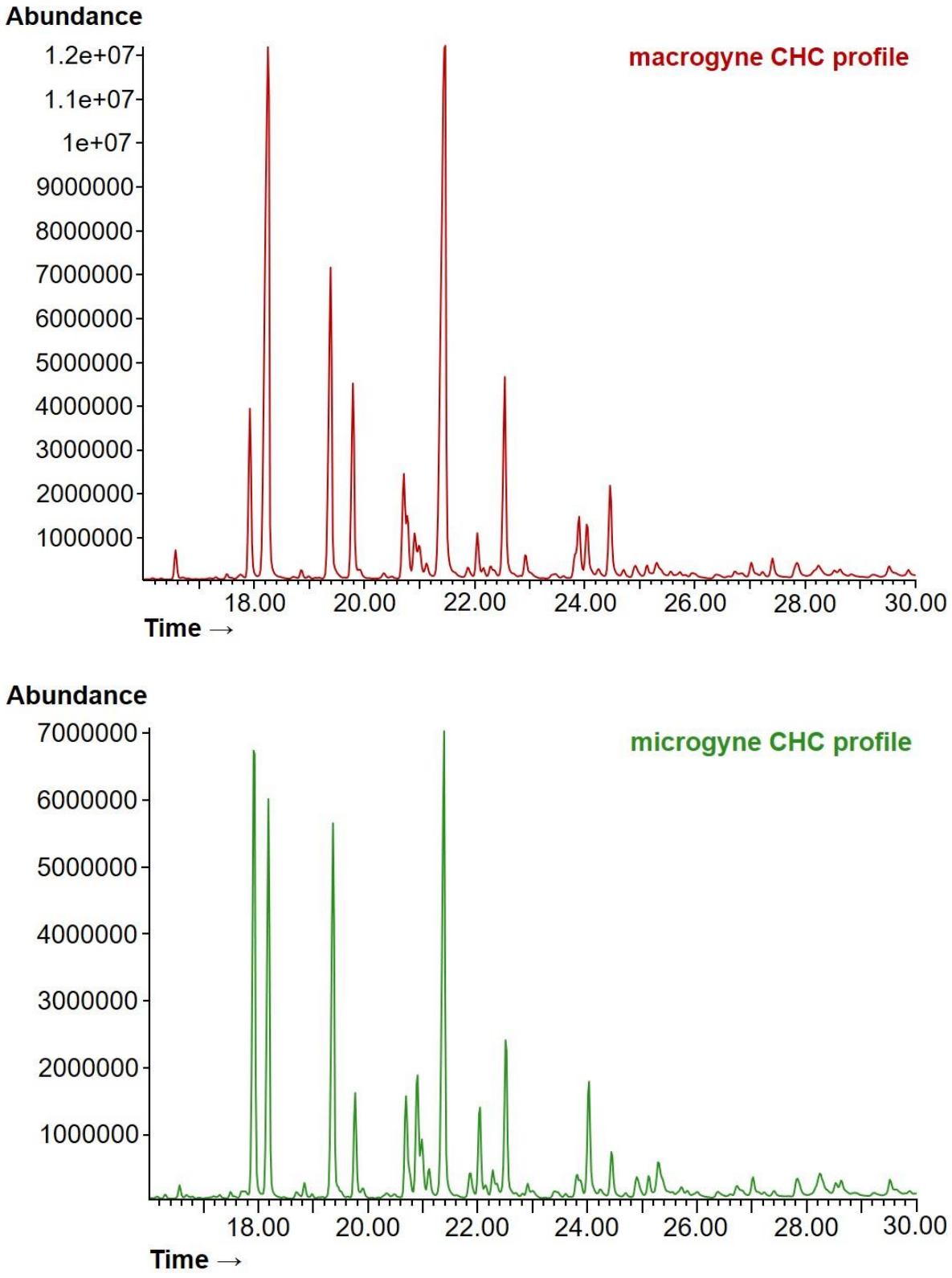
| Response variable | Explanatory variables | p-value |
|---|---|---|
| Location after 1h | Queen morph<br>Colony composition<br>Queen morph:Colony composition | 0.9685<br>0.4403<br>0.3585 |
| Aggression after 1h | Queen morph<br>Colony composition<br>Queen morph:Colony composition | 0.7119<br>0.1877<br>0.8054 |
| Grooming after 1h | Queen morph<br>Colony composition<br>Queen morph:Colony composition | 0.9096<br>0.7451<br>0.6601 |
| Survival after 24h | Queen morph<br>Colony composition<br>Queen morph:Colony composition | 0.5318<br>0.9455<br>0.5700 |
| Location after 24h | Queen morph<br>Colony composition<br>Queen morph:Colony composition | 0.4583<br>0.3633<br>0.9107 |

**Supplementary Table S8.** Proportion of queens alive after 24h and their location, depending on queen morph

| Queen morph | Proportion of queens alive after 24h | Proportion of queens found inside the nest (among the alive ones) |
|---|---|---|
| Macrogyne | 73.80% | 54.84% |
| Microgyne | 66.67% | 45.00% |

**Supplementary Table S9.** Identified chemical substances extracted from the queens

| Substance | Retention index | Relative abundance (%) |
|---|---|---|
| n-C19 | 19.11 | 0.22 ± 0.02 |
| unknown | 19.61 | 0.16 ± 0.02 |
| unknown | 19.97 | 0.05 ± 0.01 |
| n-C21 | 21.03 | 0.18 ± 0.03 |
| unknown | 21.47 | 0.11 ± 0.01 |
| unknown | 21.88 | 0.16 ± 0.01 |
| unknown | 22.05 | 0.09 ± 0.01 |
| unknown | 22.15 | 0.05 ± 0.01 |
| unknown | 22.41 | 0.04 ± 0.01 |
| unknown | 22.76 | 0.03 ± 0.01 |
| unknown | 22.79 | 0.09 ± 0.02 |
| n-C23 | 23.04 | 0.23 ± 0.03 |
| unknown | 23.24 | 0.03 ± 0.01 |
| unknown | 23.44 | 0.02 ± 0.01 |
| n-C24 | 24.05 | 0.38 ± 0.04 |
| n-C25 | 25.04 | 0.49 ± 0.04 |
| unknown | 25.09 | 0.03 ± 0.01 |
| unknown | 25.55 | 0.1 ± 0.01 |
| C26-ene | 25.87 | 0.07 ± 0.01 |
| n-C26 | 26.03 | 0.62 ± 0.05 |
| 6-MeC26 | 26.49 | 0.05 ± 0.01 |
| 4-MeC26 | 26.61 | 0.14 ± 0.01 |
| C27-ene | 26.75 | 0.21 ± 0.02 |
| C27-ene | 26.79 | 0.15 ± 0.02 |
| C27-ene | 26.87 | 11.87 ± 0.42 |
| n-C27 | 27.03 | 6.56 ± 0.4 |
| 11-MeC27 | 27.37 | 0.44 ± 0.02 |
| cf. C27-methyl alkene | 27.46 | 0.6 ± 0.03 |
| 5-MeC27 | 27.54 | 0.2 ± 0.01 |
| 11,15-DiMeC27 | 27.65 | 0.16 ± 0.01 |
| 3-MeC27 | 27.77 | 7.92 ± 0.26 |
| C28-ene | 27.87 | 0.06 ± 0.02 |
| n-C28 | 28.03 | 1.91 ± 0.14 |
| 3,7-DiMeC27 | 28.12 | 0.49 ± 0.03 |
| squalene | 28.34 | 0.23 ± 0.03 |
| 3,7,11-TriMeC27 | 28.39 | 0.36 ± 0.02 |
| unknown | 28.48 | 0.13 ± 0.01 |
| 4-MeC28 | 28.61 | 1.54 ± 0.07 |
| C29-ene | 28.74 | 4.64 ± 0.18 |
| C29-ene | 28.79 | 2.64 ± 0.13 |
| C29-ene | 28.87 | 1.11 ± 0.04 |
| nC29 | 29.02 | 6.96 ± 0.4 |
| 4,8,12-TriMeC28 | 29.18 | 0.11 ± 0.01 |
| 9-;11-;13-MeC29 | 29.35 | 1.31 ± 0.05 |
| cf C29-methyl alkene | 29.45 | 3.41 ± 0.15 |
| 11,15-DiMeC29 | 29.6 | 1.21 ± 0.08 |
| 11,19-DiMeC29 | 29.65 | 0.59 ± 0.04 |
| 3-MeC29 | 29.76 | 3.15 ± 0.19 |
| unknown | 29.82 | 0.03 ± 0.01 |
| 11,15,19-TriMeC29 | 29.87 | 0.25 ± 0.02 |
| 7,11,15-TriMeC29 | 29.96 | 0.15 ± 0.01 |
| n-C30 | 30.01 | 0.93 ± 0.09 |
| 5,11,15-TriMeC29 | 30.08 | 0.42 ± 0.03 |
| cf. 3,11,15-TriMeC29 | 30.33 | 0.07 ± 0.01 |
| 3,7,11-TriMeC29 | 30.37 | 0.42 ± 0.03 |
| C31-diene | 30.46 | 0.24 ± 0.03 |
| C31-diene | 30.61 | 0.84 ± 0.04 |
| C31-ene | 30.73 | 5.89 ± 0.26 |
| C31-ene | 30.88 | 0.45 ± 0.02 |
| n-C31 | 31.01 | 1.23 ± 0.08 |
| unknown | 31.2 | 0.17 ± 0.01 |
| 11-;13-MeC31 | 31.32 | 0.96 ± 0.04 |
| cf. C31-methyl alkene | 31.47 | 1.01 ± 0.05 |
| 11,15-DiMeC31 | 31.57 | 3.07 ± 0.15 |
| unknown | 31.75 | 0.13 ± 0.02 |
| cf. 9,15,x-TriMeC31 | 31.85 | 0.73 ± 0.05 |
| cf. 9,13,x-TriMeC31 | 31.92 | 0.33 ± 0.02 |
| unknown | 32 | 0.29 ± 0.05 |
| unknown | 32.04 | 0.39 ± 0.04 |
| 12-;13-;14-;15-;16-MeC32 | 32.31 | 0.3 ± 0.02 |
| C33-diene | 32.44 | 0.01 ± 0.01 |
| C33-diene | 32.47 | 0.2 ± 0.03 |
| C33-diene | 32.54 | 1.06 ± 0.08 |
| C33-diene | 32.61 | 0.13 ± 0.02 |
| C33-diene | 32.66 | 0.07 ± 0.02 |
| C33-ene | 32.74 | 1.21 ± 0.06 |
| C33-ene | 32.81 | 0.22 ± 0.03 |
| unknown saturated CHC | 32.88 | 0.16 ± 0.01 |
| n-C33 | 33 | 0.32 ± 0.03 |
| 11-;13-;15-;17-MeC33 | 33.29 | 0.9 ± 0.05 |
| 15,19-DiMeC33 | 33.47 | 0.03 ± 0.01 |
| 11,19-;13,19-DiMeC33 | 33.6 | 2.59 ± 0.13 |
| 11,15,x-TriMeC33 | 33.79 | 0.75 ± 0.04 |
| 11,15,x-TriMeC33 | 33.84 | 1.24 ± 0.07 |
| unknown | 33.98 | 0.39 ± 0.03 |
| unknown | 34.26 | 0.16 ± 0.02 |
| unknown | 34.35 | 0.07 ± 0.02 |
| C35-diene | 34.48 | 1.19 ± 0.08 |
| C35-diene | 34.56 | 0.45 ± 0.04 |
| C35-diene | 34.68 | 0.04 ± 0.01 |
| C35-en | 34.74 | 0.29 ± 0.03 |
| 13-;15-;17-MeC35 | 35.27 | 0.56 ± 0.04 |
| 11,x-;13,x-DiMeC35 | 35.56 | 1.46 ± 0.09 |
| 11,x,y-TriMeC35 | 35.8 | 0.55 ± 0.05 |
| unknown | 36.09 | 0.55 ± 0.07 |
| unknown | 36.16 | 0.7 ± 0.07 |
| C37-diene | 36.45 | 0.21 ± 0.03 |
| C37-diene | 36.53 | 0.1 ± 0.02 |
| 11-;13-;15-MeC37 | 37.26 | 0.21 ± 0.04 |
| 13,23-DiMeC37 | 37.52 | 0.68 ± 0.11 |
| 11-;13-MeC39 | 39.22 | 0.07 ± 0.01 |
| 11,x-;13,x-DiMeC39 | 39.47 | 0.2 ± 0.03 |




**Supplement Figure S4.** Cuticular hydrocarbon profiles from a macrogyne (red, above) and a microgyne (green, below)

## Glossary

**Queen morph:** morph of the queen (macrogyne or microgyne)

**Social structure:** number of queens in a colony (monogynous or polygynous)

**Colony morph:** morph of the queen(s) in a colony (macrogynous, microgynous or mixed if the two morphs co-occur)

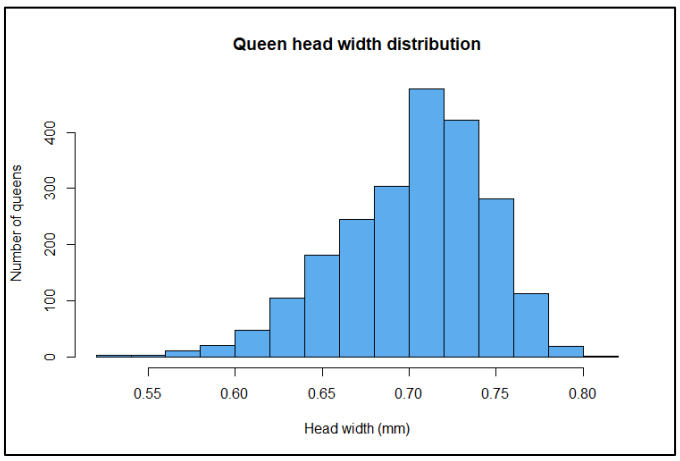
**Colony composition:** association of social structure and colony morph (monogynous macrogynous, polygynous macrogynous, monogynous microgynous, polygynous microgynous or polygynous mixed)

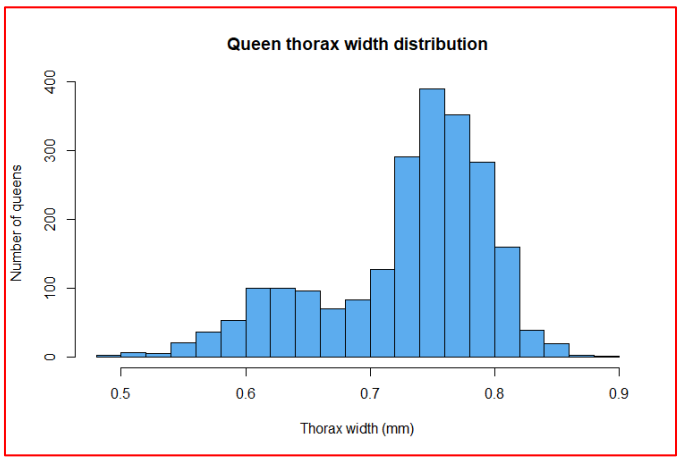
## Protocol for queen morph assignment

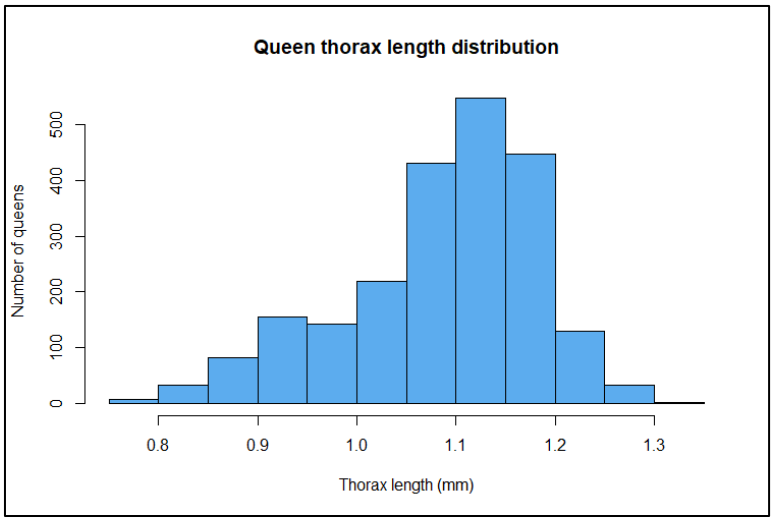
**Note:** we did not use the size index from Rüppel et al (1998) as criteria for queen morph assignment because we used this measure as a proxy for queen body size in our statistical analyses

**1.** Measure of head width, thorax width and thorax length for each queen (N = 2227)

**2.** Plot of the distribution of each measure

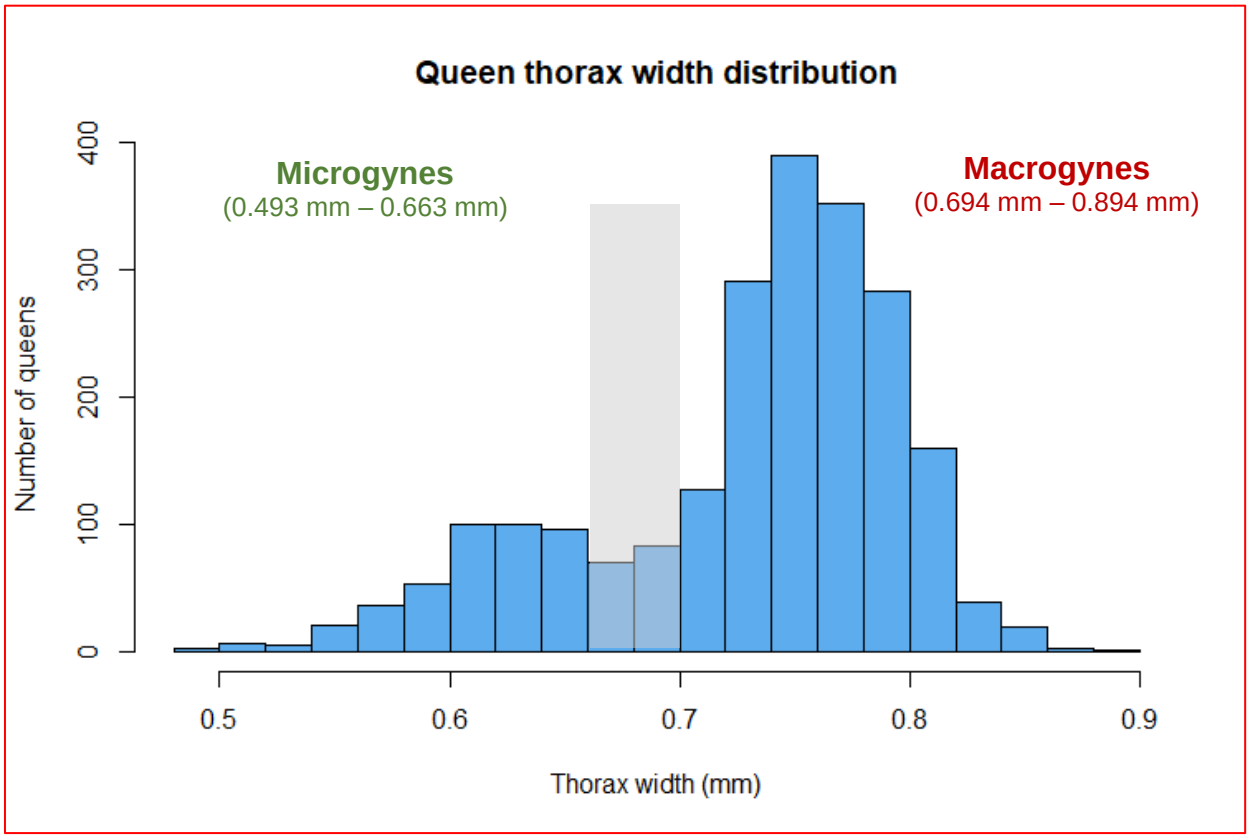





**3.** Decision on which measure has the strongest apparent binomial distribution for the initial assignment of the queens to their morph → thorax width

**4.** Initial assignment of the queens to their morph and definition of a grey zone with intermediate-sized queens (N = 106)



**5**. Calculation of the mean ± sem of each measure for both morph, excluding the intermediate-sized queens

| Queen morph | Head width (mm) | Thorax width (mm) | Thorax length (mm) |
|---|---|---|---|
| **Macrogyne** | 0.718 ± 0.035 | 0.759 ± 0.043 | 1.124 ± 0.052 |
| **Microgyne** | 0.640 ± 0.037 | 0.609 ± 0.044 | 0.919 ± 0.051 |

**6.** Based on the previous calculations, establishment of three criteria to assign the intermediate-sized queens to one of the two morphs (queens have to meet at least one criteria)

**Note:** thorax width is absent since we used this measure to define the grey zone

| **Criteria to be a macrogyne:** | **Criteria to be a microgyne:** |
|---|---|
| • 0.683 ≤ Head width ≤ 0.753<br>• 1.072 ≤ Thorax length ≤ 1.176<br>• Head width < Thorax width | • 0.603 ≤ Head width ≤ 0.677<br>• 0.868 ≤ Thorax length ≤ 0.970<br>• Head width > Thorax width |