

# Pathogen evasion of social immunity

---

In the format provided by the  
authors and unedited

## Supplementary Tables

**Supplementary Table 1) Statistical data analysis of all selection lines.** Statistical tests and their results. All p-values are two-sided and corrected following the Benjamini-Hochberg (BH) procedure. Exact p-values and contrasts in the Bayesian analysis are given (except for values <0.0001) and highlighted in bold when  $p < 0.05$  or Bayesian level of support LOS >95%. Individual selection treatment abbreviated by I, social selection treatment by S.

| Strain diversity during and after serial passage (Fig. 1b)                                                                                                                 |                                                                                      |                         |                          |                        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------|--------------------------|------------------------|
| <i>Wilcoxon Rank Sum Test (all data):</i>                                                                                                                                  |                                                                                      | W                       | Effect size ( $\eta^2$ ) | p-value                |
| strain number ~ selection treatment                                                                                                                                        | passage 5                                                                            | 21                      | 0.55                     | <b>0.015</b>           |
|                                                                                                                                                                            | passage 10                                                                           | 50                      | 0.00                     | 1.000                  |
| Strain composition after serial passage (Fig. 1b)                                                                                                                          |                                                                                      |                         |                          |                        |
| <i>Fisher's Exact Test:</i>                                                                                                                                                |                                                                                      |                         |                          | p-value                |
|                                                                                                                                                                            |                                                                                      |                         |                          | 0.629                  |
| Virulence after serial passage (Fig. 2a)                                                                                                                                   |                                                                                      |                         |                          |                        |
| <i>Bayesian multilevel model (all data):</i>                                                                                                                               |                                                                                      | Dying probability 95 CI |                          | HDI lower-upper        |
| mortality ~ selection history * current host social context + (1   strain) + (1   colony), family=bernoulli(), control = list(adapt_delta = 0.99), iter = 5000, chains = 6 | SS                                                                                   | 0.26                    |                          | 0.14-0.39              |
|                                                                                                                                                                            | II                                                                                   | 0.44                    |                          | 0.28-0.60              |
|                                                                                                                                                                            | SI                                                                                   | 0.3                     |                          | 0.16-0.43              |
|                                                                                                                                                                            | IS                                                                                   | 0.29                    |                          | 0.16-0.43              |
|                                                                                                                                                                            |                                                                                      | Contrast BH-corrected   |                          | Level of support (LOS) |
|                                                                                                                                                                            |                                                                                      | II vs SS                | <b>0.005</b>             | <b>99.82</b>           |
|                                                                                                                                                                            |                                                                                      | II vs IS                | <b>0.005</b>             | <b>99.92</b>           |
|                                                                                                                                                                            |                                                                                      | II vs SI                | <b>0.020</b>             | <b>99.15</b>           |
|                                                                                                                                                                            |                                                                                      | IS vs SI                | 0.610                    | 38.73                  |
|                                                                                                                                                                            |                                                                                      | IS vs SS                | 0.410                    | 66.05                  |
|                                                                                                                                                                            |                                                                                      | SI vs SS                | 0.300                    | 79.65                  |
| Spore production after serial passage (Fig. 2b)                                                                                                                            |                                                                                      |                         |                          |                        |
| <i>LMM Overall model (all data):</i>                                                                                                                                       |                                                                                      | $\chi^2$                | DF                       | p-value                |
| spore number ~ selection history * current host social context + (1   replicate line) + (1   laboratory stock colony)                                                      |                                                                                      | 21.68                   | 3                        | <b>&lt;0.0001</b>      |
|                                                                                                                                                                            | Effect size ( $\eta^2$ )                                                             |                         |                          |                        |
|                                                                                                                                                                            | selection history: 0.64<br>current host social context: <0.0001<br>interaction: 0.04 |                         |                          |                        |
| Interaction                                                                                                                                                                |                                                                                      | 4.37                    | 1                        | <b>0.037</b>           |
|                                                                                                                                                                            |                                                                                      | Contrast BH-corrected   |                          |                        |
|                                                                                                                                                                            |                                                                                      | II vs SS                | <b>&lt;0.0001</b>        |                        |
|                                                                                                                                                                            |                                                                                      | II vs IS                | 0.052                    |                        |
|                                                                                                                                                                            |                                                                                      | II vs SI                | <b>0.0007</b>            |                        |
|                                                                                                                                                                            |                                                                                      | IS vs SI                | <b>&lt;0.0001</b>        |                        |
|                                                                                                                                                                            |                                                                                      | IS vs SS                | <b>&lt;0.0001</b>        |                        |
|                                                                                                                                                                            |                                                                                      | SI vs SS                | 0.340                    |                        |

Supplementary Table 1 continued

| Allogrooming elicitation after serial passage (Fig. 3a) |                                |         |         |
|---------------------------------------------------------|--------------------------------|---------|---------|
| Wilcoxon Rank Sum Test (all data):                      | W                              | p-value |         |
| grooming events ~ selection history                     | 90                             | 0.0027  |         |
|                                                         | Effect size                    |         |         |
|                                                         | 0.678                          |         |         |
| Allogrooming elicitation by pure ergosterol (Fig. 3c)   |                                |         |         |
| Wilcoxon Rank Sum Test:                                 | W                              | p-value |         |
| grooming events ~ treatment                             | 155.5                          | 0.026   |         |
|                                                         | Effect size                    |         |         |
|                                                         | 0.333                          |         |         |
| Allogrooming elicitation by pure cholesterol (Fig. 3d)  |                                |         |         |
| Wilcoxon Rank Sum Test:                                 | W                              | p-value |         |
| grooming events ~ treatment                             | 345                            | 0.144   |         |
|                                                         | Effect size                    |         |         |
|                                                         | 0.214                          |         |         |
| Chemical analysis (Fig. 3b, ED Fig. 1)                  |                                |         |         |
| Permanova:                                              | F                              | DF      | p-value |
| compounds ~ selection history, method="eucl"            | 4.75                           | 1       | 0.021   |
| Random forest (RF):                                     |                                |         |         |
| Number of trees: 500                                    |                                |         |         |
| No. of variables tried at each split: 6                 |                                |         |         |
| OOB estimate of error rate: 30%                         |                                |         |         |
| Wilcoxon RS test on RF-suggested compounds              | W                              |         | p-value |
|                                                         | Ergosterol (comp. 18; RI 3269) | 82      | 0.025   |
|                                                         | compound 7 (RI2631)            | 85      | 0.007   |
|                                                         | compound 17 (RI 3226)          | 89      | 0.007   |
|                                                         | compound 19 (RI 3277)          | 82      | 0.025   |
|                                                         | compound 25 (RI 3420)          | 79      | 0.031   |
|                                                         | compound 16 (RI 3204)          | 79      | 0.031   |

**Supplementary Table 2) Statistical data analysis of the *M. robertsii* R1-dominant lines.**

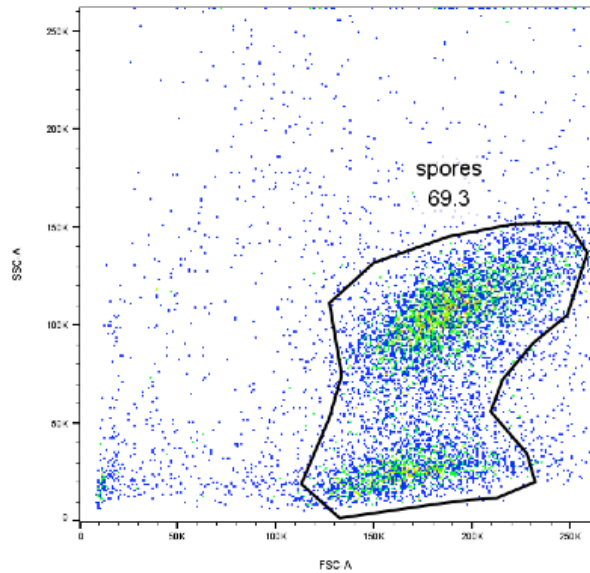
Statistical tests and their results for the subset of the 7 individual and 6 social lines, in which strain R1 was dominant. All p-values are two-sided and corrected following the Benjamini-Hochberg (BH) procedure. Significant p-values ( $p < 0.05$ ) and contrasts in the Bayesian analysis (level of support LOS >95%) shown in bold. Individual selection treatment abbreviated by I, social selection treatment by S.

| Virulence after serial passage (Fig. 2a)                                                                                                                                   |                                   |       |                        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-------|------------------------|
| Bayesian multilevel model (subset R1-dominant lines):                                                                                                                      | Dying probability 95 CI           |       | HDI lower-upper        |
| mortality ~ selection history * current host social context + (1   strain) + (1   colony), family=bernoulli(), control = list(adapt_delta = 0.99), iter = 4000, chains = 6 | SS                                | 0.28  | 0.10-0.47              |
|                                                                                                                                                                            | II                                | 0.45  | 0.24-0.68              |
|                                                                                                                                                                            | SI                                | 0.28  | 0.11-0.48              |
|                                                                                                                                                                            | IS                                | 0.28  | 0.11-0.47              |
|                                                                                                                                                                            | Contrast BH-corrected             |       | Level of support (LOS) |
|                                                                                                                                                                            | II vs SS                          | 0.007 | 99.82                  |
|                                                                                                                                                                            | II vs IS                          | 0.002 | 99.92                  |
|                                                                                                                                                                            | II vs SI                          | 0.009 | 99.14                  |
|                                                                                                                                                                            | IS vs SI                          | 0.502 | 38.73                  |
|                                                                                                                                                                            | IS vs SS                          | 0.491 | 66.05                  |
|                                                                                                                                                                            | SI vs SS                          | 0.490 | 79.65                  |
| Spore production after serial passage (Fig. 2b)                                                                                                                            |                                   |       |                        |
| LMM Overall model (subset R1-dominant lines):                                                                                                                              | $\chi^2$                          | DF    | p-value                |
| spore number ~ selection history * current host social context + (1   replicate line) + (1   laboratory stock colony)                                                      | 12.44                             | 3     | 0.006                  |
|                                                                                                                                                                            | Effect size ( $\eta^2$ )          |       |                        |
|                                                                                                                                                                            | selection history: 0.55           |       |                        |
|                                                                                                                                                                            | current host social context: 0.07 |       |                        |
|                                                                                                                                                                            | interaction: 0.004                |       |                        |
| Interaction                                                                                                                                                                | 0.95                              | 1     | 0.329                  |
| Selection history                                                                                                                                                          | 10.65                             | 1     | 0.001                  |
| Current host social context                                                                                                                                                | 0.33                              | 1     | 0.562                  |
|                                                                                                                                                                            | Contrast BH-corrected             |       |                        |
|                                                                                                                                                                            | II vs SS                          |       | 0.0009                 |
|                                                                                                                                                                            | II vs IS                          |       | 0.871                  |
|                                                                                                                                                                            | II vs SI                          |       | 0.008                  |
|                                                                                                                                                                            | IS vs SI                          |       | 0.008                  |
|                                                                                                                                                                            | IS vs SS                          |       | 0.0009                 |
|                                                                                                                                                                            | SI vs SS                          |       | 0.300                  |
| Allogrooming elicitation after serial passage (Fig. 3a)                                                                                                                    |                                   |       |                        |
| Wilcoxon Rank Sum Test (subset R1-dominant lines):                                                                                                                         | W                                 |       | p-value                |
| grooming events ~ selection history                                                                                                                                        | 37                                |       | 0.026                  |
|                                                                                                                                                                            | Effect size                       |       |                        |
|                                                                                                                                                                            | 0.638                             |       |                        |
| Chemical analysis (Fig. 3b, ED Fig.1)                                                                                                                                      |                                   |       |                        |
| Wilcoxon Rank Sum Test (subset R1-dominant lines):                                                                                                                         | W                                 |       | p-value                |
|                                                                                                                                                                            | Ergosterol (comp. 18; RI 3269)    | 34    | 0.073                  |
|                                                                                                                                                                            | compound 7 (RI 2631)              | 39    | 0.020                  |
|                                                                                                                                                                            | compound 17 (RI 3226)             | 40    | 0.020                  |
|                                                                                                                                                                            | compound 19 (RI 3277)             | 34    | 0.073                  |
|                                                                                                                                                                            | compound 25 (RI 3420)             | 37    | 0.053                  |
|                                                                                                                                                                            | compound 16 (RI 3204)             | 36    | 0.060                  |

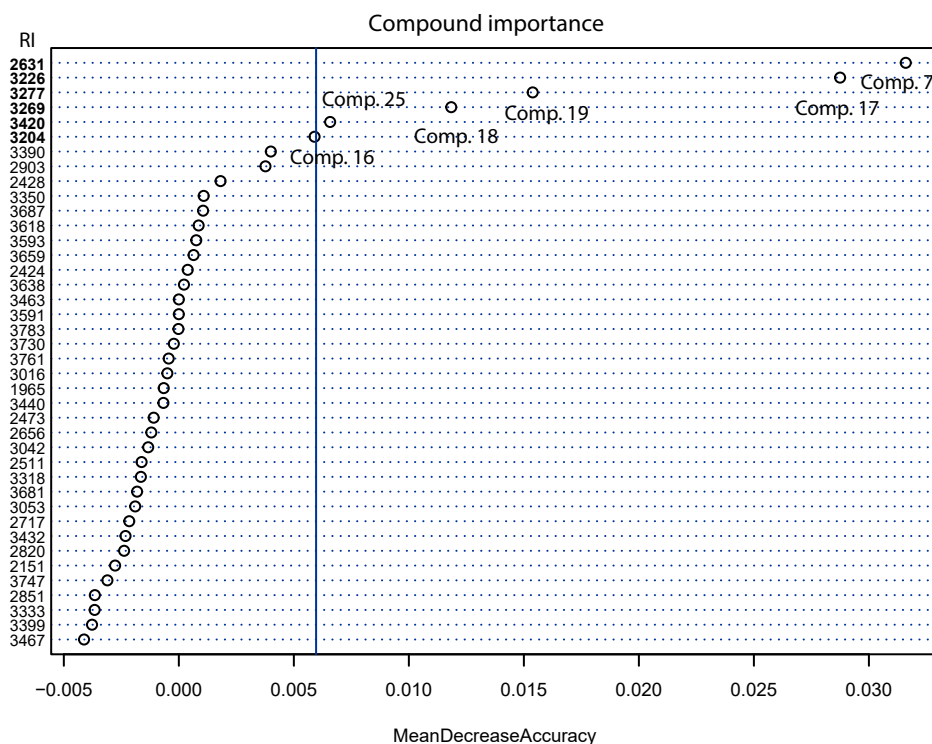
**Supplementary Table 3) Chemical spore profiles.** Identification number (ID), Kováts retention index (RI) and diagnostic ions, as well as resulting classification (Class), and the confidence level of compound annotations, following the Compound Identification work group of the Metabolomics Society (Online Methods); unknown compounds C1-3 have very similar spectra. Mass spectrum of compound 7 (Unknown A) shown in Supplementary Fig. 3. Note that not every compound was detectable above the signal to noise ratio in each sample.

| ID | RI   | Diagnostic ions [ <i>m/z</i> ] | Confidence level of annotation | Class                 |
|----|------|--------------------------------|--------------------------------|-----------------------|
| 1  | 1965 | 129, 213, 256                  | 2                              | Carboxylic acid       |
| 2  | 2151 | 55, 264, 282                   | 3                              | Carboxylic acid       |
| 3  | 2424 | 67, 262, 280                   | 3                              | Ester                 |
| 4  | 2428 | 98, 264, 265                   | 3                              | Ester                 |
| 5  | 2473 | 67, 263, 336                   | 3                              | Ester                 |
| 6  | 2511 | 134, 239, 299                  | 3                              | Ester                 |
| 7  | 2631 | 147, 187, 314                  | 4                              | Unknown A             |
| 8  | 2656 | 101, 117, 131                  | 4                              | Unknown B             |
| 9  | 2717 | 55, 262, 264                   | 3                              | Ester                 |
| 10 | 2820 | 69, 81, 410                    | 2                              | Squalene              |
| 11 | 2851 | 337, 363, 378                  | 3                              | Ergosterol derivative |
| 12 | 2903 | 253, 363, 378                  | 3                              | Ergosterol derivative |
| 13 | 3016 | 105, 251, 376                  | 2                              | Ergosterol derivative |
| 14 | 3042 | 250, 251, 376                  | 3                              | Ergosterol derivative |
| 15 | 3053 | 250, 251, 376                  | 3                              | Ergosterol derivative |
| 16 | 3204 | 253, 363, 396                  | 3                              | Ergosterol derivative |
| 17 | 3226 | 253, 363, 396                  | 3                              | Ergosterol derivative |
| 18 | 3269 | 253, 363, 396                  | 1                              | Ergosterol            |
| 19 | 3277 | 251, 376, 394                  | 3                              | Ergosterol derivative |
| 20 | 3318 | 339, 365, 398                  | 3                              | Ergosterol derivative |
| 21 | 3333 | 271, 383, 398                  | 3                              | Ergosterol derivative |
| 22 | 3350 | 189, 218, 424                  | 3                              | Lupeol derivative     |
| 23 | 3390 | 175, 218, 426                  | 3                              | Lupeol derivative     |
| 24 | 3399 | 393, 411, 426                  | 3                              | Lupeol derivative     |
| 25 | 3420 | 99, 283, 417                   | 3                              | Ester                 |
| 26 | 3432 | 253, 268, 392                  | 3                              | Ergosterol derivative |
| 27 | 3440 | 205, 313, 424                  | 3                              | Lupeol derivative     |
| 28 | 3463 | 393, 411, 426                  | 3                              | Lupeol derivative     |
| 29 | 3467 | 120, 148, 176                  | 4                              | Unknown C1            |
| 30 | 3591 | 205, 313, 424                  | 3                              | Lupeol derivative     |
| 31 | 3593 | 120, 148, 176                  | 4                              | Unknown C2            |
| 32 | 3618 | 189, 357, 426                  | 3                              | Lupeol derivative     |
| 33 | 3638 | 120, 148, 176                  | 4                              | Unknown C3            |
| 34 | 3659 | 189, 411, 440                  | 3                              | Lupeol derivative     |
| 35 | 3681 | 205, 313, 424                  | 3                              | Lupeol derivative     |
| 36 | 3687 | 205, 453, 468                  | 3                              | Lupeol derivative     |
| 37 | 3730 | 379, 422, 482                  | 2                              | Ursane derivative     |
| 38 | 3747 | 407, 422, 482                  | 3                              | Lanosterol derivative |
| 39 | 3761 | 189, 409, 440                  | 3                              | Lupeol derivative     |
| 40 | 3783 | 189, 453, 469                  | 3                              | Lupeol derivative     |

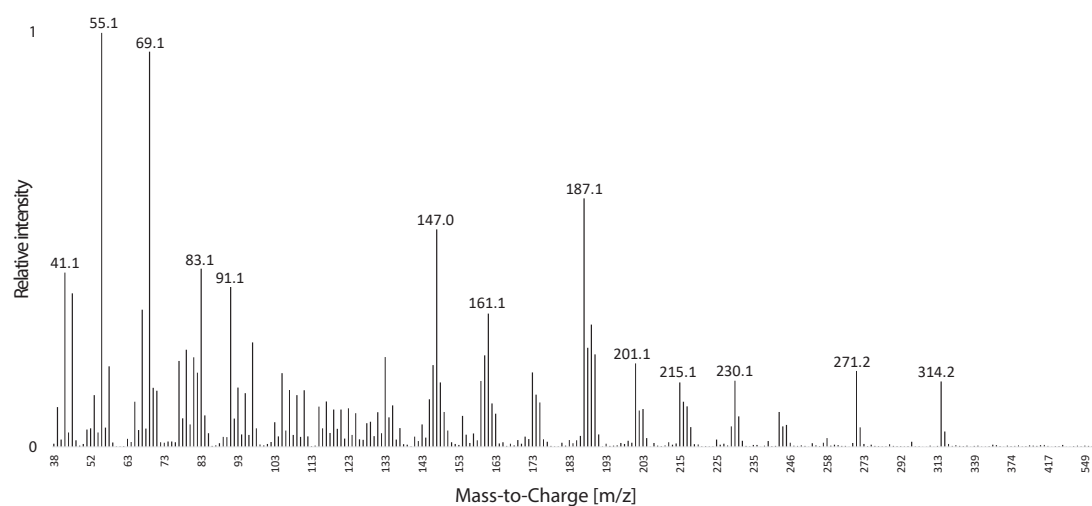
## Supplementary Figures



**Supplementary Figure 1) Gating strategy in the flow cytometry.** To obtain single clones per replicate line (and excluding spore clusters), individual spores were sorted into 96-well plates. Unstained spores were detected using the exemplified gating strategy of FSC/SSC in linear mode (70  $\mu\text{m}$  nozzle). Two spore populations appear, as *Metarhizium* spores are elongated, so that depending on their orientation (horizontal vs. vertical) in the sorter, the forward and sideward scatter values vary slightly. Confirmation that this procedure led to single spores was obtained by microscopy.



**Supplementary Figure 2) Spore compound importance as identified by random forest analysis.** Conditional random forest classification (n trees = 500, n variables per split = 6) identified 6 compounds (numbers 7, 16, 17, 18, 19 and 25; Supplementary Table 3) of the chemical spore profiles that contribute to differences between the two selection treatments. Important compounds were selected based on the absolute value of the lowest negative score of the mean decrease in accuracy as a threshold (indicated by the vertical blue line). Compounds are sorted from top to down by their importance of contribution, and named by their Retention Index (RI; Supplementary Table 3).



**Supplementary Figure 3) Mass spectrum of compound 7.** Mass spectrum of compound 7 (Unknown A, Supplementary Table 3), which is significantly lower in abundance in the social than the individual lines (Supplementary Fig. 2, Extended Data Fig. 1).