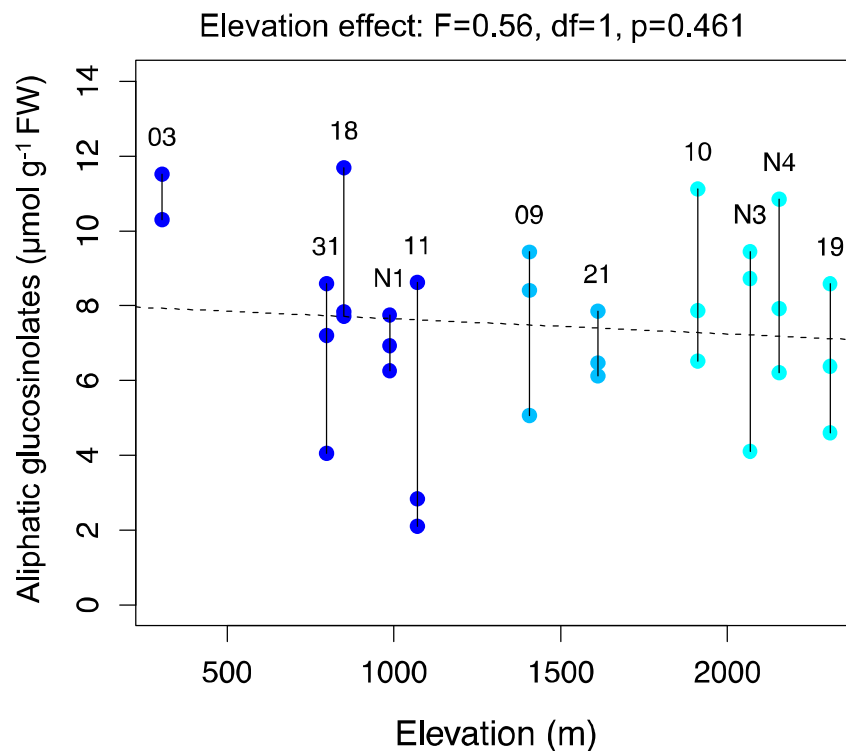


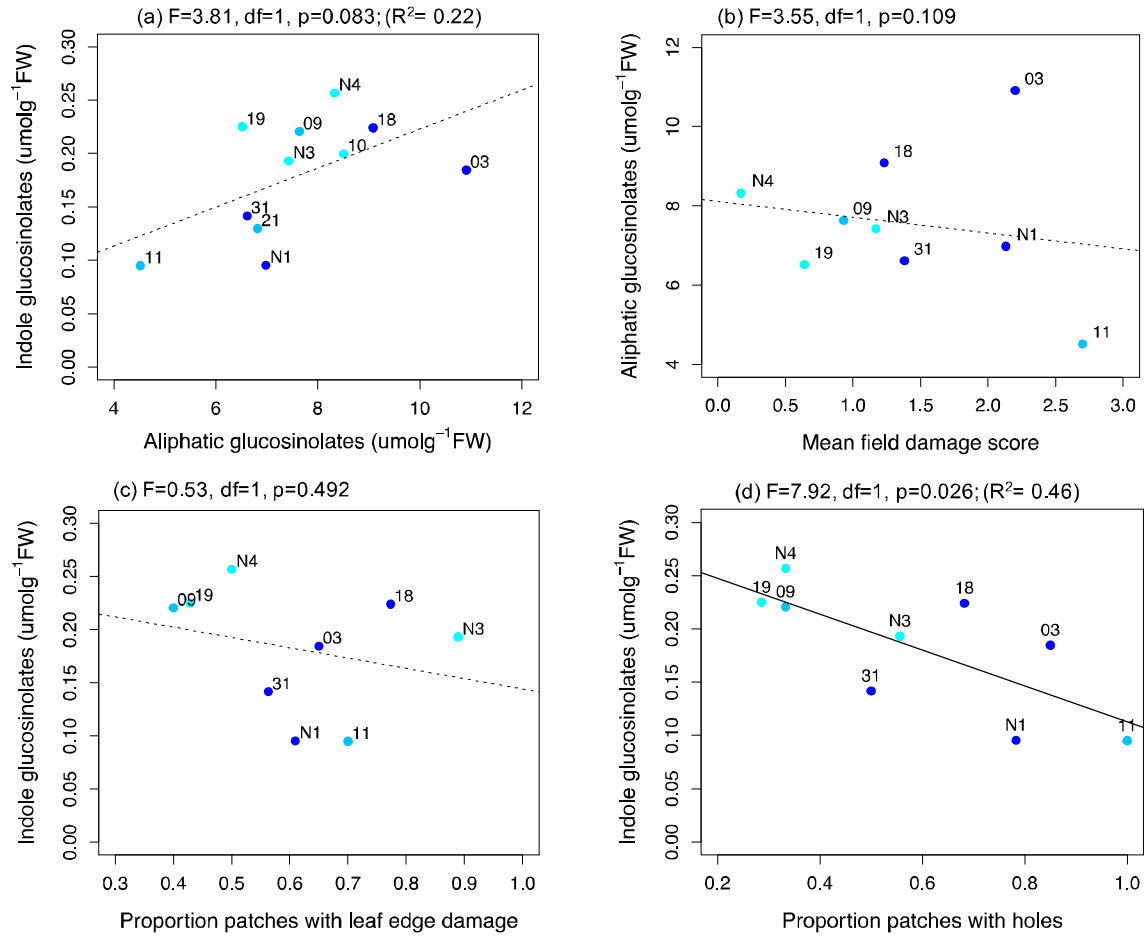
# Divergence in Glucosinolate Profiles between High- and Low-Elevation Populations of *Arabidopsis halleri* Correspond to Variation in Field Herbivory and Herbivore Behavioral Preferences

James Buckley, Foteini G. Pashalidou, Martin C. Fischer, Alex Widmer, Mark C. Mescher, Consuelo M. De Moraes\*

## SUPPLEMENTARY INFORMATION



**Figure S1.** Regression of total aliphatic glucosinolates (in micromoles per gram of fresh tissue,  $\mu\text{mol g}^{-1}$  FW) against elevation controlling for variation in plant size (with samples from the same population joined by black vertical lines). Samples came from plants growing under a common greenhouse environment. Elevation had no significant effect on aliphatic glucosinolate concentrations. Points are coloured by elevation class.



**Figure S2.** Regressions of amounts of aliphatic and indole glucosinolates (in micromoles per gram of fresh tissue,  $\mu\text{mol g}^{-1}$  FW) against each other and with respect to population-level mean field damage. (a) total indole glucosinolates against total aliphatic glucosinolates. (b) total aliphatic glucosinolates against mean damage score per population in the field surveys in 2016. (c) total indole glucosinolates against the proportion of patches in a population with leaf edge damage. (d) total indole glucosinolates against the proportion of patches in a population with hole damage. The significance of the effect and adjusted R-squared is given. Points are coloured by elevation class. A dashed line indicates a non-significant regression line, a solid line indicates a significant line.

**Table S1.** Overview of locations of study sites in the Alps, including a brief description of the habitat in which *Arabidopsis halleri* was found, as well as the dates on which damage surveys were conducted in the field and the number of genotypes (and total number of individual plants) used for preference assays and screened for constitutive variation in glucosinolates (GSLs). Elevation is given in metres above sea level (m asl).

| Site name | Swiss Canton | Coordinates (decimal degrees, °N, °E) | Elevation (m asl) | Habitat description                         | Year parent collected | Field damage survey date | Number genotypes                |                |
|-----------|--------------|---------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------|---------------------------------|----------------|
|           |              |                                       |                   |                                             |                       |                          | Preference assay (total number) | Screening GSLs |
| Aha03     | Ticino       | 46.17733, 8.70761                     | 305               | Roadside verge, forest edge                 | 2016                  | 29th April               | -                               | 2              |
| Aha31     | Graubünden   | 46.33682, 9.52171                     | 798               | Grass meadow (for cutting)                  | 2014, 2016            | 29th April               | 4 (12)                          | 3              |
| Aha01     | Ticino       | 46.16439, 9.07098                     | 804               | Grass meadow around building                | 2016                  | 28th April               | -                               | -              |
| Aha18     | Ticino       | 46.41593, 8.82605                     | 850               | Grass meadow (for cutting) and forest glade | 2012                  | 28th April               | 4 (12)                          | 3              |
| Aha02     | Ticino       | 46.16814, 9.09173                     | 944               | Grass meadow (for cutting)                  | 2016                  | 28th April               | -                               | -              |
| AhaN1     | Graubünden   | 46.27532, 10.10072                    | 987               | Next to path, grass meadow (for cutting)    | 2012                  | 30th April               | 4 (12)                          | 3              |
| Aha11     | Graubünden   | 46.27767, 10.10619                    | 1070              | Grass meadow around building                | 2012                  | 30th April               | -                               | 3              |
| Aha09     | Graubünden   | 46.36925, 9.65868                     | 1406              | Grass meadow (for cutting)                  | 2012                  | 29th April               | 4 (12)                          | 3              |
| Aha21     | Graubünden   | 46.36682, 9.63081                     | 1611              | Grass meadow around building                | 2012                  | Not done                 | 4 (11)                          | 3              |
| Aha10     | Graubünden   | 46.45919, 9.88106                     | 1910              | Grass meadow around building                | 2012                  | Not done                 | 4 (11)                          | 3              |
| AhaN3     | Graubünden   | 46.49926, 9.82731                     | 2067              | Vegetated areas around buildings            | 2012                  | 4th July                 | -                               | 3              |
| AhaN4     | Italy        | 46.49115, 10.22226                    | 2155              | Grass meadow                                | 2012, 2016            | 5th July                 | 4 (11)                          | 3              |
| Aha19     | Graubünden   | 46.41125, 10.02253                    | 2307              | Grass meadow (grazed)                       | 2012, 2016            | 5th July                 | 4 (10)                          | 3              |

**Table S2.** Glucosinolates identified in *A. halleri* in our study, with the abbreviations used throughout the manuscript, together with the retention times and m/z values ([M-H]<sup>-</sup> ion) of desulfo-glucosinolates (desulfo-GSL) on our LC/MS system. Sinigrin monohydrate was used as a standard for quantification of other glucosinolates and is therefore highlighted in bold (abbreviation 'PREN'). All the aliphatic glucosinolates listed derive from the amino acid methionine, and the listed indole glucosinolates derive from the amino acid tryptophan. All glucosinolates were provisionally identified by MS formula matches, UV spectra and retention times (elutropic series), but the identity of glucobrassicin (GB) was also confirmed through comparison to a laboratory standard.

| Abbreviation | Full name (+GSL suffix)   | Glucosinolate class | Other names             | Retention time | m/z ([M-H] <sup>-</sup> ) | Formula (desulfo-GSL)                                           |
|--------------|---------------------------|---------------------|-------------------------|----------------|---------------------------|-----------------------------------------------------------------|
| <b>PREN</b>  | <b>2-propenyl</b>         | <b>aliphatic</b>    | <b>Sinigrin</b>         | <b>4.9</b>     | <b>277.07</b>             | <b>C<sub>10</sub>H<sub>12</sub>NO<sub>6</sub>S</b>              |
| 5MSP         | 5-(methylsulfinyl)pentyl  | aliphatic           | Glucoalyssin            | 6.63           | 370.09                    | C <sub>13</sub> H <sub>25</sub> NO <sub>7</sub> S <sub>2</sub>  |
| 6MSH         | 6-(methylsulfinyl)hexyl   | aliphatic           | Glucohesperin           | 9.35           | 384.12                    | C <sub>14</sub> H <sub>27</sub> NO <sub>7</sub> S <sub>2</sub>  |
| 7MSH         | 7-(methylsulfinyl)heptyl  | aliphatic           | Glucoibarin             | 12.6           | 398.14                    | C <sub>15</sub> H <sub>29</sub> NO <sub>7</sub> S <sub>2</sub>  |
| GB           | 3-indolylmethyl           | indole              | Glucobrassicin          | 14.35          | 367.09                    | C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub> S |
| 8MSO         | 8-(methylsulfinyl)octyl   | aliphatic           | Glucohirsutin           | 16.5           | 412.15                    | C <sub>16</sub> H <sub>31</sub> NO <sub>7</sub> S <sub>2</sub>  |
| 4MeOGB       | 4-methoxy-3-indolylmethyl | indole              | 4-Methoxyglucobrassicin | 17.35          | 397.11                    | C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>7</sub> S |
| 6MTH         | 6-(methylthio)hexyl       | aliphatic           | Glucosquerellin         | 23.05          | 368.12                    | C <sub>14</sub> H <sub>27</sub> NO <sub>6</sub> S <sub>2</sub>  |
| 7MTH         | 7-(methylthio)heptyl      | aliphatic           | NA                      | 28.05          | 382.137                   | C <sub>15</sub> H <sub>29</sub> NO <sub>6</sub> S <sub>3</sub>  |
| 8MTO         | 8-(methylthio)octyl       | aliphatic           | NA                      | 32.85          | 396.15                    | C <sub>16</sub> H <sub>31</sub> NO <sub>6</sub> S <sub>2</sub>  |

**Table S3.** Mean amounts of total, aliphatic and indole glucosinolates for each population screened in this study (in micromoles per gram of fresh tissue,  $\mu\text{mol g}^{-1}\text{FW}$ ). The proportion of indole glucosinolates relative to the total glucosinolates is given and populations are ordered by increasing elevation. The values in each column are shaded red to indicate populations with relatively high amounts (darker reds) and low amounts (white/lighter reds) of the respective glucosinolate class.

| Population | N | Mean amounts in $\mu\text{mol g}^{-1}\text{FW}$ |                  |               |           |
|------------|---|-------------------------------------------------|------------------|---------------|-----------|
|            |   | Total glucosinolates                            | Total aliphatics | Total indoles | % indoles |
| Aha03      | 2 | 11.10                                           | 10.91            | 0.18          | 1.7       |
| Aha31      | 3 | 6.76                                            | 6.62             | 0.14          | 2.1       |
| Aha18      | 3 | 9.31                                            | 9.08             | 0.22          | 2.4       |
| AhaN1      | 3 | 7.08                                            | 6.98             | 0.10          | 1.3       |
| Aha11      | 3 | 4.61                                            | 4.52             | 0.09          | 2.1       |
| Aha09      | 3 | 7.86                                            | 7.64             | 0.22          | 2.8       |
| Aha21      | 3 | 6.95                                            | 6.82             | 0.13          | 1.9       |
| Aha10      | 3 | 8.71                                            | 8.51             | 0.20          | 2.3       |
| AhaN3      | 3 | 7.62                                            | 7.43             | 0.19          | 2.5       |
| Aha19      | 3 | 6.75                                            | 6.52             | 0.22          | 3.3       |
| AhaN4      | 3 | 8.58                                            | 8.33             | 0.26          | 3.0       |

**Table S4.** Overview of glucosinolate investment, field damage and *Pieris brassicae* preference for the four populations (two high-elevation, two low-elevation) used in the second preference experiment. The mean number of eggs laid after 20 h are given for the four populations used in the first and second preference experiment, as well as the mean amounts of total, aliphatic and indole glucosinolates (in micromoles per gram of fresh tissue,  $\mu\text{mol g}^{-1}\text{FW}$ ) estimated under greenhouse conditions. The percentage of indole glucosinolates relative to the total glucosinolates is given. The last column provides mean damage score per plant surveyed in the field for the four populations. .

| Population | Elevation class | Preference experiment 2 |      | Preference experiment 1 |      | Mean $\mu\text{mol g}^{-1}\text{FW}$ |                  |               | % indoles of total | Mean damage score (field) |
|------------|-----------------|-------------------------|------|-------------------------|------|--------------------------------------|------------------|---------------|--------------------|---------------------------|
|            |                 | Mean eggs laid          | SE   | Mean eggs laid          | SE   | Total glucosinolates                 | Total aliphatics | Total indoles |                    |                           |
| Aha31      | low             | 18.0                    | 7.9  | 23.3                    | 11.5 | 6.76                                 | 6.62             | 0.14          | 2.1                | 1.38                      |
| Aha18      | low             | 22.8                    | 11.6 | 27.2                    | 8.2  | 9.31                                 | 9.08             | 0.22          | 2.4                | 1.23                      |
| AhaN4      | high            | 7.3                     | 3.4  | 11.6                    | 6.2  | 8.58                                 | 8.33             | 0.26          | 3.0                | 0.17                      |
| Aha19      | high            | 0.7                     | 0.4  | 4.9                     | 2.4  | 6.75                                 | 6.52             | 0.22          | 3.3                | 0.64                      |