Chemical signals act as the main reproductive barrier between sister and mimetic *Heliconius* butterflies

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Table S1. Samples included in the quantification analysis of wing phenotype ................................................................. 3

Table S2. Female behavioural response towards males with normal (control) and altered (treatment) wing phenotype................................................................. 6

Table S3. Female behavioural responses in triads that tested female preference for males “perfumed” with a hexanic extract from five males either of *H. melpomene malleti* or *H. timareta florencia* ........................................ 7

Table S4. Amount (ng) of compounds that remained in the wings of perfumed males before evaporation................................................................. 8

Table S5. Behavioural response of F\(_1\) and backcross females towards pure males of *H. melpomene malleti* and *H. timareta florencia* ...................................................... 11

Table S6. Compounds identified in wing androconia’s extracts of males of *H. melpomene malleti*, *H. timareta florencia*, F\(_1\) and backcross......................... 12

Table S7. Compounds identified in the abdominal glands’ extracts of males of *H. melpomene malleti*, *H. timareta florencia*, F\(_1\) and backcross......................... 15

Table S8. Probability of mating in no-choice experiments.................. 19

Figure S1. Map showing the geographic distribution and wing phenotype of *H. melpomene malleti* and *H. timareta florencia* .......................................................... 20

Figure S2. Location of landmarks (LM) coordinates on the forewings and hindwings of *H. melpomene malleti* and *H. timareta florencia* .............................. 21

Figure S3. Species wing size and shape .................................................. 22

Figure S4. (A) Shape variation of forewings of *H. melpomene malleti* (light grey) and *H. timareta florencia* (dark grey) .................................................. 23

Figure S5. Colour pattern comparison between the two species ................. 24
Figure S6. Mate choice triads testing the importance of wing colour pattern in mate preference .................................................................................................................26

Figure S7. Amount (ng) of compounds that remained in the wings of perfumed males before evaporation .................................................................................................27

Figure S8. Mate choice triads testing behavioural responses in F1 and backcross (BC) females ..................................................................................................................28

Figure S9. Species differences in male androconia extracts ........................................29

Figure S10. Cluster analysis based on Euclidian distance of compound composition in the wing androconia of males of H. melpomene malleti, H. timareta florencia, F1 and backcrosses (BC) ..................................................................................................................30

Figure S11. Chromatogram patterns obtained from androconial extracts of F1 and backcross males .................................................................................................................31

Figure S12. Chromatogram pattern of the abdominal gland bouquet of males .................................................................................................................................32

Figure S13. Cluster analysis based on Euclidian distance of the compound composition of the wing androconia of males of H. melpomene malleti, H. timareta florencia, F1 and backcrosses (BC) ..................................................................................................................33

Figure S14. Chromatogram patterns obtained from abdominal gland bouquet of F1 and backcross males .................................................................................................................34

REFERENCES ......................................................................................................................35
Table S1. Samples included in the quantification analysis of wing phenotype. A total of 89 individuals were used. The wings were obtained from “Colección de Artrópodos de la Universidad del Rosario (CAUR229)”. Wild individuals of *H. timareta florencia* and *H. melpomene malleti* were collected in the localities Sucre and Doraditas in Colombia (01°48′12″ N - 75°39′19″ W, 1200 m and 01°42′39″ N - 75°42′32″ W, 1400 m). The last column specifies the analysis in which each specimen was used. D: dorsal; V: ventral; HW: hindwing; FW: forewing.

| ID Collection | ID Wing Scan | Taxon                  | Locality              | Analysis in which the sample was used |
|---------------|--------------|------------------------|-----------------------|---------------------------------------|
| M54           | LGE-WS-00351 | *H. t. florencia*      | Quebrada_Las_Doraditas | D-HW; D-FW                            |
| M63           | LGE-WS-00349 | *H. t. florencia*      | Finca_Piñacue         | D-HW; D-FW; V-HW; V-FW                 |
| M64           | LGE-WS-00350 | *H. t. florencia*      | Finca_Piñacue         | D-HW; D-FW; V-HW; V-FW                 |
| M244          | LGE-WS-00373 | *H. m. malleti*        | Florencia             | D-HW; D-FW; V-HW; V-FW                 |
| M253          | LGE-WS-00375 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M255          | LGE-WS-00326 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M257          | LGE-WS-00346 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M259          | LGE-WS-00325 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW                            |
| M415          | LGE-WS-00397 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M418          | LGE-WS-00305 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M426          | LGE-WS-00361 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M433          | LGE-WS-00390 | *H. m. malleti*        | Florencia_Sucre       | V-HW; V-FW                            |
| M434          | LGE-WS-00363 | *H. m. malleti*        | Florencia_Sucre       | V-HW; V-FW                            |
| M451          | LGE-WS-00304 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M462          | LGE-WS-00337 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M468          | LGE-WS-00383 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M471          | LGE-WS-00307 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M472          | LGE-WS-00311 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M474          | LGE-WS-00359 | *H. m. malleti*        | Florencia_Sucre       | V-HW; V-FW                            |
| M583          | LGE-WS-00367 | *H. m. malleti*        | Florencia             | D-HW; D-FW; V-HW; V-FW                 |
| M584          | LGE-WS-00357 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M587          | LGE-WS-00306 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M588          | LGE-WS-00310 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M589          | LGE-WS-00370 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M590          | LGE-WS-00395 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M592          | LGE-WS-00394 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M593          | LGE-WS-00309 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M594          | LGE-WS-00368 | *H. m. malleti*        | Quebrada_Las_Doraditas| D-HW; D-FW; V-HW; V-FW                 |
| M595          | LGE-WS-00318 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M596          | LGE-WS-00308 | *H. t. florencia*      | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| M598          | LGE-WS-00377 | *H. m. malleti*        | Florencia_Sucre       | D-HW; D-FW; V-HW; V-FW                 |
| Código   | Identificador          | Especie            | Localidad            | Habitat   |
|----------|------------------------|--------------------|----------------------|-----------|
| M602     | LGE-WS-00303           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M606     | LGE-WS-00352           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M607     | LGE-WS-00317           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M610     | LGE-WS-00379           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M611     | LGE-WS-00316           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M612     | LGE-WS-00319           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M614     | LGE-WS-00355           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M616     | LGE-WS-00334           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M618     | LGE-WS-00324           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW      |
| M620     | LGE-WS-00302           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M622     | LGE-WS-00332           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW      |
| M1009    | LGE-WS-00333           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1010    | LGE-WS-00313           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1016    | LGE-WS-00378           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1074    | LGE-WS-00315           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1075    | LGE-WS-00314           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1079    | LGE-WS-00348           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1084    | LGE-WS-00328           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1085    | LGE-WS-00329           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW      |
| M1094    | LGE-WS-00330           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1098    | LGE-WS-00396           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1196    | LGE-WS-00354           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1283    | LGE-WS-00353           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1288    | LGE-WS-00364           | H. m. malleti      | Florencia_Paraiso     | V-HW; V-FW    |
| M1321    | LGE-WS-00365           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1441    | LGE-WS-00381           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1507    | LGE-WS-00389           | H. m. malleti      | Florencia_Paraiso     | V-HW; V-FW    |
| M1511    | LGE-WS-00387           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1512    | LGE-WS-00386           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1514    | LGE-WS-00384           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1522    | LGE-WS-00385           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1754    | LGE-WS-00331           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1757    | LGE-WS-00376           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1758    | LGE-WS-00345           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1767    | LGE-WS-00391           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1769    | LGE-WS-00343           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1770    | LGE-WS-00388           | H. m. malleti      | Florencia_Sucre      | V-HW; V-FW    |
| M1771    | LGE-WS-00344           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1772    | LGE-WS-00321           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1773    | LGE-WS-00369           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1774    | LGE-WS-00360           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1805    | LGE-WS-00320           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW; V-HW; V-FW |
| M1808    | LGE-WS-00322           | H. t. florencia    | Florencia_Sucre      | D-HW; D-FW      |
| M1813    | LGE-WS-00366           | H. m. malleti      | Quebrada_Las Doraditas | D-HW; D-FW; V-HW; V-FW |
| M1814    | LGE-WS-00399           | H. m. malleti      | Florencia_Sucre      | D-HW; D-FW      |
| M1817    | LGE-WS-00323           | H. t. florencia    | Florencia_Sucre      | V-HW; V-FW    |
| M1823    | LGE-WS-00356           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
| M1845    | LGE-WS-00362           | H. m. malleti      | Florencia_Paraiso     | D-HW; D-FW; V-HW; V-FW |
|   |   |   |   |   |
|---|---|---|---|---|
| M1846 | LGE-WS-00336 | H. t. florencia | Florencia_Sucre | D-HW; D-FW; V-HW; V-FW |
| M2347 | LGE-WS-00393 | H. m. malleti | Florencia_Paraiso | D-HW; D-FW; V-HW; V-FW |
| M2360 | LGE-WS-00374 | H. m. malleti | Florencia_Paraiso | D-HW; D-FW; V-HW; V-FW |
| M2408 | LGE-WS-00382 | H. m. malleti | Florencia_Paraiso | D-HW; D-FW; V-HW; V-FW |
| M3544 | LGE-WS-00358 | H. m. malleti | Florencia_Paraiso | D-HW; D-FW; V-HW; V-FW |
| M3765 | LGE-WS-00347 | H. t. florencia | Florencia_Sucre | D-HW; D-FW; V-HW; V-FW |
| M3767 | LGE-WS-00339 | H. t. florencia | Florencia_Sucre | D-HW; D-FW; V-HW; V-FW |
| M3874 | LGE-WS-00341 | H. t. florencia | Florencia_Sucre | D-HW; D-FW; V-HW; V-FW |
| M3875 | LGE-WS-00327 | H. t. florencia | Florencia_Sucre | D-HW; D-FW |
Table S2. Female behavioural response towards males with normal (control) and altered (treatment) wing phenotype. Behaviours are classified as Acceptance or Rejection. The asterisk (*) indicates statistical significance ($\alpha$=0.01) according to the GLMM.

| Behaviour            | $H. \ m. \ malleti$          | $H. \ t. \ florencia$          |
|----------------------|-------------------------------|-------------------------------|
| Flutter              | Acceptance $\chi^2(1,326)=0.646$, $p>0.01$ | $\chi^2(1,216)=4.734$, $p>0.01$ |
| Fly towards          | Acceptance $\chi^2(1,326)=0.877$, $p>0.01$ | $\chi^2(1,216)=0.090$, $p>0.01$ |
| Slow flat            | Acceptance $\chi^2(1,326)=0.608$, $p>0.01$ | $\chi^2(1,216)=1.223$, $p>0.01$ |
| Wings open           | Acceptance $\chi^2(1,326)=2.005$, $p>0.01$ | $\chi^2(1,216)=6.905$, $p<0.01^*$ |
| Abdomen exposed      | Acceptance $\chi^2(1,326)=0.215$, $p>0.01$ | $\chi^2(1,216)=0.067$, $p>0.01$ |
| Fly away             | Rejection $\chi^2(1,326)=2.353$, $p>0.01$ | $\chi^2(1,216)=1.474$, $p>0.01$ |
| Tucked up            | Rejection $\chi^2(1,326)=1.213$, $p>0.01$ | $\chi^2(1,216)=5.500$, $p>0.01$ |
| Erratic flutter      | Rejection $\chi^2(1,326)=1.723$, $p>0.01$ | $\chi^2(1,216)=2.495$, $p>0.01$ |
| Abdomen bent         | Rejection $\chi^2(1,326)=0.075$, $p>0.01$ | $\chi^2(1,216)=5.549$, $p>0.01$ |
Table S3. Female behavioural responses in triads that tested female preference for males “perfumed” with a hexanic extract from five males either of *H. melpomene malleti* or *H. timareta florencia*. The asterisk (*) indicates statistical significance (α=0.01) according to the GLMM.

| Behaviour      | *H. m. malleti*                          | *H. t. florencia*                         |
|----------------|-----------------------------------------|------------------------------------------|
| Flutter        | Acceptance $\chi^2_{(1,473)}=51.113$, p<0.01* | $\chi^2_{(1,354)}=25.476$, p<0.01*      |
| Fly towards    | Acceptance $\chi^2_{(1,473)}=75.612$, p<0.01* | $\chi^2_{(1,354)}=32.715$, p<0.01*      |
| Slow flat      | Acceptance $\chi^2_{(1,473)}=44.195$, p<0.01* | $\chi^2_{(1,354)}=48.501$, p<0.01*      |
| Wings open     | Acceptance $\chi^2_{(1,473)}=139.04$, p<0.01* | $\chi^2_{(1,354)}=93.902$, p<0.01*      |
| Abdomen exposed| Acceptance $\chi^2_{(1,473)}=90.039$, p<0.01* | $\chi^2_{(1,354)}=60.483$, p<0.01*      |
| Fly away       | Rejection $\chi^2_{(1,473)}=44.263$, p<0.01* | $\chi^2_{(1,354)}=109.41$, p<0.01*      |
| Tucked up      | Rejection $\chi^2_{(1,473)}=13.629$, p<0.01* | $\chi^2_{(1,354)}=8.1586$, p<0.01*      |
| Erratic flutter| Rejection $\chi^2_{(1,473)}=43.539$, p<0.01* | $\chi^2_{(1,354)}=75.385$, p<0.01*      |
| Abdomen bent   | Rejection $\chi^2_{(1,473)}=27.207$, p<0.01* | $\chi^2_{(1,354)}=47.357$, p<0.01*      |
Table S4. Amount (ng) of compounds that remained in the wings of perfumed males before evaporation. We quantified the presence of the perfume applied at the beginning of the experiment, at 1 minute, at 30 minutes and at 60 minutes after spreading the perfume. RI, retention index.

| Name                             | RI    | H. melpomene malleti | H. timareta florencia |
|----------------------------------|-------|-----------------------|-----------------------|
|                                  |       | Beginning 1 minute    | 30 minutes 60 minutes | Beginning 1 minute 30 minutes 60 minutes |
| Unknown                          | 958.50| 0.14 0.26 0.00 0.00  | - 0.29 0.20 0.00 0.00  |
| Limonene                         | 1023.80| - - - -              | 1.44 1.37 0.00 0.03  |
| Phenylacetaldehyde               | 1036.10| - - - -              | 2.78 2.82 2.01 1.15  |
| Methyl salicylate                | 1187.20| - - - -              | 0.45 2.35 2.20 0.83  |
| Dodecane                         | 1177.30| 0.20 0.14 0.14 0.41  | - - - - -            |
| Unknown                          | 1174.80| 0.14 0.00 0.00 0.00  | - - - - -            |
| (Z)-3-Hexenyl isobutyrate        | 1233.30| 19.22 17.46 0.00 0.00 | - - - - -            |
| Hexyl 3-methylbutyrate           | 1239.50| 53.33 0.00 0.00 0.00  | - - - - -            |
| Unknown                          | 1243.70| 0.00 0.30 0.00 0.00  | - - - - -            |
| Alkane                           | 1265.40| - - - -              | 1.82 0.46 0.00 0.00  |
| Tridecane                        | 1300.00| 15.16 18.51 15.01 5.62 | 7.67 7.24 4.64 1.72  |
| Tetradecane                      | 1302.40| 0.58 0.30 0.00 0.00  | - - - - -            |
| 5-Decanolide                     | 1369.50| 0.00 - -              | 4.80 4.76 4.44 1.16  |
| alpha-Copaene                    | 1371.50| 1.31 0.00 0.00 0.00  | - - - - -            |
| Dihydroactinidiolide             | 1391.70| 0.00 0.73 0.41 0.00  | - - - - -            |
| Unknown                          | 1394.80| 0.00 14.13 8.36 0.00  | - - - - -            |
| Ethyl 4-ethoxybenzoate           | 1402.80| 1.56 1.68 1.16 0.45  | 12.09 15.41 14.39 14.06 |
| Homovanillic alcohol             | 1412.10| 1.00 1.03 1.00 0.00  | - - - - -            |
| Methyl 4-hydroxybenzoate         | 1449.00| 0.00 0.00 0.00 0.00  | - - - - -            |
| Methyl 3,4-                      | 1464.10| - - - -              | - - - - -            |
| Dimethoxybenzoate | Unknown | 1470.10 | 0.92  | 0.57  | 0.56  | 0.30  | -    | -    | -    | -    |
|                  | Unknown | 1488.30 | -     | -     | -     | 0.11  | 0.00 | 0.00 | 0.00 |
|                  | Unknown | 1495.20 | 0.10  | 0.16  | 0.12  | 0.06  | -    | -    | -    | -    |
|                  | Syringa aldehyde | 1519.00 | 291.80 | 293.20 | 280.37 | 251.10 | 277.15 | 263.46 | 207.00 | 174.30 |
|                  | 3,5-Dimethoxy 4-hydroxybenzyl alcohol | 1565.00 | -     | -     | -     | 7.17  | 0.00 | 0.00 | 0.00 |
| Propyl 4-hydroxybenzoate | 1614.60 | -     | -     | -     | -     | 2.70  | 0.00 | 0.00 | 0.00 |
|                  | Methyl 1H-indol-3-carboxylate | 1663.10 | -     | -     | -     | 0.24  | 0.28 | 0.26 | 0.07 |
|                  | Unknown | 1715.80 | 1.22  | 2.10  | 0.00  | 0.64  | -    | -    | -    | -    |
|                  | Tricosene | 1740.20 | -     | -     | -     | -     | 1.13 | 1.27 | 1.23 | 0.56 |
|                  | Hexadecanoic acid | 1818.00 | -     | -     | -     | -     | 1.75 | 1.46 | 0.61 | 0.31 |
|                  | Octadecanol | 1868.00 | 1094.90 | 826.74 | 202.60 | 130.10 | -    | -    | -    | -    |
|                  | Isopropyl Palmitate | 1877.50 | -     | -     | -     | -     | 1.93 | 2.62 | 2.04 | 1.60 |
|                  | Unknown | 1922.10 | 16.56 | 22.88 | 21.86 | 0.80  | -    | -    | -    | -    |
|                  | Heneicosene | 1946.10 | -     | -     | -     | -     | 12.30 | 3.45 | 0.00 | 0.00 |
|                  | Heneicosane | 1952.10 | 296.84 | 325.10 | 287.02 | 171.70 | 174.57 | 193.60 | 165.48 | 146.40 |
|                  | 1-Octadecanol | 1929.60 | 27.83 | 34.65 | 34.44 | 21.10 | 26.23 | 15.28 | 9.59 | 0.00 |
|                  | (Z)-11-Eicosenal | 2031.20 | 231.99 | 63.82 | 59.68 | 32.72 | 3.86 | 2.48 | 0.00 | 0.00 |
|                  | Docosane | 2044.40 | 0.28  | 4.70  | 4.41  | 0.00  | -    | -    | -    | -    |
|                  | Eicosanal | 2058.90 | 26.43 | 35.88 | 31.85 | 0.00  | -    | -    | -    | -    |
|                  | Unknown | 2109.90 | -     | -     | -     | -     | 0.50 | 0.00 | 0.00 | 0.00 |
|                  | Unknown | 2135.80 | 1.54  | 1.99  | 1.77  | 0.64  | 0.20 | 0.25 | 0.23 | 0.11 |
|                  | Tricosane | 2137.20 | 27.27 | 0.00  | 0.00  | 0.00  | 3.36 | 0.00 | 0.00 | 0.00 |
|                  | Isopropyl oleate | 2187.70 | -     | -     | -     | -     | 0.00 | 0.03 | 0.00 | 0.00 |
|                  | Ethyl stearate | 2190.40 | -     | -     | -     | -     | 1.77 | 1.24 | 0.00 | 0.00 |
|                  | (Z)-13-Docosenal | 2217.80 | 23.67 | 0.00  | 0.00  | 0.00  | -    | -    | -    | -    |
|                  | Eicosane | 2228.10 | 2.65  | 1.34  | 2.17  | 5.26  | 2.56 | 3.27 | 2.36 | 1.83 |
| Compound                  | MW  | 60.12 | 78.75 | 79.10 | 219.00 | 31.49 | 30.28 | 21.81 | 11.74 |
|--------------------------|-----|-------|-------|-------|--------|-------|-------|-------|-------|
| Heptacosane              | 2489.80 | -     |       |       | -      | -     | -     | -     | -     |
| Octacosane               | 2569.30 | -     | -     | -     | -      | 0.40  | 0.00  | 0.00  | 0.00  |
| Hexacosanal              | 2594.80 | -     | -     | -     | -      | 0.66  | 0.00  | 0.00  | 0.00  |
| Nonacosane               | 2652.30 | 25.34 | 26.21 | 24.79 | 0.00   | 14.03 | 11.58 | 2.88  | 1.34  |
| 13,17-Dimethylnonacosane| 2688.10 | -     | -     | -     | -      | 1.83  | 0.89  | 0.00  | 0.00  |
| Ethyl benzoate           | 2686.30 | -     | -     | -     | -      | 0.31  | 0.32  | 0.30  | 0.00  |
| Octacosanate             | 2740.80 | 12.51 | 15.64 | 13.83 | 0.00   | 28.55 | 24.31 | 11.60 | 1.18  |
| Cholesterol              | 2807.00 | 286.19 | 229.63 | 128.30 | 0.00 | 236.60 | 92.43 | 87.18 | 38.70 |
| Hentriacontane           | 2807.80 | 58.10 | 53.88 | 47.26 | 0.00 | 115.60 | 65.82 | 18.58 | 15.55 |
| 13,17-Dimethylhentriacontane | 2835.80 | 12.95 | 10.76 | 1.64 | 0.00 | 3.83 | 2.65 | 1.74 | 0.00 |
| 2-Eicosyl-5-nonyltetrahydrofuran | 3121.50 | - | - | - | - | 3.89 | 0.00 | 0.00 | 0.00 |
Table S5. Behavioural response of F₁ and backcross females towards pure males of *H. melpomene malleti* and *H. timareta florenzia*. The asterisk (*) indicates statistical significance (α=0.01) according to the GLMM.

| Behaviour       | F₁ Females | Backcross Females |
|-----------------|------------|-------------------|
| Flutter         | Acceptance | $\chi^2_{(1,561)}=2.103$, p>0.01 | $\chi^2_{(1,466)}=0.1015$, p>0.01 |
| Fly towards     | Acceptance | $\chi^2_{(1,561)}=3.184$, p>0.01 | $\chi^2_{(1,466)}=37.345$, p<0.01* |
| Slow flat       | Acceptance | $\chi^2_{(1,561)}=50.916$, p<0.01* | $\chi^2_{(1,466)}=259.21$, p<0.01* |
| Wings open      | Acceptance | $\chi^2_{(1,561)}=62.043$, p<0.01* | $\chi^2_{(1,466)}=57.215$, p<0.01* |
| Abdomen exposed | Acceptance | $\chi^2_{(1,561)}=10.650$, p<0.01* | $\chi^2_{(1,466)}=309.72$, p<0.01* |
| Fly away        | Rejection  | $\chi^2_{(1,561)}=0.600$, p>0.01 | $\chi^2_{(1,466)}=97.105$, p<0.01* |
| Tucked up       | Rejection  | $\chi^2_{(1,561)}=35.489$, p<0.01* | $\chi^2_{(1,466)}=106.42$, p<0.01* |
| Erratic flutter | Rejection  | $\chi^2_{(1,561)}=44.106$, p<0.01* | $\chi^2_{(1,466)}=72.130$, p<0.01* |
| Abdomen bent    | Rejection  | $\chi^2_{(1,561)}=24.640$, p<0.01* | $\chi^2_{(1,466)}=4.630$, p>0.01 |
Table S6. Compounds identified in wing androconia’s extracts of males of *H. melpomene malleti*, *H. timareta florencia*, F1 and backcross. RI, retention index. Mean ± SD amounts in ng.

| Name                           | RI   | *H. melpomene malleti* | *H. timareta florencia* | F1 Mean ± SD | Backcrosses Mean ± SD |
|--------------------------------|------|-------------------------|--------------------------|--------------|-----------------------|
|                                |      | Mean ± SD               | Mean ± SD                |              |                       |
| Unknown                        | 902.60 | -                       | -                        | 3.80 ± 4.91  | 0.78 ± 1.60           |
| Dimethyl sulfone               | 916.00 | -                       | -                        | 0.76 ± 1.33  | 0.50 ± 1.99           |
| Unknown                        | 958.50 | 0.13 ± 0.76             | 0.43 ± 1.69              | 7.15 ± 4.04  | 11.25 ± 5.68          |
| Limonene                       | 1023.60 | 0.28 ± 0.52             | -                        | -            | -                     |
| Phenylacetaldehyde             | 1036.10 | -                       | 0.16 ± 0.77              | -            | -                     |
| Unknown                        | 1037.60 | -                       | 1.50 ± 2.40              | 1.16 ± 1.85  | -                     |
| Nonanal                        | 1100.70 | 0.58 ± 1.51             | 1.47 ± 2.94              | 3.67 ± 4.97  | -                     |
| Dodecane                       | 1117.30 | -                       | 2.91 ± 5.72              | -            | -                     |
| Unknown                        | 1174.80 | -                       | 0.06 ± 3.09              | -            | -                     |
| Methyl salicylate              | 1187.20 | 2.44 ± 2.51             | 1.39 ± 1.99              | -            | -                     |
| Decanal                        | 1200.90 | -                       | 3.20 ± 8.32              | -            | -                     |
| (2)-3-Hexenyl isobutyrate      | 1233.30 | 19.21 ± 14.04           | -                        | -            | -                     |
| Hexyl 3-methylbutyrate         | 1239.50 | 53.33 ± 144.25          | -                        | -            | -                     |
| Alkane                         | 1265.40 | -                       | 1.76 ± 9.72              | -            | -                     |
| Tetracane                      | 1300.00 | 15.16 ± 12.07           | 8.15 ± 9.15              | -            | -                     |
| Tetradeacne                    | 1302.90 | -                       | 0.92 ± 2.54              | -            | -                     |
| 5-Decanolide                   | 1399.90 | -                       | 4.65 ± 4.40              | 1.06 ± 1.88  | 0.12 ± 0.64           |
| alpha-Copaene                  | 1371.50 | 1.31 ± 1.88             | -                        | -            | -                     |
| Dihydroactinidiolide           | 1392.90 | 17.67 ± 11.54           | 13.96 ± 15.69            | 1.71 ± 1.84  | 0.93 ± 2.02           |
| Ethyl 4-ethoxybenzoate         | 1403.40 | 8.28 ± 6.87             | 12.12 ± 16.23            | 8.28 ± 5.88  | 5.65 ± 6.65           |
| Homovanillin alcohol           | 1413.10 | 1.55 ± 2.93             | 0.249 ± 0.78             | 0.27 ± 0.53  | 0.12 ± 0.47           |
| 6,10-Dimethyl 5,9-undecadien-2-one | 1447.60 | 2.64 ± 3.10             | -                        | -            | -                     |
| Compound                                   | Mass (Da) | Retention Time (min) | Area Ratio (Ratios) | Area (nA) | Peak Area Ratio (Ratios) | Area (nA) | Peak Area Ratio (Ratios) | Area (nA) |
|-------------------------------------------|-----------|----------------------|---------------------|-----------|--------------------------|-----------|--------------------------|-----------|
| Methy 4-hydroxybenzoate                   | 1449.00   | 2.51 ± 4.83          | -                   | -         | -                        | -         | -                        | -         |
| Methyl 3,4-dimethoxybenzoate              | 1465.90   | 8.97 ± 18.25         | -                   | -         | -                        | -         | -                        | -         |
| Unknown                                   | 1470.10   | 0.19 ± 0.50          | -                   | -         | 0.33 ± 0.65              | -         | -                        | -         |
| Unknown                                   | 1488.30   | -                    | 0.09 ± 0.46         | -         | -                        | -         | -                        | -         |
| Unknown                                   | 1495.20   | -                    | -                   | 0.06 ± 0.22| -                        | -         | -                        | -         |
| Syringaldyde                              | 1519.00   | 291.8 ± 234.84       | -                   | 268.75 ± 285.36| 56.11 ± 62.01             | 34.18 ± 50.06| -                        | -         |
| 3,5-Dimethoxy 4-hydroxybenzyl alcohol     | 1565.00   | -                    | 6.95 ± 36.05        | -         | -                        | -         | -                        | -         |
| Propyl 4-hydroxybenzoate                  | 1614.60   | -                    | 3.02 ± 5.43         | -         | -                        | -         | -                        | -         |
| Methyl 1H-indol-3-acetate                 | 1663.10   | -                    | 0.23 ± 0.75         | -         | -                        | -         | -                        | -         |
| Unknown                                   | 1715.80   | 0.89 ± 1.51          | -                   | -         | 2.60 ± 3.14              | 1.57 ± 1.77| -                        | -         |
| Ethyl benzoate                            | 1765.30   | -                    | 0.30 ± 0.89         | -         | 0.19 ± 0.53              | -         | -                        | -         |
| 16-Hexadecanolide                         | 1769.10   | -                    | -                   | -         | 0.92 ± 4.01              | -         | -                        | -         |
| Unknown                                   | 1795.00   | -                    | 3.72 ± 20.66        | 0.16 ± 0.40| -                        | -         | -                        | -         |
| Hexadecanoic acid                         | 1826.50   | -                    | 2.76 ± 8.24         | -         | -                        | -         | -                        | -         |
| Octadecanol                               | 1869.80   | 1094.91 ± 519.64     | 1.40 ± 5.84         | 19.25 ± 35.04| 1.86 ± 5.38              | -         | -                        | -         |
| Unknown                                   | 1873.80   | -                    | -                   | 0.14 ± 0.48| 1.64 ± 4.85              | -         | -                        | -         |
| Isopropyl palmitate                       | 1878.30   | -                    | 1.86 ± 6.02         | -         | 3.00 ± 8.28              | -         | -                        | -         |
| Unknown                                   | 1923.40   | 16.56 ± 14.99        | -                   | -         | -                        | -         | -                        | -         |
| Unknown                                   | 1928.30   | 0.57 ± 2.42          | -                   | -         | 1.02 ± 1.39              | -         | -                        | -         |
| 1-Octadecanol                             | 1929.60   | 27.83 ± 33.07        | 29.85 ± 138.42      | 2.82 ± 0.48| -                        | -         | -                        | -         |
| Heneicosene                               | 1946.10   | 0.47 ± 1.90          | 26.80 ± 104.03      | 5.97 ± 18.76| 4.22 ± 11.20             | -         | -                        | -         |
| Heneicosane                               | 1953.70   | 298.83 ± 335.05      | 174.57 ± 225.37     | 132.51 ± 102.24| 125.07 ± 122.00         | -         | -                        | -         |
| (Z)-11-Eicosenal                          | 2032.70   | 231.98 ± 255.99      | 4.39 ± 11.48        | 22.25 ± 20.38| 3.75 ± 9.35              | -         | -                        | -         |
| Docosane                                  | 2045.50   | 0.27 ± 0.99          | 6.25 ± 22.60        | -         | -                        | -         | -                        | -         |
| Eicosanal                                  | 2058.90   | 43.43 ± 53.98        | -                   | -         | 1.46 ± 3.12              | -         | -                        | -         |
| Unknown                                   | 2088.50   | -                    | 1.93 ± 11.14        | -         | -                        | -         | -                        | -         |
| Tricosene                                 | 2106.80   | -                    | 2.84 ± 16.35        | 0.20 ± 0.68| -                        | -         | -                        | -         |
| Unknown                                   | 2109.90   | 0.91 ± 4.15          | -                   | 0.28 ± 0.72| 0.37 ± 0.86              | -         | -                        | -         |
| Unknown                                   | 2135.80   | 1.53 ± 4.23          | -                   | -         | -                        | -         | -                        | -         |
| Tricosane                                 | 2137.20   | 27.27 ± 151.83       | 8.46 ± 31.72        | -         | -                        | -         | -                        | -         |
| Compound                        | Retention Time | Relative Intensity | 1.71 ± 3.18 | 0.05 ± 0.19 |
|--------------------------------|----------------|-------------------|--------------|-------------|
| Ethyl stearate                 | 2190.40        | -                 | -            | -           |
| (Z)-13-Docosenal               | 2217.80        | 23.66 ± 68.98     | -            | 0.62 ± 2.08 |
| Docosane                       | 2227.90        | -                 | 0.19 ± 0.92  | 1.75 ± 3.75 |
| Eicosane                       | 2228.10        | 2.63 ± 5.25       | 3.43 ± 7.06  | 0.10 ± 0.35 |
| Unknown                        | 2321.80        | 0.10 ± 0.57       | -            | 0.98 ± 2.78 |
| Heptacosane                    | 2411.00        | 56.11 ± 73.64     | 12.16 ± 39.18| 23.78 ± 17.25| 36.12 ± 30.35|
| Octacosane                     | 2569.30        | -                 | 2.63 ± 15.11 | -           |
| Hexacosanal                    | 2594.80        | -                 | 5.20 ± 26.32 | -           |
| Methylheptacosane              | 2596.10        | 15.88 ± 26.13     | 12.42 ± 39.54| 4.24 ± 6.31 |
| Nonacosane                     | 2652.30        | 25.34 ± 41.2      | 13.60 ± 25.45| 31.43 ± 23.85| 31.24 ± 38.05|
| Unknown                        | 2687.70        | -                 | 0.39 ± 2.25  | 0.43 ± 1.44 |
| 13,17-Dimethylnonacosane       | 2688.10        | -                 | 1.77 ± 4.84  | 7.26 ± 7.19 |
| Octacosanal                    | 2740.80        | 12.50 ± 21.93     | 28.82 ± 52.43| 28.71 ± 28.59| 23.6 ± 24.67 |
| Unknown                        | 2794.20        | 1.21 ± 3.23       | -            | 0.15 ± 0.52 |
| Cholesterol                    | 2807.00        | 286.19 ± 445.59   | 250.36 ± 219.16| 218.74 ± 95.74| 204.56 ± 135.52|
| Hentriacontane                 | 2807.80        | 58.09 ± 100.82    | 118.43 ± 206.82| 88.60 ± 39.46| 52.32 ± 32.19 |
| 13,17-Dimethylhentriacontane   | 2835.80        | 12.95 ± 28.21     | 3.81 ± 17.50 | 16.13 ± 17.65| 4.16 ± 8.97 |
| 2-Eicosyl-5-heptyltetrahydrofuran | 2923.70    | 97.19 ± 257.04    | -            | -           |
| 2-Eicosyl-5-nonyltetrahydrofuran | 3121.50    | 10.11 ± 56.30     | 4.69 ± 13.95 | -           |

14
Table S7. Compounds identified in the abdominal glands’ extracts of males of *H. melpomene malleti*, *H. timareta florencia*, F$_1$ and backcross. RI, retention index. Mean ± SD amounts in ng.

| Name                        | RI       | H. melpomene malleti Mean ± SD | H. timareta florencia Mean ± SD | F1 Mean ± SD | Backcrosses Mean ± SD |
|-----------------------------|----------|--------------------------------|---------------------------------|--------------|-----------------------|
| Unknown                     | 903.90   | -                              | 1.58 ± 3.99                     | 7.08 ± 5.85  | 13.70 ± 12.24         |
| Dimethyl sulfone            | 919.00   | -                              | 0.34 ± 1.22                     | -            | -                     |
| (Z)-beta-Ocimene            | 1037.50  | 11899.84 ± 7633.07             | -                               | 142.08 ± 270.49 | 76.92 ± 243.28       |
| Phenylacetonitril_Benzylicyanid | 1039.80 | -                              | 62.59 ± 95.31                  | -            | -                     |
| (E)-beta-Ocimene            | 1048.20  | 12096 ± 7193.57                | -                               | -            | 2247.58 ± 3047.13    | 34.18 ± 75.04        |
| 2-sec-Butyl-3-methoxy pyrazine | 1170.10 | -                              | 74.80 ± 91.67                  | 12.45 ± 16.86 | 55.28 ± 34.86        |
| 2-isobutyl-3-methoxy pyrazine | 1177.70 | 0.02 ± 0.11                    | -                               | 1.26 ± 1.51  | 2.43 ± 3.94          |
| Methyl salicylate           | 1188.50  | -                              | 0.12 ± 0.54                     | 1.74 ± 1.28  | 1.27 ± 1.68          |
| 5-Decanolide                | 1203.00  | 0.42 ± 2.24                    | 0.18 ± 1.03                     | -            | -                     |
| Dihydroedulan II            | 1284.80  | 43.92 ± 40.09                  | 5.52 ± 14.98                   | 1.82 ± 2.26  | -                     |
| Tridecane                   | 1300.00  | 1.33 ± 1.91                    | 0.44 ± 0.87                     | -            | -                     |
| Nonadecane                  | 1302.70  | 554.93 ± 1403.34               | 721.14 ± 3082.59               | -            | -                     |
| alpha-Copaene               | 1374.10  | 0.02 ± 0.14                    | 2.53 ± 6.81                     | -            | -                     |
| GC-EAD active compound      | 1395.20  | 264.14 ± 333.29                | 622.68 ± 661.95                | 122.10 ± 124.78 | 47.67 ± 66.71       |
| Dihydroactinidiolide        | 1517.30  | 0.09 ± 0.48                    | 21.34 ± 120.35                 | -            | -                     |
| Ethyl 4-ethoxybenzoate      | 1520.20  | 8.81 ± 7.73                    | 18.26 ± 40.40                  | 9.51 ± 6.44  | 6.02 ± 7.69          |
| Homovanillyl alcohol        | 1534.00  | 1.43 ± 2.86                    | 1.40 ± 4.25                    | 0.18 ± 0.48  | 1.38 ± 2.72          |
| Unknown                     | 1542.90  | 0.18 ± 1.00                    | 0.49 ± 2.54                    | 3.83 ± 5.72  | 0.55 ± 1.05          |
| Heptadecane                 | 1700.40  | 155.78 ± 602.03                | 0.13 ± 0.76                    | 0.24 ± 0.82  | -                     |
| Benzy1_saliclylate          | 1708.20  | 0.34 ± 0.84                    | -                               | -            | -                     |
| Unknown                     | 1712.00  | -                              | 23.37 ± 69.08                  | 0.76 ± 1.33  | -                     |
| 14-Tetradecanolide          | 1721.50  | 5.86 ± 14.98                   | 30.18 ± 62.64                  | 40.03 ± 48.57 | 9.77 ± 15.12       |
| Name                          | Value   | Standard Deviation | Value   |
|-------------------------------|---------|--------------------|---------|
| Hexadecatrienolide            | 1726.10 | -                  | 3.14 ± 0.25 |
| 9,11-Hexadecadien-11-olide    | 1734.70 | -                  | 13.31 ± 25.54 |
| Ethyl benzoate                | 1764.60 | 6.86 ± 14.56       | 11.54 ± 20.45 |
| Unknown                       | 1771.70 | -                  | 1.06 ± 3.42 |
| Macrolide                     | 1773.70 | 0.5423 ± 2.86      | 0.81 ± 2.44 |
| (Z2,Z4)-C16-15-olide          | 1806.60 | -                  | 1.75 ± 6.20 |
| Hexadecadien-11-olide         | 1819.30 | -                  | 12.32 ± 16.05 |
| Octadecatrienolide            | 1823.40 | 32.10 ± 32.03      | 26.36 ± 36.51 |
| Hexadecenolide                | 1857.00 | 11.18 ± 24.31      | 126.3 ± 214.21 |
| Macrolide                     | 1923.60 | 6.98 ± 36.87       | 2.85 ± 10.88 |
| Heptadecanal                  | 1923.70 | -                  | 8.64 ± 12.30 |
| 16-Hexadecanolate             | 1924.80 | -                  | 124.38 ± 160.04 |
| Brassicalactone               | 1960.90 | -                  | 278.62 ± 496.97 |
| Octadecen-11-olide            | 2002.70 | 2.28 ± 9.61        | 242.74 ± 436.73 |
| Eicosane                      | 2005.10 | 1.04 ± 3.16        | 1354.90 ± 1833.53 |
| Isopropyl palmitate           | 2029.80 | 2.17 ± 9.03        | 75.63 ± 183.90 |
| (Z)-9-C18-11-olide            | 2032.80 | 4.33 ± 10.11       | 841.29 ± 1138.35 |
| (Z)-9-C18-13-olide            | 2038.70 | 221.76 ± 278.84    | 1933.83 ± 2671.28 |
| 12-Octadecanolate             | 2051.80 | -                  | 3.13 ± 7.20 |
| Macrolide                     | 2056.70 | 0.8512 ± 2.40      | 0.19 ± 1.12 |
| (E)-Octadec-9-en-12-olide     | 2057.20 | -                  | 64.11 ± 70.56 |
| Macrolide                     | 2058.90 | 5.31 ± 10.70       | 11.75 ± 22.80 |
| IsopropylOctadecanolate       | 2063.40 | 5.57 ± 16.67       | 277.67 ± 399.68 |
| (Z9,E11)-C18-13-olide         | 2069.60 | 0.06 ± 0.35        | 1417.08 ± 3035.70 |
| Octadeca-9-11-dien-13-olide and 11-Octadecanolate | 2070.20 | - | 29.81 ± 101.85 |
| IsopropylLinoleate            | 2073.40 | 2.53 ± 7.92        | 929.63 ± 1218.84 |
| Heneicosene                   | 2074.20 | 28.17 ± 40.01      | 20.59 ± 25.93 |
| 1-Octadecanol                 | 2081.50 | 116.92 ± 160.41    | 67.52 ± 138.34 |
| Heneicosane                   | 2101.60 | 2122.79 ± 1879.48  | 1176.11 ± 2609.26 |
| Octadecen-18-olide            | 2123.30 | 0.27 ± 1.47        | 15.88 ± 45.96 |

16
| Compound                     | M_Sum  | σ_M_Sum | M_1H_Sum | σ_M_1H_Sum |
|------------------------------|---------|---------|-----------|------------|
| 17-Octadecanole              | 2136.30 |         | 25.34 ± 143.36 | 108.12 ± 263.67 |
| Isopropyl_octadecadienolate  | 2130.20 | 1.67 ± 6.25 | 99.43 ± 369.98 |         |
| 9-Octadecen-18-olide         | 2138.10 | 44.04 ± 122.04 | 159.13 ± 217.98 | 152.97 ± 190.59 |
| Octadecanole                 | 2158.50 |         | 6.56 ± 2.35 |         |
| Ethyl oleate                 | 2156.90 | 377.55 ± 474.37 | 608.93 ± 733.31 | 101.26 ± 172.42 |
| Octadecadienolide            | 2171.70 | 29.60 ± 71.78 | 700.86 ± 919.28 | 598.20 ± 448.49 |
| Butyl hexadecanoate          | 2186.90 | 9.05 ± 13.25 | 122.77 ± 197.54 | 28.75 ± 61.82 |
| Isopentyl octadecadienolate  | 2189.30 |         | 402.26 ± 1692.33 | 32.41 ± 48.63 |
| Isopropyl oleate             | 2196.10 | 969.26 ± 826.53 | 7715.08 ± 6651.53 | 2070.673 ± 1733.78 |
| Docosane                     | 2200.90 | 3.00 ± 7.09 | 39.28 ± 124.92 | 6.32 ± 13.64 |
| Butyl_octadecanoate          | 2209.00 | 17.85 ± 27.85 | 115.71 ± 162.59 |         |
| Eicosanole                   | 2222.30 | 0.42 ± 2.24 | 1.05 ± 5.98 | 0.48 ± 1.60 |
| Unknown                      | 2248.70 | 8.56 ± 20.40 | 19.21 ± 26.10 | 0.39 ± 1.29 |
| 13-Eicosanole                | 2252.30 |         | 6.53 ± 18.44 | 33.26 ± 62.74 |
| Tricosene                    | 2274.90 | 23.00 ± 33.65 | 45.26 ± 73.63 | 19.37 ± 23.37 |
| Isobutyl oleate              | 2297.70 | 243.89 ± 916.10 | 883.52 ± 1294.80 | 146.69 ± 205.08 |
| Tricosane                    | 2303.20 | 168.89 ± 166.76 | 191.35 ± 273.22 | 61.88 ± 84.02 |
| 2-Heneicosanol               | 2310.60 |         | 196.39 ± 488.74 | 7.84 ± 26.02 |
| 11-Icosenol                  | 2317.40 | 11.18 ± 19.08 | 1260.80 ± 4099.99 | 2.66 ± 6.30 |
| Butyl oleate                 | 2359.30 | 539.28 ± 615.35 | 2633.57 ± 3949.56 | 1805.67 ± 1680.41 |
| Hexeryl hexadecanoate        | 2379.10 |         | 12.53 ± 29.20 | 4.44 ± 10.55 |
| Tetracosane                  | 2405.50 | 15.69 ± 68.45 | 104.19 ± 513.61 | 1.60 ± 5.31 |
| 1,3-Docosanediol             | 2409.10 | 0.08 ± 0.32 | 17.25 ± 42.61 |         |
| Macroleide                   | 2417.30 | 2.324 ± 8.02 | 2.17 ± 11.45 | 0.76 ± 1.33 |
| Macroleide                   | 2418.70 | 2.318 ± 3.72 | 11.22 ± 16.59 | 19.73 ± 37.04 |
| Isoprenyl octadec-11-enoate  | 2433.60 |         | 92.19 ± 313.63 |         |
| Unknown                      | 2434.60 | 5.90 ± 17.08 | 17.35 ± 51.73 | 0.39 ± 1.29 |
| (E)-13-Docosen-1-ol          | 2461.40 | 87.02 ± 176.14 | 297.51 ± 425.23 | 39.84 ± 63.69 |
| Eicosanolide                 | 2475.20 |         | 26.80 ± 44.29 | 56.17 ± 102.54 |
| 1-Docosanol                  | 2488.10 | 37.55 ± 57.04 | 140.38 ± 331.25 | 108.25 ± 126.81 |

17
| Compound                        | Retention Time (min) | Area (ng) ± SE Area (ng) | Peak Area (ng) ± SE Peak Area (ng) | Peak Height (ng) ± SE Peak Height (ng) |
|--------------------------------|----------------------|--------------------------|-----------------------------------|---------------------------------------|
| Pentacosane                    | 2500.80              | 136.3 ± 141.58           | 206.84 ± 803.27                   | 33.49 ± 39.19                        |
| (Z)-9-Tricosene                | 2514.30              | -                        | 17.94 ± 38.29                     | -                                     |
| 11-methylpentacosane           | 2532.50              | 48.70 ± 52.49            | 7.45 ± 22.8                       | -                                     |
| Docosen-22-olide               | 2537.50              | -                        | 92.32 ± 117.00                    | 38.40 ± 69.37                        |
| Hexyl octadecadienoate         | 2538.70              | -                        | 11.25 ± 35.99                     | 7.01 ± 23.26                         |
| Hexyl octadecanoate and Hexenyl octadecatrienoate | 2553.20 | 114.56 ± 243.75 | 679.70 ± 974.09 | 586.15 ± 580.31 | 1173.90 ± 1111.75 |
| Hexenyl octadecatrienoate and Hexenyl octadecanoate | 2555.40 | -                  | 16.35 ± 41.97 | 41.83 ± 96.64 | 157.85 ± 196.27 |
| Benzyl hexadecanoate           | 2571.20              | -                        | 12.51 ± 26.71                     | 1.20 ± 4.00                          |
| Hexyl octadecanoate            | 2580.30              | 6.08 ± 16.75             | 4.99 ± 9.47                       | 1.53 ± 2.67                          |
| Hexyl octadecanoate            | 2594.70              | 0.743 ± 3.93             | 81.33 ± 95.56                     | 25.69 ± 38.86                        |
| Hexacosane                     | 2601.10              | 5.54 ± 11.89             | 179.42 ± 1012.18                  | 6.31 ± 15.96                         |
| Tetracosanol                   | 2666.20              | 205.96 ± 239.41          | 220.54 ± 319.25                   | 439.32 ± 530.86                      |
| 1-Tetracosanol                 | 2691.80              | 9.04 ± 21.35             | 47.67 ± 116.63                    | 138.60 ± 309.57                     |
| Heptacosane                    | 2700.90              | 19.83 ± 47.97            | 225.04 ± 1162.37                  | 98.30 ± 85.46                       |
| Tetracosanolide                | 2735.80              | 2.96 ± 7.36              | 42.87 ± 56.81                     | 33.63 ± 55.08                       |
| 1,3-Tetracosanediol            | 2811.90              | -                        | 58.20 ± 120.24                    | 8.24 ± 19.93                        |
| Unknown                        | 2869.20              | 8.19 ± 19.09             | 0.29 ± 1.51                       | 3.56 ± 9.01                          |
| Hexacosanal                    | 2871.80              | 8.43 ± 28.03             | 28.60 ± 49.18                     | -                                     |
| Nonacosane                     | 2901.50              | 1.57 ± 6.43              | 167.65 ± 924.25                   | 15.15 ± 19.06                       |
| 13,17-Dimethylnonacosane       | 2969.20              | -                        | 32.82 ± 147.47                    | 43.77 ± 115.55                      |
| Cholesterol                    | 3099.40              | 192.05 ± 273.18          | 153.25 ± 263.26                   | 617.34 ± 599.42                     |
| Unknown                        | 3147.40              | 0.08 ± 0.33              | 0.68 ± 3.85                       | 6.26 ± 16.19                        |
| 13,17-Dimethylhentriacontane   | 3158.20              | 153.09 ± 173.73          | 395.95 ± 485.06                   | 438.67 ± 328.29                     |
| 2-Nonyl-5-octadecyloctahydrofuran | 3177.50 | 0.08 ± 0.33 | 0.20 ± 0.82 | 22.65 ± 57.78 | -                                      |
| Campestosterone or Ergostenol  | 3207.30              | 1.42 ± 5.61              | 7.37 ± 17.36                      | 16.35 ± 21.95                       |
| 13,17-Dimethyltripentacontane  | 3348.00              | 24.37 ± 39.54            | 42.23 ± 105.73                    | 93.10 ± 75.05                       |
| 2-Eicosyl-5-nonyl tetrahydrofuran | 3370.90 | 14.77 ± 35.28 | 20.68 ± 48.38 | 63.61 ± 50.82 | 39.84 ± 72.41 |
Table S8. Probability of mating in no-choice experiments. Hmm: *H. m. malleti*; Htf: *H. t. florencia*; F1: Htf x Hmm; BC: backcrosses [Htf x Hmm] x Htf. Cross type is specified as female x male. [Confidence interval at 95%]. No-choice mating data was collected as in previous studies of *Heliconius* (1,2). This information allowed us to gain a better understanding of the premating barriers operating in this species pair (3). Mating probability for interspecific and hybrid trials was obtained by maximizing the loge of the likelihood function (for details see 4,5).

| Cross type       | N trials | Mating probability | Confidence interval | Source                  |
|------------------|----------|--------------------|---------------------|-------------------------|
| Control (conspecific) |          |                    |                     |                         |
| Htf x Htf        | 45       | 0.911              | [0.82 - 0.971]      | Merot et al. 2017       |
| Hmm x Hmm        | 35       | 0.857              | [0.737 - 0.946]     | Merot et al., 2017      |
| Interspecific    |          |                    |                     |                         |
| Hmm x Htf        | 13       | 0.152              | [0.04 - 0.363]      | This study              |
| Htf x Hmm        | 16       | 0.188              | [0.157 - 0.377]     | This study              |
| Hybrid crosses   |          |                    |                     |                         |
| F1 x Hmm         | 18       | 0.249              | [0.119 - 0.4]       | Merot et al., 2017      |
| F1 x Htf         | 24       | 0.249              | [0.119 - 0.4]       | Merot et al., 2017      |
| Hmm x F1         | 8        | 0.2                | [0.04 - 0.45]       | Merot et al., 2017      |
| Htf x F1         | 10       | 0.2                | [0.04 - 0.45]       | Merot et al., 2017      |
| F1 x F1          | 4        | 0.2                | [0.024 - 0.045]     | This study              |
| BC x Htf         | 24       | 0.374              | [0.225 - 0.56]      | This study              |
| BC x Hmm         | 24       | 0.374              | [0.225 - 0.56]      | This study              |
Figure S1. Map showing the geographic distribution and wing phenotype of *H. melpomene malleti* and *H. timareta florentia*. 
Figure S2. Location of landmarks (LM) coordinates on the forewings and hindwings of *H. melpomene malleti* and *H. timareta florencia*. (A) LM used in the colour pattern analysis, (B) LM used in the shape and size analyses. Shape deformation (C) and (D) represents the shape at minimum values for PC1 and PC2, respectively. Shape deformation (E) and (F) represent maximum values for PC1 and PC2, respectively. LM3, LM4 and LM15 are those that vary the most.
**Figure S3. Species wing size and shape.** Forewing (A) and hindwing (B) size variation. Density plots showing the variation in the shape of the forewing (C) and the hindwing (D). A total of 43 *H. melpomene malleti* and 45 *H. timareta florencia* were analysed.
Figure S4. (A) Shape variation of forewings of *H. melpomene malleti* (light grey) and *H. timareta florencia* (dark grey). (B) and (C) show the PC1 and PC2 loadings, respectively.
Figure S5. Colour pattern comparison between the two species. (A) Yellow patch on the dorsal forewing ($F_{(1,80)}=0.0647$, $p>0.01$); (B) Dennis on the dorsal forewing ($F_{(1,79)}=0.0603$, $p>0.01$); (C) Ray on the dorsal hindwing ($F_{(1,79)}=0.5929$, $p>0.01$); (D) Yellow patch on the ventral forewing ($F_{(1,74)}=0.6191$, $p>0.01$); (E). Dennis on the ventral forewing ($F_{(1,74)}=1.2597$, $p>0.01$) and (F) ray on the ventral hindwing ($F_{(1,74)}=0.6584$, $p>0.01$). Only individuals with wings in good condition were used ($n=88$; Table S1). Yellow circles = *H. t. florencia*. Red triangles = *H. m. malleti*. The $\Delta$ colour scale indicates how present the colour pattern is, where positive values (red) represent higher presence and negative values (blue) represent lower presence or even absence. PC1 explains the variation in size and PC2 explains the variation in the shape of said colour pattern element.
Figure S6. Mate choice triads testing the importance of wing colour pattern in mate preference. The number of matings obtained is indicated above each bar. Control males are represented in light grey and treatment males are represented in dark grey. (exact binomial test $p=0.55$ in both cases).

![Bar chart showing number of matings for H. timareta florencia and H. melpomene malleti.](image)
Figure S7. Amount (ng) of compounds that remained in the wings of perfumed males before evaporation. We quantified the presence of the perfume applied at the beginning of the experiment, at 1 minute, at 30 minutes and at 60 minutes after spreading perfume. (A) Octadecanal; (B) Heneicosane; (C) Syringaldehyde; (D) Z-11-eicosanal.
Figure S8. Mate choice triads testing behavioural responses in F1 and backcross (BC) females. (A). The number of matings obtained is indicated above each bar. (B) Proportion of courtships that resulted in behavioural responses in F1 and backcross (BC) females. Behaviours recorded were acceptance (A) or rejection (R) towards males of *H. m. malleti* (red, left) and *H. t. florencia* (blue, right). Means are marked with a black square and boxplots mark the inter-quartile ranges. Size of datapoint is proportional to the number of courtships by that male. The asterisk (*) next to the female (F1/BC) is indicative of statistical significance (α=0.01) according to GLMM.
Figure S9. **Species differences in male androconia extracts.** Chromatogram of extract of androconial region from (A) *H. timareta florencia* and (B) *H. melpomene malleti*. IS, internal standard (2-tetradecylacetate); 1, dihydroactinidiolide; 2, unknown; 3, syringaldehyde; 4, henicosane; 5, octadecanal.
Figure S10. Cluster analysis based on Euclidian distance of compound composition in the wing androconia of males of *H. melpomene malleti, H. timareta florencia, F₁* and backcrosses (BC). Compounds highlighted in red are the most abundant.
Figure S11. Chromatogram patterns obtained from androconial extracts of $F_1$ and backcross males. (A) $F_1$ individuals and (B) backcross individuals. IS, internal standard (2-tetradecylacetate); 1, dihydroactinidiolide; 2, unknown; 3, syringaldehyde; 4, henicosane; 5, octadecanal.
Figure S12. Chromatogram pattern of the abdominal gland bouquet of males. (A) *H. timareta florencia*. (B) *H. melpomene malleti*. IS, internal standard (2-tetradecylacetate); 1, eicosane; 2, Z-9-C18,11olide; 3, heneicosane; 4, ethyl oleate; 5, isopropyl oleate; 6, isopropyl octadecanoate; 7, butyl oleate; 8, β-ocimene; 9, heneicosene.
Figure S13. Cluster analysis based on Euclidian distance of the compound composition of the wing androconia of males of *H. melpomene malleti*, *H. timareta florencia*, F₁ and backcrosses (BC). Compounds highlighted in red are the most abundant.
Figure S14. Chromatogram patterns obtained from abdominal gland bouquet of F$_1$ and backcross males. (A) F$_1$ individuals and (B) backcrosses individuals. IS, internal standard (2-tetradecylacetate); 1, eicosane; 2, Z-9-C18,11olide; 3, heneicosane; 4, ethyl oleate; 5, isopropyl oleate; 6, isopropyl octadecanoate; 7, butyl oleate; 8, β-ocimene; 9, henicose.
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