**Additional Files**

**Additional File 1**

**Table S1.** Geographic and temporal differences between *Culicoides* cohorts used in the study.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cohort** | **Region** | **Time** | **Site 1\*** | **Site 2\*** | **Site 3\*** | **Site 4\*** | **Site 5\*** |
| SES | SE England | June-August |  |  |  |  |  |
| SEA | SE England | September-October |  |  |  |  |  |
| NES | Kielder, NE England | July |  |  |  |  |  |
| SBS | Scottish Borders | July |  |  |  |  |  |

\*Sites used in each cohort are displayed by dark grey boxes, sites not used in each cohort are displayed by empty boxes.

**Additional File 2**



**Figure S1.** CDC miniature UV light-suction trap to collect live insects in the flight activity study.

The trap displayed here is located at site 2 in south east England. Collections were made into a 340ml cardboard collection cup containing a cotton pad soaked in 10% sucrose and paper towel cut into long, thin strips. Each trap was powered using a 12V lead acid sealed battery.

**Additional File 3**

1. CDC traps were set up two hours before sunset and run overnight to collect live insects into cardboard collection cups
2. Cardboard collection cups and attached mesh sleeve were collected within two hours of sunrise the following morning
3. Immediate transfer of cardboard collection cups and attached mesh sleeve from field sites to lab sites (The Pirbright Institute from sites 1-3 or Kielder Campsite from sites 4 and 5)
4. Two-hour acclimatisation from 20°C to test temperature (4°C to 14°C)
5. Live trap collections within 2 hours of sunrise
6. Cotton bung removed and pill box added at 0 hour. Pill boxes subsequently replaced at
0.5, 1, 2, 4, 6, 8, 10, 24 hours
7. All insects from each pill box and the remainder of insects left in the flight activity pots were killed by freezing at -20°C and later counted and identified.
8. Live trap collections within 2 hours of sunrise
9. Sorting of live collections to collect approx. 150 active *Culicoides* into eight flight
activity pots
10. Live trap collections within 2 hours of sunrise

**Figure S2.** Methods used to collect and sort *Culicoides* collections for the flight activity study.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Temperaturetrial | Total number of *Culicoides* tested | Mean cumulative proportion of active *Culicoides* | Subgenus *Avaritia* | *C. achrayi* | *C. impunctatus* | Other *Culicoides* | Unpigmented | Pigmented | Gravid  | Blood-fed | Male  |
| **Cohort SES – south east England – June-August** |
| 6°C | 941 | **0.4%** | 99.8% | 0.1% | 0.0% | 0.1% | 78.3% | 13.9% | 3.2% | 0.1% | 4.5% |
| 8°C | 455 | **0.6%** | 87.0% | 2.2% | 0.0% | 10.8% | 56.9% | 33.6% | 8.6% | 0.4% | 0.4% |
| 10°C | 1032 | **17.1%** | 99.5% | 0.0% | 0.0% | 0.5% | 82.6% | 14.7% | 1.5% | 1.1% | 0.2% |
| 12°C | 623 | **14.5%** | 92.0% | 5.0% | 0.0% | 3.0% | 52.6% | 38.4% | 7.9% | 0.2% | 1.0% |
| 14°C | 2535 | **72.3%** | 99.7% | 0.0% | 0.0% | 0.3% | 83.2% | 10.4% | 1.3% | 2.0% | 3.0% |
| **Cohort SEA – south east England – September-October** |
| 2°C | 1363 | **0.2%** | 100.0% | 0.0% | 0.0% | 0.0% | 40.9% | 58.0% | 0.9% | 0.1% | 0.1% |
| 4°C | 1215 | **4.8%** | 99.4% | 0.0% | 0.0% | 0.6% | 43.1% | 55.4% | 0.7% | 0.7% | 0.1% |
| 6°C | 1061 | **19.3%** | 96.2% | 0.0% | 0.0% | 3.8% | 65.8% | 15.2% | 17.3% | 0.5% | 1.2% |
| 8°C | 1129 | **35.7%** | 99.9% | 0.0% | 0.0% | 0.1% | 71.4% | 28.0% | 0.3% | 0.2% | 0.2% |
| 10°C | 541 | **66.8%** | 99.6% | 0.0% | 0.0% | 0.4% | 76.5% | 19.8% | 1.1% | 2.2% | 0.4% |
| **Cohort NES – north east England - July** |
| 8°C | 1812 | **0.0%** | 0.2% | 0.2% | 99.6% | 0.1% | 0.6% | 99.4% | 0.0% | 0.0% | 0.0% |
| 10°C | 1562 | **0.8%** | 0.0% | 0.1% | 99.7% | 0.2% | 0.5% | 99.2% | 0.2% | 0.0% | 0.1% |
| 12°C | 1864 | **1.7%** | 0.1% | 0.5% | 99.1% | 0.3% | 1.3% | 97.9% | 0.5% | 0.0% | 0.3% |
| 14°C | 1405 | **9.1%** | 0.0% | 0.1% | 99.8% | 0.1% | 0.8% | 99.1% | 0.0% | 0.0% | 0.1% |
| **Cohort SBS – Scottish Borders - July** |
| 12°C | 585 | **14.4%** | 29.7% | 35.9% | 29.1% | 5.3% | 37.9% | 44.8% | 3.2% | 0.2% | 13.8% |

**Additional File 4**

**Table S2.** Differences observed in *Culicoides* populations used in each temperature trial across all cohorts tested.

**Additional File 5**

**Table S3.** Estimated coefficients (standard errors) in binomial family GLMMs for flight activity (proportion of midges flying) of *Culicoides* biting midges.

|  |  |  |  |
| --- | --- | --- | --- |
| parameter | all midges | unpigmented females | pigmented females |
| total *Culicoides* |  |  |  |
| intercept | -11.00 (0.63) | -10.66 (0.77) | -11.51 (0.79) |
| temperature | 0.84 (0.05) | 0.83 (0.07) | 0.86 (0.06) |
| cohort |  |  |  |
| SES | baseline | baseline | baseline |
| SEA | 3.88 (0.37) | 3.59 (0.42) | 4.46 (0.46) |
| NES | -3.20 (0.33) | - | -2.99 (0.34) |
| random effect (pot)† | 0.93 | 1.04 | 0.89 |
| *Avaritia* subgenus |  |  |  |
| intercept | -10.95 (0.71) | -10.06 (0.76) | -11.62 (0.94) |
| temperature | 0.84 (0.06) | 0.83 (0.07) | 0.87 (0.07) |
| cohort |  |  |  |
| SES | baseline | baseline | baseline |
| SEA | 3.83 (0.41) | 3.56 (0.42) | 4.51 (0.54) |
| random effect (pot)† | 1.02 | 1.03 | 1.01 |
| *Culicoides impuncatatus*‡ |  |  |  |
| intercept | -14.08 (1.23) | - | -13.95 (1.17) |
| temperature | 0.84 (0.09) | - | 0.83 (0.09) |
| random effect (pot)† | 0.41 | - | 0.37 |

† standard deviation (on logit scale)

‡ NES cohort only

**Additional File 6**

**Table S4.** Estimated coefficients (standard errors) in binomial family GLMMs for flight activity (proportion of midges flying) of *Culicoides* biting midges at 12°C.

|  |  |  |  |
| --- | --- | --- | --- |
| parameter | all midges | unpigmented females | pigmented females |
| total *Culicoides* |  |  |  |
| intercept | -1.85 (0.26) | -1.53 (0.32) | -2.08 (0.28) |
| cohort |  |  |  |
| SES | baseline | baseline | baseline |
| NES | -2.26 (0.38) | - | -2.05 (0.37) |
| SBS | 0.03 (0.36) | 0.35 (0.44) | -0.30 (0.39) |
| random effect (pot)† | 0.57 | 0.62 | 0.38 |
| *Avaritia* subgenus |  |  |  |
| intercept | -1.71 (0.28) | -1.41 (0.35) | -2.01 (0.31) |
| cohort |  |  |  |
| SES | baseline | baseline | baseline |
| SBS | 0.35 (0.42) | 0.10 (0.56) | 1.44 (0.53) |
| random effect (pot)† | 0.52 | 0.69 | 0.44 |
| *Culicoides impuncatatus* |  |  |  |
| intercept | -4.20 (0.29) | - | - |
| cohort |  |  |  |
| NES | baseline | - | - |
| SBS | -1.01 (1.05) | - | - |
| random effect (pot)† | 0.42 | - | - |

† standard deviation (on logit scale)

**Additional File 7**

**Table S5.** Estimated coefficients (standard errors) in binomial family GLMs for flight activity (proportion of midges flying) of *Culicoides obsoletus* and *Culicoides scoticus*.

|  |  |  |  |
| --- | --- | --- | --- |
| parameter | all midges | unpigmented females | pigmented females |
| intercept | -14.11 (1.08) | -15.25 (1.32) | -18.81 (5.06) |
| temperature | 1.24 (0.09) | 1.33 (0.11) | 1.76 (0.49) |
| cohort |  |  |  |
| SES | baseline | baseline | baseline |
| SEA | 8.51 (1.20) | 11.21 (1.38) | 10.70 (5.21) |
| species |  |  |  |
| *C. obsoletus* | baseline | baseline | baseline |
| *C. scoticus* | 0.25 (0.32) | 0.61 (0.13) | -1.36 (1.16) |
| temperature:cohort |  |  |  |
| SES | baseline | baseline | baseline |
| SEA | -0.76 (0.10) | -0.99 (0.13) | -0.94 (0.50) |
| cohort:species |  |  |  |
| SEA, *C. scoticus* | 0.99 (0.46) | - | 2.98 (1.40) |