**Supplementary Tables and Figures**

Table A1. Primers used for generating *period* dsRNA

|  |  |  |
| --- | --- | --- |
| Primer name | Forward primer | Reverse primer |
| NV\_per\_dsRNA\_0708Region A | 5’-CCTTCTTCCAACCCATACGG-3’ | 5’-CTCAATGATCTTGGCTTCCTG-3’ |
| NV\_per\_dsRNA\_1213Region B | 5’-CTGCTGTCGTTAGATGTGAG-3’ | 5’-GTCGCCATATCAGTTATCGG-3’ |
| NV\_per\_dsRNA\_1213\_T7Region A | 5’-TAATACGACTCACTATAGGG'CCT TCTTCCAACCCATACGG-3’ | 5’-TAATACGACTCACTATAGGG'CTCAATGATCTTGGCTTCCTG-3’ |
| NV\_per\_dsRNA\_1213\_T7Region B | 5’-TAATACGACTCACTATAGGG'CTGCTGTCGTTAGATGTGAG-3’ | 5’-TAATACGACTCACTATAGGG'GTCGCCATATCAGTTATCGG-3’ |

Table A2. Primers used for period qPCR

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | NCBI Ref. seq. | Forward primer | Reverse primer |
| *per* | XM\_008211021.1 | 5’-GCCTTCATTACACGCATCTC-3’ | 5’-ACCATTCGCACCTGATTGAC-3’ |
| *ef1 α* | XM\_008209960.1 | 5’-CACTTGATCTACAAATGCGGTG-3’ | 5’-CCTTCAGTTTGTCCAAGACC-3’ |
| *ak* | XM\_016986045.1 | 5’-AATTCAATCGGGTTCTGCTC-3’ | 5’-CAGCATCTCATCTAACTTCTCTG-3’ |

**Table A3.** Number of head-nods and duration of the first 5 cycles of wild type southern and northern wasps. Asterisks indicate significant differences (\*\*\* *p* < 0.05 by Dunn’s multiple-comparison)

|  |  |  |  |
| --- | --- | --- | --- |
|  | Southern(*n*=15) | *X*2df; *p* value | Northern(*n*=14) |
| Number of |  |  |  |
| 1st Head-nods | 4.46 ± 0.20 | \*\*\*H1 = 4.35; *p = 0.03* | 5.31 ± 0.36 |
| 2nd Head-nods | 2.95 ± 0.18 | \*\*\*H1 = 13.4; *p =* 0.002 | 4.23 ± 0.27 |
| 2nd minus 1st | -1.51 ± 0.2 | \*\*\*H1 = 5.09; *p* = 0.02 | -1.08 ± 0.36 |
| 3rd Head-nods | 3.44 ±0.23 | \*\*\*H1 = 8.16; *p* = 0.004 | 4.30 ± 0.22 |
| 4th Head-nods | 3.63 ± 0.20 | \*\*\*H1 = 8.18; *p* = 0.003 | 4.90 ± 0.37 |
| 5th Head-nods | 3.81 ± 0.16 | \*\*\*H1 = 7.8; *p* = 0.005 | 5.20 ± 0.44 |
|  |  |  |  |
| Duration (in s) of |  |  |  |
| 1st cycle | 9.15 ± 0.44 | H1 = 0.05; *p* = 0.8 | 8.57 ± 0.40 |
| 2nd cycle | 8.83 ± 0.45 | H1 = 0.70; *p* = 0.4 | 9.31 ± 0.50 |
| 2nd minus 1st | - 0.32 ± 0.45 | H1 = 1.27; *p* = 0.3 | -0.74 ± 0.50 |
| 3rd cycle | 9.67 ± 0.48 | H1 = 1.26; *p* = 0.3 | 10.36 ± 0.54 |
| 4th cycle | 11.13 ± 0.65 | H1 = 0.14; *p* = 0.8 | 10.82 ± 0.30 |
| 5th cycle | 11.73 ± 0.31 | H1 = 0.14; *p* = 0.8 | 12.08 ± 0.43 |

Table A4. Free running rhythms and arrhythmicity of control and RNAi-treated southern and northern wasps

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| DD treatment | Average τ(h) | Standard deviation | *n* | Standard error | % of arrhythmic wasps | % of rhythmic wasps |
| S\_Control | 24.98 | 0.79 | 57 | 0.11 | 20.83 | 79.17 |
| S\_RNAi\_A | 23.31 | 0.71 | 41 | 0.11 | 8.89 | 91.11 |
| S\_RNAi\_B | 23.48 | 0.59 | 37 | 0.10 | 24.00 | 76.00 |
| N\_Control | 23.96 | 0.37 | 53 | 0.05 | 8.62 | 91.38 |
| N\_RNAi\_A | 22.97 | 0.76 | 47 | 0.11 | 7.84 | 92.16 |
| N\_RNAi\_B | 23.00 | 0.80 | 44 | 0.12 | 13.73 | 86.27 |

DD = constant darkness, S = southern, N = northern, τ = tau

Table A5. Timing of locomotor activity under LD16:08 of controls and RNAi-treated, southern and northern wasps

|  |  |  |  |
| --- | --- | --- | --- |
| LD16:08 treatment | Onset ± SEZT(h) | Peak ± SEZT (h) | Offset ± SEZT (h) |
| S\_Control | 23.32 ± 0.11 | 1.89 ± 0.19 | 9.87 ± 0.28 |
| S\_RNAi\_A | 23.63 ± 0.13 | 1.44 ± 0.17 | 6.99 ± 0.35 |
| S\_RNAi\_B | 23.69 ± 0.09 | 1.36 ± 0.17 | 6.12 ± 0.35 |
| N\_Control | 23.85 ± 0.05 | 3.53 ± 0.30 | 11.81 ± 0.26 |
| N\_RNAi\_A | 23.75 ± 0.11 | 3.76 ± 0.32 | 11.63 ± 0.30 |
| N\_RNAi\_B | 23.66 ± 0.11 | 3.39 ± 0.23 | 11.61 ± 0.30 |

**Table A6.** Courtship of control and RNAi-treated southern and northern wasps. Different letters indicate significant differences *p* < 0.05 by Dunn’s multiple-comparison.

|  |  |  |
| --- | --- | --- |
|  | Southern | Northern |
| Control*n*=43 | RNAi\_A*n*=28 | RNAi\_B*n*=28 | *X*2df;*p* value | Control*n*=28 | RNAi\_A*n*=27 | RNAi\_B*n*=24 | *X*2df;*p* value |
| Number |  |  |  |  |  |  |  |  |
| 1st Head-nods | 4.88 ± 0.15a | 5.9 ± 0.32b | 6.08 ± 0.30b | H2=7.32;*p* = 0.025 | 5.51 ± 0.18a | 6.80 ± 0.36b | 6.91 ± 0.23b | H2=5.47;*p* = 0.011 |
| 2nd Head-nods | 3.38 ± 0.13a | 4.63 ± 0.25b | 4.52 ± 0.29b | H2=9.53;*p* = 0.008 | 4.19 ± 0.12a | 4.95 ± 0.23b | 4.65 ± 0.20b | H2=6.53;*p* = 0.015 |
| 2nd minus 1st | -1.54 ± 0.15a | -1.27 ± 0.32b | -1.73 ± 0.39b | H2=9.16;*p* = 0.01 | -1.34 ± 0.17a | -1.85 ± 0.34b | -2.26 ± 0.17b | H2=4.41;*p* = 0.02 |
| 3rd Head-nods | 3.50 ± 0.15a | 4.40 ± 0.18b | 4.30 ± 0.24b | H2=9.50;*p* = 0.0003 | 4.39 ± 0.17a | 5.00 ± 0.27b | 4.61 ± 0.16b | H2=5.58;*p* = 0.03 |
| 4th Head-nods | 3.62 ± 0.11a | 4.62 ± 0.21b | 4.91 ± 0.29b | H2=9.54;*p* = 0.01 | 4.60 ± 0.17a | 5.44 ± 0.27b | 5.13 ± 0.23b | H2=6.54;*p* = 0.04 |
| 5th Head-nods | 3.80 ± 0.15a | 5.00 ± 0.24b | 5.05 ± 0.48b | H2=7.39;*p* = 0.019 | 5.00 ± 0.17a | 5.69 ± 0.33b | 5.30 ± 0.21b | H2=5.39;*p* = 0.031 |
|  |  |  |  |  |  |  |  |  |
| Duration (sec) |  |  |  |  |  |  |  |  |
| 1st cycle | 9.44 ± 0.21a | 10.46 ± 0.37b | 10.93±0.28b | H2=15.62;*p* = 0.0002 | 9.22 ± 0.20a | 10.30 ± 0.38b | 10.04 ± 0.20b | H2=20.47;*p* = 3.588e-05 |
| 2nd cycle | 9.58 ± 0.19a | 10.80 ± 0.51b | 10.57±0.26b | H2=29.58;*p* = 0.0008 | 9.96 ± 0.27a | 10.87 ± 0.38b | 11.38± 0.35b | H2=9.27;*p* = 0.008 |
| 2nd minus 1st | 0.15 ± 0.17a | -0.04 ± 0.29b | -0.36 ± 0.30b | H2=25.15;*p* = 3.45e-06 | 0.56 ± 0.33a | 0.62 ± 0.39b | 1.33 ± 0.36b | H2=9.16;*p* = 0.009 |
| 3rd cycle | 10.43 ± 0.28a | 11.52 ± 0.34b | 11.25 ± 0.38b | H2=14.76;*p* = 0.0006 | 10.94 ± 0.23a | 12.7 ± 0.43b | 12.20 ± 0.24b | H2=6.99;*p* = 0.02 |
| 4th cycle | 11.17 ± 0.22a | 12.00 ± 0.34b | 12.16 ± 0.45b | H2=26.7;*p* = 1.593e-06 | 11.59 ± 0.22a | 13.25 ± 0.45b | 13.08 ± 0.35b | H2=9.67;*p* = 0.004 |
| 5th cycle | 11.82 ± 0.25a | 12.67 ± 0.40b | 12.73 ± 0.45b | H2=20.31;*p* = 4.045e-05 | 12.55 ± 0.28a | 14.47 ± 0.43b | 14.09 ± 0.40b | H2=9.68;*p* = 0.009 |

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**Figure A1.** *Period* gene structure and location of dsRNAs. Schematic representation of *period* in *Nasonia vitripennis*. Exons are indicated with boxes and introns with lines. The total length is 22.4 kb. Red boxes indicate the PAS domains, green the PAC domain and blue the Period\_C domain. DsRNA\_A and dsRNA\_B indicate the region targeted by RNAi.



**Figure A2.** Expression levels of the reference gene*, Ak3*, in southern and northern lines: controls and RNAi-treated wasps.The average relative expression of *ak3* normalized against *ef1α* is compared between the southern and northern lines in control and RNAi-treated wasps by two-way ANOVA.

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**Figure A3.** Locomotor activity of control and RNAi –treated southern and northern wasps. Locomotor activity profile of (A–C) northern wasps (control and RNAi, respectively) and of (D–F) southern wasps (control and RNAi respectively) are shown as average of bin crosses/minute of 25–32 individuals each over 24-h periods at LD 16:08. Grey shading indicates the night phase, and white indicates the day phase. *Zeitgeber time* (ZT) is given in hours on the *x*-axis where ZT=0 represents light on. Dots indicate respectively the average onset, the average peak ± SE and the average offset ± SE of activity.