

**Table S1.** Summary statistics from weighted, grouped logistic regressions of SNP frequency against latitude.

| Gene             | SNP number | $\beta_0$ ( $\pm$ S. E.)     | q-value* | pseudo-R <sup>2</sup> |
|------------------|------------|------------------------------|----------|-----------------------|
|                  |            | $\beta_{1\#}$ ( $\pm$ S. E.) |          |                       |
| <i>BRh</i> opsin | 6          | 0.133 (0.046)                | 0.008    | 0.624                 |
|                  | 39         | 0.139 (0.060)                | 0.028    | 0.479                 |
|                  | 41         | 0.147 (0.049)                | 0.006    | 0.663                 |
|                  | 152        | 0.193 (0.049)                | 0.0008   | 0.851                 |
|                  | 172        | 0.210 (0.047)                | 7.35E-05 | 0.923                 |
|                  | 186        | 0.035 (0.094)                | NS       | 0.015                 |
|                  | 189        | -0.063 (0.043)               | NS       | 0.188                 |

$\beta_0$ : linear regression coefficient;  $\beta_{1\#}$ : quadratic regression coefficient, #:  $\beta_1$  only shown where relevant

\* FDR-adjusted q-values associated with regression coefficients, NS: not significant at  $q < 0.05$  threshold

**Table S1.** Contd.

| Gene             | SNP number | $\beta_0$ ( $\pm$ S. E.),                     | q-value      | pseudo-R <sup>2</sup> |
|------------------|------------|-----------------------------------------------|--------------|-----------------------|
|                  |            | $\beta_{1\#}$ ( $\pm$ S. E.)                  |              |                       |
| <i>BRh</i> opsin | 375        | 1.573 (0.604),<br>-0.021 (0.008) <sub>#</sub> | 0.016, 0.011 | 0.861                 |
|                  | 405        | -0.045 (0.048)                                | NS           | 0.081                 |
|                  | 543        | -0.034 (0.036)                                | NS           | 0.085                 |
|                  | 553        | 0.196 (0.052)                                 | 0.001        | 0.833                 |
|                  | 564        | -0.082 (0.042)                                | NS           | 0.297                 |
|                  | 570        | -0.022 (0.037)                                | NS           | 0.035                 |
|                  | 606        | 0.033 (0.043)                                 | NS           | 0.055                 |

**Table S1.** Contd.

| Gene             | SNP number | $\beta_0$ ( $\pm$ S. E.),                     | q-value      | pseudo-R <sup>2</sup> |
|------------------|------------|-----------------------------------------------|--------------|-----------------------|
|                  |            | $\beta_{1\#}$ ( $\pm$ S. E.)                  |              |                       |
| <i>BRh</i> opsin | 615        | 0.013 (0.035)                                 | NS           | 0.013                 |
|                  | 647        | 0.451 (0.097)                                 | 4.00E-0.5    | 0.971                 |
|                  | 846        | 0.140 (0.051)                                 | 0.012        | 0.587                 |
|                  | 901        | 0.262 (0.056)                                 | 4.00E-0.5    | 0.950                 |
|                  | 930        | -0.049 (0.035)                                | NS           | 0.174                 |
|                  | 942        | -0.070 (0.042)                                | NS           | 0.262                 |
|                  | 948        | -1.885 (0.834),<br>0.025 (0.011) <sub>#</sub> | 0.033, 0.027 | 0.658                 |

**Table S1.** Contd.

| Gene              | SNP number | $\beta_0$ ( $\pm$ S. E.),              | q-value      | pseudo- $R^2$ |
|-------------------|------------|----------------------------------------|--------------|---------------|
|                   |            | $\beta_{1\#}$ ( $\pm$ S. E.)           |              |               |
| <i>BRh</i> opsin  | 972        | 2.821 (1.052),<br>-0.038 (0.014) $\#$  | 0.013, 0.010 | 0.770         |
| <i>LWRh</i> opsin | 328        | 14.372 (4.545),<br>-0.166 (0.054) $\#$ | 0.005, 0.005 | 0.999         |
|                   | 330        | 14.372 (4.545),<br>-0.166 (0.054) $\#$ | 0.005, 0.005 | 0.999         |
|                   | 334        | 14.546 (4.550),<br>-0.168 (0.054) $\#$ | 0.005, 0.005 | 0.999         |
|                   | 429        | 14.546 (4.551),<br>-0.168 (0.054) $\#$ | 0.005, 0.005 | 0.999         |
|                   | 435        | 14.546 (4.550),<br>-0.168 (0.054) $\#$ | 0.005, 0.005 | 0.999         |
|                   | 489        | 12.654 (3.876),<br>-0.146 (0.046) $\#$ | 0.005, 0.005 | 0.999         |

**Table S1.** Contd.

| Gene              | SNP number | $\beta_0$ ( $\pm$ S. E.),              | q-value      | pseudo-R <sup>2</sup> |
|-------------------|------------|----------------------------------------|--------------|-----------------------|
|                   |            | $\beta_{1\#}$ ( $\pm$ S. E.)           |              |                       |
| <i>LWRh</i> opsin | 510        | 14.114 (4.545),<br>-0.163 (0.054) $\#$ | 0.005, 0.006 | 0.999                 |
|                   | 528        | 15.024 (4.385),<br>-0.174 (0.052) $\#$ | 0.005, 0.005 | 0.999                 |
|                   | 582        | 0.050 (0.040)                          | NS           | 0.156                 |
|                   | 726        | -0.010 (0.033)                         | NS           | 0.009                 |
|                   | 933        | 14.309 (4.568),<br>-0.165 (0.054) $\#$ | 0.005, 0.005 | 0.999                 |
| <i>UVRh</i> opsin | 189        | -0.437 (0.061)                         | 2.24E-11     | 0.999                 |
|                   | 660        | -0.391 (0.040)                         | 7.20E-06     | 0.981                 |

**Table S1.** Contd.

| Gene              | SNP number | $\beta_0$ ( $\pm$ S. E.),    | q-value  | pseudo-R <sup>2</sup> |
|-------------------|------------|------------------------------|----------|-----------------------|
|                   |            | $\beta_{1\#}$ ( $\pm$ S. E.) |          |                       |
| <i>UVRh</i> opsin | 699        | -0.537 (0.055)               | 1.18E-14 | 1                     |
| <i>wingless</i>   | 42         | -0.016 (0.067)               | NS       | 0.006                 |
|                   | 150        | -0.109 (0.108)               | NS       | 0.117                 |
|                   | 219        | -0.032 (0.041)               | NS       | 0.064                 |
|                   | 240        | -0.047 (0.076)               | NS       | 0.041                 |
|                   | 327        | -0.061 (0.073)               | NS       | 0.075                 |
| <i>EF-1 alpha</i> | 274        | -0.087 (0.036)               | 0.024    | 0.475                 |

**Table S1.** Contd.

| Gene              | SNP number | $\beta_0$ ( $\pm$ S. E.),    | q-value | pseudo-R <sup>2</sup> |
|-------------------|------------|------------------------------|---------|-----------------------|
|                   |            | $\beta_{1\#}$ ( $\pm$ S. E.) |         |                       |
| <i>EF-1 alpha</i> | 286        | -0.087 (0.036)               | 0.025   | 0.469                 |
|                   | 421        | 0.107 (0.083)                | NS      | 0.180                 |
|                   | 649        | -0.087 (0.036)               | 0.024   | 0.475                 |
|                   | 721        | -0.084 (0.036)               | 0.028   | 0.449                 |
|                   | 934        | 0.101 (0.040)                | 0.018   | 0.514                 |
|                   | 949        | -0.125 (0.042)               | 0.007   | 0.560                 |
|                   | 967        | 0.010 (0.031)                | NS      | 0.009                 |

**Table S2.** A list of the white admiral butterfly (*Limenitis arthemis*) populations which were sampled for an AFLP genome scan analysis through two parts of the hybrid zone (*L. a. arthemis* x *L. a. astyanax*).

| Transect | Site | Code | Locality                              | Latitude | Longitude | Wing pattern       | Subspecies               | N  | $F_{ST}$ |
|----------|------|------|---------------------------------------|----------|-----------|--------------------|--------------------------|----|----------|
| East     | 1    | NB   | Holtville, New Brunswick, Canada      | 46.5653° | -66.4619° | white-banded       | <i>arthemis</i>          | 19 | 0.43     |
|          | 2    | ON   | Algonquin, Ontario, Canada            | 44.7037° | -75.6695° | white-banded       | <i>arthemis</i>          | 16 | 0.45     |
|          | 3    | ME   | Hancock Co., Maine, USA               | 44.5770° | -68.3567° | white-banded       | <i>arthemis</i>          | 14 | 0.64     |
|          | 4    | VT   | Addison Co., Vermont, USA             | 44.0495° | -72.9600° | white-banded       | <i>arthemis</i>          | 24 | 0.34     |
|          | 5    | FL   | Finger Lakes National Forest, NY, USA | 42.4987° | -76.8133° | intergrades        | <i>arthemis-astyanax</i> | 11 | 0.21     |
|          | 6    | AF   | Allegheny National Forest, PA, USA    | 42.0483° | -78.8751° | intergrades        | <i>arthemis-astyanax</i> | 19 | 0.26     |
|          | 7    | RG   | Ricketts Glen, Columbia Co., PA, USA  | 41.3788° | -76.2662° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.27     |
|          | 8    | PO   | Poconos, Luzerne Co., PA, USA         | 41.1022° | -75.6864° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.37     |
|          | 9    | BM   | Blue Mtns, Carbon Co., PA, USA        | 40.7658° | -75.7338° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.40     |
|          | 10   | VA   | Shenandoah Co., Virginia, USA         | 38.8800° | -78.4303° | red-spotted purple | <i>astyanax</i>          | 24 | 0.51     |
|          | 11   | GA   | Putnam Co., Georgia, USA              | 33.3066° | -83.4816° | red-spotted purple | <i>astyanax</i>          | 24 | 0.43     |
| West     | A    | TB   | Thunder Bay, Ontario, Canada          | 48.4068° | -89.2455° | white-banded       | <i>arthemis</i>          | 16 | 0.55     |
|          | B    | HO   | Houghton County, Michigan, USA        | 47.0500° | -88.6148° | white-banded       | <i>arthemis</i>          | 24 | 0.52     |
|          | C    | BA   | Bayfield County, Wisconsin, USA       | 46.6651° | -91.1222° | white-banded       | <i>arthemis</i>          | 24 | 0.49     |
|          | D    | TA   | Taylor County, Wisconsin, USA         | 45.2231° | -90.5299° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.66     |
|          | E    | JU   | Juneau County, Wisconsin, USA         | 43.8673° | -90.0747° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.75     |
|          | F    | JO   | Jo Daviess County, Illinois, USA      | 42.3149° | -90.2245° | intergrades        | <i>arthemis-astyanax</i> | 24 | 0.36     |
|          | G    | MA   | Mason County, Illinois, USA           | 40.2271° | -89.9253° | red-spotted purple | <i>astyanax</i>          | 24 | 0.40     |
|          | H    | KY   | Jefferson Co., Kentucky, USA          | 38.1938° | -85.6435° | red-spotted purple | <i>astyanax</i>          | 19 | 0.58     |
|          | I    | MS   | Calhoun Co., Mississippi, USA         | 33.8839° | -89.3227° | red-spotted purple | <i>astyanax</i>          | 15 | 0.41     |

These study samples correspond to Fig. S6, where each population is identified by a site label in the map. General collection locality information, along with the wing pattern characterizations and sample sizes are listed for each sampled region. Butterflies characterized as red-spotted purples (*L. a. astyanax*) are known Batesian mimics of the chemically-defended pipevine swallowtail, *Battus philenor*.

**Table S3.** Primer combinations (showing only the selective nucleotides) used to isolate AFLP loci during the final selective amplification of DNA from *Limenitis arthemis*, and the number of the resulting monomorphic and polymorphic bands generated from each combination ( $N = 417$  individuals).

| <b>EcoRI</b> | <b>MseI</b> | <b>Loci</b> | <b>Polymorphic Loci</b> |
|--------------|-------------|-------------|-------------------------|
| ACT          | CTC         | 307         | 59                      |
|              | CTG         | 157         | 31                      |
| ACA          | CTC         | 249         | 36                      |
|              | CTG         | 258         | 28                      |
| AAC          | CAA         | 174         | 35                      |
|              | CAT         | 236         | 47                      |
|              | CTA         | 274         | 50                      |
|              | CTT         | 273         | 48                      |
| AT           | CTG         | 115         | 13                      |
| AGG          | CAC         | 267         | 48                      |
| AG           | CC          | 263         | 61                      |
| TT           | CC          | 150         | 34                      |

In total there were 2723 loci, of which 490 were variable across the populations. Of the 490 variable AFLP loci, 424 were estimated as neutral and 66 were detected as outliers potentially under the influence of selection. Estimates of population differentiation for all 490 variable AFLP markers ( $F_{ST} = 0.21$ ) was driven by inclusion of outlier loci. After analyzing the data separately,  $F_{ST} = 0.09$  for the 424 neutral loci, and  $F_{ST} = 0.51$  for the 66 outlier loci.

**Table S4.** Pairwise population  $F_{ST}$  calculated for 424 neutral and 66 outlier loci, below and above diagonal, respectively.

|    |    | 1<br>NB | 2<br>ON | 3<br>ME | 4<br>VT | 5<br>FL | 6<br>AF | 7<br>RG | 8<br>PO | 9<br>BM | 10<br>VA | 11<br>GA | A<br>TB | B<br>HO | C<br>BA | D<br>TA | E<br>JU | F<br>JO | G<br>MA | H<br>KY | I<br>MS |
|----|----|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1  | NB | —       | 0.12    | 0.13    | 0.70    | 0.09    | 0.29    | 0.65    | 0.68    | 0.68    | 0.10     | 0.12     | 0.04    | 0.71    | 0.61    | 0.69    | 0.55    | 0.03    | 0.03    | 0.10    | 0.01    |
| 2  | ON | 0.04    | —       | 0.02    | 0.63    | 0.17    | 0.14    | 0.57    | 0.60    | 0.60    | 0.18     | 0.15     | 0.05    | 0.64    | 0.51    | 0.61    | 0.42    | 0.12    | 0.12    | 0.09    | 0.11    |
| 3  | ME | 0.06    | 0.05    | —       | 0.62    | 0.19    | 0.09    | 0.56    | 0.60    | 0.60    | 0.17     | 0.11     | 0.09    | 0.64    | 0.50    | 0.61    | 0.39    | 0.21    | 0.14    | 0.12    | 0.21    |
| 4  | VT | 0.18    | 0.17    | 0.18    | —       | 0.67    | 0.65    | 0.07    | 0.06    | 0.08    | 0.70     | 0.67     | 0.65    | 0.02    | 0.18    | 0.18    | 0.43    | 0.71    | 0.67    | 0.68    | 0.70    |
| 5  | FL | 0.07    | 0.10    | 0.10    | 0.16    | —       | 0.25    | 0.63    | 0.66    | 0.65    | 0.09     | 0.06     | 0.04    | 0.69    | 0.58    | 0.67    | 0.54    | 0.13    | 0.05    | 0.16    | 0.08    |
| 6  | AF | 0.10    | 0.08    | 0.07    | 0.17    | 0.04    | —       | 0.60    | 0.63    | 0.62    | 0.30     | 0.27     | 0.17    | 0.67    | 0.54    | 0.65    | 0.49    | 0.34    | 0.31    | 0.21    | 0.32    |
| 7  | RG | 0.14    | 0.14    | 0.15    | 0.03    | 0.12    | 0.13    | —       | 0.02    | 0.07    | 0.66     | 0.62     | 0.60    | 0.08    | 0.21    | 0.04    | 0.29    | 0.67    | 0.62    | 0.63    | 0.65    |
| 8  | PO | 0.16    | 0.15    | 0.15    | 0.04    | 0.16    | 0.15    | 0.02    | —       | 0.03    | 0.68     | 0.65     | 0.63    | 0.07    | 0.21    | 0.07    | 0.36    | 0.69    | 0.65    | 0.65    | 0.68    |
| 9  | BM | 0.16    | 0.15    | 0.14    | 0.06    | 0.16    | 0.16    | 0.04    | 0.02    | —       | 0.68     | 0.64     | 0.63    | 0.13    | 0.22    | 0.15    | 0.36    | 0.69    | 0.65    | 0.65    | 0.68    |
| 10 | VA | 0.11    | 0.10    | 0.09    | 0.17    | 0.12    | 0.09    | 0.12    | 0.12    | 0.11    | —        | 0.04     | 0.10    | 0.72    | 0.62    | 0.70    | 0.58    | 0.09    | 0.08    | 0.09    | 0.07    |
| 11 | GA | 0.24    | 0.27    | 0.29    | 0.32    | 0.18    | 0.26    | 0.29    | 0.33    | 0.33    | 0.29     | —        | 0.10    | 0.68    | 0.57    | 0.66    | 0.50    | 0.13    | 0.06    | 0.11    | 0.12    |
| A  | TB | 0.08    | 0.06    | 0.09    | 0.20    | 0.11    | 0.13    | 0.18    | 0.19    | 0.18    | 0.12     | 0.29     | —       | 0.66    | 0.55    | 0.64    | 0.50    | 0.10    | 0.08    | 0.11    | 0.08    |
| B  | HO | 0.10    | 0.11    | 0.12    | 0.10    | 0.12    | 0.14    | 0.09    | 0.11    | 0.10    | 0.14     | 0.26     | 0.13    | —       | 0.25    | 0.15    | 0.44    | 0.72    | 0.68    | 0.69    | 0.71    |
| C  | BA | 0.16    | 0.18    | 0.20    | 0.17    | 0.11    | 0.18    | 0.16    | 0.20    | 0.20    | 0.21     | 0.20     | 0.18    | 0.09    | —       | 0.33    | 0.33    | 0.63    | 0.58    | 0.59    | 0.62    |
| D  | TA | 0.13    | 0.14    | 0.17    | 0.15    | 0.10    | 0.16    | 0.12    | 0.16    | 0.16    | 0.19     | 0.21     | 0.15    | 0.05    | 0.04    | —       | 0.32    | 0.70    | 0.66    | 0.67    | 0.69    |
| E  | JU | 0.10    | 0.10    | 0.13    | 0.15    | 0.10    | 0.13    | 0.11    | 0.15    | 0.14    | 0.15     | 0.22     | 0.14    | 0.05    | 0.08    | 0.04    | —       | 0.59    | 0.51    | 0.53    | 0.57    |
| F  | JO | 0.06    | 0.04    | 0.06    | 0.17    | 0.08    | 0.07    | 0.13    | 0.14    | 0.13    | 0.06     | 0.24     | 0.07    | 0.09    | 0.15    | 0.13    | 0.09    | —       | 0.06    | 0.10    | 0.01    |
| G  | MA | 0.10    | 0.10    | 0.12    | 0.18    | 0.08    | 0.12    | 0.15    | 0.17    | 0.16    | 0.10     | 0.25     | 0.11    | 0.10    | 0.10    | 0.08    | 0.07    | 0.05    | —       | 0.10    | 0.02    |
| H  | KY | 0.06    | 0.04    | 0.07    | 0.17    | 0.08    | 0.08    | 0.12    | 0.12    | 0.11    | 0.05     | 0.23     | 0.07    | 0.10    | 0.14    | 0.11    | 0.09    | 0.03    | 0.06    | —       | 0.06    |
| I  | MS | 0.11    | 0.13    | 0.16    | 0.25    | 0.11    | 0.17    | 0.20    | 0.23    | 0.21    | 0.16     | 0.27     | 0.14    | 0.17    | 0.15    | 0.14    | 0.15    | 0.12    | 0.11    | 0.08    | —       |

Values derived using pre-defined  $F_{IS}$  for both neutral (0.69) and outlier (0.72) datasets. Refer to Table S2 for population numbers and locations.

**Table S5.** Analysis of molecular variance (AMOVA) using pairwise genetic distances ( $F_{ST}$ ) for five gene datasets ( $BRh$ ,  $LWRh$ ,  $UVRh$ , *wingless*, and  $EF-1\alpha$ ) and ten populations of *Limenitis arthemis*.

| Gene            | Source of variation                          | Variance component | Percentage variation | Fixation Index | Sum of squares |
|-----------------|----------------------------------------------|--------------------|----------------------|----------------|----------------|
| $BRh$           | Among groups ( $F_{CT}$ )                    | 0.5                | 2.5                  | 0.02           | 1340.8         |
|                 | Among populations within groups ( $F_{SC}$ ) | 10.0               | 46.2                 | 0.47           | 7969.1         |
|                 | Within populations ( $F_{ST}$ )              | 11.1               | 51.3                 | 0.49           | 10961.0        |
| $LWRh$          | Among groups ( $F_{CT}$ )                    | 0.2                | 9.7                  | 0.10           | 125.7          |
|                 | Among populations within groups ( $F_{SC}$ ) | 0.2                | 8.0                  | 0.09           | 159.5          |
|                 | Within populations ( $F_{ST}$ )              | 2.0                | 82.4                 | 0.18           | 1882.4         |
| $UVRh$          | Among groups ( $F_{CT}$ )                    | 0.1                | 21.4                 | 0.21           | 92.5           |
|                 | Among populations within groups ( $F_{SC}$ ) | 0.0                | 5.0                  | 0.06           | 24.6           |
|                 | Within populations ( $F_{ST}$ )              | 0.5                | 73.6                 | 0.26           | 452.0          |
| <i>wingless</i> | Among groups ( $F_{CT}$ )                    | 0.0                | 0.1                  | 0.00           | 3.1            |
|                 | Among populations within groups ( $F_{SC}$ ) | 0.0                | 1.9                  | 0.02           | 10.0           |
|                 | Within populations ( $F_{ST}$ )              | 0.5                | 98.0                 | 0.02           | 479.2          |
| $EF-1\alpha$    | Among groups ( $F_{CT}$ )                    | 0.0                | 0.6                  | 0.01           | 16.5           |
|                 | Among populations within groups ( $F_{SC}$ ) | 0.0                | 1.6                  | 0.02           | 32.0           |
|                 | Within populations ( $F_{ST}$ )              | 1.8                | 97.9                 | 0.02           | 1759.0         |

Populations were grouped to maximize the among group variance component ( $F_{CT}$ ), which would indicate whether there is spatial genetic structure across the sampled populations. With noteworthy exceptions in the three opsin genes, the genetic variance is largely attributable to genetic diversity within populations but  $F_{ST}$  values are quite low ( $F_{ST} = 0.02$ ), suggesting little evidence for population

structure. The high  $F_{ST}$  estimates for the three opsin genes suggest significant population genetic variation, and it is regionally distributed among populations within geographic clusters. All AMOVA results are highly significant for  $\alpha = 0.05$ .

**Table S6.** Comparisons of population pairwise genetic distances ( $F_{ST}$ ) for the five gene datasets, *BRh*, *LWRh*, *UVRh*, *wingless*, and *EF-1 alpha*.

| <b><i>BRh</i></b> | WB    | NH    | VT    | MA    | RG    | PO    | BM    | WAI   | VA    | GA |
|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| WB                |       | +     | +     | +     | +     | +     | +     | +     | +     | +  |
| NH                | 0.141 |       | +     | +     | +     | +     | +     | +     | +     | +  |
| VT                | 0.448 | 0.345 |       | +     | +     | +     | +     | +     | +     | +  |
| MA                | 0.624 | 0.541 | 0.332 |       | +     | +     | +     | +     | +     | +  |
| RG                | 0.417 | 0.355 | 0.105 | 0.249 |       | +     | +     | +     | +     | -  |
| PO                | 0.504 | 0.421 | 0.146 | 0.310 | 0.044 |       | +     | +     | +     | +  |
| BM                | 0.501 | 0.474 | 0.285 | 0.175 | 0.197 | 0.263 |       | +     | +     | +  |
| WAI               | 0.835 | 0.753 | 0.659 | 0.898 | 0.710 | 0.771 | 0.825 |       | +     | +  |
| VA                | 0.553 | 0.464 | 0.175 | 0.233 | 0.073 | 0.061 | 0.216 | 0.837 |       | +  |
| GA                | 0.444 | 0.402 | 0.156 | 0.146 | 0.050 | 0.085 | 0.131 | 0.784 | 0.032 |    |

  

| <b><i>LWRh</i></b> | WB     | NH     | VT    | MA     | RG    | PO    | BM    | WAI   | VA    | GA |
|--------------------|--------|--------|-------|--------|-------|-------|-------|-------|-------|----|
| WB                 | -      | -      | -     | -      | -     | -     | +     | +     | +     | +  |
| NH                 | 0.000  |        | +     | -      | -     | -     | +     | +     | +     | +  |
| VT                 | 0.002  | 0.017  |       | -      | -     | +     | +     | +     | +     | +  |
| MA                 | 0.007  | -0.003 | 0.041 |        | -     | -     | +     | +     | +     | +  |
| RG                 | -0.007 | -0.003 | 0.002 | 0.005  |       | -     | +     | +     | +     | +  |
| PO                 | 0.024  | 0.006  | 0.059 | -0.008 | 0.016 |       | +     | +     | +     | +  |
| BM                 | 0.187  | 0.128  | 0.247 | 0.100  | 0.173 | 0.076 |       | -     | +     | +  |
| WAI                | 0.208  | 0.142  | 0.260 | 0.123  | 0.191 | 0.095 | 0.005 |       | +     | +  |
| VA                 | 0.365  | 0.272  | 0.428 | 0.273  | 0.356 | 0.232 | 0.053 | 0.054 |       | -  |
| GA                 | 0.346  | 0.256  | 0.403 | 0.262  | 0.336 | 0.224 | 0.065 | 0.053 | 0.015 |    |

  

| <b><i>UVRh</i></b> | WB    | NH    | VT    | MA     | RG    | PO    | BM    | WAI   | VA    | GA |
|--------------------|-------|-------|-------|--------|-------|-------|-------|-------|-------|----|
| WB                 |       | +     | +     | +      | +     | +     | +     | +     | +     | +  |
| NH                 | 0.034 |       | -     | +      | +     | +     | +     | +     | +     | +  |
| VT                 | 0.022 | 0.003 |       | +      | +     | +     | +     | +     | +     | +  |
| MA                 | 0.061 | 0.164 | 0.133 |        | -     | -     | -     | +     | +     | +  |
| RG                 | 0.025 | 0.113 | 0.089 | -0.005 |       | +     | -     | +     | +     | +  |
| PO                 | 0.164 | 0.294 | 0.253 | 0.035  | 0.064 |       | -     | -     | +     | +  |
| BM                 | 0.102 | 0.231 | 0.192 | 0.017  | 0.030 | 0.002 |       | +     | +     | +  |
| WAI                | 0.233 | 0.360 | 0.315 | 0.065  | 0.111 | 0.013 | 0.045 |       | -     | +  |
| VA                 | 0.295 | 0.416 | 0.376 | 0.118  | 0.168 | 0.024 | 0.073 | 0.007 |       | +  |
| GA                 | 0.524 | 0.594 | 0.566 | 0.378  | 0.415 | 0.221 | 0.292 | 0.225 | 0.118 |    |

| <i>wingless</i> | WB    | NH    | VT    | MA    | RG    | PO    | BM     | WAI   | VA    | GA |
|-----------------|-------|-------|-------|-------|-------|-------|--------|-------|-------|----|
| WB              |       | +     | +     | +     | -     | +     | -      | -     | +     | +  |
| NH              | 0.040 |       | +     | +     | -     | -     | +      | +     | -     | +  |
| VT              | 0.031 | 0.016 |       | +     | +     | -     | +      | +     | +     | +  |
| MA              | 0.024 | 0.019 | 0.022 |       | -     | -     | -      | -     | +     | -  |
| RG              | 0.010 | 0.006 | 0.018 | 0.003 |       | -     | -      | -     | -     | -  |
| PO              | 0.029 | 0.003 | 0.002 | 0.007 | 0.002 |       | +      | -     | -     | -  |
| BM              | 0.009 | 0.046 | 0.030 | 0.014 | 0.013 | 0.024 |        | -     | +     | -  |
| WAI             | 0.006 | 0.033 | 0.021 | 0.005 | 0.007 | 0.011 | -0.004 |       | +     | -  |
| VA              | 0.053 | 0.003 | 0.024 | 0.020 | 0.022 | 0.012 | 0.056  | 0.040 |       | +  |
| GA              | 0.029 | 0.015 | 0.022 | 0.008 | 0.000 | 0.004 | 0.015  | 0.011 | 0.029 |    |

| <i>EF-1 alpha</i> | WB     | NH    | VT    | MA    | RG    | PO    | BM    | WAI   | VA    | GA |
|-------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|----|
| WB                |        | -     | +     | -     | +     | +     | -     | +     | -     | +  |
| NH                | -0.003 |       | -     | -     | -     | -     | -     | +     | +     | +  |
| VT                | 0.019  | 0.010 |       | +     | -     | -     | -     | +     | -     | -  |
| MA                | -0.001 | 0.003 | 0.030 |       | +     | +     | +     | +     | +     | +  |
| RG                | 0.012  | 0.006 | 0.000 | 0.024 |       | -     | -     | +     | -     | -  |
| PO                | 0.016  | 0.010 | 0.002 | 0.032 | 0.002 |       | -     | +     | -     | -  |
| BM                | 0.011  | 0.008 | 0.005 | 0.027 | 0.001 | 0.000 |       | +     | -     | -  |
| WAI               | 0.100  | 0.081 | 0.038 | 0.125 | 0.043 | 0.038 | 0.035 |       | +     | +  |
| VA                | 0.009  | 0.009 | 0.012 | 0.026 | 0.007 | 0.006 | 0.001 | 0.057 |       | +  |
| GA                | 0.041  | 0.031 | 0.008 | 0.065 | 0.009 | 0.002 | 0.007 | 0.019 | 0.017 |    |

Significance of pairwise comparisons ( $P < 0.05$ ) is indicated (+) above the diagonal. Population labels correspond to localities in Figure 1, and are in order of decreasing latitude from WB to GA.

**Table S7.** List of outlier loci detected by the hierarchical island model (Excoffier et al. 2009).

| Gene       | SNP (bp)   | $H_o$ | $F_{ST}$ | P-value  | Threshold | Pattern |
|------------|------------|-------|----------|----------|-----------|---------|
| <i>BRh</i> | <b>6</b>   | 0.274 | 0.038    | 1.00E-07 | ***       | <       |
| 1028 bp    | <b>39</b>  | 0.178 | 0.032    | 1.00E-07 | ***       | <       |
|            | <b>41</b>  | 0.254 | 0.041    | 1.00E-07 | ***       | <       |
|            | 141        | 0.004 | 0.002    | 2.80E-02 | *         | <       |
|            | <b>152</b> | 0.311 | 0.074    | 1.00E-07 | ***       | <       |
|            | <b>172</b> | 0.350 | 0.085    | 1.00E-07 | ***       | <       |
|            | <b>186</b> | 0.051 | 0.018    | 1.00E-02 | *         | <       |
|            | <b>189</b> | 0.189 | 0.010    | 1.00E-07 | ***       | <       |
|            | 216        | 0.051 | 0.030    | 3.00E-02 | *         | <       |
|            | <b>375</b> | 0.472 | 0.050    | 1.00E-07 | ***       | <       |
|            | <b>405</b> | 0.152 | 0.043    | 1.00E-07 | ***       | <       |
|            | 468        | 0.059 | 0.022    | 6.00E-03 | **        | <       |
|            | 474        | 0.004 | 0.002    | 2.80E-02 | *         | <       |
|            | 519        | 0.004 | 0.002    | 2.80E-02 | *         | <       |
|            | <b>543</b> | 0.289 | 0.017    | 1.00E-07 | ***       | <       |
|            | 549        | 0.070 | 0.042    | 1.10E-02 | *         | <       |
|            | <b>553</b> | 0.278 | 0.067    | 1.00E-07 | ***       | <       |
|            | <b>564</b> | 0.178 | 0.016    | 1.00E-07 | ***       | <       |
|            | <b>570</b> | 0.289 | 0.016    | 1.00E-07 | ***       | <       |
|            | <b>606</b> | 0.219 | 0.016    | 1.00E-07 | ***       | <       |
|            | <b>615</b> | 0.360 | 0.004    | 1.00E-07 | ***       | <       |
|            | <b>647</b> | 0.184 | 0.081    | 1.00E-07 | ***       | <       |
|            | 735        | 0.521 | 0.772    | 8.00E-03 | **        | >       |
|            | 736        | 0.521 | 0.772    | 8.00E-03 | **        | >       |
|            | 737        | 0.521 | 0.772    | 8.00E-03 | **        | >       |
|            | 738        | 0.521 | 0.772    | 8.00E-03 | **        | >       |
|            | 739        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 740        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 741        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 742        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 743        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 744        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 745        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 746        | 0.520 | 0.783    | 7.00E-03 | **        | >       |
|            | 748        | 0.517 | 0.767    | 1.00E-02 | *         | >       |
|            | 749        | 0.517 | 0.767    | 1.00E-02 | *         | >       |
|            | 750        | 0.517 | 0.767    | 1.00E-02 | *         | >       |
|            | 751        | 0.517 | 0.767    | 1.00E-02 | *         | >       |
|            | 811        | 0.070 | 0.054    | 2.20E-02 | *         | <       |
|            | <b>846</b> | 0.243 | 0.035    | 2.40E-05 | ***       | <       |

|                   |             |              |              |          |     |   |
|-------------------|-------------|--------------|--------------|----------|-----|---|
|                   | <b>901</b>  | 0.289        | 0.095        | 4.08E-03 | *** | < |
|                   | <b>930</b>  | 0.310        | 0.005        | 1.62E-04 | *** | < |
|                   | <b>942</b>  | 0.296        | 0.036        | 1.19E-05 | *** | < |
|                   | <b>948</b>  | 0.280        | 0.028        | 1.00E-07 | *** | < |
|                   | 966         | 0.066        | 0.029        | 4.53E-03 | *** | < |
|                   | <b>972</b>  | 0.172        | 0.042        | 3.63E-04 | *** | < |
|                   | 987         | 0.092        | 0.045        | 2.07E-03 | *** | < |
|                   | <b>4.6%</b> | <b>0.305</b> | <b>0.287</b> |          |     |   |
| <i>LWRh</i>       | 355         | 0.004        | 0.002        | 2.20E-02 | *   | < |
| 800 bp            | <b>582</b>  | 0.345        | 0.008        | 3.40E-06 | *** | < |
|                   | 621         | 0.017        | 0.004        | 5.00E-02 | ms  | < |
|                   | <b>726</b>  | 0.447        | 0.019        | 4.00E-03 | **  | < |
|                   | <b>0.5%</b> | <b>0.203</b> | <b>0.008</b> |          |     |   |
| <i>UVRh</i>       | <b>189</b>  | 0.377        | 0.154        | 2.61E-01 | ns  | < |
| 241 bp            | <b>660</b>  | 0.232        | 0.089        | 7.89E-02 | ns  | < |
|                   | <b>699</b>  | 0.519        | 0.338        | 1.09E-01 | ns  | < |
|                   | <b>1.2%</b> | <b>0.376</b> | <b>0.194</b> |          |     |   |
| <i>wingless</i>   | 143         | 0.004        | 0.017        | 3.50E-02 | ms  | < |
| 402 bp            | 162         | 0.025        | 0.057        | 2.70E-02 | ms  | > |
|                   | 198         | 0.004        | 0.002        | 3.50E-02 | ms  | < |
|                   | <b>0.7%</b> | <b>0.011</b> | <b>0.025</b> |          |     |   |
| <i>EF-1 alpha</i> | 145         | 0.004        | 0.002        | 4.30E-02 | ms  | < |
| 1066 bp           | 349         | 0.004        | 0.002        | 4.30E-02 | ms  | < |
|                   | 376         | 0.004        | 0.002        | 4.30E-02 | ms  | < |
|                   | 421         | 0.085        | 0.002        | 4.00E-02 | ms  | < |
|                   | 457         | 0.012        | 0.002        | 4.94E-02 | ms  | < |
|                   | 718         | 0.020        | 0.063        | 1.75E-02 | *   | > |
|                   | 778         | 0.004        | 0.002        | 4.34E-02 | ms  | < |
|                   | 808         | 0.004        | 0.002        | 4.30E-02 | ms  | < |
|                   | 967         | 0.422        | 0.003        | 3.78E-02 | ms  | < |
|                   | 1048        | 0.004        | 0.002        | 4.34E-02 | ms  | < |
|                   | <b>0.9%</b> | <b>0.056</b> | <b>0.008</b> |          |     |   |

For the five gene datasets, each SNP is identified in base pair position, observed heterozygosity ( $H_O$ ), observed  $F_{ST}$ ,  $P$ -value, significance threshold (non-significant – ns, marginally significant – ms, and asterisks indicate the threshold:  $P < 0.05$  \*,  $P < 0.01$  \*\*, and  $P < 0.001$  \*\*\*), and pattern of outlier detection (observed  $F_{ST} <$  expected, or observed  $F_{ST} >$  expected). Also listed for each gene dataset are the percent outlier loci, and average observed  $H_O$  and  $F_{ST}$ . SNP loci in bold typeface correspond to the markers identified in Figure 2.