**SUPPLEMENTAL MATERIALS (File S1: Tables S1-S6; Figures S1-S9)**

**Table S1. Factor loadings for PC1 and PC2 from a principal components analysis of 22 summary statistics derived from spectrometry of *N. lecontei* larvae.** Summary statistics are as described in the Colour Analysis Programs v1.05 manual (Montgomerie 2008). Percentages are the percent variance explained by PC1 and PC2. For each PC, the five loadings with the largest magnitude are bolded.

| Summary statistic | PC1 (48.2%) | PC2 (25.5%) |
|---|---|---|
| B1 | **0.274** | -0.162 |
| B2 | **0.274** | -0.163 |
| B3 | 0.228 | -0.260 |
| S1R | -0.203 | -0.224 |
| S1G | **-0.284** | -0.028 |
| S1B | 0.240 | 0.223 |
| S1U | 0.243 | -0.092 |
| S1Y | **-0.292** | -0.076 |
| S1V | 0.257 | -0.072 |
| S3 | -0.263 | -0.120 |
| S5a | -0.032 | **-0.410** |
| S5b | 0.129 | **-0.352** |
| S5c | 0.155 | **-0.330** |
| S6 | 0.207 | **-0.272** |
| S7 | -0.130 | -0.009 |
| S8 | **-0.276** | -0.020 |
| S9 | 0.243 | 0.211 |
| H1 | 0.027 | -0.072 |
| H3 | -0.238 | -0.084 |
| H4a | -0.054 | -0.261 |
| H4b | 0.125 | 0.247 |
| H4c | 0.162 | **0.290** |

**Table S2. Results of ANOVA tests for phenotypic covariates.** Results are given for each trait/covariate combination (head = head capsule area). For each comparison, degrees of freedom (*d.f.*), *F*-statistic (*F*), and *P*-value (*P*) are given. Covariates significant at $\alpha$=0.05 are bolded.

| Trait | Covariate | *d.f.* | *F* | *P* |
|---|---|---|---|---|
| Yellow | Mother | 9 | 1.151 | 0.325 |
| Yellow | Head | 1 | 0.223 | 0.637 |
| **PC1** | **Mother** | **9** | **3.207** | **0.000906** |
| **PC1** | **Head** | **1** | **5.61** | **0.0183** |
| **PC2** | **Mother** | **9** | **4.991** | **2.14E-06** |
| PC2 | Head | 1 | 2.004 | 0.158 |
| **SpotNum** | **Mother** | **9** | **4.835** | **3.66E-06** |
| **SpotNum** | **Head** | **1** | **10.41** | **0.00135** |
| **SpotArea** | **Mother** | **9** | **4.303** | **2.28E-05** |
| **SpotArea** | **Head** | **1** | **33.14** | **1.64E-08** |

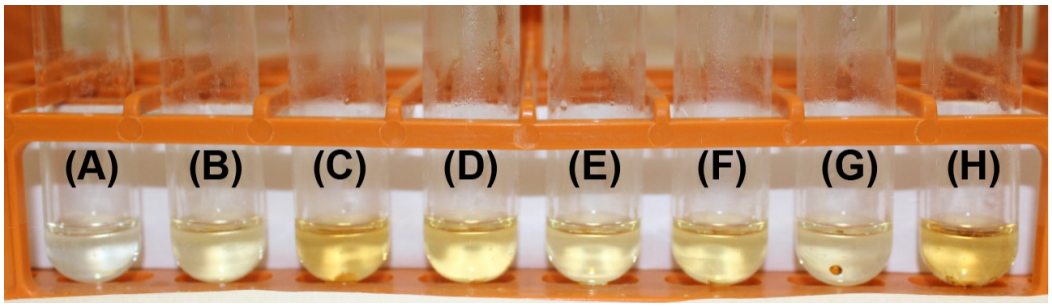


**Figure S1. Heated pyridine assay is consistent with carotenoid-based larval pigmentation.** To test for carotenoid pigments, we employed the heated pyridine test protocol outlined in McGraw *et al.* 2005. Briefly, we added 1mL of acidified pyridine to 4-10mg of tissue and incubated the samples at 95°C for 4 hours. After cooling, we photographed samples. When carotenoid pigments are present, this procedure produces a colorful pigmented solution. In contrast, pteridines, hemoglobin, and eumelanins do not release colored pigments into heated pyridine. For a positive control (**H**), we used elytra from *Hippodamia convgens* (ladybugs); our negative control (**A**) did not include any tissue. Consistent with carotenoid-based yellow and red pigmentation, tissue derived from the bodies and heads of redheaded, yellow-bodied *N. lecontei* larvae from Michigan produced a colorful pigmented solution (bodies: **B-F**; heads: **G**). We note, however, that this assay does not rule out pheomelanins, which have recently been confirmed in insects (Galván *et al.* 2015) and occasionally produce a pigmented solution under this assay (McGraw *et al.* 2005).

**Table S3. Results of Welch's two-tailed *t*-tests for five color phenotypes and three generations.** Comparisons are between white, dark-spotted larvae from VA; yellow, light-spotted larvae from MI; $F_1$ female larvae; and $F_2$ male larvae. For each comparison, test statistic (*t*), degrees of freedom (d.f.), and *P*-value (*P*) are given. *P*-values are Bonferroni-corrected for multiple comparisons (N = 6 per trait).

| Trait type | Trait | Comparison | *t* | d.f. | *P* |
|---|---|---|---|---|---|
| Body color | Yellow | VA vs. MI | 63.52 | 42.00 | <1.32E-15 |
| Body color | Yellow | VA vs. $F_1$ | -18.26 | 66.29 | <1.32E-15 |
| Body color | Yellow | MI vs. $F_1$ | 15.41 | 51.65 | <1.32E-15 |
| Body color | Yellow | VA vs. $F_2$ | -8.05 | 167.05 | 8.89E-13 |
| Body color | Yellow | MI vs. $F_2$ | 42.55 | 427.06 | <1.32E-15 |
| Body color | Yellow | $F_1$ vs. $F_2$ | 11.61 | 91.69 | <1.32E-15 |
| Body color | PC1 | VA vs. MI | -9.05 | 40.96 | 1.52E-10 |
| Body color | PC1 | VA vs. $F_1$ | 3.34 | 43.07 | 1.05E-02 |
| Body color | PC1 | MI vs. $F_1$ | -6.53 | 61.97 | 8.44E-08 |
| Body color | PC1 | VA vs. $F_2$ | 2.86 | 21.10 | 5.64E-02 |
| Body color | PC1 | MI vs. $F_2$ | -10.13 | 29.97 | 2.06E-10 |
| Body color | PC1 | $F_1$ vs. $F_2$ | -1.61 | 49.36 | 6.85E-01 |
| Body color | PC2 | VA vs. MI | -8.70 | 35.84 | 1.37E-09 |
| Body color | PC2 | VA vs. $F_1$ | 8.68 | 29.83 | 7.03E-09 |
| Body color | PC2 | MI vs. $F_1$ | -0.95 | 53.03 | 2.09E+00 |
| Body color | PC2 | VA vs. $F_2$ | 5.48 | 21.06 | 1.17E-04 |
| Body color | PC2 | MI vs. $F_2$ | -6.80 | 31.93 | 6.68E-07 |
| Body color | PC2 | $F_1$ vs. $F_2$ | -7.30 | 59.59 | 4.72E-09 |
| Spot pattern | Spot number | VA vs. MI | -26.03 | 28.42 | <1.32E-15 |
| Spot pattern | Spot number | VA vs. $F_1$ | 7.04 | 46.57 | 4.51E-08 |
| Spot pattern | Spot number | MI vs. $F_1$ | -12.34 | 72.34 | <1.32E-15 |
| Spot pattern | Spot number | VA vs. $F_2$ | 27.56 | 451.46 | <1.32E-15 |
| Spot pattern | Spot number | MI vs. $F_2$ | -12.47 | 40.06 | 1.36E-14 |
| Spot pattern | Spot number | $F_1$ vs. $F_2$ | 3.96 | 62.04 | 1.16E-03 |
| Spot pattern | Spot area | VA vs. MI | -23.13 | 41.68 | <1.32E-15 |
| Spot pattern | Spot area | VA vs. $F_1$ | 2.92 | 68.37 | 2.85E-02 |
| Spot pattern | Spot area | MI vs. $F_1$ | -10.52 | 52.33 | 9.77E-14 |
| Spot pattern | Spot area | VA vs. $F_2$ | 8.20 | 53.49 | 2.98E-10 |
| Spot pattern | Spot area | MI vs. $F_2$ | -20.85 | 165.28 | <1.32E-15 |
| Spot pattern | Spot area | $F_1$ vs. $F_2$ | 1.87 | 56.79 | 3.99E-01 |

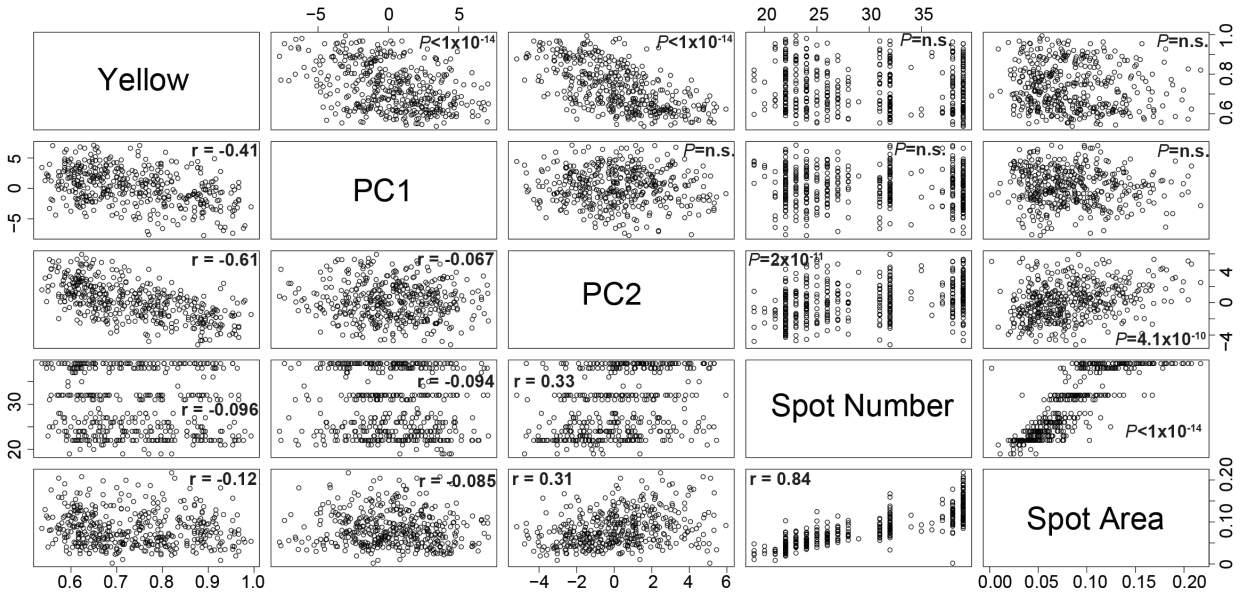


**Figure S2. Pairwise correlations between five color traits.** Pearson's *r* and Bonferroni-corrected *P*-values are given above and below the diagonal, respectively. Non-significant *P*-values (at $\alpha = 0.05$) are listed as "n.s.".

**Table S4. Linkage map summaries for all individuals ("All") and each of four families derived from a single grandparental pair ("F7" – "F14").**

| Map (N) | LG | # SNPs | Length (cM) | Avg. spacing (cM) | Max. spacing (cM) |
|---|---|---|---|---|---|
| All (429) | 1 | 101 | 188.8 | 1.9 | 18.2 |
| All (429) | 2 | 88 | 195.1 | 2.2 | 15 |
| All (429) | 3 | 75 | 181.9 | 2.5 | 19.3 |
| All (429) | 4 | 74 | 197.4 | 2.7 | 23.6 |
| All (429) | 5 | 65 | 159.2 | 2.5 | 20.3 |
| All (429) | 6 | 51 | 139.9 | 2.8 | 18.9 |
| All (429) | 7 | 49 | 106.7 | 2.2 | 24.3 |
| All (429) | overall | 503 | 1169.1 | 2.4 | 24.3 |
| F7 (161) | 2 | 493 | 466.5 | 0.9 | 38.3 |
| F7 (161) | 1 | 496 | 545.8 | 1.1 | 27.7 |
| F7 (161) | 4 | 380 | 393.1 | 1 | 19.8 |
| F7 (161) | 3 | 443 | 288.7 | 0.7 | 11.2 |
| F7 (161) | 5 | 361 | 523.6 | 1.5 | 45.2 |
| F7 (161) | 6 | 274 | 427.6 | 1.6 | 21.1 |
| F7 (161) | 7 | 241 | 418.4 | 1.7 | 27.3 |
| F7 (161) | overall | 2688 | 3063.7 | 1.1 | 45.2 |
| F8 (54) | 2 | 360 | 174.2 | 0.5 | 7.7 |
| F8 (54) | 1 | 368 | 179.4 | 0.5 | 11.3 |
| F8 (54) | 3 | 356 | 169.4 | 0.5 | 13.3 |
| F8 (54) | 4 | 294 | 178.7 | 0.6 | 9.4 |
| F8 (54) | 5 | 285 | 134.8 | 0.5 | 10.1 |
| F8 (54) | 6 | 224 | 137.5 | 0.6 | 7.5 |
| F8 (54) | 7 | 162 | 98.1 | 0.6 | 9.4 |
| F8 (54) | overall | 2049 | 1072 | 0.5 | 13.3 |
| F10 (73) | 1 | 471 | 309.9 | 0.7 | 9.5 |
| F10 (73) | 2 | 462 | 304 | 0.7 | 8.6 |
| F10 (73) | 3 | 461 | 283.7 | 0.6 | 12.2 |
| F10 (73) | 4 | 429 | 404.2 | 0.9 | 20.6 |
| F10 (73) | 5 | 365 | 201.6 | 0.6 | 7.1 |
| F10 (73) | 6 | 268 | 269.4 | 1 | 37.1 |
| F10 (73) | 7 | 202 | 194.2 | 1 | 10.3 |
| F10 (73) | overall | 2658 | 1967 | 0.7 | 37.1 |
| F14 (120) | 1 | 577 | 339.3 | 0.6 | 23.8 |
| F14 (120) | 3 | 503 | 507.3 | 1 | 22 |
| F14 (120) | 2 | 557 | 435.2 | 0.8 | 30.9 |
| F14 (120) | 4 | 491 | 290.8 | 0.6 | 7.3 |
| F14 (120) | 5 | 389 | 297.2 | 0.8 | 16.2 |
| F14 (120) | 6 | 378 | 271.2 | 0.7 | 17.9 |
| F14 (120) | 7 | 260 | 262.8 | 1 | 18.2 |
| F14 (120) | overall | 3155 | 2403.9 | 0.8 | 30.9 |

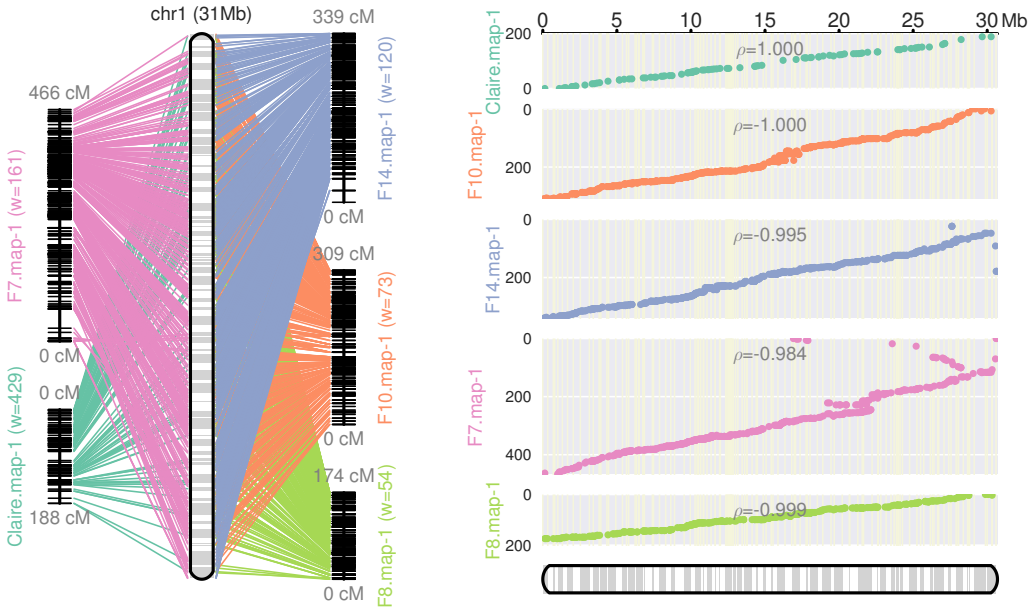


**Figure S3. Scaffold ordering for *N. lecontei* chromosome 1.** The left side of the figure gives side-by-side comparisons of the chromosome and five linkage maps used to anchor scaffolds. Maps were weighted by sample size ("w" in parentheses). Scatter plots on the right depict physical location on the chromosome (in Mb, x-axis) versus map location (in cM, y-axis) for each genetic map, with the corresponding correlation coefficient (ρ) between the two locations. Overall, there is good agreement among maps, as indicated by high ρ. Figures prepared by ALLMAPS (Tang *et al.* 2015).

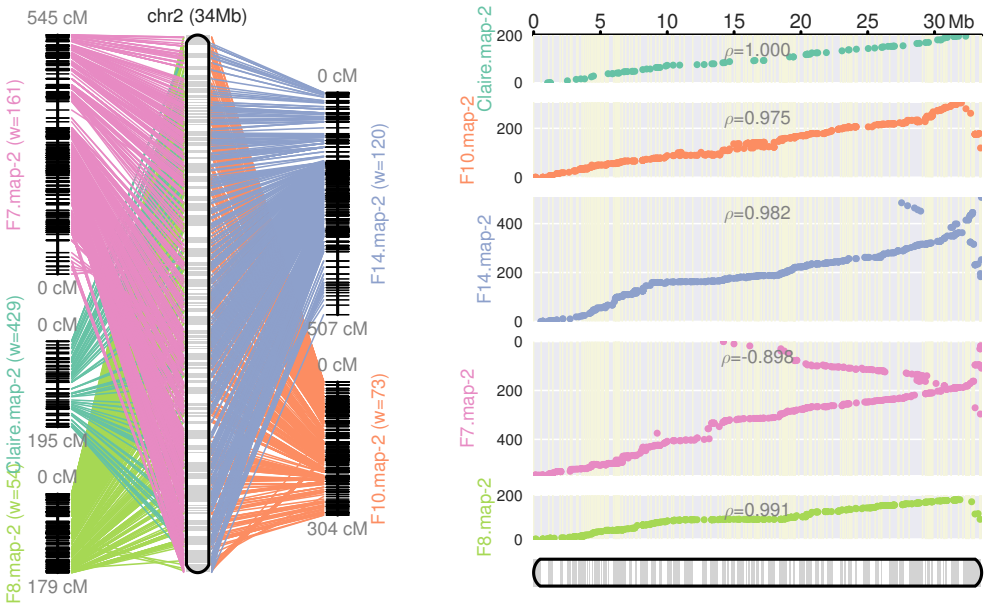


**Figure S4. Scaffold ordering for *N. lecontei* chromosome 2.** Details as in Figure S3.

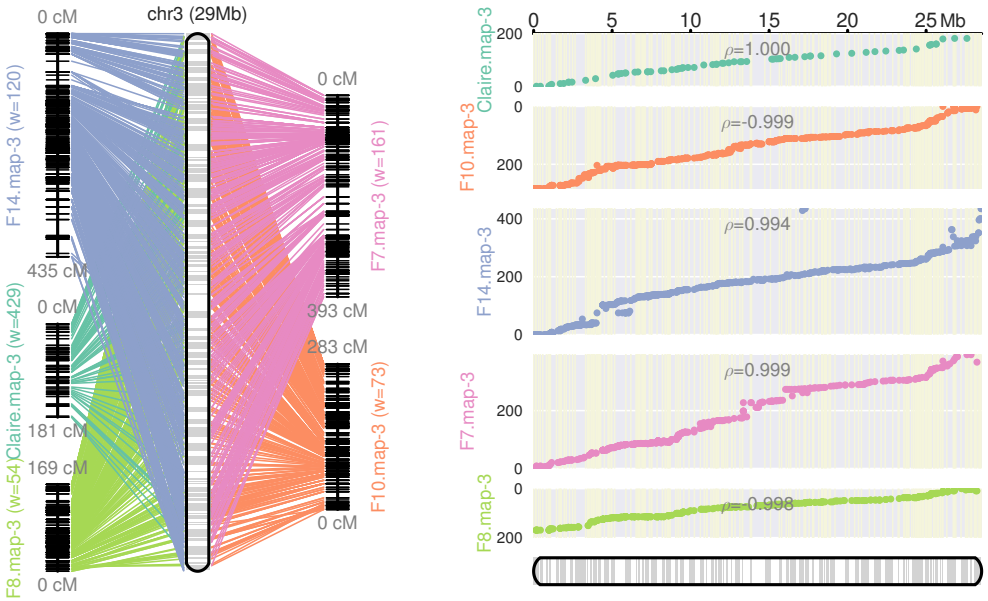


**Figure S5. Scaffold ordering for *N. lecontei* chromosome 3.** Details as in Figure S3.

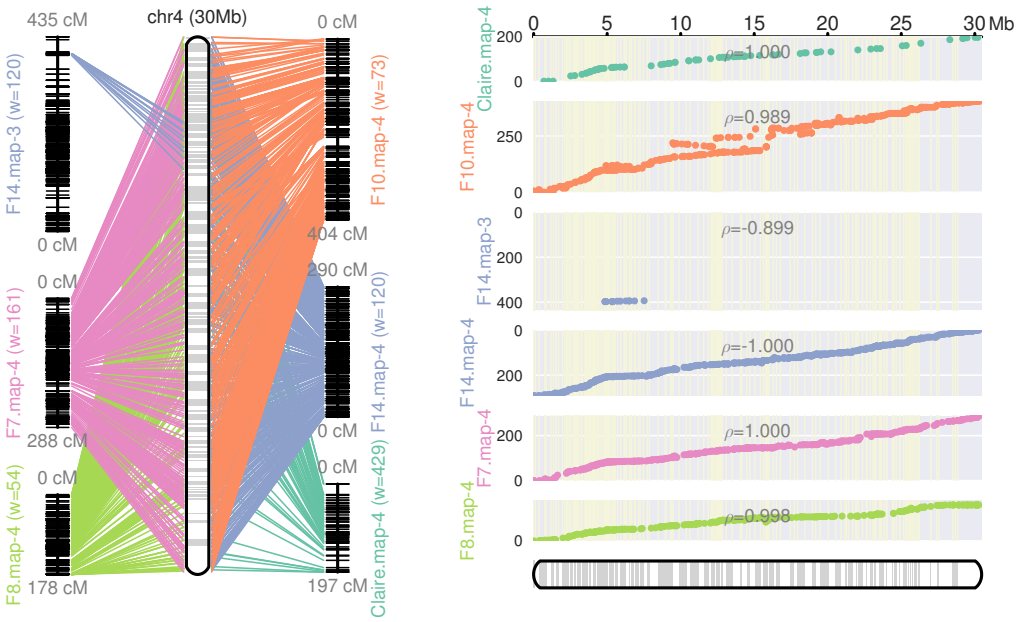


**Figure S6. Scaffold ordering for *N. lecontei* chromosome 4.** Details as in Figure S3.

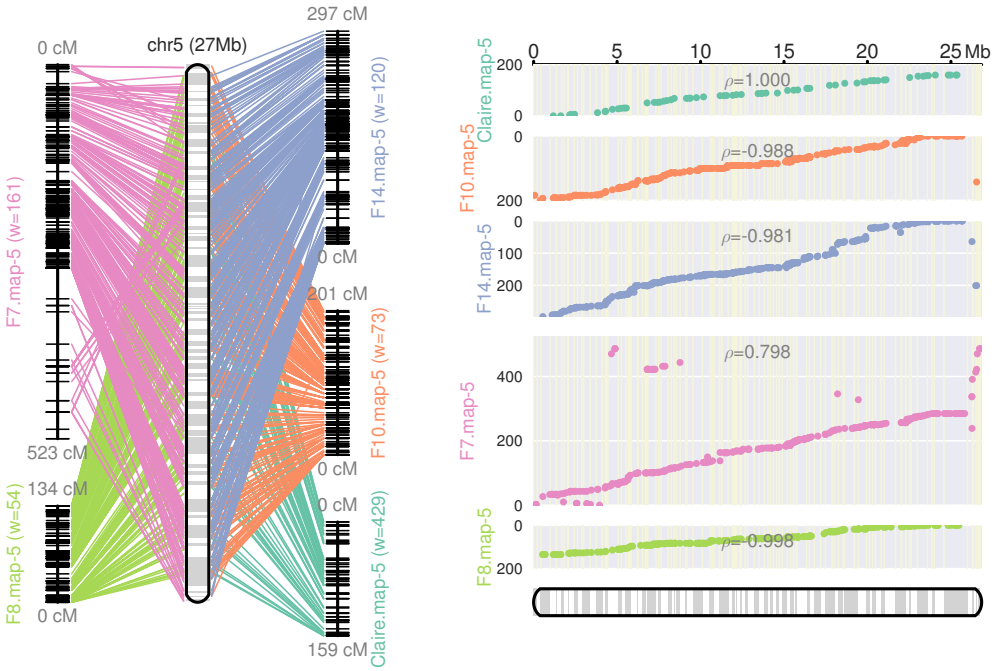


**Figure S7. Scaffold ordering for *N. lecontei* chromosome 5.** Details as in Figure S3.

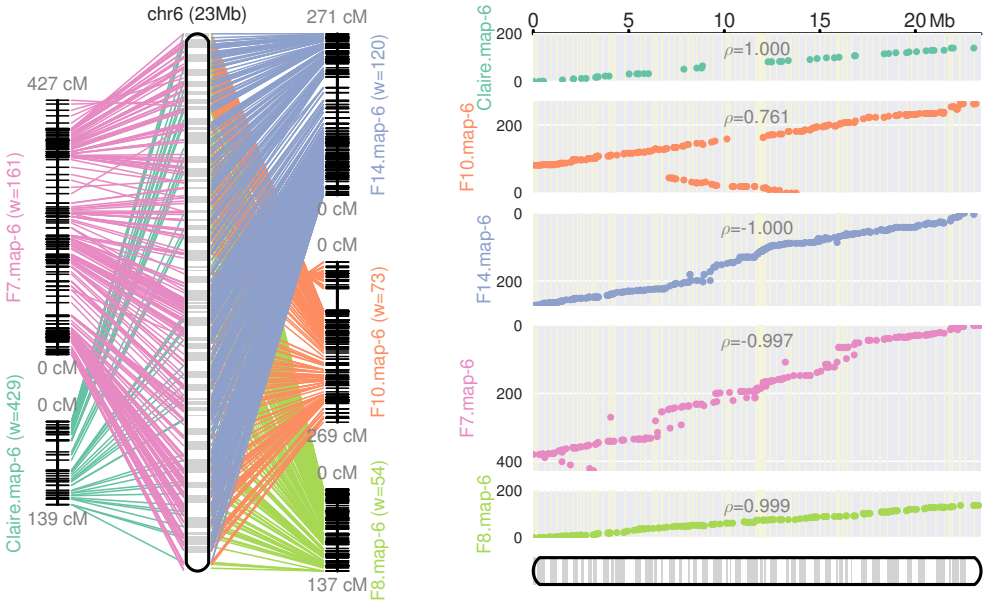


**Figure S8. Scaffold ordering for *N. lecontei* chromosome 6.** Details as in Figure S3.

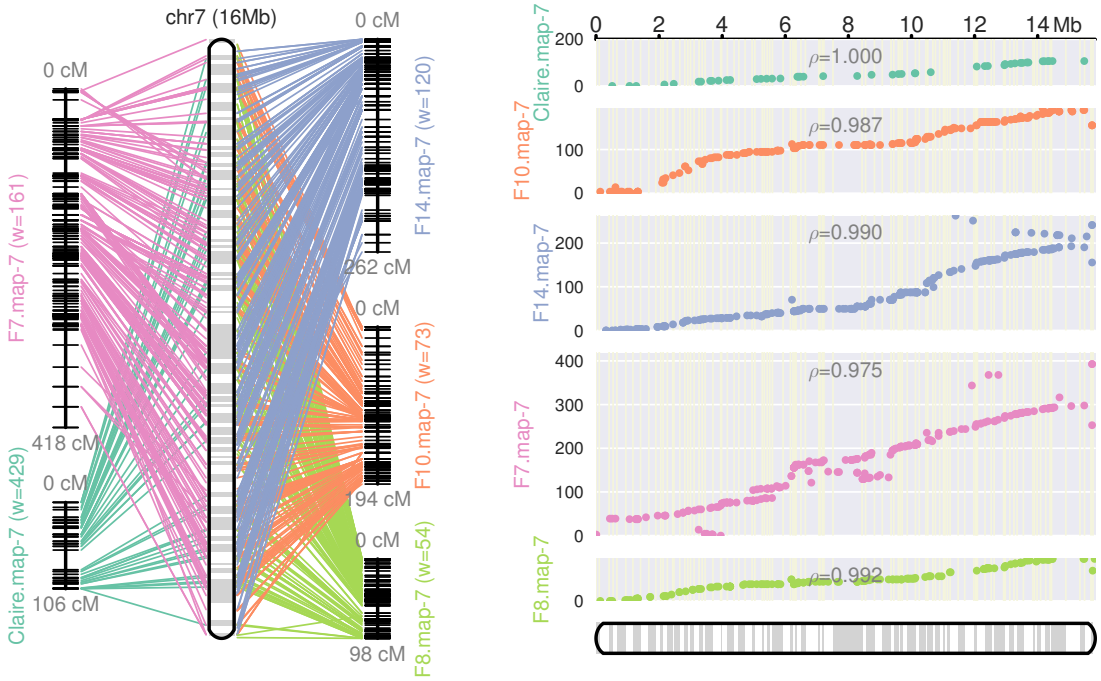


**Figure S9. Scaffold ordering for *N. lecontei* chromosome 7.** Details as in Figure S3.

**Table S5. Scaffolding analysis summary for five individual maps.** "All" map includes all $F_2$ males; remaining maps (F10, F14, F7, and F8) represent four families each derived from a unique set of grandparents.

| | ALL | F10 | F14 | F7 | F8 |
|---|---|---|---|---|---|
| # individuals | 429 | 73 | 120 | 161 | 54 |
| Linkage groups | 7 | 7 | 7 | 7 | 7 |
| Markers (unique) | 501 | 2,658 | 3,155 | 2,688 | 2,049 |
| Markers per Mb | 5.1 | 15.6 | 17.8 | 15.9 | 13.1 |
| N50 Scaffolds | 184 | 291 | 299 | 285 | 273 |
| Scaffolds | 358 | 807 | 867 | 798 | 704 |
| Scaffolds with 1 marker | 256 | 283 | 273 | 294 | 279 |
| Scaffolds with 2 markers | 78 | 157 | 174 | 161 | 145 |
| Scaffolds with 3 markers | 14 | 107 | 119 | 101 | 88 |
| Scaffolds with ≥ 4 markers | 10 | 260 | 301 | 242 | 192 |
| Total bases | 98,645,377 | 170,357,426 | 176,897,565 | 168,757,901 | 156,857,940 |
| % of bases | 41.20% | 71.10% | 73.80% | 70.40% | 65.40% |

**Table S6. Scaffolding analysis summary for consensus of five individual maps.**

| | Anchored | Oriented | Unplaced |
|---|---|---|---|
| Markers (unique) | 5,091 | 2,510 | 2 |
| Markers per Mb | 26.9 | 29 | 0 |
| N50 Scaffolds | 306 | 166 | 4 |
| Scaffolds | 1,005 | 282 | 3,518 |
| Scaffolds with 1 marker | 250 | 0 | 2 |
| Scaffolds with 2 markers | 165 | 31 | 0 |
| Scaffolds with 3 markers | 121 | 29 | 0 |
| Scaffolds with ≥ 4 markers | 469 | 222 | 0 |
| Total bases | 189,218,649 | 86,510,569 | 50,458,544 |
| % of bases | 78.90% | 36.10% | 21.10% |

## References Cited in Supplemental Materials

Galván I., Jorge A., Edelaar P., Wakamatsu K., 2015 Insects synthesize pheomelanin. Pigment Cell Melanoma Res. 28: 599–602.

McGraw K. J., Hudon J., Hill G. E., Parker R. S., 2005 A simple and inexpensive chemical test for behavioral ecologists to determine the presence of carotenoid pigments in animal tissues. Behav. Ecol. Sociobiol. 57: 391–397.

Montgomerie R., 2008 CLR, version 1.05.

Tang H., Zhang X., Miao C., Zhang J., Ming R., *et al.*, 2015 ALLMAPS: robust scaffold ordering based on multiple maps. Genome Biol. 16: 3.