

## Supplemental Information for:

### Genetic architecture of a body colour cline in *Drosophila americana*

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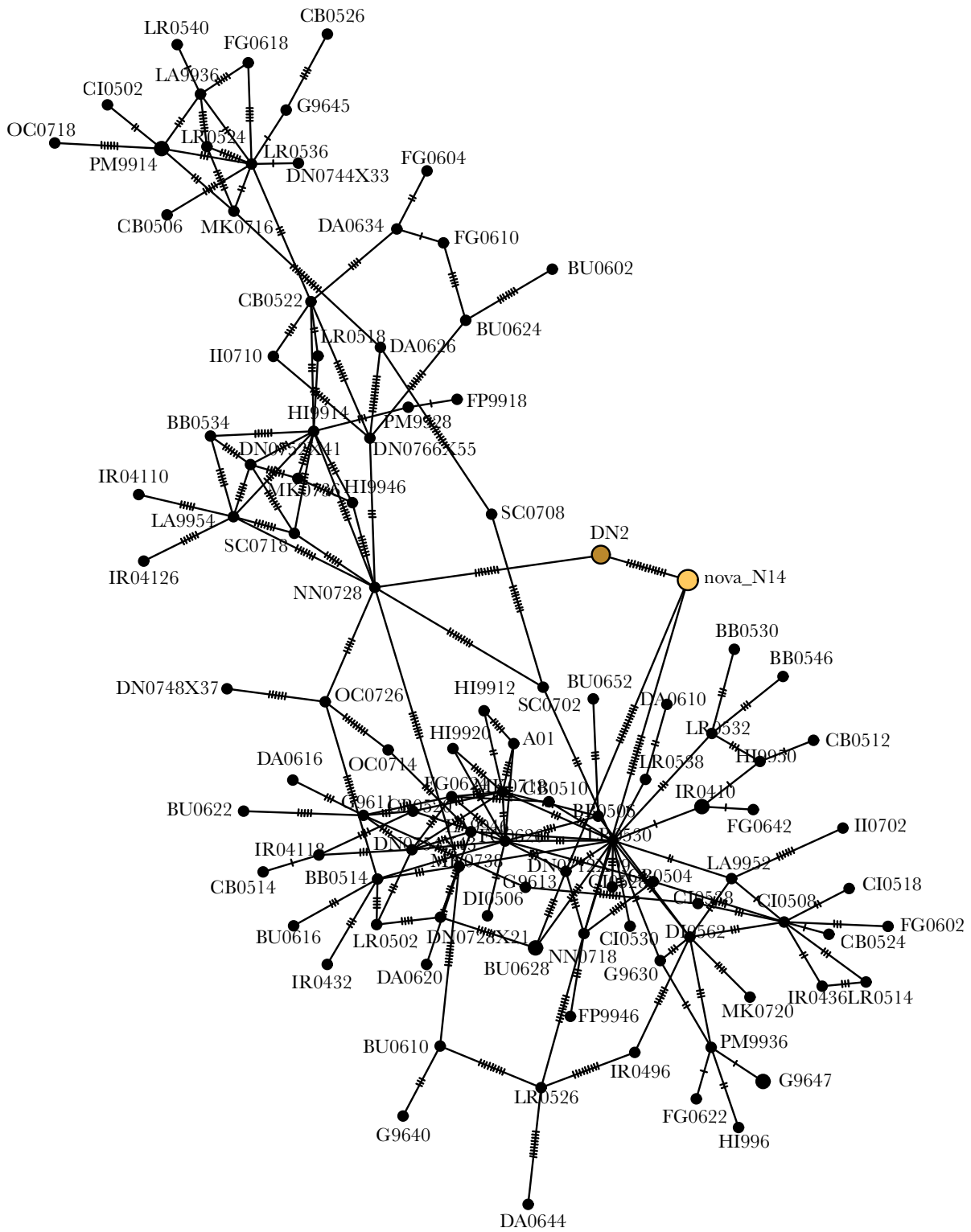
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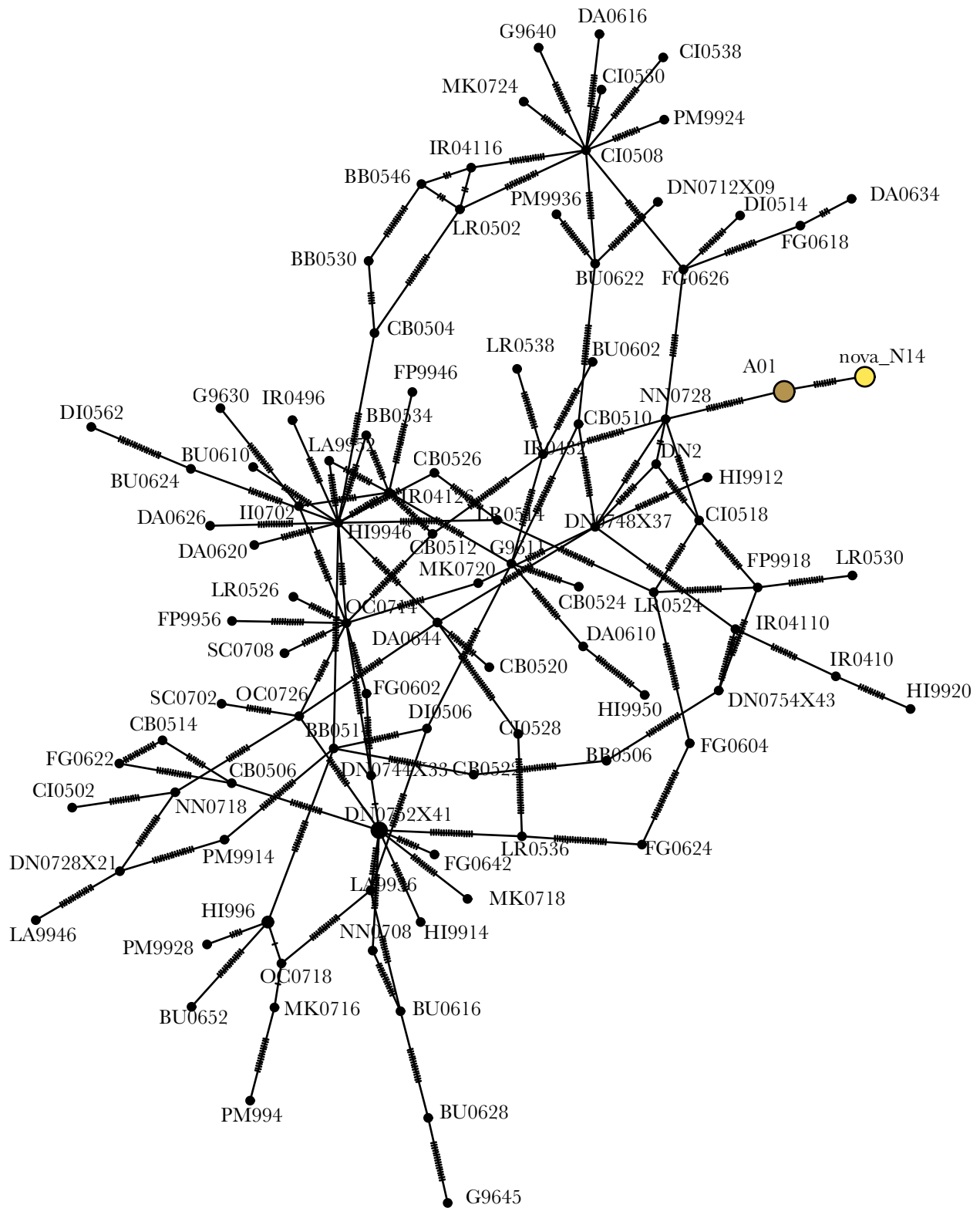
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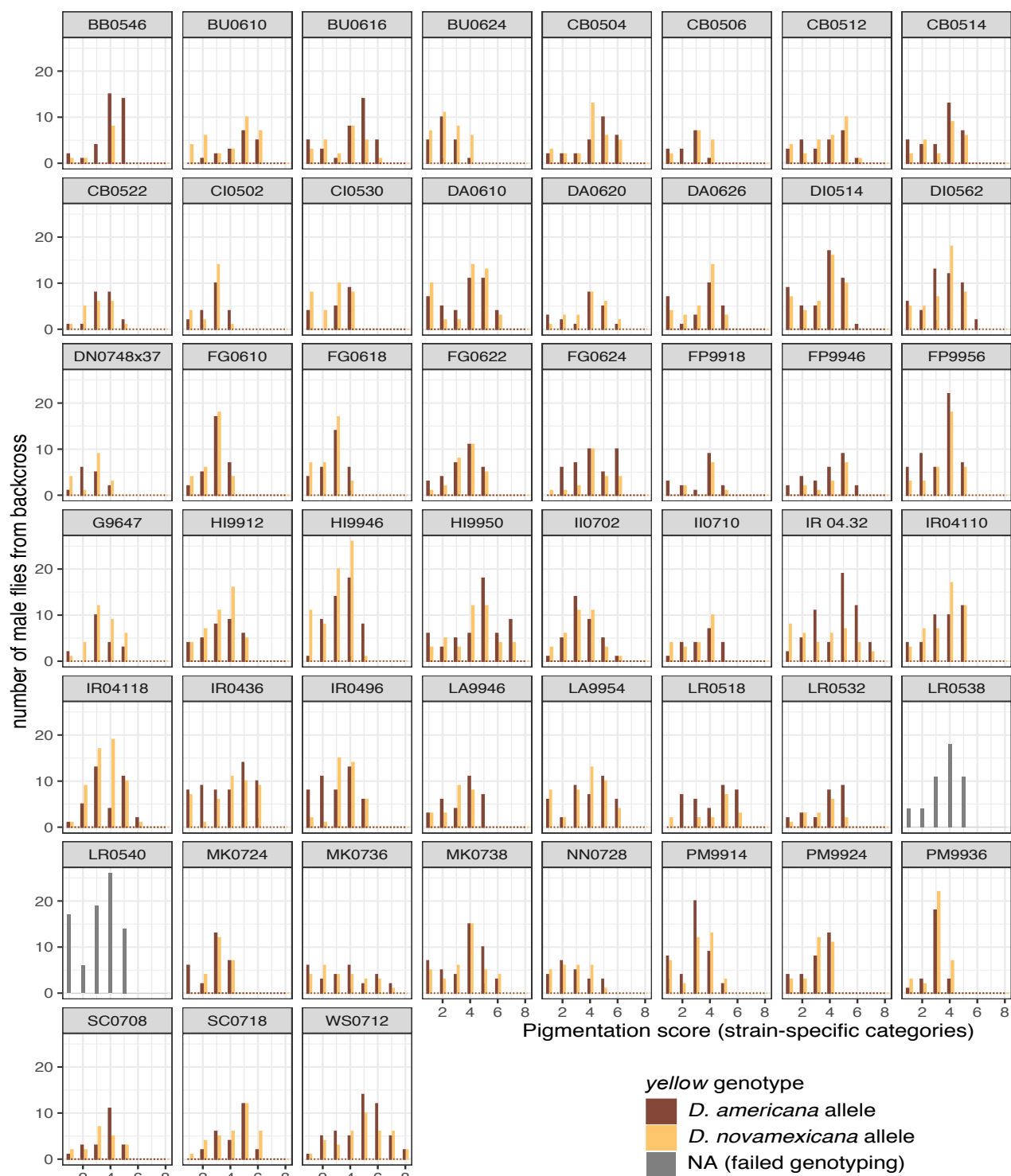
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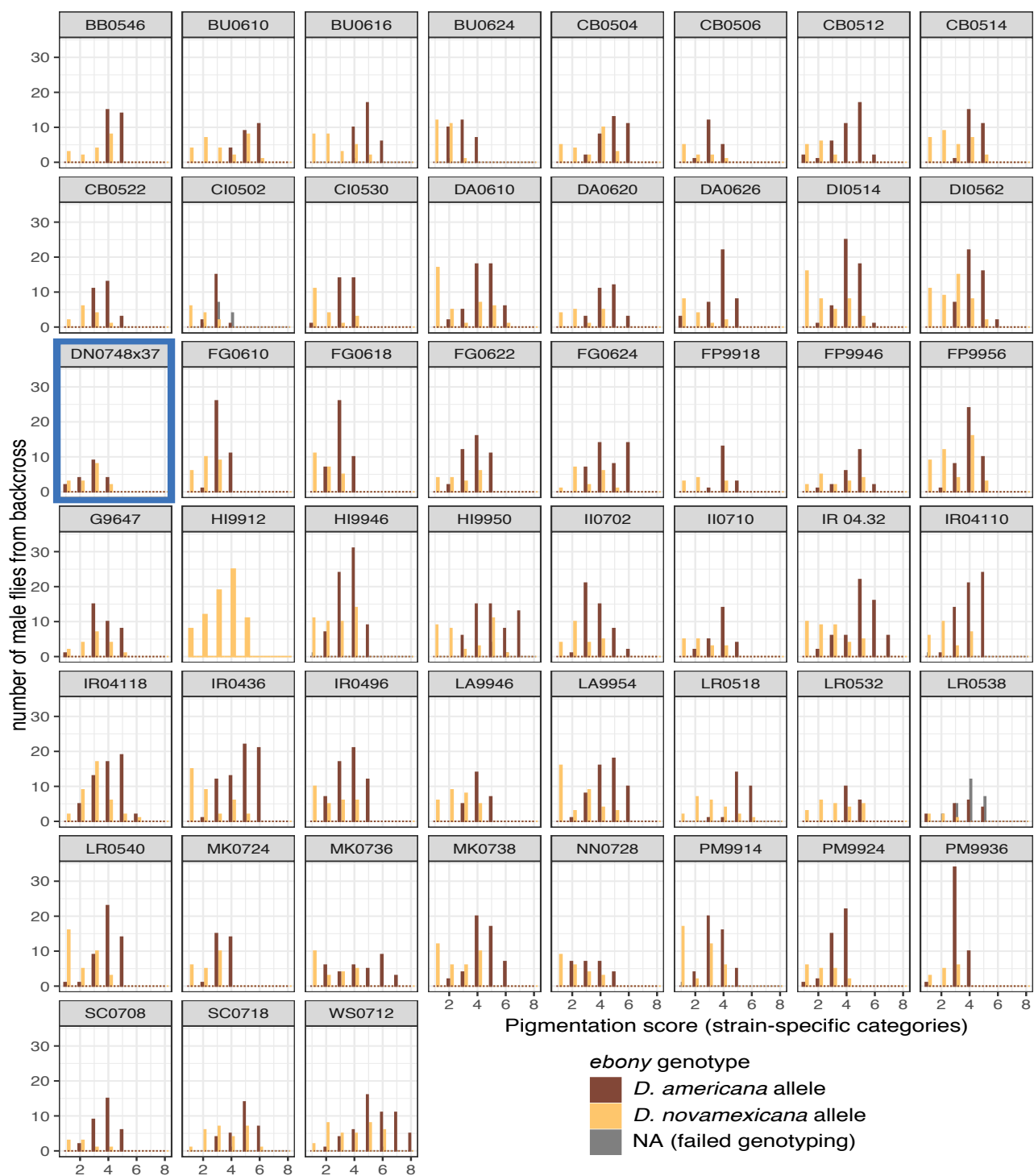
**Supplementary Figure 1. Haplotype network for *ebony*.** A Minimum Spanning Network built from the same *ebony* sequences used to construct the phylogenetic tree shown in Figure 1D is shown. Note that the DN2 allele from *D. americana* previously shown to share similarity in sequence and function with *D. novamexicana* (brown) is most similar to the *D. novamexicana* (“nova\_N14”) allele (yellow).



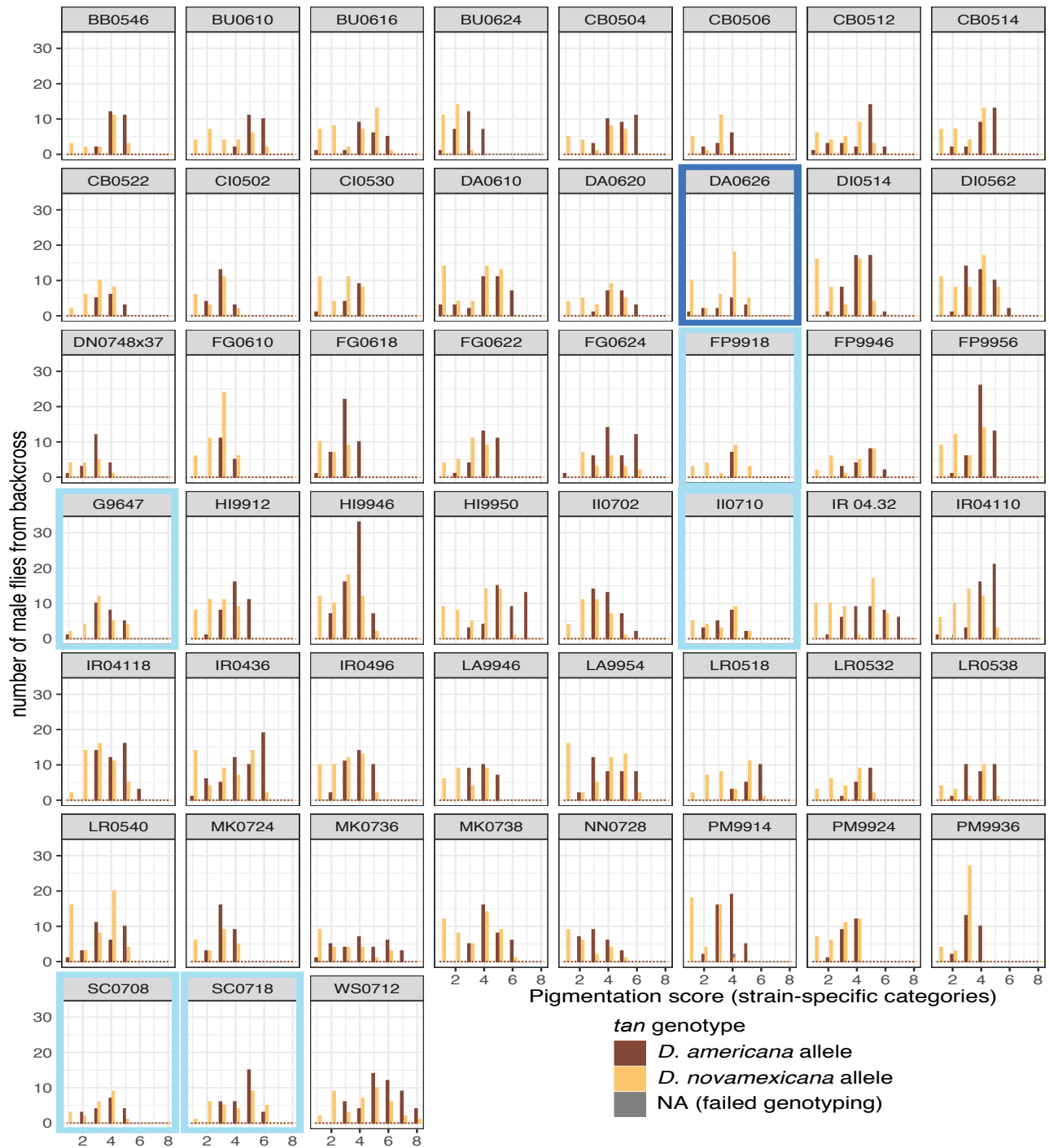
**Supplementary Figure 2. Haplotype network for *tan*.** A Minimum Spanning Network built from the same *tan* sequences used to construct the phylogenetic tree shown in Figure 1E is shown. Note that the A01 allele from *D. americana* previously shown to share similarity in sequence and function with *D. novamexicana* (brown) is most similar to the *D. novamexicana* (“nova\_N14”) allele (yellow).



**Supplementary Figure 3. Testing for function divergence of *yellow*.** Distributions of pigmentation phenotypes for backcross progeny inheriting the *D. americana* (brown) or *D. novamexicana* allele (yellow) of the *yellow* gene from their F<sub>1</sub> hybrid mother are shown for each strain of *D. americana* tested, with the strain name shown at the top of each panel. Numbers of males in each pigmentation class are shown rather than proportions to communicate sample sizes. Grey bars are shown for strains LR0538 and LR0540 because the *yellow* genotyping assay failed for all flies, presumably because of sequence differences in these *yellow* alleles.



**Supplementary Figure 4. Testing for function divergence of *ebony*.** Distributions of pigmentation phenotypes for backcross progeny inheriting the *D. americana* (brown) or *D. novamexicana* allele (yellow) of the *ebony* gene from their F<sub>1</sub> hybrid mother are shown for each strain of *D. americana* tested, with the stain name shown at the top of each panel. Numbers of males in each pigmentation class are shown rather than proportions to communicate sample sizes. Grey bars indicate samples with failed genotyping reactions, which were most common for *ebony* with flies from strain LR0538. The genotyping assay seemed to fail to differentiate alleles in the backcross with strain HI9912. Dark blue box indicates no significant difference between *D. americana* and *D. novamexicana* alleles.



**Supplementary Figure 5. Testing for function divergence of *tan*.** Distributions of pigmentation phenotypes for backcross progeny inheriting the *D. americana* (brown) or *D. novamexicana* allele (yellow) of the *tan* gene from their F<sub>1</sub> hybrid mother are shown for each strain of *D. americana* tested, with the stain name shown at the top of each panel. Numbers of males in each pigmentation class are shown rather than proportions to communicate sample sizes. Grey bars indicate samples with failed genotyping reactions, of which very few were observed for *ebony*. Dark blue box indicates no significant difference between *D. americana* and *D. novamexicana* alleles. Light blue boxes indicate marginal evidence of equivalent alleles (P-values = 0.05 or 0.06).

## **Supplementary Tables**

**Supplementary Table 1.** Summary of strains used for sequence analysis and/or functional testing, including details of sites where their progenitors were collected.

**Supplementary Table 2.** Median pigmentation measure for each fly sampled from each strain of *D. americana* obtained using a custom-built fiber optic probe to measure light reflected off the fly's abdominal cuticle.

**Supplementary Table 3.** Standardization of pigmentation classes among all backcrosses.

**Supplementary Table 4.** *yellow*, *tan*, and *ebony* genotypes for male progeny of F<sub>1</sub> hybrids backcrossed to *D. novamexicana*.

**Supplementary Table 5.** Results of permutation tests used to identify functional differences between *D. americana* and *D. novamexicana* alleles of *yellow*, *tan*, and *ebony*.

**Supplementary Table 6.** Genotypes of sites in *tan* used to test for an association with body pigmentation.

**Supplementary Table 7.** Genotypes of sites in *ebony* used to test for an association with body pigmentation.

**Supplementary Table 8.** Results from general linear models used to test for associations between body pigmentation and variable sites in *tan* and *ebony*.

## **Supplementary Files**

**Supplementary File 1.** FASTA format summary of *ebony* allele sequences analyzed.

**Supplementary File 2.** FASTA format summary of *tan* allele sequences analyzed.

**Supplementary File 3.** Text file containing R code used for all analyses presented in the manuscript.