**Supplementary Materials for Brand, Wright, and Presgraves, 2019**

**Table S1: Strategy to obtain coding sequences for PAML analyses**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | | |  |  |  |
| Species | Group | | | Gene | CDS source | Notes |
|  |  | | |  |  |  |
| *D. simulans* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. sechellia* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. mauritiana* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. melanogaster* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. yakuba* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. erecta* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. eugracilis* | *melanogaster* | | | *mei-218* | PCR Amplicon | Genomic DNA1 was extracted with DNeasy Blood and Tissue Kit (Qiagen Inc., Hilden, CA). Primers were anchored in the conserved 5' upstream *RpS5a* gene and an inferred conserved region at the 3' end of *mei-217*. Using iProof Taq polymerase (Bio-Rad, Hercules, CA) these primers produced a ~3kb amplicon that was subsequently Sanger sequenced.4 |
| *D. biarmipes* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. suzukii* | *melanogaster* | | | *mei-218* | SpottedWingFlyBase | Chiu et al. 2013 |
| *D. takahashii* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. elegans* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. rhopaloa* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. ficusphila* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. kikkawai* | *melanogaster* | | | *mei-218* | Flybase |  |
| *D. pseudoobscura* | *obscura* | | | *mei-218* | Flybase |  |
| *D. persimilis* | *obscura* | | | *mei-218* | Flybase |  |
| *D. miranda* | *obscura* | | | *mei-218* | Flybase |  |
| *D. affinis* | *obscura* | | | *mei-218* | R. Unckless |  |
| *D. obscura* | *obscura* | | | *mei-218* | NCBI |  |
| *D. americana* | *virilis* | | | *mei-218* | Y. Ahmed-Braimah |  |
| *D. lummei* | *virilis* | | | *mei-218* | Y. Ahmed-Braimah |  |
| *D. novamexicana* | *virilis* | | | *mei-218* | Y. Ahmed-Braimah |  |
| *D. virilis* | *virilis* | | | *mei-218* | Flybase |  |
| *D. simulans* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. sechellia* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. mauritiana* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. melanogaster* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. yakuba* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. erecta* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. eugracilis* | *melanogaster* | | | *mei-217* | Genomic Walking Kit | Genomic DNA1 was extracted with a DNeasy Blood and Tissue Kit. The *mei-218* gene region was amplified with APAgene Gold Genome Walking Kit (Bio S&T Inc., Montreal, QC) using nested primers in the *mei-217* gene region and degenerately binding primers. The resulting amplicons were subsequently Sanger sequenced.4 |
| *D. biarmipes* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. suzukii* | *melanogaster* | | | *mei-217* | SpottedWingFlyBase | Chiu et al. 2013 |
| *D. takahashii* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. elegans* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. rhopaloa* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. ficusphila* | *melanogaster* | | | *mei-217* | Flybase |  |
| **Table S1: continued** | |  | |  |  |  |
|  |  | | |  |  |  |
| Species | Group | | | Gene | CDS source | Notes |
|  |  | | |  |  |  |
| *D. kikkawai* | *melanogaster* | | | *mei-217* | Flybase |  |
| *D. pseudoobscura* | *obscura* | | | *mei-217* | Flybase |  |
| *D. persimilis* | *obscura* | | | *mei-217* | Flybase |  |
| *D. miranda* | *obscura* | | | *mei-217* | Flybase |  |
| *D. affinis* | *obscura* | | | *mei-217* | R. Unckless |  |
| *D. obscura* | *obscura* | | | *mei-217* | NCBI |  |
| *D. americana* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. lummei* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. novamexicana* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. virilis* | *virilis* | | | *mei-217* | Flybase |  |
| *D. simulans* | *melanogaster* | | | *rec* | Flybase |  |
| *D. sechellia* | *melanogaster* | | | *rec* | Flybase |  |
| *D. mauritiana* | *melanogaster* | | | *rec* | Flybase |  |
| *D. melanogaster* | *melanogaster* | | | *rec* | Flybase |  |
| *D. yakuba* | *melanogaster* | | | *rec* | Flybase |  |
| *D. erecta* | *melanogaster* | | | *rec* | Flybase |  |
| *D. eugracilis* | *melanogaster* | | | *rec* | Flybase |  |
| *D. biarmipes* | *melanogaster* | | | *rec* | Flybase |  |
| *D. suzukii* | *melanogaster* | | | *rec* | SpottedWingFlyBase | Chiu et al.2013 |
| *D. takahashii* | *melanogaster* | | | *rec* | Flybase |  |
| *D. elegans* | *melanogaster* | | | *rec* | Flybase |  |
| *D. rhopaloa* | *melanogaster* | | | *rec* | Flybase |  |
| *D. ficusphila* | *melanogaster* | | | *rec* | Flybase |  |
| *D. kikkawai* | *melanogaster* | | | *rec* | Flybase |  |
| *D. pseudoobscura* | *obscura* | | | *rec* | Flybase |  |
| *D. persimilis* | *obscura* | | | *rec* | Flybase |  |
| *D. miranda* | *obscura* | | | *rec* | Flybase |  |
| *D. affinis* | *obscura* | | | *rec* | R. Unckless |  |
| *D. obscura* | *obscura* | | | *rec* | NCBI |  |
| *D. americana* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. lummei* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. novamexicana* | *virilis* | | | *mei-217* | Y. Ahmed-Braimah |  |
| *D. virilis* | *virilis* | | | *mei-217* | Flybase |  |
| *D. simulans* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. sechellia* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. mauritiana* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. melanogaster* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. yakuba* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. erecta* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. eugracilis* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. biarmipes* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. suzukii* | *melanogaster* | | | *Mcm5* | SpottedWingFlyBase | Chiu et al. 2013 |
| *D. takahashii* | *melanogaster* | | | *Mcm5* | Flybase |  |
| **Table S1: continued** | | |  |  |  |  |
|  |  | | |  |  |  |
| Species | Group | | | Gene | CDS source | Notes |
|  |  | | |  |  |  |
| *D. elegans* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. rhopaloa* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. ficusphila* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. kikkawai* | *melanogaster* | | | *Mcm5* | Flybase |  |
| *D. pseudoobscura* | *obscura* | | | *Mcm5* | Flybase &PCR Amplicon | The 5’ end of *Mcm5* was missing in the Flybase assembly. To obtain the complete sequence, genomic DNA2 was extracted with a DNeasy Blood and Tissue Kit. Primers were anchored in the inferred 5’ flanking region and the known *Mcm5* gene region. Using iProof Taq polymerase these primers produced a ~650bp amplicon that was subsequently Sanger sequenced.5 |
|  |  | | |  |  |  |
| *D. persimilis* | *obscura* | | | *Mcm5* | Flybase &PCR Amplicon | The 3’ end of *Mcm5* contained errors in the Flybase assembly. To obtain the complete sequence, genomic DNA2 was extracted with a DNeasy Blood and Tissue Kit. Primers were anchored the known *Mcm5* gene region and 3’ flanking region. Using iProof Taq polymerase these primers produced a ~450bp amplicon that was subsequently Sanger sequenced.4 |
| *D. miranda* | *obscura* | | | *Mcm5* |  |  |
| *D. affinis* | *obscura* | | | *Mcm5* |  |  |
| *D. obscura* | *obscura* | | | *Mcm5* | NCBI |  |
| *D. americana* | *virilis* | | | *Mcm5* | Y. Ahmed-Braimah |  |
| *D. lummei* | *virilis* | | | *Mcm5* | Y. Ahmed-Braimah |  |
| *D. novamexicana* | *virilis* | | | *Mcm5* | Y. Ahmed-Braimah |  |
| *D. virilis* | *virilis* | | | *Mcm5* | Flybase |  |
| *D. simulans* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. sechellia* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. mauritiana* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. melanogaster* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. yakuba* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. erecta* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. eugracilis* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. biarmipes* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. suzukii* | *melanogaster* | | | *Blm* | SpottedWingFlyBase | Chiu et al.2013 |
| *D. takahashii* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. elegans* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. rhopaloa* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. ficusphila* | *melanogaster* | | | *Blm* | PCR Amplicon | Genomic DNA3 was extracted with DNeasy Blood and Tissue Kit. Primers were anchored in the conserved flanking genes CG14721 and CG14722. Using iProof Taq polymerase these primers produced a ~5.5kb amplicon that was subsequently Sanger sequenced.4 |
| *D. kikkawai* | *melanogaster* | | | *Blm* | Flybase |  |
| *D. pseudoobscura* | *obscura* | | | *Blm* | Flybase |  |
| *D. persimilis* | *obscura* | | | *Blm* | Flybase |  |
| *D. miranda* | *obscura* | | | *Blm* | Flybase |  |
| *D. affinis* | *obscura* | | | *Blm* | R. Unckless |  |
| *D. obscura* | *obscura* | | | *Blm* | NCBI |  |
| *D. americana* | *virilis* | | | *Blm* | Y. Ahmed-Braimah |  |
| **Table S1: continued** | | | |  |  |  |
|  |  | | |  |  |  |
| Species | Group | | | Gene | CDS source | Notes |
|  |  | | |  |  |  |
| *D. lummei* | *virilis* | | | *Blm* | Y. Ahmed-Braimah |  |
| *D. novamexicana* | *virilis* | | | *Blm* | Y. Ahmed-Braimah |  |
| *D. virilis* | *virilis* | | | *Blm* | Flybase |  |

1San Diego Stock Center strain: 14026-0451.10

2Presgraves lab stock: genome reference strain

3San Diego Stock Center strain: 14025-0441.05

4Sequence now available on NCBI

5see Supplemental File 1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | Crossover interval | *mel* | *pse* | *vir* | *mei-218*1 |
|  |  |  |  |  |  |
| **Total**  **Parental** |  | **1208** | **1330** | **1221** | **323** |
|  | 1 | 37 | 56 | 3 | 0 |
| 2 | 102 | 112 | 19 | 0 |
| 3 | 421 | 233 | 34 | 2 |
| 4 | 214 | 133 | 14 | 2 |
| 5 | 61 | 81 | 4 | 3 |
| **Total Single COs** |  | **835** | **615** | **74** | **7** |
|  | 1/2 | 0 | 6 | 0 | 0 |
| 1/3 | 4 | 7 | 1 | 0 |
| 1/4 | 2 | 11 | 1 | 0 |
| 1/5 | 2 | 2 | 0 | 0 |
| 2/3 | 6 | 5 | 1 | 0 |
| 2/4 | 6 | 10 | 0 | 0 |
| 2/5 | 2 | 2 | 0 | 0 |
| 3/4 | 13 | 10 | 3 | 0 |
| 3/5 | 21 | 18 | 1 | 0 |
| 4/5 | 3 | 7 | 1 | 0 |
| **Total Double COs** |  | **59** | **78** | **8** | **0** |
|  | 1/2/3 | 1 | 2 | 0 | 0 |
| 1/2/4 | 0 | 1 | 0 | 0 |
| 1/2/5 | 0 | 0 | 0 | 0 |
| 1/3/4 | 0 | 1 | 0 | 0 |
| 1/3/5 | 0 | 0 | 0 | 0 |
| 1/4/5 | 0 | 0 | 0 | 0 |
| 2/3/4 | 0 | 0 | 0 | 0 |
| 2/3/5 | 0 | 0 | 0 | 0 |
| 2/4/5 | 0 | 1 | 1 | 0 |
| 3/4/5 | 0 | 0 | 0 | 0 |
| **Total Triple COs** |  | **1** | **5** | **1** | **0** |
| **Total COs** |  | **956** | **786** | **93** | **7** |
| **Total progeny** |  | **2103** | **2028** | **1304** | **330** |

**Table S2: Crossovers across the *net*-*cn* region among genotypes**

Each row lists the total progeny with either parental, single, double, or triple-crossover chromosomes among the three genotypes: *mei-217/mei-218*mel (*mel*), *mei-217/mei-218*pse (*pse*), *mei-217/mei-218*vir (*vir*), and *mei-218*1. Crossovers were scored among six visible markers spanning the left arm of chromosome *2* and the centromere, creating five genetic intervals: *net*-*ho* (1); *ho*-*dp* (2); *dp*-*b* (3); *b*-*pr* (4); *pr*-*cn* (5). The numbers of crossovers per recovered chromosome differ significantly among all genotypes (**2 test, *df* = 3, *p* ≤1.29e-16).

**Table S3. The distribution of crossovers among intervals of the *net*-*cn* region differs among genotypes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
|  | *mel* | *pse\** | *vir*†‡ | *mei-218*1 | Uniform |
|  |  |  |  |  |  |
| *net-ho* | 0.048 | 0.109 | 0.054 | 0.000 | 0.076 |
| *ho-dp* | 0.122 | 0.177 | 0.226 | 0.000 | 0.067 |
| *dp-b* | 0.487 | 0.351 | 0.43 | 0.286 | 0.297 |
| *b-pr* | 0.249 | 0.221 | 0.215 | 0.286 | 0.200 |
| *pr-cn* | 0.093 | 0.141 | 0.075 | 0.429 | 0.360 |

Proportions = average number of crossovers divided by total number of crossovers

Uniform = crossover frequency proportional to physical length of chromosome interval

\**mei-217/-218*pse is significantly different from *mei-217/-218*mel (χ2 test, *df* = 4, *p* = 9.52e-28) and from *mei-217/-218*vir (χ2 test, *df* = 4, *p* = 7.53 e-23).

†*mei-217/-218*vir is marginally significantly different from *mei-217/-218*mel (χ2 test, *df* = 4, *p* = 0.05)

‡*mei-217/-218*vir significantly differs from uniform expectation (χ2 test, *df* = 4, *p* = 1.2e-12)

**File S1. *D. pseudoobscura* MCM5 CDS**

ATGGAGGGATTTGATGATGCTGGTGTTTTTTTTTCTGATAATTTCGGAAACGATCAGCAGTCGGACGGTGCGCAAATAAATTTGCAAGCGGTCAAGAAAAGATACAAGGAGTTTATTCGGACATTCAACGAAGACAACTTTTTTTATAAATACCGTGACACATTGAAACGCAACTACCTCAATGGACGCTATTTTCTGGAAATCGAAATGGAAGATTTGGTGGGATTTGATGAGGCCCTTGCCGATAAAATGAACAAACAACCAACTGAACATCTGCAAATTTTTGAAGAGGCTGCTCTGGAAGTGGCTGACGAAATTACAGCTCCACGTCCCGAGCATGAAGAGCACATGCACGATATCCAAATACTATTGAGATCCAATGCCAACCCCACCAATATACGTGAACTAAAATCAGAGTGCGTGTCGCGCTTAGTTAAAATTGCAGGCATAATTGTGGCCGCATCCGGCATTAGTGCAAAGGCAACAAAAATGTCCATTATGTGCTTGTCGTGCAGCACTGTTATACCAAATCTCAGAGTAAATCCCGGCCTAGAAGGCTACGCCCTGCCACGAAAATGCACAACGGAACAGGCGGGACGACCAAAGTGTCCCCTAGATCCTTTCTTTGTAATGCCGGACAAGTGCAAATGCGTGGATTTTCAAACGCTAAAATTGCAAGAGCTACCAGACTTTGTCCCTCAAGGAGAGATTCCTCGACATTTGCAGTTATTCTGTGACCGTTCCCTGTGCGAACGTGTTGTGCCAGGAAATCGTGTTCTCATACAAGGTATTTATTCAATTCGAAAAGTGGGTAAACCCTCTCGTCAGGATGGTCGAGAGAAGGCCGTTGTGGGTGTTCGCGCGCCCTATATGCGGGTGGTGGGCATAACAGTGGATGCAGAGGGGGCCGGTGCCATTTCTCGCTATAACAATATAACCACCGATGAGGAGGACAACTTCCGACGCATGGCTGCCTCGGGAGATATATACGAACGCCTTTCTCAATCATTGGCTCCCAGTATATTCGGCTCTCGAGACATTAAAAAGGCTATAACTTGTATGCTTTTTGGTGGGTCCCGCAAGCGGCTGCCAGATGGACTGTGTCGACGTGGGGACATCAACGTACTGCTGTTGGGGGATCCAGGTACGGCAAAGTCGCAGCTACTAAAGTTTGTCGAGAAGGTAGCCCCCATTGGCGTGTATACATCAGGAAAGGGATCTAGCGCGGCTGGTCTTACAGCATCGGTTATGAAGGATCCCCAAACGCGCAATTTCGTCATGGAAGGAGGTGCCATGGTATTGGCTGACGGTGGTGTCGTTTGCATCGACGAATTTGACAAAATGCGTGAAGATGACCGGGTGGCCATACACGAGGCAATGGAACAGCAAACCATCTCCATTGCAAAAGCCGGCATTACAACCACATTAAATTCTCGATGTTCGGTTTTAGCAGCTGCTAATTCTATATTTGGCCGGTGGGACGATACAAAGGGCGAGGAAAATATCGATTTCATGCCCACAATATTATCTCGTTTTGACATGATATTTATTGTTAAGGACGTACACGATGAGACACGTGATATTACTCTTGCAAAGCACATCATAAACGTCCATCTTAGTTCAAATAAATCTGCACCAACCGAGCCAGCCGAAGGTGAAATATCTTTGTCCACATTTAAAAAGTACATACACTACTGTCGCACCCATTGTGGGCCACGATTAAGTGAAGCTGCTGGCGAGAAGCTTAAGAGCCGCTATGTGCTGATGCGAAGTGGTGCCGGTCAGCAGGAAAAAAATGCAGACAAACGGCTGAGTATTCCGATTACAGTGCGCCAGCTAGAGGCTGTTATTCGAATTTCAGAGTCCTTAGCAAAAATACGCTTGCTTCCTTTTGTCGCCGATGATCATGTCAATGAGGCCCTTCGACTTTTCCAAGTATCGACTCTTGATGCAGCTATGACTGGAAGCCTGGCCGGGGCTGAGGGATTCACCACAGAGGAAGACCAGGAAACTCTTAACCGTATTGAAAAACAATTAAAGCGTCGCTTTGCTATCGGATCTCAAGTTTCTGAACAGAATATCTTACAGGACTTTTTACGTCAAAAGTACGAGGAGCGAACTGTACTCAAAGTCATTCACACGATGATTCGACGTGGTGAACTTCAGCACAGAATGCAACGCAAGATGCTATACCGCATATGCTAG