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Supplementary Information for

Sexual conflict drives male manipulation of female post-mating responses in *Drosophila melanogaster*

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Supplementary Information Text

Supplementary methods

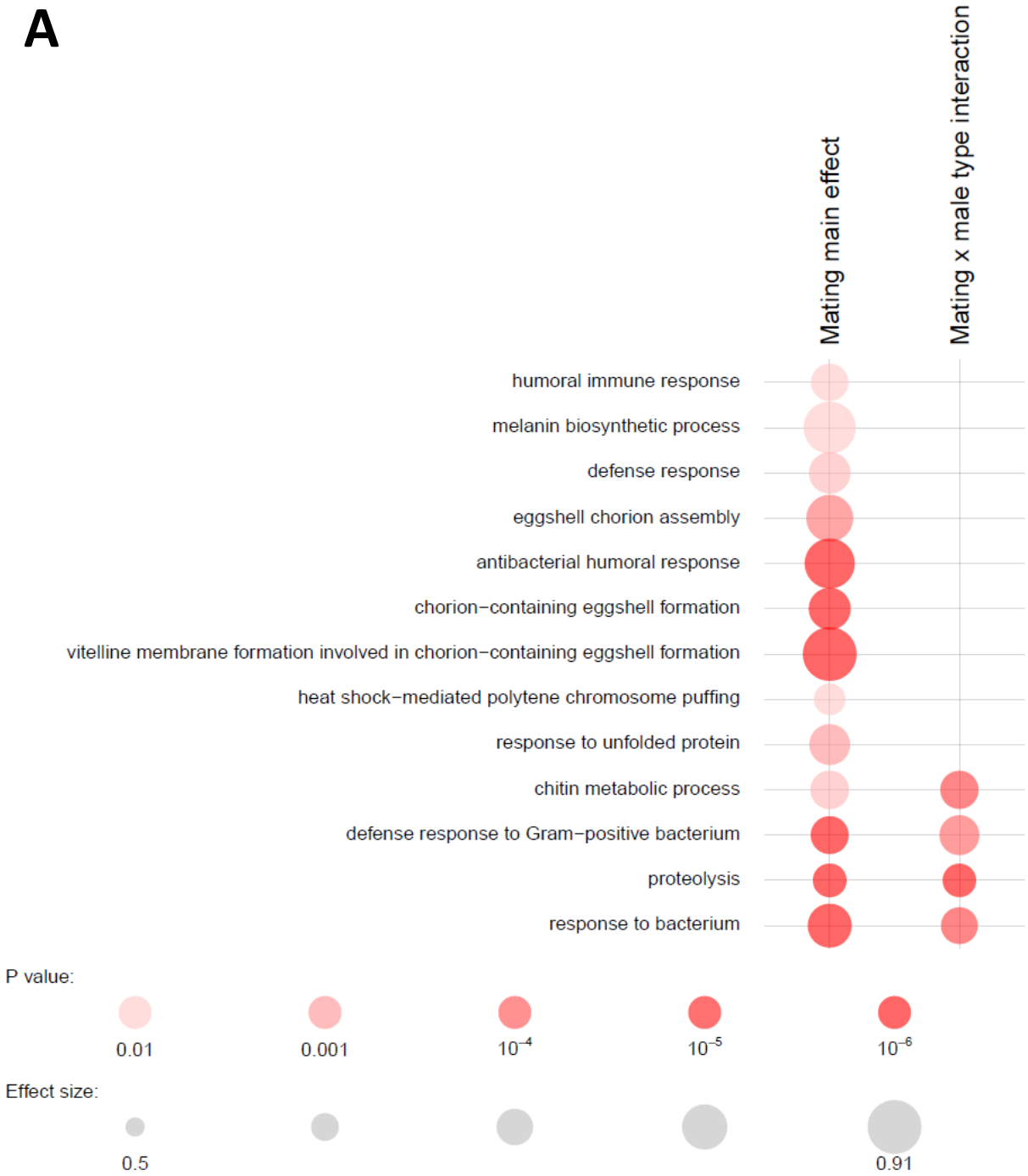
RNA extraction, library preparation, sequencing, and qPCR validation

For female heads and abdomens, RNA was obtained using a phenol-chloroform extraction, cleanup and concentration with RNeasy MinElute spin columns (Qiagen), and then treatment with Turbo DNase (Ambion). Stranded libraries were generated using TruSeq Stranded Total RNA Library Prep kit (Illumina) and multiplexed. Libraries were then sequenced as paired-end reads (2 x 150 bp) in eight lanes, across two flow cells, on an Illumina HiSeq4000. Reads were first inspected visually using FastQC (1). Quality trimming, filtering, and adapter clipping was done with Trimmomatic 0.33 (2). TruSeq adapters were removed from read ends and bases with a quality score smaller than 20 (less than 99% base call accuracy) were removed from both read ends. Further quality trimming was done using a 5 bp sliding window requiring an average quality score of 20. Finally, only reads at least 50 bp long after trimming were retained. Quality-trimmed reads were mapped to the *D. melanogaster* transcriptome with TopHat2 2.1.0 (3) using genome assembly BDGP6 (4) and genome annotation BDGP6.84 downloaded from the *Ensembl* website (5). Mapped reads were then counted using *HTSeq* 0.6.1 (6).

For male reproductive tract gene expression measurements, RNA was extracted from pooled male reproductive tracts using the RNeasy Mini Kit (Qiagen). Stranded libraries were generated using TruSeq Stranded Total RNA Library Prep kit (Illumina) and multiplexed. Libraries were then sequenced as single-end reads (100 bp) in two lanes on an Illumina HiSeq2500. Reads were first inspected visually using FastQC (1). Quality trimming, filtering, and adapter clipping was done with Trimmomatic 0.33 (2). TruSeq adapters were removed from read ends and bases with a quality score smaller than 20 (less than 99% base call accuracy) were removed from both read ends. Further quality trimming was done using a 5 bp sliding window requiring an average quality score of 20. Finally, only reads at least 50 bp long after trimming were retained. Quality-trimmed reads were mapped to the *D. melanogaster* transcriptome with TopHat2 2.1.0 (3) using genome assembly BDGP6 (4) and genome annotation BDGP6.84 downloaded from the *Ensembl* website (5). Mapped reads were then counted using *HTSeq* 0.6.1 (6).

In order to gain more detailed insight into SFP gene expression dynamics in maturing accessory glands, and validate detected differences in gene expression, we followed up our whole-transcriptome profiling of male reproductive tracts with a targeted qPCR approach after 19 more generations of experimental evolution. RNA extractions were performed with the Qiagen RNeasy Mini Kit (Qiagen) and included an on-column DNA digestion step (Qiagen RNase-Free DNase Set). RNA concentration and quality were then estimated using a NanoPhotometer (Pearl, Implen) and a Bioanalyzer (Agilent 2100). Approximately 300 ng RNA was transcribed to cDNA using the Thermo Fisher Revert Aid First Strand cDNA Synthesis Kit and Oligo $(dT)_{18}$ primer. Before the qPCR, cDNA was diluted 1:5. The qPCR was performed on a Light Cycler 480 (Roche) using KAPA SYBR Fast qPCR Master Mix in a 15 µl reaction (7.5 µl SYBR Fast, 0.4 µl

forward primer, 0.4 µl reverse primer, 4.7 µl water and 2 µl cDNA template) in 96-well plates with the following settings: preincubation (3 min at 95° C), 40 amplification cycles (15 s at 95° C then 1 min at 60° C), melting curve (heating samples up to 97° C). For all target genes and the reference genes *Ef1α48D* and *RpL13A*, primer efficiencies had already been determined in an earlier experiment with similar RNA samples (7) and here we added efficiency values for primers for two additional reference genes *αTub84B* and *Act42A* (Table S3). Crossing point cycle values (Ct) were obtained using the Second Derivative Maximum Method. The mean Ct value of two technical replicates was used to calculate relative gene expression as the efficiency calibrated relative expression ratio (8) using a calibrator sample from the base IV population collected 24 h post-eclosion and the geometric mean of the four reference genes.


A
Mating main effect
Mating x male type interaction
humoral immune response
melanin biosynthetic process
defense response
eggshell chorion assembly
antibacterial humoral response
chorion−containing eggshell formation
vitelline membrane formation involved in chorion−containing eggshell formation
heat shock−mediated polytene chromosome puffing
response to unfolded protein
chitin metabolic process
defense response to Gram−positive bacterium
proteolysis
response to bacterium
P value:
0.01
0.001
10−4
10−5
10−6
Effect size:
0.5
0.91

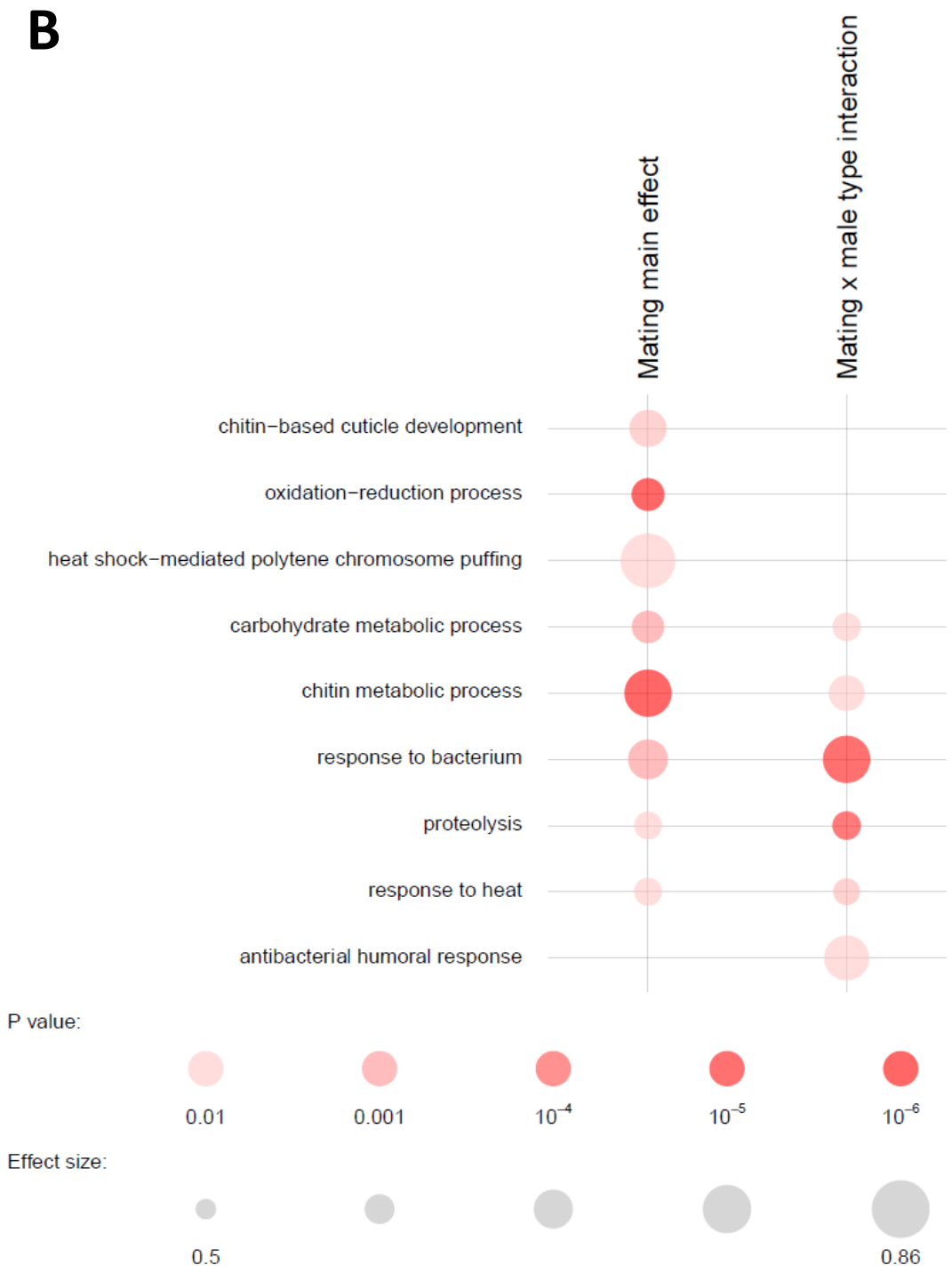


Fig. S1. Gene ontology (GO) terms that were significantly enriched for the main effect of mating and the interaction between mating and male type (monogamous versus polygamous) for (A) female abdomens and (B) female heads. The size of the circles indicates effect size of the enrichment while intensity of the color indicates adjusted p values.

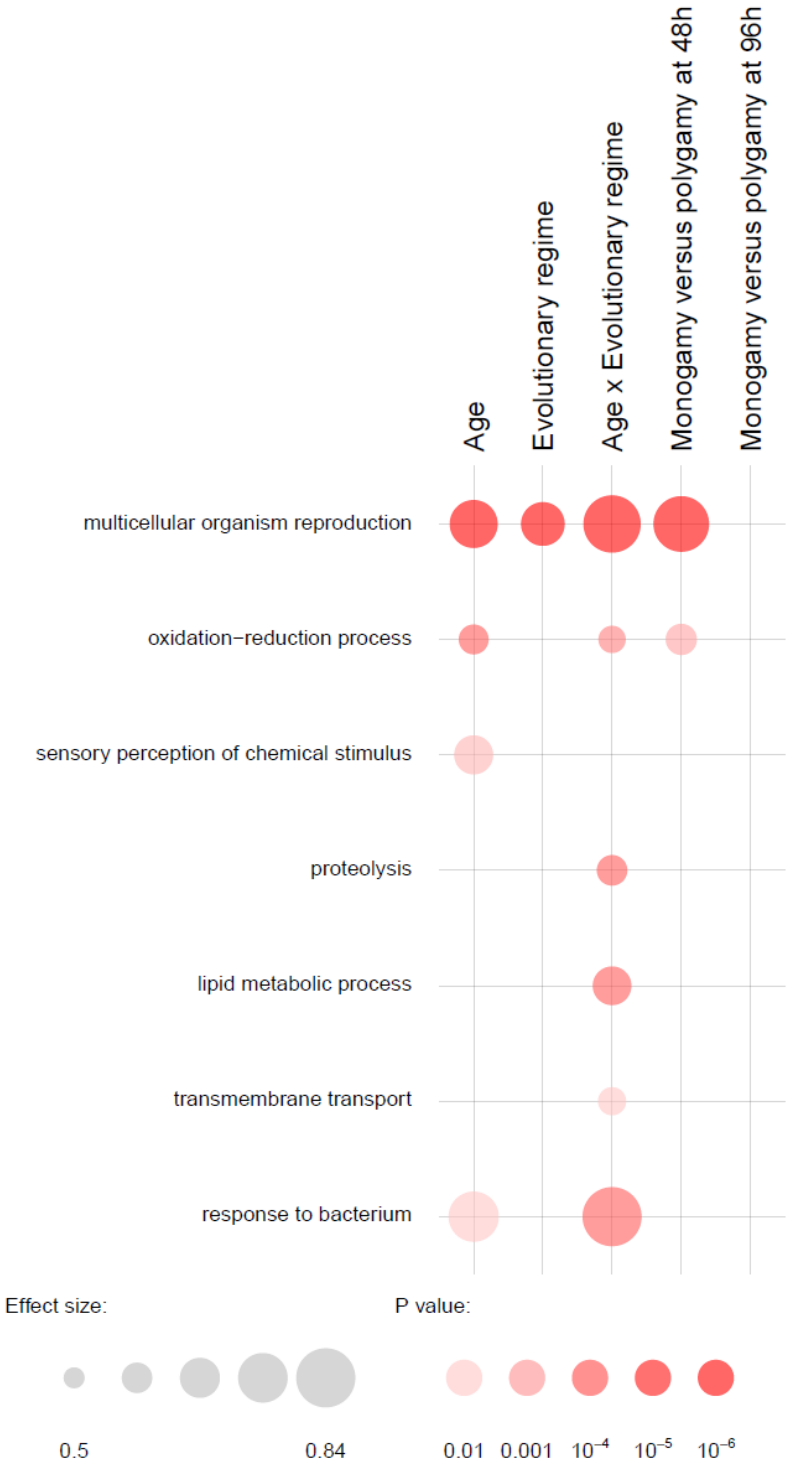


Fig. S2. Gene ontology (GO) terms that were significantly enriched in at least one comparison. The size of the circles indicates effect size of the enrichment while intensity of the color indicates adjusted p values.

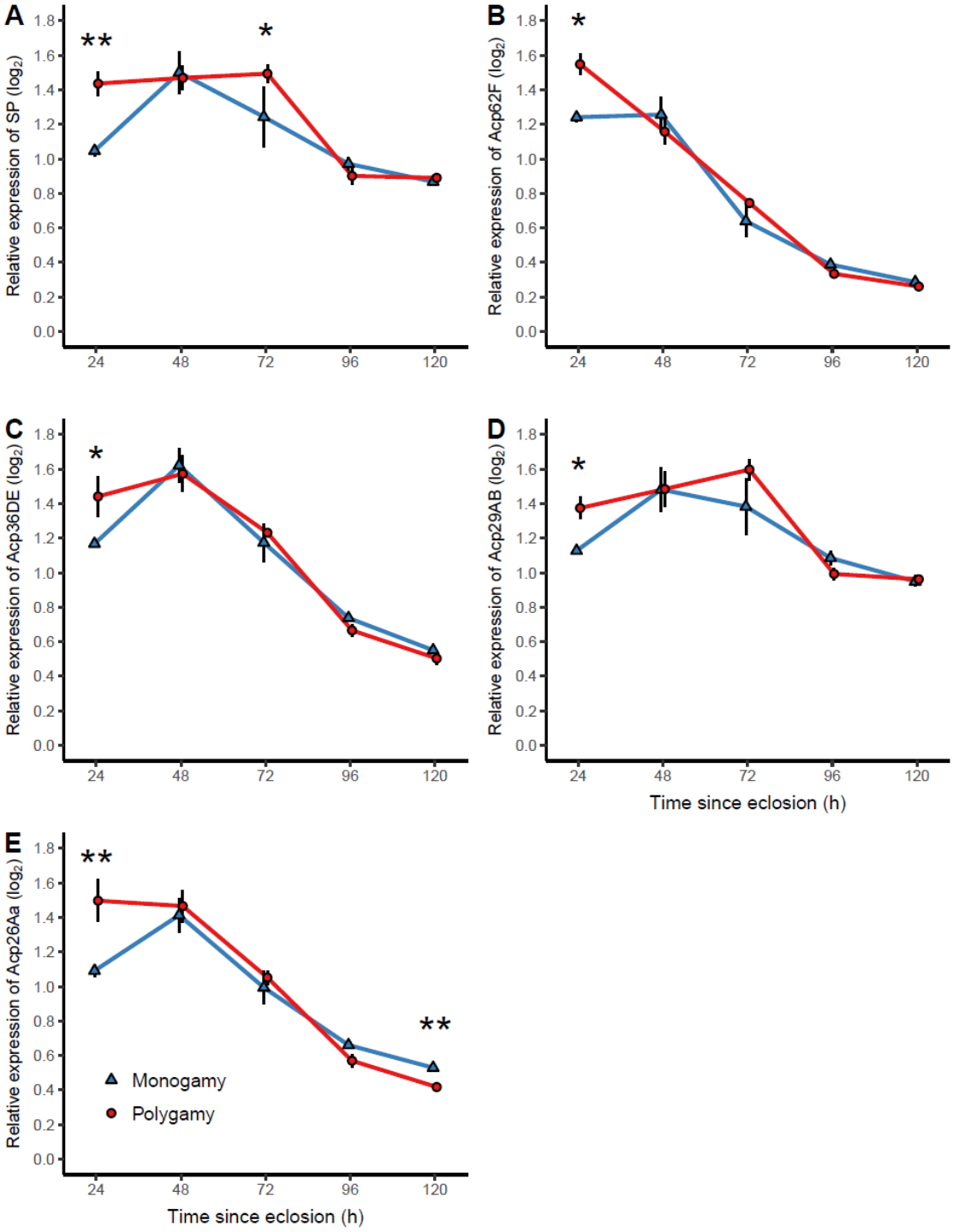


Fig. S3. (A – E) Expression levels of five SFP genes in the reproductive tracts of males with different evolutionary histories across the first five days after eclosion. Values are evolutionary regime means (± SE) log2 transformed relative expression, normalized against four reference genes and a calibrator sample, for the monogamous evolutionary regime (blue triangles) and polygamous evolutionary regime (red circles). All five genes are nominally significantly different ($p < .05$) between monogamous and polygamous males at 24 hours; only two genes (SP and Acp26Aa) remain significant after Bonferroni correction ($\alpha = .01$). * $p < .05$, ** $p < .01$.

Additional data table S1 (separate file)

Effects of mating and mate evolutionary regime on gene expression in female heads and abdomens.

Additional data table S2 (separate file)

Effects of time since eclosion and evolutionary regime on gene expression in the reproductive tracts of males.

References

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