

SUPPLEMENTARY MATERIALS

Brain microRNAs among social and solitary bees

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Table S1. Genome assemblies used for alignment of small RNA sequences or genome scans for known small RNAs. The last six species were used only for genome scans.

Species	Genome assembly	Reference
<i>Apis mellifera</i>	Amel v4.5	Elsik et al. 2014
<i>Bombus impatiens</i>	Bimp v2.0	Sadd et al. 2015
<i>Bombus terrestris</i>	Bter v1.0	
<i>Megalopta genalis</i>	Mgen v1.0	
<i>Megachile rotundata</i>	Mrot v1.0	Kapheim et al. 2015
<i>Nomia melanderi</i>	Nmel v1.0	Kapheim et al. 2019
<i>Apis florea</i>	Aflo v1.0	Baylor College of Medicine, unpublished (NCBI PRJNA45871)
<i>Dufourea novaeangliae</i>	Dnov v1.0	Kapheim et al. 2015
<i>Eufriesea mexicana</i>	Emex v1.0	Kapheim et al. 2015
<i>Habropoda laboriosa</i>	Hlab v1.0	Kapheim et al. 2015
<i>Lasioglossum albipes</i>	Lalbi v2	Kocher et al. 2013
<i>Melipona quadrifasciata</i>	Mqua v1.0	Kapheim et al. 2015

Table S2. Sample acquisition and library preparation methods. Samples were collected into liquid nitrogen and stored at -80 °C until dissection. All libraries were prepared from RNA isolation from whole brain tissue. All libraries were sequenced for 51 cycles on a HiSeq 2500. *B. impatiens*, *M. genalis*, *N. melanderi* libraries were pooled and sequenced in one lane. *A. mellifera*, *B. terrestris*, and *M. rotundata* libraries were pooled and sequenced in one lane.

Species	Collection	Sample type	RNA Isolation	RNA Quality Assessment	Sequencing Center	Library Prep	# reads	Reads mapped to genome
<i>Bombus impatiens</i>	Commercial colony (BioBest, Romulus, MI, USA)	Worker	mirVana miRNA Isolation kit with phenol (Ambion)	TapeStation (Agilent) – USU Center for Integrated Biosystems	University of Illinois Roy J. Carver Biotech Center	Illumina TruSeq Small RNA Sample Preparation kit	18,173,120	11,147,865 (61.3%)
<i>Megalopta genalis</i>	Barro Colorado Island, Panama [‡]	Lab-reared female					17,273,381	14,459,264 (83.7%)
<i>Nomia melanderi</i>	Touchet, WA, USA	Reproductive female					21,916,316	16,681,729 (76.1%)
<i>Apis mellifera</i>	Urbana-Champaign, IL; Tyson Research Station, St. Louis, MO, USA	Worker	TRIzol reagent (Thermo Fisher Scientific)	Bioanalyzer (Agilent) - Washington University Genome Technologies Access Center	Washington University Genome Tech Access Center	Illumina TruSeq, Clontech SMARTer small RNA library kit	12,793,471	7,638,748 (59.7%)
<i>Bombus terrestris</i>	Commercial colony (Pollination Services Yad-Mordechai, Kibbutz Yad-Mordechai, Israel)	Worker					16,270,644	11,205,739 (68.9%)
<i>Megachile rotundata</i>	Logan, UT, USA	Reproductive female					19,160,796	15,357,334 (80.1%)

[‡]*M. genalis* samples were exported under permit SEX/A-37-15

Table S3. Known microRNAs used in miRDeep2 microRNA detection protocol.

Species	Source	Reference
<i>Apis mellifera</i>	miRBase v21	Kozomara and Griffiths-Jones 2014
<i>Drosophila melanogaster</i>		
<i>Nasonia vitripennis</i>		
<i>Tribolium castenum</i>		
<i>Bombyx mori</i>		
<i>Apis mellifera</i>	Small RNA sequencing	Ashby et al. 2016 (Table S1)

Table S4. Gene models used for localization of microRNAs and predicted target analysis.

Species	Genome annotation	Reference
<i>Apis mellifera</i>	Amel OGS v3.2	Elsik et al. 2014
<i>Bombus impatiens</i>	Bimp OGS v1.0	Elsik et al. 2016
<i>Bombus terrestris</i>	Bter v1.3	Sadd et al. 2015
<i>Megalopta genalis</i>	Mgen v1.0	Kapheim et al. 2020
<i>Megachile rotundata</i>	Mrot v1.1	Kapheim et al. 2015
<i>Nomia melanderi</i>	Nmel v1.0	Kapheim et al. 2019

Table S5 (separate file). Enrichment results for predicted targets of lineage-specific miRs and “social genes”. Includes gene lists, conversion lists based on reciprocal blastp results, and overlap test statistics. Column head descriptions are in ‘ColumnDetails’ sheet.

Table S6 (separate file). Results from genome scans for miRNA seed matches in Rfam. Descriptions for each sheet and column header are provided in the ‘Metadata’ sheet.

Table S7 (separate file). Final miRNA sets for each species. Descriptions for each sheet and column header are provided in the ‘Metadata’ sheet.

Table S8 (separate file). Predicted targets and orthogroup ages of lineage-specific microRNAs in each species. Descriptions for each sheet and column header are provided in the ‘Metadata’ sheet.

Figure S1 (separate file). Predicted targets of lineage-specific miRNAs in relation to social behavior. Genes that are both predicted targets of lineage-specific miRNAs and genes with differential expression in a social context (solid outlines) or genes under selection (dashed outlines) are represented by overlapping circles for each study and species. Numbers of lineage-specific miRNA targets are given for each species. Colors indicate different studies. Overlaps not significantly different from random (representation factor, RF=1) are unlabeled, while significant over- or under-enrichments are marked with asterisks with RF and p-value as indicated.

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