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                                      |
|--------------------------|-----------------|------------------------------------------------|
| *Apis mellifera*         | Amel v4.5       | Elsik et al. 2014                              |
| *Bombus impatiens*       | Bimp v2.0       | Sadd et al. 2015                               |
| *Bombus terrestris*      | Bter v1.0       |                                                 |
| *Megalopta genalis*      | Mgen v1.0       | Kapheim et al., 2020                           |
| *Megachile rotundata*    | Mrot v1.0       | Kapheim et al. 2015                            |
| *Nomia melanderi*        | Nmel v1.0       | Kapheim et al. 2019                            |
| *Apis florea*            | Aflo v1.0       | Baylor College of Medicine, unpublished (NCBI PRJNA45871) |
| *Dufourea novaeangliae*  | Dnov v1.0       | Kapheim et al. 2015                            |
| *Eufriesea mexicana*     | Emex v1.0       | Kapheim et al. 2015                            |
| *Habropoda laboriosa*    | Hlab v1.0       | Kapheim et al. 2015                            |
| *Lasioglossum albipes*   | Lalbi v2        | Kocher et al. 2013                             |
| *Melipona quadrisociata* | 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 |
|------------------|-------------------------------------------------|-------------|------------------------|------------------------|------------------------------------|--------------|---------------|------------------------|
| *Bombus impatiens* | 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%) |
| *Megalopta genalis* | Barro Colorado Island, Panama*                  | Lab-reared female |            |                        |                                    |              | 17,273,381 | 14,459,264 (83.7%) |
| *Nomia melanderi*  | Touchet, WA, USA                                | Reproductive female |            |                        |                                    |              | 21,916,316 | 16,681,729 (76.1%) |
| *Apis mellifera*   | 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%) |
| *Bombus terrestris* | Commercial colony (Pollination Services Yad-Mordechai, Kibbutz Yad-Mordechai, Israel) | Worker      |            |                        |                                    |              | 16,270,644 | 11,205,739 (68.9%) |
| *Megachile rotundata* | 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                                         |
|-------------------|------------------|---------------------------------------------------|
| *Apis mellifera*  | miRBase v21      | Kozomara and Griffiths-Jones 2014                |
| *Drosophila melanogaster* |               |                                                   |
| *Nasonia vitripennis* |               |                                                   |
| *Tribolium castenum* |               |                                                   |
| *Bombyx mori*     | Small RNA sequencing | Ashby et al. 2016 (Table S1)                   |
| *Apis mellifera*  | 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                                         |
|-------------------|-------------------|---------------------------------------------------|
| *Apis mellifera*  | Amel OGS v3.2     | Elsik et al. 2014                                 |
| *Bombus impatiens*| Bimp OGS v1.0     | Elsik et al. 2016                                 |
| *Bombus terrestris*| Bter v1.3         | Sadd et al. 2015                                 |
| *Megalopta genalis*| Mgen v1.0         | Kapheim et al. 2020                              |
| *Megachile rotundata* | Mrot v1.1         | Kapheim et al. 2015                              |
| *Nomia melanderi* | 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.
REFERENCES
Ashby R, Forêt S, Searle I, Maleszka R. 2016. MicroRNAs in honey bee caste determination. Sci. Rep. 6:1–15.
Elsik CG, Tayal A, Diesh CM, Unni DR, Emery ML, Nguyen HN, Hagen DE. 2016. Hymenoptera Genome Database: integrating genome annotations in HymenopteraMine. Nucleic Acids Res. 44:D793–D800.
Elsik CG, Worley KC, Bennett AK, Beye M, Camara F, Childers CP, de Graaf DC, Debyser G, Deng J, Devreese B, et al. 2014. Finding the missing honey bee genes: Lessons learned from a genome upgrade. BMC Genomics 15:1–29.
Kapheim KM, Pan H, Li C, Blatti C, Harpur BA, Ioannidis P, Jones BM, Kent CF, Ruzzante L, Sloofman L, et al. 2019. Draft genome assembly and population genetics of an agricultural pollinator, the solitary alkali bee (Halictidae: Nomia melanderi). G3. 9:625–634.
Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T, Robertson HM, Hudson ME, Venkat A, Fischman BJ, et al. 2015. Genomic signatures of evolutionary transitions from solitary to group living. Science 348:24–32.
Kapheim KM, Jones BM, Pan H, Harpur BA, Kent CF, Zayed A, Ioannidis P, Waterhouse RW, Kingwell C, Stolle E, Avalos A, Zhang G, McMillan WO, Wcislo WT. 2020. Developmental plasticity shapes social traits and selection in a facultatively eusocial bee. Proc Nat Acad Sci USA. 117:13615-13625.
Kočer SD, Li C, Yang W, Tan H, Yi S V, Yang X, Hoekstra HE, Zhang G, Pierce NE, Yu DW. 2013. The draft genome of a socially polymorphic halictid bee, Lasioglossum albipes. Genome Biol. 14:R142.
Kozomara A, Griffiths-Jones S. 2014. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 42:D68-73.
Sadd BM, Barribeau SM, Bloch G, de Graaf DC, Dearden P, Elsik CG, Gadau J, Grimmelekhuijzen CJ, Hasselmann M, Lozier JD, et al. 2015. The genomes of two key bumblebee species with primitive eusocial organization. Genome Biol. 16:76.