Characterization of sex-biased genes from the transcriptome of a male-dimorphic mite

Michał T. Stuglik[1], Wiesław Babik[1], Jacek Radwan[2]

[1]Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland
[2]Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Introduction & aim

The different sexes experience different selective pressures, which can lead to highly divergent phenotypes [Fig. 1] that are achieved via sex-biased gene expression. As a result of sexual selection and sexual conflict, sex-biased genes are expected to evolve at a faster rate than other genes in the genome. Furthermore, because sexual selection acts more strongly on males, male-biased genes are expected to evolve faster than female-biased genes.

We aimed to test these predictions in a model species for sexual selection and conflict research, the bulb mite Rhizoglyphus robini (Acari, Acaridae) [Fig. 1] , in which armored, aggressive fighter males coexist with unarmored scrambler males.

Fig. 1A. Fighter.

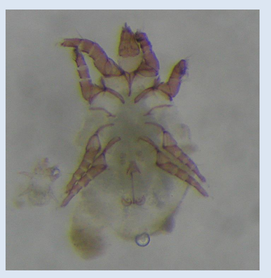

Fig. 1B. Scrambler.

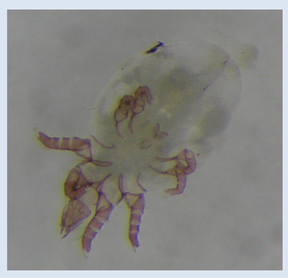

Fig. 1C. Female.

Photos: Ania Skrzynecka

materials & methods

* RNA was extracted from adult females, two male morphs (fighters & scramblers) and tritonymphs in about 100 individuals in each group. Sequencing libraries were prepared from polyA+ RNA with the TrueSeq RNA kit and 2 x 100 bp paired-end (PE) sequencing was performed in a single Illumina HiSeq2000 lane.
* Paired-end reads were cleaned, de-novo assembled and transformed into transcriptome-based gene models (TGMs) [Fig. 2] .
* Predicted likely coding sequences (ORFs) were identified in the TGM database.
* Gene expression analysis: for each sample, reads were mapped to TGMs with Bowtie (Langmead et al., 2009), then transcript abundance was estimated by RSEM (Li & Dewey, 2011). Next, we identified and analyzed differentially expressed genes and performed all pairwise comparisons between females, fighters, and scramblers, using edgeR (Robinson et al., 2010), with TMM normalization, which accounts for differences between samples in the number of reads.
* Amino acid distance (dA) between T. urticae and R. robini orthologous sequences were measured as the rate of evolution of protein-coding sequences.

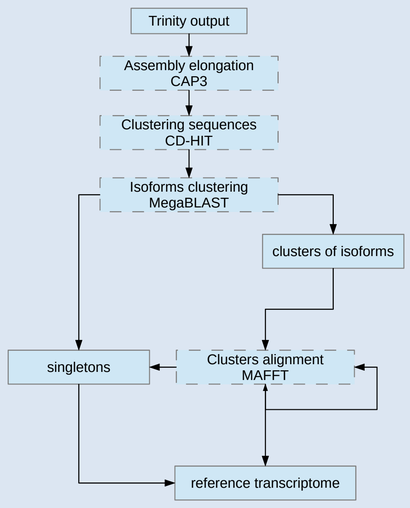

Fig. 2. Pipeline for the construction of transcriptome-based gene models (TGM).

acknowledgements

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results

* 134.3 million of paired-end reads were assembled into 114,456 transcriptome-based gene models (TGMs) (including protein coding and non-coding expressed sequences).
* Expression of 4.0% of TGMs was male-biased, whereas 1.3% of TGMs had female-biased expression and this difference was highly significant [Fig 3, Fig 4].
* TGMs that were overexpressed in one, but not both male morphs (compared to expression in females), were more biased genes in fighters (2.4% of all TGMs) than in scramblers (0.5%). The genes that differed in expression level between male morphs constituted only 0.26% of all TGMs [Fig 3].
* For TGMs with protein-coding capacity, the proportion with orthologs identified in the spider mite (Tetranychus urticae) genome was lower for male-biased than for unbiased genes, but was very similar for female-biased and unbiased genes.

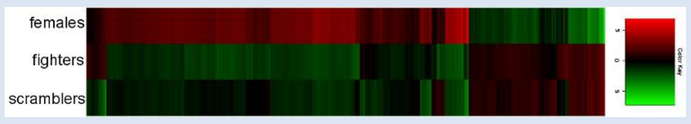

Fig. 4. Heat map showing genes with significantly different expression levels in females, fighter males, and scrambler males in R. robini.

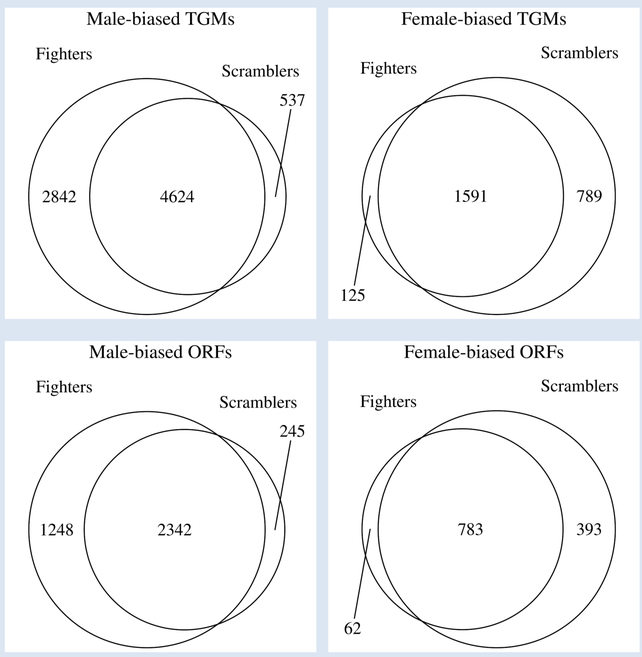

Fig. 3. Venn diagram of TGMs (upper graphs) and TGMs containing ORFs (lower graphs with higher expression in males (fighters only, scramblers only, both male morphs) in comparison to females (left) and lower expression in males (fighters only, scramblers only, both male morphs) in comparison to females (right).

conclusions

1) Comparison of male and female transcriptomes in a species expressing alternative mating tactics provided support for the hypothesis that dimorphism driven by sexual selection is responsible for sex-bias in expression of a significant part of the bulb mite genome.
2) Our data are consistent with those reported for other species in showing that male-biased genes evolved at a faster rate than female-biased genes. This faster evolution was reflected both in a higher gene-turnover rate and amino acid substitution rate.
3) Fighter-only-biased genes showed different patterns of evolution than male-biased and scrambler-only-biased genes, in that a lower fraction of fighter-only-biased genes had orthologs in T. urticae, but these orthologs were characterized by lower amino acid divergence. This suggest that genes associated with the distinct morphology, behavior and physiology of fighter males evolve in different way compared to genes which are involved in processes common to both morphs, such as sperm competition, possibly because the latter are involved in inter-locus conflict, resulting in continual evolution of male-biased genes.