

Polymorphic house fly male-determining proto-Y chromosomes affect male behaviors in ways that are consistent with their distributions in natural populations

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BACKGROUND

Sex determination - developmental process by which genetic differences or environmental cues initiate sexually dimorphic phenotypes between males and females.

Evolutionary transitions in sex determination pathway are often accompanied by evolutionary turnover of the sex chromosomes.

Understanding the factors that drive transitions in sex determination and turnover of sex chromosomes has been a long-standing goal in evolutionary biology.

House fly, *Musca domestica*, is a well-suited model to those ends because a male-determining locus can be found on all six chromosomes.

Polygenic sex determination in house fly has been maintained as a stable polymorphism along a latitudinal cline since at least the mid-20th century between the two most common genotypes, in which the proto-Y chromosome is either the third (**III^M**) or Y (**Y^M**) chromosome.

Examining general differences in gene expression profile between III^M and Y^M male genotypes can help identify specific phenotypes under selection.

Previous research revealed mating advantages for III^M males (Hamm et al. 2009), but the relative roles of temperature and inter- vs. intra-sexual mating dynamics in shaping this outcome are unknown.

OBJECTIVE

Use gene expression data and conduct behavior trials to identify phenotypes that may contribute to stable polymorphism between III^M and Y^M male proto-Y chromosome genotypes in house fly.

RESULTS

Y^M male house flies show upregulation of a large number of obp56h genes

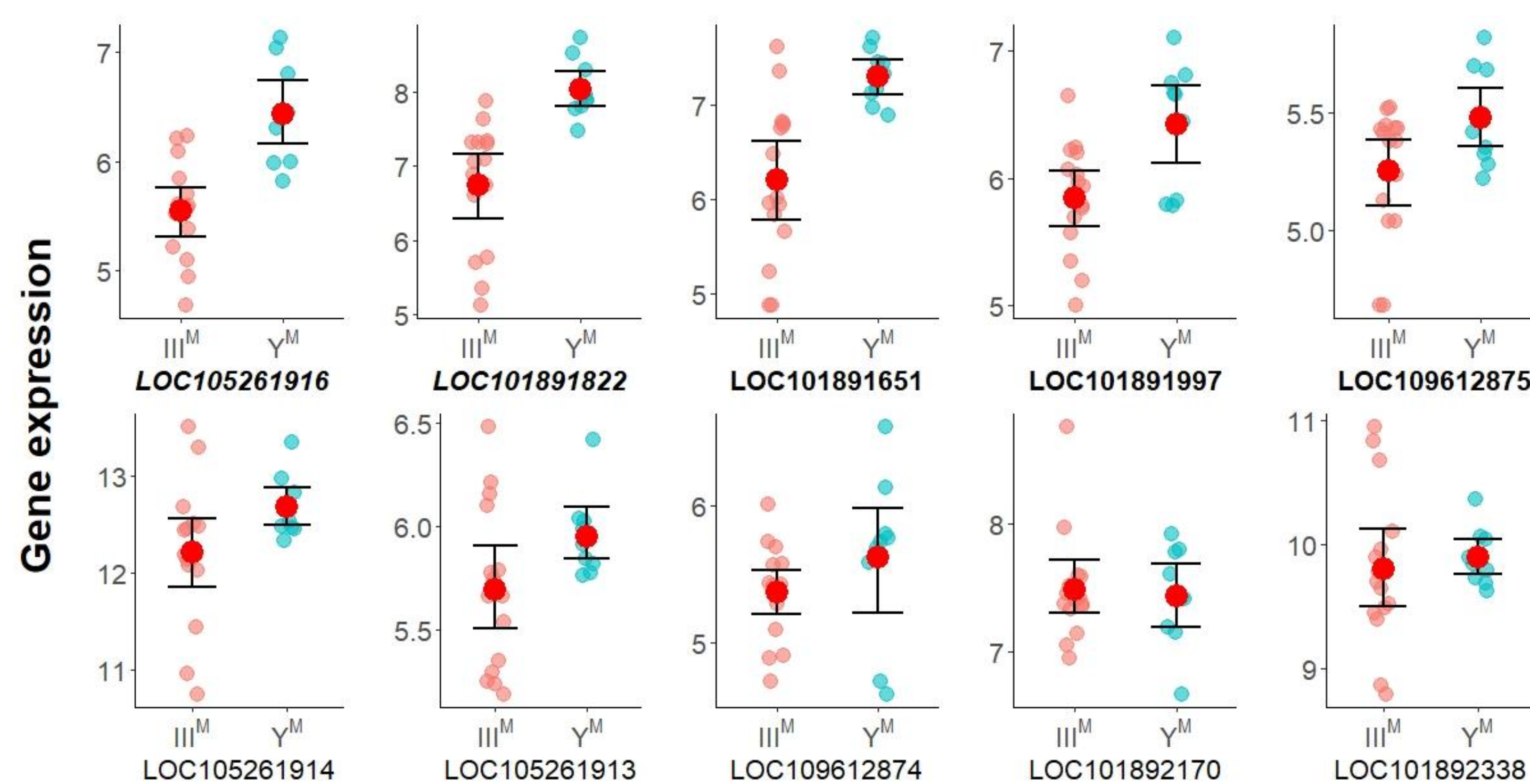


Fig 1 - Expression of *Obp56h* gene family according to male genotype (III^M v Y^M) and gene. Solid red data points and error bars denote means and standard error of mean. Bold italic label : significant difference ($p < 0.05$) between male types after FDR adjustment. Bold : significant before FDR adjustment.

- **Meta-analysis** on previously published RNA-Seq data from male house fly heads (Meisel et al. 2015 and Son et al. 2019, $N = 9$ Y^M samples, 15 III^M samples). Data transformation (DESeq2) → batch effects correction (sva) → Differential expression (limma, FDR < 0.05).
- 45 differentially expressed (DE) genes between III^M and Y^M males.
- Two members of the *obp56h* gene family were upregulated in Y^M males. Upon further inspection, we found general upregulation of nearly the entire gene family in Y^M males (Fig. 1).
- *Obp56h* gene family experienced rapid evolutionary expansion in muscid flies compared to fruit flies (Olafson et al. 2019, Fig. 2).
- In *Drosophila melanogaster*, knockdown of *obp56h* reduces copulation latency in males (Shorter et al. 2016).

House flies have an expanded obp56h gene family relative to D. melanogaster

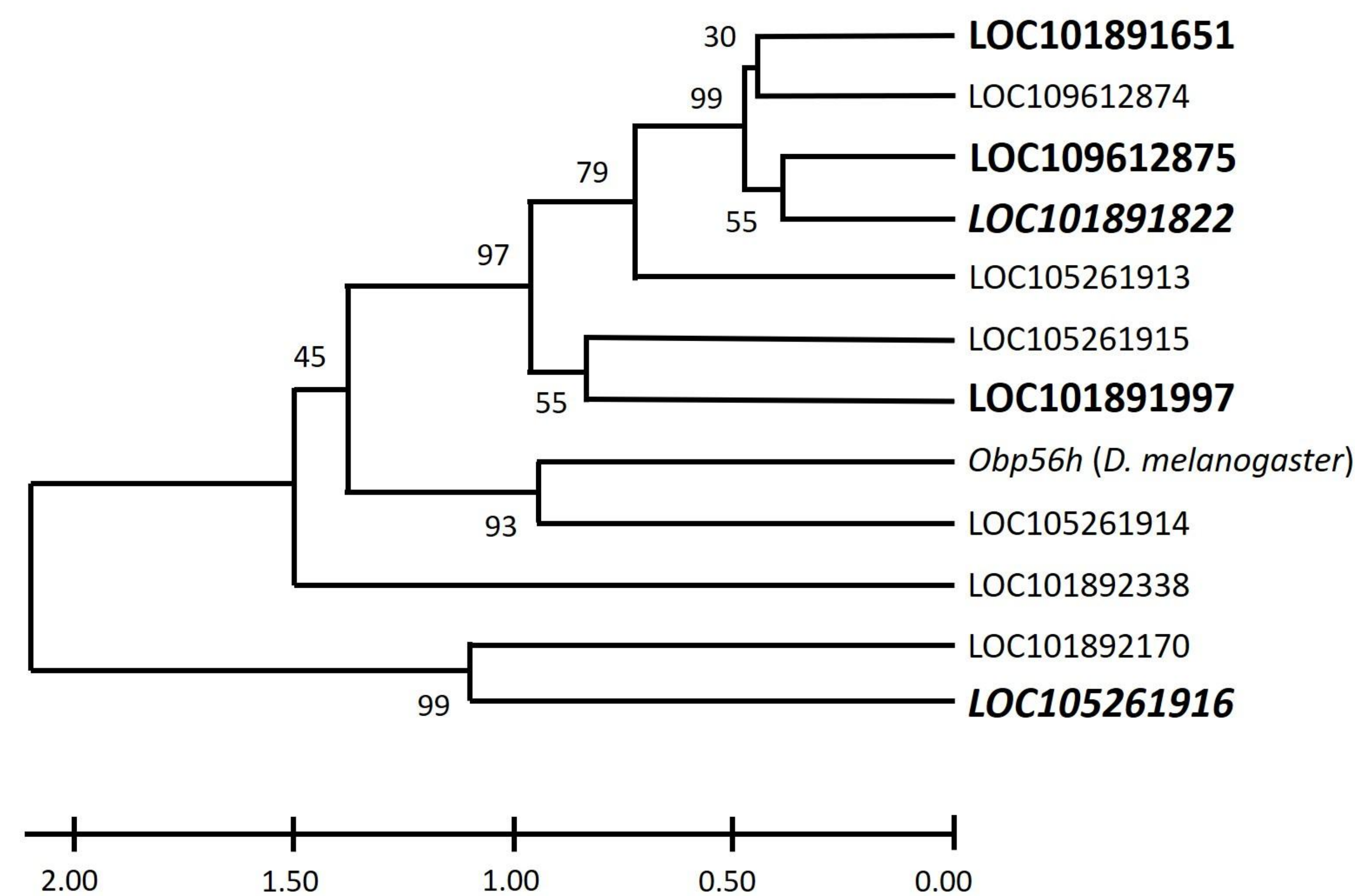


Fig 2 - Unrooted phylogenetic tree of the *Obp56h* gene family in *M. domestica* and *D. melanogaster* based on protein sequences. Genes are identified based on gene IDs (Bold: $p < 0.05$ before correction for multiple comparisons, Bold italic: adjusted $p < 0.05$). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown at each node. Branch lengths and scale are in the units of number of amino acid substitutions per site. Evolutionary analysis conducted in MEGA X.

Obp56h expression correlates predictably with male mating behavior between male genotypes

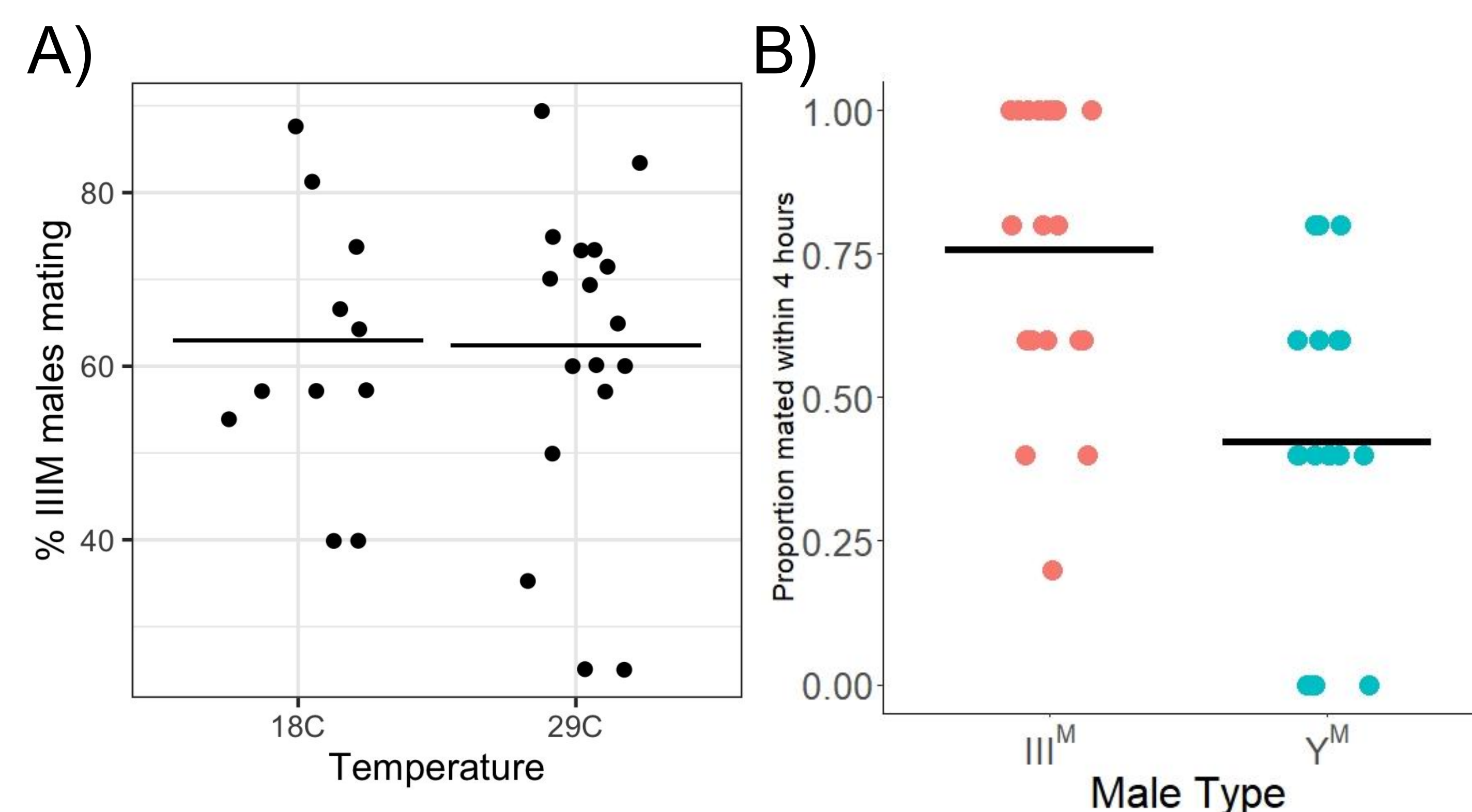


Fig 3 - Summary of differential mating behaviors between III^M and Y^M male house flies. A) Outcomes of competitive mating assays between III^M ($N = 11$ trials) and Y^M males at 18 °C ($N = 11$ trials) and 29 °C ($N = 17$ trials). Data points represent experimental batches. Horizontal lines denote the median. B) Outcomes of single-choice mating assays. Proportion mated refers to the proportion of males that mated with females within 4 hours within each experimental trial. Males are grouped according to group (III^M : $N = 19$ trials), Y^M : $N = 18$ trials). Lines denote means within male group.

- III^M males, which experience lower *obp56h* levels on average, outcompete Y^M males for mates (Fig. 3A), regardless of rearing temperature.
- This mating advantage is at least partly influenced by their ability to mate more quickly with females (Fig. 3B).
- Results mirror previous study showing that *obp56h* knockdown reduces copulation latency in male *D. melanogaster* (Shorter et al. 2016).

Obp56a plays an important role in the regulation of gene modules that are differentially expressed between male genotypes

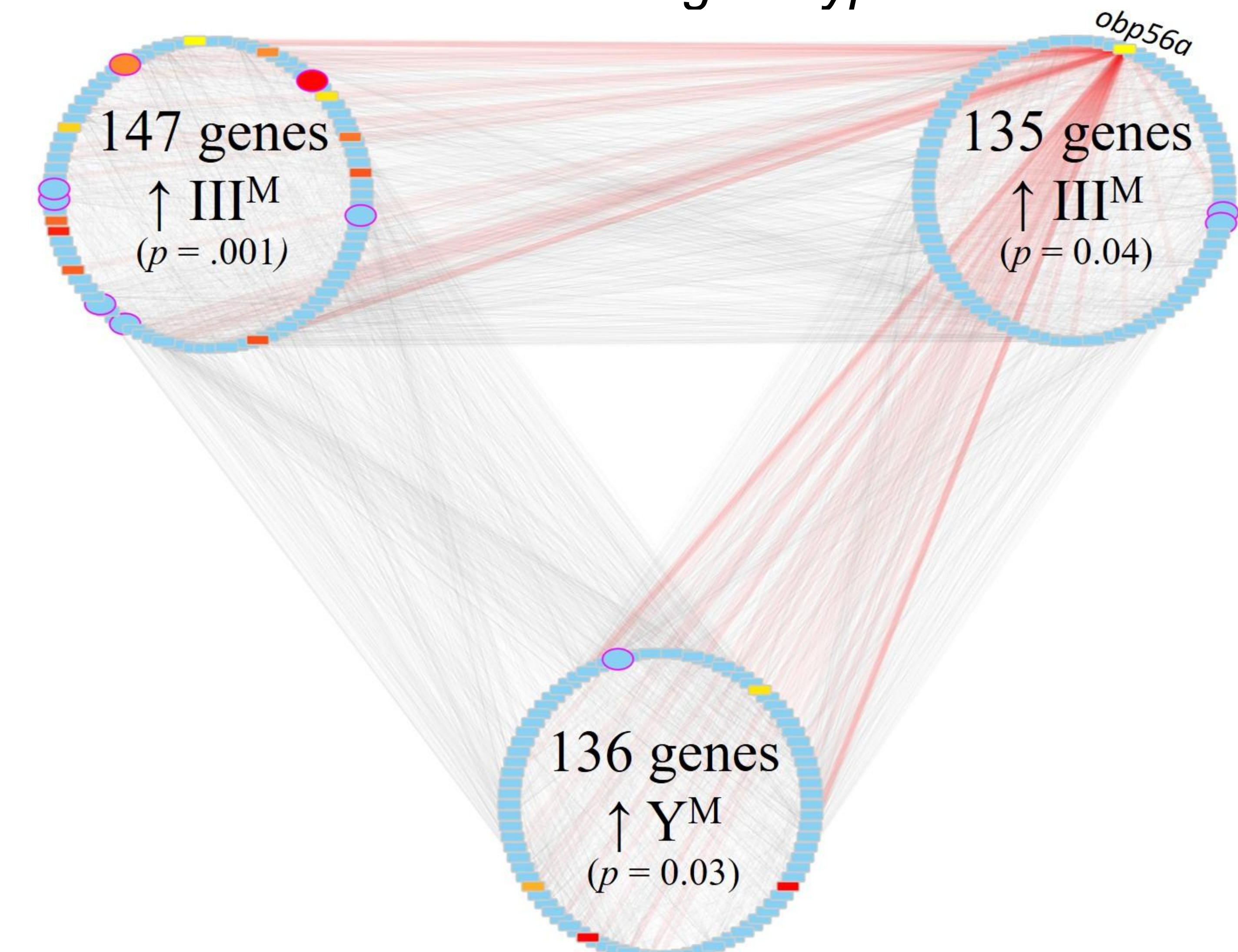


Fig 4 - Coexpression network overview of modules significantly associated with a given male type. Each circle represents a given module, and the cells along each module's border represents a gene. Ellipsoid cells with purple borders denote genes within the most enriched GO biological process (long-chain fatty acid metabolic process). Lines represent edge connections between genes (connections with the most highly connected or “hub” gene, *Obp56a*, highlighted in red). Genes found to be differentially expressed between male types in limma analysis are filled with colors following a continuum from yellow ($p = 0.05$) to red ($p < 0.001$).

- Using weighted gene co-expression network analysis (Langfelder & Horvath 2005), we identified 25 modules of highly covaried genes, 3 of which were significantly differentially expressed, on average, between genotypes (Fig. 4).
- Modules were enriched for genes found to be differentially expressed between genotypes and had similar functional roles based on GO analysis.
- *Obp56a* was identified as the most central gene based on number and strength of among-gene connections within the modules.

CONCLUSIONS

- Differential expression results highlight *Obp56h* as a candidate family of genes under differential selection between male genotypes.
- WGCNA further implicated odorant-binding proteins as targets of selection, as *obp56a* was identified as a “hub” gene.
- As in *D. melanogaster*, *obp56h* may modulate male copulation latency in *M. domestica*, a trait that differs according to male proto-Y chromosome genotype.
- Lower copulation latency in III^M male house flies may contribute to their ability to directly outcompete Y^M males for mates, regardless of rearing temperature.
- The differences in mating behaviors and gene expression described here may contribute to the maintenance of these two male genotypes in nature, although further research is needed.

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