Identifying gene regulatory interactions associated with hybrid male sterility in *Drosophila pseudoobscura*

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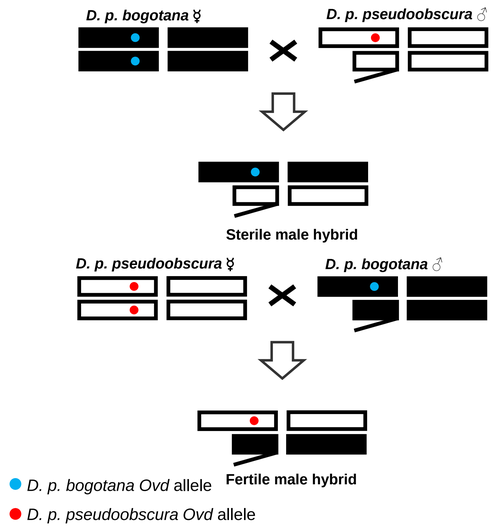
BACKGROUND

Incompatibilities between divergent genomes lead to reproductive barriers that promote speciation. The *Drosophila pseudoobscura* subspecies pair is representative of the earliest stages of speciation and is a useful system in understanding the genetics leading to it. They exhibit unidirectional hybrid male sterility:

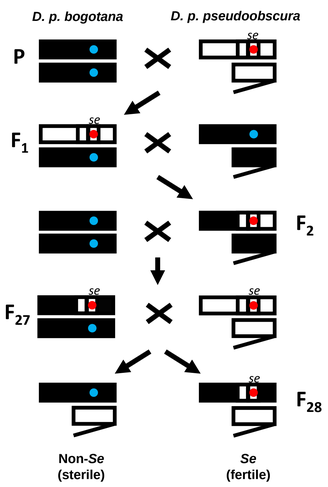


Hybrid male sterility is due to X-autosomal incompatibilities. *Overdrive* (*Ovd*) has a major contribution and is found within the X chromosome, this gene is predicted to have a DNA binding domain making it a possible transcription factor[1].

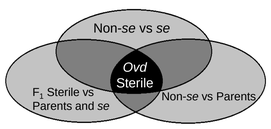
Objective: To identify the set of genes whose expression is regulated by the state of the *Ovd* alleles.

METHODS

To identify the interacting partners of *Ovd*, we took advantage of the fact that *Ovd* is tightly linked to the *sepia* (*se*) eye colour mutation in *D. p. pseudoobscura*[1]. This allowed us to replace the sterile *D. p. bogotana* allele with the fertile *D. p. pseudoobscura* one using the introgression design below:



Triplicate samples of RNA were extracted from the testes of the parental subspecies, F_1 sterile male hybrids, *se* males and non-*se* males. Differential expression analysis was performed using DESeq2 and edgeR. Results were filtered using a log fold change threshold of 1 and significance (FDR corrected $p < 0.05$).



The overlap of all differentially expressed genes from these comparisons are targets of *Ovd* related to sterility.

RESULTS

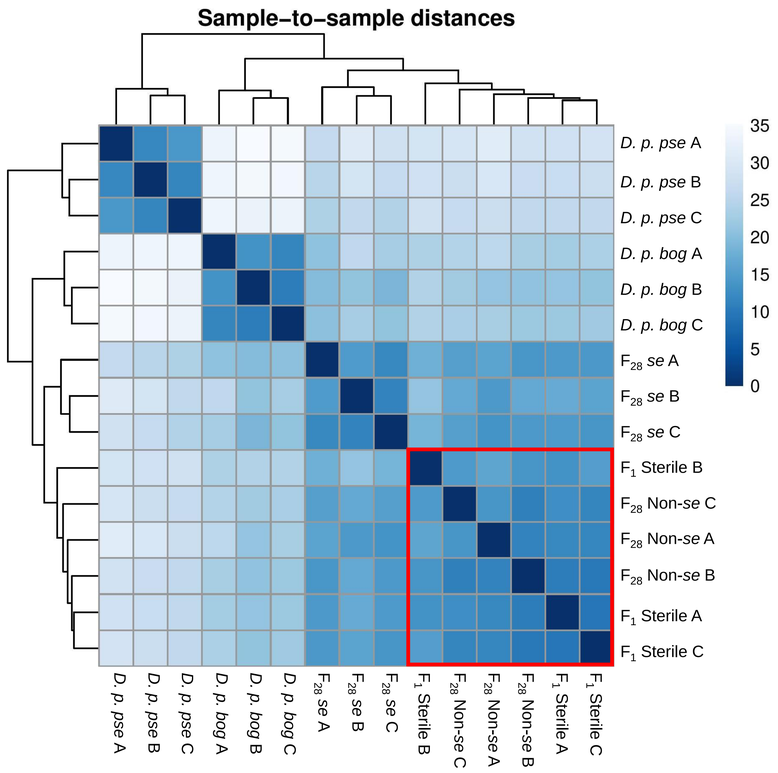


Figure 1: DESeq2 sample clustering based on normalised data. The replicates from the parental subspecies as well as *se* males cluster closely with each other while replicates from the sterile samples (F_1 sterile male hybrids and non-*se* males) form one big cluster.

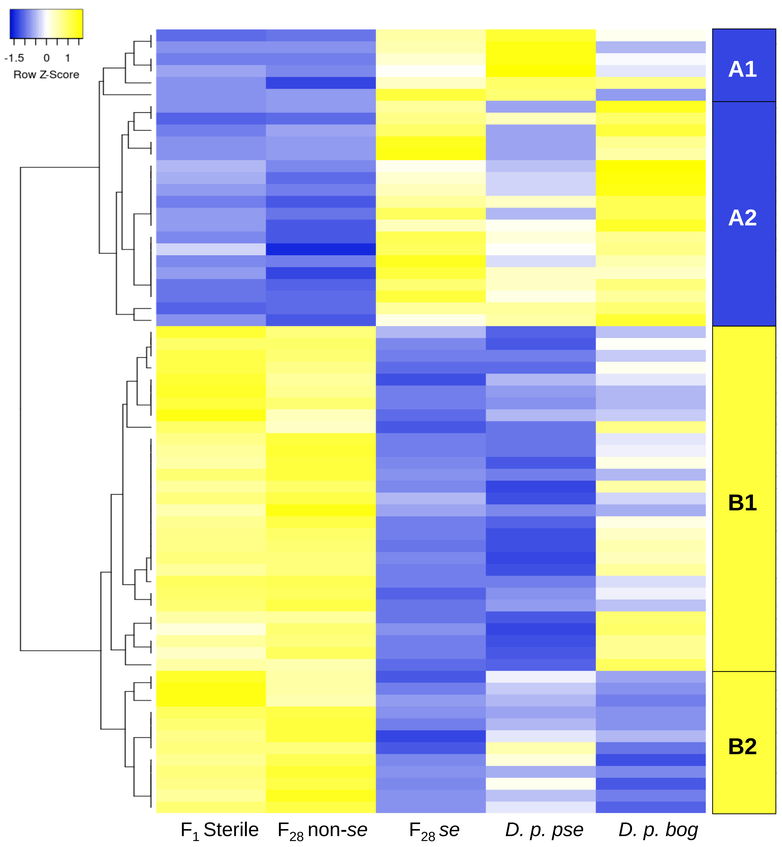


Figure 3: Expression correlation of the 66 targets of *Ovd* across all samples. The two major clusters (A and B) are based on under- and over- expression of the sterile samples relative to the fertile samples. Two sub-clusters (1 and 2) based on differential expression between the parental subspecies and F_{28} *se* are within the main clusters. The proportion of sterility targets of *Ovd* in each sub-cluster are: A1 (17%), A2 (16%), B1 (10%), and B2 (25%).

Table 1: Number of differentially expressed genes for each of the pairwise comparisons from both edgeR and DESeq2 along with the number of consensus genes from the two tools.

Pairwise comparison	edgeR	DESeq2	Consensus
D. p. bogotana - *D. p. pseudoobscura*	1720	1098	1096
D. p. bogotana - F_1 sterile male hybrids	703	357	357
D. p. bogotana - F_{28} *se* male hybrids	544	278	277
D. p. bogotana - F_{28} non-*se* male hybrids	685	375	374
D. p. pseudoobscura - F_1 sterile male hybrids	1115	546	546
D. p. pseudoobscura - F_{28} *se* male hybrids	935	452	452
D. p. pseudoobscura - F_{28} non-*se* male hybrids	1142	572	572
F_1 sterile male hybrids - F_{28} *se* male hybrids	125	58	58
F_1 sterile male hybrids - F_{28} non-*se* male hybrids	1	2	1
F_{28} *se* male hybrids - F_{28} non-*se* male hybrids	110	66	66

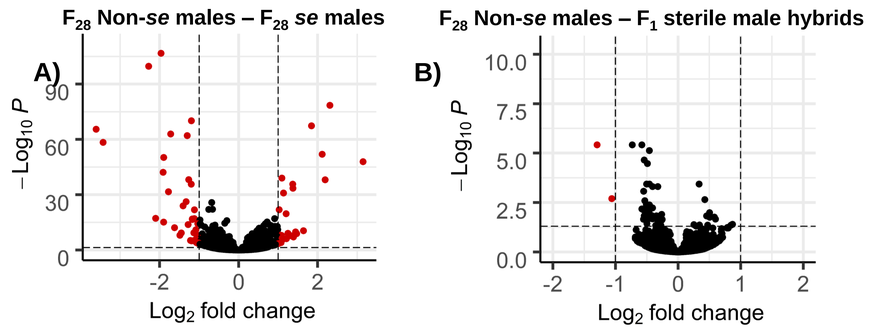


Figure 2: Volcano plot based on DESeq2 results for the comparisons between A) non-*se* vs *se* males. This comparison shows all the genes whose expression is regulated by the state of the *Ovd* alleles. These are potential *Ovd* targets. B) non-*se* vs F_1 sterile male hybrids: shows that after 28 generations of backcrosses the non-*se* males are now nearly identical to the F_1 sterile male hybrids.

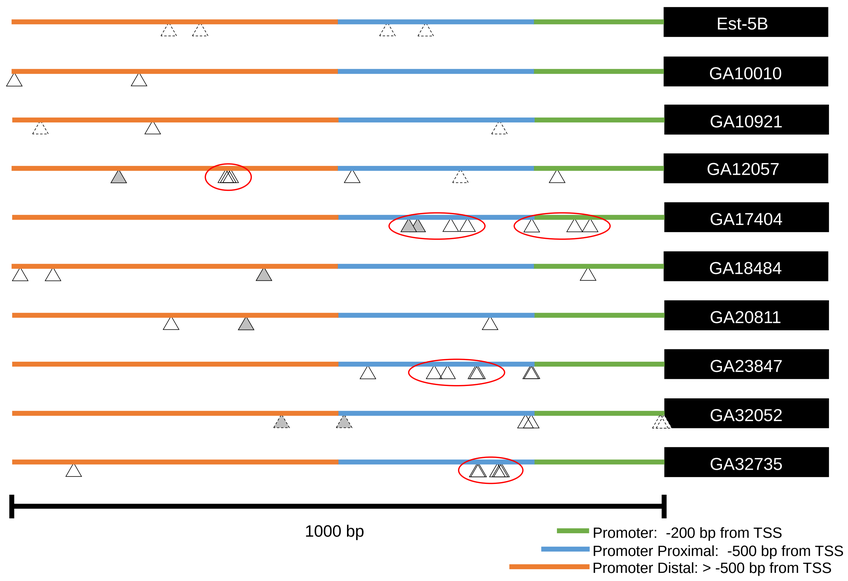


Figure 4: 43 *D. p. pseudoobscura* strains[2] were used to examine polymorphisms and substitutions 1000 bp upstream of the transcription start site of the sterility *Ovd* targets. Δ represent the relative positions of the fixed substitutions between *D. p. bogotana* and *D. p. pseudoobscura*. Grey Δ show *D. p. bogotana* allele frequency of less than 5% while those with dotted lines reflect the scarcity of *D. p. pseudoobscura* sequences (n<10). Red circles show clusters of fixed substitution as putative alternative binding sites for *Ovd* alleles.

CONCLUSIONS

- The non-*se* males are roughly equivalent to the F_1 sterile male hybrids. 66 differentially expressed genes between *se* and non-*se* males are potential targets of *Ovd*. 10 of these genes are sterility targets of *Ovd*.
- The 66 targets of *Ovd* group into 4 networks of correlated expression with significant enrichment for genes involved in *proteolysis*, *regulation of proteolysis*, *cell adhesion*, *cuticle development*, and *mitotic cell cycle checkpoint* based on gene ontology.
- The 10 sterility targets of Ovd will be validated by qPCR and clusters of fixed nucleotide changes upstream of these genes are possible Ovd binding sites which can be tested using ChIP-PCR.

REFERENCES

1. Phadnis, N., & Orr, H. A. (2009). A single gene causes both male sterility and segregation distortion in Drosophila hybrids. *science*, *323*(5912), 376-379.
2. Fuller, Z. L., Haynes, G. D., Zhu, D., Batterton, M., Chao, H., Dugan, S., ... & Ongeri, F. (2014). Evidence for stabilizing selection on codon usage in chromosomal rearrangements of Drosophila pseudoobscura. *G3: Genes, Genomes, Genetics*, *4*(12), 2433-2449.