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Resolving the Gene Expression bases for the Convergent Evolution of a Pigmentation Trait

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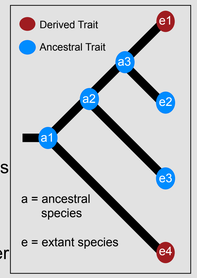
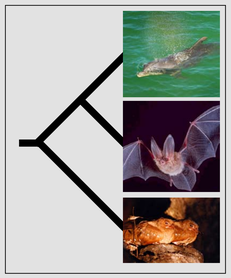
ABSTRACT

The genetic basis by which organisms adapt to an ever changing world remains a topic of great interest to the fields of evolution,development, and conservation biology. It is understood that animal genomes contain over ten thousand genes and distantly related species possess many of the same genes due to common ancestry. What is less well understood is how new traits evolve using these shared genes and whether the genetic basis for evolution favors certain genes over others. At the heart of trait development are genes that encode proteins that regulate the expression of other genes, notably transcription factors and chromatin modifying proteins. Traits can evolve through changes in the expression patterns for these genes or through changes in which target genes they regulate. However, case studies connecting gene expression changes to trait evolution remain few in number. Additionally, it is unclear whether gene expression evolution favors alterations in certain genes over others. In order to understand how a novel trait evolves and to determine whether evolution can prefer certain gene targets for modification, we are studying the convergent evolution of fruit fly pigmentation in the lineages of *Drosophila melanogaster* and *Drosophila funebris*. These two species can be considered biological replicates for the evolution of male-specific pigmentation on the A5 and A6 abdominal segments. To understand the genes involved in the formation and evolution of these similar pigmentation patterns, we are utilizing candidate gene and comparative transcriptomic approaches. Completion of this work will provide novel insights on the genetic changes responsible for a trait's origin,and whether development constrains evolutionary paths to certain genes.

Converging towards similar phenotypes

Convergent evolution refers to instances where similar phenotypic traits evolve in independent lineages. For example, the ability to echolocate evolved indepen--dently in bats, dolphins, and oilbirds (1).





A question that has long captured the interests of geneticists is how many genes are suitable mutational targets for a certain trait to evolve?

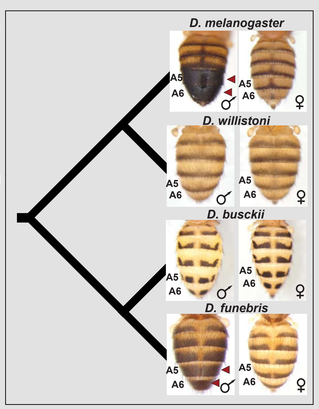
One possible answer is that a large number of genes present equally useful targets for evolutionary change.

A second possible answer is that only one or very few genes are suitable targets for evolution to act upon.

A convergent male-specific pattern of fruit fly pigmentation

One way to distinguishing between these possibilities is by deep genetic investigations into cases of convergent evolution.

Our research utilizes the independent evolution of male-specific pigmentation on the posterior abdominal segments of fruit fly abdomens, including in the lineage of *D. melanogaster* and *D. funebris*.



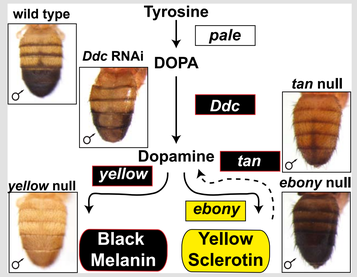
ESTABLISHING THE PIGMENTATION GENE NETWORK IN A MODEL ORGANISM SPECIES

We are using the wealth of genetic tools available in *D. melanogaster* to rigorously characterize the pigmentation pathway and network of transcriptional regulators that make this species male-limited pigmentation.

The *D. melanogaster* pigmentation pathway and the effects of its disruption

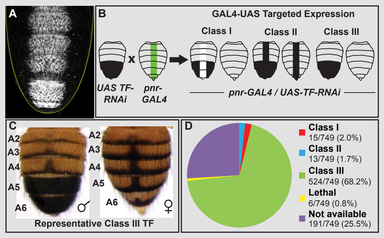
The wild type pattern of black pigmentation requires the expression of the genes *Ddc*, *tan*, and *yellow*, and absence of *ebony* expression.

Disruption of either *Ddc*, *tan* or *yellow* results in reduction/loss of black pigmentation.



Numerous transcription factors populate the regulatory tier of the *D. melanogaster* network

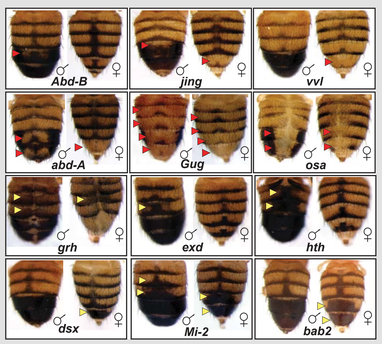
An RNAi screen identified 15 and 13 genes respectively whose suppressed expression resulted in pigmentation reductions and ectopic pigmentation.



RNAi phenotypes for select transcription factors

Red and yellow arrowheads indicate phenotypes implicating TFs in pigmentation suppression and formation respectively.

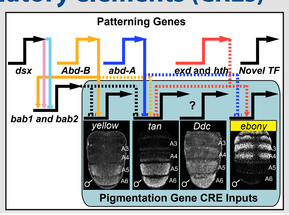
Screen highlights the role of *Hox* genes and *Hox* cofactor genes in patterning male pigmentation and *dsx* and the *bab* genes in repressing pigmentation in females.



Network structure as revealed by transcription factor binding sites within pigmentation gene cis-regulatory elements (CREs)

yellow and *tan* expression is regulated by distinct mechanisms of Hox-regulation (3).

Male-limited pigmentation involves a female-specific regulatory circuit involving *dsx* and the *bab* genes that shuts down enzyme expression.



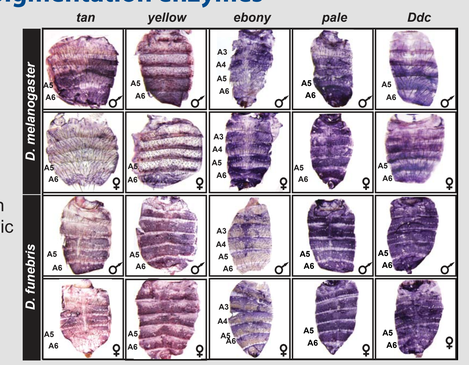
COMPARING THE MODEL NETWORK TO THAT OPERATING IN A NON-MODEL SPECIES WITH A CONVERGENT TRAIT

D. funebris is an easy to culture species that has received little genetic scrutiny to date. We want to know whether male-specific pigmentation evolved by a network similar to that of *D. melanogaster* or one that is highly dissimilar.

Pigmentation convergence involves the similar deployment of key pigmentation enzymes

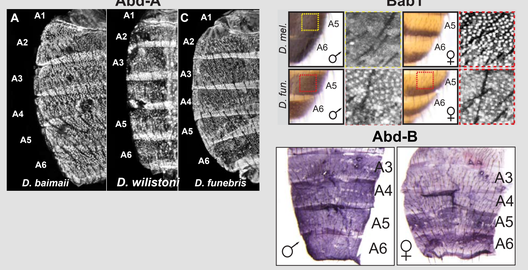
mRNA expression for *tan*, *yellow*, and *ebony* reveal similar sex-specific patterns between *D. melanogaster* and *D. funebris*.

Preliminary data indicates that *pale* expression is monomorphic in both species, but *Ddc* expression is dimorphic in *D. melanogaster*.



Regulatory tier of *D. funebris* network appears to be distinct

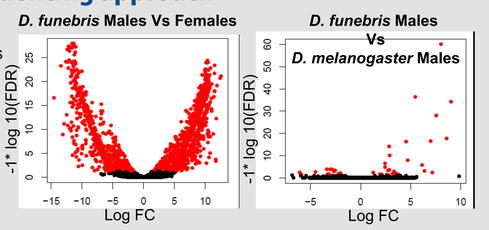
While Abd-A is expressed similarly, the Bab proteins appear to exhibit a monomorphic pattern of expression while Abd-B appears dimorphic.



Finding candidate pigmentation network genes via RNA sequencing approach

RNA was extracted from the epidermis underlying A5 and A6 segment tergites from males and females. RNA-seq libraries were then deep sequenced (Illumina Rapid Paired End 150).

Volcano Plot reveals few genes that are expressed significantly different between tissue from males of the distantly related species.

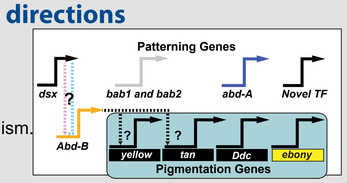


Conlusions and future directions

Inferring the struture of *D. funebris* pigmentation network.

Convergent pigmentation appears to have involved the similar deployment of genes involved in pigment metabolism.

Convergence appears to have co-opted unique loci for generating sexual dimorphism.



While Hox genes seem to be a general part of patterning pigmentation along the A-P axis, how Hox-inputs were integrated differs between genes in the same network and between the convergent networks.

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