Understanding the evolution of a fruit fly pigmentation gene network from the vantage point of the *Ddc* gene

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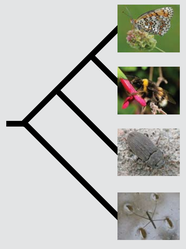
ABSTRACT

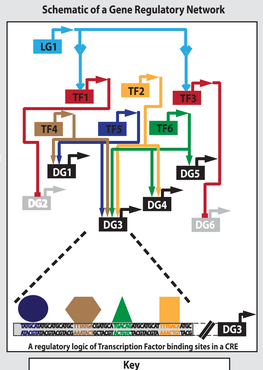
Understanding the genetic and molecular underpinnings for morphological diversity remains a central goal of evolutionary and developmental biology research. While it is now understood that these traits arise by the orchestrated expression of numer--ous genes, a so called gene regulatory network, what remains poorly understood is how these networks of genes and their expression patterns are initially assembled and subsequently diversify. Gene expression is controlled by DNA sequences that are often referred to as cis-regulatory elements (CREs). Each CRE possesses binding sites for transcription factor proteins whose cumulative binding results in a specific pattern of gene expression. It is anticipated that gene expression evolution frequently occurs through the formation, modification, and destruction of CREs, presumably through changes that create or remove binding sites for transcription factor proteins. However the binding site level of CRE evolution has been worked out in very few cases. The fruit fly species *Drosophila melanogaster* has a male specific pattern of abdominal pigmentation for which the enzyme encoding genes and several of their upstream transcription factor regulators are known. However, the details of how these regulators interact with CREs remain largely uncharacteri-zed. One such enzyme gene that is necessary for this species' pattern of pigmentat ion is *Dopa decaboxylase (Ddc)*. Here we share the results of our efforts to uncover the CRE-basis this gene's expression pattern, and how this regulation and pattern of expression has evolved during the origin and diversification of this male-specific trait. Success here will advance a leading model for the CRE and gene network basis for morphological diversity.

Gene Networks and CREs in Animal Development and its Evolution



Body plans and body plans features are the outcomes of networks of genes whose expression are controlled by transcription factors that ultimately illicit the expression of differentiation genes.

Expression is encoded in the combination of binding sites for transcription factors in *cis*-regulatory elements (CREs).



A central finding of evo-devo research has been that animal diversity has evolved around genomes that share a strikingly similar inventory of genes.

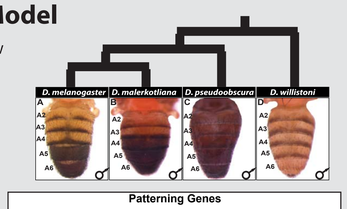
Often what has changed is the manner in which these conserved genes are expressed.

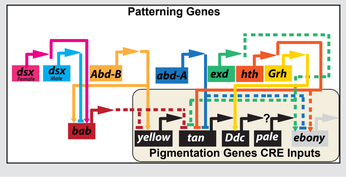
Fruit Fly Pigmentation as a Network and CRE Evolutionary Model

A major challenge to evo-devo research is to understand how gene expression evolves at the level of CREs.

A convenient trait to study is the gain of male tergite pigmentation in the lineage of *D. melanogaster* and its close relatives.

The network of patterning and pigmentation enzyme genes has received considerable attention, resulting in connections being found in the CREs of key genes such as *yellow* and *tan*. However, it remains unclear as to whether these CREs evolved *de novo* or through co-option of existing CREs.

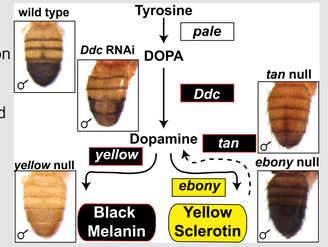




Dopa Decarboxylase (Ddc) is Essential for Male Tergite Pigmentation

While the mutant pigmentation phenotypes of *yellow*, *tan*, and *ebony* have received considerable attention, other pigmentation genes such as *Ddc* has received far less attention.

RNA-interference of *Ddc* in the abdomen midline region resulted in a dramatic reduction in black pigmentation. Thus, *Ddc* appears as important to pigmentation as *yellow* and *tan*.

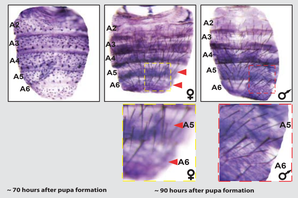


The Spatial, Temporal, and Sex-Specific Expression Pattern of *Ddc*

Ddc expression can be prominently seen in the developing bristle cells ~70 hours into pupal development.

Towards the end of pupal development, ~95 hours, expression has shifted to the epidermal cells underlying the tergite regions that will be colored black.

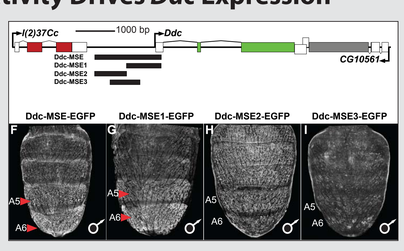
Preliminary data indicates that expression is expanded in the male A5 and A6 segments compared to females.



A CRE with Male-Specific Activity Drives *Ddc* Expression

We identified the upstream region of Ddc exon 1 (Ddc-MSE) as capable of driving a pattern of GFP reporter expression that corresponds with that characterized *in vivo*.

This upstream region includes a distal region (Ddc-MSE2) with enhancer activity spanning the dorsal abdomen, and a promoter-proximal region (Ddc-MSE1) that drives prominent reporter expression in the posterior male A5 and A6 segments.

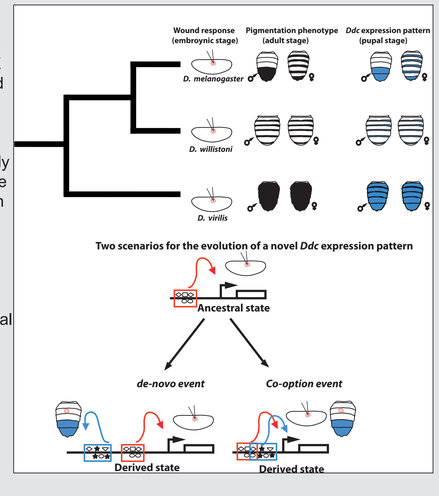


Working Model for the Origin of a Novel Gene Usage

In distantly related fruit fly species *Ddc* expression is induced in response to wounding under the regulation of a wound response CRE proximal to the *Ddc* promoter. This function and CRE can be considered conserved ancestral features.

Male-specific pigmentation evolved more recently in the *D. melanogaster* lineage and it seems the same would be true of the male-specific pattern of *Ddc* expression.

Two evolutionary scenarios can be imagined for the origin of this new pattern of expression. Evolving the CRE activity from scratch or *"de novo"*, or from the "co-option" of an ancestral CRE for a new activity.



Mutational Approach to Find Functional Ddc-MSE1 Sequences

If the Ddc-MSE1 evolved *de novo*, we suspect that it would not share functional sequences with the wound response CRE.

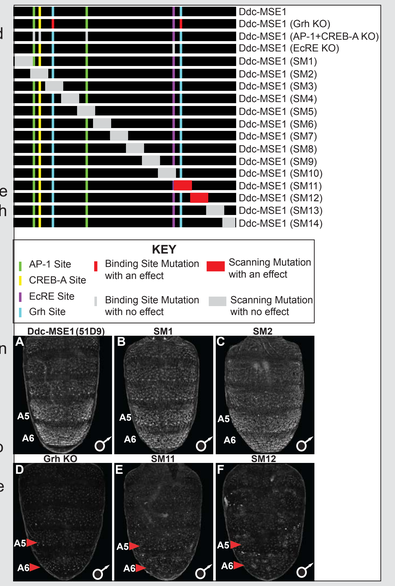
Several transcription factor binding sites had been characterized for the wound response CRE. Most important among these are two sites for the Grh transcription factor.

We created mutant forms of the Ddc-MSE1 that include binding site mutants, and scanning mutants that each possess an ~70 base pair mutation.

The mutant CREs were tested for their ability to direct reporter transgene expression in the male pupal abdomen.

Only 3 of the mutants resulted in dramatic reductions in Ddc-MSE1 activity. Two scanning mutants which narrowed the necessary sequence to an ~150 bp region, and the Grh sites mutant.

This 150 bp region and its Grh site are sequences also required for the wound response activity. Thus, our data supports the co-option model for the origin of the Ddc-MSE1 CRE activity.

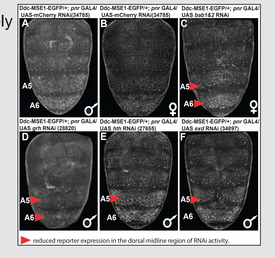


Mapping the Upstream Regulators of the Ddc-MSE1 by an RNAi Approach

Though the sequence necessary for Ddc-MSE1 activity is small, it is likely to involve regulatory inputs in addition to Grh.

We are using an RNAi approach to find the upstream genetic regulators of Ddc-MSE1 activity.

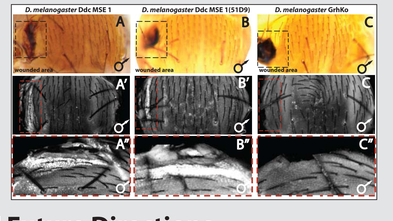
Here RNAi hairpins are expressed along the midline of the pupal abdomen and a change in reporter expression indicates a regulatory relationship. We confirmed a role for *grh* and also implicate regulation by *bab, exd, and hth*.



Tracing the Ancestry of Novel Ddc-MSE1 Activity

In order to trace the ancestry of Ddc-MSE1 activity, we performed a Wound response assay on adult flies. It was found that Ddc MSE1 CRE is activated in response to wounding.

An intact Grh binding site is required for a strong response to wounding as found previously by *Mace et al* (Science 2005).



Conclusions and Future Directions

The novel pattern of male-pigmentation in *D. melanogaster* requires the patterned expression of Ddc, and this expression pattern seemingly evolved from an pleiotropic CRE that functions in response to wounding.

We plan to characterize the expression patterns of *Ddc* in monomorphic species to see if expression corresponds with the pattern of pigmentation.

We plan to compare the activities of orthologous Ddc-MSE1 sequences to better evaluate the relative contributions of CRE sequence and *trans*-regulator evolution.

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ACKNOWLEDGEMENTS

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