

Inspecting the role for the trans-regulatory landscape to the origin, diversification, and loss of a sexually dimorphic fruit fly pigmentation trait

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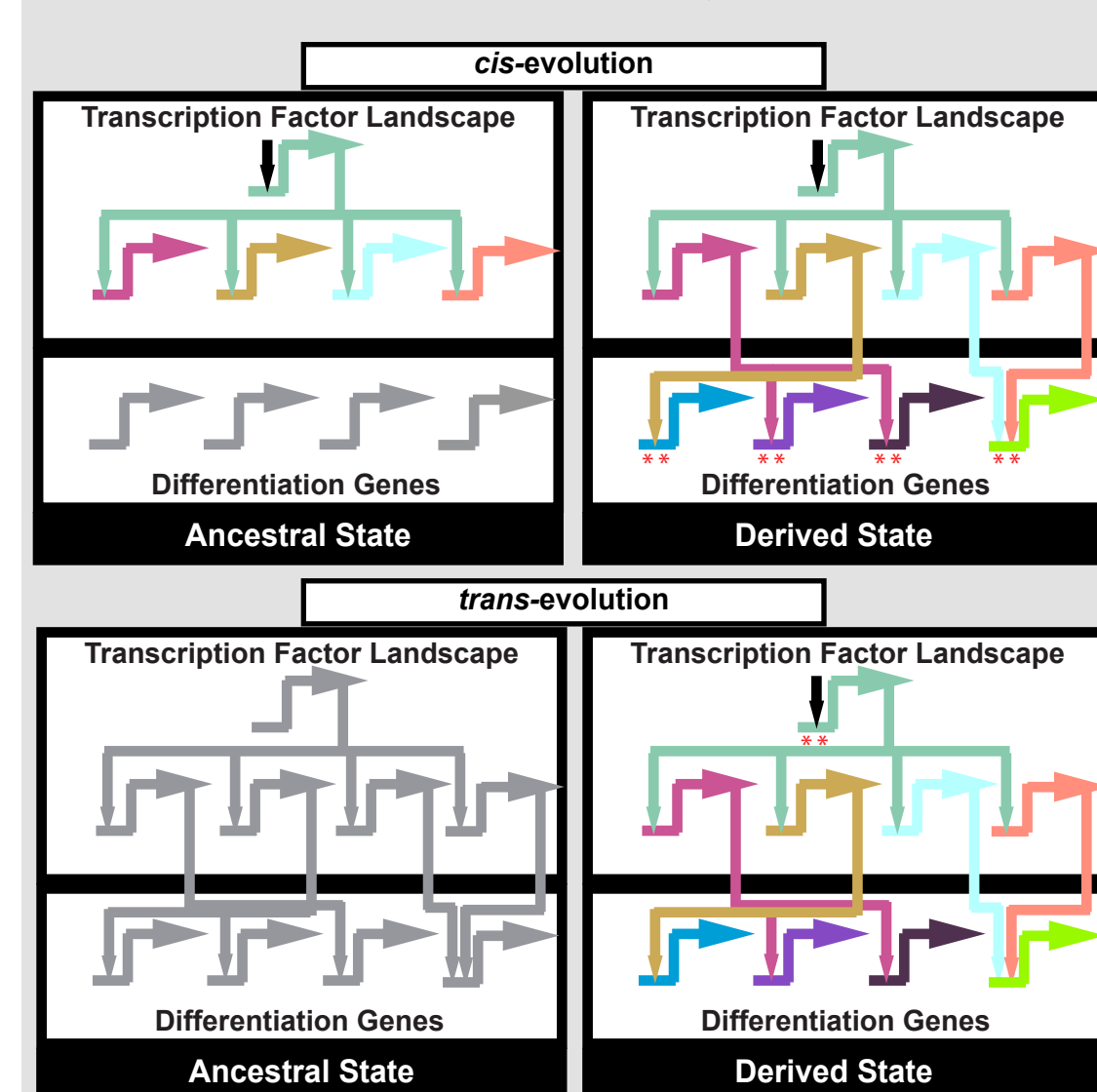
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ABSTRACT

A major goal for evolutionary-developmental biology research is to identify the genetic changes underlying the origins, diversification, and loss of morphological traits. Such traits are built by the spatial and temporal regulation of gene expression, and thus the evolution of gene expression is often involved in their evolutionary histories. Gene expression is under the control of a network of transcription factors (*trans*-landscape) that ultimately impinge on the *cis*-regulatory elements (CREs) of differentiation genes whose encoded proteins produce particular traits. Transcription factor genes are often highly pleiotropic, as they can regulate the expression of multiple genes for multiple traits. Thus, it seems reasonable to expect that evolutionary changes in gene expression more frequently occurred by mutations altering the CREs for differentiation genes than changes to the *trans*-landscape. Our research aims to test whether this expectation for a conserved *trans*-landscape applies to the origin, diversification, and loss of a well-studied fruit fly pigmentation trait in the *Sophophora* subgenus. The origin of a male-specific pattern of abdominal tergite pigmentation involved the gain of CREs controlling the expressions of pigmentation enzyme genes responsive to the prevailing *trans*-landscape of body plan patterning and sexual dimorphism transcription factors. Here, we share our results from tests of these CREs in transgenic hosts that represent the ancestral sexually monomorphic trait, diverse forms of the derived dimorphic trait, and a secondary loss of the dimorphic trait. The outcomes from these tests will reveal the extent to which this particular *trans*-landscape has remained conserved while the pigmentation phenotype has widely evolved. This project has received funding from the National Science Foundation as grant to TMW (IOS-1555906) and a Graduate Research Fellowship to JTH (DGE-1439647).

Gene Regulatory Networks in Trait Development and Evolution



Traits are constructed by orchestrated patterns of gene expression that make up a gene regulatory network (GRN).

- GRNs include a “*trans*-landscape” of transcription factors that drive subordinate differentiation gene expression by interactions with binding sites in “*cis*-regulatory elements” (CREs).

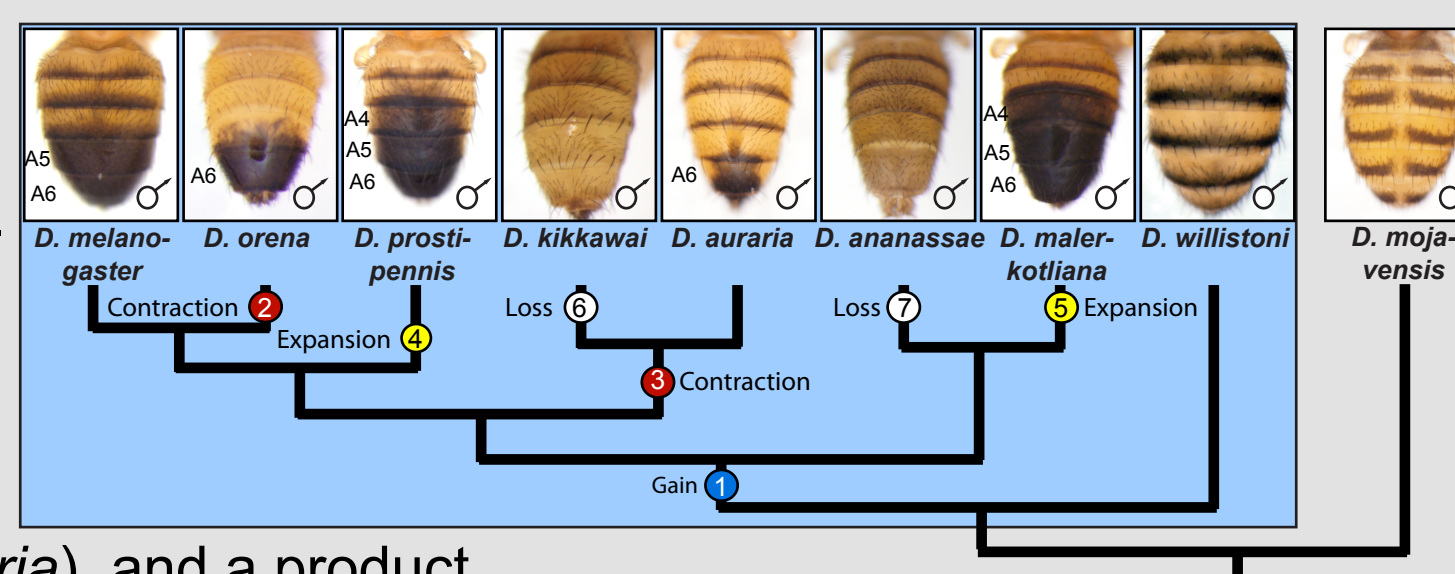
Altered expression for differentiation genes plays a key role in evolution.

- An open question is whether trait origins, modifications, and losses favor changes to the *trans*-landscape (here “*trans*-evolution”) or the CREs controlling differentiation gene expression (here “*cis*-evolution”)

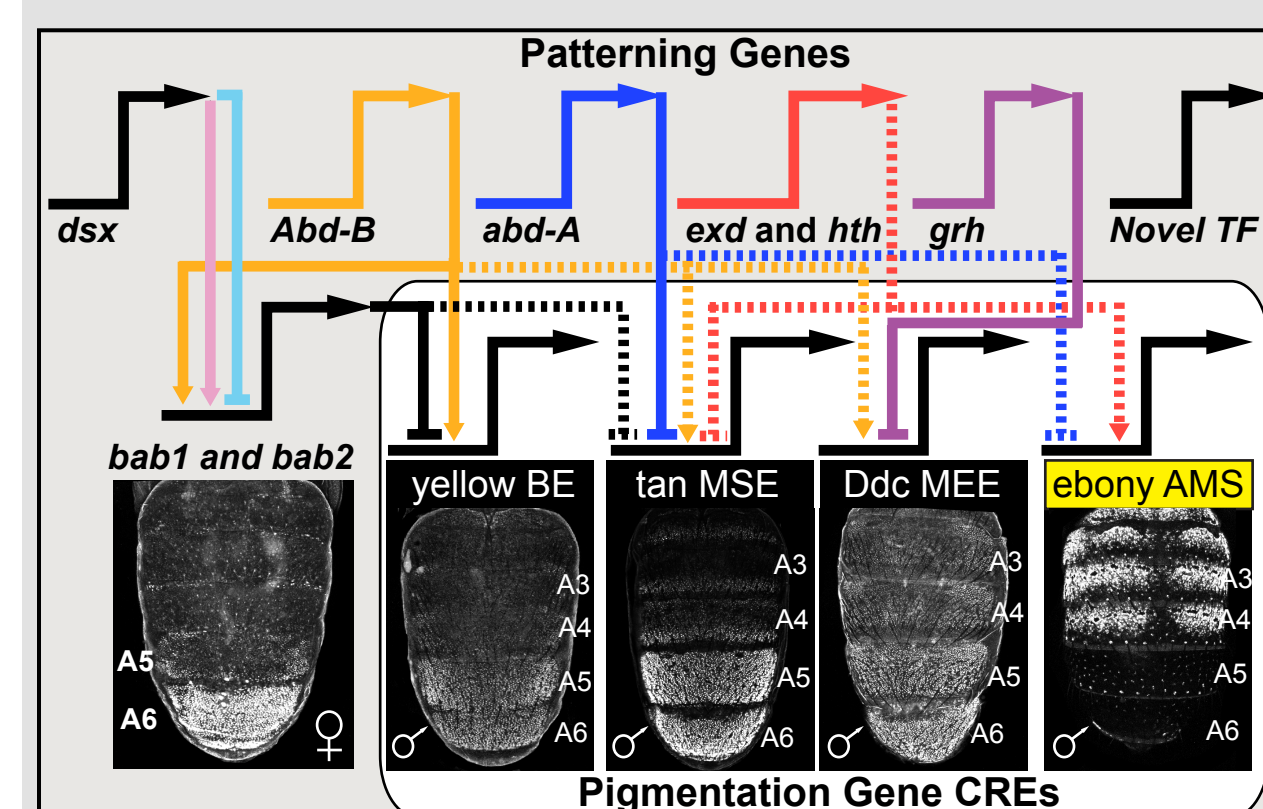
The Gain, Modification, and Loss of Fruit Fly Abdomen Pigmentation Patterns

- The *Sophophora* subgenus provides an evo-devo model in which male-limited tergite pigmentation originated, subsequently modified, and was lost on separate occasions.

- Extant species can be used as a surrogate for the ancestral monomorphic state (*D. willistoni*), variations of the derived dimorphic state (*D. melanogaster* and *D. auraria*), and a product of trait loss (*D. ananassae*).



The *cis* and *trans* of the *D. melanogaster* Pigmentation Gene Regulatory Network

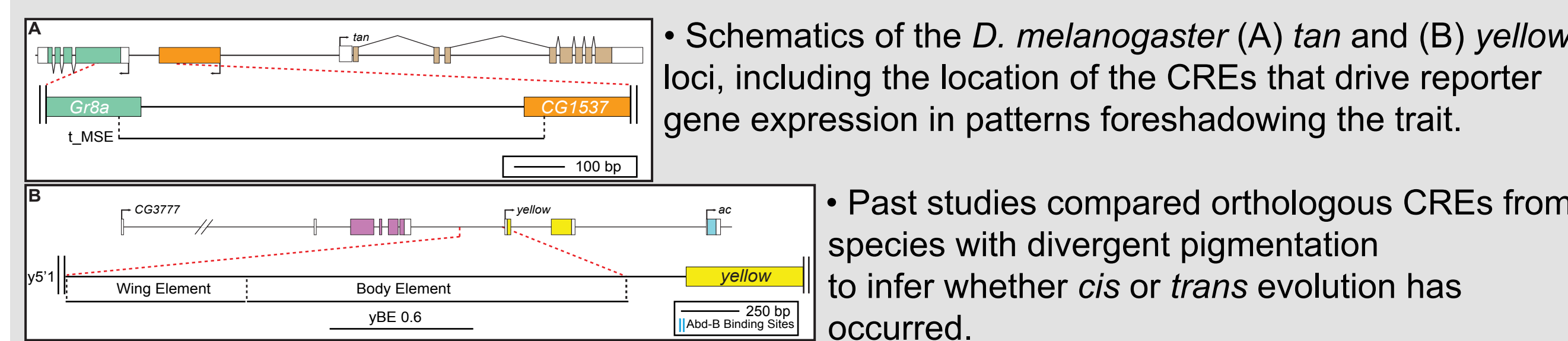


- The gene regulatory network responsible for the male-limited pattern of pigmentation has been resolved to some extent for *D. melanogaster*.

- Two key melanistic pigmentation genes that are expressed in the male A5 and A6 abdomen segments of *D. melanogaster*, and whose CREs and regulative *trans*-landscape has been resolved are *yellow* and *tan*.

- This GRN can be compared to that for other species to understand the *cis* and *trans* underpinning for trait gain, modification, and loss.

CREs Controlling *D. melanogaster* *yellow* and *tan* Expression

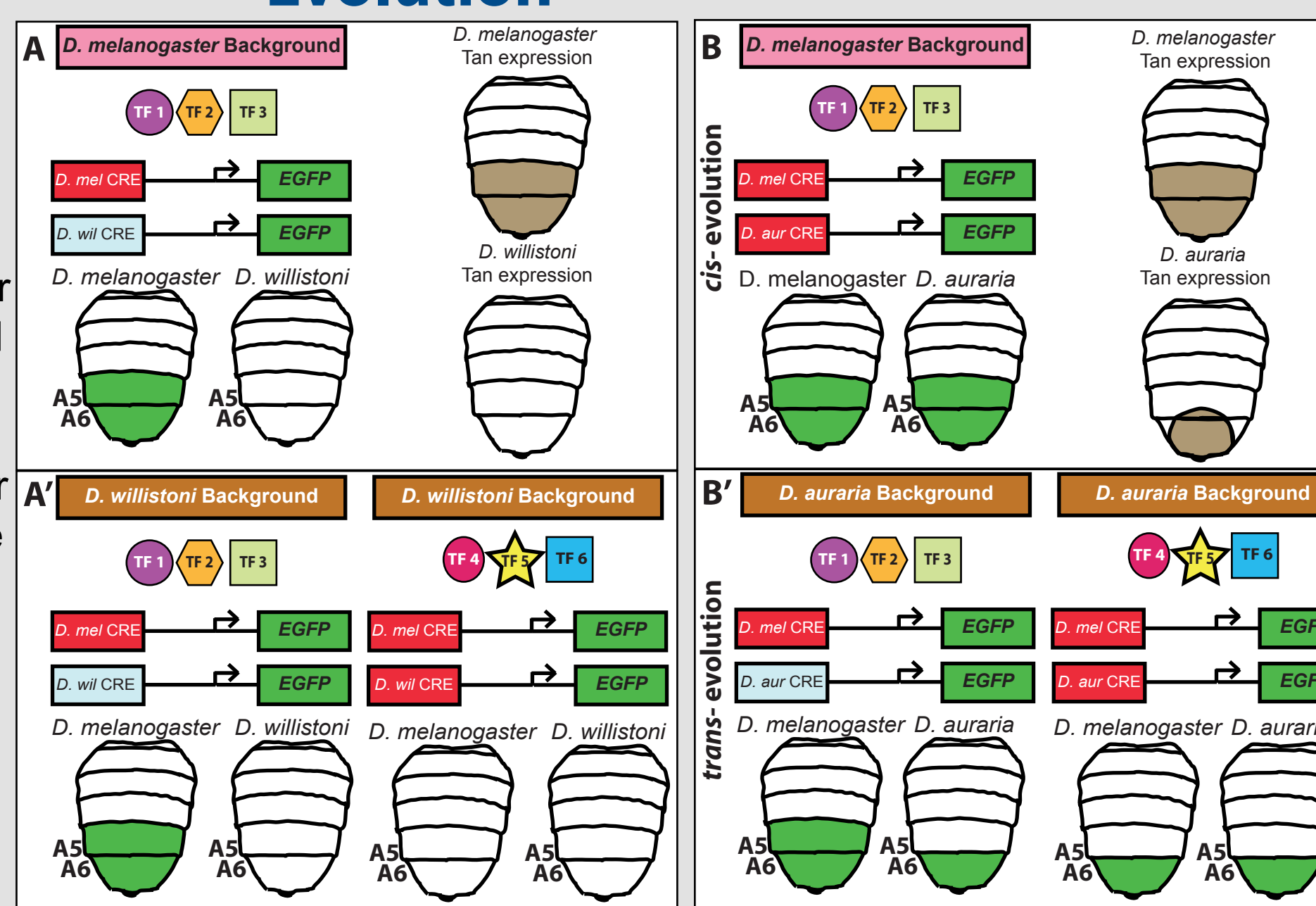


- Schematics of the *D. melanogaster* (A) *tan* and (B) *yellow* loci, including the location of the CREs that drive reporter gene expression in patterns foreshadowing the trait.

- Past studies compared orthologous CREs from species with divergent pigmentation to infer whether *cis* or *trans* evolution has occurred.

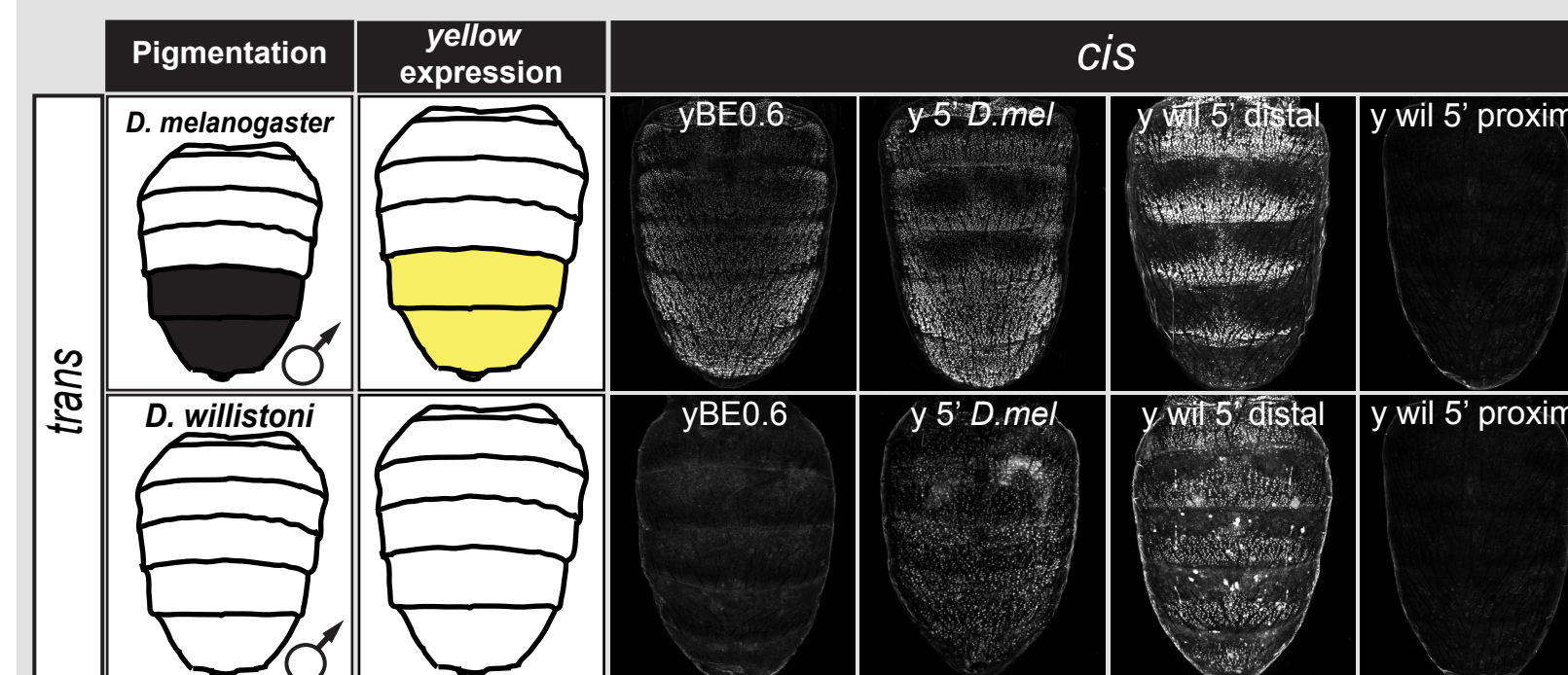
Reciprocal Host Reporter Transgenesis Reveals *cis* and *trans* Evolution

(A) Reporter transgene activity in *D. melanogaster* revealed *cis*-evolution between these species *tan* gene regulatory sequences. This did not rule out a role for *trans*-evolution in the derived *D. melanogaster* *tan* pattern. (A') Transgenesis in *D. willistoni* can resolve whether *trans*-evolution helped shape the derived *D. melanogaster* *tan* expression pattern.



(B) Reporter transgene activity in *D. melanogaster* suggested *trans*-evolution shaped the activity of orthologous CREs. However, this data could be explained by *cis*-evolution occurring outside the orthologous CRE region. (B') Transgenesis in *D. auraria* can resolve whether *cis* or *trans* evolution shaped the diverse *D. melanogaster* and *D. auraria* *tan* expression patterns.

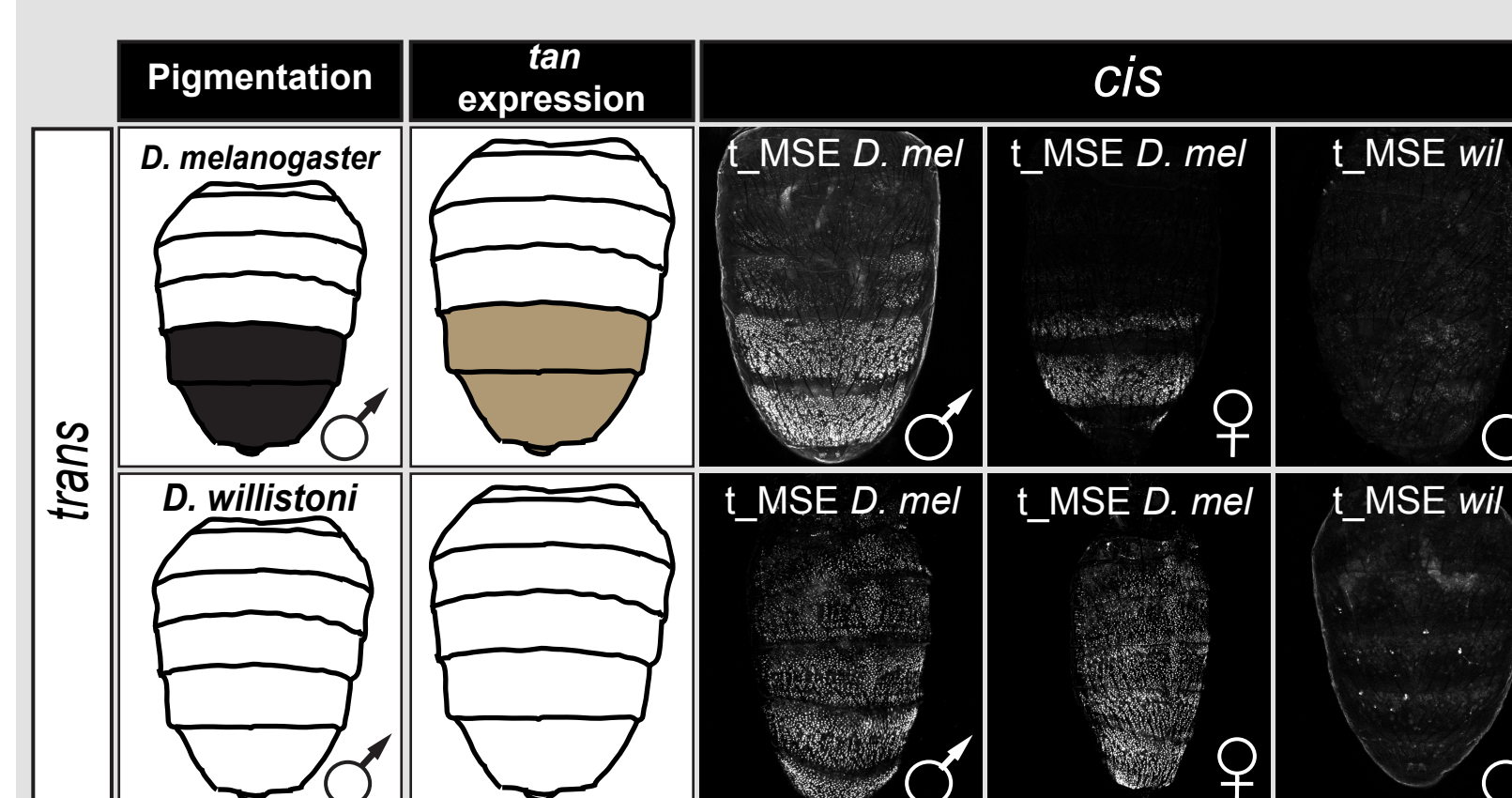
The Gain of Derived *yellow* Expression Required *cis* and *trans* Evolution



- The failure of the *D. willistoni* *yellow* sequences to activate expression in *D. melanogaster* indicates the occurrence of *cis*-evolution.

- Failure of the *D. melanogaster* *yellow* CRE to activate expression in *D. willistoni* indicates *trans*-evolution between these host species.

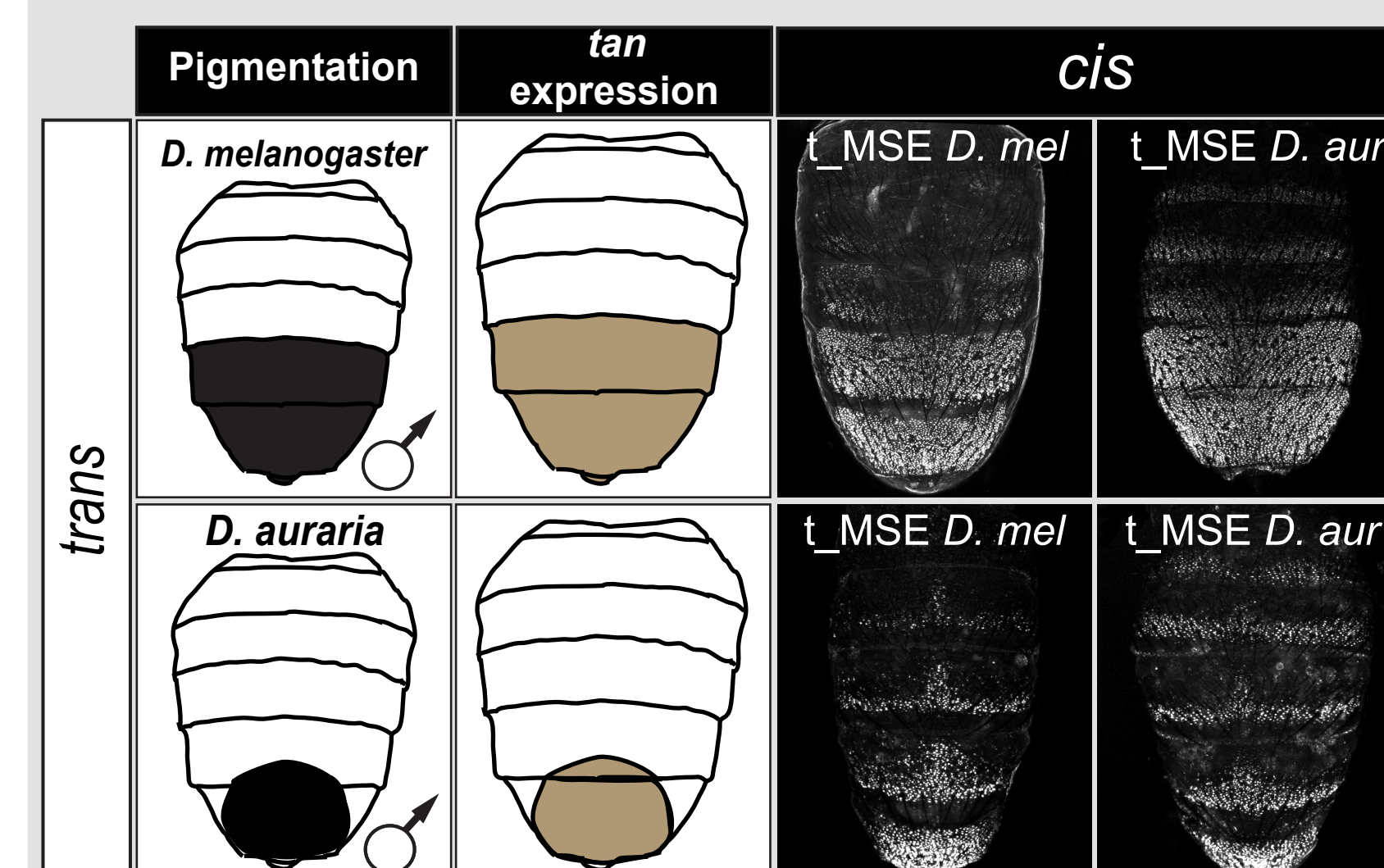
The Gain of Derived *tan* Expression Required *cis* and *trans* Evolution



- Failure of the orthologous *D. willistoni* *tan* sequences to activate expression in *D. melanogaster* indicates *cis*-evolution.

- The broad monomorphic activity of the *D. melanogaster* *tan* CRE in *D. willistoni* indicates *trans*-evolution between these host species.

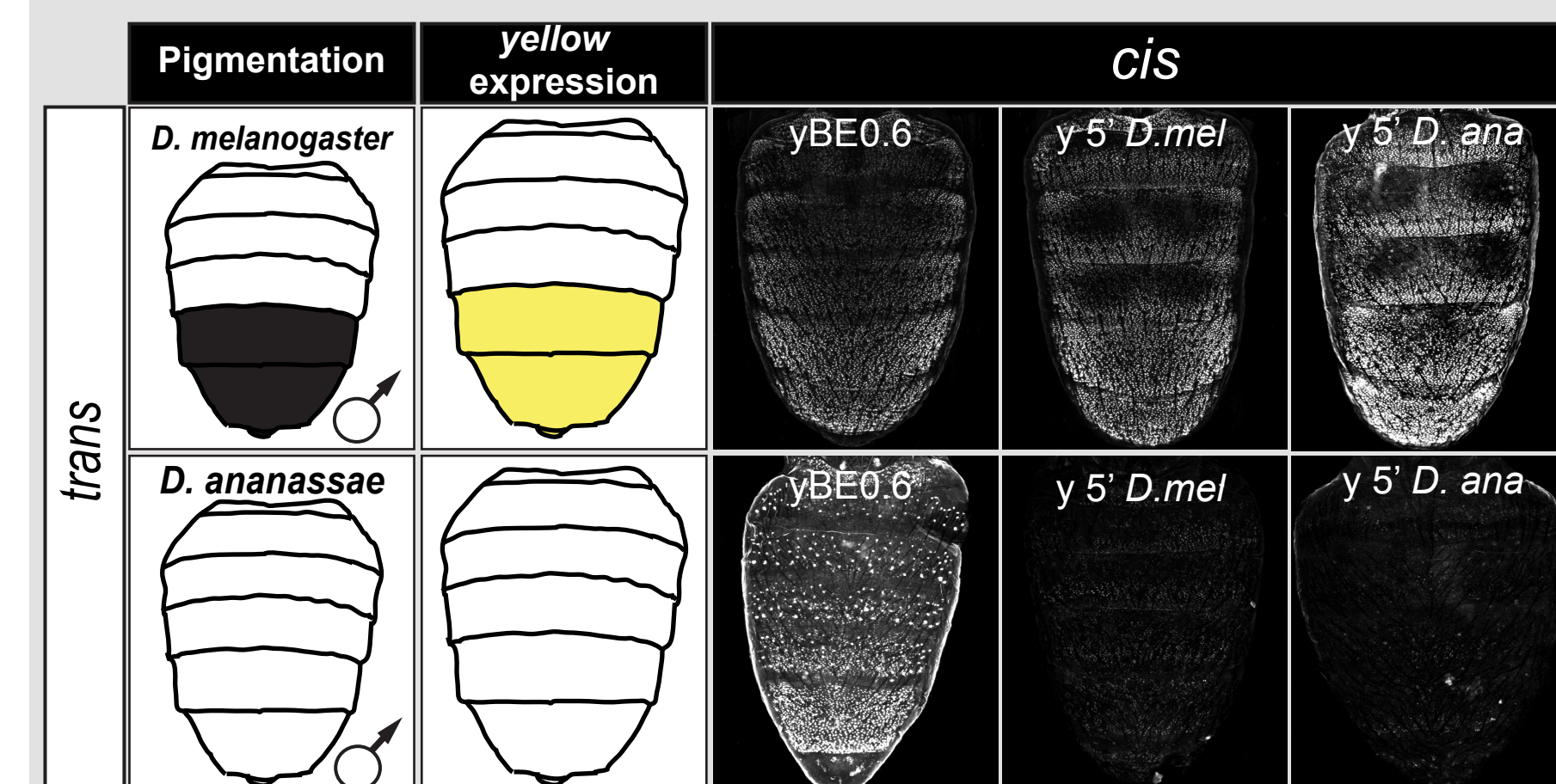
Trans Evolution is Responsible for Divergent *tan* Expression



- *D. melanogaster*-like regulatory activity for the *D. auraria* CRE in *D. melanogaster* hints at a *trans*-evolution explanation.

- *D. auraria*-like regulatory activity for the *D. melanogaster* CRE in *D. auraria* confirms that the diverse *tan* expressions stem from *trans*-evolution. A similar story explains the divergent patterns of *yellow* expression between these two species.

Trans Evolution and the Loss of *yellow* Expression

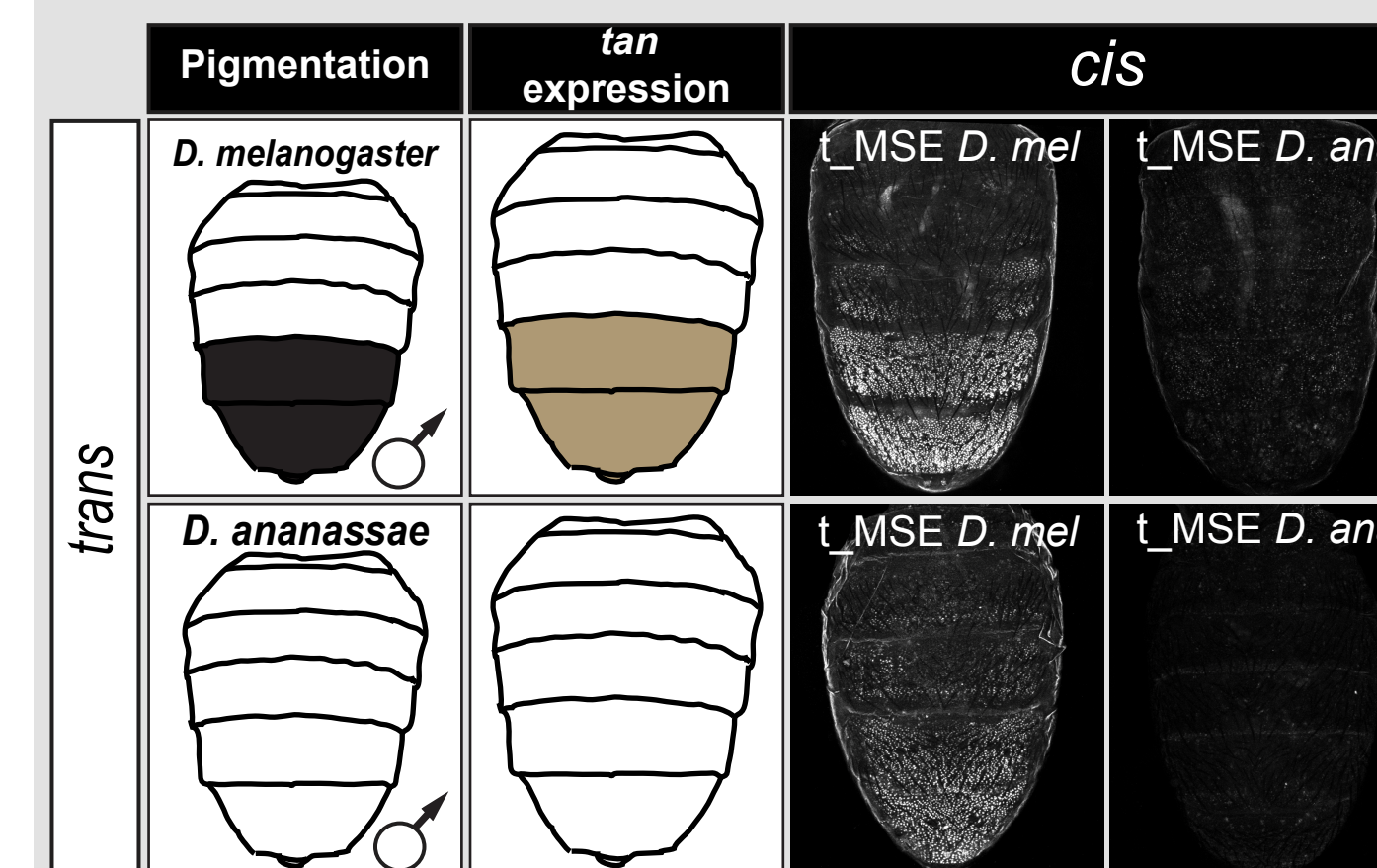


- Although *D. ananassae* does not express *yellow*, its regulatory region drove patterned expression in *D. melanogaster*. Suggesting the absence of expression required a *trans*-landscape change.

- Reporter transgenes with the larger orthologous 5' regions of *yellow* do not drive reporter activity in *D. ananassae*, though the minimal *yellow* CRE does.

- These results validate a role for *trans*-evolution and indicates that the *trans*-responding sequence resides outside the minimal CRE region.

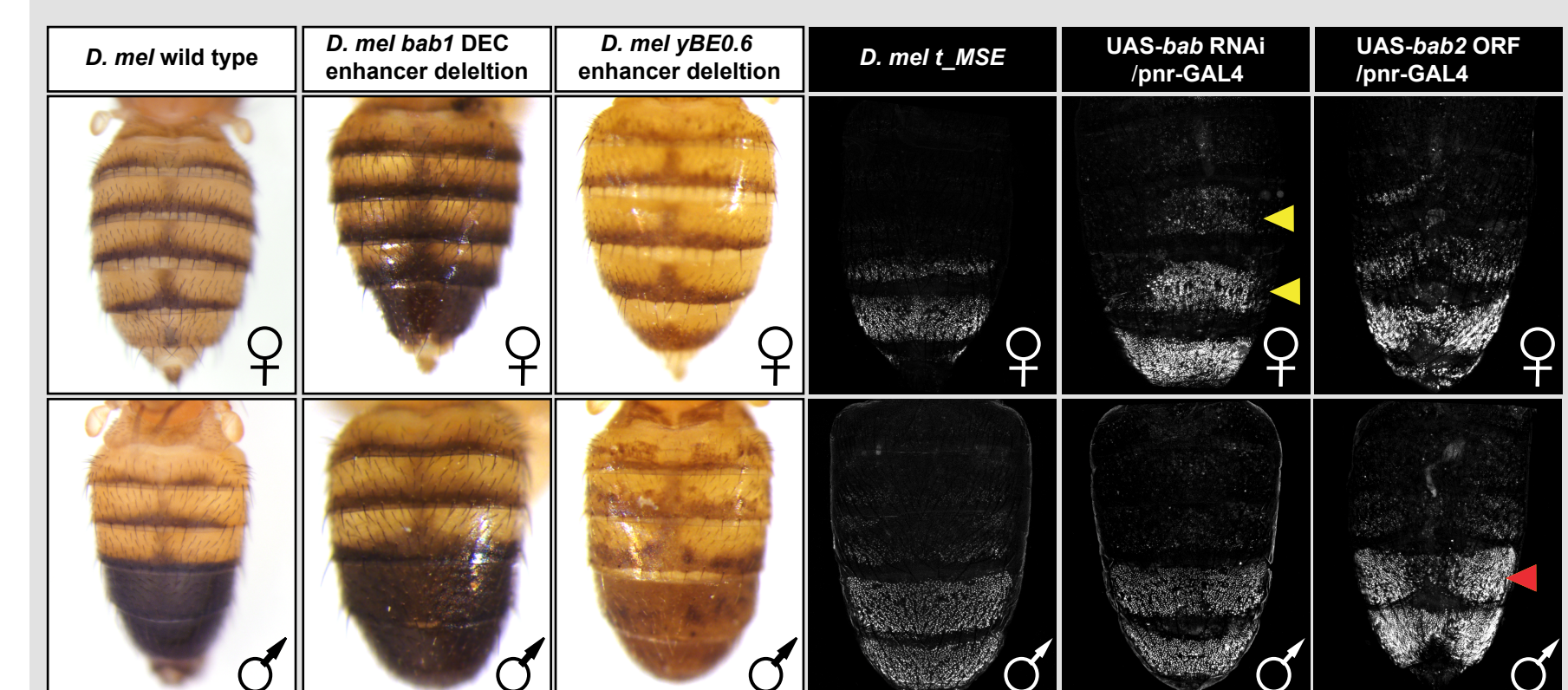
Cis Evolution and the loss of *tan* Expression



- *D. ananassae* does not express *tan* and its CRE region fails to drive expression in *D. melanogaster*, suggesting the expression loss involved *cis*-evolution in the t_MSE.

- The *D. melanogaster* but not the *D. ananassae* t_MSE drove expression in *D. ananassae*. These outcomes suggest *cis*-evolution inactivated the *D. ananassae* CRE while the *trans*-landscape remained conserved.

Conclusions and Future Directions



- *Cis*-evolution was necessary to create *yellow* and *tan* CREs that respond to a pigmentation GRNs regulative *trans*-landscape, and to disable a CRE in a case of trait loss.

- We find compelling evidence for *trans*-evolution in the origin, diversification, and loss of this trait. Thus, the pigmentation GRN's *trans*-landscape has been a dynamic entity.

- We aim to identify which genes comprise the evolving *trans*-landscape and validate the phenotypic necessity for instances of *cis*-evolution by removing and replacing orthologous CRE regions in the *D. melanogaster*

REFERENCES

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TMW was supported by funding from the American Heart Association (11BGIA7280000) and National Science Foundation (IOS-1146373 and IOS-1555906). Corresponding Authors Contact Info: twilliams2@udayton.edu; hughesj7@udayton.edu