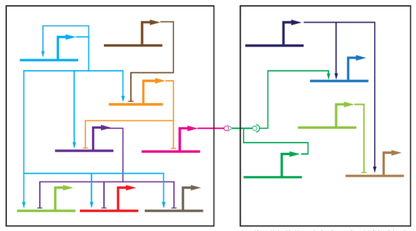
Gene regulatory network evolution during *Drosophila melanogaster* and *Aedes aegypti* nervous system development

Isabella Schember[1,2] and Marc S. Halfon[1,2,3]

Gene regulatory networks

Metazoan development proceeds through coordinated gene expression programs governed by specific gene regulatory networks (GRNs). Changes in GRN structure and function underlie phenotypic diversity. Here we investigate the *cis*- and *trans*-regulatory changes in a GRN involved in nervous system development in both flies (*D. mel*) and mosquitos (*A. aeg*).

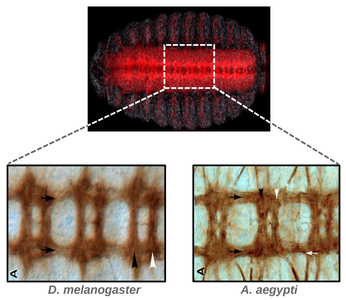


Halfon (2017) *Trends in Genetics* ***33(7)***:436-47

Diverged *single-minded* expression

***A. aeg sim* expression has diverged in the late midline.** In *D. mel*, *sim* is expressed at the ventral midline during mid (A) and late (B) embryogenesis and in muscle progenitor (arrowheads in A). Unlike other mosquitos, which have a fly-like pattern of *sim* throughout embryogenesis, in *A. aeg*, *sim* is expressed in the midline until mid embryogenesis (C), but then shifts laterally (D).

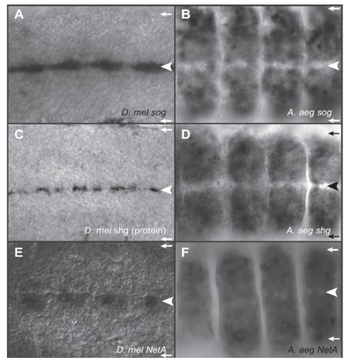
Developmental system drift



This is a prime example of developmental system drift, where the phenotype appears essentially unchanged despite significant genetic rewiring.

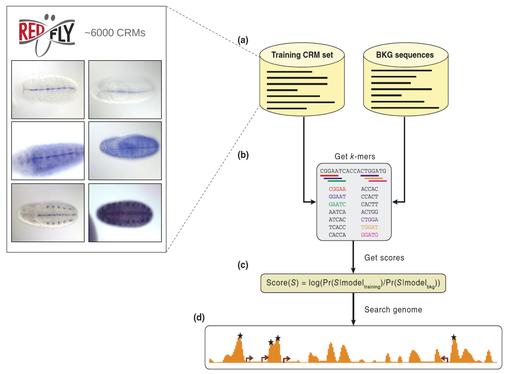
Redeployment of the midline GRN

Additional *D. mel* genes downstream of *sim* and expressed in the midline have been redeployed laterally in *A. aeg*. This demonstrates that not just *sim* but substantial portions of the GRN has been redeployed. Downstream genes include *sog* (A,B), *shg* (C,D), and *NetA* (E,F). In each panel, the midline is marked with an arrowhead and small arrows indicate the approximate boundary of the central nervous system. **In order to probe the mechanisms responsible for these changes in the *sim* GRN, we have started identifying enhancers regulating the expression of the component genes in both species.**



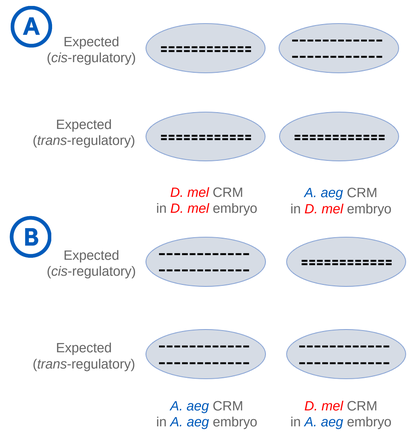
Supervised *cis*-Regulatory Module Prediction

SCRMshaw, our computational approach, is used to identify transcriptional *cis*-regulatory modules (CRMs) in both *D. mel* and *A. aeg*. We are currently evaluating midline enhancers in transgenic *D. mel* and *A. aeg*. After revising and updating our input sequences, we will re-run SCRMshaw and select new candidates for *in vivo* validation.

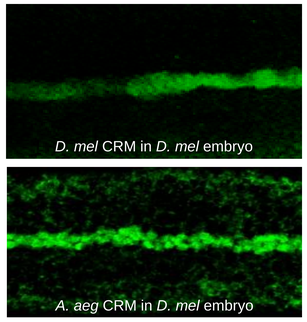


Reciprocal reporter gene assays test for *cis*- vs. *trans*-mediated changes in gene expression

The altered gene expression that we observed is due to either *cis*- or *trans*-mediated changes. We can test this through reciprocal reporter gene assays. (A) We have tested *sog* enhancers in transgenic *D. mel* (see next panel). (B) Testing in transgenic *A. aeg* is currently in progress.

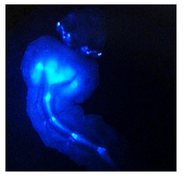


Testing homologous *sog* enhancers suggests a primarily trans-regulatory change



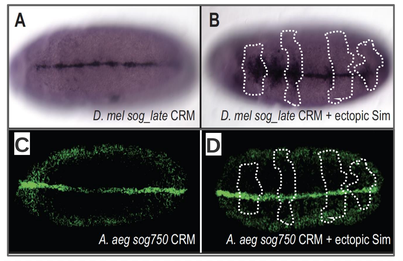
The expression driven by both the *D. mel* and *A. aeg sog* enhancers, in *D. mel* embryos, is at the midline, suggesting a *trans*-regulatory event. This is consistent with the change that we see in *sim*, which itself likely results from a *cis*-mediated change.

Evaluation of the expression driven by the *sog* enhancers in transgenic *A. aeg* is currently in progress.



Ectopic Sim expression also reveals a role for *cis*-acting changes in the *sog* enhancer

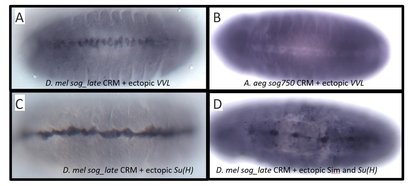
Transgenic embryos with *D. mel* or *A. aeg sog* reporter genes. The *D. mel sog* CRM expands in response to ectopic Sim expression (A, B). The *A. aeg sog* CRM is non-responsive to ectopic Sim (C, D). This may be due to a need for additional activators.



Phenocopying *A. aegypti* gene expression to interrogate the *trans*-regulatory landscape

Late midline gene expression is only partly dependent on *sim*. Multiple transcription factors are known to act in the midline in *D. mel*. By ectopically expressing combinations of these we can determine what is required for the shift in *A. aeg sog* CRM expression.

Both the *D. mel sog* CRM and *A. aeg sog* CRM are non-responsive to D, activated Notch, activated Ras, SoxN, or VVL (represented by A,B). We are currently testing *sim* with D, activated Notch, activated Ras, SoxN, or VVL combined activity. Preliminary results suggest a repression of *D. mel sog* CRM at the midline in response to Sim and activated Notch activity (C,D).



References

Duman-Scheel, M., Patel, N.H. (1999). *Development*, 126:2327-34.

Kazemian, M., Suryamohan, K., Chen, J.Y., Zhang, Y., Samee, M.A., Halfon, M.S., Sinha S. (2014). *Genome Biology and Evolution*, 6(9):2301-20.

Suryamohan, K., Hanson, C., Andrews, E., Sinha, S., Duman-Scheel, M., Halfon, M.S. (2016). *Developmental Biology*, 416(2):402-13.

University at Buffalo The State University of New York

[1]Department of Biochemistry, University at Buffalo – State University of New York
[2]NY State Center of Excellence in Bioinformatics & Life Sciences
[3]Roswell Park Comprehensive Cancer Center