Cofactor-dependent and -independent functions of Hox reveal two distinct evolutionary lineages of insect wing tissue

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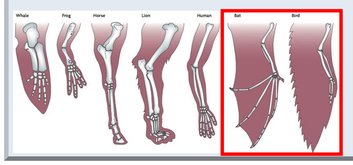
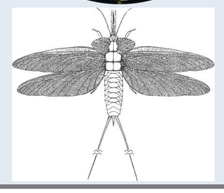
1. Insect wings are a morphological novelty

Insects are incredibly evolutionarily successful – they are the dominant clade of life on this planet. This feat is often attributed to the acquisition of wings.

While vertebrate wings share homology to known structures, insect wings arose independently, begging the question:

What is the origin of this structure?

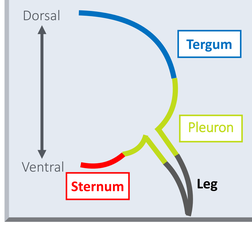




2. The wing origin debate: Tergal vs. Pleural origin

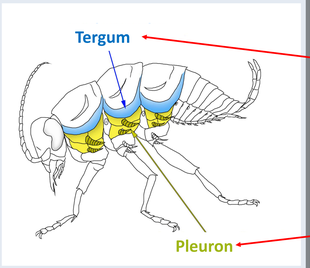
Winged insects have 3 thoracic segments: **T1 (wingless)**, and **T2-T3 (winged)**.

Dorsoventrally, there are three major compartments in each thoracic segment: Tergum, Pleuron, Sternum



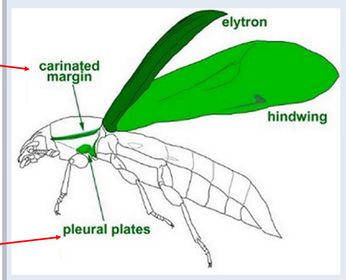
Tergal origin hypothesis:
Attributes wing origin to expansion of dorsal (tergal) body wall

Pleural origin hypothesis:
Attributes wing origin to ancestral proximal leg segments and the associated exite branches



Both hypotheses have been debated heavily for centuries, but **an evo-devo approach to wing origin studies could provide new insight**

3. Potential dual origin of insect wings



Previous work in *Tribolium castaneum* suggested:

1. Wing gene-dependent tissues in T1 (associated with tergum and pleuron) are "wings" maintained in more ancestral state → wing serial homologs (WSHs)
2. Both WSHs contribute to wing formation upon Hox-induced **homeotic transformation**

It remains unclear to what degree each tissue contributes to wing formation

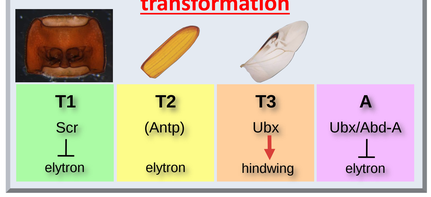
Dual Origin "Spectrum"

4. Both WSHs transform into wing upon Hox-induced homeotic transformation

Hox genes determine segmental identity along the body axis:

- **T1**: *Scr* prevents elytron formation
- **T2**: "Default" state; no Hox modification in elytron
- **T3**: *Ubx* controls hindwing identity

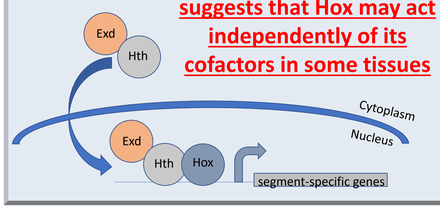
Removing Hox results in homeotic transformation



5. Hox proteins are known to interact with cofactor complex Hth/Exd

Proper Hox function is thought to require the Hth/Exd cofactor complex, together acting as a transcription factor complex to activate segment-specific genes.

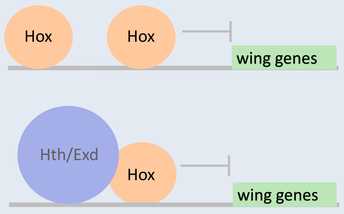
Previous work in *Drosophila melanogaster* suggests that Hox may act independently of its cofactors in some tissues



6. Investigating the different modes Hox cofactor dependency

Hox genes are necessary for proper segmental identity, but the extent to which the cofactor complex, Hth/Exd, is necessary for individual tissue assignment has not been studied.

We propose that among the WSHs there are tissues which are Hox cofactor-dependent or –independent.

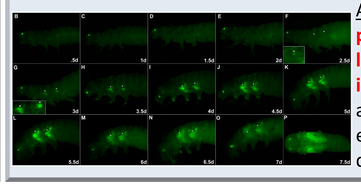


Here, I seek to investigate this cofactor dependence in each of the WSHs of:
T1: Scr and Hth/Exd function in preventing elytron formation
T2: Hox-independent action of Hth/Exd
T3: Ubx and Hth/Exd function in hindwing differentiation

7. RNAi allows us to target Hox and the cofactor gene *hth* for genetic knockdown

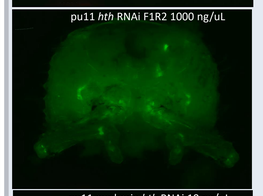
Using RNA interference (RNAi) techniques, we knock down *hth* to determine what role this gene plays in the maintenance of the WSHs.

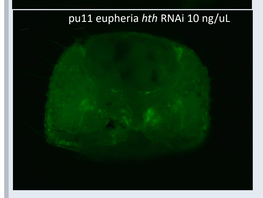
Comparison to Hox RNAi or reduction (via hypomorphic alleles) phenotypes can reveal distinct modes of action in each WSH.



An important tool: **pu11 enhancer trap line expresses EYFP in wing tissues**, allowing us to track ectopic wing tissue development.

8. Scr and Hth/Exd have different roles in maintaining the T1 WSHs



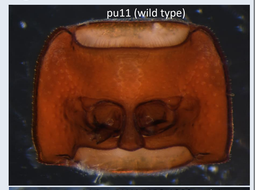


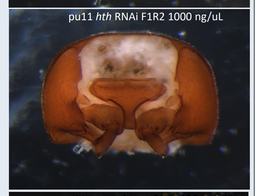
Knockdown of *hth* induced transformation in T1 at different stages:

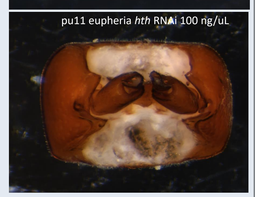
- **Pupae** (left): Ectopic eYFP expression was found around one of the pleural WSHs (trochantin)
- **Adults** (right): Several pleural tissues affected (trochantin, sternum, epimera)

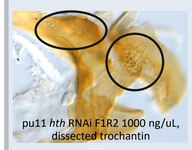
Overall, **T1 is losing its identity as the wingless thoracic segment** as it begins to take on a phenotype that appears to be closer to that of T2.

These transformations appear to be limited to the pleural WSHs, **indicating that Scr works independently of Hth/Exd in the tergal WSHs**.

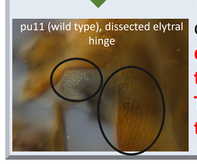








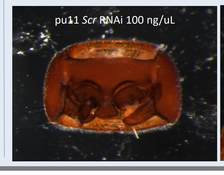
The trochantin was dissected out from adult beetles (top), revealing sensory structures unique to elytral hinge (bottom).

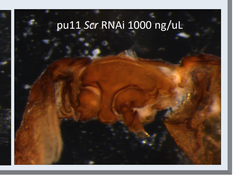


Together with above data, **this supports a distinct segmental transformation from T1 to T2 in the pleural tissues**.

hth RNAi phenotype is distinctly different from that of *Scr* RNAi, and supports our hypothesis that Hth/Exd and Hox have different modes of action in the tergal and pleural WSHs:

- **Tergal WSH: Scr is cofactor-independent**
- **Pleural WSHs: Scr is cofactor-dependent**

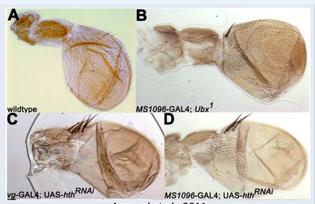




9. The cofactor dependency of T3 Hox gene *Ubx*

Ubx is the Hox gene responsible for T3 (hind)wing differentiation.

Previous work in Drosophila showed Hth/Exd dependence in the T3 "wing" hinge only, suggesting that Hth/Exd is important for distinguishing T3 proximal wing structures.

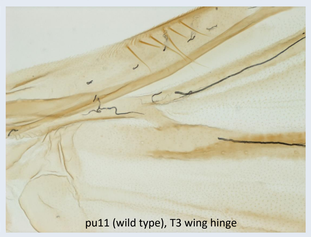


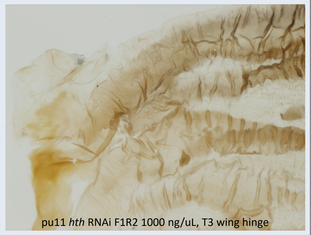
Agrawal et al., 2011

We find that upon *hth* RNAi, proximal T3 sensory structures (wing hinge) are transforming at much higher frequencies, suggesting that:

- **landmark proximal wing structures are cofactor-dependent**
- **landmark distal wing structures may form independently of Hth/Exd**.

A comparison to *Ubx* RNAi phenotype will help determine tissue-specific cofactor-dependence.





Transformation (pu11 hth F1R2 1000 ng/uL)	Frequency (%, n=26)
Fewer sensory bristles along anterior proximal edge of wing (normally 6-8 bristles) (**proximal**)	100.0
Missing 1-2 hairs at vein junction (**distal**)	3.8
Ectopic sensory structures along vein leading to vein junction (**proximal**)	88.5
Ectopic sensory structures along vein leading to vein junction (**distal**)	26.9
Reduced sensory hairs on anterior half	7.1
Reduced sensory hairs on posterior half	50.0
Potential sclerotization or tanning	57.1

11. Conclusions thus far

Separating the functions of Hth/Exd from those of the Hox genes in each segment is a necessary step that will reveal genetic mechanisms behind wing formation:

- In T1, *hth* functions with *Scr* to provide pleural identity, while *Scr* can function without Hth/Exd in tergal tissues.
- In T2, *hth* functions without Hox input in elytron development.
- In T3, *hth* functions with *Ubx* to provide hinge identity.

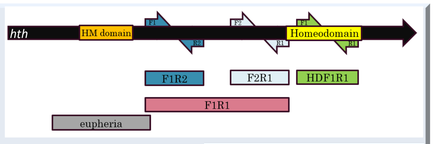
The pleural tissues are often associated with the musculature (hinge) of the wing. So far, we have found that hth is important in the pleuron (T1) and the wing hinge (T3), so Hth/Exd appears to be important in the development of only the pleural WSH.

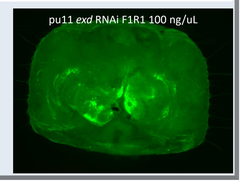
Across all three thoracic segments, *hth* functions in the proximal-most wing structures (T1 pleuron, T2-T3 hinge).

In sum, **we have been able to separate segmental cofactor-dependent and –independent functions of Hox** as they relate to insect wing, and thus wing origin, but further investigation is still necessary.

12. Future directions

- ***Ubx* RNAi** to separate the T3 Hox function from Hth/Exd function in this segment (as with *Scr* in T1)
- **Antp RNAi** to determine its effect (if any) in forming elytron
- Further **investigation of *exd* RNAi phenotype** to elucidate its mechanisms in relation to *hth*, Hox, and wing origin.
- Investigation of **potential *hth* isoforms** (previously found in Drosophila) using different primers, targeting different regions of the gene (top figure).
- **Further use of Sal4 line** to investigate the tandem transformation of T1 pleural plates with T2/T3 wing hinge upon *hth* RNAi.
- **Investigation of ancestral ground state** through analysis of T2/T3 body wall.





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