

Genetic Mechanisms Governing the Development of Polyphagan Prothorax and Their Implications to the Evolutionary Origin of Insect Wings*

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The evolutionary origin of insect wings has been the subject of heated debates for centuries. These debates have culminated into two contrasting hypotheses: the tergal origin hypothesis and the pleural origin hypothesis (Clark-Hachtel and Tomoyasu, 2016). However, despite accumulating efforts to unveil the origin of insect wings (including developmental, taxonomical, and paleontological analyses), neither hypothesis has been able to surpass the other. Through investigations into the development of wing-related tissues in a wide taxonomy of organisms (including beetles, cockroaches, and crustaceans), we previously obtained evidence supporting a third hypothesis, the dual origin hypothesis (Clark-Hachtel et al., 2013, 2021; Linz and Tomoyasu, 2018; Clark-Hachtel and Tomoyasu, 2020). Although this hypothesis has the potential to unify the two competing wing origin hypotheses, it requires rigorous testing from various fields. We reasoned that a detailed genetic analysis of the tissues that are serially homologous to wings (wing serial homologs, WSHs) will help us further decipher the evolutionary origin of insect wings. Our genetic dissection of the prothoracic (T1) body wall tissues in the red flour beetle (*Tribolium castaneum*) revealed several intriguing aspects of WSHs. First, we obtained evidence for the unique nature of the T1 pleural plates that we previously identified as WSHs; they are genetically distinct from the tergal tissue but are not serially homologous to the pleural plates in other thoracic segments (such as the episternum and epimeron). This outcome further supports the idea that these pleural plates are serially homologous to wings. Second, we found that the mode of Hox action operating is distinct between the tergal and pleural WSHs, with the identity of pleural WSHs depending on Hox cofactors while tergal WSH identity is independent of Hox cofactors. Interestingly, Hox cofactors are

known to be important for the proximal wing identity, suggesting that the evolutionary contribution of pleural tissues might be predominantly to the proximal portion of the wing. Third, through an enhancer analysis in *Drosophila* and *Tribolium*, we identified several wing enhancers that are also active in either terga or pleura of prothorax in *Tribolium*. The overlapping expressions of these enhancers between wings and T1 WSHs suggest a shared transcriptional landscape between wings and the two proposed origin tissues, while the non-overlapping aspect of these enhancers within T1 supports distinct identities between the tergal and pleural T1 WSHs. Taken together, these genetic differences between the two T1 WSH identified here provide a further support for the evolutionary contribution of the two distinct lineages of thoracic tissues to the emergence of insect wings.

References

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