

Developmental Origin of Wings in the Two-spotted Cricket *Gryllus bimaculatus* Suggests an Evolutionary Mechanism of the Insect Wing*

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The classical hypotheses about the evolutionary origin of the insect wing have claimed tergal or pleural origin and have drawn mutually exclusive evolutionary scenarios (Clark-Hachtel and Tomoyasu, 2016). The third hypothesis, the dual origin hypothesis, proposes the evolution of the wing through the combined contribution of the morphological elements of both the tergum and pleuron, but there is no unified view on the degree of contribution of each element (Clark-Hachtel and Tomoyasu, 2016). Where does the insect wing come from? To answer this question, we investigated the developmental origin of the wing in the hemimetabolous insect, *Gryllus bimaculatus*. Hemimetabolous insects develop into adults without major changes in body plans formed during embryogenesis. This feature could clarify the contribution of the tergum and pleuron to wing development.

In order to find the homologous structure of wings, we first investigated the expression pattern of *vestigial* (*vg*), *apterous* (*ap*), and *wingless* (*wg*), which play a decisive role in the wing development process of *Drosophila*. In *Gryllus* embryos, *ap* was widely expressed in the tergal region, and *vg* and *wg* were expressed at the edge of the tergal plate. In addition, *ap* and *vg* were also expressed in the pleural region. Since *vg* is involved in determining the fate of wing primordial cells in *Drosophila* embryos (Fuse et al., 1996), the fate of *vg*-expressing cells is important for understanding the developmental origin of cricket wings. Therefore, a reporter line of *vg* expression was created by genome editing, and the fate of *vg*-expressing cells was traced in *Gryllus*. Subsequently, we performed functional analysis of *vg* and *ap* using genome editing technology. In the *vg* mosaic knockout individual, the formation of the nymphal tergal edge was inhibited. Adult wing formation was significantly

inhibited in crickets lacking the nymphal tergal edge. This result suggests that nymphal tergal cells contribute considerably to adult wing formation. Consistently, physical ablation of the nymphal tergal edge significantly inhibited adult wing growth. On the other hand, we found that *ap* is required for *vg* expression at the tergal margin by creating a mosaic knockout individual of *ap* in the *vg* reporter cricket strain. From the above results, we conclude that *vg* induced by *ap* in the embryonic stage is required to form the tergal edge, and a part of the tergal edge leads the growth of the wing in the postembryonic stage.

How do the cells of tergal edge grow wings in the cricket? To further understand this point, the nymphal tergal margin was divided into two regions and a comparative transcriptome analysis was performed. The analysis revealed that the components of multiple signaling pathways were highly expressed in the lateral region of the tergal margin, suggesting that this region could serve as a signaling center and control wing growth. In fact, RNAi analysis revealed that the Wnt/Ft (Fat)/Hippo pathway is involved in cricket wing growth. These pathways, along with *vg*, have been reported to play a collaborative role in *Drosophila* wing growth (Zecca and Struhl, 2010), suggesting a correspondence between cricket lateral tergal margin and *Drosophila* wing regions.

This study showed the instructive role of tergal cells in the wing development of the hemimetabolous insect. Together with a previous study (Niwa et al., 2010), this study suggests that the tergal edge of apterygote and hemimetabolous insects are homologous. Therefore, we conclude that activation of the growth mechanism mediated by Wnt/Ft/Hippo pathways in the tergal margin cells of a wingless ancestor might be a key step in the wingless-to-winged transition.

* Contribution to the symposium “Acquisition of Functional Diversity of Insects” in the 57th Annual Meeting of the Arthropodan Embryological Society of Japan, July 9–10, 2021, Ushiku-numa, Ibaraki, Japan.

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