

# Exploring the origin of insect wings from an evo-devo perspective

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Although insect wings are often used as an example of morphological novelty, the origin of insect wings remains a mystery and is regarded as a major conundrum in biology. Over a century of debates and observations have culminated in two prominent hypotheses on the origin of insect wings: the tergal hypothesis and the pleural hypothesis. However, despite accumulating efforts to unveil the origin of insect wings, neither hypothesis has been able to surpass the other. Recent investigations using the evolutionary developmental biology (evo-devo) approach have started shedding new light on this century-long debate. Here, we review these evo-devo studies and discuss how their findings may support a dual origin of insect wings, which could unify the two major hypotheses.

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## Introduction

Insects compose nearly 80% of all known animal species, making them one of the most diverse metazoan groups [1]. The success of this group can partially be credited to the acquisition and subsequent diversification of wing structures [1,2]. However, despite accumulating efforts to unveil the origin of this novel structure (including morphological, taxonomical, and paleontological analyses), the origin of insect wings remains a mystery and is regarded as a major conundrum in biology. The difficulty in deciphering the origin of the insect wing stems from the lack of transitional fossils between apterygote (non-winged) and pterygote (winged) insects in the fossil record [3–6]. The earliest known insect fossils (apterygotes) date from the Lower Devonian (390 million years ago, MYA) [4,5,7,8]. Although there is no fossil record of the event, insect wings are believed to have evolved only

once (i.e. are monophyletic) sometime during the Upper Devonian or Lower Carboniferous (370–330 MYA) [3,5,9]. By the early Permian (~300 MYA), winged insects had diversified into at least 10 orders [4]. Therefore, there is quite a large gap in the fossil record between apterygote and diverged pterygote lineages, which has resulted in a long-running debate over where insect wings have come from and how they have evolved.

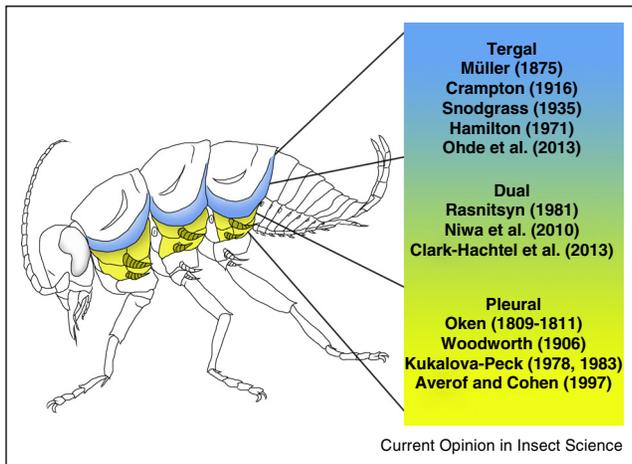
## Two proposed wing origins

The insect wing origin debate can be broken into two main groups of thought; wings evolved from the tergum of ancestral insects or wings evolved from pleuron-associated structures (Figure 1. See Box 1 for insect anatomy) [10<sup>\*</sup>]. *The tergal origin hypothesis* (a.k.a. paranotal hypothesis) proposes that wings evolved from lateral extensions of the thoracic tergum, allowing ancient insects to glide and, when eventually articulated, to fly (blue in Figure 1) [11–13]. *The pleural origin hypothesis* has many variations, but essentially proposes that wings formed from an outgrowth and/or branch of pleural tissue (yellow in Figure 1). Depending on the variation of this hypothesis, the proposed origin can be a de novo projection of the pleural plate or the articulated branches (exites and/or endites) that stemmed from the pleural plate. An influential variation of the pleural hypothesis is the exite hypothesis, which states that insect wings originated from exites of ancestral proximal leg segments (such as the epicoxa) (Figure 1) [14<sup>\*</sup>,15]. According to this hypothesis, the exites migrated dorsally and flattened to eventually become the wing blade recognized today [13,14<sup>\*</sup>,16], while ancestral proximal leg segments fused into the body wall and provided a series of sclerotized plates along with preexisting muscle attachment, enabling the quick acquisition of insect wing articulation [14<sup>\*</sup>].

## Application of evo-devo to insect wing origin studies

As mentioned earlier, difficulty in revealing the origin of insect wings stems from the lack of transitional fossils between apterygotes and pterygotes. The absence of ‘proto-wings’ in the fossil record has necessitated the search for wing serial homologs in wingless segments (such as T1 or abdominal segments) of winged insects or wing homologs in wingless species (apterygotes and non-insect arthropods), in hopes that they will provide clues to the transitional state in the evolution of insect wings (Box 2). The tergal lobe of silverfish (apterygotes), T1 pronotal lobes of several extant insect species (such as mantises and cockroaches), and the T1 winglet of

Figure 1



Overview of wing origin hypotheses. The locations of two proposed wing origins, terga (blue) and pleura (yellow), in an ancestral insect ground plan. Some articles in support of each hypothesis are also listed. Note that this model is a summary of the two hypotheses and not the reconstruction of an ancestral state of insects. In addition, the presence of pleural exites in some fossil insects is currently under debate among the paleontology community (see [36,37] for evidence in opposition to the pleural exites, but also see [38] for a possible confirmation of some of these exites).

Palaeodictyoptera (an extinct palaeopteran order), have been proposed to be wing (serial) homologs in support of the paranotal hypothesis (Figure 2) [10\*,17]. In contrast, the abdominal gills/gill plates in palaeopteran nymphs and the leg branches in apterygotes and other non-insect arthropods (such as bristletail styli and crustacean endites/exites) have been proposed to be homologous to wings in support of the pleural origin hypothesis (Figure 2) (reviewed in [16]). However, most of these proposed wing homologs (both tergal and pleural) are morphologically quite distinct from the actual wing, making it difficult to argue that any of these tissues reflects a transitional state in the evolution of wings.

Only recently have we been able to use the combination of molecular and developmental approaches (i.e. evo-devo) to investigate questions related to the origin of insect wings. The accumulated knowledge on genes and pathways involved in insect wing development (mainly from *Drosophila* studies) opens the possibility of identifying wing homologs without relying on morphology, providing us with a new angle from which to tackle this century-long conundrum. In fact, the main switch in popular opinion from a tergal to a pleural origin of wings in the late 1900s came with the advent of molecular tools. Averof and Cohen discovered that the critical wing genes *apterous* (*ap*) and *nubbin* (*nub*) are expressed in the exites of the brine shrimp (Figure 2) [18\*\*]. In *Drosophila*, *ap* is expressed in the dorsal compartment of the wing primordium [19], while *nub* is expressed in the entire developing

wing [20]. Therefore, the expression of these genes in the exites of the brine shrimp provided the first molecular support for the homology between crustacean exites and insect wings. As the first molecular evidence, this finding was quite influential on popular opinion at the time (Box 2). This study illuminates the potential impact that the evo-devo approach can have on the insect wing origin debate.

### Dual origin hypothesis supported by an evo-devo study

In 2010, Niwa *et al.* reported their expression analyses of several wing genes in two basal insect orders, Archaeognatha (bristletails) and Ephemeroptera (mayflies) [21\*\*]. They found that both the tergal margin and pleural branches (stylus of bristletails and abdominal gill of mayflies) share a part of the wing gene network operating during *Drosophila* wing development (Figure 2). Through this finding, they proposed that the fusion of these two fields facilitated the evolution of the insect wing, thus suggesting a dual origin of insect wings.

Although this is the first time a dual origin was proposed from the evo-devo perspective, this type of combinational model (i.e. dual origin hypothesis) has been proposed in the past (Figure 1). Crampton may be the first to mention the possibility of the combinational model, although he was in favor of the paranotal hypothesis [17]. Later, Rasnitsyn proposed that the wing originated from the fusion of notal lobes and proximal leg segments (coxopodite) [11]. Nonetheless, Niwa *et al.* provide an important turning point in the insect wing origin debate by hinting at the possibility of unifying the two hypotheses on insect wing origin based upon molecular evidence.

### Two distinct T1 wing serial homologs identified through evo-devo analyses

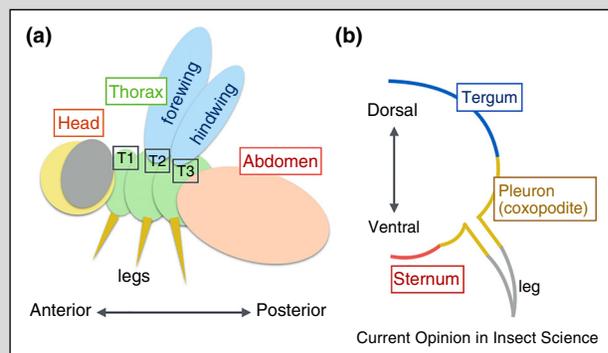
Our previous work provided another important piece of evidence in support of a dual origin hypothesis. Through the functional analysis of *vestigial* (*vg*), a critical wing gene, we found that there are two *vg*-dependent tissues in the 'wingless' T1 of the *Tribolium* beetle (the carinated margin and two pleural plates) (Figure 2) [22\*\*]. *vg* is known as the wing master gene due to its ability to induce wings in non-wing contexts when ectopically expressed (with some limitations) [23,24]. The ectodermal function of *vg* appears to be specific to wing formation (at least in *Drosophila*), making this gene an interesting candidate for the identification of wing-related structures.

The identification of two *vg*-dependent tissues in the T1 segment of *Tribolium* was quite surprising because it is generally assumed that wing-related tissues are never induced in T1 of most (if not all) insects. Interestingly, the only *vg*-dependent tissues we identified in T2 and T3 were the wings (except for a small region at the base of the

**Box 1 Insect body plan**

Insects have segmented bodies organized into three distinct sections (or tagma), namely head, thorax and abdomen (a). All locomotor appendages (legs and wings) are associated with the thorax, which is composed of three segments (T1, T2, and T3). Generally, insects possess two pairs of wings, one pair on T2 and the other pair on T3. Although T1 possesses a pair of walking legs, it is 'wingless' in all extant insects. Abdominal segments are generally appendage-less, with the exception of some appendages retained in basal orders (such as *Collembola*) or secondarily acquired appendages in some insect lineages [2].

Dorsoventrally, the insect thoracic body wall can be subdivided into three distinct regions; dorsal, lateral, and ventral. The dorsal body wall is composed of a large plate called the notum or thoracic tergum. The lateral portion consists of several pleural plates collectively referred to as the pleuron. The ventral body wall is also often sclerotized and forms the sternum (b). The pleural plates are thought to have stemmed from ancestral proximal leg segments that have fused into the body wall of extant insects (b) [39]. Recent molecular analyses also support the proximal leg segment origin of the pleural plates [22\*\*,40,41].



T3 leg), suggesting that the T1 *vg*-dependent tissues may be serially homologous to the wings on T2 and T3.

The serial homology between the T1 *vg*-dependent tissues and the wing is further supported by analysis of homeotic transformations. In insects, segmental identities are determined by a cluster of genes, called Hox genes (reviewed in [25]). Serially homologous but distinct structures are often produced by the action of Hox genes (such as with wings and halteres in flies [26,27], also see Box 3). In T1, a Hox gene, *Sex combs reduced* (*Scr*), has been shown to repress wing development [28,29]. We found that reducing *Scr* function in *Tribolium* leads to the merger of the two *vg*-dependent tissues and the transformation of the merged tissues into an ectopic wing, strongly suggesting that these two *vg*-dependent tissues are wing serial homologs in T1 (Figure 2) [22\*\*].

Intriguingly, these two tissues appear to be homologous to the two proposed wing origins (Figure 2). The carinated margin is tergal in nature and therefore can be related to the tergal/paranotal lobe hypothesis. In contrast, the

**Box 2 History of the wing origin debate**

Throughout the history of the insect wing origin debate, popular opinion has shifted between variations of the two prominent hypotheses without reaching any consensus. The first mention of a possible insect wing origin in the literature appears to be by Oken in 1809 [3,6,42,43], which predates the Darwinian era. In his 1809 publication, Oken claimed that insect wings were analogous to gills of aquatic arthropods [44,45\*]. Despite the fact that there was no concept of evolution at the time, Oken proposed that insect wings and arthropod gills share some similarity (although he also claimed that the insect wing and the bird feather are related, which is completely false). This idea was expanded to explain that ancestral wings are derived from pleural associated tracheal gills or tracheated gill accessory structures (gill plates) (reviewed in [17,42,43,46]). A shift away from a pleural-associated origin of wings came with Müller in 1875. Müller observed that the thoracic lobes of immature termites resemble wings, but are not initially tracheated and therefore cannot be of tracheal gill origin (mentioned in [17,42,43]). This marked the early beginning of the reign of the paranotal hypothesis, which was expanded and supported by many scientists [11,12,17,47]. In 1978, through examination of Paleozoic pterygote nymphs, Kukalova-Peck proposed that wings are of subcoxal (pleural) origin. Later she amended this hypothesis to link the origin of wings to the exite of a hypothetical proximal-most segment of the ancestral arthropod leg (termed epicoxa) [14\*]. Although the presence of pleural exites in fossil insects has been under debate [36–38], this exite hypothesis gained momentum when an evo-devo study provided evidence supporting the homology between crustacean exites and insect wings [18\*\*].

two pleural plates can be related to the pleural/exite hypothesis. Therefore, the two T1 *vg*-dependent tissues could be wing serial homologs present in (or reverted to) a more ancestral state, and their merger to form a complete wing upon homeotic transformation provides compelling functional evidence for the dual origin of insect wings.

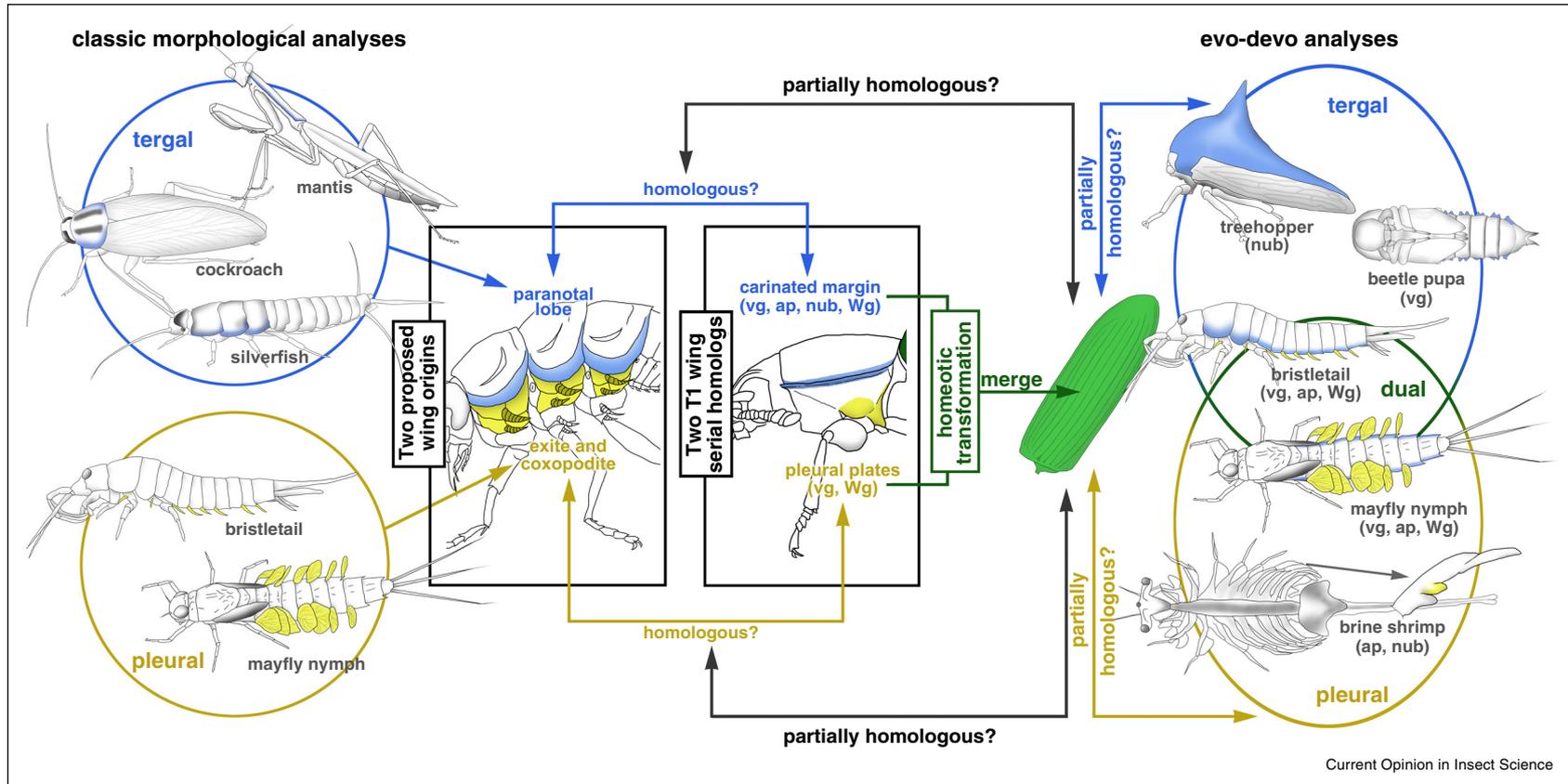
**Abdominal *vg*-dependent wing serial homologs in beetles**

Ohde *et al.* also found that there are *vg*-dependent tissues in T1 as well as in other non-winged segments of the *Tenebrio* beetle [30\*\*]. The hypomeron in the T1 segment identified as *vg*-dependent in this study corresponds to the carinated margin in our study (the carinated margin is a part of the hypomeron [22\*\*]). Additionally, they found that defensive structures in the pupal abdomen, called gin traps, are also *vg*-dependent. Further analysis of homeotic transformations led them to propose that gin traps are wing serial homologs in the abdominal segments (Figure 2). This study along with our own led to the realization that wing serial homologs in the wingless segments are more common than initially anticipated and also signifies the potential usefulness of identifying *vg*-dependent tissues to reveal wing serial homologs in other insects.

**Treehopper helmets: 'partial' wing serial homologs?**

Before the above-mentioned two studies in beetles, Prud'homme *et al.* found that treehopper helmets have high *nub* expression and proposed that these impressively

Figure 2



Visual overview of wing serial homologs proposed by classical and evo-devo studies, along with a conceptual framework of the dual origin hypothesis. The locations of proposed wing homologs in various arthropods are indicated by blue (tergal) and yellow (pleural). The elytron resulting from the merger of two wing serial homologs via homeotic transformation is indicated by green. Note that 'partially homologous' in the figure refers to a structure being homologous to a part of the wing, and is not meant to imply gradual degrees of homology.

**Box 3 Serial homology and Hox mutant analysis in insects.**

**Serial homology:** Segmentally organized bodies of insects are a reflection of the ancestral arthropod body plan, which consists of repeats of a fairly uniform segmental unit (a) [48]. Each of these ancestral segments is thought to have possessed a set of common structures, such as a pair of legs. These structures on one segment are considered to be serially homologous to the corresponding structures on the other segments, as they share the same developmental and evolutionary origin.

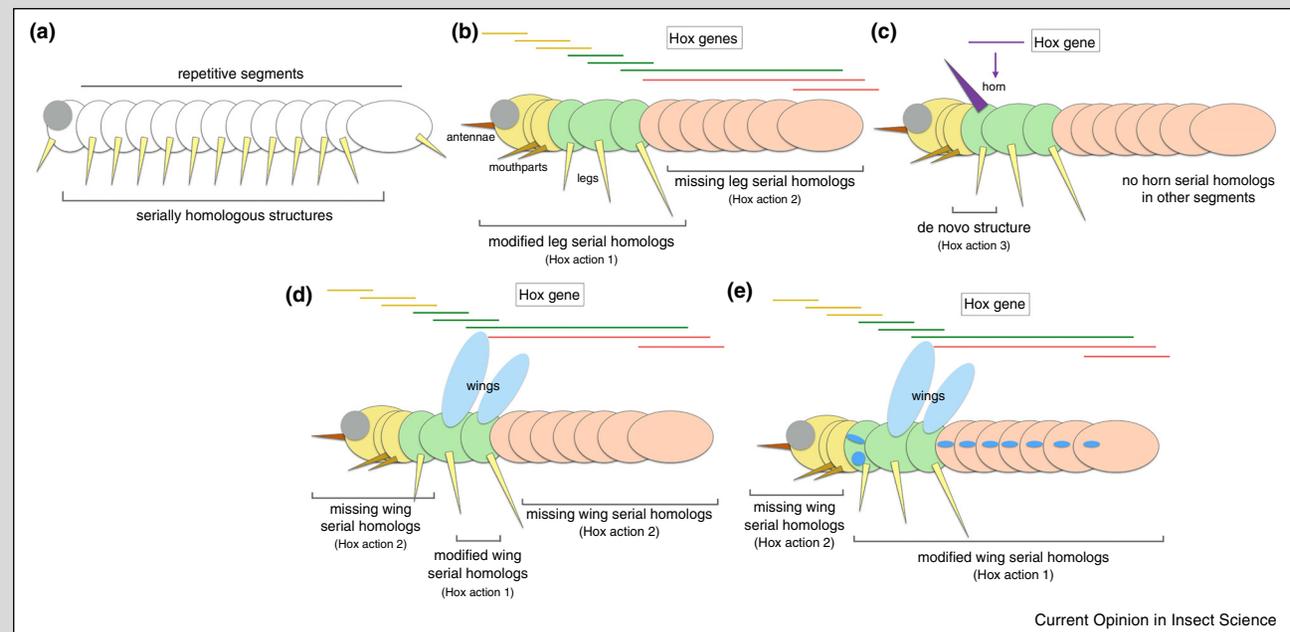
**Hox function:** Hox genes are region selector genes that provide individuality to the otherwise uniform segments and segmentally repetitive structures [48–50]. Hox achieves this task via at least three distinct modes of action, (i) modification of preexisting structures, (ii) suppression of preexisting structures, and (iii) induction of de novo structures. Through these actions, serially homologous structures are modified uniquely in each segment. Sometimes the modification is so drastic, that it is difficult to recognize homology based on morphology.

**Tissue marker genes:** Some genes are expressed predominantly in a certain tissue. These genes can be used to identify serial homologs, as serially homologous tissues are expected to share the expression of marker genes. *vg* is one of the wing marker genes, while *Distalless (Dll)* is often used as a marker for leg-related identity [51]. Although, caution must be taken when identifying serial homologs based on marker genes, as these genes could be coopted into a new context. An example of cooption is *Dll* expression in the beetle horn [34].

**Identifying serial homologs:** Removing Hox function will allow us to strip away the modifications applied to the serially homologous structures, revealing serial homologs that are otherwise morphologically distinct and difficult to recognize. In addition, marker genes will be useful to gain more information regarding the mode of Hox action, and also to determine the precise cell population that is transformed and/or ectopically induced in Hox mutations. Some potential outcomes when Hox function is removed are as follows:

- **Transformation of one structure to another.** This happens when Hox acts to modify the structure (Hox action 1), thus these two structures are likely serially homologous. Insect mouthparts and legs are in this category (b) (reviewed in [48]). These structures share *Dll* marker gene expression, further supporting their serial homology.
- **Induction of ectopic structures.** This happens when Hox acts as a suppressor (Hox action 2). The absence of leg serial homologs in the abdominal segments belongs to this category (b) [48]. A key difference compared to the above transformation is that there are no *Dll* expressing cells in the abdomen unless Hox is removed.
- **Loss of the structure examined.** This happens when Hox itself acts as an inductive signal for the structure (Hox action 3), suggesting that the structure was evolved de novo, and that there is no serial homolog of this structure in other segments. Beetle horns may belong to this category (c) [52].

**Wing serial homologs:** Wings are unique to the two thoracic segments (T2 and T3) in extant insects (d and e). Since there are no apparent wing-like structures in other segments, and removing Hox genes could induce wing formation in these segments, it has been considered that induction of wing-related structures is suppressed by Hox in these segments (Hox action 2, d). However, recent evo-devo studies have revealed that there are some tissues outside of T2 and T3 that express *vg*, a wing marker gene [22\*\*,30\*\*]. Furthermore, in beetles, these tissues transform into the wing upon Hox mutation. These results suggest that the *vg*-positive tissues in the wingless segments found in beetles are wing serial homologs modified by Hox (thus this is Hox action 1 instead of action 2, e).



exaggerated structures are modified wings of the T1 segment (Figure 2) [31\*]. Unfortunately, this report was disputed rather intensely due to errors in their morphological interpretation [32,33]. Further morphological analyses have revealed that the treehopper helmet is an extreme posterior expansion of T1 tergum and not an appendage on T1 [32,33]. As we discussed in our previous report [22\*\*], the recognition of the two wing serial homologs in T1 of *Tribolium* may provide an alternative explanation for the identity of the treehopper helmet. Since both the treehopper helmet and the beetle carinated margin are an expansion of T1 tergum, it is likely that these two tissues are homologous, suggesting the possibility that the treehopper helmet is not an appendage, yet may be serially homologous to a part of the wing (i.e. partially serially homologous to the wing).

This treehopper helmet discussion offers an interesting (and perhaps rather thought provoking) view to the insect wing origin debate; the wing serial homologs in wingless segments may be more widespread among insects than initially anticipated. In 1916, Crampton suggested that pronotal expansions seen in various insects (such as silverfish, mantises, hemipterans, and several more species) could be wing serial homologs [17] (and more insects are mentioned in [10\*]). We propose that most (if not all) tergal margins may actually be ‘partially’ serially homologous to the wing (i.e. to the portion that is tergal in origin). A comprehensive survey for wing serial homologs in various insects through an evo-devo approach will shed light on the widespread occurrence of wing serial homologs in the wingless segments of insects.

### An evo-devo based dual origin hypothesis

Two important insights obtained from evo-devo studies to date on the origin of insect wings are (i) a possible widespread nature of wing serial homologs in the wingless segments, and (ii) the presence of two distinct types of tissues individually serially homologous to a part of the wing (tergal and pleural). Another critical observation we made in our previous study is that the tergal and pleural wing serial homologs in T1 merge to form a complete wing upon homeotic transformation in *Tribolium* [22\*\*]. Together, these findings allow us to revise the dual origin hypothesis from an evo-devo perspective, namely, *insect wings have a dual origin, and the merger of two unrelated tissues (tergal and pleural) was a key step in developing this morphologically novel structure during evolution*. In this hypothesis, we support and expand the idea that was initially put forward by Niwa *et al.* [21\*\*]; that tergal expansion has provided a genetic mechanism responsible for a large and flat wing blade structure, while pleural plates (potentially with exite-like branches) have provided a complex articulation mechanism along with muscle attachment.

The dual origin hypothesis unifies the two major hypotheses by overcoming some limitations of each hypothesis.

For example, the acquisition of a complex articulation mechanism has been difficult to explain by the paranotal hypothesis, but this can be explained by a coxopodite segment (possibly bearing exites) merging into the tergal expansion. On the other hand, the striking similarities between wings and pronotal expansions in some insects (such as vein-like structures in lace bugs) have been used to argue against a pleural origin of insect wings. However, the dual origin hypothesis allows for a pleural contribution to the wing while maintaining the similarities between lobes and wings.

A possible genetic mechanism that facilitated the merger of tergal and pleural tissues is largely unknown. The fusion of the proximal coxopodites into the body wall (i.e. the formation of the pleural plates) that occurred early in the hexapod lineage could have been a key step in placing the two distinct *vg*-dependent tissues in close proximity. Subsequently, the induction of two *vg*-dependent tissues adjacent to each other may have caused a ‘cross-wiring’ of the two distinct developmental pathways, resulting in one fused *vg*-dependent tissue, namely a wing. It is worth mentioning that we have detected two distinct *vg*-positive cell populations in all three thoracic segments during embryogenesis in *Tribolium* [22\*\*]. This observation suggests that the two types of wing serial homologs are initially induced separately even in the winged segments (T2 and T3) of extant insects (i.e. partial recapitulation of phylogeny in ontogeny). An in-depth analysis of how the two distinct *vg*-positive cell populations induced during embryogenesis contribute to the wing imaginal tissues in *Tribolium* may help us understand how the merger occurred during the evolution of insect wings.

### Testing the dual origin hypothesis

Identification of wing serial homologs based on morphological analysis has been the main approach in the attempt to elucidate the origin of insect wings. However, this approach can detect tissues potentially homologous to wings only when these tissues resemble wings. As described above, identifying *vg*-dependent ectodermal tissues appears to be an effective alternative way to find wing serial homologs, regardless of their morphological similarity to wings. Comprehensive identification of *vg*-dependent tissues throughout insect taxa will provide further clues to the identity and nature of these tissues and how these tissues (or more precisely, the tissues in the winged segments that are homologous to them) have contributed to the evolution of wings. A quick survey of *vg* orthologs in publicly available insect genome sequences revealed that *vg* is conserved throughout arthropod taxa (Clark-Hachtel and Tomoyasu, unpublished data). Thus, a survey of *vg*-dependent tissues via expression and/or functional analyses should be applicable to many insect species and possibly even to non-insect arthropods, such as crustaceans.

The dual origin hypothesis will be supported if we find two distinct sets of *vg*-positive cells, one in the tergum and the other in the pleuron, in a diverse array of insects. It is also important that these serial homologs are present in some of the wingless segments (such as T1), but not in the winged segments (T2 and T3), as the wings in these segments should correspond to the two wing serial homologs in the wingless segments.

It will be of great interest to also identify *vg*-dependent tissues from non-insect arthropods, such as crustacean species. The *vg*-dependent tissues in the tergum and/or proximal legs may represent crustacean tissues homologous to insect wings, though further analysis will be required to reach a conclusion. Identification of potential wing homologs in non-insect arthropods will have a significant impact on the insect wing origin debate by allowing us to identify the tissues that possibly gave rise to wings in the hexapod lineage. Presence of wing homologs both in the tergum and the proximal leg would strongly support a dual origin hypothesis.

Coooption is always a significant caveat when identifying homologous tissues using a marker gene. For example, it is possible that a tissue unrelated to wings gained *vg* expression via cooption, which could hinder identification of true wing serial homologs. Using more than one gene as a wing identity marker (such as *nub* and *ap*) may allow us to identify true wing serial homologs, although it has been shown that even a battery of genes can be coopted together in some contexts (e.g. several leg genes coopted into horn development in *Onthophagus* beetles [34]). Analyzing more species will also help trace the history of the *vg*-expressing tissues, which will allow us to distinguish newly coopted *vg*-expressing tissues from wing-related tissues. In addition to this standard ‘more genes, more species’ approach, we believe that inducing homeotic transformation is very useful to identify serially homologous structures. As mentioned, Hox genes are often responsible for modifying segmentally repeated structures and providing a unique identity to each segment. Thus, if a *vg*-expressing tissue transforms into a wing upon homeotic transformation, that will strongly support that the tissue in question is serially homologous to wing (see Box 3 for further explanation about serially homologous structures and Hox mutations). Identifying *vg*-expressing tissues, followed by testing the potential of these tissues to transform into a wing upon homeotic transformation, will be a powerful way to identify wing serial homologs in various insects.

## Conclusion

Insect wings are a core example of morphological novelty, yet their acquisition remains a biological mystery. A series of evo-devo studies, including our analysis in *Tribolium*, appears to point toward a dual origin of insect wings. However, the contribution of evo-devo to the insect

origin debate is still in its infancy. Detailed expression and functional analyses for more wing genes in various winged and wingless species will be critical to obtain further insights into insect wing origin from an evo-devo point of view.

The evo-devo approach is promising, but has its own limitations. For example, evo-devo analyses do not help determine the selective pressures or the Paleozoic environment that molded the wing from apterygote insect tissues. Evo-devo also does not help reveal the mechanical aspect of the evolution of wings that allowed for efficient flight, nor does it have the ability to reveal the actual shape of the ancient and extinct insects as paleontology can. Instead, evo-devo can provide a new angle to the wing origin debate, which, in combination with other approaches, will provide us with a more comprehensive view of insect wing evolution.

The wing origin studies can also impact evolutionary biology in general. Morphological innovation is a fundamental process in evolution, yet the molecular mechanism underlying this process remains elusive. Cooption is often implicated in the evolution of morphologically novel structures [35]. As discussed, we think that the evolution of insect wings might have been facilitated via a distinct mechanism, i.e. ‘cross wiring’ between two developmental systems that relied on a similar set of genes. The application of evo-devo to the study of insect wing origin will broaden the scope of evo-devo by leading to a more comprehensive view of the molecular mechanisms underlying the evolution of morphologically novel structures.

## Note added in proof

After the acceptance of this manuscript, Medved *et al.* reported the contribution of tergal and pleural tissues to the wing in a hemimetabolous insect, further supporting a dual origin of insect wings [53].

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