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3 **Exploring the origin of insect wings from an evo-devo**
4 **perspective**

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

19

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 the pleuron-associated structures (Figure 1. See Box 1 for insect anatomy) [10]. 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 epicoxa) (Figure 1) [14,15]. According to this hypothesis, the exites migrated dorsally and flattened to eventually become the wing blade recognized today [13,14,16], 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].

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 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, all 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 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 1900's 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 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 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 hypomerion [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?

Prior to the previous two studies in beetles, Prud'homme et al. found that treehopper helmets have high *nub* expression and proposed that these impressively 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 (dorsal 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 fusion 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 on 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

204 detect tissues potentially homologous to wings only when these tissues resemble wings. As
205 described above, identifying *vg*-dependent ectodermal tissues appears to be an effective
206 alternative way to find wing serial homologs, regardless of their morphological similarity to
207 wings. Comprehensive identification of *vg*-dependent tissues throughout insect taxa will provide
208 further clues to the identity and nature of these tissues and how these tissues (or more precisely,
209 the tissues in the winged segments that are homologous to them) have contributed to the
210 evolution of wings. A quick survey of *vg* orthologs in publicly available insect genome
211 sequences revealed that *vg* is conserved throughout arthropod taxa (Clark-Hachtel and Tomoyasu,
212 unpublished data). Thus, a survey of *vg*-dependent tissues via expression and/or functional
213 analyses should be applicable to many insect species and possibly even to non-insect arthropods,
214 such as crustaceans.

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

220 It will be of great interest to also identify *vg*-dependent tissues from non-insect
221 arthropods, such as crustacean species. The *vg*-dependent tissues in the tergum and/or proximal
222 legs may represent crustacean tissues homologous to insect wings, though further analysis will
223 be required to reach a conclusion. Identification of potential wing homologs in non-insect
224 arthropods will have a significant impact on the insect wing origin debate by allowing us to
225 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.

Cooption 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.

Acknowledgement

We thank Drs. Pick and Extavour for inviting us to contribute to this special issue, the two anonymous reviewers for their thoughtful comments, and members of Tomoyasu lab for

272 discussion. This work was supported by National Science Foundation (YT: NSF-IOS0950964)
273 and an NSF Graduate Research Fellowship (CCH).

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Figure Legends

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 relevant to this review 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 the results in opposition to the pleural exites, but also see [38] for a possible confirmation of some of these exites).

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 1: Insect body plan

Insects have segmented bodies organized into three distinct sections (or tagma), namely head, thorax and abdomen (A). All locomotion 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, each on T2 and 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].

Box 2: History of the wing origin debate

Throughout the history of the insect wing origin debate, the 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 tracheated 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 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 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].

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 segment, 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 the leg-related identity [51]. Though caution must be taken when identifying serial homologs based on marker genes, as these genes could be co-opted into a new context. An example is *Dll* expression in the beetle horn [52].

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 leg serial homologs 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, 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 are 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).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Grimaldi D, Engel MS: *Evolution of the Insects*: Cambridge University Press; 2005.
2. Division of Entomology CSIRO: *The Insects of Australia; a textbook for students and research workers* edn second edition. Carlton, Vic.: Melbourne University Press; 1991.
3. Dudley R: **Evolution of Flight and Flightlessness**. In *The Biomechanics of Insect Flight*. Edited by: Princeton University Press; 2000:261-301.
4. Kukalova-Peck J: **Fossil History and the Evolution of Hexapod Structures**. In *The Insects of Australia: A textbook for students and research workers*, edn second. Edited by Naumann ID: Melbourne University Press; 1991:141-179. vol 1.]
5. Grimaldi D, Engel MS: **Fossil Insects**. In *Evolution of the Insects*. Edited by: Cambridge University Press; 2005:42-92.
6. La Greca M: **Origin and evolution of wings and flight in insects**. *Bolletino di zoologia* 1980, **47:sup1**:65-82.
7. Labandeira CC, Beall BS, Hueber FM: **Early Insect Diversification: evidence from a Lower Devonian bristletail from Quebec**. *Science* 1988, **242**:913-916.
8. Labandeira CC: **Rise and Diversification of Insects**. In *Palaeobiology II* Edited by Briggs DEG, Crowther PR: Wiley-Blackwell; 2001:82-88.
9. Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, et al.: **Phylogenomics resolves the timing and pattern of insect evolution**. *Science* 2014, **346**:763-767.
10. Quartau JA: **An overview of the paranotal theory on the origin of the insect wings**. *Publicações do Instituto de Zoologia "Dr. Augusto Nobre" Faculdade de Ciencias do Porto* 1986, **194**:1-42.
 - This article comprehensively summarizes the arguments for both tergal and pleural wing origin at a time just prior to the use of evo-devo to study wing origin.
11. Rasnitsyn AP: **A Modified Paranotal Theory of Insect Wing Origin**. *Journal of Morphology* 1981, **168**:331-338.
12. Hamilton KGA: **The insect wing, Part 1. Origin and development of wings from notal lobes**. *Journal of the Kansas Entomological Society* 1971, **44**:421-433.
13. Grimaldi D, Engel MS: **Insects Take to the Skies**. In *Evolution of the Insects*. Edited by: Cambridge University Press; 2005:155-187.

- 412 14. Kukalova-Peck J: **Origin of the insect wing and wing articulation from the arthropodan**
 413 **leg.** *Canadian Journal of Zoology* 1983, **61**:1618-1669.
- 414 •The revised exite theory proposed in this article sparked a reconsideration of pleural tissue
 415 involvement in insect wing evolution.
- 416 15. Kukalova-Peck J: **Phylogeny of Higher Taxa in Insecta: Finding Synapomorphies in the**
 417 **Extant Fauna and Separating Them from Homoplasies.** *Evol Dev* 2008, **35**:4-51.
- 418 16. Kukalova-Peck J: **Origin and evolution of insect wings and their relation to**
 419 **metamorphosis as documented by the fossil record.** *Journal of Morphology* 1978,
 420 **156**:53-125.
- 421 17. Crampton G: **The Phylogenetic Origin and the Nature of the Wings of Insects**
 422 **According to the Paranotal Theory.** *New York Entomological Society* 1916, **24**:1-39.
- 423 18. Averof M, Cohen SM: **Evolutionary origin of insect wings from ancestral gills.** *Nature*
 424 1997, **385**:627-630.
- 425 ••This article is the first to apply evo-devo to the insect wing origin debate and was influential in
 426 changing the popular opinion from a tergal origin to a pleural origin.
- 427 19. Cohen B, McGuffin ME, Pfeifle C, Segal D, Cohen SM: **apterous, a gene required for**
 428 **imaginal disc development in Drosophila encodes a member of the LIM family of**
 429 **developmental regulatory proteins.** *Genes Dev* 1992, **6**:715-729.
- 430 20. Ng M, Diaz-Benjumea FJ, Cohen SM: **Nubbin encodes a POU-domain protein required**
 431 **for proximal-distal patterning in the Drosophila wing.** *Development* 1995, **121**:589-
 432 599.
- 433 21. Niwa N, Akimoto-Kato A, Niimi T, Tojo K, Machida R, Hayashi S: **Evolutionary origin of**
 434 **the insect wing via integration of two developmental modules.** *Evol Dev* 2010,
 435 **12**:168-176.
- 436 ••This is the first evo-devo study to perform expression analyses for wing genes in basal
 437 insects.
- 438 22. Clark-Hachtel CM, Linz DM, Tomoyasu Y: **Insights into insect wing origin provided by**
 439 **functional analysis of vestigial in the red flour beetle, Tribolium castaneum.** *Proc*
 440 *Natl Acad Sci U S A* 2013, **110**:16951-16956.
- 441 ••This article identifies tergal and pleural wing serial homologs in T1 of *Tribolium*, and provides
 442 the first functional evidence for a dual origin of insect wings.
- 443 23. Baena-Lopez LA, Garcia-Bellido A: **Genetic requirements of vestigial in the regulation of**
 444 **Drosophila wing development.** *Development* 2003, **130**:197-208.
- 445 24. Halder G, Polaczyk P, Kraus ME, Hudson A, Kim J, Laughon A, Carroll S: **The Vestigial**
 446 **and Scalloped proteins act together to directly regulate wing-specific gene**
 447 **expression in Drosophila.** *Genes Dev* 1998, **12**:3900-3909.
- 448 25. Carroll S, Grenier JK, Weatherbee SD: *From DNA to Diversity* edn second edition: Blackwell
 449 publishing; 2005.

- 450 26. Lewis EB: **A gene complex controlling segmentation in *Drosophila***. *Nature* 1978,
451 **276**:565-570.
- 452 27. Weatherbee SD, Halder G, Kim J, Hudson A, Carroll S: **Ultrabithorax regulates genes at**
453 **several levels of the wing-patterning hierarchy to shape the development of the**
454 ***Drosophila* haltere**. *Genes Dev* 1998, **12**:1474-1482.
- 455 28. Tomoyasu Y, Wheeler SR, Denell RE: **Ultrabithorax is required for membranous wing**
456 **identity in the beetle *Tribolium castaneum***. *Nature* 2005, **433**:643-647.
- 457 29. Carroll SB, Weatherbee SD, Langeland JA: **Homeotic genes and the regulation and**
458 **evolution of insect wing number**. *Nature* 1995, **375**:58-61.
- 459 30. Ohde T, Yaginuma T, Niimi T: **Insect morphological diversification through the**
460 **modification of wing serial homologs**. *Science* 2013, **340**:495-498.
- 461 ••This article identifies wing serial homologs in T1 and the abdomen of a *Tenebrio* beetle,
462 signifying the use of wingless segments in the investigation of wing origin.
- 463 31. Prud'homme B, Minervino C, Hocine M, Cande JD, Aouane A, Dufour HD, Kassner VA,
464 Gompel N: **Body plan innovation in treehoppers through the evolution of an extra**
465 **wing-like appendage**. *Nature* 2011, **473**:83-86.
- 466 •This article challenges a classic view regarding the identity of the treehopper helmet, proposing
467 that it is a wing homolog. But see [32] and [33] for an alternative interpretation.
- 468 32. Miko I, Friedrich F, Yoder MJ, Hines HM, Deitz LL, Bertone MA, Seltmann KC, Wallace MS,
469 Deans AR: **On dorsal prothoracic appendages in treehoppers (Hemiptera:**
470 **Membracidae) and the nature of morphological evidence**. *PLoS ONE* 2012,
471 **7**:e30137.
- 472 33. Yoshizawa K: **The treehopper's helmet is not homologous with wings (Hemiptera:**
473 **Membracidae)**. *Systematic Entomology* 2012, **37**:2-6.
- 474 34. Moczek AP, Rose DJ: **Differential recruitment of limb patterning genes during**
475 **development and diversification of beetle horns**. *Proc Natl Acad Sci U S A* 2009,
476 **106**:8992-8997.
- 477 35. Shubin N, Tabin C, Carroll S: **Deep homology and the origins of evolutionary novelty**.
478 *Nature* 2009, **457**:818-823.
- 479 36. Bethoux O, Briggs DEG: **How *Gerarus* lost its head: stem-group Orthoptera and**
480 **Paraneoptera revisited**. *Systematic Entomology* 2008, **33**:529-547.
- 481 37. Boxshall GA: **The evolution of arthropod limbs**. *Biological Reviews* 2004, **79**:253-300.
- 482 38. Haug JT, Haug C, Garwood RJ: **Evolution of insect wings and development - new**
483 **details from Palaeozoic nymphs**. *Biol Rev Camb Philos Soc* 2014.
- 484 39. Snodgrass RE: **The Thorax**. In *Principles of Insect Morphology*. Edited by: Cornell
485 University Press; 1935:157-192.
- 486 40. Coulcher JF, Edgecombe GD, Telford MJ: **Molecular developmental evidence for a**
487 **subcoxal origin of pleurites in insects and identity of the subcoxa in the gnathal**
488 **appendages**. *Sci Rep* 2015, **5**:15757.

- 489 41. Linz DM, Tomoyasu Y: **RNAi screening of developmental toolkit genes: A search for**
490 **novel wing genes in the red flour beetle *Tribolium castaneum*.** *Dev Genes Evol*
491 2015, **218**.
- 492 42. Wigglesworth VB: **Evolution of insect wings and flight.** *Nature* 1973, **246**:127-129.
- 493 43. Wigglesworth VB: **The evolution of insect flight.** *Symposia of the Royal Entomological*
494 *Society of London* 1976, **7**:255-269.
- 495 44. Oken L: *Lehrbuch der Naturphilosophie*, vol 1-3; 1809-1811.
- 496 45. Oken L: *Elements of Physiophilosophy*: Ray Society; 1847.
- 497 •This manuscript is the English translation of [39]. The author attempts to make comparisons
498 between various organisms and is the first to speculate on an origin of insect wings.
- 499 46. Woodworth CW: **The wing veins of insects.** *University of California publications technical*
500 *bulletins* 1906, **1**:1-152.
- 501 47. Snodgrass RE: **The Wings.** In *Principles of Insect Morphology*. Edited by: Cornell University
502 Press; 1935:211-245.
- 503 48. Hughes CL, Kaufman TC: **Hox genes and the evolution of the arthropod body plan.** *Evol*
504 *Dev* 2002, **4**:459-499.
- 505 49. Angelini DR, Kaufman TC: **Comparative developmental genetics and the evolution of**
506 **arthropod body plans.** *Annu Rev Genet* 2005, **39**:95-119.
- 507 50. Pearson JC, Lemons D, McGinnis W: **Modulating Hox gene functions during animal**
508 **body patterning.** *Nat Rev Genet* 2005, **6**:893-904.
- 509 51. Cohen SM: **Specification of limb development in the *Drosophila* embryo by positional**
510 **cues from segmentation genes.** *Nature* 1990, **343**:173-177.
- 511 52. Wasik BR, Rose DJ, Moczek AP: **Beetle horns are regulated by the Hox gene, *Sex***
512 **combs reduced, in a species- and sex-specific manner.** *Evol Dev* 2010, **12**:353-362.

Figure 1

Figure 2