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Ultrabithorax and the evolution of insect forewing/hindwing differentiation

Yoshinori Tomoyasu

Decades have passed since the stunning four-winged phenotype of the *Drosophila Ultrabithorax* (*Ubx*) mutant was reported, and accumulating knowledge obtained from studies on *Ubx* in *Drosophila* has provided a framework to investigate the role of *Ubx* during insect wing evolution. With several new insights emerging from recent studies in non-*Drosophila* insects, along with the outcomes of genomic studies focused on identifying Ubx targets, it appears to be an appropriate time to revisit the *Drosophila* paradigm regarding insect wing development and evolution. Here, I review the recent findings related to Ubx during wing development, and discuss the impact of these findings on the current view of how *Ubx* came to regulate wing differentiation in the evolution of insect flight structures.

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Introduction

Insect wings display an astonishing array of diversity in their shape, size, and color, which has captivated scientists and nonscientists alike for centuries [1]. Although the origin of insect wings remains a mystery and is regarded as a chief conundrum in biology [2], it is generally assumed that insect wings have evolved only once in the hexapod lineage (i.e. are monophyletic) [3–5]. Based on the fossil record, the common ancestor of all winged-insects appears to have had two (or perhaps three) pairs of large membranous flight wings on its second and third thoracic segments (T2 and T3) [6], similar to the situation seen in the extant paleopteran insects, such as dragonflies. Those two pairs of wings have been modified uniquely in each lineage throughout the evolution of winged insects, allowing them to pursue various niches. In addition, modification often occurs differently between the two pairs of wings (i.e. forewing/hindwing differentiation), creating further diversity in insect wings. Diversification of wings has been a major driving force for the successful radiation of insects, which has made them one of the dominant taxa on this planet. Although wing development has been extensively studied in the fruit fly, *Drosophila melanogaster*, the molecular and developmental mechanisms driving the production of diverse wings in the vast majority of insect orders have just begun to be investigated. In this review, I focus on the evolution of forewing/hindwing differentiation mechanisms (with a particular emphasis on the Hox gene *Ultrabithorax*, *Ubx*), and discuss how new insights obtained from recent studies in *Drosophila* and other insects impact our current view of the molecular mechanisms responsible for the creation of some of the most beautiful examples of diversity seen on this planet.

Forewing/hindwing differentiation in *Drosophila*

As is true in many other developmental contexts, the forewing/hindwing differentiation mechanism has been most intensively studied in *Drosophila*. In dipteran insects, such as *Drosophila*, the forewing (T2 wing) is used for flight, while the hindwing (T3 wing) is highly reduced to form a balancing structure called a haltere. The key factor that controls this forewing/hindwing differentiation is the Hox gene, *Ubx*. Since the removal of *Ubx* alone is sufficient to induce a transformation of haltere into forewing, *Ubx* is generally regarded as the selector gene of haltere identity [7]. Intrigued by the stunning four-winged phenotype of the *Ubx* mutant fly that was first reported by Lewis [8], many studies have sought to uncover the function of *Ubx* in regard to forewing/hindwing differentiation in *Drosophila*. Those studies have revealed that Ubx controls the transcription of many genes involved in wing development (wing genes). Ubx acts as a repressor of the expression of several known wing genes [9,10], however, it also activates some haltere-specific genes [11•,12•]. An unexpected outcome of these studies was the realization that, instead of repressing at a single point at the top of the wing gene network, Ubx appears to act as a micromanager in haltere, repressing multiple steps in the wing gene network [9,13]. Among the wing genes that are repressed by Ubx are *spalt* (*sal*), *vestigial* (*vg*), *serum response factor* (*srf*), *knirps* (*kn*), and *achaete|scute* (*ac|sc*) [9]. These genes code for transcription factors essential for wing patterning and development. For *sal* and *kn*, it was shown that Ubx directly binds to the *cis*-regulatory elements of these

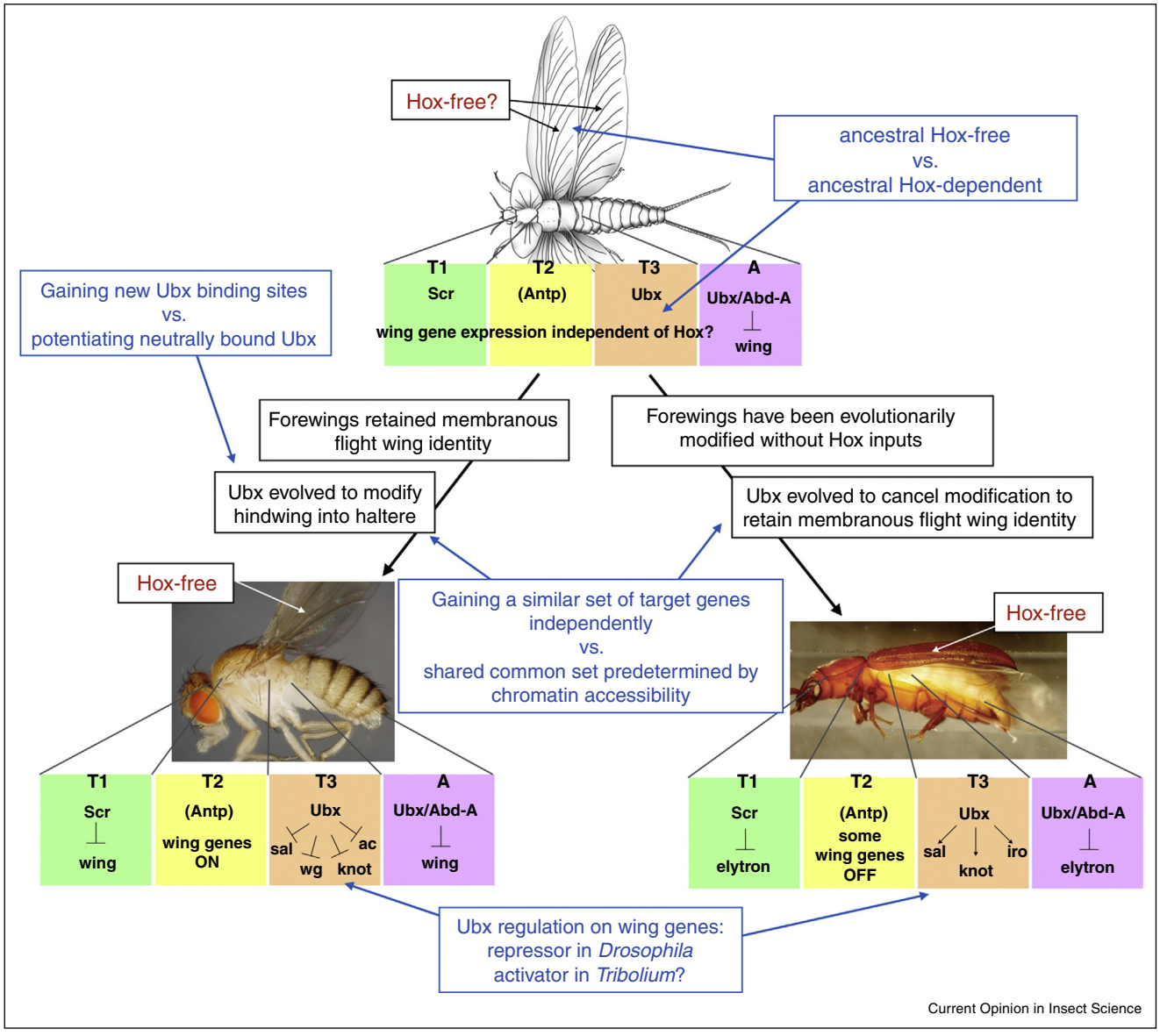
genes and suppresses their expression [14,15]. In addition, Ubx also regulates the activity of some growth signals (such as Dpp, Wg, and Hippo signals) by controlling the expression of some of their signal transducer genes, and promotes the haltere identity in a cell non-autonomous manner [10,11•,16–21].

In contrast to the *Ubx*-dependent state of the dipteran hindwing, the forewing in Diptera is thought to be a Hox-free state, since removing the function of the only Hox gene expressed in the forewing, *Antennapedia* (*Antp*), has no effect on wing morphology [22,23].

Evolutionary implication of *Drosophila* findings

An interesting view of the evolution of insect forewing/hindwing emerges when we consider the evolutionary implications of the findings obtained from *Drosophila* studies (Figure 1). As mentioned, fossil evidence suggests that an ancestral insect had two (or three) pairs of relatively unmodified wings on their thoracic segments. Since *Drosophila* forewings (which maintain relatively ancestral morphology) are Hox-free structures, the assumption is that all of the ancestral wings were Hox-free structures, and *Ubx* (or any other Hox genes) did not have any

Figure 1



The current view of the role of Ubx in the evolution of insect wings and the questions raised by recent genomics and genetics studies in various insects (blue box).

significant function during wing development [7,22]. In the lineage leading to *Drosophila*, *Ubx* has come to modify hindwings to form halteres by gaining an array of target genes (Figure 1). The acquisition of target genes by Ubx has likely been achieved through the evolution of Ubx binding sites in the *cis*-regulatory elements of wing genes [9,12•,14,15]. In contrast, *Drosophila* forewings have maintained a Hox-free state, namely the membranous flight wing. The micromanager role of Ubx may imply that the evolution of the haltere happened gradually, through Ubx sequentially acquiring control over various wing genes.

This view fits well with the situation in butterflies, as Ubx is also expressed in the hindwing and determines hindwing specific traits [24,25]. Therefore, it is possible to think that, in butterflies, the lineage specific modifications have occurred on both forewings and hindwings (such as the scales that cover butterfly wings [26] and eye spots [27]), while Ubx has come to regulate a set of wing genes different from that of *Drosophila*, orchestrating forewing/hindwing differentiation in a butterfly-specific manner [24].

Forewing/hindwing differentiation in beetles

The two pairs of insect wings have been modified uniquely in various lineages, some of which may not be easily explained with the *Drosophila* paradigm described above. One such example is found in Coleoptera. Forewing/hindwing differentiation in beetles is, in a way, reversed compared to that of dipteran insects; it is the forewing (elytron) that is modified, while the hindwing retains more ancestral flight wing characteristics. One possible explanation for this 'reversed' modification is that *Ubx* plays a role in the forewing instead of hindwing in beetles, and is responsible for the modification of the beetle forewing into the elytron. Some earlier observations indeed supported this view. For example, an allele of classic *Ubx* mutants in the red flour beetle, *Tribolium castaneum*, exhibits morphological abnormalities on their elytra [28,29]. In addition, the *Ubx* expression domain expands into T2 in the *Tribolium* embryo [29] (cf. *Ubx* is expressed in T3 and posterior in *Drosophila* [7,30]). These observations led to the idea that Ubx might be regulating the modification of the forewing in beetles [29].

However, a later analysis revealed that this is not the case [31,32]. Through RNA interference (RNAi) for various Hox genes during the post-embryonic stage of *Tribolium*, the beetle elytron is shown to be the Hox-free state despite its diverged morphology (Figure 1). Ubx is expressed specifically in the beetle hindwing, but instead of modifying wings, Ubx cancels the modifications in the hindwing to retain the more ancestral wing morphology. The beetle orthologs of wing genes that are repressed by Ubx in *Drosophila*, such as *sal*, are positively regulated by *Ubx* in *Tribolium* [31]. It is also worth mentioning that *Ubx* is dispensable for wing development in dipteran and lepidopteran insects [9,24], but is essential for wing development in beetles (i.e. the membranous flight wing of beetle cannot be formed without *Ubx*). These results suggest that *Ubx* has integrated into a basic wing gene network to promote wing development in the beetle lineage, while forewing modification into elytra took place without any Hox input.

Box 1 Exoskeletalization

This is a word introduced by Tomoyasu *et al.* [35•]. Although 'sclerotized' is the word most often used to describe the hardened elytron cuticle, this hardening is not achieved by simple sclerotization. The elytra are indeed highly sclerotized (a biochemistry term describing chemical bridging between proteins [52]). However, this sclerotization is accompanied by several additional processes in elytra, including the induction of more chitin, induction of various cuticle proteins, and pigmentation. Thus, the formation of elytron-type cuticle is achieved by a process that coordinates these independent cuticular processes, namely 'exoskeletalization'.

Evolution of the Hox free state and the *Ubx*-dependent dual functions of wing genes

Subsequent studies in *Tribolium* have provided further insights into how the evolution of elytra took place. The beetle elytra are not simply hardened forewings, but rather display highly diverged morphologies in their shape, size, texture, and color [33,34]. Regarding the acquisition of the 'exoskeletalized' surface of elytra (see Box 1), the exoskeletalization pathway appears to be co-opted into the conserved wing gene network at least three times, first under the vein patterning genes, then under a sensory formation gene (*achaete-scute homolog*, *ASH*) and under the dorsal selector, *apterous* (*ap*, there are two *ap* paralogs in *Tribolium*) [35•,36]. Regarding the unique shape of the elytra, another wing gene, *abrupt* (*ab*), appears to have gained a new function to control the forewing shape in the beetle lineage [37]. The molecular basis underlying the evolution of other elytron unique traits is currently under investigation.

It is important to mention that the genes identified as gaining new functions to induce elytron specific traits (such as *ap*, *ASH*, and *ab*) are also required for the formation of the membranous hindwings. However, in the hindwing, these genes do not induce the elytron-specific traits and instead execute evolutionarily conserved wing functions, such as vein pattern determination and sensory organ induction [35•,37–41]. How, then, does Ubx suppress only the elytron unique functions of these genes without compromising their hindwing functions? A clue is found in the outcome of the *ap* RNAi in *Tribolium*, which resulted in not only the loss of an elytron unique trait in the elytron, but also the ectopic induction of elytron traits in the hindwing [35•]. This result suggests that Ap switches its function depending on the presence

and absence of Ubx; it acts as an elytron-trait inducer when Ubx is absent, but acts as an elytron-trait repressor when Ubx is present. *ap* encodes a LIM homeodomain protein, which is known to interact with another homeodomain protein [42]. Therefore, it is intriguing to think that Ubx physically interacts with Ap to achieve the switching of Ap function in the hindwing. More recently, *optomotor blind* (*omb*), another wing gene, is shown to have similar dual functions [43•]. Omb is well-established as a critical transcription factor promoting cell proliferation and survival during wing development [44]. Surprisingly, Simon and Guerrero showed that, in haltere where Ubx is present, Omb suppresses cell proliferation [43•]. This finding implies that Ubx collaborates with various wing-related transcription factors instead of acting by itself. This mode of action may have allowed Ubx to have another level of regulation over the wing gene network; namely, Ubx not only controls the expression of wing genes, but also modulates the function of the wing related transcription factors. These two levels of regulation might be a hint as to how Ubx has come to regulate wing development very differently among various insects, from modifying the Hox-free state into a more derived structure in flies, to canceling the modifications on the Hox-free state to maintain the more ancestral flight wing characteristics in beetles.

Genome-wide identification of Ubx targets during haltere development in *Drosophila*

Most of the above mentioned studies were focused only on a handful of candidate genes. However, advances in molecular biology techniques have allowed genome-wide analyses, which provided a more comprehensive view of Ubx function during forewing/hindwing differentiation in *Drosophila*.

One such analysis is a microarray-based identification of genes that are differentially expressed between the *Ubx*− and *Ubx*+ conditions (e.g. a comparison between wing and haltere, *Ubx* conditional misexpression versus control, or a comparison between wild-type wing and wing of a *Ubx* gain-of-function allele *Cbx*). Three research groups independently performed these analyses, with each study identifying hundreds of genes potentially regulated by Ubx during *Drosophila* haltere development [11•,12•,45•]. Many known wing genes were identified as potential Ubx targets, but also identified were genes that are involved in fundamental cellular processes and metabolism. These results provided a first look at the pervasiveness of Ubx regulation over the wing gene network. Another interesting finding is that Ubx target genes appear to be largely distinct at different stages of wing development [45•], suggesting a dynamic nature of the regulation by Ubx over the course of haltere development.

The genes identified through the above microarray analyses are candidates for primary Ubx targets, however, some of these genes might be regulated by Ubx indirectly. Three studies sought to identify the direct targets of Ubx using CHIP (CHromatin ImmunoPrecipitation)-chip analysis in *Drosophila* [46•,47•,48•]. This technique allows genome-wide identification of *in vivo* Ubx binding sites during haltere development. These studies identified hundreds of additional genes that can be directly regulated by Ubx. The overlap between the CHIP-chip identified Ubx targets and the Ubx targets from microarray ranges from ~5% (26/542 in [46•] and 191/3400 in [48•]) to ~20% (294/1488 in [47•]). Interpreting this overlap is difficult due to the dynamic nature of the haltere transcriptome throughout the course of development [45•]. Despite this difficulty, the broad consensus is that Ubx binding may not necessarily be associated with the genes whose expression is regulated differentially between wings and halteres. It is still possible that Ubx regulates target gene expression only slightly, at the level that is below detection of current differential gene analyses, or in a spatially distinct manner in the haltere compared to the wing, without affecting the total expression level of the gene in these two tissues. Nonetheless, the large number of gene loci associated with Ubx binding that are expressed similarly between wings and halteres suggests that the binding of Ubx to DNA itself might not be sufficient for the function of Ubx (i.e. having Ubx bound in the vicinity does not mean that the gene is regulated by Ubx). Additional factors may be required for Ubx to be functional, such as other wing related transcription factors, as postulated in the previous section. Agrawal *et al.* also proposed that Trithorax-like (Trl or GAF) might work with Ubx during haltere development [46•]. Chromatin accessibility is also shown to largely influence where Ubx binds in the genome [46•,47•,48•]. For example, Choo *et al.* noticed that the binding profiles of two transcription factors (Ubx and Hth) are very similar (if not identical) in haltere, even though Hth is not functional or expressed in the pouch region of the haltere disk, where Ubx has a major role in determining the identity of haltere [47•]. This suggests that the binding of these transcription factors to the genome may not be as functionally relevant as we initially anticipated. The authors discussed that the Ubx binding loci they identified may be related to chromatin accessibility and may not necessarily be the bona fide Ubx target binding sites. In addition, Slattery *et al.* observed that Ubx binding profiles are similar between halteres and legs (although the authors also saw tissue specific bindings) [48•], even though the sets of genes regulated by Ubx are likely distinct between these two tissues. These observations raise the possibility that Ubx might have a tendency toward binding to open chromatin regions, but some (most?) of these bindings may not be functionally significant on their own.

Later, McKay and Lieb performed Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE)-sequencing to profile genome-wide chromatin accessibility

in various tissues, including developing wings and halteres [49••]. Their finding was rather surprising, as the chromatin accessibility turned out to be almost identical among related tissues, such as wings, halteres, and legs, except at the loci encoding the 'master regulators' [49••]. This result indicates that chromatin accessibility is predetermined by a factor other than the master regulators, and the master regulators (e.g. Ubx in haltere) mainly access these open chromatin regions to activate a tissue specific developmental program. The authors also found that open chromatin profiles for the related tissues (leg, haltere, and wing) change coordinately and significantly over developmental time, which is consistent with the earlier finding obtained from the microarray analysis regarding the dynamic nature of the Ubx target-gene transcriptomes over developmental time [45•].

Taken together, genome-wide analyses in *Drosophila* have revealed several new aspects of Ubx action during haltere development: first, a surprisingly large number of genes (hundreds, if not thousands) might be controlled by Ubx during haltere development, second, Ubx binds to hundreds of loci in the genome during haltere development, but the act of Ubx binding to these loci alone might not be sufficient for Ubx to regulate the expression of nearby genes, third, the sites that Ubx binds in the genome might, in part, be controlled by chromatin accessibility, which is predetermined by a factor other than Ubx, and fourth, additional factors might partner with Ubx to promote haltere development.

New insights obtained from a genome-wide Ubx target identification study in other holometabolous insects

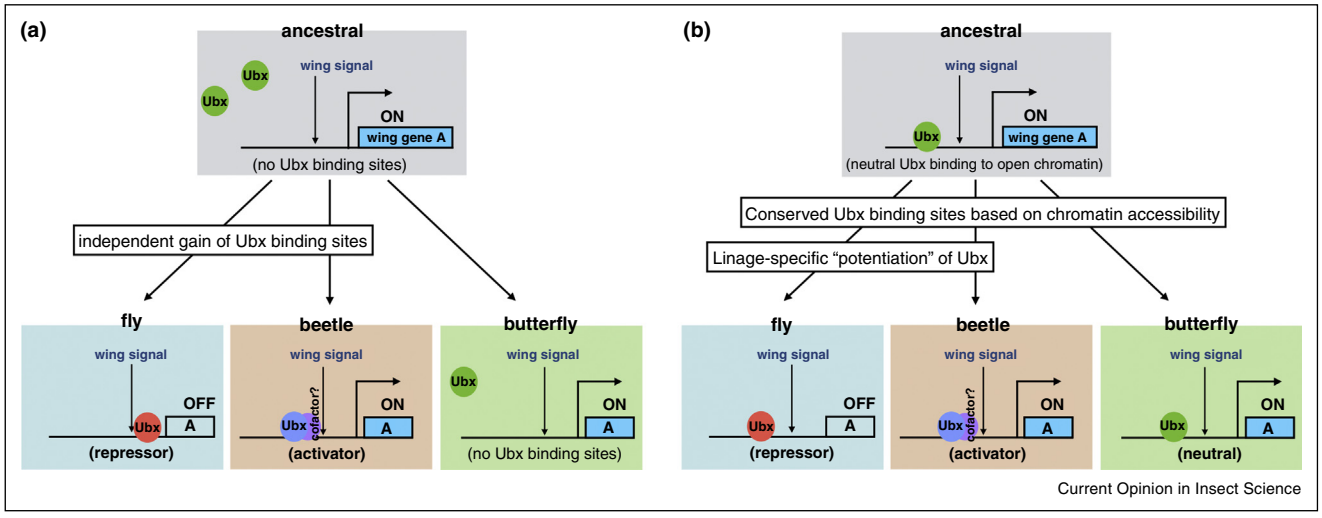
More recently, Prasad and Tarikere *et al.* analyzed the involvement of Ubx during wing development in two additional holometabolous insects, *Bombyx mori* (silkmoth, Lepidoptera) and *Apis mellifera* (honeybee, Hymenoptera) [50••]. Unlike flies and beetles, these two orders of species possess two pairs of flight wings, making comparisons of the Ubx function in these insects to that in *Drosophila* very interesting. The authors performed a series of expression analyses, as well as CHIP-sequencing (CHIP-seq) to identify Ubx targets during wing development genome-wide. This study made several surprising findings. First, although Ubx is expressed specifically in the hindwing in *Bombyx* (consistent with the earlier report in another lepidopteran species [24,25]), Ubx was found to be expressed BOTH in the forewing and hindwing in *Apis* (though Ubx expression in the forewing might be slightly lower). This finding has a significant impact on our current model regarding the involvement of *Ubx* in the evolution of insect forewing/hindwing differentiation. Second, their CHIPseq analysis identified a large number of possible direct Ubx targets that are shared among *Drosophila*, *Apis*, and *Bombyx*. This rather contradicts the current view of insect wing evolution, as the current model proposes that Ubx has gained a distinct set of target genes in each lineage, which has promoted the diversification of wing morphologies among different species. Third, a significant number of genes are found to be putative Ubx targets in both the forewing and the hindwing of *Apis*, even though the morphology of these two wings are quite similar (which implies that the gene regulation between these two wings should also be similar). This again suggests that Ubx binding itself might not be functionally significant. Most notably, *vg*, an important wing gene, is found to be a target of Ubx in both forewings and hindwings. The authors identified a possible wing enhancer from the *Apis vg* locus (which contains Ubx binding sites), and assessed its activity in *Drosophila*. Surprisingly, this enhancer was found to be active in both wings and halteres in *Drosophila*, despite the presence of Ubx binding sites in the enhancer. Although it is important to confirm that Ubx indeed binds to this *Apis vg* enhancer during haltere development in *Drosophila*, this result further supports the idea that Ubx binding to DNA might not be sufficient to confer transcriptional regulatory function upon Ubx. Taken together, the findings from other holometabolous insects provide several intriguing new insights, which may necessitate a revision of the current view of the role of *Ubx* in insect wing evolution.

Re-thinking the involvement of *Ubx* in the evolution of forewing/hindwing differentiation

Studies in *Drosophila* have provided us with a detailed view of how *Ubx* coordinates forewing/hindwing differentiation by acting as the haltere selector gene, which in turn has given us a framework to understand the evolution of forewing/hindwing differentiation in other insects. Now, with new insights from studies in non-*Drosophila* insects, along with the outcomes from some genome-wide studies on Ubx function in *Drosophila*, it appears to be an appropriate time to revisit the *Drosophila* paradigm and re-think the involvement of Ubx in the evolution of forewing/hindwing differentiation.

After *Ubx* was confirmed to be the hindwing selector gene not only in flies, but also in beetles and butterflies [24,31,32], the consensus was that, even in other insects, the forewing represents the Hox-free state (whether it is a membranous flight wing or a modified structure), and *Ubx* changes the Hox-free morphology into the hindwing. However, the Ubx expression in both forewings and hindwings of *Apis* poses a significant challenge to this view. Is the *Apis* situation unique to Hymenoptera (or even just to the lineage leading to *Apis*), or should we expect to find a similar situation in other insects? Since Hymenoptera is considered to be a basal lineage in holometabola [51], it is even possible that Ubx expression in both forewings and hindwings represents an ancestral state. It will be critical to investigate Ubx expression in the forewing and the hindwing of various other insect orders to further assess the ancestral status of Ubx expression.

Figure 2



Overview of the traditional and new models describing how *Ubx* came to regulate wing differentiation in the evolution of insect flight structures. Note that the expression of gene A depends on the presence of Ubx in beetles, while gene A expression is independent of Ubx in butterflies.

The idea that all wings were Hox-free in the ancestral state was based on the fact that *Drosophila* forewing (which is a membranous flight wing) represents a Hox-free state. This view was, in a way, already challenged when the highly modified beetle forewing was found to be a Hox-free state [31]. However, the beetle situation was explainable with the *Drosophila* paradigm by considering that Ubx acts in the opposite way to that in *Drosophila*; namely, the evolutionary modification of wings in beetles occurred without Hox input, and Ubx evolved to cancel the modifications in the hindwing to maintain the more ancestral flight wing characteristics. Again, the *Apis* situation now brings another challenge to the traditional view regarding the Hox-independent nature of the ancestral wings. One interesting point to consider is the origin of insect wings. Although the exact tissues that served as the origin of insect wings are still a mystery (reviewed in [2]), those tissues could have already been under the control of Hox genes. This view could significantly challenge the current *Drosophila* paradigm, as Hox genes would have been a part of the wing gene network in the ancestral situation and forewings became Hox-free in some lineages.

Whether the *Apis* situation is ancestral or specific to this lineage, it will be interesting to investigate what will happen when *Ubx* is removed from the *Apis* wing. Will that reveal a hidden default state of hymenopteran wings? Or perhaps *Ubx* is an essential part of the wing gene network in *Apis*, and the removal of *Ubx* may prevent wings (both forewings and hindwings) to form. Yet another possibility is that, despite the expression of Ubx in the forewing, Ubx may not be functional in this tissue in *Apis*. Detailed functional analyses for *Ubx* in *Apis* will provide a new view on the role of Ubx during insect wing development.

Genome-wide surveys for Ubx targets in *Drosophila* using various genomic techniques also provided some intriguing new insights that may encourage a revision of the current view on how Ubx has come to control hindwing development (note that whether the following discussion is applicable to a wider insect taxonomy, including both hemimetabola and holometabola, or limited to specific holometabolous lineages, depends on how far back the hindwing specific Ubx expression goes in insect evolution). The main mechanism in the current view of the evolution of forewing/hindwing differentiation is that Ubx has gained binding sites to a distinct set of genes in each lineage, allowing Ubx to modify the Hox-free state of the wing in a lineage-specific manner (Figure 2a). However, the presence of a large number of shared Ubx targets among three insect orders suggest that Ubx might have had binding sites to a certain set of genes even in the common ancestor of these orders. The CHIP analyses in *Drosophila* and other insects suggest that these binding sites may correspond to open chromatin regions that contain genes important for wing development in general. Thus, it is intriguing to speculate that Ubx was binding to various sites on the genome even in the ancestral state. Most of the Ubx binding to DNA could have been neutral in regard to forewing/hindwing differentiation, since the two pairs of wings in the ancestral state had relatively similar morphologies. In this model, the binding sites would have been largely determined by chromatin accessibility (i.e. gene loci important for wing development are

more open), thus restricting most of the Ubx binding sites to the loci important for wing development (Figure 2b). Then, in each lineage, some factors evolved to potentiate Ubx (either as an activator or repressor) at a distinct set of loci, resulting in *Ubx*-dependent hindwing differentiation in a lineage specific manner (Figure 2b). The factors that potentiate Ubx can be new binding partners of Ubx or chromatin modification factors that could further modulate the chromatin status. This model could explain why Ubx has a tendency to regulate a similar set of genes among different insects, and also why Ubx binding at some (many?) loci appears to be functionally neutral in *Drosophila* and other insects.

This 'potentiation of Ubx' could be facilitated by changes in either *cis* or *trans*. It can be achieved through cofactor/binding partner proteins gaining binding sites near the Ubx site (*cis* changes), or through the Ubx protein gaining an ability to bind to other proteins (*trans* changes). Identifying the factors that potentiate Ubx will be critical to test this model. As mentioned, Ap and Omb appear to be good candidates [35•,43•], and Trl (GAF) is also promising as a potentiation factor [50••].

Conclusion

The four-winged phenotype of the *Drosophila Ubx* mutant has been one of the most symbolic figures in developmental biology, epitomizing how influential one gene can be to the development of an organism. The same *Ubx* phenotype has been quite iconic in evolutionary biology as well, since this phenotype exemplifies that, through genetic manipulation, we might be able to strip away evolutionary modifications from extant organisms to understand the molecular changes that have facilitated morphological evolution. Decades have passed since the four-winged phenotype has been reported, and accumulating knowledge obtained from studies on *Ubx* in *Drosophila* has provided us with a framework to investigate the role of *Ubx* in the evolution of insect wings. Until recently, studies in other insects heavily relied on this framework (i.e. *Drosophila* paradigm), testing the function of a handful of genes learned from *Drosophila* studies. However, with the recent advances of molecular biology techniques, we are finally reaching the stage where *Drosophila* type genetics and genomics are possible even in other non-*Drosophila* species. By analyzing the function of *Ubx* in various insects at a level that is currently only achievable in *Drosophila*, we will be able to obtain a less biased view of the function of *Ubx*, which will in turn lead to a more comprehensive understanding of the molecular mechanisms that have facilitated the diversification of insect wings.

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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 M: *Evolution of the Insects*. Cambridge University Press; 2005.

2. Clark-Hachtel CM, Tomoyasu Y: **Exploring the origin of insect wings from an evo-devo perspective**. *Curr Opin Insect Sci Opin Insect Sci* 2016, **13**:77-85.

3. Dudley R: **Evolution of flight and flightlessness**. *The Biomechanics of Insect Flight*. Princeton Unversity Press; 2000:: 261-301.

4. Grimaldi D, Engel MS: **Fossil insects**. *Evolution of the Insects*. Cambridge University Press; 2005:: 42-92.

5. 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.

6. Grimaldi D, Engel MS: **Insects take to the skies**. *Evolution of the Insects*. Cambridge University Press; 2005:: 155-187.

7. Carroll S, Grenier JK, Weatherbee SD: *From DNA to Diversity*. Blackwell Publishing; 2005.

8. Lewis EB: **A gene complex controlling segmentation in Drosophila**. *Nature* 1978, **276**:565-570.

9. Weatherbee SD, Halder G, Kim J, Hudson A, Carroll S: **Ultrabithorax regulates genes at several levels of the wing-patterning hierarchy to shape the development of the Drosophila haltere**. *Genes Dev* 1998, **12**:1474-1482.

10. Shashidhara LS, Agrawal N, Bajpai R, Bharathi V, Sinha P: **Negative regulation of dorsoventral signaling by the homeotic gene Ultrabithorax during haltere development in Drosophila**. *Dev Biol* 1999, **212**:491-502.

11. • Mohit P, Makhijani K, Madhavi MB, Bharathi V, Lal A, Sirdesai G, Reddy VR, Ramesh P, Kannan R, Dhawan J *et al.*: **Modulation of AP and DV signaling pathways by the homeotic gene Ultrabithorax during haltere development in Drosophila**. *Dev Biol* 2006, **291**:356-367.
See annotation to Ref. [45•].

12. • Hersh BM, Nelson CE, Stoll SJ, Norton JE, Albert TJ, Carroll SB: **The UBX-regulated network in the haltere imaginal disc of *D. melanogaster***. *Dev Biol* 2007, **302**:717-727.
See annotation to Ref. [45•].

13. Akam M: **Hox genes: from master genes to micromanagers**. *Curr Biol* 1998, **8**:R676-R678.

14. Galant R, Walsh CM, Carroll SB: **Hox repression of a target gene: extradenticle-independent, additive action through multiple monomer binding sites**. *Development* 2002, **129**:3115-3126.

15. Hersh BM, Carroll SB: **Direct regulation of knot gene expression by Ultrabithorax and the evolution of cis-regulatory elements in Drosophila**. *Development* 2005, **132**:1567-1577.

16. Crickmore MA, Mann RS: **Hox control of morphogen mobility and organ development through regulation of glypican expression**. *Development* 2007, **134**:327-334.

17. Crickmore MA, Mann RS: **Hox control of organ size by regulation of morphogen production and mobility**. *Science* 2006, **313**:63-68.

18. de Navas LF, Garaulet DL, Sánchez-Herrero E, Sanchez-Herrero E: **The ultrabithorax Hox gene of Drosophila controls haltere size by regulating the Dpp pathway**. *Development* 2006, **133**:4495-4506.

19. Makhijani K, Kalyani C, Srividya T, Shashidhara LS: **Modulation of decapentaplegic gradient during haltere specification in Drosophila**. *Dev Biol* 2007, **302**:243-255.

20. Pallavi SK, Kannan R, Shashidhara LS: **Negative regulation of Egfr/Ras pathway by Ultrabithorax during haltere development in Drosophila**. *Dev Biol* 2006, **296**:340-352.

21. Singh S, Sánchez-Herrero E, Shashidhara LS: **Critical role for Fat/Hippo and IIS/Akt pathways downstream of Ultrabithorax during haltere specification in Drosophila**. *Mech Dev* 2015, **138(Pt 2)**:198-209.

22. Carroll SB, Weatherbee SD, Langeland JA: **Homeotic genes and the regulation and evolution of insect wing number**. *Nature* 1995, **375**:58-61.

23. Struhl G: **Genes controlling segmental specification in the Drosophila thorax**. *Proc Natl Acad Sci U S A* 1982, **79**:7380-7384.

24. Weatherbee SD, Nijhout HF, Grunert LW, Halder G, Galant R, Selegue J, Carroll S: **Ultrabithorax function in butterfly wings and the evolution of insect wing patterns**. *Curr Biol* 1999, **9**:109-115.

25. Tong X, Hrycaj S, Podlaha O, Popadic A, Monteiro A: **Over-expression of Ultrabithorax alters embryonic body plan and wing patterns in the butterfly *Bicyclus anynana***. *Dev Biol* 2014, **394**:357-366.

26. Galant R, Skeath JB, Paddock S, Lewis DL, Carroll SB: **Expression pattern of a butterfly achaete-scute homolog reveals the homology of butterfly wing scales and insect sensory bristles**. *Curr Biol* 1998, **8**:807-813.

27. Monteiro A: **Origin, development, and evolution of butterfly eyespots**. *Annu Rev Entomol* 2015, **60**:253-271.

28. Beeman RW, Stuart JJ, Haas MS, Denell RE: **Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum***. *Dev Biol* 1989, **133**:196-209.

29. Bennett RL, Brown SJ, Denell RE: **Molecular and genetic analysis of the Tribolium Ultrabithorax ortholog, Ultrathorax**. *Dev Genes Evol* 1999, **209**:608-619.

30. Hueber SD, Lohmann I: **Shaping segments: Hox gene function in the genomic age**. *Bioessays* 2008, **30**:965-979.

31. Tomoyasu Y, Wheeler SR, Denell RE: **Ultrabithorax is required for membranous wing identity in the beetle *Tribolium castaneum***. *Nature* 2005, **433**:643-647.

32. Deutsch J: **Hox and wings**. *Bioessays* 2005, **27**:673-675.

33. Crowson RA: *The Biology of the Coleoptera*. Academic Press; 1981.

34. Bouchard P, Bouchard P: *The Book of Beetles*. The University of Chicago Press; 2014.

35. • Tomoyasu Y, Arakane Y, Kramer KJ, Denell RE: **Repeated co-options of exoskeleton formation during wing-to-elytron evolution in beetles**. *Curr Biol* 2009, **19**:2057-2065.
See annotation to Ref. [43•].

36. Fédrigo O, Wray GA: **Developmental evolution: how beetles evolved their shields**. *Curr Biol* 2010, **20**:R64-R66.

37. Ravisankar P, Lai YT, Sambrani N, Tomoyasu Y: **Comparative developmental analysis of Drosophila and Tribolium reveals conserved and diverged roles of abrupt in insect wing evolution**. *Dev Biol* 2016, **409**:518-529.

38. Campuzano S, Carramolino L, Cabrera CV, Ruíz-Gómez M, Villares R, Boronat A, Modolell J: **Molecular genetics of the achaete-scute gene complex of *D. melanogaster***. *Cell* 1985, **40**:327-338.

39. Hu S, Fambrough D, Atashi JR, Goodman CS, Crews ST: **The Drosophila abrupt gene encodes a BTB-zinc finger regulatory protein that controls the specificity of neuromuscular connections**. *Genes Dev* 1995, **9**:2936-2948.

40. Brook WJ, Diaz-Benjumea FJ, Cohen SM: **Organizing spatial pattern in limb development**. *Annu Rev Cell Dev Biol* 1996, **12**:161-180.

41. Tomoyasu Y, Denell RE: **Larval RNAi in Tribolium (Coleoptera) for analyzing adult development**. *Dev Genes Evol* 2004, **214**:575-578.

42. Dawid IB, Breen JJ, Toyama R: **LIM domains: multiple roles as adapters and functional modifiers in protein interactions**. *Trends Genet* 1998, **14**:156-162.

43. • Simon E, Guerrero I: **The transcription factor optomotor-blind antagonizes Drosophila haltere growth by repressing decapentaplegic and Hedgehog targets**. *PLOS ONE* 2015, **10**:e0121239.
This study along with [35•] shows Ubx-dependent dual functions of wing genes (*omb* in [43•] and *ap* in [35•]).

44. Grimm S, Pflugfelder GO: **Control of the gene optomotor-blind in Drosophila wing development by decapentaplegic and wingless**. *Science* 1996, **271**:1601-1604.

45. • Pavlopoulos A, Akam M: **Hox gene Ultrabithorax regulates distinct sets of target genes at successive stages of Drosophila haltere morphogenesis**. *Proc Natl Acad Sci U S A* 2011, **108**:2855-2860.
This study with [11•] and [12•] are the first three studies uncovering the pervasiveness of Ubx regulation over the wing gene network.

46. • Agrawal P, Habib F, Yelagandula R, Shashidhara LS: **Genome-level identification of targets of Hox protein Ultrabithorax in Drosophila: novel mechanisms for target selection**. *Sci Rep* 2011, **1**:205.
This and the following two studies ([47•] and [48•]) used CHIP-chip and performed genome-wide identification of Ubx binding sites during haltere development in *Drosophila*.

47. • Choo SW, White R, Russell S: **Genome-wide analysis of the binding of the Hox protein Ultrabithorax and the Hox cofactor Homothorax in Drosophila**. *PLoS ONE* 2011, **6**:e14778.
See annotation to Ref. [46•].

48. • Slattery M, Ma L, Négre N, White KP, Mann RS: **Genome-wide tissue-specific occupancy of the Hox protein Ultrabithorax and Hox cofactor Homothorax in Drosophila**. *PLoS ONE* 2011, **6**:e14686.
See annotation to Ref. [46•].

49. •• McKay DJ, Lieb JD: **A common set of DNA regulatory elements shapes Drosophila appendages**. *Dev Cell* 2013, **27**:306-318.
This is the first study comprehensively profiling chromatin accessibility in various tissues in *Drosophila*, providing a surprising finding that chromatin accessibility is almost identical among related tissues, such as wings, halteres, and legs.

50. •• Prasad N, Tarikere S, Khanale D, Habib F, Shashidhara LS: **A comparative genomic analysis of targets of Hox protein Ultrabithorax amongst distant insect species**. *Sci Rep* 2016, **6**:27885.
In this work, the authors used two non-*Drosophila* insects, and performed an impressive series of experiments from expression and reporter analyses to CHIPseq-based identification of Ubx target sites.

51. Savard J, Tautz D, Richards S, Weinstock GM, Gibbs RA, Werren JH, Tettelin H, Lercher MJ: **Phylogenomic analysis reveals bees and wasps (Hymenoptera) at the base of the radiation of Holometabolous insects**. *Genome Res* 2006, **16**:1334-1338.

52. Hopkins TL, Kramer KJ: **Insect Cuticle Sclerotization**. *Annu Rev Entomol* 1992, **37**:273-302 http://dx.doi.org/10.1146/annurev.en.37.010192.001421.