16. Szabadkai, G., Simoni, A.M., Chami, M., Wieckowski, M.R., Youle, R.J., and Rizzuto, R. (2004). Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+-mediated apoptosis. Mol. Cell *16*, 59–68.

17. Bianchi, K., Vandecasteele, G., Carli, C., Romagnoli, A., Szabadkai, G., and Rizzuto, R. (2006). Regulation of Ca2+ signalling and Ca2+-mediated cell death by the transcriptional coactivator PGC-1alpha. Cell Death Differ. *13*, 586–596.

18. Youle, R.J., and van der Bliek, A.M. (2012). Mitochondrial fission, fusion, and stress. Science *337*, 1062–1065.

19. Bravo, R., Vicencio, J.M., Parra, V., Troncoso, R., Munoz, J.P., Bui, M., Quiroga, C., Rodriguez, A.E., Verdejo, H.E., Ferreira, J., *et al*. (2011). Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. J. Cell Sci. *124*, 2143–2152.

20. Hoitzing, H., Johnston, I.G., and Jones, N.S. (2015). What is the function of mitochondrial networks? A theoretical assessment of hypotheses and proposal for future research. Bioessays *37*, 687–700.

Evo–Devo: The Double Identity of Insect Wings

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Sometime in the Devonian, perhaps about 400 million years ago, insects became the first clade to conquer the sky. Recent evo-devo studies have begun to unravel the mysterious origin of the flight structure that made insects into extraordinary six-legged fliers.

Although the immense success of insects can be attributed to various traits, the wing is the signature character of this clade, which has allowed insects to explore a variety of niches via powered flight, and has also served as an evolutionary medium for the emergence of new traits (such as camouflage, mimicry, armoring, and communication). So, considering the significance of wings during insect evolution, it is somewhat surprising that the origin of this evolutionarily prominent structure is still a hotly debated mystery. One of the first passages in the science literature hinting at a possible origin of insect wings can be found in a book published over two hundred years ago [1].

> *Wings and feet* [of insects] *are dependent from the same ring of the body, and thus like the branchiae and feet of the Crabs. Let the Crab's branchiae elongate and dry, and they will thus wings*
>
> *Lorenz Oken, Elements of Physiophilosophy, 1809*
>
> *(Quoted from the English version published in 1847)*

As a scientist in the pre-Darwinian era, Oken probably did not imply an evolutionary relationship between the insect wing and the crustacean branchia (i.e. gill). Nonetheless, various homologizations made in his book have no doubt inspired many scientists in subsequent generations. A variety of ideas on the origin of insect wings have been proposed in the past two hundred years. Through sometimes intense intellectual battles, two schools of thought have emerged, namely the tergal origin hypothesis (also called the paranotal hypothesis) and the pleural origin hypothesis (also known as the exite hypothesis) (reviewed in [2]). The tergal origin hypothesis connects the origin of insect wings to the dorsal epidermal plate (tergum), while the pleural origin hypothesis states that the insect wing has originated from a branch (such as a gill) of the ancestral proximal leg segment (corresponding to the pleural plates in modern insects). Interestingly, several recent studies point toward a unification of these two seemingly incompatible hypotheses [3–9].

With two distinct origin tissues, the dorsal body wall and the pleural plates, how can both be the origin of insect wings? The third hypothesis, the dual origin hypothesis, states that the 'merger' of the two unrelated tissues may have been a key step in the evolution of this efficient flight device. The idea of a dual origin of insect wings is not new. It can date back as early as the beginning of the wing origin debate [10], although Rasnitsyn, in his modified paranotal hypothesis, may have been the first to clearly favor the possibility of a dual origin [11]. This idea has been mentioned by several scientists in the past ([12] for example); however, it did not gain significant support until more recently with evidence from evo-devo studies [3–6]. Several key findings from these studies include (i) the identification of 'wing serial homologs' (i.e. tissues that share an evolutionary ancestry with wings) in non-winged segments via an evo–devo approach [13], (ii) the identification of two distinct sets of wing serial homologs, one tergal and the other pleural, in a non-winged segment [4], and (iii) the evolutionary conservation of (i) and (ii) in diverse insects [5,6]. An example of evo–devo support for a dual insect wing origin is found in the first thoracic segment (T1, a wingless segment) of the beetle, where wing serial homologs are maintained as two distinct sets of tissues (of the tergal and pleural nature) [4]. When a set of wings is ectopically induced in T1 of this beetle

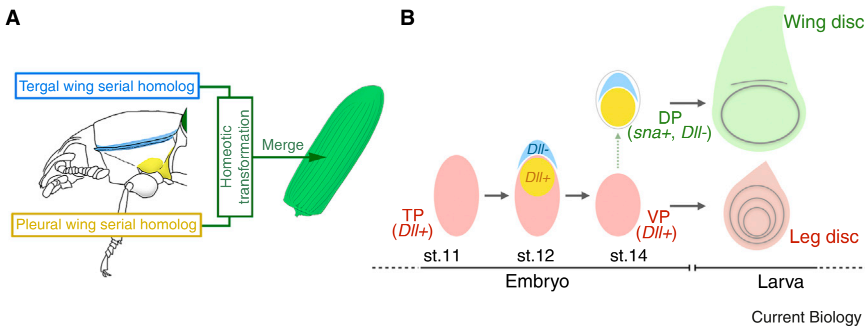

Figure 1. Two distinct sets of cell populations that contribute to the formation of insect wings.

(A) Two distinct sets of wing serial homologs in the first thoracic segment (T1) of the red flour beetle, *Tribolium castaneum*. Upon homeotic transformation, the tergal (blue) and pleural (yellow) wing serial homologs merge to form a complete wing (green) [4]. A similar situation has been found in the milkweed bug (*Oncopeltus fasciatus*) [5] and the German cockroach (*Blattella germanica*) [6]. (B) Wing disc development in the fruit fly, *Drosophila melanogaster*. The dorsal primordium (DP) is composed of two separate groups of cells (yellow and blue). TP: thoracic primordium, VP: ventral primordium.

through the genetic alteration of segment identity (homeotic transformation), both the tergal and pleural wing serial homologs contribute to the formation of a complete wing, thus supporting the idea that insect wings have a dual developmental (and evolutionary) origin (Figure 1A) [4]. Now, as reported in a recent issue of *Current Biology*, through a very thorough analysis of the *cis*-element that is responsible for the wing-specific expression of the *snail* (*sna*) gene in *Drosophila*, Requena *et al.* have revealed that the tissue that gives rise to wings in *Drosophila* (wing imaginal disc) is composed of two distinct sets of cells (Figure 1B) [9], which provides further support for the dual evolutionary origin of insect wings.

Insect wing development has been studied most thoroughly in *Drosophila*, which has led to a very detailed understanding of the underlying molecular mechanisms. Interestingly, however, prior to Requena *et al.*, the evo-devo support for the dual origin model came mostly from 'non-*Drosophila*' insects. Some major shortcomings of using *Drosophila* for studying the origin of insect wings include their extremely unique mode of development and the resulting diverged adult morphology (reviewed in [14]). The wingless T1 segment studied in the above-mentioned evo-devo studies is almost entirely missing from adult *Drosophila*, making it a challenge to study wing serial homologs in wingless segments in this insect. In addition, almost the entirety of the *Drosophila* adult epidermis is formed from a set of unique tissues called imaginal discs [15]. This highly derived mode of development, found only in certain holometabolous orders (such as Diptera and Hymenoptera), makes *Drosophila* a tough system to use to gain insight into the ancestral state of insect wings. But the imaginal disc development in *Drosophila* is the exact process where Requena *et al.* found a clue to the evolutionary identity of the insect wing [9].

During *Drosophila* embryogenesis, six clusters of thoracic epidermal cells positive for *Distalless* (*Dll*) expression, each cluster corresponding to a hemi-segment, invaginate into the embryo, and form thoracic primordia (TP, Figure 1B) [15–18]. The TP then splits into two populations of cells, producing the wing and the leg imaginal discs [17,18]. The names of these discs are somewhat deceiving, as the wing disc also gives rise to the entire dorsal body wall (i.e. tergum), while the leg disc contains the pleural region. To avoid possible confusion, Requena *et al.* call them dorsal and ventral primordium (DP and VP), respectively. The VP cells continue to express *Dll*, while the DP cells are marked by the expression of the wing master gene, *vestigial* (*vg*), as well as by the expression of a transcription factor gene, *sna* [17,18] (Figure 1B). It has been assumed that the DP (i.e. wing disc) is entirely formed from the TP. However, a careful lineage tracing analysis using the DP specific enhancer of *sna* has revealed that not all DP cells are coming from the TP. There appears to be a second set of cells outside of the TP that join into the DP. Therefore, the DP is composed of two separate groups of cells. The discovery that the *Drosophila* wing disc is formed from the mixture of two distinct cell populations has important implications for the insect wing origin debate, as it demonstrates that wings have a dual developmental origin even in a very diverged dipteran mode of development. This finding complements previous demonstrations of the merger of tergal and pleural tissues to form the T1 ectopic wing upon homeotic transformation [4–6], which collectively further supports a dual evolutionary origin of insect wings.

Several important questions remain unanswered. For example, it is largely elusive how the two sets of cells that make the DP in *Drosophila* correspond to the two proposed wing origin tissues (the tergum and the pleural plates). At a glance, it makes sense to think that the TP-originating DP cells (yellow in Figure 1B) would correspond to the most proximal leg tissue (pleural plates in modern insects), while non-TP DP cells (blue in Figure 1B) may be of a more dorsal identity. However, the dipteran mode of development poses an important caveat to this interpretation, as the lineage tracing experiment demonstrated that some TP-originating DP cells (yellow in Figure 1B) also contribute to the formation of the adult tergum in *Drosophila*. Therefore, it is crucial to further investigate the nature of these two cell populations that contribute to the formation of the DP in *Drosophila*, and also to assess how these two cell populations correspond to the two wing serial homologs found in other insects.

An evo–devo-based approach has been central to the recent advancement of the dual origin hypothesis [2,14]; however, this approach also harbors some limitations. For instance, most evo–devo interpretations assume that analyses of the developmental origin of a tissue can inform us on the evolutionary origin of this tissue. However, what we see in each organism is an evolutionary 'snapshot', which has the potential to be highly diverged from an ancestral mode of development. Therefore, it is of paramount importance to analyze wing development in a wide taxonomy of insects and to facilitate collaboration with other fields to gain a more comprehensive view of the evolutionary origin of insect wings. In fact, the inclusion of other fields has already proven fruitful, as a recent study investigating an ancestral mode of wing development in an extinct insect order (Palaeodictyoptera) has provided novel support for a dual origin of insect wings from a paleontological perspective [7].

The molecular basis underlying the evolution of insect wings has just begun to be unraveled. A 'cross-wiring' of the two similar gene regulatory networks (GRNs) operating in the tergal and pleural wing serial homologs is one possible mechanism that has been proposed [2,4], while co-option of a GRN capable of inducing a certain characteristic (such as a flat outgrowth) into the lateral body wall is another [19]. The application of *cis* analyses to the investigation of wing origin (such as Requena *et al.*) and the comparison of *cis* regulation of wing genes between various insects [20] will be quite powerful in elucidating the processes that facilitated the evolution of insect wings at the molecular level. The origin of insect wings is a fascinating mystery that has captivated scientists for many years. With new molecular techniques rapidly emerging, we are entering an exciting period where we may be able to put this century-old conundrum to rest.

REFERENCES

1. Oken, L. (1847). Elements of Physiophilosophy (Ray Society).
2. Clark-Hachtel, C.M., and Tomoyasu, Y. (2016). Exploring the origin of insect wings from an evo-devo perspective. Curr. Opin. Insect Sci. *13*, 77–85.
3. Niwa, N., Akimoto-Kato, A., Niimi, T., Tojo, K., Machida, R., and Hayashi, S. (2010). Evolutionary origin of the insect wing via integration of two developmental modules. Evol. Dev. *12*, 168–176.
4. Clark-Hachtel, C.M., Linz, D.M., and Tomoyasu, Y. (2013). Insights into insect wing origin provided by functional analysis of vestigial in the red flour beetle, Tribolium castaneum. Proc. Natl. Acad. Sci. USA *110*, 16951–16956.
5. Medved, V., Marden, J.H., Fescemyer, H.W., Der, J.P., Liu, J., Mahfooz, N., and Popadić, A. (2015). Origin and diversification of wings: Insights from a neopteran insect. Proc. Natl. Acad. Sci. USA *112*, 15946–15951.
6. Elias-Neto, M., and Belles, X. (2016). Tergal and pleural structures contribute to the formation of ectopic prothoracic wings in cockroaches. R. Soc. Open Sci. *3*, 160347.
7. Prokop, J., Pecharová, M., Nel, A., Hörnschemeyer, T., Krzemińska, E., Krzemiński, W., and Engel, M.S. (2017). Paleozoic nymphal wing pads support dual model of insect wing origins. Curr. Biol. *27*, 263–269.
8. Mashimo, Y., and Machida, R. (2017). Embryological evidence substantiates the subcoxal theory on the origin of pleuron in insects. Sci. Rep. *7*, 12597.
9. Requena, D., Alvarez, J.A., Gabilondo, H., Loker, R., Mann, R.S., and Estella, C. (2017). Origins and specification of the Drosophila wing. Curr Biol. *27*, 3826–3836.e5.
10. Crampton, G. (1916). The phylogenetic origin and the nature of the wings of insects according to the paranotal theory. New York Entomol. Soc. *24*, 1–39.
11. Rasnitsyn, A.P. (1981). A modified paranotal theory of insect wing origin. J. Morphol. *168*, 331–338.
12. Grimaldi, D., and Engel, M.S. (2005). Insects take to the skies. In Evolution of the Insects (Cambridge University Press), pp. 155–187.
13. Ohde, T., Yaginuma, T., and Niimi, T. (2013). Insect morphological diversification through the modification of wing serial homologs. Science *340*, 495–498.
14. Tomoyasu, Y., Ohde, T., and Clark-Hachtel, C. (2017). What serial homologs can tell us about the origin of insect wings. F1000Res. *6*, 268.
15. Cohen, S.M. (1993). Imaginal disc development. In The Development of Drosophila melanogaster, M. Bate, and A. Martinez-Arias, eds. (Plainview, N.Y: Cold Spring Harbor Laboratory Press), pp. 747–841.
16. Cohen, S.M. (1990). Specification of limb development in the Drosophila embryo by positional cues from segmentation genes. Nature *343*, 173–177.
17. Carroll, S.B., Weatherbee, S.D., and Langeland, J.A. (1995). Homeotic genes and the regulation and evolution of insect wing number. Nature *375*, 58–61.
18. Goto, S., and Hayashi, S. (1997). Specification of the embryonic limb primordium by graded activity of Decapentaplegic. Development *124*, 125–132.
19. Shiga, Y., Kato, Y., Aragane-Nomura, Y., Haraguchi, T., Saridaki, T., Watanabe, H., Iguchi, T., Yamagata, H., and Averof, M. (2017). Repeated co-option of a conserved gene regulatory module underpins the evolution of the crustacean carapace, insect wings and other flat outgrowths. bioRxiv https://doi.org/10.1101/160010.
20. Lai, Y.-T., Deem, K.D., Borras-Castells, F., Sambrani, N., Rudolf, H., Suryamohan, K., El-Sherif, E., Halfon, M.S., McKay, D.J., and Tomoyasu, Y. (2017). Enhancer identification and activity evaluation in the red flour beetle, Tribolium castaneum. bioRxiv https://doi.org/10.1101/199729.

Chromosome Biology: The Sight of DNA, at Last!

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Chromosomes are shaped by the combined function of the condensin and cohesin Smc-kleisin complexes. After more than two decades of research in this field, a new study finally sheds light on how these machines might interact with their DNA substrates.

We have all marvelled at cartoons, still found in textbooks, of 'beads-on-a-string' nucleosomes magically folding into ever-bigger structures, forming a 30 nm-wide structure that will pack histone–DNA complexes into chromosomes. No need for anything else, nucleosomes and thermodynamics would do the job. However, there is growing evidence