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THINK AGAIN

Insights & Perspectives

Distinguishing serial homologs from novel traits: Experimental limitations and ideas for improvements

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Abstract

One of the central but yet unresolved problems in evolutionary biology concerns the origin of novel complex traits. One hypothesis is that complex traits derive from pre-existing gene regulatory networks (GRNs) reused and modified to specify a novel trait somewhere else in the body. This simple explanation encounters problems when the novel trait that emerges in a body is in a region that is known to harbor a latent or repressed trait that has been silent for millions of years. Is the novel trait merely a re-emerged de-repressed trait or a truly novel trait that emerged via a novel deployment of an old GRN? A couple of new studies sided on opposite sides of this question when investigating the origin of horns in dung beetles and helmets in treehoppers that develop in the first thoracic segment (T1) of their bodies, a segment known to harbor a pair of repressed/modified wings in close relatives. Here, I point to some key limitations of the experimental approaches used and highlight additional experiments that could be done in future to resolve the developmental origin of these and other traits.

KEYWORDS
atavism, Hox genes, novel trait, serial homolog, sex combs reduced

INTRODUCTION

Much body plan evolution has been hypothesized to originate through the use and modification of the same GRNs indifferent locations in the body.[10,21–22] This is because re-use followed by modification of a network is more likely to produce a functioning trait, in a shorter amount of time, than creation of completely new networks from individual genes. There are, however, two distinct routes by which GRNs are re-used in the same body: in the differentiation of repeated traits, or serial homologs, or in the differentiation of novel traits.

In the serial homolog route, the GRN is activated at similar developmental coordinates within a body, and these coordinates have been generated by a homologous patterning mechanism. An example would be the flight wings and haltere balancing organs of flies that despite looking considerably different, share the same embryonic coordinates and marker genes and are considered bona fide serial homologs.[5,9] The similar embryonic coordinates of wings and halteres ultimately result from the use of the same set of early patterning positional information mechanisms[5] and segmentation GRN that generate the serial homologous segments of arthropods.[34]

In the novel trait route, the GRN is activated at a novel developmental coordinate in the body that is spatially distinct from the coordinates of all other pre-exiting traits or putative serial homologs. Some examples are the proposed activation of the limb GRN in the heads of dung beetles and rhinoceros beetles to generate horns;[21,24] the activation of the same limb GRN in the wings of butterflies to generate the first eyespots;[2,6] or the activation of a larval breathing spiracle GRN to produce a new genital lobe in the genitalia of flies.[10]

Over the course of evolution, the GRNs that generated members of a system of serial homologs as well as novel traits can become modified at each of these body locations beyond recognition so the origin of the trait, that is, the GRN that begot its developmental origin, becomes obscured. What often allows the recognition of traits as being derived from the same ancestral GRN is the presence of an homologous core set of genes underlying their development, whose cobbling together from scratch at that same location would be unlikely.[3,25]

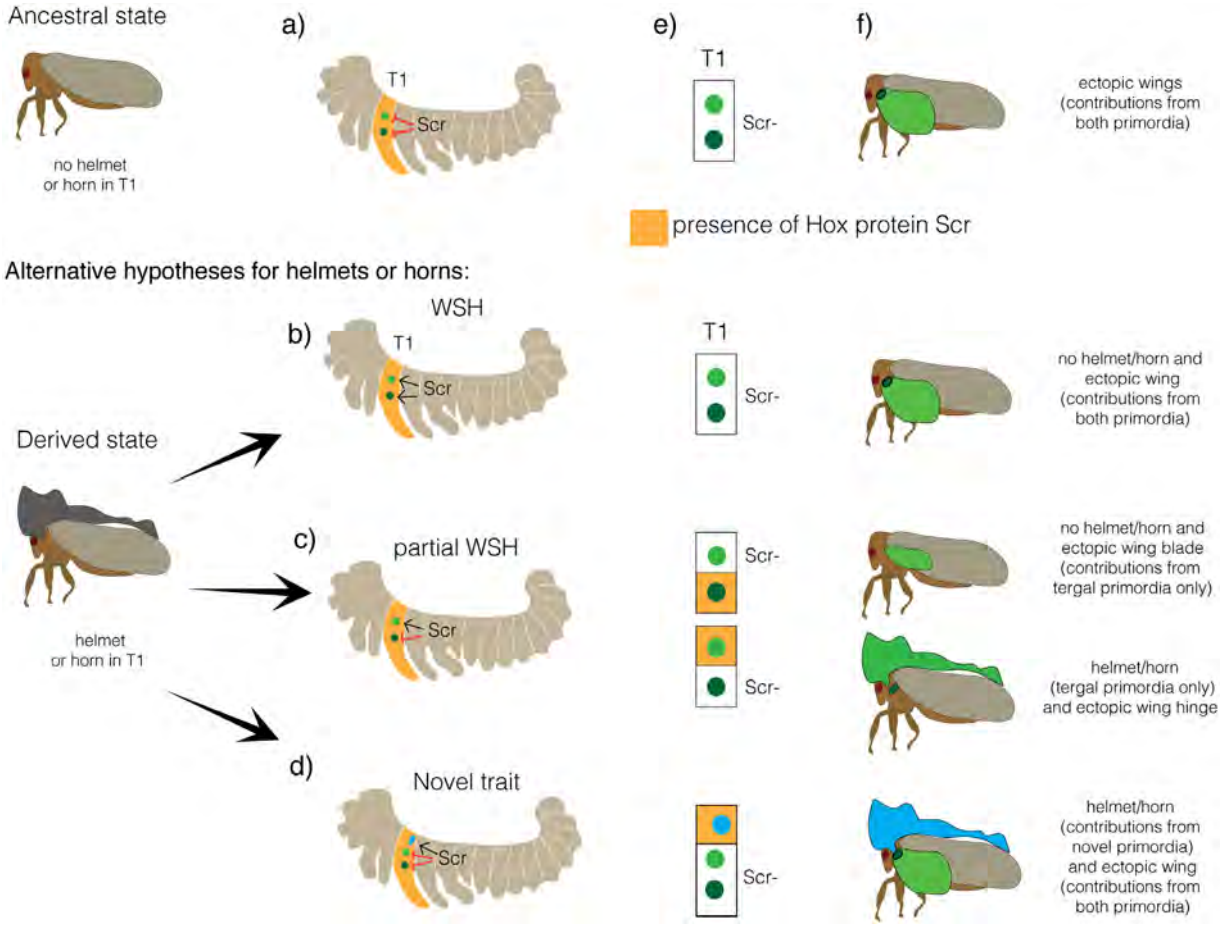

FIGURE 1 Different hypotheses for the origin of helmets in treehoppers and horns in horned beetles. (a) The ancestral state for how Scr is interacting with distinct cell primordia in T1 in species that have no helmets or horns: Scr is modifying/repressing the tergal and pleural cell primordia (WSHs) into components of the body wall. (b-d) Derived states for Scr interactions under three different hypothesis of helmet/horn origins. (b) Helmets/horns are WSHs and Scr no longer represses the development of wings but rather modifies them into helmets or horns; (c) Helmets/horns are partial WSHs. The dorsal embryonic cells that give rise to the wing blade (tergal primordia, light green) are no longer repressed by Scr and instead require Scr to develop into helmets/horns. The ventral cells are still repressed by Scr; (d) Helmets/horns are novel traits. Tergal and pleural wing primordia continue to be repressed/modified by Scr in T1, but Scr now also activates the Wing GRN in a novel cluster of dorsal cells that will produce helmets and horns. (e) Testing these alternative hypotheses with Crispr-Cas9 will depend on the chance (or targeted) knock-out of Scr expression (presence of Scr is in orange) in only parts of the T1 segment, where the candidate WSHs reside (Light green – tergalWSH primordia; Dark green – pleural WSH primordia; Blue – novel helmet/horn primordia). (f) Expected phenotypes under each of the alternative hypotheses (colors of traits correspond to the colors of the primordia in E that build them)

Conversely, what often distinguishes a serial homolog from a novel trait is the distinct developmental coordinates from where the two traits develop, as mentioned above. However, when a new trait emerges in a general body region known to harbor the potential to develop a serial homolog that has been repressed at that location for millions of years, it becomes unclear whether the trait is truly novel or merely a re-emerged and modified serial homolog.

HORNS AND HELMETS: PREMATURE CONCLUSIONS FROM LATEST STUDIES

Because of these difficulties, two recent studies have drawn distinct conclusions about the developmental origins of helmets and horns that originated in thoracic segment T1 of treehoppers and beetles, respectively. The first thoracic segment is a region of the body where wings have been modified into body wall, or completely repressed in different insect lineages.[19,25,30,33] In beetles and hemipterans with a nonmodified T1 segment, when the locally acting Hox gene *Sex combs reduced* (*Scr*) is knocked-down, wings re-emerge in T1from bits of the body wall, which in turn disappear[19,25,33] (Figure 1). But what has happened in species that have modified T1 segments with horns and helmets? Which GRN is involved in building these traits? And are they novel traits or re-emerged and modified wing serial homologs (WSH)?

In the study with the dung beetles,[11] the authors took a functional approach (among others) and asked what would happen if *Scr* was knocked down in those beetles. They used RNAi for this experiment and found thatwings re-emerged in T1, as in previous experiments

with *Tribolium* and *Tenebrio* (non-horned) beetles, but the hornthat was present in that same T1 wildtype segment also dissapeared.[11] The study concluded that the horn had transformed intoa pair of wings. In other words, the study concluded that the horn is an atavistic and reshaped pair of WSHs. However, the study did not investigate whether the traditional bits of the body wall that normally become transformed into wings had also dissapeared.

The helmet study took a different approach to investigate the origins of the helmet GNR. The authors compared transcriptomes of different developing body parts of two hemipteran species, with and without a helmet, and showed that the helmet's tissue transcriptome was most similar to that of the wing in the species with a helmet, but most similar to the dorsal T2 plate (the tergum) in the species without the helmet.[7] Previous data had showed the presence of wing markers in developing helmet tissue,[27] but the lack of any type of articulation between the helmet and the rest of the segment, as expected for a WSH.[20,36] These previous data together with the new transcriptome data led the authors to conclude that the helmet was instead a novel trait derived from the wing GRN having been co-opted to a novel location in that same T1 body segment.

I believe that the conclusions from both studies are not as clear cut as presented and could still support either hypothesis, that is, that thoracic horns in dung beetles are truly novel traits, rather than WSHs, and that helmets in tree-hoppers may be partial WSHs.

HOX GENES CAN BOTH MODIFY AS WELL AS PROMOTE THE DEVELOPMENT OF NOVEL TRAITS

Because serial homologs can look quite distinct from each other, they have been identified at the experimental level by their ability to convert into another via the manipulation of region-specific selector genes such as Hox genes.[32] Hox genes are transcription factors that regulate the expression of downstream target genes. By virtue of being restricted to just a few regions (or segments) along the anterior-posterior body axis of animals, hox genes are often involved in repressing or modifying traits, such as serial homologous traits, along that axis. For instance, mutations in most of the head and trunk Hox genes in *Tribolium* beetles produced embryos with a pair of identical antennal-like limbs developing from each segment.[1] These and other experiments[15] have shown that Hox genes are used for modifying serial homologs along an arthropod body into a unique morphology, or for repressing serial homologs altogether from particular body regions. In these latter instances, when the Hox gene is disrupted, a trait that has been repressed (or modified beyond recognition) for millions of years can re-emerge.

Hox genes, however, can also promote the origin of novel traits. In particular, when novel traits are restricted to specific regions of the body, their development is often dependent on a locally expressed Hox gene. Examples include the development of the rows of sex combs in fly legs used for mating,[31] the pollen basket in worker bees,[18] head horns in dung beetles[35]s, thoracic horns in rhinoceros beetles,[24] and eyespots in nymphalid butterflies.[17] In these examples, and quite distinctly from their function as modifiers or repressors of serial homologs, such as arthropod limbs, Hox genes promote the development of novel traits, and when a Hox gene is disrupted the novel trait is severely disrupted or lost.

Given that hox genes are general transcription factors that can either promote the development of novel traits or repress/modify pre-existent traits, the observed loss of thoracic horns paired with the simultaneous gain of wings in T1 of dung beetles, when the Hox gene *Scr* was downregulated with RNAi, can simply reflect two distinct actions of the same Hox gene on two separate traits, both derived from the same ancient GRN. Specifically, *Scr*RNAi could have led to the loss of horns that depend on *Scr* for their development, and to the gain of wings, a different trait, that depends on *Scr* for its repression in T1 of beetles. Under this scenario, when T1 horns originated, *Scr* kept its repressor/modifier role toward the wing GRN in that segment, but perhaps, and in combination with a regulatory protein expressed more dorsally (perhaps *pannier*, see below), Scr gained a novel activating role for the same wing GRN. RNAi downregulation experiments targeting the *Scr*Hox gene by diffusion of dsRNA molecules across the whole body cannot, thus, distinguish whether thoracic horns in these beetles are WSHs or truly novel traits developing from distinct embryonic/larval coordinates on the same T1 segment.

The authors tried to address the possible dual function of *Scr* by using a double gene knockdown, involving *Scr* and a gene, *pannier (pnr)*, that when knocked-down in isolation showed horn-specific effects but no effects on T2 or T3 wings. *Pannier* is expressed in the dorsal region of the thorax of *Drosophila*,[8] is also differentially expressed between male and female T1 thoracic horn primordia of rhinoceros beetles, and leads to horn loss in these beetles upon knock-down.[24]The double *Scr/prn* knock-down experiments showed thatT1 ectopic wings were smaller than when *Scr* alone was used, suggesting that horn tissue was prevented from converting completely into wing tissue.[11] However, an alternative interpretation, such as the two genes having a positive interactive effect in the promotion of T1 wing growth(not observed in T2 or T3 wings given that *Scr* is not expressed in those segments) is also possible, making this experiment inconclusive. Furthermore, *pnr* appears to have distinct isoforms in *Drosophila* that either activate or repress growth genes such as *wingless*, side by side in the thoracic body wall, depending on the presence of other proteins,[8] calling for additional research on *pannier* in dung beetles.

FUTURE DIRECTIONS: EMBRYONIC MARKER INVESTIGATIONS AND USE OF CRISPR-Cas9

I believe the developmental origins of both horns and helmets are still open for debate, and below I propose two approaches that can help further distinguish serial homologs from novel traits experimentally in these as well as other systems.

First, the developmental origins of thoracic horns or helmets (or of any focal trait of interest) should be more closely investigated at the cellular level during embryonic and/or early larval/nymphalstages. It is important to examine whether the homologs of cells known to produce wings in T2 and T3 segments are now producing hornor helmet

FIGURE 2 Body appendages that appear in segments where wing serial homologs have been repressed in close relatives. (a) Treehopper (*Hybanda* sp.) with helmet in T1. (b) Brown rhinoceros beetle (*Xylotrupesgideon*) with horns in head and T1 segment. (c) Plain nawab caterpillar (*Polyurahebeplautus*) with horns in head segments (no wing serial homologs are known to develop in head segments). (d) Bull horn stinkbug (*Pygoplatys lancifer*) with body projections in T1 segment; (e) Spiky grouse grasshopper (*Discotettixbelzebuth*) with multiple protrusions along its trunk segments. (f) Hickory horned devil (*Citheroniaregalis*) with horn-like projections in T1-T3 segments. These are likely not wing serial homologs as they appear in up to 4 units per segment and wings are also developing simultaneously inside the body of these larvae in T2-T3 segments. (a-d) Photos by Nicky Bay, (www.nickybay.com).(e) Photo by A. Monteiro. (f) Photo by CDC, public domain

primordia in T1. Wing development is increasingly viewed as the merging of two-cell populations[4,23] where the wing blade derives from dorsal (tergal) cells and the hinge from more ventral (pleural) cells.[16] These two-cell populations, which express the wing marker gene *vestigial*, but which have not yet been observed fusing, have each been proposed to give rise to a part of the wing, and, thus, to be a partial WSH.[4,23] So, it is important to follow the fate of serial homologs of these cells in the T1 segment of treehoppers and horned beetle species. This involves perhaps following the expression of genetic markers of these cells during early embryonic development as was done for other wing marker genes such as *snail*-expressing-cells in *Drosophila*[29] or *nubbin*-expressing cells in *Tribolium*.[13] It is important to note here that an important wing marker gene, *vestigial*, expressed in both dorsal and ventral cells that merge to form a wing, and investigated by Hu and colleagues in pre-pupae (but not in embryos), was expressed in horn tissue and also in the carinated margin, the proposed *dorsal* component of a wing, suggesting that this component together with the more ventral (pleural) component of the wing are still present (and modified or repressed) in the T1 segment of dung beetles, and that a third novel expression domain of *vestigial* is present in a more dorsal position of the body (see Figure 1d) that may underlie horn development in these beetles. Really pinning down embryonic or even nymphal or larval coordinates for the trait under investigation (horns/helmets, etc) and showing that these coordinates are distinct from those of a pre-existent trait should be a litmus test in distinguishing a novel trait from a serial homolog.

Second, CRISPR/Cas9, instead of RNAi, should be used to target the locally expressed Hox gene where the focal trait is present. This approach takes advantage of the natural mosaic of wild-type and mutant cells that, by chance, will be found side by side in the same segment. If the trait in question is a WSH, or even just a partial WSH (deriving perhaps only from the dorsal cluster of cells that are used

for building wings[4]), it should be impossible to obtain both traits developing together in the same segment (Figure 1b,c). This represents one of Patterson's tests for homology, who proposed that if a human arm and a wing of a bird were ever to be found in the same body (as in an angel) they could never be homologous.[26] However, if the trait is a novel trait, with a separate embryonic coordinate from the previously modified/silenced trait, it should be possible to obtain both traits emerging from the same segment simultaneously, namely wing and horn or wing and helmet (Figure 1d). For treehoppers and dung beetles, and under the hypothesis that helmets and thoracic horns are truly novel traits, this would happen if *Scr* gene function is removed from cells where it works as a trait repressor (e.g., the wing primordia), and maintained in the cells where it works as a trait promotor (e.g., helmetor horn primordia) (Figure 1d).

Finally, while both studies reviewed here have added exciting new data to the resolution of old problems, there is still scope for future studies to shed additional light on these and similar evo-devo mysteries. These include horns in the head and in the thoracic segments of lepidopteran larvae (Figure 2c,f), lateral projections in the T1 segment of hemipterans (Figure 2d), and similar projections in the trunk segments of grasshoppers (Figure 2e). Similar mysteries exist in the abdomen of insects and spiders, where different types of ventral appendage have emerged after abdominal legs had been repressed for millions of years. Here, we have to address the origin of spider spinnerets,[14] abdominal appendages of lepidopterans, sawflies, or scorpion fly larvae,[12] and sepsid fly male abdominal appendages.[28] All these traits still need to be approached experimentally using novel powerful genetic technologies.

CONCLUSIONS

Here, I propose the use of early (embryonic) gene expression investigations of maker genes of any suspected candidate GRN that may have been either co-opted or resurrected to develop a new trait at a general body location that is known to have the potential to develop a serial homolog of a pre-existent trait. A single domain of marker gene expression should point to a serial homolog, whereas two domains should point to a novel trait. In addition, using CRISPR (but not RNAi) against the locally expressed hox gene might allow the development of the old repressed/modified serial homolog (in clones where CRISPR was effective), as well as the development of the novel trait (in clones not affected by CRISPR), if co-option is responsible for the origin of the novel trait.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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REFERENCES

1. Brown, S. J., Shippy, T. D., Beeman, R. W., & Denell, R. E. (2002). Tribolium Hox genes repress antennal development in the gnathos and trunk. *Mol. Phylogenet. Evol.*, *24*(3), 384–387. https://doi.org/10.1016/s1055-7903(02)00205-1
2. Carroll, S. B., Gates, J., Keys, D. N., Paddock, S. W., Panganiban, G. E. F., Selegue, J. E., & Williams, J. A. (1994). Pattern formation and eyespot determination in butterfly wings. *Science*, *265*, 109–114.
3. Clark-Hachtel, C. M., Linz, D. M., & 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*(42), 16951–16956. https://doi.org/10.1073/pnas.1304332110
4. Clark-Hachtel, C. M., & Tomoyasu, Y. (2016). Exploring the origin of insect wings from an evo-devo perspective. *Curr. Opin. Insect Sci.*, *13*, 77–85. https://doi.org/10.1016/j.cois.2015.12.005
5. Cohen, B., Simcox, A. A., & Cohen, S. M. (1993). Allocation of the thoracic imaginal primordia in the Drosophila embryo. *Development*, *117*(2), 597–608.
6. Connahs, H., Tlili, S., Creij, J. v., Loo, T. Y. J., Banerjee, T. D., Saunders, T. E., & Monteiro, A. (2019). Activation of butterfly eyespots by Distal-less is consistent with a reaction-diffusion process. *Development*, *146*, dev169367. https://doi.org/10.1242/dev.169367
7. Fisher, C. R., Wegrzyn, J. L., & Jockusch, E. L. (2020). Co-option of wing-patterning genes underlies the evolution of the treehopper helmet. *Nat. Ecol. Evol.*, *4*(2), 250–260. https://doi.org/10.1038/s41559-019-1054-4
8. Fromental-Ramain, C., Vanolst, L., Delaporte, C., & Ramain, P. (2008). Pannier encodes two structurally related isoforms that are differentially expressed during Drosophila development and display distinct functions during thorax patterning. *Mech. Dev.*, *125*(1-2), 43–57. https://doi.org/10.1016/j.mod.2007.10.008
9. Fuse, N., Hirose, S., & Hayashi, S. (1996). Determination of wing cell fate by the escargot and snail genes in Drosophila. *Development*, *122*(4), 1059–1067.
10. Glassford, W. J., Johnson, W. C., Dall, N. R., Smith, S. J., Liu, Y., Boll, W., … Rebeiz, M. (2015). Co-option of an ancestral hox-regulated network underlies a recently evolved morphological novelty. *Dev. Cell*, *34*(5), 520–531. https://doi.org/10.1016/j.devcel.2015.08.005
11. Hu, Y. G., Linz, D. M., & Moczek, A. P. (2019). Beetle horns evolved from wing serial homologs. *Science*, *366*(6468), 1004–1007. https://doi.org/10.1126/science.aaw2980
12. Kou, L. X., & Hua, B. Z. (2016). Comparative embryogenesis of Mecoptera and Lepidoptera with special reference to the abdominal prolegs. *J. Morphol.*, *277*(5), 585–593. https://doi.org/10.1002/jmor.20521
13. Linz, D. M., & Tomoyasu, Y. (2018). Dual evolutionary origin of insect wings supported by an investigation of the abdominal wing serial homologs in Tribolium. *Proc. Natl. Acad. Sci. USA*, *115*(4), E658–E667. https://doi.org/10.1073/pnas.1711128115
14. Mariano-Martins, P., Lo-Man-Hung, N., & Torres, T. (2020). Evolution of spiders and silk spinning: Mini review of the morphology, evolution, and development of spiders' spinnerets. *Front. Ecol. Evol.*, *8*, 109. https://doi.org/10.3389/fevo.2020.00109
15. Martin, A., Serano, J. M., Jarvis, E., Bruce, H. S., Wang, J., Ray, S., … Patel, N. H. (2016). CRISPR/Cas9 mutagenesis reveals versatile roles of Hox genes in crustacean limb specification and evolution. *Curr. Biol.*, *26*(1), 14–26. https://doi.org/10.1016/j.cub.2015.11.021

16. Mashimo, Y., & Machida, R. (2017). Embryological evidence substantiates the subcoxal theory on the origin of pleuron in insects. *Sci. Rep.*, *7*. https://doi.org/10.1038/s41598-017-12728-2
17. Matsuoka, Y., & Monteiro, A. (2020). *Hox genes are essential for the development of novel serial homologous eyespots on the wings of Bicyclus anynana butterflies*. BioRxiv. https://doi.org/10.1101/814848
18. Medved, V., Huang, Z. Y., & Popadic, A. (2014). Ubx promotes corbicular development in Apis mellifera. *Biol. Lett.*, *10*(1), 20131021. https://doi.org/10.1098/rsbl.2013.1021
19. Medved, V., Marden, J. H., Fescemyer, H. W., Der, J. P., Liu, J., Mahfooz, N., & Popadic, A. (2015). Origin and diversification of wings: Insights from a neopteran insect. *Proc. Natl. Acad. Sci. USA*, *112*(52), 15946–15951. https://doi.org/10.1073/pnas.1509517112
20. Miko, I., Friedrich, F., Yoder, M. J., Hines, H. M., Deitz, L. L., Bertone, M. A., … Deans, A. R. (2012). On dorsal prothoracic appendages in treehoppers (Hemiptera: Membracidae) and the nature of morphological evidence. *PLoS One*, *7*(1), e30137. https://doi.org/10.1371/journal.pone.0030137
21. Moczek, A. P., & Rose, D. J. (2009). Differential recruitment of limb patterning genes during development and diversification of beetle horns. *Proc. Natl. Acad. Sci. USA*, *106*(22), 8992–8997. https://doi.org/10.1073/pnas.0809668106
22. Monteiro, A., & Podlaha, O. (2009). Wings, horns, and butterfly eyespots: How do complex traits evolve? *PLoS Biol.*, *7*(2), e1000037.
23. Niwa, N., Akimoto-Kato, A., Niimi, T., Tojo, K., Machida, R., & Hayashi, S. (2010). Evolutionary origin of the insect wing via integration of two developmental modules. *Evol. Dev.*, *12*(2), 168–176.
24. Ohde, T., Morita, S., Shigenobu, S., Morita, J., Mizutani, T., Gotoh, H., … Niimi, T. (2018). Rhinoceros beetle horn development reveals deep parallels with dung beetles. *PLoS Genet.*, *14*(10), e1007651. https://doi.org/10.1371/journal.pgen.1007651
25. Ohde, T., Yaginuma, T., & Niimi, T. (2013). Insect morphological diversification through the modification of wing serial homologs. *Science*, *340*, 495–498, https://doi.org/10.1126/science.1234219
26. Patterson, C. (1988). Homology in classical and molecular biology. *Mol. Biol. Evol.*, *5*(6), 603–625.
27. Prud'homme, B., Minervino, C., Hocine, M., Cande, J. D., Aouane, A., Dufour, H. D., … Gompel, N. (2011). Body plan innovation in treehoppers through the evolution of an extra wing-like appendage. *Nature*, *473*(7345), 83–86. https://doi.org/10.1038/nature09977
28. Rajaratnam, G., Supeinthiran, A., Meier, R., & Su, K. F. Y. (2018). CRISPR/Cas9 deletions in a conserved exon of Distal-less generates gains and losses in a recently acquired morphological novelty in flies. *Iscience*, *10*, 222–233. https://doi.org/10.1016/j.isci.2018.11.036
29. Requena, D., Álvarez, J. A., Gabilondo, H., Loker, R., Mann, R. S., & Estella, C. (2017). Origins and specification of the Drosophila wing. *Curr. Biol.*, *27*(24), 3826–3836.e5. https://doi.org/10.1016/j.cub.2017.11.023
30. Rogers, B. T., Peterson, M. D., & Kaufman, T. C. (1997). Evolution of the insect body plan as revealed by the Sex combs reduced expression pattern. *Development*, *124*(1), 149–157.
31. Tanaka, K., Barmina, O., Sanders, L. E., Arbeitman, M. N., & Kopp, A. (2011). Evolution of sex-specific traits through changes in HOX-dependent double sex expression. *PLoS Biol.*, *9*(8), e1001131. https://doi.org/10.1371/journal.pbio.1001131
32. Tomoyasu, Y. (2018). Evo-devo: the double identity of insect wings. *Curr. Biol.*, *28*(2), R75–R77. https://doi.org/10.1016/j.cub.2017.12.004
33. Tomoyasu, Y., Wheeler, S. R., & Denell, R. E. (2005). Ultrabithorax is required for membranous wing identity in the beetle Tribolium castaneum. *Nature*, *433* (7026), 643–647.
34. von Dassow, G., Meir, E., Munro, E. M., & Odell, G. M. (2000). The segment polarity network is a robust developmental module. *Nature*, *406*, 188–192.
35. Wasik, B. R., Rose, D. J., & Moczek, A. P. (2010). Beetle horns are regulated by the Hox gene, Sex combs reduced, in a species- and sex-specific manner. *Evol. Dev.*, *12*(4), 353–362. https://doi.org/10.1111/j.1525-142X.2010.00422.x
36. Yoshizawa, K. (2012). The treehopper's helmet is not homologous with wings (Hemiptera: Membracidae). *Syst. Entomol.*, *37*(1), 2–6. https://doi.org/10.1111/j.1365-3113.2011.00606.x

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