



Original research article

Taking flight!

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ABSTRACT

Understanding the origins of novel complex traits, the evolutionary transitions they enabled, and how those shaped the subsequent course of evolution, are all foundational objectives of evolutionary biology. Yet how developmental systems may transform to yield the first eye, limb, or placenta remains poorly understood. Seminal work by Courtney Clark-Hachtel, David Linz, and Yoshinori Tomoyasu published in the *Proceedings of the National Academy of Sciences* in 2013 used the origins of insect wings - one of the most impactful innovations of animal life on Earth - to provide both a case study and a new way of thinking of how novel complex traits may come into being. This paradigm-setting study not only transformed the way we view insect wings, their origins, and their affinities to other morphological structures; even more importantly, it created entryways to envision innovation as emerging gradually, not somehow divorced from ancestral homology, but *through it* via the differential modification, fusion, and elaboration of ancestral component parts. In a conceptual universe of *descent with modification*, where everything new must ultimately emerge from the old, this work thereby established a powerful bridge connecting ancestral homology and novelty through a gradual process of innovation, sparking much creative and groundbreaking work to follow since its publication just a little over a decade ago.

The origins of novelty constitute both a foundational and a largely unresolved challenge in biology (Raff, 1996; Shubin et al., 2009; Wagner, 2014). It is foundational because it is at the heart of what motivates our fascination with the evolutionary process, and how - under the right circumstances - it may yield the first limb, eye, placenta, etc., and how once in existence these innovations forever altered subsequent diversification on this planet. In our quest to understand why and how evolution unfolds the way it does, the nature of innovation rightfully deserves to take center stage. But the origin of novelty also remains a largely unresolved challenge, in large part because it resists easy accessibility through conceptual frameworks otherwise central to evolutionary biology, such as the evolution of adaptation by means of natural selection, or neutral evolution via drift and historical accidents, or the role played by genetic and developmental constraints in prioritizing certain routes of phenotype construction over others. While these and other conceptualizations of the evolutionary process remain valuable, none offers much help when it comes to addressing the origins of novelty, because selection can only sort among pre-existing variation, and likewise drift and constraints only eliminate options, but by themselves cannot create new ones (Moczek, 2008). As such, traditional evolutionary biology has yet to illuminate how evolutionary novelties come into being (Wagner, 2014).

Fortunately, evolutionary developmental biology - often abbreviated

as *evo devo* - has begun to come to the rescue over the past three decades by revolutionizing the way we think about the genesis of organismal diversity: away from organisms as uniquely evolved each on their own, and toward organisms as uniquely *assembled* from the same and finite pool of building blocks, be those genes, signal transduction pathways, cell types, or morphogenetic processes (Carroll et al., 2004; Shubin et al., 2009). We now understand that fruit flies and humans share about 65% of genes executing similar functions in both organisms (Ugur et al., 2016), that many of the same pathways instruct the formation of cephalopod, vertebrate, and arthropod eyes even though all three originated independently of each other (Koenig and Gross, 2020), and that outgrowths as diverse as the tail of a mouse, the horn of a beetle, the tube feet of echinoderms or the siphons of ascidians all establish their proximo-distal axis using homologous genetic and developmental tools and processes (Panganiban et al., 1997; Moczek and Rose, 2009). Yet all these extraordinary insights notwithstanding, how *reassembly* may eventually beget *novelty* has remained unclear. In fact, *evo devo* defined novelty as a discrete category early on, i.e. as traits that lack homology to traits in other organisms (homology) or to other structures in the same organisms (serial homology or homonymy; sensu Müller and Wagner, 1991). But in doing so *evo devo*, too, cut itself off from being able to offer starting points to interrogate the beginnings of innovation. It was in this conceptual gridlock that work by Courtney Clark-Hachtel, David Linz,

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and Yoshinori Tomoyasu published in the *Proceedings of the National Academy of Science* in 2013 began to create a critical and consequential opening, using the origins of insect wings as a focal innovation to provide both a case study and eventually a new way of thinking for how novel complex traits come into being (Clark-Hachtel et al., 2013).

1. The origins of insect wings - a 200-year-old debate

The wings of insects are one of the most extraordinary evolutionary innovations (Grimaldi and Engel, 2005). Unlike the three other independent origins of sustained flight observed in bats, birds and pterosaurs whose wings derive from modifications of the ancestral quadruped forelimb (Dudley, 2000), insect wings emerged in the context of an entirely different bauplan, yet how they did so has been the subject of a debate dating back to the early 19th century (reviewed in Clark-Hachtel et al., 2013; Clark-Hachtel and Tomoyasu, 2016; see also Oken, 1809; Crampton, 1916; Wigglesworth, 1973; Kukalova-Peck, 1983). Insects possess a three-segmented thorax, of which the second (T2) and third segment (T3) each support a pair of wings. While these may subsequently be modified into various forms - e.g. forewings to elytra in beetles, hindwings to halteres in true flies - this basic rule holds across insect orders: T2 and T3 bear wings, while T1 never does (Snodgrass, 1935).

The significance of wings for the evolutionary success of insects cannot be overstated. Wings did not simply allow insects to take to the sky, they also allowed them to hunt, disperse, pollinate, seek mates, shelter, and evade dangers in ways previously impossible, opening up myriad novel niches for insects to radiate into (Grimaldi and Engel, 2005). So how were insect wings thought to have originated? Two conflicting hypotheses dominated the debate over the past 200 years. First, the *paranotal lobe* hypothesis posits that insect wings originated via extensions of the dorsal-most sclerite that makes up each thoracic segment - the notum (Quartau, 1986; Hamilton, 1971; Rasnitsyn, 1981). This hypothesis envisions that over evolutionary time an ever larger, bilateral notal extension was eventually joined by a hinge and associated muscular and nervous support system to arrive at the exquisitely articulated wings we observe today. Supported primarily by fossil data, the chief weakness of this hypothesis, however, lies in the difficulty of explaining where from - and how - as complex a component as a well supported hinge mechanism may derive from. Here a second main hypothesis - the *pleural* (aka *gill flap/exite*) hypothesis claimed an advantage. This hypothesis has various articulations, but in essence it posits that insect wings originated from gills or other appendages that branched off of proximal leg regions (so-called “exite bearing leg segments”) that prior to the origin of insects became incorporated into the pleural side walls of thoracic segments (Kukalova-Peck, 1978, 1983). This last part - the incorporation of ancestral leg segments into what we now view as the side wall or *pleuron* - is well supported by comparative morphological data, while shared gene expression between crustacean exites and insect wings lends additional support for the hypothesis as a whole (Averof and Cohen, 1997; Bruce and Patel, 2020; Clark-Hachtel and Tomoyasu, 2020). Still, it remained hard to envision functional intermediate stages that would permit transition from e.g. a highly specialized gill to a highly specialized wing, without having to traverse a valley of forbiddingly low fitness.

Lastly, some also made room for a kind of *dual origin* hypothesis, whereby both notal and pleural compartments may somehow contribute jointly to the formation of insect wings (Crampton, 1916; Rasnitsyn, 1981). This somewhat neglected hypothesis offered a possible resolution of the conflict between the two dominant explanations, and it began to gain significant momentum through a study on two basal hexapod orders, the primitively wingless Archaeognatha (bristletails) and the palaeopteran order Ephemeroptera (mayflies; Niwa et al., 2010). This study found that the tergal margin (a reinforced bilateral edge of the notum) and the unique pleural outgrowths of these two orders *both* express some of the same genes known to instruct wing formation during

Drosophila development. However, by that time *evo devo* had already learned the hard way that shared gene expression alone can be misleading and ought to be interpreted with caution. Still, the work by Niwa et al. (2010) reinvigorated calls to resolve a centuries long gridlock through modern comparative developmental genetic approaches. The stage was thus set for a critical study in another powerful model system in insect *evo devo* - the red flour beetle *Tribolium castaneum* - to forever transform the way we think of insect wings, their origins, and their affinities to other morphological structures.

2. Wing formation through the lens of gene regulatory networks and serial homology

As already hinted at above, the most influential definition of evolutionary novelty in *evo devo* equates novelty with the absence of homology or homonymy (serial homology) to other traits (Müller and Wagner, 1991). However, since the articulation of this definition *evo devo* has systematically forced a revision of how we think of homology. Traditional criteria (location relative to other traits, intermediate stages in development and/or fossil record, and special qualities; Remane, 1952) allowed a binary assessment: traits either were or were not homologous - done! But *evo devo* taught us that homologous genes, pathways, and tissues may give rise to non-homologous morphological features, and conversely, that homologous morphologies can be underlain by clearly non-homologous developmental genetic means. In other words, homology became layered and partial, a matter of degree. It also raised the question: where does homology end and novelty begin? Is this even a meaningful way to interrogate innovation in evolution? The work by Clark-Hachtel et al. (2013) advanced this discussion like few before.

First, the author team documented that components of the wing gene regulatory network (as known from a large body of work in *Drosophila* but also *Tribolium*), not only underlie the formation of *bona fide* fore and hind wings, but in *Tribolium* also seemed to be required for the formation of several somewhat unimpressive morphological structures on the first thoracic segment (T1): the carinated margin (a component of the notum) and the trochantin and epimeron (components of the pleural side walls). Specifically, downregulation of the critical wing patterning gene *vestigial* (*vg*) was found to compromise not only wing formation, but also resulted in defects in all three of the above-listed T1 structures (Clark-Hachtel et al., 2013). Further, proper formation of the carinated margin in particular also depended on other factors essential for wing development (e.g. Wingless signal, *apterous* expression; *nubbin* enhancer activity). This first set of results therefore critically reinforced the notion that overlapping gene networks may be responsible for the formation of both T2/3 wings and select T1 structures.

Most importantly, however, Clark-Hachtel et al. (2013) took a closer look at a well-known homeotic transformation. The Hox gene *Sex combs reduced* (*Scr*; *Cephalothorax* (*cx*) in *Tribolium*) inhibits wing formation in T1, and when compromised the *Tribolium* T1 segment produces a pair of ectopic elytra on T1 (fore wings; Carroll et al., 1995; Tomoyasu et al., 2005; Wasik et al., 2010). Strong transformations are spectacular testimony to the powerful selector function of homeotic genes, but Clark-Hachtel et al. (2013) chose to look more closely at hypomorphic phenotypes instead - partial and largely incomplete transformations. Doing so revealed that ectopic T1 elytra actually originate from two distinct T1 locations, the carinated margin as well as the much more ventrally located base of the epimeron. In more strongly transformed individuals, these two outgrowths then merged into one, which in the strongest cases then yielded the fully transformed ectopic elytra previously documented. Clark-Hachtel et al. (2013) thus showed that the T1 tissues whose normative development necessitates regulation by parts of the wing gene regulatory network (GRN) can be transformed into wings, or in other words that the carinated margin and pleural plates together appear to be serially homologous to wings. Work on *Tenebrio molitor*, a beetle species in the same family as *Tribolium*, by Ohde et al. (2013) independently arrived at largely similar conclusions.

Such serial homologs are everywhere in development and evolution, and most are easy to spot: cervical, thoracic and lumbar vertebrae are serially homologous (McShea, 1991), as are the antennae, mouthparts, legs, and genitalia of insects' (Snodgrass, 1935). However, the realization that insects fore and hind wings are serially homologous to completely non-wing-like structures was a huge surprise. And it did not stop there. Additional work investigated whether corresponding tissues may also exist in the abdominal segments of insects, and thus outside the wing-bearing thorax. This work was in part motivated by the observation that insect abdomens often produce serially repeated bilateral projections such as stylets, pupal support structures, or so-called gin-traps (toothed defensive structures found in many pupae), even though none of them look remotely wing-like. But neither did the carinated margin and epimeron. Studies on a series of beetle systems (*Tenebrio*: Ohde et al., 2013; *Tribolium*: Linz and Tomoyasu, 2018; Hu et al., 2018; *Onthophagus*: Hu and Moczek, 2021) ended up supporting - to varying degrees - the existence of notal and/or pleural wing serial homologs in each segment, with notal homologs in particular able to instruct the formation of diverse abdominal outgrowths. Under the right circumstances, such as an abdomen-wide knockdown of homeotic selector genes, these abdominal wing serial homologs could even be transformed once again to form pairs of wings, one on each abdominal segment (Ohde et al., 2013; Linz and Tomoyasu, 2018). Further, Clark-Hachtel et al. (2013) also helped advance our understanding of several other traits often considered textbook examples of evolutionary innovations, such as the extraordinary helmets of membracid treehoppers (Hemiptera, e.g. Prud'Homme et al., 2011; Fisher et al., 2020) and the enormous and highly diversified prothoracic horns of scarab beetles (Moczek et al., 2006). The work of Yonggang Hu and David Linz on horned dung beetles in particular (Hu et al., 2019) illustrated the power of the wing GRN in providing conserved starting points to localize, initiate, and pattern early trait development, to be then followed by the establishment of trait specific transcriptional landscapes enabling the extraordinary diversity of traits found within and outside wing-bearing segments (see also Linz et al., 2020; Linz and Moczek, 2020).

Collectively, this impressive body of work not only provided additional support for the dual origin hypothesis across a growing taxonomic range (see also work by Elias-Neto and Belles (2016) on cockroaches and Medved et al. (2015) on milkweed bugs), but also began to make room for two novel considerations: first, wing serial homologs outside wing bearing segments emerged as so commonplace that they may simply represent an ancestral feature of insect segments, possibly predating the origin of wings; and second, that differential collaboration between two ancestrally distinct tissues may be a powerful avenue in the genesis of novelty: the notal gene network appeared especially effective in enabling the formation of projections, whereas the pleural network could contribute key substrate for articulations and hinges. Building gin-traps, pupal support structures, or beetle horns was supported by the notal homolog alone, but by collaborating within the confines of T2 and T3, both serial homologs synergized to form the insect wing. As such, this work was beginning to create an entryway to envision innovation as emerging not somehow divorced from ancestral homology, but through it.

3. The innovation gradient

How does the work by Courtney Clark-Hachtel, David Linz, and Yoshinori Tomoyasu, as well as the body of work that followed, help us reconcile descent with modification with the origins of novelty? First, these discoveries underscore that *evo devo* would do well to once and for all let go of the notion that morphological novelty must somehow emerge or exist in the absence of ancestral homologies, and that we instead should focus our attention on *how* ancestral homologies scaffold and bias the process of innovation (Wake, 1999, 2003). In particular, this work invites us to imagine evolutionary innovation as a *gradient*, contributed to by a diversity of processes including of course the long

recognized pervasiveness of evolutionary changes in timing, location, amount, and governance of gene expression, but also the power of combining ancestrally distinct GRNs and corresponding tissues into novel functional units, able to undergo collective diversification in ways each component part alone was previously precluded from. Doing so would put us in a position to investigate how the nature of the innovation gradient may facilitate hotspots of innovation in some anatomical locations or developmental circumstances, yet deep conservation elsewhere. In the process, we may also well recognize that important early stages of innovation may already be discernible in the development of diversity within and among closely related taxa. This then may put us on a path to eventually confront what many consider the final frontier in *evo devo*: to integrate the processes that produce novelty and diversity in developmental evolution with those that sort it within natural populations.

CRedit authorship contribution statement

Armin P. Moczek: Writing – review & editing, Writing – original draft, Conceptualization.

Data availability

No data was used for the research described in the article.

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