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Contrasting paths to evolutionary novelty: insect wings and wing hearts

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Abstract: Contrasting paths to evolutionary novelty: insect wings and wing hearts. Some organs in evolutionary history appear to have emerged without clear precursors and transitional forms, challenging the gradualist assumptions central to Darwinian theory. Strikingly, studies focusing on such evolutionary novelties remain limited to a few well-characterized cases. This review examines two instructive examples in insects that illustrate distinct pathways to fundamental phenotypic innovation. The first concerns the origin of insect wings, undoubtedly one of the most significant and extensively studied morphological novelties in arthropods. Since the early post-Darwinian era, two competing explanatory models have dominated the debate: the paranotal lobe hypothesis and the leg-exite hypothesis. Recent evo-devo research has reshaped this discourse, culminating in the integrative dual origin hypothesis. However, emerging evidence has reignited the controversy, suggesting that wing evolution may be more complex than previously thought. The second example focuses on wing-associated circulatory organs, evolutionary novelties that have received comparatively little attention. These small pumping units drive hemolymph flow through the wing vein network, a mechanism essential for maintaining sensory structures on the wings and preserving cuticular hydration, which is critical for their biomechanical performance. While the ancestral condition is represented by modifications of the dorsal vessel, fully independent wing hearts have evolved multiple times across various insect lineages as novel pulsatile organs. Their emergence appears to be a consequence of spatial constraints imposed by the progressive evolution of the insect flight apparatus. Research in *Drosophila* revealed that these organs originate from four pericardial precursor cells and represent evolutionary novelties in the strictest sense, as they not only lack homology with any known ancestral structure but also develop as discrete modules under the control of unique gene regulatory networks. Wing hearts thus represent a versatile and experimentally accessible model system for studying the emergence and integration of novel traits — a central, yet still underexplored theme in evolutionary biology.

Keywords: circulatory organs, pericardial cells, organ evolution, spatial constraint, wing heart, accessory pulsatile organs, *Drosophila*, pericardial cells

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Introduction

Darwin's concept of "descent with modification" offers a compelling framework for explaining how new organs may arise through the transformation of pre-existing body structures. But what about traits that lack any clear morphological precursor? This question posed a major challenge for Darwin, as his theory of natural selection struggled to account for the origin of such evolutionary novelties. Critics, especially from theological backgrounds, highlighted this difficulty as evidence of a fundamental limitation in Darwin's theory, often invoking the notion of divine intervention to fill the explanatory gap (Wagner & Lynch 2010). Even today, while microevolutionary changes are broadly accepted, skepticism remains over whether current scientific theories can fully explain the emergence of complex evolutionary novelties, a concern not confined to religious communities. Given the profound implications for both evolutionary theory and the broader public acceptance of evolutionary explanations, it is striking how long this topic remained underexplored (Mayr 1960, Müller & Wagner 1991). In the past decade, however, the study of evolutionary novelty has gained considerable momentum, emerging as a central topic in contemporary evolutionary biology (for overviews see Wagner 2014, 2015, Peterson & Müller 2016, Almudí & Pascual-Anaya 2019, Erwin 2021, Müller 2021, DiFrisco et al. 2023a, 2023b, Moczek 2023). Nevertheless, despite substantial progress, the concept of novelty remains far from resolved. This is not only due to methodological challenges but also to deeper epistemological concerns. Defining evolutionary novelty is inherently complex, with different authors applying varying and sometimes conflicting criteria (for an overview see Müller 2021). A central difficulty lies in establishing boundaries: some definitions are so broad that even the addition of a single new bristle on an insect's body qualifies as novelty, while others are so restrictive that only a few extraordinary structures are recognized as such (Pigliucci 2008). Beyond these definitional debates, broader conceptual challenges further complicate discussions about evolutionary novelty, as outlined in the supplementary textbox (Fig. 1). Importantly, such lack of consensus is not unusual in biology, other foundational terms such as homology and species face similar difficulties. Against this background, the epistemologists Brigandt & Love (2012) argue that the study of evolutionary novelty should not become entangled in overly rigid definitions. Instead, they advocate for a broad conceptual framework that can be refined over time.

Building on this perspective, we review the current state of research on two closely related examples of evolutionary novelty in insects. The first is insect wings, whose evolutionary origins have been studied intensively across various disciplines for nearly two centuries. The second concerns the circulatory organs associated with insect wings, which are a primary focus of our research team. Despite their shared evolutionary history and close functional integration, these two structures emerge through fundamentally different developmental and evolutionary trajectories. Together, they offer a valuable comparative framework to explore the diverse mechanisms underlying the emergence of evolutionary novelty.

Insect wings: a landmark example of evolutionary novelty

Among evolutionary novelties, the emergence of insect wings is one of the most frequently cited examples in biology textbooks. Given their profound significance for insect evolu-

Evolutionary novelty: different perspectives and fuzzy concepts

Focus on functional perspective:

„Any newly acquired structure or property that permits the performance of a new function, which, in turn, will open a new adaptive zone, is a novelty“ (Mayr 1963)

Focus on structural perspective:

„A morphological novelty is a structure that is neither homologous to any structure in the ancestral species nor homonomous to any other structure of the same organism“ (Müller & Wagner 1991)

Wagner (2014) emphasized that a morphological novelty must be an individualized trait that is quasi-independent. At the genetic level, this implies large-scale reorganizations of cooperative gene regulatory networks (GRN), referred to as **Character Identity Networks (ChINs)**

DiFrisco et al. (2023a) further expanded this concept → **Character Identity Mechanisms (ChIMs),** which include all critical regulatory mechanisms determining a trait`s identity.

Wagner (2014) further suggests distinguishing between:

- **type I novelties**: *„new traits that arise without any clear precursor in the ancestral lineage and represent entirely new structures or functions that were not present in any form in the ancestors*”
- **type II novelties**: “*new traits that arise as modifications of pre-existing structures. They involve significant changes to an existing trait, leading to a new function or form”*

Fig. 1: Conceptual approaches to evolutionary novelty and their historical development. Two fundamentally distinct perspectives guide this discussion: one emphasizing the function, the other the structure. While defining evolutionary novelty through function is intuitively appealing, it remains difficult to operationalize in research. In contrast, structure-based definitions offer a more concrete framework, but involve conceptual challenges related to elusive concepts such as homology, individuality, and character identity. At the genetic level, character-specific gene regulatory networks (ChINs), or more precisely character identity mechanisms (ChIMs), provide a mechanistic basis for addressing these issues. For alternative terminology and classification of novelty types, see Müller (2021).

tion, it is not surprising that this topic has fascinated both entomologists and the broader scientific community for long time (Brodsky 1994, Dudley 2000, Alexander 2015, Glaeser et al. 2017). Yet, despite being one of the most extensively studied questions in insect evolution, the origin of wings remains the subject of ongoing debate. Our overview highlights the diverse research approaches that have shaped the current understanding and outlines the challenges that remain. We begin by reviewing paleontological evidence and theories based on the limited fossil record. Subsequent sections examine the origin of insect wings from both functional and structural perspectives. Functionally, insect wings represent an exceptional example of novelty, as few organs have taken on such a wide range of essential roles throughout insect evolution. Structurally, their origin has long been a central focus of inquiry, fueling debates that have helped to shape not only our understanding of insect evolution but also broader conceptual frameworks surrounding the emergence of evolutionary novelty. In recent decades, evolutionary developmental biology (evo-devo) has provided new insights into wing evolution by uncovering key developmental and genetic mechanisms underlying this novel structure. Yet even with these advances, recent findings continue to challenge established views, underscoring that fundamental aspects of wing evolution remain unresolved.

Enigmatic wing origin: fossil evidence and interpretative challenges

Compelling evidence suggests that insect wings evolved only once in evolutionary history (Engel et al. 2013, Misof et al. 2014). The oldest unambiguous fossils of pterygote insects date to the Lower Carboniferous, approximately 325 million years ago (Braukmann et al. 1996). Molecular data, however, place the origin of Pterygota around 400 million years ago (Misof et al. 2014). This substantial discrepancy is often attributed to the limited preservation potential of fragile insect bodies and the global scarcity of freshwater sedimentary deposits from that period (Engel et al. 2013). However, a recent study challenges this view, arguing that the presumed early origin of wings may result from analytical artifacts, particularly misleading calibrations of fossil reference points in molecular clocks (Schachat et al. 2023). If correct, this would imply that wings evolved over a significantly shorter timespan than previously assumed. Regardless of the precise timing, wings and powered flight emerged early in insect evolution, granting them a long-standing monopoly in the skies until the arrival of pterosaurs – the first actively flying vertebrates – around 230 million years ago.

The earliest known fossils of Pterygota belong to the Palaeodictyoptera, a group of medium- to giant-sized insects that went extinct at the end of the Paleozoic era, around 250 million years ago (Prokop et al. 2023a). These insects exhibited a pair of fully developed wings on both the meso- and metathorax, almost certainly enabling active flight. Intriguingly, they also displayed small lateral lobes on the prothorax, often referred to as "winglets" (Fig. 2B). Whether these structures were wing precursors or vestigial structures remains unclear. Since they lack clearly defined joints with the thorax, most researchers believe they functioned as stabilizers, possibly enhancing aerodynamic control during gliding (Prokop et al. 2017). Despite extensive fossil searches, no specimens have been found so far that exhibit clear precursor structures of wings on the pterothoracic segments. Consequently, fundamental questions on the structure, articulation, and primary function of ancestral protowings remain speculative. In the absence of transitional fossils — and given the limited explanatory power of fossil evidence for certain functional questions — researchers rely on alternative approaches, such as studies of extant species (e.g. Yanoviak et al. 2009) or experiments with physical models (Kingsolver & Koehl 1985, Wootton & Ellington 1991, Zeng et al. 2023; Fig. 2C).

A central issue in the debate about wing origins concerns the habitat and lifestyle of early Pterygota (Fig. 2). The prevailing view holds that these ancestors were terrestrial, much like all extant primarily wingless hexapods (Wipfler et al. 2019). However, the functional role of wing precursors in this scenario remains unresolved, with various hypotheses proposing protective, thermoregulatory or aerodynamic advantages, or perhaps a combination thereof (Dudley et al. 2007). The evolution of flight is widely discussed in relation to jumping behavior as an escape strategy. In this context, arboreal bristletails (Archaeognatha; Fig. 2A) have received particular attention. These hexapods use directed aerial descent, a form of controlled gliding, to return to tree trunks when dislodged or to evade predators by jumping (Yanoviak et al. 2009). By adjusting the position of their legs, abdomen, and caudal appendages, they achieve a controlled descent. Their thoracic

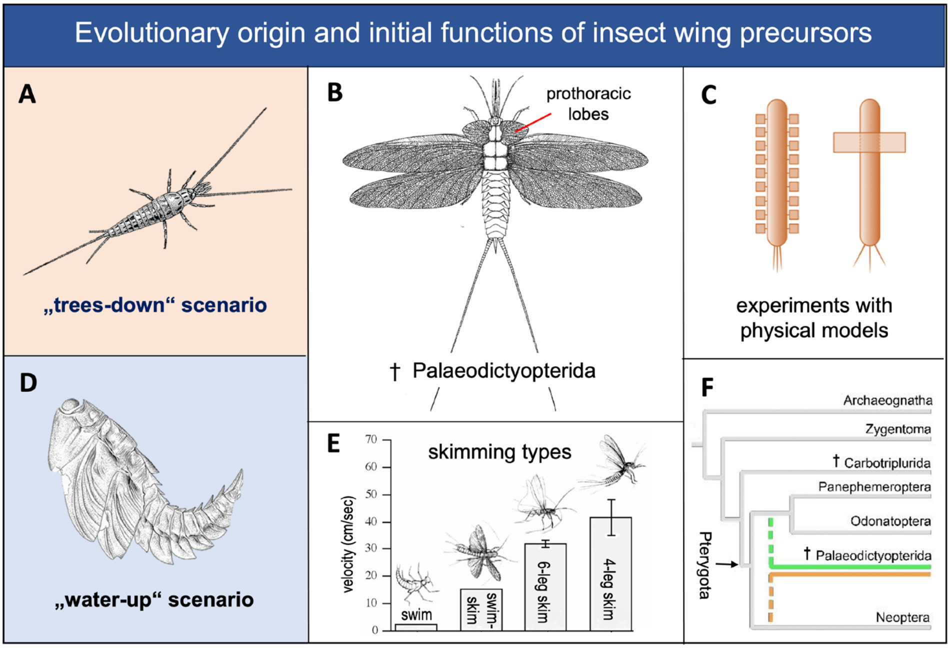


Fig. 2: Approaches to reconstructing the evolutionary origin of insect wing precursors. A–C: *Evidence supporting a terrestrial origin.* A: During directed descent from trees (observed in arboreal bristletails), expanded paranotal lobes in wingless insect ancestors may have enhanced gliding performance. **B:** Prothoracic lobes ("winglets") in adult Palaeodictyoptera may have served a similar aerodynamic function. **C:** Experiments with physical models provide insights into the aerodynamic and structural constraints that likely shaped early wing evolution. **D–E: *Evidence supporting an aquatic origin*. D:** Larvae of certain palaeodictyopterans possessed presumed wing serial homologs on the abdomen, likely functioning as gills. **E:** Diverse aquatic and semi-aquatic locomotion strategies – such as surface skimming in stoneflies and mayflies – highlight the potential functional value of lateral appendages in aquatic environments **F:** Phylogenetic relationships at the base of Pterygota remain unresolved, especially regarding the position of the extinct Palaeodictyoptera. Sources: A, online; B, Kukalová-Peck (1978); C, redrawn from Wootton & Ellington (1991); D, Prokop et al. 2022; E, Marden et al. 2000; F, Ohde & Prokop (2025).

lateral extensions (paranota), which are even more pronounced in certain fossil forms, might have served a stabilizing role, somewhat akin to the proposed function of winglets in Palaeodictyoptera. Directed gliding appears widespread among wingless arthropods, including spiders, insect nymphs, and ants (Dudley & Yanoviak 2011). A key insight from this phenomenon is that arboreal animals evidently experience strong selection pressures to enhance aerial control, providing a compelling model for the evolutionary origins of wings and powered flight.

Nonetheless, some paleontologists have recently argued that an aquatic or semi-aquatic origin of insect wings cannot be ruled out (Prokop et al. 2022, 2023b, Ohde & Prokop 2025; Fig. 2D). This revives older hypotheses that have been reconsidered multiple times in the past (reviewed by Crampton 1916). The recent studies emphasize that phylogenetic reconstructions based on molecular data have inherent limitations in accounting for extinct lineages and that the stem group of Pterygota may have had an aquatic lifestyle.

This interpretation gains further plausibility from the widespread occurrence of aquatic larvae among nearly all major extant basal pterygote lineages. For Palaeodictyoptera, most species had terrestrial larvae, but some were aquatic. There is also evidence suggesting that certain adult Palaeodictyoptera may have lived amphibiously, as indicated by the presence of rudimentary or functional tracheal gills on their abdomen (Prokop et al. 2022). Furthermore, transcriptomic studies of the mayfly *Cloeon dipterum* revealed striking genetic similarities between larval tracheal gills and wings, pointing to a shared developmental program (Almudí et al. 2020). This supports the hypothesis that flattened thoracic and abdominal outgrowths in larval Palaeodictyoptera may be serial homologs of wings (Prokop et al. 2019, 2022). Primarily serving probably respiratory functions, these structures may also have contributed to locomotion and served as platforms for sensory organs. It appears plausible that such larval outgrowths could have been retained into adulthood in modified forms, potentially enabling surface-skimming locomotion across the water surface through enhanced steering and propulsion (Marden & Kramer 1994, Marden et al. 2000; Fig. 2E). Such a transitional stage may have paved the way for the evolution of fully powered flight, which requires the additional capability of supporting the entire body weight in the air. We eagerly await further paleontological discoveries — perhaps even the "*Archaeopteryx* of insects" — to help resolve some of these enduring and fundamental questions.

Functional perspectives: wings as catalysts of novel adaptations and life history transformations

Although the function of ancestral protowings remains debated, the roles of fully developed insect wings are well understood. They have been extensively investigated across a wide range of disciplines (reviews: Brodsky 1994, Dudley 2000, Grimaldi & Engel 2005, Treidel et al. 2024, Lehmann & Wehmann 2025). In a broader evolutionary context, the emergence of wings is widely regarded as one of the most transformative innovations in the insect body plan. Yet, their pivotal contribution to the subsequent diversification of insects is often underappreciated, despite the immense ecological and adaptive advantages they confer. The full impact of wings and powered flight becomes strikingly evident when we consider the evolutionary trajectories that would have remained inaccessible without them.

Of the many functions that insect wings serve, powered flight stands out as arguably the most transformative. Enhanced mobility allows them to explore new habitats and access food sources that are scarce or patchily distributed. Some species even undertake long-distance migrations to overcome seasonal shortages, thereby increasing reproductive success and overall evolutionary fitness (Chapman et al. 2015). Greater mobility has also promoted dietary specialization — a key driver of insect diversification (Albrecht et al. 2023). In addition, wings shape both intra- and interspecific interactions. Improved dispersal capabilities facilitate mating across larger distances, thereby profoundly influencing population structure and reproductive dynamics (García-Berro et al. 2023). Flight also alters predator-prey relationships, enhancing both predatory efficiency and prey evasion. Similarly, while mobility helps insects escape parasites and pathogens, it can also contribute to their spread.

Wings are not only indispensable for flight but also serve a wide array of additional functions. Heavily sclerotized wings provide protection against mechanical damage and desiccation (LINZ et al. 2016; GOCZAŁ et al. 2025), while their coloration — whether cryptic or conspicuous — can reduce predation risk (TENG & ZHANG 2024). They play central roles in intraspecific communication and sexual selection, acting as visual displays, sound-producing structures (SONG et al. 2020, HALL & ROBINSON 2021), or as tools to disperse pheromones through vigorous fanning (LOUDON & KOEHL 2000). In various insect groups, wings also contribute to thermoregulation (LAHONDÈRE 2023). Moreover, they function as multimodal sensory platforms essential for flight control and spatial orientation, integrating mechanoreceptors, chemoreceptors, and thermoreceptors into a complex sensory network (PASS 2018).

The evolutionary significance of wings and powered flight extends far beyond insects themselves. Their emergence enabled far-reaching mutualistic interactions, particularly with flowering plants. Recent phylogenetic analyses confirm that entomophily is the ancestral pollination mode in angiosperms and remains dominant today, accounting for approximately 70% of all species (STEPHENS et al. 2023). However, insect flight activity is not universally beneficial to their plant partners, as insects may also serve as vectors for pathogens (HECK 2018). According to traditional views, these intricate co-evolutionary relationships have profoundly shaped the diversification of both insects and flowering plants — two lineages that stand out not only for their remarkable species richness, but also for their unparalleled impact on terrestrial ecosystems (BENTON et al. 2022, HERNÁNDEZ-HERNÁNDEZ & WIENS 2020). Although the concept of tightly coupled co-evolution has been called into question by recent studies (PERIS & CONDAMINE 2024), the origin of insect wings and the advent of powered flight should nonetheless be regarded not merely as pivotal milestones in insect evolution, but as catalysts for major evolutionary developments in angiosperms. These innovations thus profoundly shaped — and in certain respects constrained — the entire subsequent evolutionary trajectory of life on Earth.

Taken together, these observations vividly illustrate MAYR's (1960) concept that evolutionary novelties can open entirely new "adaptive zones". Wings allowed insects to exploit a vast range of ecological niches, laying the foundation for extensive diversification. This is reflected in the fact that only about 1% of described hexapod species are primarily wingless, underscoring the overwhelming dominance of winged forms (ZHANG 2013). Yet, contrary to long-held assumptions, recent research suggests that the origin of wings did not immediately trigger explosive diversification (CONDAMINE et al. 2016). Instead, major radiation events occurred later, shaped by a complex interplay of ecological possibilities and evolutionary constraints (PERIS & CONDAMINE 2024). Thus, while wings undoubtedly represent a key innovation, their evolutionary importance lies less in initiating immediate diversification than in providing the structural and functional foundations for the spectacular radiations that followed.

Structural perspectives: advances in understanding wing morphogenesis and its evo-devo framework

The evolutionary origin of insect wings has been the subject of intense debate since the early post-Darwinian era (Alexander 2018). At that time, two primary hypotheses were proposed (Fig. 3): wings evolved either from appendage-derived structures (the leg-exite or gill hypothesis) or from lateral extensions of the dorsal body wall (the paranotal lobe hypothesis). These competing models sparked heated discussions among leading zoologists, with insect wings serving as a focal example in the broader debate on the evolutionary origin of novel structures. Crampton (1916) summarized these perspectives in a landmark review, framing wings as either "entirely new structures (or organs *sui generis*)" or as modifications of preexisting ones. After reviewing all available evidence, he concluded that the paranotal hypothesis was the most plausible. This view was later endorsed by leading authorities, such as Comstock (1918) and Snodgrass (1935), and dominated entomological textbooks throughout much of the twentieth century. Although later contributions by Wigglesworth (1973), Kukalová-Peck (1978, 1983), and Rasnitsyn (1981) proposed more intricate scenarios, the debate gained widespread traction only with the advent of the evo-devo approach.

A pivotal breakthrough came with the study by Averof & Cohen (1997), who demonstrated that key regulatory genes governing wing development in *Drosophila* are also expressed in the branched limbs of the crustacean *Artemia*. At that time, these findings were widely interpreted as conclusive evidence that insect wings evolved from gill-like appendages of a common aquatic ancestor, prompting revisions in many textbooks. However, a decade later, Niwa et al. (2010) marked a decisive turning point by expanding the genetic framework and incorporating taxa more closely related to the origin of wings. They revealed that both tergal and pleural tissues contribute to wing formation. Shortly thereafter, the discovery of serially homologous wing-related gene regulatory networks (GRNs) in non-winged segments (Clark-Hachtel et al. 2013, Ohde et al. 2013, Moczek 2025) profoundly reshaped and substantially advanced the discourse once more. These deeply conserved GRNs govern the development of a range of structures with striking morphological and functional diversity, illustrating how genetic continuity can give rise to profound phenotypic divergence. Notably, two distinct sets of wing-related serial homolog GRNs — one tergal, one pleural — were identified in the prothoracic and abdominal segments of *Tribolium castaneum*, indicating that this pattern may be a fundamental feature of each body segment (Clark-Hachtel et al. 2013, Linz & Tomoyasu 2018). These discoveries lent strong support to the integrative dual-origin hypothesis, a concept which had already been anticipated in principle by the paleoentomologist Rasnitsyn (1981). Further corroboration came from embryological studies on *Gryllus bimaculatus*, which revealed that the wing proper originates exclusively from the tergum, whereas the wing hinge and direct muscles may be appendicular in origin (Mashimo & Machida 2017). Taken together, this body of evidence has elevated the dual-origin model to a widely accepted framework for explaining insect wing evolution.

To deepen the phylogenetic understanding of wing-related GRNs, subsequent studies have expanded their scope beyond pterygote insects to include primarily wingless hexa-

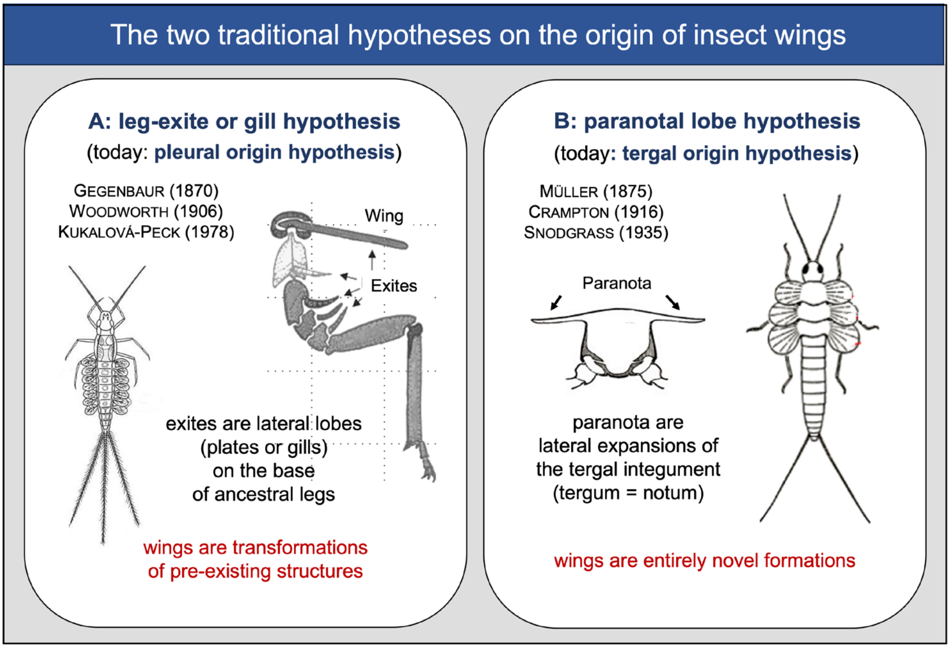


Fig. 3: The two traditional schools of thought on the origin of insect wings based on morphological evidence. A: *Leg-exite (or gill) hypothesis*: proposes that wings evolved from the lateral thoracic pleura, believed to derive from ancestral leg segments. In this view, wings are modified appendages, with some interpretations tracing their origin to tracheal gills. **B: *Paranotal lobe hypothesis*:** suggests that wings originated as lateral extensions of the dorsal body wall, developing independently of any pre-existing appendages. This model treats wings as evolutionary novelties. Sources: diagrams adapted from Kukalová-Peck (1983) and online sources.

pods and crustaceans as outgroups (Clark-Hachtel & Tomoyasu 2016, Tomoyasu et al. 2017). These analyses revealed homologous GRNs in crustaceans, demonstrating that the genetic framework for wing development had already emerged long before the evolution of wings themselves (Shiga et al. 2017). In two pivotal studies on the amphipod *Parhyale hawaiensis*, wing-related GRNs were identified in proximal leg segments (Bruce & Patel 2020, Clark-Hachtel & Tomoyasu 2020; Fig. 4). Although both studies concluded that leg-derived tissues contributed to the dorsolateral body wall during insect evolution, their interpretations of wing formation differ significantly. Bruce & Patel (2020) argue that wings evolved entirely from transformed leg segments, particularly the precoxa, and therefore represent modified, rather than genuinely novel, structures. In contrast, Clark-Hachtel & Tomoyasu (2020), along with Clark-Hachtel et al. (2021), propose that wings constitute evolutionary novelties arising from the fusion of leg-derived tissues and non-appendicular tergal components. This integrative process likely involved the co-option of previously unrelated GRNs. Tomoyasu (2021) synthesizes the various perspectives, illustrating how evo-devo research has broadened our understanding of insect wing origins and the mechanisms underlying evolutionary novelties in general. More recent studies have added further aspects to the debate. In *G. bimaculatus*, a species

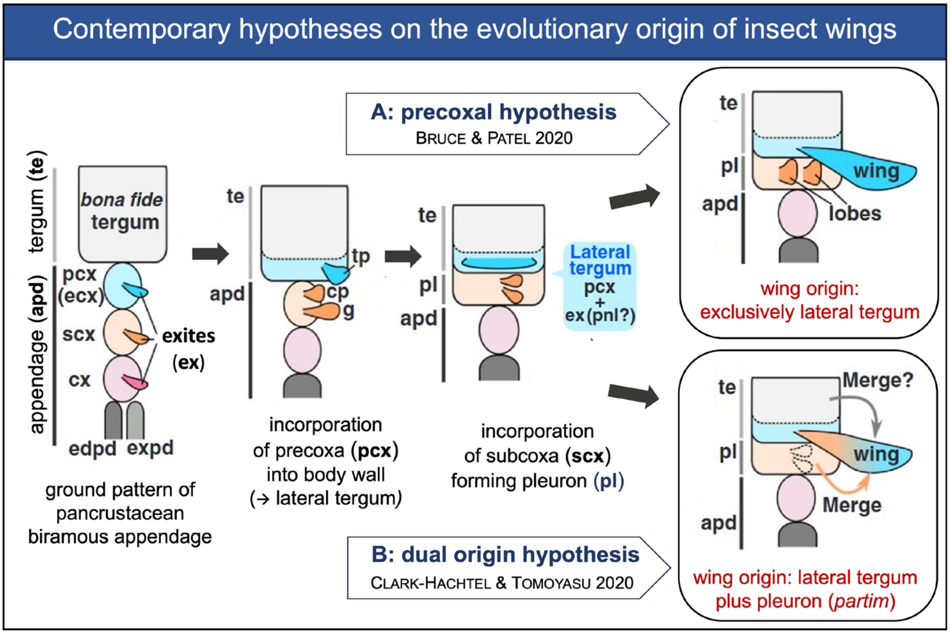


Fig. 4: Contemporary alternative hypotheses on insect wing evolution based on gene expression and knockout studies in the crustacean *Parhyale hawaiensis*. Both studies compared their results with previously published data from *Drosophila* and concluded that proximal leg segments of the pancrustacean ancestor were incorporated into the insect body wall. **The two models diverge, however, in how these segments contributed to wing formation. A: *Precoxal hypothesis*:** the precoxa, the most proximal leg segment, is proposed to have fused with the lateral body wall. The wing is thought to have evolved from an associated exite (tergal plate), suggesting an entirely appendicular (leg-derived) origin. **B: *Dual origin hypothesis*:** wings evolved through the integration of two components: a tergal plate (from the lateral body wall) and a pleural plate (of appendicular origin). This model views wings as evolutionary novelties emerging from the merger of previously unrelated tissue types. Abbreviations: apd, appendage; cp, coxal plate; cx, coxa; ecx, epicoxa; edpd, endopodite; ex, exite; expd, exopodite; g, gill; pcx, precoxa; pl, pleuron; pnl, paranotal lobe; pro, pro-wing; scx, subcoxa; te, tergum; tp, tergal plate. Source: adapted and modified from Ohde & Prokop (2025).

representative of the ancestral hemimetabolous mode of wing development, Ohde et al. (2022) identified a genetic growth organizer in the tergal region that plays a key role in wing formation, but found no evidence for pleural contributions. Using a different approach, Kobayashi et al. (2022, 2025) employed classical anatomical methods to examine thoracic muscle arrangements in larvae of the beetle *Carabus insulicola* and concluded that the paranotal lobes are entirely of appendicular origin. Their findings align with the model proposed by Bruce & Patel (2020), which was likewise developed using a holometabolous species (*Drosophila*).

These partly conflicting findings suggest that wing development may be more complex and less uniform across insect lineages than previously assumed (Yamashita et al. 2023). Moreover, it has become apparent that regulatory elements once thought to be wing-specific are more broadly distributed. Genes previously regarded as definitive wing markers are also expressed in various other flat, bi-layered outgrowths throughout the body, irrespective of anatomical location. This indicates that, although these genes are essential for wing development, they are part of an ancient, versatile GRNs involved in shaping integumental structures across multiple evolutionary contexts, including crustacean and mayfly gills (Shiga et al. 2017, Fisher et al. 2021).

Moreover, there is widespread consensus that insect wings evolved through a gradual, multi-step process. Treidel et al. (2024) recently proposed a model based on the precoxal hypothesis that reconciles the leg-exite and paranotal models by situating them at different evolutionary stages. Rather than a direct fusion of tergal and pleural tissues, their model posits that wing-forming tissues first evolved from a precoxal exite at the base of the ancestral leg, consistent with the leg-exite hypothesis. With subsequent integration of the leg base into the lateral body wall, the precoxal exite expanded into the paranotal region and ultimately gave rise to the wing — thus aligning with the paranotal model in a later phase.

In conclusion, recent progress in evo-devo research on insect wings has redefined our understanding of morphological innovation. A widely held view maintains that novel structures arise primarily through the co-option of originally unrelated GRNs (True & Carroll 2002, McQueen & Rebeiz 2020). However, Bruce & Patel (2022) challenge this view, arguing that morphological innovations more often stem from the reconfiguration of conserved, serially homologous gene cassettes, which persist in cryptic forms over deep evolutionary timescales. According to their model, this intrinsic flexibility — rather than GRN co-option — serves as the primary driver of morphological diversity in arthropods. Resolving this foundational debate will require high-resolution methods, such as transcriptomic single-cell analyses to map developmental trajectories and evolutionary pathways across taxa.

Wing circulatory organs: evolution between modification and innovation

Until recently, research on the evolutionary origins of insect wings has focused almost exclusively on the epidermal folds from which wings develop. However, a comprehensive account of wing evolution must also consider their integration with other organ systems, particularly the circulatory system (Pass 2018). Hemolymph is transported to these appendages via the hollow wing veins, with circulation sustained by specific pumping organs. The following four sections examine the structure and evolutionary trajectories of these accessory circulatory organs, whose sole function is to maintain hemolymph flow through the wing vein network.

The first section explores how the circulatory system contributes to wing formation and maturation, and highlights its vital role in maintaining the biomechanical integrity of mature wings. The second section presents a comparative analysis of wing circulatory organs across major insect lineages, revealing striking diversity in both functional morphology and evolutionary origin. While modifications of the dorsal cardiac tube (commonly referred to as “the insect heart,” despite the presence of multiple distinct pumping organs in insects) represent the plesiomorphic condition, autonomous wing hearts have independently evolved in several insect groups as novel structures. Building on these

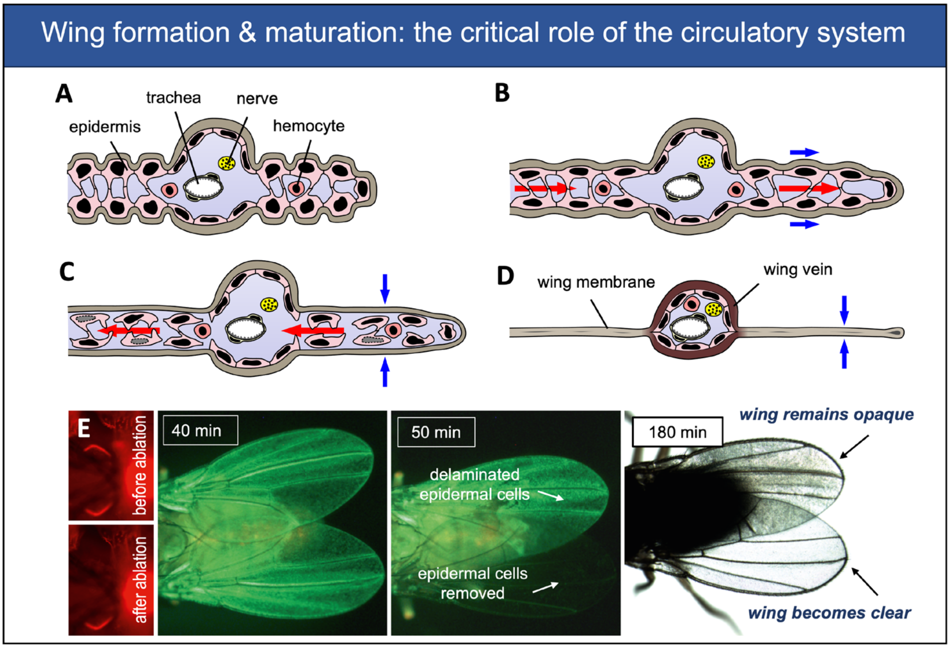


Fig. 5: Critical functions of the circulatory system during wing formation and maturation. A–D: Schematic cross-sections illustrating key stages of wing maturation (see main text for details). Red arrows indicate hemolymph flow; blue arrows show passive changes in the wing integument. **E:** Laser ablation of the wing hearts in *Drosophila* results in severe maturation defects. On the treated side, the wing remains opaque due to retained hemolymph containing floating apoptotic cell fragments. In contrast, the untreated side becomes transparent as the wing hearts effectively suck out the hemolymph. Time markers refer to minutes after wing unfolding. Sources: A–D, Pass et al. (2015); E, modified after Tögel et al. (2008).

findings, the third section provides detailed — and somewhat unexpected — insights into the evolutionary origin of wing hearts from a developmental genetic perspective, using *Drosophila* as a model system. The final section places these insights into a broader evolutionary context, considering how developmental and functional constraints may have facilitated the emergence of autonomous wing hearts. It concludes by outlining why wing circulatory organs constitute a promising model system for investigating general principles of evolutionary innovation.

Wing formation and maturation: crucial roles of the circulatory system

Insect wings develop from dorsolateral outgrowths of the integument in the meso- and metathoracic segments. Immediately after imaginal ecdysis, the wings are soft, folded, and crumpled. To become fully functional, they must be unfolded, stretched, and hardened (Hadjaje et al. 2024), a process that follows a general pattern shared by all insects, progressing through several phases that may last from hours to days, depending on the species (Fig. 5A–D). Wing expansion is driven by a substantial influx of hemolymph into these appendages (Salcedo et al. 2023a). This is achieved by increased hemolymph pressure within the thorax, facilitated by air intake into the gut and rhythmic abdominal

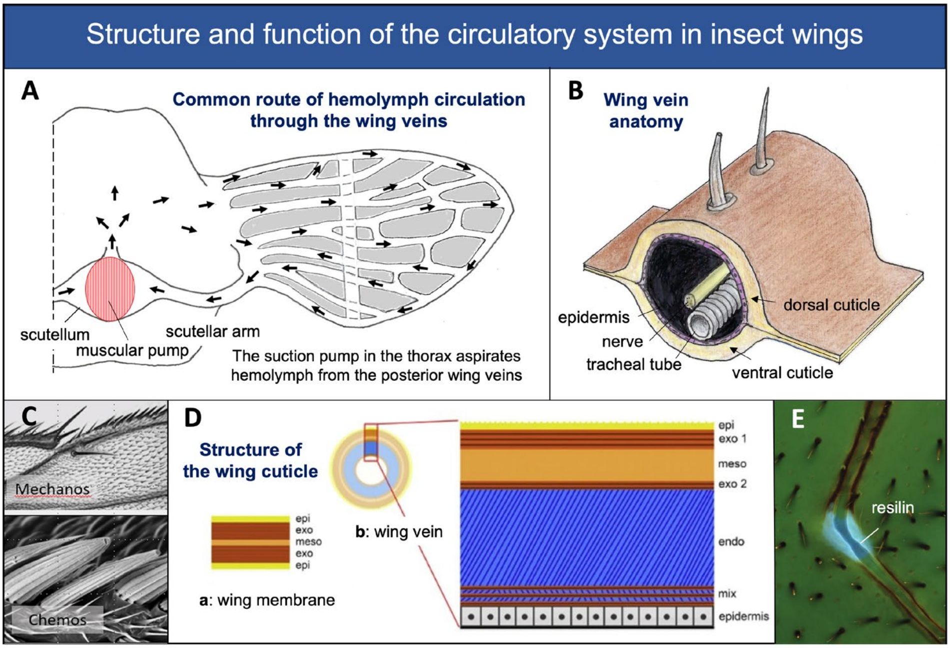


Fig. 6: Structure and function of the insect wing circulatory system. A: Schematic representation of hemolymph circulation in an insect wing. Hemolymph enters through anterior veins (Costa, Subcosta, Radius, Media) and returns via posterior veins (Cubitus, Analis) in a loop-like pattern. The flow is driven by a thoracic muscular pump associated with the scutellum (red hatched area). For anatomical details and structural variations, see Section 3.2. and Fig. 7. **B:** Cross-sectional diagram of a wing vein. Formed by the dorsal and ventral cuticle of the integumental fold that gives rise to the wing, the hollow wing veins serve multiple functions. In addition to providing structural support to the wing blade, they act as conduits for hemolymph, which is essential for supplying the numerous sensilla aligned along the veins and for maintaining continuous hydration of the vein cuticle; tracheal tubes within the veins ensure adequate oxygen supply to the associated tissues. **C:** Multimodal sensilla located at the leading edge of a *Calliphora* wing: top, mechanosensory hairs; bottom, chemosensory tip-pore sensilla (SEM). **D:** Schematic of the layered structure of the wing cuticle. a: wing membrane; b: wing vein with underlying epidermis. While wing membranes are nearly free of water, most wing water is localized in the endocuticle of the veins. **E:** Detail of the forewing of *Apis mellifera*. Blue autofluorescence reveals resilin in a flexible vein joint region (confocal laser scanning microscopy). Sources: A,B, originals; C, Pass (2018); D, modified from Appel et al. (2015); E, Appel et al. (2024).

muscle contractions (Hughes 1980). External forces also contribute, as demonstrated by experiments showing that insect wings possess a certain degree of autonomous expansion capability (Glaser & Vincent 1997, Salcedo et al. 2023b).

The subsequent maturation phase critically depends on the function of wing circulatory organs (Tögel et al. 2008). *Drosophila* flies lacking functional wing hearts, due to genetic mutation or laser ablation, display malformed wings (Fig. 5E). These flies struggle with flight and often crash during initial attempts, likely due to excessive hemolymph retention in the wings. In contrast, untreated individuals gradually remove hemolymph and cellular debris from the wings via the pumping action of the wing hearts. This withdrawal reduces the wing lumen and brings the dorsal and ventral lamellae into close contact. Once these lamellae meet, they fuse via extracellular matrix, forming the thin wing membrane (Kiger et al. 2001). Regions containing wing veins, however, remain unfused and develop into hollow tubes. The final stage of wing maturation involves cuticle sclerotization, imparting the necessary biomechanical properties for flight.

The circulatory system is not only essential for the proper formation and maturation of insect wings, but also remains critical for maintaining their health and functionality throughout the adult life stage. Contrary to the common misconception that insect wings are composed solely of inert cuticle, they actually contain living tissues, including sensory receptors and other metabolically active cells. These require a continuous supply of nutrients and efficient removal of metabolic waste — functions enabled by hemolymph circulation through the wing vein network (Arnold 1964, Chintapalli & Hillyer 2016, Salcedo & Socha 2020, Salcedo et al. 2023c, Ryu et al. 2025; Fig. 6 A, B, C).

Hemolymph circulation is also essential for maintaining cuticle hydration (Fig. 6D). Although every entomologist has likely observed how quickly insect wings dry out and become brittle after death, this fundamental aspect of wing biology remained surprisingly understudied until recently (Pass 2018). While the thin wing membranes contain almost no water, the cuticle of the wing veins retains nearly all of the wing's water content. Hydration is especially critical in the flexible regions between heavily sclerotized veins, which are rich in the elastic protein resilin — a hydrogel whose mechanical properties are highly sensitive to hydration levels (Lietz et al. 2021, Appel et al. 2024; Fig. 6E).

Morphological disparity and evolutionary pathways: unraveling the enigmatic origin of autonomous wing hearts

The organs responsible for hemolymph circulation within the wing veins have long remained poorly understood, with earlier literature offering only sparse and fragmentary descriptions (Jones 1977). To address this gap, our team conducted a comprehensive comparative study on the functional and evolutionary morphology of these structures across nearly all insect orders (Krenn 1993, Krenn & Pass 1993, 1994, 1995). A central theme of this research — one that has particularly captured our interest — is the recognition that these organs represent evolutionary novelties of the Pterygota. This perspective forms the primary focus of the present review. Notably, the concept of evolutionary novelty is not restricted to circulatory structures associated with the wings. It also applies to a wide range of accessory pulsatile organs involved in hemolymph supply to other insect appendages, including antennae, legs, cerci, and ovipositors (reviews: Pass 1998, 2000, Hillyer & Pass 2020).

Hemolymph flow within the wing veins is not driven by mechanisms intrinsic to the wings themselves, but rather by accessory pumps located in the thorax. These circulatory organs are composed of both cuticular and muscular elements. The scutellum, a dome-shaped structure of the pterothoracic tergites, forms the external casing of the pump and connects to the posterior wing veins via lateral cuticular tubes (Krenn & Pass 1993; Fig. 7A). Rhythmic contractions of the associated musculature draw hemolymph from the posterior veins into the thoracic hemocoel, establishing a looped flow through the wing vein system. This represents the general mode of wing circulation in almost all insects (Arnold 1964). Notable exceptions include Lepidoptera and scarabaeid beetles, which

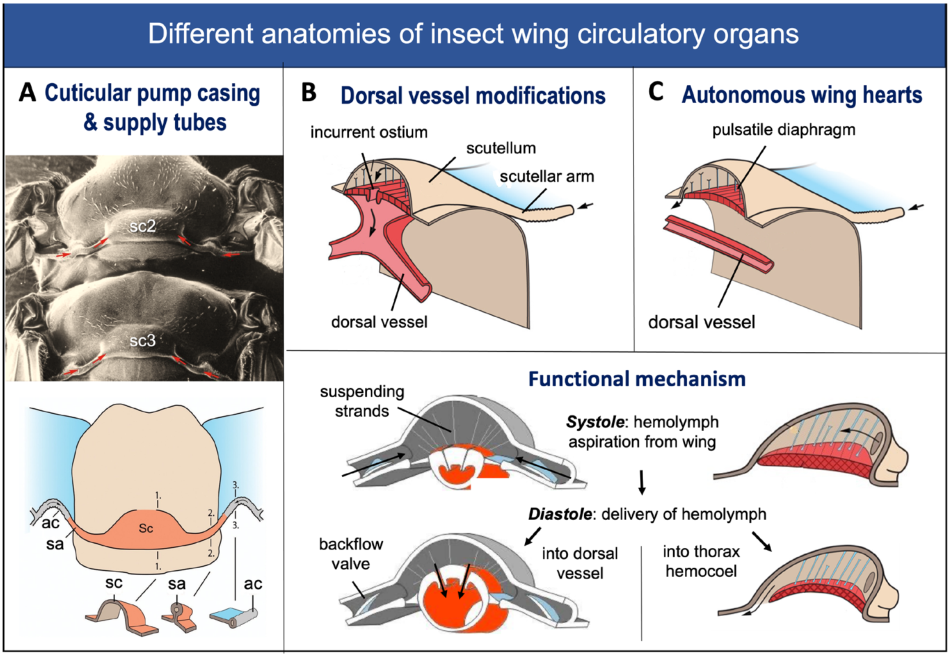
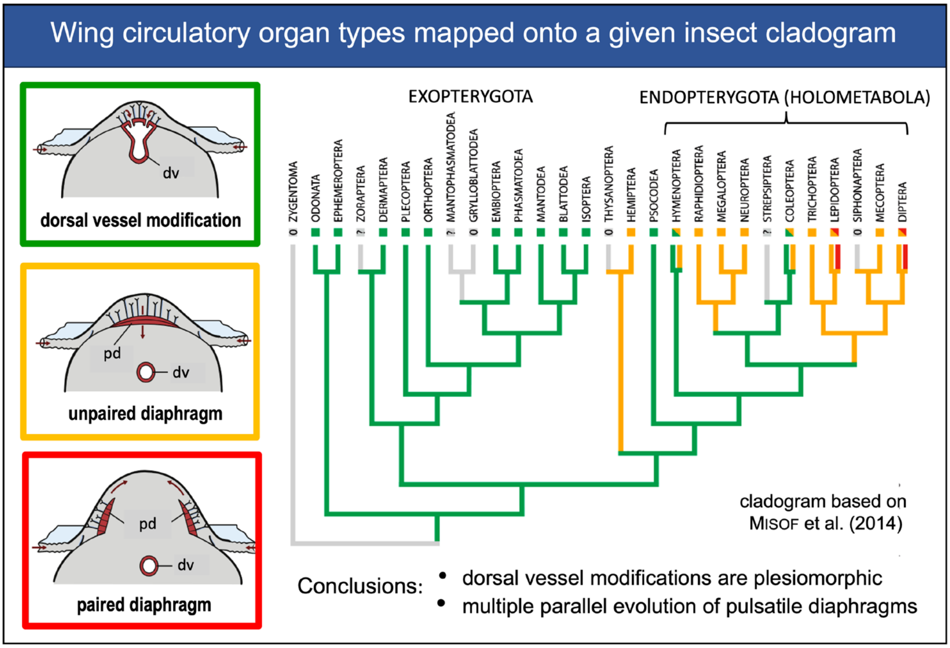


Fig. 7: Structural components and functional mechanisms of the various wing circulatory organ types. A: *Cuticular pump casing and supply tubes.* Dorsal view of the meso- and metathorax of *Panorpa* sp. (SEM), showing dome-shaped scutella (sc2, sc3) and lateral supply tubes connecting to the posterior wing veins. Red arrows indicate hemolymph flow. The schematic illustrates the scutellum acting as a pump casing, with the supply tubes consisting of a rigid section called the scutellar arm (sa) and a flexible section known as the axillary cord (ax). Lower panels: sagittal sections at the levels of the scutellum (1–1), scutellar arm (2–2), and axillary cord (3–3). **B: *Dorsal vessel modification*.** An enlarged and modified section of the dorsal vessel is attached to the scutellum's basal ridge, isolating the subscutellar space from the thoracic hemocoel. During systole, contraction of the dorsal vessel reduces pressure, drawing hemolymph from posterior wing veins through the supply tubes. During diastole, the dorsal vessel expands via elastic suspensory strands, and hemolymph is pushed through incurrent ostia into the vessel lumen; valves in the scutellar arms prevent retrograde flow. **C: *Autonomous wing heart.*** A muscular diaphragm, separate from the dorsal vessel, generates pulsation. During systole, the relaxed diaphragm arches dorsally; during diastole, it flattens upon contraction. Although functionally similar to B, hemolymph is here released directly into the thoracic cavity through an anterior slit-like opening. Sources: A, photo from Krenn & Pass (1993); all diagrams, Pass et al. (2015). Animations illustrating the functional mechanisms of these organs are available in the supplementary data of Pass (2000): https://www.annualreviews.org/content/journals/10.1146/annurev.ento.45.1.495#supplementary_data

exhibit a derived pattern of hemolymph exchange within the wing veins, known as tidal or oscillatory circulation (Wasserthal 1980). In these insects, hemolymph alternately flows into and out of the veins, synchronized with periodic reversals in the flow direction of the dorsal vessel and intermittent activity of the wing-associated circulatory organs. While the cuticular components of thoracic pumps are largely conserved across insects, the pulsatile elements show considerable variation. These elements may include enlarged, modified portions of the dorsal vessel or separate muscular structures referred to as pulsatile diaphragms (Fig. 7B, C). In most taxa with such diaphragms, a single unit occurs per pterothoracic segment. However, in some groups, paired diaphragms are present,



Conclusions:
- dorsal vessel modifications are plesiomorphic
- multiple parallel evolution of pulsatile diaphragms

Fig. 8: Evolutionary trajectories of insect wing circulatory organs. The three principal anatomical configurations of wing-associated circulatory organs are shown in schematic cross-sections and mapped onto the insect cladogram from Misof et al. (2014): dorsal vessel modification (green), unpaired pulsatile diaphragm (yellow), and paired pulsatile diaphragms (red). Abbreviations: dv, dorsal vessel; pd, pulsatile diaphragm. Source: modified from Pass et al. (2015).

allowing each wing to be served by an independent organ (Krenn & Pass 1995). These structures function as autonomous pumping units, contracting independently of the dorsal vessel's rhythm, and are therefore commonly referred to as wing hearts.

Tsai et al. (2020) observed a pulsating region within the wing vein network of butterflies and referred to it as a "wing heart". However, in the absence of detailed structural and functional analyses, it remains unclear whether this region truly functions as an autonomous pumping organ, as the term "heart" implies. Descriptions of similar pulsating wing vein regions in various other species are cited in Arnold's (1964) comprehensive review of insect wing circulation. However, based on his own investigations, he found no evidence that these structures operate independently. Instead, Arnold (1964) attributed their pulsations to pressure changes generated by the dorsal vessel and the thoracic wing hearts. This interpretation is supported by our own study on a pulsating region in the wings of *Panorpa* (Krenn & Pass 1993). In semithin sections, we did not find any indication of contractile structures. However, the cuticle in this area is less sclerotized and therefore more flexible, which may explain the rhythmic bulging in response to externally generated pressure. These findings suggest that such wing vein regions, while potentially aiding hemolymph distribution, do not constitute autonomous contractile organs. We therefore propose the designation "pulsating wing vein region" rather than "wing heart", as it better captures the structural and functional characteristics of these formations.

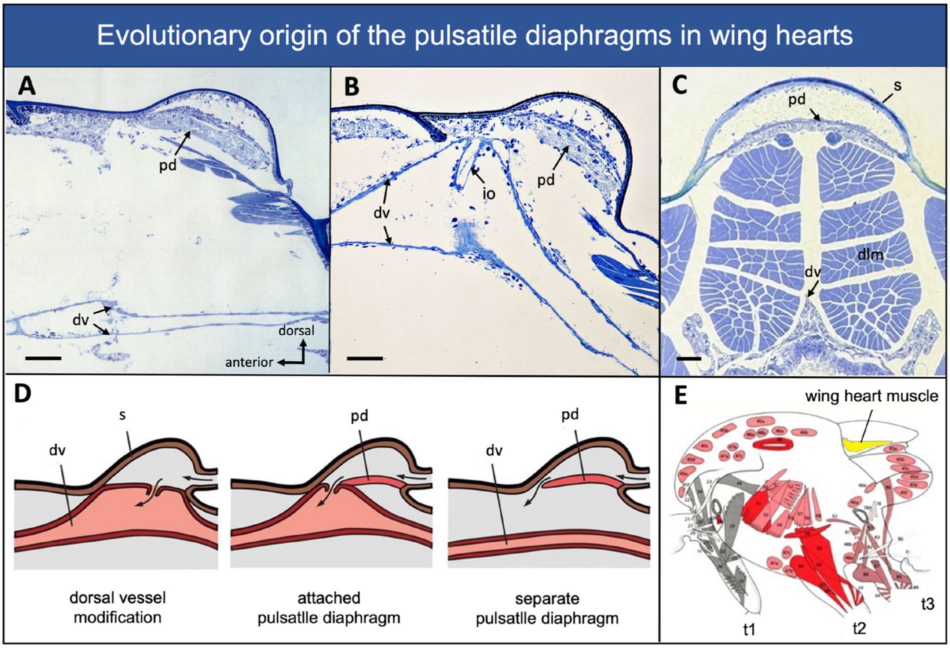


Fig. 9: Evolutionary origin of the pulsatile diaphragms in autonomous wing hearts. A, B: Longitudinal semithin sections of the thorax in *Phaeostigma notata* (Raphidioptera) reveal two configurations of pulsatile diaphragms: one independent of the dorsal vessel in the mesothorax (**A**), and another anteriorly attached to the dorsal vessel in the metathorax (**B**), near an incurrent ostium. **C:** Transverse section through the thorax of *Panorpa* sp. (Mecoptera) shows the pulsatile diaphragm in an arched (diastolic) position, attached to the lateral margin of the scutellum. The dorsal vessel appears as a narrow tube located dorsally above the intestine. **D:** Schematic longitudinal sections illustrate three evolutionary stages of wing circulatory organs: (left) ancestral condition with hemolymph flow driven by the dorsal vessel, entering via an incurrent ostium; (middle) intermediate stage in which an attached pulsatile diaphragm drives flow into the vessel lumen through the ostium; (right) derived state with an autonomous pulsatile diaphragm that pumps hemolymph directly into the thoracic cavity. **E:** Lineage origins of the thoracic muscles in *Drosophila*. Each color indicates a distinct founder cell lineage; notably, the wing heart muscle (yellow) is the only thoracic muscle that does not derive from somatic muscle precursors, suggesting that it derives from the cardiac mesoderm. Abbreviations: dlm, dorsal longitudinal muscle; dv, dorsal vessel; i, intestine; io, incurrent ostium; pd, pulsatile diaphragm; s, scutellum; t1–t3, thoracic legs; scale bar: 100 µm. Sources: A–C, originals; D, Pass et al. (2015); E, modified from Lawrence (1992).

The functional diversity of wing circulatory organs (Fig. 7B, C) raises compelling evolutionary questions. Comparative analyses across insect orders indicate that dorsal vessel modifications represent the plesiomorphic condition, whereas autonomous wing hearts are apomorphic features that evolved independently in multiple lineages (Krenn & Pass 1994, 1995; Fig. 8). This leads to the key issue of the evolutionary origin of the muscular components of wing hearts. Anatomical comparisons suggest that pulsatile diaphragms may be derived from the myocardial wall of the dorsal vessel. This hypothesis is supported by observations in certain Raphidioptera and Lepidoptera, where diaphragms are structurally connected with the dorsal vessel — possibly representing an intermediate stage of individuation into autonomous units (Krenn & Pass 1995; Fig. 9A–D). Additional support comes from developmental studies in *Drosophila*, which identified the pulsatile diaphragms of the wing hearts as the only thoracic muscles not derived from somatic muscle precursors, suggesting a probable origin in the cardiac mesoderm (Lawrence 1982; Fig. 9E).

Evo-devo insights from *Drosophila*: a paradigm shift in understanding wing heart origin

Two decades later, advanced genetic tools in *Drosophila* finally enabled us to test our hypothesis about the origin of the wing hearts at a more detailed developmental level. In a collaborative project between the Universities of Osnabrück and Vienna, we employed an integrative approach to investigate wing heart development in fruit flies (for anatomy of the paired wing hearts in *Drosophila* see Fig. 10A–C). A key starting point was the generation of a reporter line in which expression of the gene *hand* was visualized via the green fluorescent marker GFP (Sellin et al. 2006). At that time, *hand* was believed to be expressed in the myocardium of *Drosophila*, and not in skeletal or any other muscle tissue. Intriguingly, this new reporter line revealed *hand* expression in the paired wing hearts of the fruit fly (Fig. 10D), at first glance strongly supporting our hypothesis that the pulsatile diaphragms originate from myocardial tissue. However, further findings – described in detail below – led us to reassess this interpretation. This research thus highlights how even the expression of a highly specific regulatory gene can mislead homology inferences, underscoring the pitfalls of relying on single-gene expression patterns to determine structural homology.

A crucial factor in uncovering the developmental origin of the wing heart muscle was the persistent expression of *hand* throughout the fly's life cycle, which made it possible to trace the entire developmental trajectory (Tögel et al. 2008, 2013). It was found that the wing hearts originate from four pairs of precursor cells that are already specified during embryogenesis. These cells remain quiescent until the third larval instar, at which point they begin to proliferate. Initially forming a central cluster during prepupal development, they subsequently split into two groups that migrate laterally during pupal stages to give rise to the wing hearts (Fig. 10F–I). By 45 hours after puparium formation, the wing hearts are fully formed and begin to beat. To place these findings in context, it is essential to consider the broader development of the entire dorsal vessel complex in *Drosophila*. This process begins with mesodermal precursor cells arranged in bilateral rows along the dorsal midline of the embryo. They differentiate into cardioblasts, which eventually form the cardiomyocytes, and so-called pericardial cells comprising several subtypes (Alvarez et al. 2003). In the postembryonic stages, the majority of pericardial cells differentiate into nephrocytes, essential for hemolymph filtration, osmoregulation, detoxification, and immune responses (Weavers et al. 2009, Meyer et al. 2024).

Unexpectedly, our developmental genetic analyses revealed that the wing hearts do not originate from cardioblasts, as we had initially assumed, but rather from one of the pericardial subtypes, the *eve*-positive pericardial cells (EPCs) which arise in bilateral pairs per segment. However, only the EPCs corresponding to the second and third thoracic segment become wing heart progenitors (Fig. 10E). The restriction to thoracic segments is facilitated by the Hox gene *Ulltrabithorax* who inhibits wing heart fate in

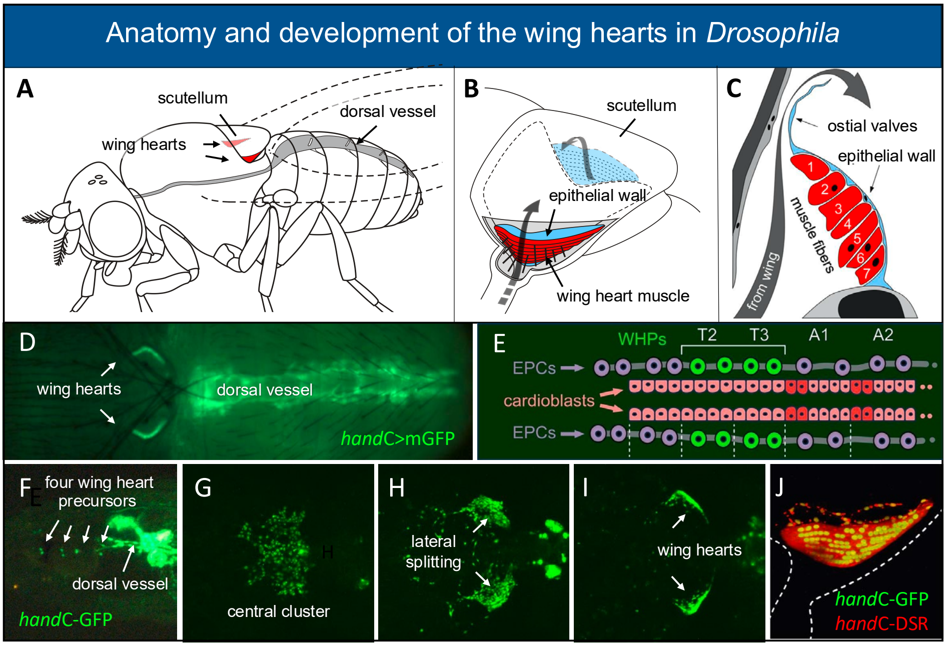


Fig. 10: Functional morphology and evolutionary, genetic, and developmental basis of wing hearts in *Drosophila*. **A:** Whole-animal diagram showing the paired wing hearts at the lateral margins of the scutellum. **B:** Close-up of the scutellum: wing hearts consist of a muscular diaphragm (red) and a connective tissue epithelium (blue). Arrows indicate hemolymph flow from the wings into the scutellum. **C:** Cross section showing muscle cells bordered by a thin epithelial layer. The dorsal part of this epithelium acts as an ostial valve directing hemolymph flow (arrow) from wings into the thoracic cavity. **D:** Epifluorescence image of dorsal view of thorax and abdomen, showing hand reporter expression in both the wing hearts and dorsal vessel (*handC*-GFP). **E:** Scheme of *eve*-positive pericardial cells (EPCs) flanking the developing dorsal vessel. EPCs in thoracic segments T2 and T3 form the wing hearts (WHPs, green); remaining EPCs become nephrocytes or other cell types (violet). Red cardioblasts differentiate into ostial cells. **F–I:** Time-lapse images of postembryonic wing heart formation: The four wing heart progenitors proliferate and combine into a central cluster, later splitting and migrating laterally. J: Confocal image showing the seven syncytial myocytes forming the wing heart with aligned nuclei (yellow). The thin epithelial layer is visible above the muscles. Sources: A–C, Pass et al. (2015); D, original; E, Tögel et al. (2022); F–J, Tögel et al. (2008).

abdominal EPCs (Tögel et al. 2022). Further surprising insights emerged from studying *tinman*, a master regulatory gene in *Drosophila* heart development. While Tinman is essential for specifying both cardiomyocytes and pericardial cells, its expression must be downregulated for wing heart development to proceed. Ectopic expression of Tinman in wing heart progenitors blocks their development, underscoring its pivotal role in early lineage specification (Tögel et al 2008).

Another striking discovery was the difference in ultrastructure between wing heart myocytes and cardiomyocytes (Lehmacher et al. 2009). Wing heart myocytes are multinucleated syncytia (Fig.10J), anchored to the cuticle via tendon cells, similar to somatic myocytes. In contrast, cardiomyocytes of the dorsal vessel are mononucleated and lack such attachments (see Fig. 11 for a synopsis comparing somatic, wing heart, and dorsal vessel myocyte development).

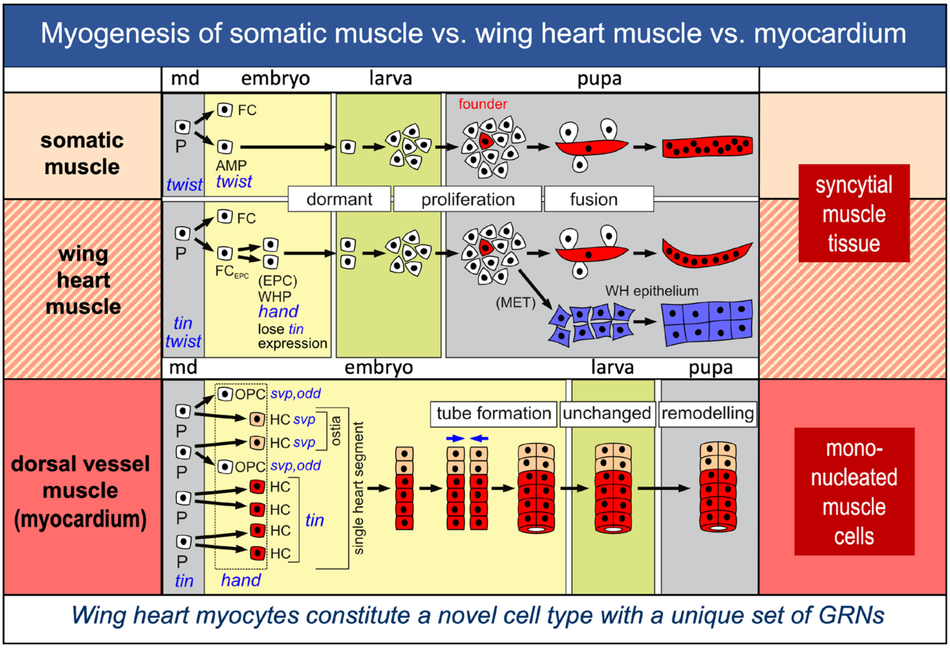


Fig. 11: Comparative developmental trajectories of adult somatic muscle, wing heart muscle, and dorsal vessel (myocardium) in *Drosophila*. Wing heart muscle development closely parallels somatic muscle formation, including specification, proliferation, and syncytium formation, while dorsal vessel cardiomyocytes remain mononucleated throughout development. Importantly, expression of the *hand* gene is restricted to cardiac lineages and is absent in somatic muscles. In the wing heart, *hand* plays crucial roles in determining attachment site positioning, muscle alignment, and terminal differentiation. These findings suggest that wing heart myocytes represent a novel hybrid cell type that follows a somatic muscle-like developmental program, but integrates cardiac-specific GRNs. AMP, adult muscle precursor; P, progenitor; FC, founder cell; EPC, *eve*-positive pericardial cell; OPC, *odd*-positive pericardial cell; HC, heart cell; WHP, wing heart progenitor; MET, mesenchymal-epithelial transition. Source: Pass et al. (2015). Cell lineages are derived from literature. Somatic muscle: Carmena et al. (1998a, b); de Joussineau et al. (2012); Bate et al. (1991); Dutta et al. (2004). Wing heart muscle: Halfon et al. (2000); Carmena et al. (1995, 2002), Tögel et al. (2008, 2013). Dorsal vessel muscle: Ward & Skeath (2000); Alvarez et al. (2003); Han & Bodmer (2003).

Based on all these findings, we revised our initial hypothesis and draw the following conclusions: (i) the wing hearts in *Drosophila* are not individualized parts of the dorsal vessel myocardium; (ii) wing heart myocytes and cardiomyocytes are not homologous; and (iii) wing heart myocytes represent a distinct muscle cell type, governed by a unique GRN that combines features of somatic muscle development with elements of cardiac gene expression.

From a broader perspective, the multiple subtypes of *Drosophila* pericardial precursors are particularly striking for their capacity to generate a wide array of structurally diverse and developmentally flexible cell types. In addition to giving rise to the wing hearts, the anterior-most EPCs also contribute to another component of the cardiac complex, the so-called outflow-hanging structure (Zmojdzian et al. 2018). Furthermore, nephrocytes derived from pericardial cells undergo extensive remodeling during development. Initially lacking hallmark filtration features such as slit diaphragms and membrane invaginations (Psathaki et al. 2018), they acquire these characteristics only at a later developmental

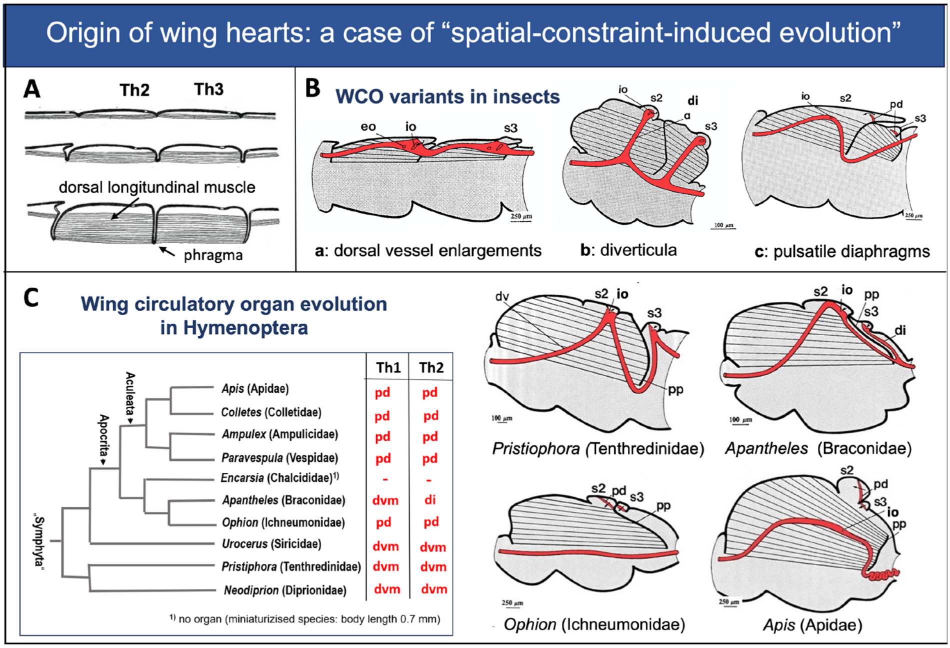


Fig. 12: Evolution of wing circulatory organs under spatial constraints imposed by the progressive enlargement of flight musculature. A: Schematic longitudinal sections through the tergal region of pterothoracic segments illustrate the evolutionary trend towards enlarged dorsal longitudinal muscles (indirect flight muscles) and expanded phragmata (cuticular infoldings serving as muscle attachment sites). **B:** Structural diversity of wing circulatory organs (WCO) shown in relation to increasing muscle volume and phragma size. **a:** Dorsal vessel enlargements (*Ectobius sylvestris*); **b:** Dorsal vessel diverticula (*Graphopsocus cruciatus*); **c:** Autonomous pulsatile diaphragms (*Cercopis sanguinolenta*). **C:** Evolution of wing circulatory organs in Hymenoptera. The selected taxa illustrate how spatial constraints due to enlarged flight muscles shape circulatory system architecture. Traits are mapped onto a cladogram based on Peters et al. (2017). Dorsal vessel modifications represent the ancestral condition, typically associated with a complex vessel trajectory shaped by a deeply invaginated postphragma (pp). Autonomous pulsatile diaphragms represent a derived condition, correlating with further enlargement of the dorsal longitudinal muscles and more deeply extending phragmata, resulting in a straight vessel course through the lower thoracic region. These structures evolved independently at least twice within Apocrita. **Abbreviations:** di, diverticulum; dvm, dorsal vessel modification; io, incurrent ostium; pd, pulsatile diaphragm; pp, postphragma; s2,s3 scutellum of thorax segment 2 and 3; th2, th3 thorax segment 2 and 3; wco, wing circulatory organs. Sources: A, Weber 1933; B, C, Krenn & Pass (1994, 1995).

stage. This transformation resembles transdifferentiation or cellular reprogramming, as observed during the regeneration of somatic striated muscles (Frasch 2016) or in the formation of muscle-derived intracardiac valves (Meyer et al. 2023). Notably, the number of pericardial cells in *Drosophila* embryos far exceeds that found in larvae or adults. However, the fate of these “missing” cells remains unclear, raising compelling questions about their potential roles or ultimate destinations (Rotstein & Paululat 2016). Collectively, these findings support the idea that embryonic pericardial cells may function as an evolutionary reservoir or cellular toolbox, capable of generating novel cell types and structures during insect development due to their inherent multipotency and dynamic developmental potential.

From dorsal vessel modifications to autonomous wing hearts: evolutionary scenarios and developmental constraints

From an evolutionary perspective, the tight functional coupling between wings and their associated circulatory organs supports the hypothesis of structural coevolution. Wing circulatory organs are unique to pterygote insects and lack homologous counterparts in apterygote ancestors, defining them as genuine evolutionary novelties (Pass et al. 2015). Modifications of the dorsal vessel represent the ancestral condition, arising through transformations of preexisting structures, and are classified as Type II novelties (Wagner 2014). In contrast, wing hearts, at least in *Drosophila*, are independent organs with pulsatile components representing a novel muscle type derived from pericardial precursors (Tögel et al. 2008), thus qualifying as Type I novelties within Wagner's framework.

This raises a central question: what factors led to the evolutionary emergence of autonomous wing hearts, and which constraints shaped their development? A comparison of wing heart distribution across pterygote lineages revealed no consistent correlation with wing shape or size. However, a notable pattern emerged: the presence of wing hearts regularly coincides with changes in the course of the dorsal vessel within the thorax. In the ancestral condition, the dorsal vessel is attached to the dorsal body wall. In taxa possessing wing hearts, however, the dorsal vessel runs deeper within the thorax, detached from the dorsal body wall. This internal displacement of the dorsal vessel was likely a consequence of evolutionary modifications to the flight musculature. In more advanced fliers, the dorsal longitudinal muscles are greatly enlarged, an evolutionary transformation of the flight motor described in detail by Brodsky (1994). These muscles attach to phragmata, deep invaginations of the tergal cuticle, which likely imposed spatial constraints on the course of the dorsal vessel (Krenn & Pass 1995; Fig. 12).

Given this context, we propose the following scenario. The cuticular structures of wing circulatory organs, such as the scutellum and scutellar arms, coevolved with the wings and can be regarded as synapomorphies of the Pterygota. Although these structures remained largely conserved, their pulsatile components underwent significant evolutionary modification. Spatial constraints likely caused the dorsal vessel to detach from the tergal cuticle, thereby disconnecting the original pulsatile components of the wing circulatory organ. These were replaced by muscular diaphragms derived – at least in *Drosophila* – from recruited pericardial precursors with a distinct developmental origin. In summary, the evolution of individualized wing hearts was likely driven not by circulatory necessity, but by spatial constraints imposed by the progressive modification of the thoracic flight musculature during adaptations optimized for flight. Their origin thus represents a case of compensatory structural innovation triggered by body plan modifications without direct functional relevance – a phenomenon we propose to term "spatial-constraint-induced evolution".

Concluding remarks and outlook

In exploring the origins of evolutionary novelties, it is often – somewhat paradoxically – assumed that explanatory factors beyond established evolutionary theory must be invoked (Linz et al. 2020). Yet, Darwin's principle of descent with modification remains fundamentally sound: every new structure must, in some way, derive from a pre-existing one. This principle also applies to insect wings, for which numerous ancestral elements and morphogenetic determinants are now well documented. Even so, the dichotomy of whether wings evolved through transformation of existing structures or arose as genuinely novel elements persists in various interpretations within current discourses.

From our perspective, Wagner's (2014) classification of evolutionary novelties provides a useful conceptual framework that enhances clarity in such discussions. Type I novelties emerge from pre-existing elements, such as specific cells or developmental modules, and result in new organs with distinct morphological or developmental identities. These novelties contribute to the individuation of body parts, for instance by modifying serially repeated structures. This framework is particularly applicable to the dual-origin hypothesis of wing evolution. In contrast, the precoxal hypothesis posits that insect wings arose from the proximal appendage segment of a pancrustacean ancestor via a complex, multistage transformation. This model corresponds to a Type II novelty, where an existing morphological identity is transformed into a new form without the addition of an entirely new body part.

The key to evaluating these scenarios lies in resolving questions of homology. Traditional morphological criteria have largely failed to provide conclusive answers, while molecular and genetic approaches introduce their own challenges. The GRNs involved in wing development are broadly conserved and overlap with multiple traits and lineages. Further complicating the issue is the inconsistent use of the term "homology", which varies widely among researchers. These challenges are comprehensively addressed in a recent thematic issue of invited papers in the Journal of Morphology, featuring contributions that range from conceptual refinements to arguments for abandoning the term altogether (see editorial by Stach & Starck 2023).

A particularly critical issue in these debates is the inherently hierarchical nature of homology, which permits comparisons only within the same organizational level of biological structure. Unfortunately, this principle is often overlooked in discussions on the origin of insect wings, where shared GRN expression is frequently misinterpreted as direct evidence of structural homology. In such cases, the concept of partial homology might offer a more appropriate explanatory model. However, this concept remains controversial, as introducing degrees of similarity without clear delimitations risks blurring the distinction between homology and analogy, thereby complicating both classification and evolutionary inference (Hall 2003). Nonetheless, there is growing consensus that homology should not be treated as a strict binary, all-or-nothing category (Minelli 2022). Recent frameworks, such as those incorporating the hierarchical organization of serial homologs (DiFrisco et al. 2023a), support a graded approach and represent a promising avenue for reconciling morphological, developmental, and molecular data.

A comparison of wings and wing hearts reveals striking differences in both developmental complexity and evolutionary patterning. While wings may seem like simple lateral outgrowths of the integument, they are, in fact, intricate structures composed of multiple developmental units, each with its own distinct evolutionary trajectory. This complexity has made their evolutionary origins challenging to reconstruct. In contrast, wing hearts provide a much more straightforward model. Yet, even here, our early assumptions proved misleading, and only a comprehensive, integrative approach ultimately allowed us to clarify their developmental and evolutionary origins.

An often-overlooked aspect of novelty evolution is the necessity for newly arising traits to be compatible with existing developmental and functional systems. Any evolutionary novelty must be integrated into the organism as a whole without disrupting its viability. This principle is clearly illustrated in insect wing evolution, where research has typically focused on the formation of the wing itself. In this regard, the co-evolution of wings and their associated circulatory organs provides an illustrative case: the emergence of one structure introduced spatial and functional constraints that shaped the evolution of the other. Studying such reciprocal dependencies is essential for a more comprehensive understanding of organ evolution.

Wing hearts, in particular, offer an accessible model system for investigating evolutionary novelties. Their anatomical simplicity and experimental tractability make them well suited for multi-level analyses across genetic, cellular, and organ scales. Importantly, wing hearts have evolved independently multiple times in different insect lineages, making them an excellent subject for studying convergent evolution. Expanding this research beyond *Drosophila* to include a broader range of taxa could yield critical insights. Moreover, pericardial precursors represent a promising entry point for exploring the evolution of cell type diversity (Arendt et al. 2016). As serial homologs, these cells exhibit remarkable developmental plasticity, giving rise to both nephrocytes and various specialized muscle cells, a versatility that makes them an ideal model for investigating mechanisms of cell type specification and diversification.

In conclusion, we advocate for a broader perspective in future research on evolutionary novelties. While deepening our understanding of established model systems remains essential, equal emphasis should be placed on discovering and characterizing new examples. Arthropods, with their extraordinary diversity, continue to offer rich opportunities for investigating how novel traits emerge and become integrated into existing biological systems.

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