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Hypothesis

Ghost Hunting: Insects Retain Ancestral Abdominal Legs in a Truncated Form

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Abstract: An iconic feature of insects is the apparent lack of legs on the abdomen, which is believed to be due to the repression of the leg-patterning gene Distalless (Dll) by abdominal Hox genes. However, in contrast to these molecular observations, it is not widely appreciated that the embryos of most insect groups do in fact form paired protrusions on most abdominal segments, which degenerate to form the abdominal exoskeleton[1,2]. These embryonic abdominal legs appear to be homologous to the thoracic legs. To resolve this discordance between molecular and morphological observations, the expression patterns of *pannier* and *araucan*, genes known to distinguish proximal leg segments in all arthropods[3–5], are examined in embryos of the flour beetle *Tribolium castaneum*. In *Tribolium* embryos, paired protrusions are observed on most abdominal segments and the stripes of *pnr* and *ara* expression that delineate the proximal leg segments of the thorax are seen to continue unabated through all abdominal segments. Thus, insects retain abdominal legs in a truncated form that were inherited from their crustacean ancestors. These cryptic, truncated abdominal legs appear to serve as an important wellspring of new structures and functions in insects, such as caterpillar prolegs, gills, and leaf-like camouflage structures.

Keywords: arthropod; leg; evo-devo; exite

1. Introduction

Insects are the most successful group of animals on the planet, due in part to the plethora of outgrowths that decorate their bodies with functions including flight, camouflage, and respiration. An iconic feature of the insect body plan is the presence of six walking legs, which gives the group its name, Hexapoda. It is commonly assumed in molecular and evo devo circles that insects lack legs on the abdomen other than the pleuropod on the first abdominal segment of some insect embryos[6–10]. In insect groups where larvae or adults have abdominal appendages, such as caterpillars or male sepsid flies, it has been proposed that these abdominal legs arose by re-evolution of the leg program[11]. However, crustaceans, from which insects evolved, generally have a pair of legs on all abdominal segments. The loss of these ancestral abdominal legs in the insect lineage is thought to have evolved when posterior Hox genes such as Ultrabithorax (Ubx) and Abdominal-A (AdbA) gained the ability to suppress the leg-patterning gene Distalless (Dll) in the insect abdomen[6,12].

However, morphologists since 1844[1] have noted for nearly a century that, in the embryos of most insect groups, a pair of nubs form on most abdominal segments which appear to be homologous to the thoracic legs[1,2,13–18]. These abdominal nubs flatten into the body wall before hatching to form the abdominal exoskeleton (sternites)[2,19]. But how could abdominal legs form in insects when Dll is suppressed by Hox genes?

By comparing the expression and function of several leg- and wing-patterning genes between insects, crustaceans, and arachnids – representing three of the four main groups of arthropods – Bruce and Patel 2020[3], Bruce and Patel 2021[4], and Bruce 2022[5] concluded that arthropods ancestrally have a total of 8 leg segments, but many arthropods have incorporated proximal leg segments into the body wall (Fig. 1). Insects, for example, have incorporated the proximal leg segments 7 and 8, which now form the entire body wall (pleura and lateral tergum) except for a narrow dorsal (and perhaps ventral) stripe[20], resulting in the familiar six (free) leg segments of insects: pretarsus/claw (1), tarsus (2), tibia (3), femur (4), trochanter (5), and coxa (6). This work and others found that, in the embryos of all arthropods examined to date, representing three of the four living arthropod groups — *Drosophila melanogaster* (fruit fly; insect)[21–24], *Tribolium castaneum* (flour beetle; insect)[3–5], *Parhyale hawaiensis* (amphipod; crustacean) [3–5], *Daphnia* magna (water flea; crustacean)[5], and *Acanthoscurria geniculata* (tarantula; chelicerate)[4] — the Iroquois complex gene *araucan* (*ara*) is expressed in a dorsal and ventral stripe bracketing the incorporated 8th leg segment, and the GATA factor *pannier* (pnr) is expressed in the dorsal-most tissue and marks the true body wall (Figs. 1, 2). Thus, in contrast to other leg patterning genes[25], the expression patterns of pnr and *ara* are highly conserved across arthropods. As such, they can be used to identify proximal leg segments even if the leg segments now function as body wall.

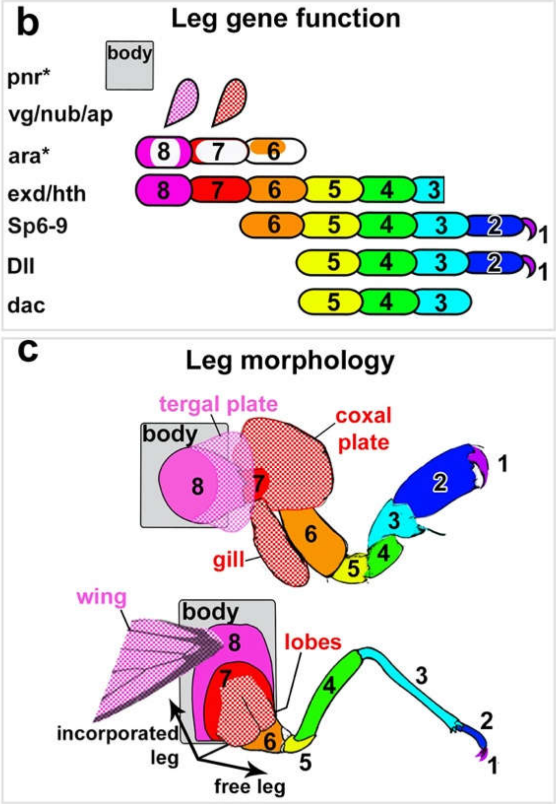

Figure 1. Leg segment alignment of arthropod legs based on expression and function of leg genes. From Bruce 2020 and 2021.

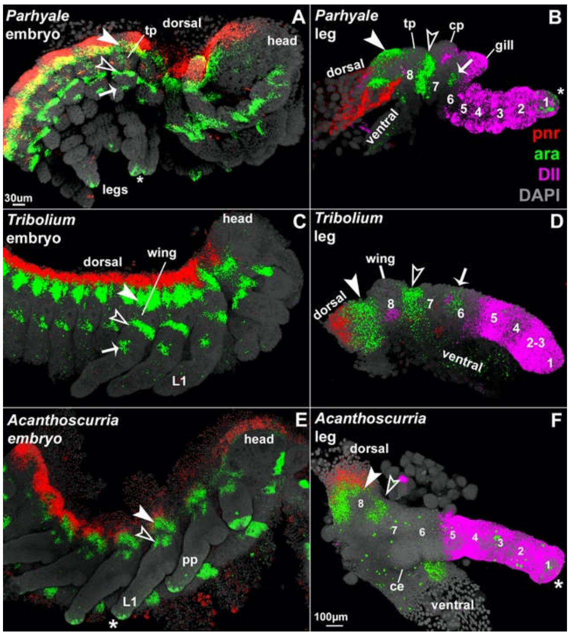

Figure 2. In all arthropods examined, *araucan* (*ara*) brackets the incorporated 8th leg segment, and *pannier* (pnr) is expressed in the dorsal-most tissue and marks the true body wall. A, B. Parhyale, crustacean. C, D. Tribolium, insect. E, F. Acanthoscurria, chelicerate. From Bruce 2021.

3. Results

When this approach is applied to the insect abdomen, the stripes of *pnr* and *ara* expression that delineate the proximal leg segments of the thorax are seen to continue unabated through all abdominal segments (Figs. 2, 3). Leg segment 8 in both thorax and abdomen bears the vg-positive tergal plates and presumptive wings, as well as the spiracle[3,5,20], and it is bracketed dorsally and ventrally by the two stripes of *ara* expression. Given that that insect abdomen does not express *ara*, which marks leg segments 1 – 5 (Fig. 1), the small abdominal protrusions ventral to leg segment 8 may represent just leg segment 7, or leg segments 6 and 7. Thus, rather than being completely limbless, the insect abdomen has a pair of legs on most or all abdominal body segments, but these abdominal legs are truncated, consisting of just the proximal-most two or three leg segments 6, 7, and 8. Based on their similar positioning, embryonic development[1,2,13–18], and gene expression, these abdominal leg nubs appear to be serially homologous with the thoracic legs.

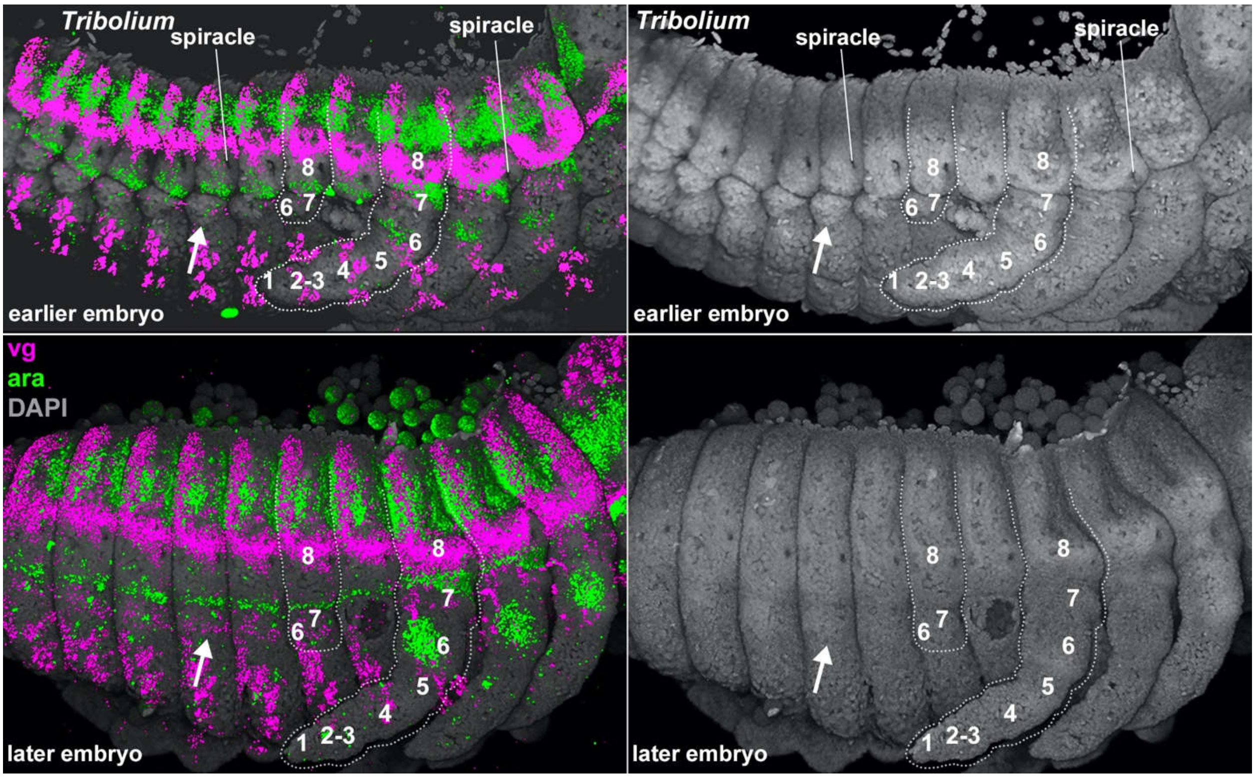

Figure 3. Segment identity of abdominal leg nubs in Tribolium embryos. Top: approximately stage NS14.1 (Klann 2021)[26]. Bottom: Stage NS15.4 (Klann 2021). Arrow points to 4th abdominal leg nub that later degenerates into the body wall. *araucan* (*ara*, green) is expressed in two stripes down the length of the embryo, one dorsal stripe and one lateral stripe, as well as a circular patch on leg segment 6 (coxa) of each thoracic leg. The two stripes bracket the proximal-most 8th leg segment that carries both the wing and the spiracle. *vestigial* (*vg*, pink) marks the future wing serial homologs: the wing, elytra, and tergal plates, as well as certain cells in the ventral nerve cord. Note that cells in the ventral nerve cord are larger and less compact than cells of the leg nub, thus, in addition to their differences in shape and position, the two are readily distinguished. Gray, DAPI, marks all cell nuclei.

4. Discussion

The results presented here answer the question of how legs can form on the insect abdomen despite the repression of *Dll* by Hox genes in the insect lineage: only the distal leg, represented by leg segments 1-5 (claw to trochanter), is repressed by the Hox genes; the three proximal leg segments that do not depend on *Dll* function[27,28], leg segments 6 – 8, are still generated. This is consistent with previous findings that a) loss of *Dll* does not delete the entire insect leg[29–33]; and more importantly, b) *Dll* is not sufficient to initiate leg development[27,28,34], which means that leg initiation is achieved by other, more upstream genes, such as *btd*[34] and *Sp6-9* (*Sp1* in *Drosophila*)[27,28], and the interaction of dorsal *dpp* and ventral *wg*[10,35,36]. Notably, *btd* is expressed in leg-like paired circular domains in both the thorax and the abdomen of insects[37], and the intersecting domains of *dpp* and *wg* that initiate leg development in the thorax are similarly expressed in the abdomen, which further supports the existence of cryptic insect abdominal legs. Given that the insect abdomen expresses markers of body wall (*pnr*), leg segments 7 and 8 (*ara* and *vg*), and leg segments 1 – 6 (*btd*)[37], but not *Dll*, which marks leg segments 1 – 5, it is deduced that the truncated insect abdominal leg is comprised of leg segments 6 – 8.

Why truncate these ancestral abdominal legs instead of simply deleting the whole structure? One reason is that several essential structures develop from these proximal leg segments, such as the respiratory system (the spiracle and tracheae)[2,38,39] as well as various exocrine glands like defensive scent glands[40] and oenocytes (lipid processing, pheromone secretion, and developmental signaling)[41]. In addition to these essential structures, many other useful structures are also carried on this leg-derived abdominal body wall, including

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tergal plates, gin traps[42], knob-like pupal support structures[43], rod-like sensory organs in certain hemipterans[44], and larval gills. In addition, in some insect lineages, the embryonic abdominal legs do not degenerate and instead form prolegs in caterpillars[45,46], sawflies[47], and watersnipes[7], as well as the adult sepsid fly male sternal brushes used in courtship[11]. Many of these insect abdominal structures have been called novel structures, which are commonly defined as structures that are not derived from, or homologous to, any structure in the ancestor nor any other structure in the individual[48]. However, rather than lacking homology, all of these structures likely derived from cryptic abdominal leg exites (multi-functional leg lobes like gills and tergal plates) and legs inherited from their crustacean ancestors[3,5,49]. A similar molecular approach should be used to assay for cryptic abdominal legs in the paraphyletic "entomostracan" crustaceans which, like insects, also appear to lack abdominal legs[50,51].

Given that insect abdominal legs were inherited from their crustacean ancestors, the functional structures on these legs may also have been inherited from crustaceans. Insect tracheae may be internalized crustacean gills (Fig. 4)[34]; insect wings, tergal plates, helmets, horns, and other ectodermal outgrowths likely evolved from crustacean plate-type outgrowths[3,5,52]; and insect secretory glands (salivary, endocrine, exocrine, etc) may have evolved from similar glands in crustaceans[40,53,54]. Surprisingly, respiratory organs and secretory glands can be homeotically transformed into each other[40,53,54] and plate-type outgrowths arise from the same tissue as respiratory organs[55], therefore all three types of structures may have arisen from a common embryonic exite-like structure on the lateral side of the proximal 8th leg segment[3–5] that was inherited from the ancestor of all arthropods. This perspective, rather than concepts like "partial homology", explains why structures that have clearly different functions, such as wings and gills, often share some genes but not others: they are homologous as exites, but not as wings, horns, tracheae, etc[1,43,56–61]. Similarly, it is likely that familiar exite genes such as *vestigial*, *trachealess*, *ventral veins lacking*, *blistered*, and *apterous* confer specific functions and shapes to exites rather than positional identity[34,52,62–64]. While they are useful for determining whether a structure is derived from an exite, these and other exite-specifying genes are probably less informative for determining positional homology between different arthropods[34,52,62,63], in contrast to the well-conserved proximal-distal positional markers *pnr* and *ara*[3–5].

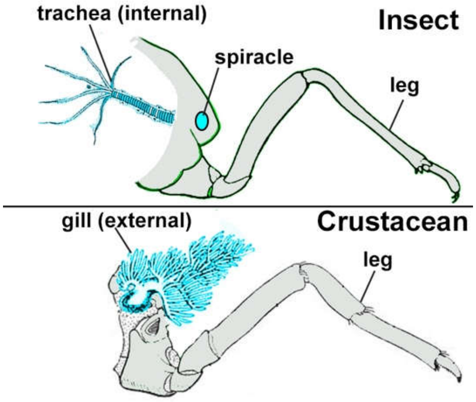

Figure 4. Similarity of internal insect tracheae and external crustacean gills. Modified from Snodgrass 1935 and Boxshall 2009.

An example of the above is the posterior lobe on the genitalia of male flies, which has been proposed as a novel structure that resulted when spiracle genes became co-opted into an unrelated structure, the genitalia[65]. However, given that genitals appear to be serially homologous to legs[66–69], and respiratory structures like spiracles/tracheae are likely derived from the leg, then perhaps the genital "leg" retains the ability to generate exites that express spiracle/tracheae genes. Given that spiracles/tracheae need not be internal (crustacean gills are external lobes and Drosophila larval posterior spiracle is external), it is plausible that the posterior lobe is an external spiracle structure. Rather than arising through gene co-option by an unrelated tissue, the posterior lobe may be the result of de-repression or re-activation of a serial homolog. This hypothesis would be supported if the posterior lobe emerges from the lateral side of the genital "leg" and if Iroquois genes like *ara* are expressed dorsal and ventral to the lobe.

In summary, the retention of the proximal leg segments in the insect abdomen for essential functions like respiration and secretion appears to have allowed the non-essential plate-like outgrowths to become elaborated into new useful structures like gin traps and camouflage. Thus, cryptic, truncated abdominal legs appear to serve as an important wellspring of new structures and functions in insects.

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