10. McLean, C.Y., Reno, P.L., Pollen, A.A., Bassan, A.I., Capellini, T.D., Guenther, C., Indjeian, V.B., Lim, X., Menke, D.B., Schaar, B.T., *et al*. (2012). Human-specific loss of regulatory DNA and the evolution of human-specific traits. Nature *471*, 216–219.
11. Cotney, J., Leng, J., Yin, J., Reilly, S.K., DeMare, L.E., Emera, D., Ayoub, A.E., Rakic, P., and Noonan, J.P. (2013). The evolution of lineage-specific regulatory activities in the human embryonic limb. Cell *154*, 185–196.
12. Visel, A., Taher, L., Girgis, H., May, D., Golonzhka, O., Hoch, R.V., McKinsey, G.L., Pattabiraman, K., Silberberg, S.N., Blow, M.J., *et al*. (2013). A high-resolution enhancer atlas of the developing telencephalon. Cell *152*, 895–908.
13. Blow, M.J., McCulley, D.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., *et al*. (2010). ChIP-Seq identification of weakly conserved heart enhancers. Nat. Genet. *42*, 806–810.
14. Shen, Y., Yue, F., McCleary, D.F., Ye, Z., Edsall, L., Kuan, S., Wagner, U., Dixon, J., Lee, L., Lobanenkov, V.V., *et al*. (2012). A map of the cis-regulatory sequences in the mouse genome. Nature *488*, 116–120.
15. Ahituv, N., Zhu, Y., Visel, A., Holt, A., Afzal, V., Pennacchio, L.A., and Rubin, E.M. (2007). Deletion of ultraconserved elements yields viable mice. PLoS Biol. *5*, e234.
16. King, M.C., and Wilson, A.C. (1975). Evolution at two levels in humans and chimpanzees. Science *188*, 107–116.

Harvard Medical School, Department of Genetics, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
*E-mail: tabin@genetics.med.harvard.edu

http://dx.doi.org/10.1016/j.cub.2013.11.026

Development: A Deep Breath for Endocrine Organ Evolution

Developmental biologists have made surprising discoveries on the evolutionary origins of cell types, organs and body plans. Now, an elegant study in *Drosophila* raises interesting questions about the origin of two major endocrine organs of insects.

Marco Grillo[1], Jordi Casanova[2], and Michalis Averof[1,*]

Although evolution is a continuous process, when we take a large-scale view certain evolutionary transitions stand out as milestones in the evolution of body form and function. We recognize events, such as the invention of muscles and neurons, or the evolution of segmented bodies, hormonal control systems and centralized brains as major changes, and use them to categorize the animals into discrete phyla with more or less distinctive ‘body plans’. While we are certain that these transitions did take place in deep branches of the animal tree, we often do not understand how they occurred and have difficulty in reconstructing functional intermediate steps taken during these transitions. We know that vertebrates, insects, mollusks and sea anemones are all related to each other, but we cannot yet picture what their common ancestors looked like and how they lived. The deepest ancestral forms and functions of animals are the ‘known unknowns’ of animal evolution. New work by Sánchez-Higueras, Sotillos and Castelli-Gair Hombría [1], published in this issue of *Current Biology*, raises new questions about one of these unknowns — the evolution of arthropod endocrine systems — by revealing an unexpected link between two major endocrine glands and the respiratory organs (tracheae) of insects.

Arthropods — including insects, crustaceans, spiders, centipedes and the extinct trilobites — are among the most successful animals on earth: insects alone comprise more than half of all known living species (Haldane once said “the Creator, if he exists, has a special preference for beetles”); ants represent the largest part of animal biomass in rainforests, as do copepods and krill in the oceans. All arthropods are characterized by the possession of a hardened exoskeleton and a modular body consisting of repeated (but not necessarily identical) segments. The exoskeleton is likely to have been a key feature in the establishment of the arthropod body plan — it provides protection and leverage for muscles, but also constrains growth and the exchange of respiratory gases and ions with the environment. The arthropod ancestors evolved solutions to these constraints, which involved the endocrine and respiratory organs that are the focus of the Sánchez-Higueras *et al.* [1] paper.

To escape the constraints on growth, arthropods exploited moulting, which allows them to replace the exoskeleton by a larger one as the body grows. Two hormones became tightly associated with growth through moulting: ecdysone, whose levels in the blood (hemolymph) provide the signal for moulting, and juvenile hormone, whose action maintains the juvenile characteristics and prevents metamorphosis during successive larval moults [2–4]. In insects, two endocrine glands produce and release these hormones into the hemolymph: the prothoracic gland releases ecdysone and the corpus allatum releases juvenile hormone.

To escape the constraints imposed by the impermeable cuticle, many arthropods also evolved specialized surfaces for gas and ion exchange. In the aquatic ancestors of arthropods and in today’s crustaceans, these functions are often carried out by specialized appendages called gills. In terrestrial arthropods, such as centipedes, spiders and insects, the respiratory function is carried out by internal respiratory organs, called book lungs and tracheae, respectively. There is evidence suggesting that the book lungs and tracheae of some terrestrial arthropods evolved from the gills of their aquatic ancestors, by internalization of these respiratory surfaces into the body [5–7].

Until now endocrine glands and tracheae were supposed to be unrelated; populations of cells that evolved independently in response to different adaptive pressures on the arthropod body plan. The results of Sánchez-Higueras *et al.* [1] question this view, by demonstrating that the embryonic primordia of the corpus allatum and the prothoracic gland are serially homologous to the primordia of tracheal cells. Serial homology means that these structures originate from identical groups of cells located in successive segments of the body, which are defined by a common set of developmental instructions; as is the case for successive limbs in insects or vertebrae in mammals. Serial

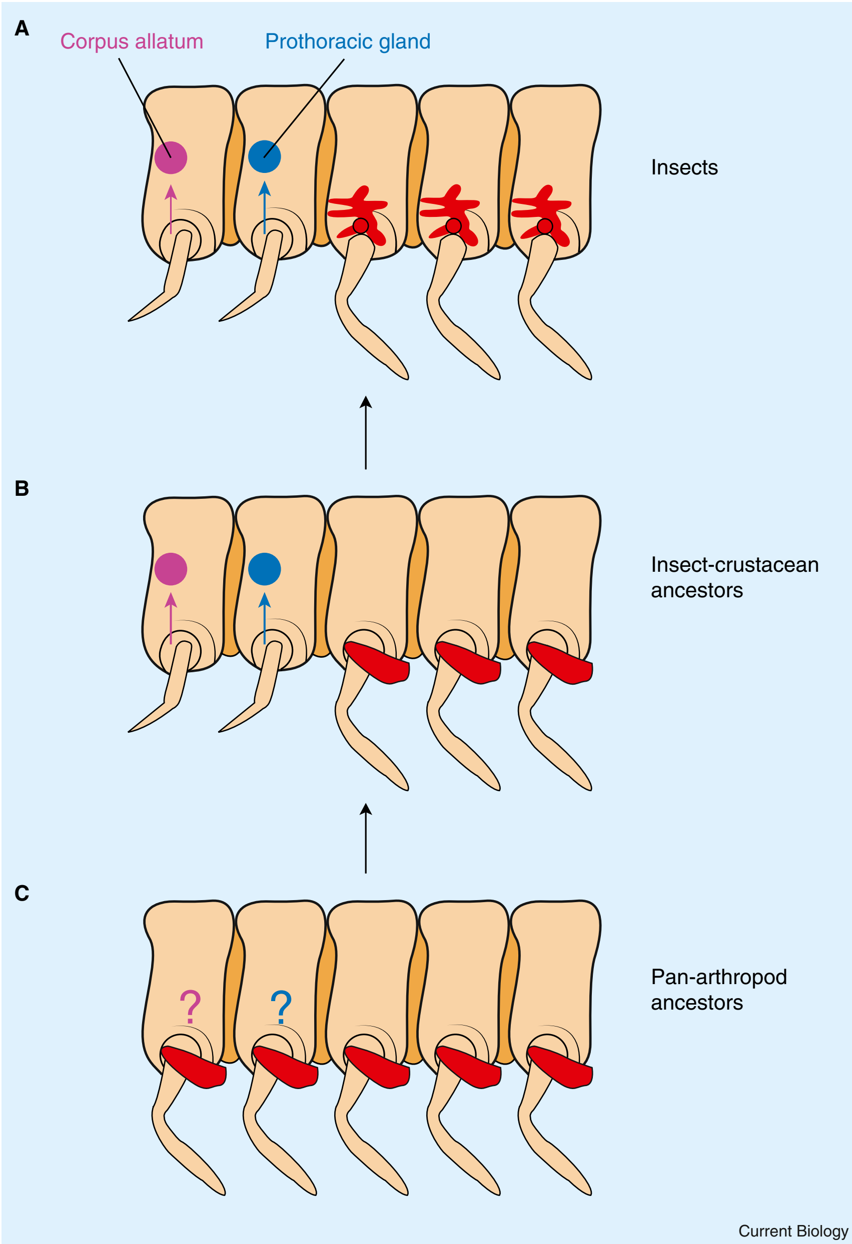

Figure 1. Hypotheses on the origin of insect endocrine glands and tracheae.

(A) In insects, as shown by Sánchez-Higueras *et al*. [1], the corpus allatum (magenta) and prothoracic gland (blue) and the tracheae (red) arise from serially homologous primordia in successive head and trunk segments. In some insects, the corpus allatum and prothoracic gland primordia fuse to form part of the ring gland. (B) Homologues of these organs are likely to have been present in the last common ancestors of crustaceans and insects; insect tracheae may derive from external respiratory surfaces (gills, in red) of these ancestors. (C) The corresponding head and trunk segments had not acquired distinct identities and probably carried gills in the last common ancestors of all arthropods. It is not known whether these gills performed any of the endocrine functions that were later associated with the corpus allatum and the prothoracic gland (see text for alternative hypotheses).

homologues are not necessarily identical. Their shared developmental programme may be modified through the action of Hox genes to generate different structures in successive segments [8].

Sánchez-Higueras *et al.* [1] show that in *Drosophila melanogaster* the corpus allatum, prothoracic gland and tracheal primordia arise at the same location in successive segments of the head and trunk: the corpus allatum in the maxillary segment, the prothoracic gland in the labial segment and the tracheae in successive thoracic and abdominal segments (Figure 1A). By performing elegant genetic experiments, they dissect the genetic requirements for corpus allatum and prothoracic gland primordium specification, invagination, epithelial-mesenchymal transition and migration. The establishment of the endocrine and tracheal primordia relies on the expression of the transcription factor Vvl, driven by the same enhancer, JAK/STAT signaling and the action of Hox genes. Once specified, the primordia invaginate in a strikingly similar fashion. Consistent with the notion that they represent serial homologues, the corpus allatum and prothoracic gland primordia can be transformed into each other and into tracheal primordia by mis-expressing the appropriate Hox genes. The study clearly establishes the common developmental origin of the corpus allatum, prothoracic gland and tracheal primordia, in successive segments of the *Drosophila* body. What are the evolutionary implications of this finding?

Comparative studies suggest that homologues of the corpus allatum, the prothoracic gland and the tracheae may exist in the closest relatives of insects, the crustaceans: the mandibular organ and the Y-gland in the head, which secrete methyl farnesoate (closely related to juvenile hormone) and ecdysone, respectively [2,9,10], and the gills in thoracic segments [7]. These organs may develop from serially homologous precursors in crustaceans — a hypothesis that could be tested in modern crustaceans — and, if so, it will be reasonable to assume that the same was true in the common ancestors of crustaceans and insects (Figure 1B). That common ancestor, an aquatic creature, would have formed external gills and internalized endocrine glands, which suggests that the invagination process was perhaps first implemented in the endocrine glands, and then co-opted to the gill/trachea precursors.

Can we trace the fate of these primordia further back in time, to the origin of all arthropods? How can we envision the origins of the developmental link between endocrine glands and tracheae? Cambrian fossils, comparative morphology and

Hox gene expression patterns suggest that at the origin of arthropods, the segments that correspond to the maxillary and the labial segment of insects had not yet acquired a distinct identity, bearing legs and gills like those of other trunk segments (Figure 1C) [11,12]. Did the precursors of the corpus allatum and the prothoracic gland exist on these segments in the last common ancestors of all arthropods?

Three possible scenarios come to mind: first, some components of the endocrine system may not have been present in these distant ancestors, but arisen later in evolution. Thus, juvenile hormone could have arisen in the crustacean/insect lineage and the corresponding endocrine organs may not have been present outside that clade. This is unlikely to be the case for ecdysone, whose role in moulting is widespread in arthropods [2]. Second, some of the relevant hormones may have been produced by other tissues. For example, in some arthropods ecdysone is also produced by the ovaries, the male accessory glands, or the epidermis [13–15]. It is possible that other tissues were the major sources of ecdysone ancestrally, and that this role was taken over by a new endocrine organ, the prothoracic gland, later in evolution. Third, endocrine functions may have been already associated with the presumed evolutionary precursors of the corpus allatum and the prothoracic gland, the gills, in anterior trunk segments or throughout the body (Figure 1C). The latter hypothesis, if true, could have had profound implications for the physiology of the animals that carried them. Oxygen concentration and osmotic pressure may influence ecdysone and juvenoid hormone levels in modern-day insects and crustaceans [16,17] and there is evidence to suggest that oxygen availability in the body could be one of the sensors of growth that trigger moulting in insects [18]. In ancestral arthropods, the physical unity of endocrine organs and gills may have helped integrate cues from the environment into a system regulating body size, respiration and growth.

References

1. Sánchez-Higueras, C., Sotillos, S., and Castelli-Gair Hombría, J. (2014). Common origin of insect trachea and endocrine organs from a segmentally repeated precursor. Curr. Biol. *24*, 76–81.
2. Nijhout, F. (2013). Arthropod developmental endocrinology. In Arthropod Biology and Evolution, A. Minelli, G. Boxshall, and G. Fusco, eds. (Springer-Verlag), pp. 123–148.
3. Yamanaka, N., Rewitz, K.F., and O'Connor, M.B. (2013). Ecdysone control of developmental transitions: lessons from Drosophila research. Annu. Rev. Entomol. *58*, 497–516.
4. Jindra, M., Palli, S.R., and Riddiford, L.M. (2013). The juvenile hormone signaling pathway in insect development. Annu. Rev. Entomol. *58*, 181–204.
5. Lankester, E.R. (1881). Limulus an Arachnid. Quart. J. Microscopical Sci. *21*, 504–548.
6. Damen, W.G.M., Saridaki, T., and Averof, M. (2002). Diverse adaptations of an ancestral gill: a common evolutionary origin for wings, breathing organs, and spinnerets. Curr. Biol. *12*, 1711–1716.
7. Franch-Marro, X., Martín, N., Averof, M., and Casanova, J. (2006). Association of tracheal placodes with leg primordia in Drosophila and implications for the origin of insect tracheal systems. Development *133*, 785–790.
8. Lewis, E.B. (1978). A gene complex controlling segmentation in Drosophila. Nature *276*, 565–570.
9. Laufer, H., Borst, D., Baker, F.C., Reuter, C.C., Tsai, L.W., Schooley, D.A., Carrasco, C., and Sinkus, M. (1987). Identification of a juvenile hormone-like compound in a crustacean. Science *235*, 202–205.
10. Lachaise, F., Le Roux, A., Hubert, M., and Lafont, R. (1993). The molting gland of crustaceans: localization, activity, and endocrine control. J. Crustacean Biol. *13*, 198–234.
11. Waloszek, D., Chen, J., Maas, A., and Wang, X. (2005). Early Cambrian arthropods—new insights into arthropod head and structural evolution. Arthropod Struct. Dev. *34*, 189–205.
12. Eriksson, B.J., Tait, N.N., Budd, G.E., Janssen, R., and Akam, M. (2010). Head patterning and Hox gene expression in an onychophoran and its implications for the arthropod head problem. Dev. Genes Evol. *220*, 117–122.
13. Hagedorn, H.H., O'Connor, J.D., Fuchs, M.S., Sage, B., Schlaeger, D.A., and Bohm, M.K. (1975). The ovary as a source of alpha-ecdysone in an adult mosquito. Proc. Natl. Acad. Sci. USA *72*, 3255–3259.
14. Zhu, X.X., Oliver, J.H., and Dotson, E.M. (1991). Epidermis as the source of ecdysone in an argasid tick. Proc. Natl. Acad. Sci. USA *88*, 3744–3747.
15. Hentze, J.L., Moeller, M.E., Jørgensen, A.F., Bengtsson, M.S., Bordoy, A.M., Warren, J.T., Gilbert, L.I., Andersen, O., and Rewitz, K.F. (2013). Accessory gland as a site for prothoracicotropic hormone controlled ecdysone synthesis in adult male insects. PLoS ONE *8*, e55131.
16. Yamanaka, N., and O'Connor, M.B. (2011). Nitric oxide directly regulates gene expression during Drosophila development: need some gas to drive into metamorphosis? Genes Dev. *25*, 1459–1463.
17. Nagaraju, G.P.C. (2007). Is methyl farnesoate a crustacean hormone? Aquaculture *272*, 39–54.
18. Callier, V., and Nijhout, H.F. (2011). Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. Proc. Natl. Acad. Sci. USA *108*, 14664–14669.

[1]Institut de Génomique Fonctionnelle de Lyon (IGFL), Centre National de la Recherche Scientifique and École Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon, France. [2]Institut de Biologia Molecular and Institut de Recerca Biomèdica de Barcelona, C/Baldiri Reixac 10, 08028 Barcelona, Catalonia, Spain.
*E-mail: michalis.averof@ens-lyon.fr

http://dx.doi.org/10.1016/j.cub.2013.11.033