

Molecular Evolutionary Biology of Carapace Formation in Crustaceans*

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More than 60,000 species belonging to the subphylum Crustacea are known to date and, in many species, a characteristic flat structure consisting of two epithelial cell layers is formed on the dorsal side. These flat structures, called carapaces, show high levels of morphological variation and are known to have various functions, depending on subgroups, including provision of hydrodynamic advantages, protection of the body, formation of a feeding chamber, a respiration chamber, and a brood (egg) chamber (Olesen, 2013).

In 1909, a Scottish zoologist William Thomas Calman first suggested that the carapace is a primitive structure in the crustacean lineage (Calman, 1909). Since then, the evolutionary origin of the carapace has been the subject of extensive study and debate, however, extensive morphological diversity in the dorsal part of crustacean head (cephalothorax) prevents conclusive answer from morphological comparisons among lineages. Several questions on the origin of the carapace, including whether the carapace is a primitive structure with a single origin or whether it had evolved multiple times, from which segment(s) it originated, and whether it is related to structures in the body of other arthropods, remains unresolved for over a century (Fryer, 1996; Olesen, 2013). The EvoDevo studies may provide good opportunities to address these questions, by exploring the molecular mechanisms that underlie the development of crustacean carapaces and examining how these mechanisms evolved.

Fossil records suggest that the bivalved carapaces of crustaceans (and crustaceanomorphs) have evolved in the early Cambrian, approximately 525 million years ago (Xian-guang et al., 2004), long before the emergence of the insect wings. The water flea *Daphnia magna* Straus, 1820 (Branchiopoda: Anomopoda) has a bivalved carapace which consists of a double epidermal cellular layer decorated with bristles on its margin.

These morphological features are also found in the wings of insects, leading us to hypothesize that some of the regulatory genes that play key roles in patterning the insect wings may have similar roles in the margin of the *Daphnia* carapace.

To test this hypothesis, full length cDNAs for *D. magna* orthologs of a TEA-domain transcriptional factor *scalloped* (*sd*) and a transcriptional cofactors *vestigial* (*vg*), both often considered to be the master genes of insect wings, and an Wnt-family secretory signaling factor *wingless* (*wg*) were cloned, and their expressions during embryogenesis were analyzed. Double immunostaining and *in situ* hybridization revealed that the expression domains of Wg, Vg and Sd fully overlap in developing *Daphnia* carapace margin and co-expression of these was not observed in other parts of the embryo. RNAi knockdown experiments demonstrated that Sd and Wg play essential roles in carapace outgrowth. The sole Hox protein expressed in developing *Daphnia* carapace was Sex combs reduced (*Scr*) and CRISPR/Cas9-mediated *Scr* gene knockout resulted in severely reduced carapace, suggesting strongly that the *Daphnia* carapace is derived from the first and second maxillary segments (Mx1 and 2).

Taken together, our data suggest that *Daphnia* carapace is formed in *Scr*-expressing domains (Mx1 and 2 segments) and is folded at the marginal Wg/Vg/Sd co-expressing cells before extending posteriorly. If Calman's hypothesis on evolution of crustacean carapaces is the case, then one can expect the existence of similar genetic mechanism for carapace development in other modern crustaceans. To address this, my group has extended our research to study on other crustacean species, including the American tadpole shrimp *Triops longicaudatus* (LeConte, 1846) (Branchiopoda: Notostraca), the American brine shrimp *Artemia franciscana* Kellogg, 1906 (Branchiopoda: Anostraca),

* Contribution to the symposium "Acquisition of Functional Diversity of Insects" in the 57th Annual Meeting of the Arthropodan Embryological Society of Japan, July 9–10, 2021, Ushiku-numa, Ibaraki, Japan.

and the red swamp crawfish *Procambarus clarkii* (Girard, 1852) (Malacostraca: Decapoda).

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