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Molecular genetics of crustacean feeding appendage development and diversification

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Arthropods dominate our seas, land, and air and have done so for hundreds of millions of years. Among the arthropods, crustaceans present us with a rich history of morphological change, much of which is still represented among extant forms. Crustacea largely interact with their environment via their appendages; thus vast amounts of variation exist among the different appendages of a single individual and between appendages from different species. Comparative studies of crustacean appendage development present us with an important story regarding the evolution of morphology over both relatively short (a few million years) and relatively long (a few hundred million years) evolutionary time scales. Recent studies have used the genetic and molecular data from Drosophila development to try to understand the molecular basis for some of the variations seen in crustacean limbs. Here we review some of these data based on the expression patterns of the genes Ultrabithorax, abdominal-A, Sex combs reduced, and Distal-less.

Key words: crustacean / evolution / maxilliped / mandible / gene expression

Crustacean limb morphology

Arthropod segments, and their associated appendages, represent serially homologous units that lie along the anterior–posterior axis of the animal. Arthropod limbs are subjected to selective pressures from diverse environments and exhibit high levels of morphological variation. Among extant arthropod groups, crustaceans display the greatest diversity of body plans and limb morphologies.

Generally, crustacean limbs fall between two morphological extremes (Figure 1). At one extreme is the lobed phyllopodous appendage composed of limb branches that are broad and laterally compressed [Figure 1(a)]. At the other extreme is the seemingly uniramous appendage which appears to be one multi-articulated rod where all other limb branches have been eliminated or greatly reduced [Figure 1(c)]. The ancestral state of the crustacean limb most likely was neither a strictly phyllopodous limb nor a strictly uniramous limb but a biramous limb composed of two primary branches [Figure 1(b)].[1]

Despite the variations seen in crustacean limbs, enough similarities remain that we can establish a consistent nomenclature that allows us to compare the different morphologies (Figure 1). The region of the limb most proximal to the body wall is referred to as the coxopodite,[2] historically termed the 'protopod'[1] (dark shading in Figure 1). The coxopodite may consist of up to three articulating elements [Figure 1(b) and (c)] or be a simple fused structure [Figure 1(a)]. Distal to the coxopodite is the telopodite (light shading in Figure 1). The telopodite includes the main limb branches known as the endopod and exopod [Figure 1(b)]. The principal ventral branch is the endopod. The principal dorsal branch is the exopod. Additional cuticular structures may be present on the coxopodite, however they are not multi-jointed structures. Cuticular structures arising ventral and medial to the endopod are termed endites [Figure 1(a)], for example the crustacean gnathobase is often thought to be an elaborated endite. Cuticular structures arising dorsal and lateral to the exopod are termed exites [Figure 1(a) and (b)], a common exite structure is the epipod which usually serves a respiratory function.[1,3–5]

Crustacean limbs can also be grouped according to their organization along the anterior–posterior

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axis of the body. Different regions, or tagmata, of the body possess characteristic types of limbs with characteristic functions. Cephalic appendages typically include two pairs of antennae involved in sensory functions. The gnathal region contains the mandibles and two pairs of maxillary appendages which are primarily associated with feeding functions. Thoracic and abdominal appendages are of variable numbers and morphologies and are involved in feeding, respiration, and locomotion.

Sticking your foot in your mouth: the maxilliped story

The serially repeated nature of the crustacean limb along the anterior–posterior axis allows us to explore morphological variation both within an individual and between related species. While descriptive studies of this morphological variation have been available for quite some time, we have only recently had insight into the possible molecular mechanisms underlying morphological diversity in crustaceans. This insight is only possible because the genetic and molecular data from several 'model' systems (primarily, in this case, *Drosophila* and mice) have provided us with a partial understanding of how regional identities are established during development. A group of key players in this process are the homeotic, or Hox genes. These genes, generally located as a clustered set in the genome, are comprised of a closely related group of transcription factors expressed in specific, spatially restricted, domains along the anterior–posterior axis of the embryo. Altering the expression pattern of these genes in *Drosophila* typically results in homeotic transformation—causing one or more segments to adopt the morphology of adjacent segments (reviewed in Lawrence, Reference 6). While these homeotic genes can clearly cause changes in the body plan when mutated or experimentally manipulated in *Drosophila* and mice, only recently have we seen evidence that alterations in the expression of these genes may also play a role in normal evolutionary processes of morphological change. Some of this evidence comes from the study of the expression of two Hox genes, *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*), in crustaceans.

Ubx and *abd-A* hox gene expression has been characterized in a broad range of crustacean taxa utilizing a cross-reactive antibody that recognizes a shared epitope in both proteins.[7] This study revealed that changes in the anterior boundary of *Ubx/abd-A* in a range of crustaceans correlates with a transition

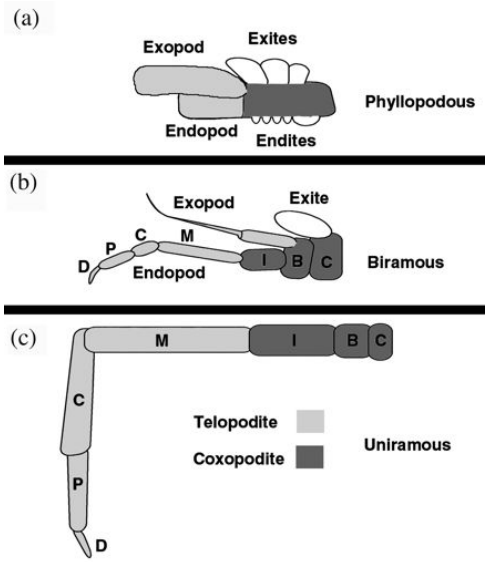

Figure 1. Crustacean appendage morphology. Ventral is down, dorsal is up. Dark shading indicates the coxopodite which may be a fused structure (a) or composed of up to three jointed, articulating elements [(b) and (c)]. Proximal to distal, these three elements are defined as: C, coxa; B, basis; and I, ischuim. Light shading indicates the telopodite, which can be unbranched (c) or include two major distal branches emanating from the coxopodite [(a) and (b)]. The ventral-most branch is the endopod, the dorsal-most branch is the exopod. While these branches can exhibit immense variation, the endopod is typically constructed from four jointed, articulating elements. Proximal to distal, these four elements are defined as: M, merus; C, carpus; P, propodus; and D, dactyl. The coxopodite may also possess a number of cuticular projections that may articulate at their junctions with the coxopodite but are non-jointed. Cuticular projections arising ventral and medial to the endopod are collectively termed endites (a). Cuticular projections arising dorsal and lateral to the exopod are collectively termed exites [(a) and (b)].

in limb identity: from what is primarily a feeding appendage to an appendage whose morphology is generally associated with locomotion.[8] While the thoracic appendages of insects all serve some primarily locomotor function, the anterior trunk appendages of many crustaceans have been recruited and modified to assist in feeding. These modified thoracic appendages are termed maxillipeds. As diagrammed in Figure 2, various crustacean species possess anywhere from zero to three pairs of maxillipeds (blue thoracic appendages in Figure 2). While the literature often refers to the maxilliped as primarily a feeding appendage, and the remaining thoracic appendages as primarily locomotary, it is probably

more accurate, especially with regard to homeosis, to refer to maxillipeds as 'gnathal-like' and the remaining thoracic appendages as 'non-gnathal-like', but for the sake of simplicity we will use the generic distinction of feeding appendage versus locomotary appendage.

Maxillipeds (and their associated segments) do not express either *Ubx* or *abd-A* gene products during early embryonic development. In contrast, more posterior trunk appendages that play a role in locomotion do express *Ubx/abd-A* proteins (orange shading in Figure 2). Branchiopod crustaceans are thought to retain a number of ancestral characters including an absence of maxillipeds. Of the two principal branchiopod lineages examined, both the anostracan *Artemia* and the notostracan *Triops* showed *Ubx/abd-A* expression in all trunk segments and limbs.

In contrast to the branchiopods, members of the monophyletic malacostracan lineage can have up to three pairs of maxillipeds. In the basal leptostracan *Paranebalia belizensis*, embryonic *Ubx/abd-A* expression is seen in all thoracic segments, and in this species, there are no maxillipeds. In amphipods, lobsters, and cleaner shrimp, there are one, two, and three pairs of maxillipeds, respectively. This pattern of maxillipeds correlates with earlier embryonic expression of *Ubx/abd-A* in these embryos; starting from T2 in amphipods (*Parhyale*), T3 in lobsters (*Homarus*), and T4 in cleaner shrimp (*Periclimenes*). Copepods (Maxillopoda) posses a single pair of maxillipeds, but these are thought to have been independently evolved from the maxillipeds found in malacostracans. However, just as with malacostracans such as amphipods, the presence of maxillipeds in copepods correlates with the lack of *Ubx/abd-A* expression in the first thoracic segment (Figure 2).

It is clear that the evolution of maxillipeds represents an 'anteriorization' of appendage morphology, with thoracic appendages taking on the morphology of more anterior gnathal appendages. Therefore, it can be thought of as a homeotic type of transformation. The correlation of this morphology with *Ubx/abd-A* expression is particularly suggestive given that mutational analysis of these genes in *Drosophila* indicates that changes in their pattern of expression are capable of precisely this type of change in limb morphology. For example, removing *Ubx* expression from the third thoracic segment of *Drosophila* transforms this segment into a duplicate copy of the second thoracic segment (which does not express *Ubx*, reviewed in Lawrence, Reference 6). Thus it is reasonable to suggest that changing patterns of *Ubx/abd-A* expression do indeed form part of the developmental basis of this evolutionary process. We must also stress that it is clear that homeotic genes such as *Ubx* and *abd-A* are not themselves responsible for any specific morphology when comparing between species—the *Ubx/abd-A* expressing locomotary appendages of *Artemia* look quite different from the *Ubx/abd-A* expressing locomotary appendages of a lobster. What these genes can do is specify a point along the body axis where there is a clear change in morphology. Evolutionary changes in the position of this boundary in limb morphology, then, may indeed be controlled by changes in homeotic gene expression.

It is also interesting to note that a similar shift in *Ubx/abd-A* expression appears to underlie the evolution of maxillipeds in both maxillipodan and malacostracan lineages, where maxillipeds are thought to have evolved independently. This result would seem to suggest a limited number of ways to generate a maxilliped. Also of particular interest are limbs that retain some intermediate character between a maxilliped and a walking appendage (as seen in the second thoracic segment appendage of the peracarid *Mysidium columbiae*). This appendage has a mosaic pattern of *Ubx/abd-A* expression. Those regions of the limb most like a maxilliped, the distal regions, do not express *Ubx* or *abd-A*. Those regions most like a locomotary limb, the proximal regions, do express *Ubx/abd-A*.

Recent studies of the expression of another homeotic gene, *Sex combs reduced* (*Scr*), in the terrestrial isopod *Porcellio scaber* also reveal an interesting correlation.[9] Embryos in late stages of development (50–80% of development) were found to express *Scr* transcripts strongly in the maxilliped limb primordia of the first thoracic segment. Expression of *Scr* was also detected in both the first and second maxillary segments and their associated maxillary appendages (green shading in Figure 2). This would suggest that the *Scr* product may act to directly activate a 'gnathal' (MxI and MxII) type of appendage development program. Additional comparative data on *Scr* expression over a broad range of crustacean taxa and additional information from early embryonic stages would serve to fill an important gap with respect to the potential general influence of the hox cluster on the evolution of maxillipeds.

While the correlations of *Ubx/abd-A* and *Scr* expression with maxilliped evolution are certainly intriguing, we still will need data from experimental manipulations of gene expression to test this hypothesis. In addition, we still do not know whether changes

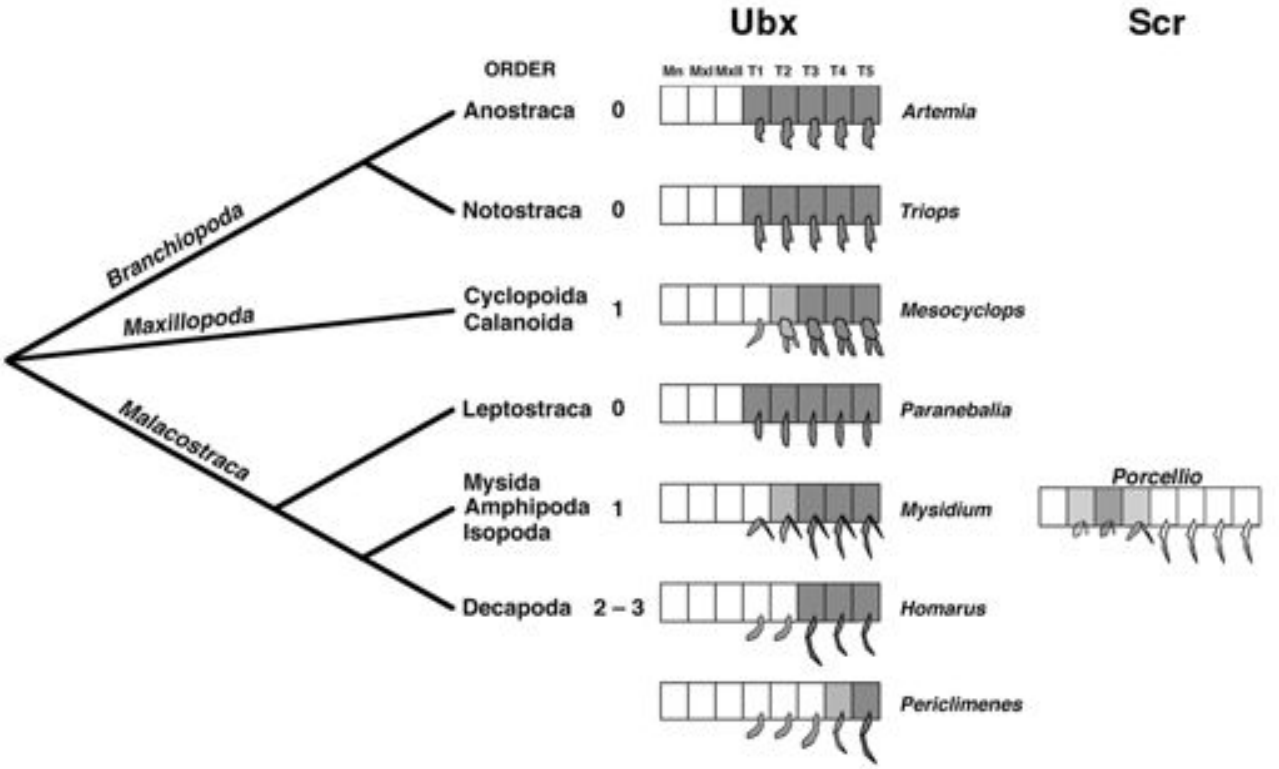

Figure 2. Distribution of maxillipeds and the expression of *Ubx/Abd-A* and *Scr*. The numbers of maxillipeds possessed by particular orders of Crustacea are indicated adjacent to the phylogeny. *Ubx/abd-A* antibody expression data are represented by orange shading (adapted from Reference 7). Darker shading indicates higher levels of expression. Maxillipeds are shaded blue. *Scr* mRNA *in situ* data are represented by green shading. Darker shading indicates higher levels of expression.

in the expression of Hox genes are due to changes in the regulatory elements of these Hox genes, or due to changes in their upstream regulators. This is an important distinction for trying to understand how evolution has actually acted at a molecular and genetic level. Furthermore, the current data on homeotic gene expression in crustaceans should not necessarily be taken as evidence in support of the Goldschmidtian view of 'hopeful monsters'. The reduced levels and mosaic patterns of expression seen in mysids suggest that changes in hox gene expression and limb morphology may be occurring gradually through the accumulation of small changes which become fixed in a population over time.

Derived states: the Mandible and Distal-less expression

Genetic experiments in *Drosophila melanogaster* have shown the homeobox gene *Distal-less (Dll)* to be required for proper distal appendage development.[10] *Dll* gene expression patterns in other arthropod taxa suggest that expression of *Dll* along the proximal–distal axis of developing limbs differentiates cells fated to become proximal coxopodite elements (*Dll* protein absent) from cells fated to become distal telopodite elements (*Dll* protein present).[2] Additional comparative studies demonstrate that *Dll* is expressed in distal outgrowths in a large number of phyla and can serve as a molecular marker of distal limb development.[11]

Morphologically, ancestral arthropod head and gnathal appendages are considered to have been composed of a whole limb;[12] that is, coxopodite + telopodite, and this ancestral arthropod was an aquatic organism. In crustaceans, the mandibular segment is the third appendage bearing segment (counting from the anterior end) and sits between the second antennal segment and the first maxillary segment. The mandibles serve as the main food processing appendages and perform their function at the entrance to the mouth. The mandible represents a highly modified appendage composed of

a coxopodite and, variably, a telopodite. Over time the mandible is thought to have been reduced to only a coxopodite in some arthropod taxa. Therefore it has been proposed that *Dll* expression patterns could be used to explore the possible correlation between regulation of a candidate gene's expression and changes in limb morphology.[13–15]

While derived crustacean mandibles are constructed of the coxopodite only [Figure 3(g)], less derived crustacean mandibles are constructed of both a proximal coxopodite and a distal telopodite [Figure 3(d)]. The crustacean mandibular telopodite, historically termed the 'mandibular palp',[1,3,16,17] can either be absent or present, uniramous or biramous, and each branch can be composed of one, two, or three articles. While the biramous telopodite consists of endopod and exopod branches, the uniramous mandibular telopodite is thought to represent a reduced endopod.[2] The distal telopodite of the mandible has, in the course of evolution, undergone multiple independent reduction and loss events. When present, however, the mandibular telopodite is adapted to assist in one or more of the following functions: feeding, grooming, and sensory feedback.[4,18] The mandibular coxopodite in crustaceans typically consists of a single expanded element composed variably of an inner molar surface and an outer incisor. The molar and incisor surfaces combined constitute a functional gnathobase,[3,8] or gnathal lobe.[16] These surfaces have been thought to represent reduced non-articulating endites.

A number of recent studies have examined mandibular *Dll* expression in crustaceans. The initial data[19] from two divergent crustacean taxa gave a conflicting result; *Dll* present in branchiopod (*Artemia*) mandibles, but *Dll* absent in isopod (*Armadilidium*) mandibles. Further studies[20,21] and those from Browne and Patel (unpublished results), examined a wide range of crustacean taxa with regard to mandibular morphology (embryonic, juvenile, and adult) and timing (both embryonic and post-embryonic) of *Dll* expression. The results suggest that arthropod mandibular structure is quite labile, and likely intimately associated with habitat, principally terrestrial versus aquatic adaptations, and food choice. Expression of *Dll* in the mandible foreshadows this morphological variation and is consequently labile in both spatial and temporal expression.

The larvae of *Artemia*, an anostracan branchiopod, possess mandibular telopodites. *Artemia* larvae hatch with only the cephalic and most anterior gnathal segments and appendages. In this case it is the larval mandibles that deliver food to the mouth by sweeping and scraping the antennae setae, which act as food collectors.[22] As larvae begin feeding, the mandibular gnathobase begins to enlarge. Following the ninth molt, distal elements are transformed to a reduced state in adults.[22] *Dll* is expressed in the larval mandible of *Artemia*, but immediately preceding reduction of the mandibular telopodite, *Dll* expression is reduced.[20] The anostracan *Eubranchipus* exhibits a similar course of mandibular development (Browne and Patel, unpublished results).

In *Triops*, a notostracan branchiopod, the larval mandible also possesses a telopodite. As development proceeds there is a similar enlargement of the mandibular gnathobase. In contrast to the two anostracans, however, the reduction of distal regions of the mandible is progressive.[22] Again, *Dll* is expressed in the larval mandible, but as distal elements are progressively reduced and the gnathobase enlarged, *Dll* expression is likewise reduced (Browne and Patel, unpublished results).

Several peracaridian taxa (mysids, amphipods, and isopods) have also been analyzed for embryonic expression of *Dll*. The direct developing mysid *Mysidium columbiae* has well-developed mandibular palps in both juveniles and adults [Figure 3(a)]. *Dll* expression encompasses the entire mandibular limb primordia early [Figure 3(b)]. As development proceeds, *Dll* expression becomes progressively restricted to the developing telopodite of the mandible [Figure 3(c)]. Hatchling *Mysidium* continue to express *Dll* in distal regions of limbs including the mandibular telopodite.

Among amphipods, gammarids share a suite of ancestral characters including presence of well-developed distal elements on their mandibles.[23,24] Morphological analysis shows both juveniles and adults to possess similar mandibular morphologies [Figure 3(d) and (e)]. Early in mandibular limb development, *Dll* protein is detected throughout the mandibular limb primordia of *Gammarus pulex*.[21] As development proceeds, *Dll* expression is restricted to distal regions of the developing mandible[21] [Figure 3(f)]. These results are indicative of a whole-limb mandible, consisting of both coxopodite and telopodite. This pattern of *Dll* expression, starting with expression in the mandibular primordium and then restricting to the developing telopodite, is also seen in a basal malacostracan, the leptostracan *Paranebalia* (Browne and Patel, unpublished results). More phylogenetically derived amphipods of the family Hyalidae, such as *Parhyale hawaiensis* [Figure 3(g)

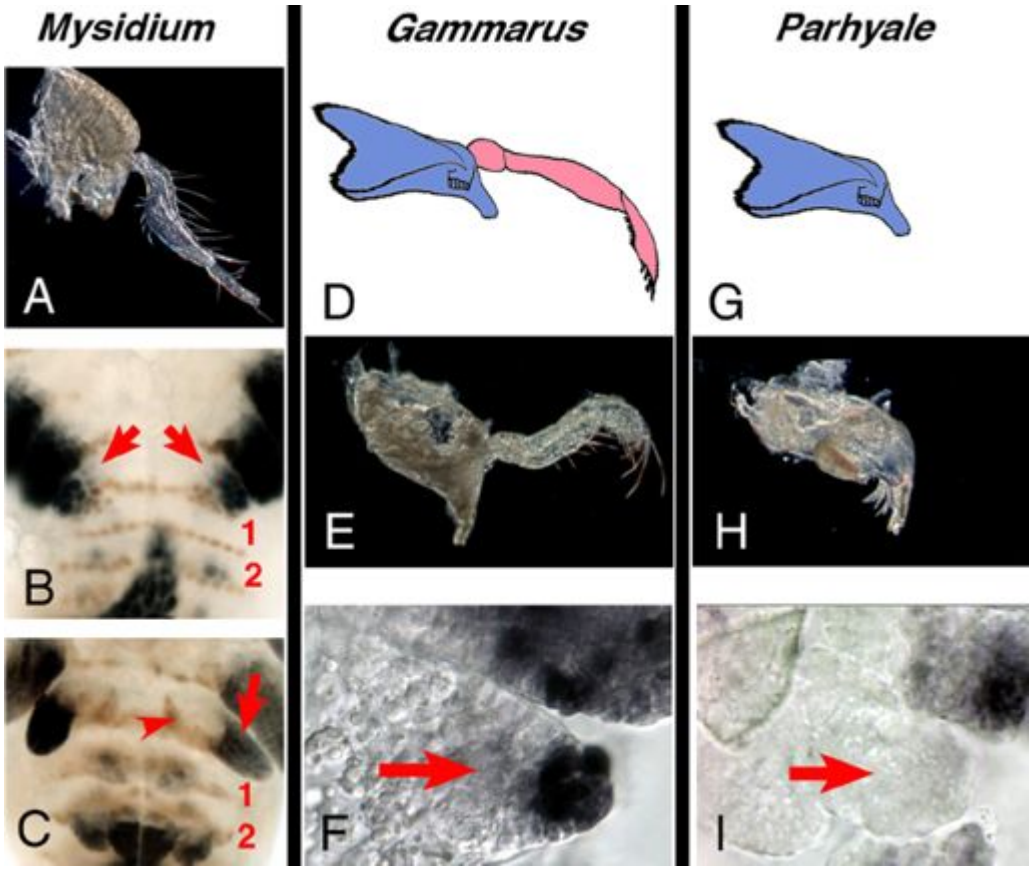

Figure 3. Crustacean mandible development. The ancestral crustacean mandible is thought to have been composed of both a coxopodite and telopodite. Within the Amphipoda, gammarids retain this ancestral character [(d) and (e), in (d) blue shading indicates the gnathobasic coxopodite and red shading indicates the distal telopodite possessing three articulating, jointed, elements]. Multiple independent reduction events have occurred throughout the Crustacea, including within the amphipods, where more derived mandibles are composed of a gnathobasic coxopodite only [(g) and (h), in (g) blue shading indicates the gnathobasic coxopodite]. The expression of *Dll* protein in amphipod mandibles appears early in development (see Reference 21). Maintenance of *Dll* expression, however, appears to be correlated with the later presence of a distal telopodite. In amphipod species where a telopodite does form, *Dll* continues to be expressed in the development of this structure as shown in *Gammarus sp* [(f), the arrow indicates the mandibular primordia]. In amphipod species where a telopodite does not form, *Dll* expression is eliminated from the mandibular appendage as shown in the more derived *Parhyale hawaiensis* [(i), the arrow indicates the mandibular primordia]. An example of progressive exclusion of *Dll* protein from the mandibular base is shown in *Mysidium columbiae* [(a), (b), and (c)]. The mysid mandible is composed of both a gnathobasic coxopodite and a telopodite palp (a). *Dll* protein is initially detected throughout the mandibular limb primordia [(b), the arrows indicate mandibular limb primordia, *Dll* protein expression is in black, *engrailed* (*en*) protein expression is in brown which marks the posterior portion of developing segments and limbs]. Later in development, *Dll* expression in the mandible becomes restricted to the developing telopodite only [(c), the arrowhead indicates the coxopodite, the arrow indicates the telopodite, *Dll* expression is in black, *en* expression is in brown]. The morphological variation between the two maxillary appendages (MxI is reduced relative to MxII, not shown) of *Mysidium columbiae* also correlates with the differential expression of *Dll*. *Dll* expression is present in MxI appendages but at reduced levels relative to MxII appendages [(c), MxI segment is indicated by 1, MxII segment is indicated by 2].

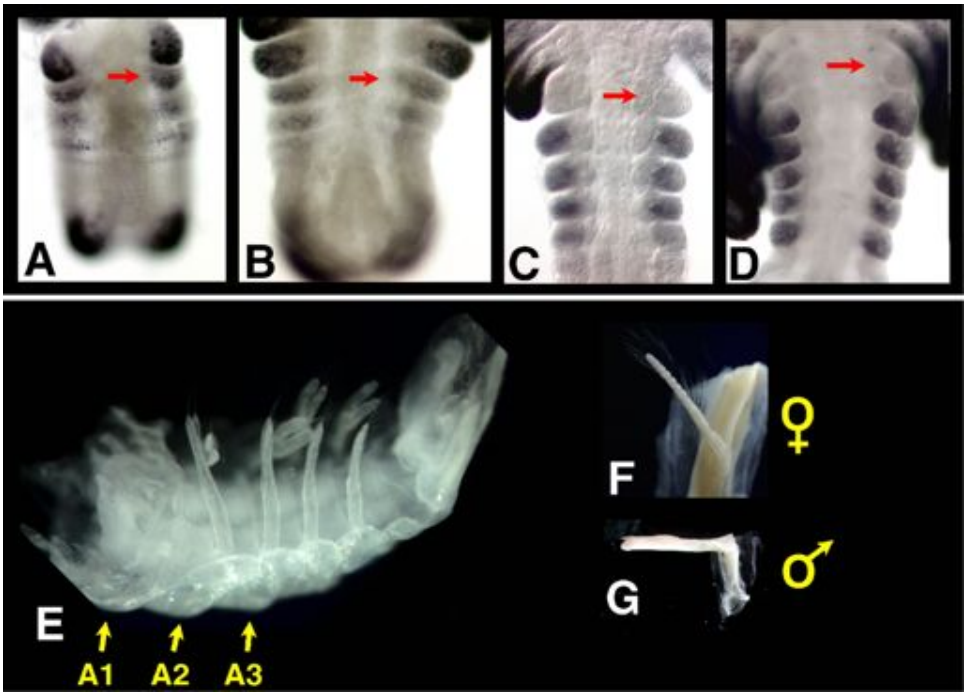

Figure 4. Cambarid crayfish first abdominal appendage (A1). Early in abdomen development, *Dll* is expressed in a one to two cell wide domain which is progressively restricted to the limb primordia [(a) and (b), *Dll* protein expression in black]. At approximately 50% of development *Dll* expression is eliminated in the A1 appendage primordia but continues at elevated levels in all thoracic appendages and abdominal appendages posterior to A1 [(c) and (d)]. Note that the highest levels of late abdominal *Dll* expression are associated with continued cell proliferation along the ventral medial lobes of developing appendages except for the arrested A1 primordia [(d), the arrow indicates the A1 appendage primordia]. In correlation with this loss of Dll expression in A1 during embryogenesis, hatchling crayfish do not possess an appendage on A1 (e). In adult crayfish, however, there is an A1 appendage which is sexually dimorphic [(f) and (g)]. In adult females A1 is reduced (f), and in adult males A1 is a distalized structure modified for copulation and spermatophore transfer [(g); the A2 appendage of adult males has distal specializations as well]. No data are currently available for *Dll* expression in A1 between hatching and sexual maturity.

and (h)], *Hyale azteca*, and the family Talitridae, such as *Orchestia cavimana*,[23] do not possess mandibular palps either as juveniles or adults. In the case of *Orchestia*, *Dll* is expressed in the early primordia of the mandible, but expression is subsequently lost.[21]

Three species of decapods have also been assayed for embryonic *Dll* expression and mandibular structure. In a palaemonid shrimp no mandibular palps are observed in juveniles or adults. In these shrimp, no *Dll* expression in the mandible is detected at later stages of embryogenesis. Early stages, however, have not been examined. In both a cambarid crayfish and a brachyuran crab, *Stenorhynchus*, well-developed mandibular palps are observed in both juveniles and adults. In both of these decapods, embryonic *Dll* expression is detected in developing distal regions of the mandible (Browne and Patel, unpublished results).

The temporal dynamics of *Dll* expression in these various crustaceans suggest that *Dll* is required for the initiation of the mandibular primordia. Subsequently, if the mandibular appendage does not possess a distal telopodite, the expression of *Dll* is progressively reduced and eliminated in the developing mandible. Conversely, if the mandibular appendage possesses a distal telopodite, *Dll* continues to be expressed but becomes restricted to the developing telopodite only.

Another particularly interesting example of temporally dynamic *Dll* expression is observed in the first abdominal (A1) appendage of cambarid crayfish (Figure 4). Cambarid hatchlings do not possess an appendage on A1 [Figure 4(e)], and *Dll* expression is transient in the A1 segment of all embryos. In abdominal segments, *Dll* is initially detected weakly in a one to two cell wide domain. These domains are progressively resolved into limb primordia [Figure 4(a)

and (b)]. By 50% of embryonic development, *Dll* protein is eliminated from the A1 limb primordia. All other abdominal limb primordia continue to express elevated levels of *Dll* [Figure 4(c) and (d)]. As with the data from crustacean mandibles, these data from crayfish A1 appendages would also seem to suggest that the expression of *Dll* is required for the initial specification of limb primordia. Subsequent elimination of *Dll* protein is concomitant with a developmental arrest of the A1 appendage until a later post-hatch growth cycle. These conclusions stand in stark contrast to the observations from *Dll* expression in insect mandibles. In all insects examined, *Dll* expression is never seen in the developing mandibles, suggesting that in this case *Dll* is not needed for the initiation of the primordia. If it becomes possible to manipulate *Dll* expression in crustaceans, it will be interesting to determine whether *Dll* expression is truly required, not only for the formation of specific distal structures in some crustacean mandibles but additionally for mandible primordia formation in all crustaceans, including those without any obvious telopodite.

The data from comparative analysis of *Dll* expression clearly highlight the labile nature of *Dll* expression, and this expression correlates with the variable morphology of the mandible, among both stages of development within a species as well as between species. The combination of *Dll* expression patterns and analyses of both juvenile and adult morphologies of mandibles amongst a range of taxa lead to a number of conclusions. Among Crustacea, embryonic development terminates either indirectly as an intermediate larval form or directly as a fully formed adult. The distinction between indirect and direct development with regard to terminal adult appendage morphologies is variable. Neither the same segments nor the same appendages in the taxa surveyed are modified similarly (attributable to differing larval life histories). The adult morphology of mandibles can be patterned during embryogenesis while other appendages have larval morphologies that are lost post-embryonically, conversely the mandible may be radically reorganized post-embryonically while other appendage morphologies remain relatively static in form. *Dll* expression and concomitant presence or absence of the mandibular palp is clearly a labile character within the Crustacea. The direct correlation of telopodite presence or absence with *Dll* protein presence or absence in the mandible of the taxa sampled would appear to provide us with an example of the integration of developmental, genetic, and structural modules to generate a specific morphology. Mandibular morphology appears to be under selection within the Crustacea and thus this developmental process, in part, may play a role in the high levels of morphological diversity observed in the group. The spatial and temporal dynamics in the expression of other genes involved in limb development are likely to play a role in the diversity of limb types observed in crustaceans and may serve as a model for general principles governing the evolution and generation of arthropod limb morphologies.

Conclusion

Clearly we have only begun to untangle the complex web of connections between genes and morphology. We need more expression studies from a number of time points during the development of individual organisms, as well as expression data from a diverse range of taxa, to be secure in our inferences. For example, we presume that differential expression of members of the hox cluster have played an important role in the evolution of limb morphology, however this inference at the present time is based on a single comparative study of *Ubx/abd-A*. Expression data from other hox cluster members, such as *Scr*, are tantalizing, however data bridging multiple crustacean taxa are needed. With regard to mandibular proximal–distal variation and the expression of *Dll*, we infer that *Dll* may be required for the initiation of the crustacean mandibular appendage primordia and that continued expression is required to generate a distal telopodite. Additional expression data from transcription factors and signaling molecules thought to play a role in the specification of limbs and growth axes are of critical importance for evaluating this argument. Ultimately, however, we will need to test the validity of our inferences and assumptions. This will require the ability to directly perturb gene function outside of the traditional model systems for which these techniques already exist.

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