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Non-insect crustacean models in developmental genetics including an encomium to *Parhyale hawaiensis*

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The impressive diversity of body plans, lifestyles and segmental specializations exhibited by crustaceans (barnacles, copepods, shrimps, crabs, lobsters and their kin) provides great material to address longstanding questions in evolutionary developmental biology. Recent advances in forward and reverse genetics and in imaging approaches applied in the amphipod *Parhyale hawaiensis* and other emerging crustacean model species have made it possible to probe the molecular and cellular basis of crustacean diversity. A number of biological and technical qualities like the slow tempo and holoblastic cleavage mode, the stereotypy of many cellular processes, the functional and morphological diversity of limbs along the body axis, and the availability of various experimental manipulations, have made *Parhyale* a powerful system to study normal development and regeneration.

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Introduction

Arthropods exhibit an enormous diversity and provide ample material for comparative developmental studies (Figure 1a) [1]. Among arthropod groups, insects have attracted disproportionately more attention than crustaceans, myriapods and chelicerates, understandably so because of the great contributions of *Drosophila* research in developmental biology and genetics. Although insects outnumber all metazoans in terms of species number, the nearly 70 000 described crustacean species are unrivalled in terms of form and lifestyle [2,3].

Crustacean model systems in biological research

Crustaceans have had a long history in other fields of biological research, including ecology, neurobiology, anatomy and physiology. Over the last years, a large body of evidence has indicated that insects have evolved from crustaceans [1,4•]. This realization motivated several comparisons of gene expression between insect and crustacean embryos, but also seeded the idea of developing one or more suitable crustacean species as experimental systems for developmental genetic studies.

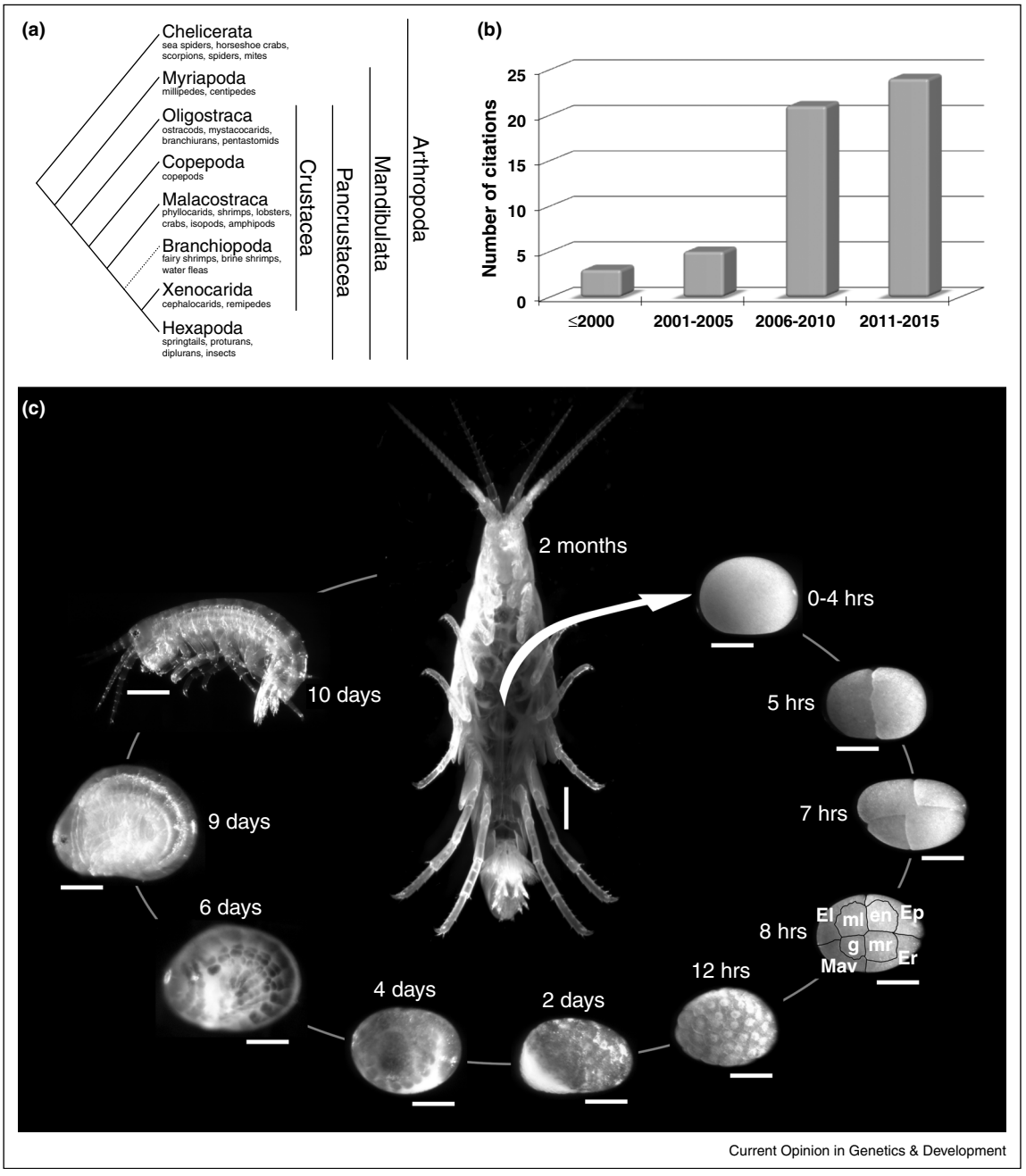
The water flea *Daphnia pulex* has served as a valuable model for environmental, evolutionary and developmental research, and was the first crustacean to have its genome sequenced [5]. Another branchiopod, the brine shrimp *Artemia franciscana*, has been used more extensively in developmental studies to understand the organization and evolution of arthropod body plans [6•,7]. The genetic and cellular basis of embryonic pattern formation has been also probed in representatives from other crustacean classes like barnacles [8,9] and malacostracans [10,11], merely at a descriptive level.

The advent of next generation sequencing technologies, the application of transgenesis and RNAi for functional genetics and, lately, the revolution of the CRISPR/Cas genome editing system have started closing the technological gap between established and emerging arthropod systems [12]. The crustacean model that has benefited the most from these technologies has been arguably the malacostracan amphipod *Parhyale hawaiensis* and will be the focus of the rest of this article.

Paving the way for malacostracans in modern developmental biology

In 2004, Dohle, Scholtz and colleagues remarked: 'Unfortunately, not many investigators take advantage of the fact that in the developing germband of malacostracans, expression of genes can be described with the resolution of single cells of which the origin is known.' [13•]. Malacostracans comprise the well-known and culinary delightful decapods (crabs, lobsters, shrimps, crayfish) and other less-recognizable orders like amphipods (scuds), isopods (woodlice) among others. (Figure 1a) [2,3]. A salient feature of malacostracan embryos that cannot be found in the rest of crustaceans and arthropods is the stereotypy of cell lineages observed during early cleavage and later germband stages [13•,14,15]. This invariance in many cell patterning events, together with

Figure 1



Parhyale hawaiensis, an emerging crustacean model system for developmental genetic research. **(a)** Phylogeny of arthropod and crustacean groups. The depicted relationships among major arthropod groups reflect most current phylogenies. There are still alternative hypotheses regarding the position of certain crustacean lineages like the barnacles (not shown here) and the branchiopods (shown with dotted line). **(b)** Number of citations in PubMed per indicated time period with the keyword 'Parhyale' in their title or abstract. **(c)** *Parhyale* life cycle. *Parhyale* eggs can be dissected from the female's ventral brood pouch at any stage of development and can be cultured in seawater. During the first 8 hours after egg lay, each egg undergoes three total cleavages producing a stereotyped arrangement of four macromeres and four micromeres with restricted cell fates: the three El, Er and Ep macromeres give rise to the ectoderm, the fourth Mav macromere gives rise to the visceral and anterior mesoderm, the ml and mr micromeres form the rest somatic mesoderm, the en and g micromeres give rise to the endoderm and germline, respectively. Later divisions produce yolk-free cells (12 h) that aggregate ventrally and anteriorly to form the embryo rudiment (2 days). During subsequent segmentation stages, the embryo elongates posteriorly and the appendage buds develop in an anterior to posterior progression (4 days). Appendages continue to grow as the yolk gets sequestered in the developing midgut and the head region separates from the trunk (6 days). Organogenesis appears complete during the last days of embryogenesis when the pigmented compound eyes form (9 days). The hatchling that emerges from the egg looks like a miniature adult (day 10). It increases in size through successive molts and reaches sexual maturation about 2 months after egg lay. All scale bars are 200 μm except in the adult female that is 1000 μm.

the amenability of *Parhyale* to a large and ever-increasing number of experimental manipulations, are some of the biological and technical qualities that make this species an increasingly popular model system for modern biological research (Figure 1b).

The biology of *Parhyale hawaiensis*

Parhyale is a marine amphipod crustacean that was introduced in the lab by Browne and Patel in the late 1990s [16••,17]. It has a worldwide tropical distribution living in shallow aquatic habitats and feeding on detritus. This lifestyle makes *Parhyale* a robust experimental organism that thrives under standard culturing conditions.

Parhyale has a life cycle of 7–8 weeks at 26 °C (Figure 1c). Embryogenesis takes about 10 days and the juvenile that emerges from the egg looks like a miniature adult [16••,17]. Thus, almost all aspects of body patterning, growth and differentiation — with the exception of a few traits associated with sexual maturation — can be studied during embryogenesis that is well-described and comprehensively staged [16••,17]. Sexually mature females are distinct from males by their conspicuous gonads and their smaller grasping appendages (limbs) in the thorax (Figure 2a). Male *Parhyale* seize and retain hold of the female until copulation occurs [15,16••]. After sperm transfer, the female molts, gets released from the male and oviposits her fertilized eggs in a ventral brood pouch (Figure 1c). Parhyalists take advantage of these ethological features to streamline the collection of embryos from gravid females [18]. Considering that thousands of animals can be raised routinely in small containers and that adults breed year-round, hundreds of fertilized eggs can be obtained daily for experimental manipulations.

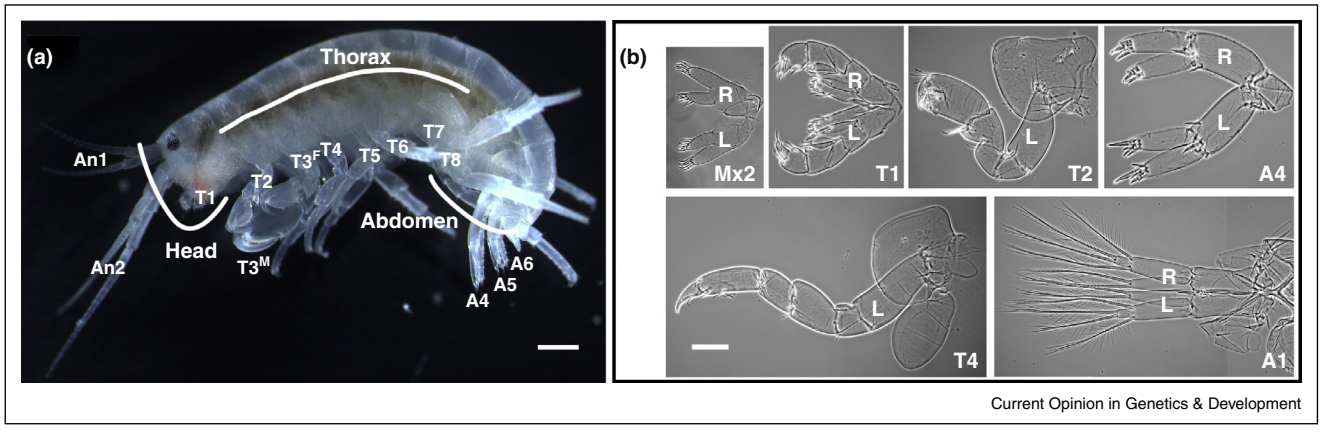
The *Parhyale* body plan

The body of *Parhyale*, like in the rest malacostracans, consists of appendage-bearing segments that are organized into the head, thorax (pereon) and abdomen (pleon) (Figure 2a) [16••]. The lateral compression of the body together with the orientation of the thoracic limbs that are directed forwards and backwards give amphipods their characteristic appearance and name. Many malacostracan groups, including amphipods, exhibit a striking specialization in their appendages that have been adapted for different functions like sensation, feeding, locomotion and others (Figure 2b) [2,3]. The evolution of these groups into living Swiss army knives has no equal among metazoans, and offers excellent material to investigate the molecular, cellular and biophysical basis of organ morphogenesis [16••,19••,20••].

Experimental tools and resources for *Parhyale* research

The *Parhyale* community has developed various experimental approaches and standardized resources that

Figure 2



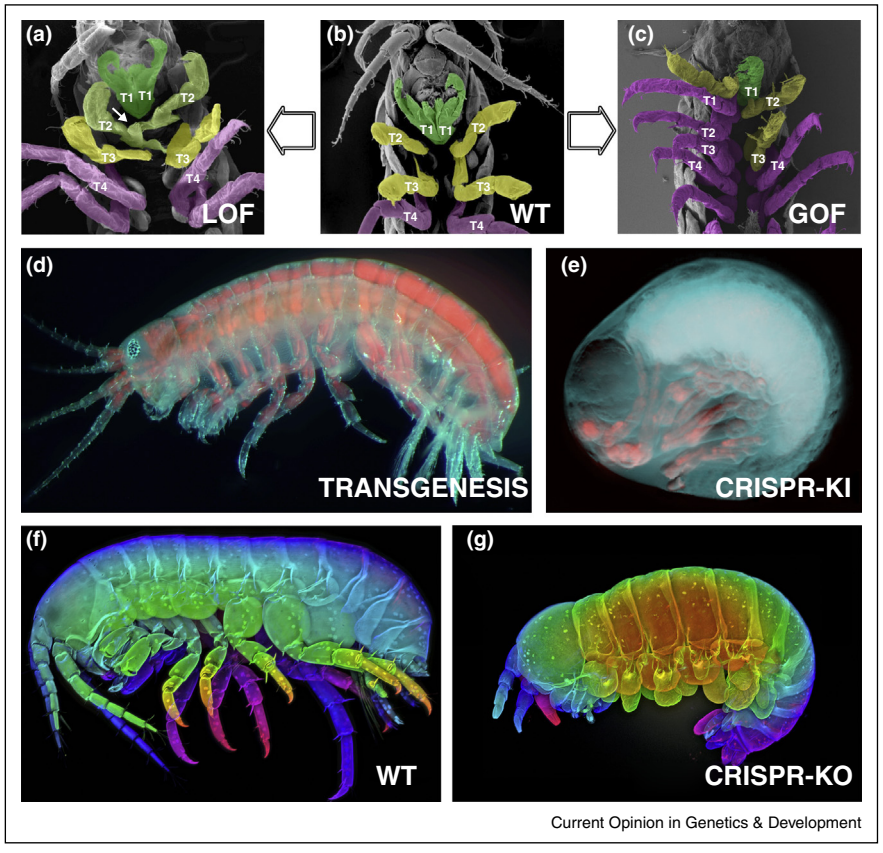
Parhyale body plan and appendage diversity. **(a)** Lateral view of adult *Parhyale* with anterior towards the left and dorsal towards the top. The head region is made up of six segments and carries two pairs of sensory antennae (An1 and An2) and three pairs of feeding appendages (mandibles, maxillae 1 and maxillae 2 concealed in this view). The first thoracic segment (T1) is fused to the head and bears another pair of feeding appendages, known as maxillipeds. T2 and T3 bear clawed appendages known as gnathopods that are used for grasping, while T4 to T8 bear elongated appendages used for walking. The abdomen is composed of six segments bearing three pairs of paddling appendages (A1–A3 pleopods not visible here) followed by three pairs of thickened appendages used for anchoring and jumping (A4–A6 uropods). This particular adult is a spontaneous mutant carrying a small female-like gnathopod ($T3^F$) on its left side and an enlarged male-like gnathopod ($T3^M$) on its right side. **(b)** Cuticle preparations of dissected appendages shown in scale with distal towards the left: maxillae 2 (Mx2), T1 maxillipeds, T2 gnathopod, T4 leg, A1 pleopods and A4 uropods. The feeding appendages like Mx2 and T1, and the abdominal pleopods and uropods are medially fused; both the left (L) and right (R) appendages are shown. Thoracic appendages like T1, T2 and T4 have a single segmented limb branch (uniramous), while abdominal appendages like A1 and A4 are branched distally (biramous). Appendages are uniquely identifiable by the distinct size and shape of their segments, as well as the presence/absence of characteristic pattern elements like cuticle plates, gills and setae. Scale bars are 100 μm.

have advanced *Parhyale* into a powerful system to tackle fundamental questions in developmental biology (Figure 3). The foundational reports describing the early cell fate map and embryonic development of *Parhyale* demonstrated that embryos were amenable to various embryological manipulations and gene expression studies using whole-mount in situ hybridization and immunohistochemistry [21••,22•,23–25]. To facilitate and advance molecular genetic research, a number of genomic and transcriptomic resources were generated by high-throughput sequencing of BAC clones and cDNA libraries [26–30]. Most recently, the huge *Parhyale* genome that resembles the human genome in terms of size and chromosome count was also sequenced, assembled *de novo* and annotated (http://www.ncbi.nlm.nih.gov/genome/15533).

The first transposon-based functional studies also indicated that *Parhyale* embryos could be genetically transformed with high efficiency (Figure 3d) [18,31•]. In these and all subsequent experiments, it was evident that transgene expression was not only detected in transgenic animals (F1, F2 generations), but also in a large fraction of injected embryos (F0 generation). Injection of F0s at the 1-cell stage often resulted in bilateral transgene expression, while single-blastomere injection at the 2-cell stage produced unilateral expression due to early lineage restrictions [19••,20••,31•]. These features are extremely useful for experimentation in *Parhyale*, first, because they enable fast and reliable F0 genetic approaches, and second, because they allow the comparison of wild-type versus the genetically perturbed conditions in the same embryo (Figure 3c).

The transgenesis toolkit in *Parhyale* was further expanded with a site-specific integrase system [32]. The establishment of transposon and integrase-based transformation systems has increased the sophistication and versatility of

Figure 3



Functional genetic manipulations in *Parhyale*. **(a)** Scanning electron microscopy analysis after RNAi knocked-down of gene activity (loss-of-function), **(b)** of a wild-type juvenile, and **(c)** after trangenesis-based misexpression of a gene (gain-of-function). In (a), the T2 gnathopods (yellow) have been partially transformed into T1 maxillipeds (green), while in (c), the T1 maxilliped and T2/T3 gnathopods have acquired the T4 identity (magenta) on one side but are wild-type on the contralateral side. **(d)** Transgenic animal expressing a muscle-specific fluorescent reporter construct (in red) merged with the auto-fluorescence of the cuticle (in cyan). **(e)** CRISPR-mediated knock-in (KI) of a construct driving expression of a fluorescent reporter in the appendages (shown in red) merged with the corresponding bright field image (in cyan). **(f)** Wild-type juvenile stained for cuticle and color-coded by depth, and **(g)** similarly stained mutant with truncated appendages after CRISPR-based gene knock-out (KO). Panels a–c are ventral views with anterior upwards, while d–g are lateral views with anterior towards the left and dorsal towards the top. *Source*: Panels a and b were reproduced from [34] and panel c from [19••].

genetic manipulations in *Parhyale* with unbiased gene trapping screens and the redeployment of gene traps for various applications [32,33]. The characterization of endogenous heat-inducible promoters allowed the development of conditional misexpression systems for gain-of-function genetic studies [18,19••], while RNA interference and morpholino-mediated gene knock-down were employed for complementary loss-of-function approaches (Figure 3a–c) [34,35]. The inherent limitations of gene knock-down approaches, like transient and incomplete reduction in gene function, were recently mitigated with the application of the revolutionary CRISPR/Cas system for targeted genome editing [12]. More specifically, CRISPR/Cas editing has been adapted to completely knock-out gene function in *Parhyale* embryos [20••], as well as for knock-in approaches to generate fluorescent reporters of gene expression (Figure 3e–g) [36•]. As with all previously tested functional genetic manipulations, the slow tempo and complete early cleavage mode of *Parhyale* embryogenesis resulted in very high targeting efficiencies and low levels of mosaicism in treated embryos [20••].

Finally, *Parhyale* has stood up to the challenge of making the link between the genetic and cellular basis of development. The advent of genetic tools for live imaging, in combination with the transparency and low autofluorescence of *Parhyale*, have allowed detailed microscopic inspections of cellular dynamics with exceptional spatiotemporal resolution. Different types of light microscopy, including bright field, confocal and multi-view light-sheet microscopy have been used successfully to image embryonic and post-embryonic processes over several days of development ([37•,38,39]; http://www.cell.com/pictureshow/lightsheet2).

The genetic basis of *Parhyale* appendage specialization

Most of these techniques have been applied primarily to study appendage development and diversification in *Parhyale*, and elucidate the role of patterning genes in body plan evolution. Hox genes have been long linked to segmental specialization in arthropods [40,41], but *Parhyale* and other crustacean species are extreme illustrations of this association (Figure 2b). Comprehensive studies have suggested that *Parhyale* Hox genes are clustered in the genome and expressed collinearly along the anterior–posterior body axis [36•]. The different appendage types are specified by a remarkable Hox code that involves distinct combinations of Hox genes together with intrasegmental modulation in the patterns and levels of Hox gene expression [36•]. Homeotic transformations of one appendage type into another have been generated systematically using complementary gain-of-function and loss-of-function approaches (Figure 3a–c) [19••,20••,34]. These functional studies, together with the comparison of the morphological transitions and expression domains of Hox homologues between *Parhyale* and other crustacean groups have provided compelling evidence that changes in Hox genes are causally related with phenotypic variation and evolution [42••,43]. The stage is set in *Parhyale* to investigate the differential cell behaviors and target genes modulated by Hox genes to control morphogenesis and diversification of serially homologous structures.

Lineage restriction and cell fate specification

Parhyale fertilized eggs undergo a series of complete, stereotyped cleavages. Formed blastomeres are uniquely identifiable based on their size, position and contacts (Figure 1c) [21••]. Cell lineage studies in *Parhyale* and other amphipods have revealed very early restrictions in the fate of these blastomeres with important implications for various experimental manipulations [21••,44•]. The first cleavage separates the left from the right side for most of the ectodermal and mesodermal tissues. Just two cleavages later, at the 8-cell stage, each blastomere is further restricted to a single germ layer (Figure 1c), although a certain capacity for regulation within each germ layer is observed after blastomere ablation [45•]. These properties of the early *Parhyale* embryo make it an excellent model system to study longstanding questions in developmental biology: What is the role of invariant cell lineages and how plastic are they? What is the relative contribution of cell history versus cell communication in different processes and at different stages of development? What is the identity of cell fate determinants and how conserved are they? A number of embryological, genetic and genomic approaches have started addressing these issues in the *Parhyale* embryo revealing both mosaic as well as regulative patterns of development [30,38,45•].

Parhyale germband formation and maturation

The malacostracan germband is composed of an early-forming anterior head (naupliar) region and a posterior (post-naupliar) region that gives rise to the posterior head and all trunk segments sequentially in anterior-to-posterior progression [13•]. The ectodermal cells in this post-naupliar region become organized in a highly ordered grid of cells where each row of cells corresponds to one parasegment. The majority of malacostracan groups generate the parasegmental rows through sequential asymmetric divisions of stem cell-like cells called ectoteloblasts. Ectoteloblasts are absent in amphipods where the grid is self-organized by scattered cells that become aligned in parasegmental rows [14,16••,38]. The post-naupliar germband grows in size through a series of stereotyped events including the progressive addition of new rows at the posterior end and invariant rounds of mitotic divisions in each formed parasegment. During germband morphogenesis, the metameric organization transitions from parasegmental to segmental with the establishment of segmental boundaries and formation of paired appendage buds in each segment. Contrary to ectoderm development, post-naupliar mesoderm formation follows the stem cell-based

teloblastic mode in all malacostracan groups [38,46,47]. Significant effort has been and continues to be invested in understanding the genetic and cellular mechanisms underlying (para)segment formation, neurogenesis, myogenesis and appendage outgrowth in *Parhyale*. For example, oscillations in the expression of segmentation genes appear to be a common theme during axial elongation in *Parhyale*, insects (but not *Drosophila*), other arthropods and vertebrates (RJ Parchem, PhD thesis, University of California, Berkeley, 2008; [48–50]).

Tissue and organ regeneration in *Parhyale*

Ongoing studies in *Parhyale* are also offering a fresh look at the molecular and cellular basis of regeneration. *Parhyale* are able to regenerate their appendages after amputation [32]. It has been possible to systematically label all different lineages and identify lineage-specific progenitors contributing to the regenerating muscles, epidermis and neurons [51••]. Furthermore, the availability of transgenic lines labeling specific cell types led to the discovery of satellite-cell-like muscle progenitors in *Parhyale* which resemble muscle regeneration in vertebrates [51••]. Another extraordinary case under investigation is the ability of *Parhyale* to regenerate its germline. Normally, the germline segregates from the soma at the 8-cell stage and is formed by one blastomere (Figure 1c) [21••,22•]. Surprisingly, ablation of this blastomere did not result in sterile animals but in fertile animals that were somehow able to replace their lost germ cells (MS Modrell, PhD thesis, University of California, Berkeley, 2007). In all these studies, researchers have benefited from the early cell fate restriction in the *Parhyale* embryo and the availability of various embryological and functional genetic tools to identify, track and manipulate the cells involved.

Concluding remarks

In their updated classification of the crustaceans, Martin and Davis wrote: 'No group of plants or animals on the planet exhibits the range of morphological diversity seen among the extant Crustacea' [52]. We are now able to actively pursue the developmental genetic basis of this diversity. This is what makes studies in *Parhyale* and other emerging crustacean models so exciting.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Akam M: **Arthropods: developmental diversity within a (super) phylum**. *Proc Natl Acad Sci U S A* 2000, **97**:4438-4441.

2. Brusca RC, Brusca GJ: *Invertebrates*. edn second. Sinauer Associates; 2003.

3. VanHook AM, Patel NH: **Crustaceans**. *Curr Biol* 2008, **18**: R547-R550.

4. • Regier JC, Shultz JW, Zwick A, Hussey A, Ball B, Wetzer R, Martin JW, Cunningham CW: **Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences**. *Nature* 2010, **463**:1079-1083.
This comprehensive molecular phylogeny of the arthropods has provided strong support for the monophyly of Pancrustacea (crustaceans and insects), has resolved crustacean relationships, and has identified the Xenocarida (cephalocarids and remipedes) as the crustacean sister group to insects.

5. Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, Tokishita S, Aerts A, Arnold GJ, Basu MK *et al.*: **The ecoresponsive genome of *Daphnia pulex***. *Science* 2011, **331**:555-561.

6. • Averof M, Akam M: **Hox genes and the diversification of insect and crustacean body plans**. *Nature* 1995, **376**:420-423.
This is one of the seminal studies linking changes in Hox gene expression patterns — rather than changes in the number of Hox genes — with the evolution of body plans and segmental specializations in arthropods.

7. Copf T, Schroder R, Averof M: **Ancestral role of caudal genes in axis elongation and segmentation**. *Proc Natl Acad Sci U S A* 2004, **101**:17711-17715.

8. Blin M, Rabet N, Deutsch JS, Mouchel-Vielh E: **Possible implication of Hox genes Abdominal-B and abdominal-A in the specification of genital and abdominal segments in cirripedes**. *Dev Genes Evol* 2003, **213**:90-96.

9. Scholtz G, Ponomarenko E, Wolff C: **Cirripede cleavage patterns and the origin of the Rhizocephala (Crustacea: Thecostraca)**. *Arthropod Syst Phylo* 2009, **67**:219-228.

10. Abzhanov A, Kaufman TC: **Crustacean (malacostracan) Hox genes and the evolution of the arthropod trunk**. *Development* 2000, **127**:2239-2249.

11. Hejnol A, Schnabel R, Scholtz G: **A 4D-microscopic analysis of the germ band in the isopod crustacean *Porcellio scaber* (Malacostraca Peracarida) — developmental and phylogenetic implications**. *Dev Genes Evol* 2006, **216**:755-767.

12. Gilles AF, Averof M: **Functional genetics for all: engineered nucleases CRISPR and the gene editing revolution**. *Evodevo* 2014, **5**:43.

13. • Dohle W, Gerberding M, Hejnol A, Scholtz G: **Cell lineage, segment differentiation, and gene expression in crustaceans**. In *Evolutionary Developmental Biology of Crustacea*, vol. 15. Edited by Scholtz GAA. Balkema Publishers; 2004 Crustacean Issues.
This is a scholarly review on crustacean development, where the authors provide histological descriptions and cell lineage reconstructions of ectoderm and mesoderm formation combined with gene expression data.

14. Dohle W, Scholtz G: **Clonal analysis of the crustacean segment: the discordance between genealogical and segmental borders**. *Development* 1988, **104(Suppl.)**:147-160.

15. Wolff C, Gerberding M: **"Crustacea": comparative aspects of early development**. In *Ecdysozoa II: Crustacea. Evolutionary Developmental Biology of Invertebrates.* Edited by Wanninger A. Springer-Verlag; 2015:4.

16. •• Browne WE, Price AL, Gerberding M, Patel NH: **Stages of embryonic development in the amphipod crustacean *Parhyale hawaiensis***. *Genesis* 2005, **42**:124-149.
This detailed staging system of Parhyale embryogenesis is a must-read for anyone working with crustaceans. The authors used a plethora of morphological, anatomical and molecular markers to classify 30 reference stages of Parhyale embryogenesis.

17. Rehm EJ, Hannibal RL, Chaw RC, Vargas-Vila MA, Patel NH: **The crustacean *Parhyale hawaiensis*: a new model for arthropod development**. *Cold Spring Harb Protoc* 2009, **2009** (pdb emo114).

18. Kontarakis Z, Pavlopoulos A: **Transgenesis in non-model organisms: the case of Parhyale**. *Methods Mol Biol* 2014, **1196**:145-181.

19. •• Pavlopoulos A, Kontarakis Z, Liubicich DM, Serano JM, Akam M, Patel NH, Averof M: **Probing the evolution of appendage specialization by Hox gene misexpression in an emerging model crustacean**. *Proc Natl Acad Sci U S A* 2009, **106**:13897-13902.
This article describes a transgenesis-based conditional misexpression system in Parhyale for gain-of-function analyses. This approach was used to demonstrate that the spatiotemporal pattern and levels of Hox gene expression specify distinct types of thoracic and gnathal appendage identities in Parhyale.

20. •• Martin A, Serano JM, Jarvis E, Bruce HS, Wang J, Ray S, Barker CA, O'Connell LC, Patel NH: **CRISPR/Cas9 mutagenesis reveals versatile roles of Hox genes in crustacean limb specification and evolution**. *Curr Biol* 2016, **26**:14-26.
This article describes a complemetary CRISPR-based knock-out system in Parhyale for loss-of-function gene studies. By systematically perturbing the activity of Hox genes expressed in the Parhyale mouth and trunk, the authors were able to identify the Hox codes underlying appendage specialization and evolution in crustaceans.

21. •• Gerberding M, Browne WE, Patel NH: **Cell lineage analysis of the amphipod crustacean *Parhyale hawaiensis* reveals an early restriction of cell fates**. *Development* 2002, **129**:5789-5801.
This is the first report of Parhyale development describing its early fate map. Lineage tracing of the blastomeres produced during the first three cleavages revealed the early lineage restrictions to particular axial positions and germ layers.

22. • Extavour CG: **The fate of isolated blastomeres with respect to germ cell formation in the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2005, **277**:387-402.
This article provides a beautiful demonstration of the embryological manipulations available in Parhyale. The author combined classic embryological manipulations with gene expression approaches to study the developmental potential of isolated blastomeres.

23. Rehm EJ, Hannibal RL, Chaw RC, Vargas-Vila MA, Patel NH: **Injection of *Parhyale hawaiensis* blastomeres with fluorescently labeled tracers**. *Cold Spring Harb Protoc* 2009, **2009** (pdb prot5128).

24. Rehm EJ, Hannibal RL, Chaw RC, Vargas-Vila MA, Patel NH: **Antibody staining of *Parhyale hawaiensis* embryos**. *Cold Spring Harb Protoc* 2009, **2009** (pdb prot5129).

25. Rehm EJ, Hannibal RL, Chaw RC, Vargas-Vila MA, Patel NH: **In situ hybridization of labeled RNA probes to fixed *Parhyale hawaiensis* embryos**. *Cold Spring Harb Protoc* 2009, **2009** (pdb prot5130).

26. Parchem RJ, Poulin F, Stuart AB, Amemiya CT, Patel NH: **BAC library for the amphipod crustacean *Parhyale hawaiensis***. *Genomics* 2010, **95**:261-267.

27. Zeng V, Villanueva KE, Ewen-Campen BS, Alwes F, Browne WE, Extavour CG: **De novo assembly and characterization of a maternal and developmental transcriptome for the emerging model crustacean *Parhyale hawaiensis***. *BMC Genomics* 2011, **12**:581.

28. Zeng V, Extavour CG: **ASGARD: an open-access database of annotated transcriptomes for emerging model arthropod species**. *Database (Oxford)* 2012, **2012**:bas048.

29. Blythe MJ, Malla S, Everall R, Shih YH, Lemay V, Moreton J, Wilson R, Aboobaker AA: **High through-put sequencing of the *Parhyale hawaiensis* mRNAs and microRNAs to aid comparative developmental studies**. *PLoS One* 2012, **7**:e33784.

30. Nestorov P, Battke F, Levesque MP, Gerberding M: **The maternal transcriptome of the crustacean *Parhyale hawaiensis* is inherited asymmetrically to invariant cell lineages of the ectoderm and mesoderm**. *PLoS One* 2013, **8**:e56049.

31. • Pavlopoulos A, Averof M: **Establishing genetic transformation for comparative developmental studies in the crustacean *Parhyale hawaiensis***. *Proc Natl Acad Sci U S A* 2005, **102**:7888-7893.
This work represents the first report of stable genetic transformation and reporter construct expression in a crustacean species. This study paved the way for establishing Parhyale as a powerful model system for functional genetic studies.

32. Kontarakis Z, Pavlopoulos A, Kiupakis A, Konstantinides N, Douris V, Averof M: **A versatile strategy for gene trapping and trap conversion in emerging model organisms**. *Development* 2011, **138**:2625-2630.

33. Kontarakis Z, Konstantinides N, Pavlopoulos A, Averof M: **Reconfiguring gene traps for new tasks using iTRAC**. *Fly (Austin)* 2011, **5**:352-355.

34. Liubicich DM, Serano JM, Pavlopoulos A, Kontarakis Z, Protas ME, Kwan E, Chatterjee S, Tran KD, Averof M, Patel NH: **Knockdown of Parhyale Ultrabithorax recapitulates evolutionary changes in crustacean appendage morphology**. *Proc Natl Acad Sci U S A* 2009, **106**:13892-13896.

35. Ozhan-Kizil G, Havemann J, Gerberding M: **Germ cells in the crustacean *Parhyale hawaiensis* depend on Vasa protein for their maintenance but not for their formation**. *Dev Biol* 2009, **327**:230-239.

36. • Serano JM, Martin A, Liubicich DM, Jarvis E, Bruce HS, La K, Browne WE, Grimwood J, Patel NH: **Comprehensive analysis of Hox gene expression in the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2016, **409**:297-309.
This article provides a comprehensive analysis of the genomic organization and expression dynamics for all Hox genes in Parhyale. It also describes the first application of the CRISPR/Cas technology for genome editing to generate a fluorescent reporter of Hox gene activity.

37. • Alwes F, Hinchen B, Extavour CG: **Patterns of cell lineage, movement, and migration from germ layer specification to gastrulation in the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2011, **359**:110-123.
This study provides a powerful illustration of live imaging and lineage reconstruction in Parhyale. The authors compared patterns of cell division and cell movement from early cleavage to germ disk formation between wild-type and experimentally perturbed embryos to elucidate mosaic and regulative aspects of early embryogenesis.

38. Hannibal RL, Price AL, Patel NH: **The functional relationship between ectodermal and mesodermal segmentation in the crustacean, *Parhyale hawaiensis***. *Dev Biol* 2012, **361**:427-438.

39. Chaw RC, Patel NH: **Independent migration of cell populations in the early gastrulation of the amphipod crustacean *Parhyale hawaiensis***. *Dev Biol* 2012, **371**:94-109.

40. Hughes CL, Kaufman TC: **Hox genes and the evolution of the arthropod body plan**. *Evol Dev* 2002, **4**:459-499.

41. Pavlopoulos A, Averof M: **Developmental evolution: Hox proteins ring the changes**. *Curr Biol* 2002, **12**:R291-R293.

42. •• Averof M, Patel NH: **Crustacean appendage evolution associated with changes in Hox gene expression**. *Nature* 1997, **388**:682-686.
This is a landmark study in the field of Evolutionary Developmental Biology that suggested a causal association between a recurrent change in Hox gene expression and a particular appendage transformation during crustacean evolution.

43. Averof M, Pavlopoulos A, Kontarakis Z: **Evolution of new appendage types by gradual changes in Hox gene expression — the case of crustacean maxillipeds**. *Palaeodiversity* 2010, **3(Suppl.)**:141-146.

44. • Wolff C, Scholtz G: **Cell lineage, axis formation, and the origin of germ layers in the amphipod crustacean *Orchestia cavimana***. *Dev Biol* 2002, **250**:44-58.
This article describes the early fate map of another amphipod model species, the beachhopper *Orchestia cavimana*. This analysis revealed an early lineage separation like in Parhyale, as well as slight differences in the fate and dynamics of individual blastomeres between the two species.

45. • Price AL, Modrell MS, Hannibal RL, Patel NH: **Mesoderm and ectoderm lineages in the crustacean *Parhyale hawaiensis* display intra-germ layer compensation**. *Dev Biol* 2010, **341**:256-266.
In this tour de force of Parhyale embryology, authors have systematically labeled and ablated all possible blastomere combinations to study developmental plasticity in the Parhyale embryo. Despite the early lineage restrictions, blastomeres have the ability for intra-germ layer compensation during early but not later stages of development.

46. Price AL, Patel NH: **Investigating divergent mechanisms of mesoderm development in arthropods: the expression of Ph-twist and Ph-mef2 in *Parhyale hawaiensis***. *J Exp Zoolog B Mol Dev Evol* 2008, **310**:24-40.

47. Hannibal RL, Price AL, Parchem RJ, Patel NH: **Analysis of snail genes in the crustacean *Parhyale hawaiensis*: insight into snail gene family evolution**. *Dev Genes Evol* 2012, **222**:139-151.

48. Sarrazin AF, Peel AD, Averof M: **A segmentation clock with two-segment periodicity in insects**. *Science* 2012, **336**:338-341.

49. Dequeant ML, Pourquie O: **Segmental patterning of the vertebrate embryonic axis**. *Nat Rev Genet* 2008, **9**:370-382.

50. Benton MA, Pechmann M, Frey N, Stappert D, Conrads KH, Chen YT, Stamataki E, Pavlopoulos A, Roth S: **Toll genes have an ancestral role in axis elongation**. *Curr Biol* 2016, **26**:1609-1615.

51. •• Konstantinides N, Averof M: **A common cellular basis for muscle regeneration in arthropods and vertebrates**. *Science* 2014, **343**:788-791.
In this seminal study, the authors probed the cellular basis of limb regeneration in Parhyale and found key similarities to the vertebrate paradigm, including the involvement of lineage-specific progenitors like satellite cells for muscle regeneration.

52. Martin JW, Davis GE: *An Updated Classification of the Recent Crustacea*. Natural History Museum of Los Angeles County; 2001.